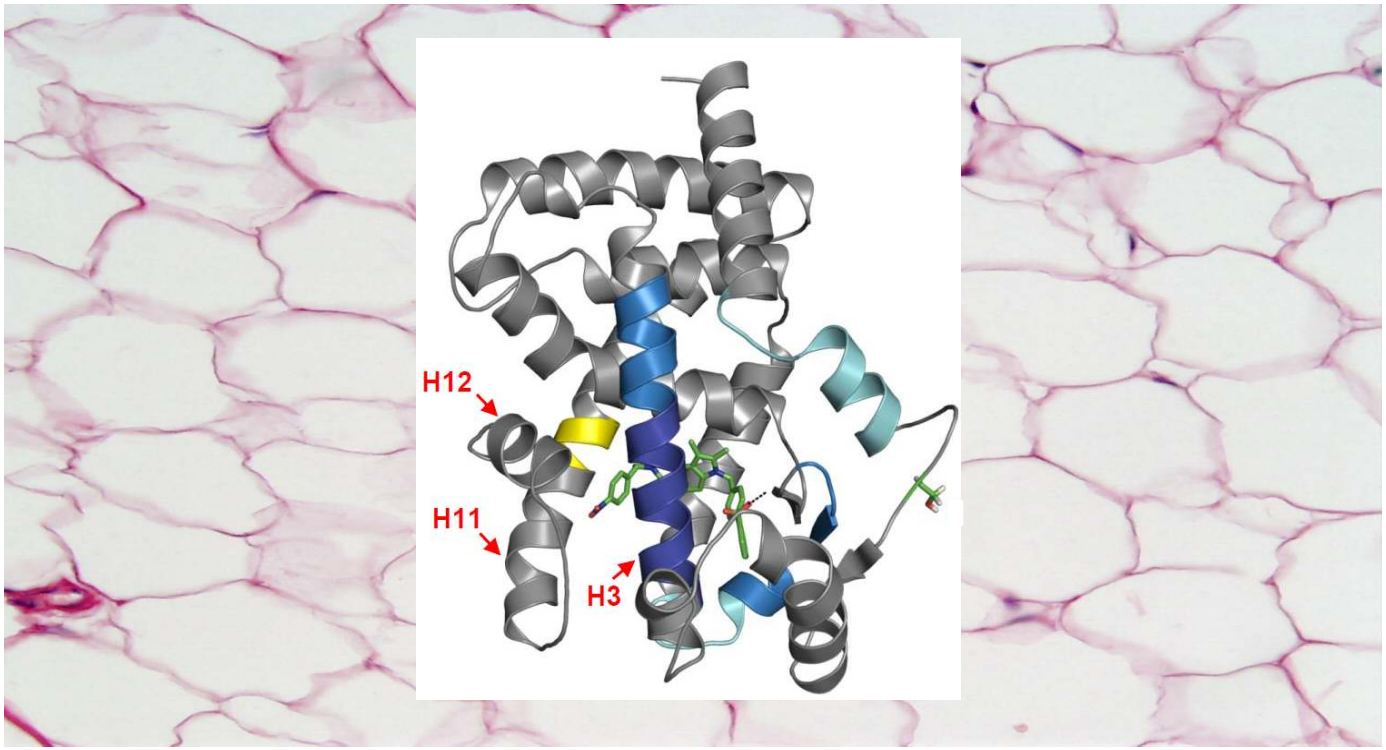
PPARγ, phosphorylation and the anti-diabetic PPARγ ligands

a new look at an old friend



Jang Hyun Choi Ph.D.

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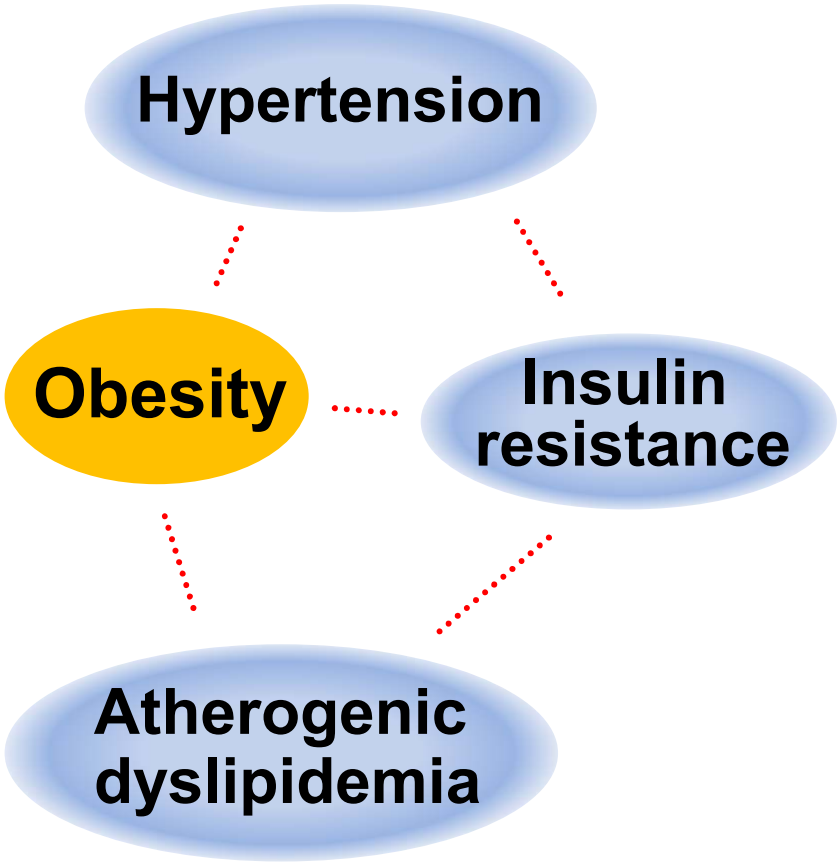
Metabolic Syndrome



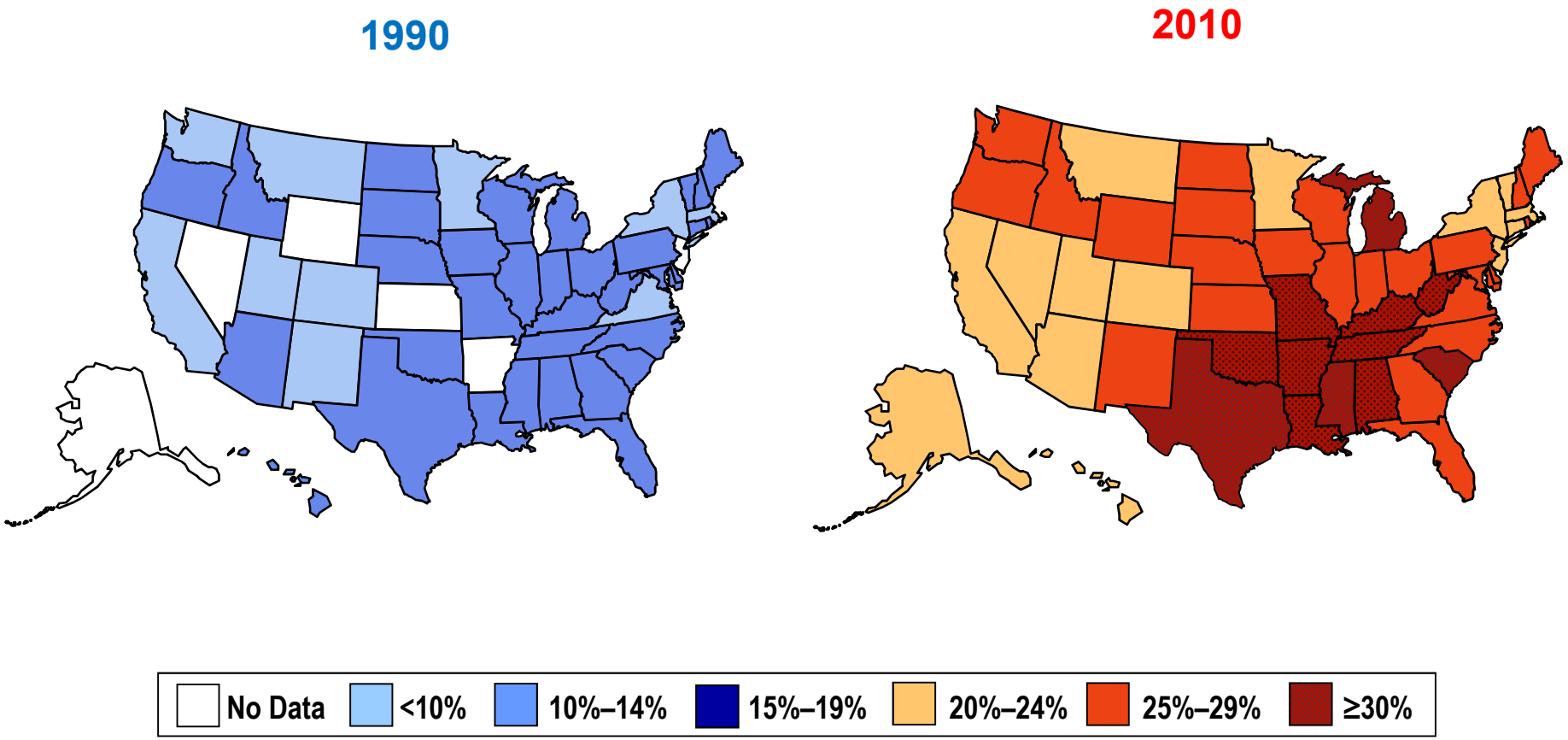
Type 2 diabetes

Cardiovascular disease

Certain cancers

Obesity trends among U.S. adults

(*BMI ≥30, or about 30 lbs. overweight for 5'4" person)



CDC (2011)

David, after a short stay in America

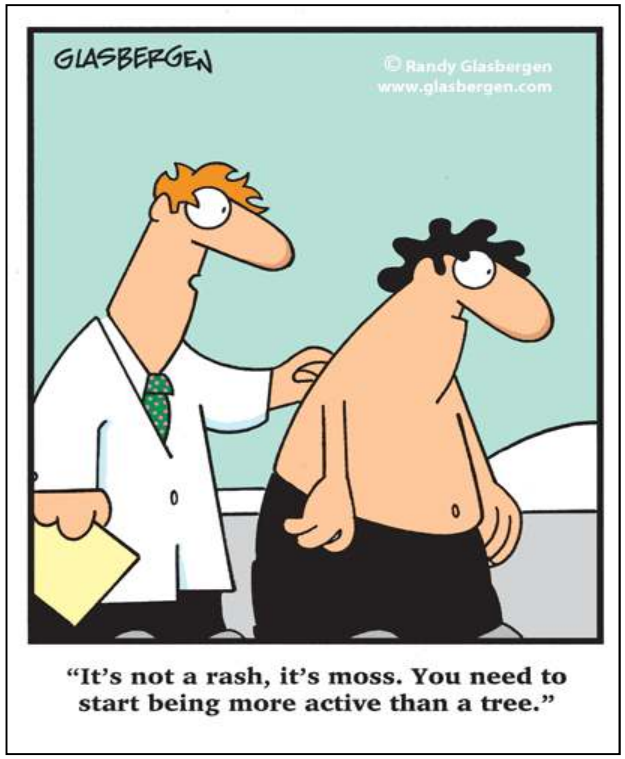
What should we do for managing metabolic syndrome?

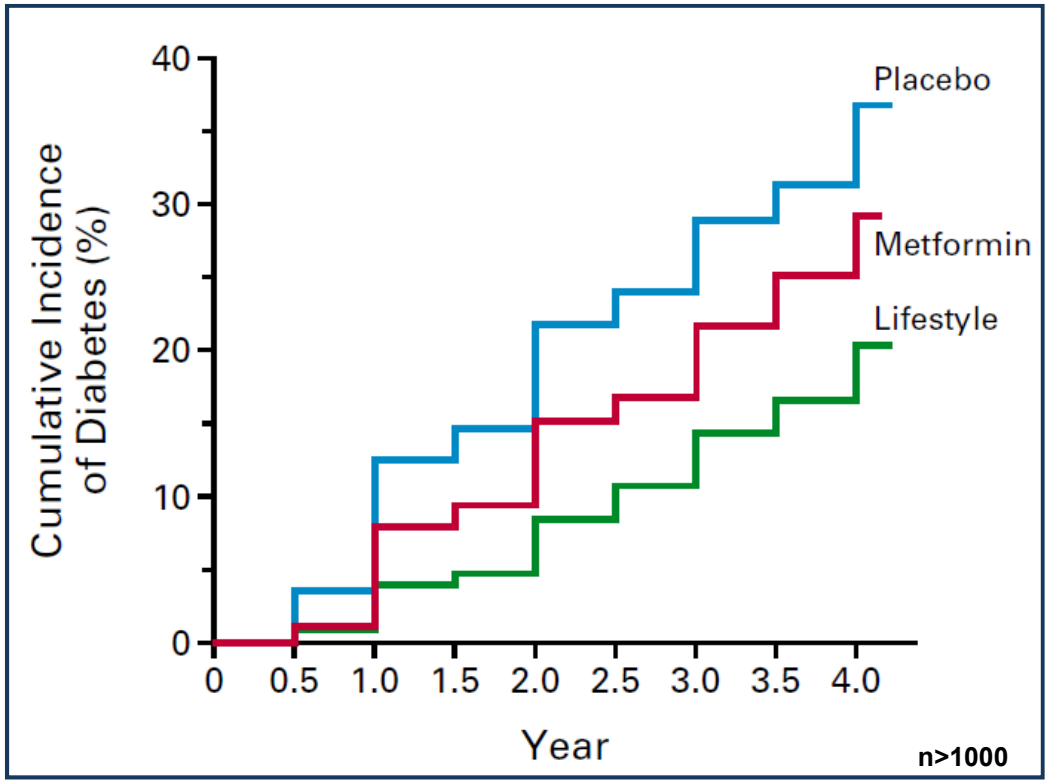
Management of diabetes

- **The major strategies for treating diabetes are:**

A	• Diet and Exercise
B	• Oral hypoglycaemic therapy
C	• Insulin Therapy

Exercise and diet





N. Eng. J. Med (2002)

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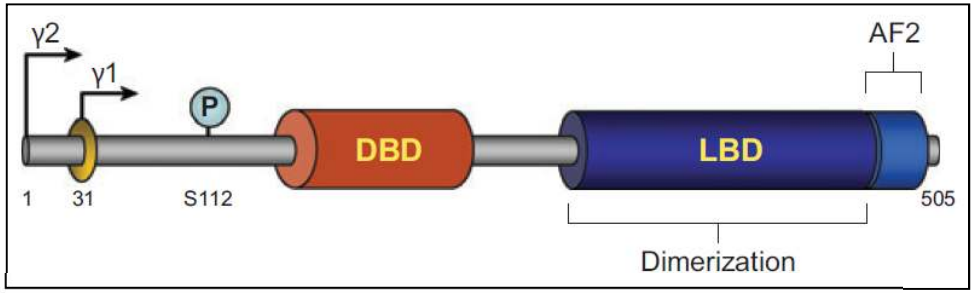
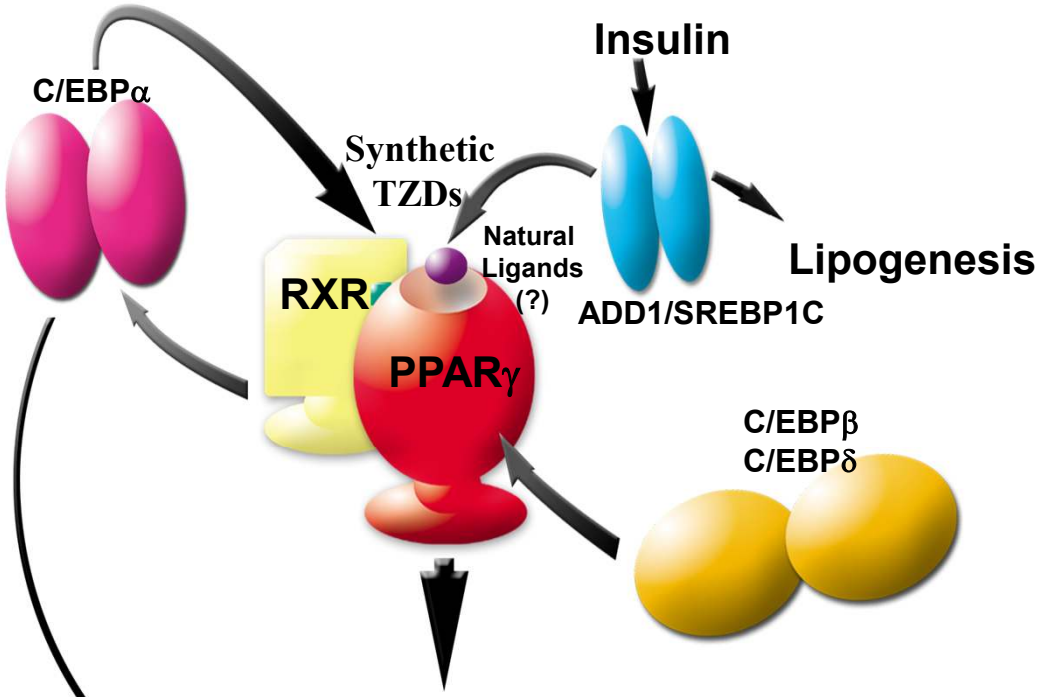
Oral hypoglycemic medications

Drug Class	Drug Name	Brand Name	Mechanism of Action
Biguanides	Metformin	Glucophage	Inhibit glucose production by the liver
Sulfonylureas (second-generation)	Glimepiride Glipizide Glyburide	Amaryl Glucotol Diabeta Glynase PresTab Micronase	Increase insulin secretion by pacreatic beta cells
Meglitinides	Repaglinide Nateglinide	Prandin Starlix	Increase insulin secretion by pancreatic beta cells
Thiazolidinediones (PPARγ specific full agonist ligands)	Pioglitazone Rosiglitazone	Actos Avandia	Increase glucose uptake by skeletal muscle
Alpha-glucosidase inhibitors	Acarbose Miglitol	Precose Glyset	Inhibit carbohydrate absorption in the small intestine

What is PPARγ?





Fat Cell-Specific Gene Expression
Fat Differentiation
Insulin Sensitivity

Annu. Rev. Biochem. (2008)

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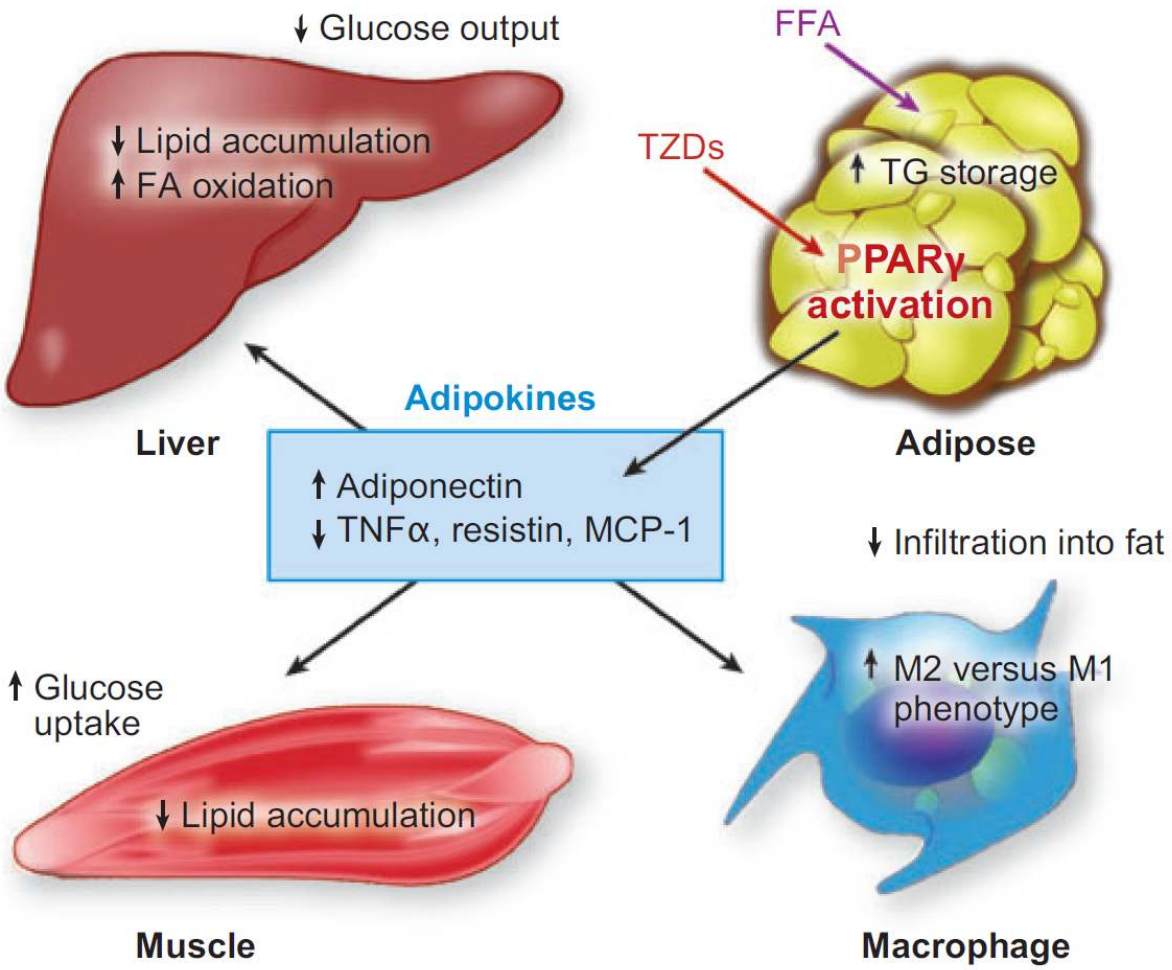
Role of TZDs in PPARγ-mediated glucose metabolism



Annu. Rev. Biochem. (2008)

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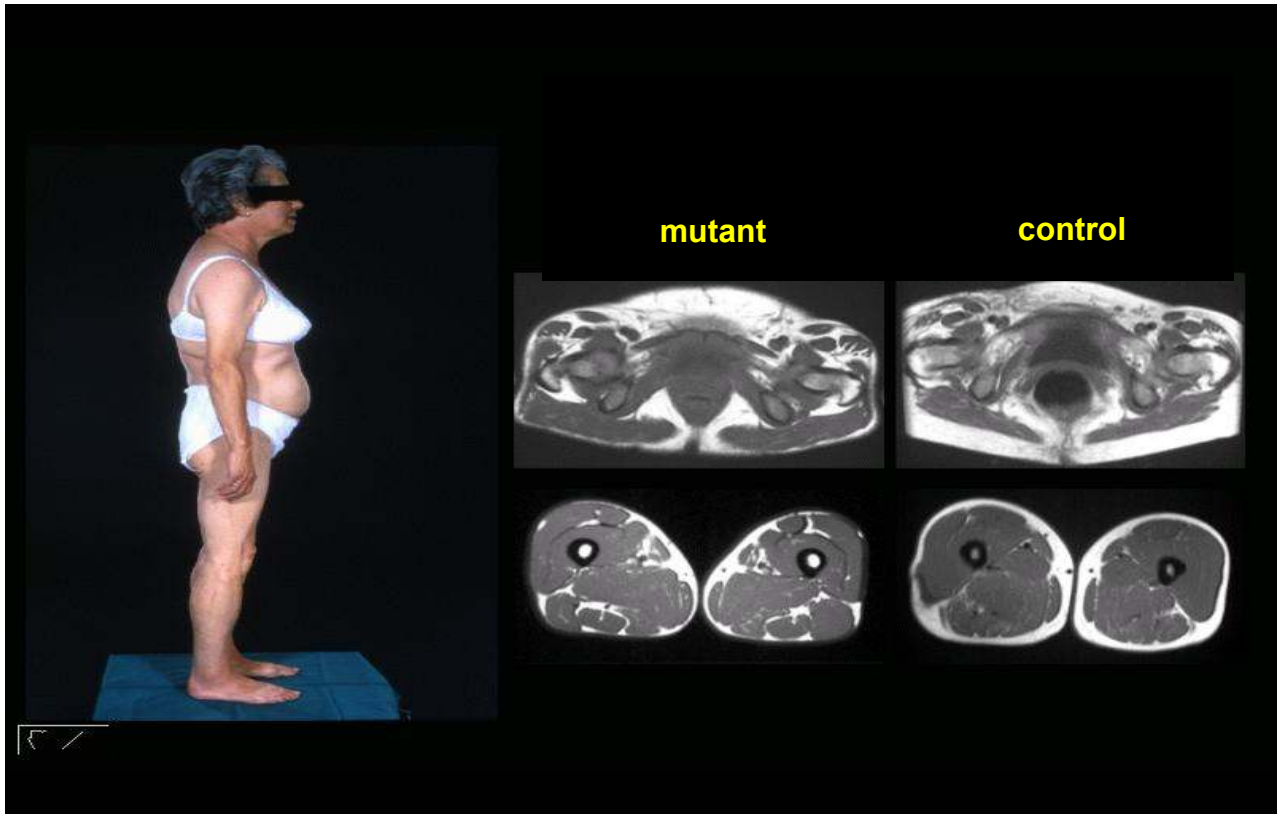
Dominant-negative PPARγ mutation in human



P467L mutation
MRI images
56-year-old female

Severe Insulin Resistance

Limb and buttock lipodystrophy

Severe dyslipidaemia

Early Onset Hypertension

Nature (1999)
Diabetes (2003)

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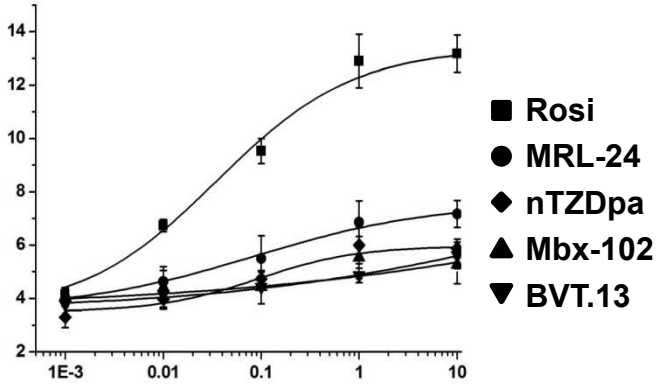
Paradoxes regarding PPARγ and insulin-resistance

1. **Partial loss of function mutations in PPARγ in humans unambiguously cause severe insulin resistance.**
2. **PPARγ agonists improve insulin-resistance and diabetes.**
3. **Most PPARγ target genes are already fully "ON" in obesity.**
4. **Severe side effects of PPARγ full agonists (TZDs) such as heart failure, weight gain, fluid retention.**
5. **Some PPARγ ligands with poor agonist activity (partial agonists) still have *marked* anti-diabetic actions.**

PPARγ full agonist ligands : TZDs (rosiglitazone etc.)
PPARγ partial agonist ligands : MRL24, nTZDpa etc.



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Paradoxes regarding PPARγ and insulin-resistance

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4. Severe side effects of PPARγ full agonists (TZDs) such as heart failure, weight gain, fluid retention.
5. Some PPARγ ligands with poor agonist activity (partial agonists) still have *marked* anti-diabetic actions.

Can we separate PPARγ agonism from anti-diabetic actions?

Are there novel mechanisms linking PPARγ to insulin-resistance?

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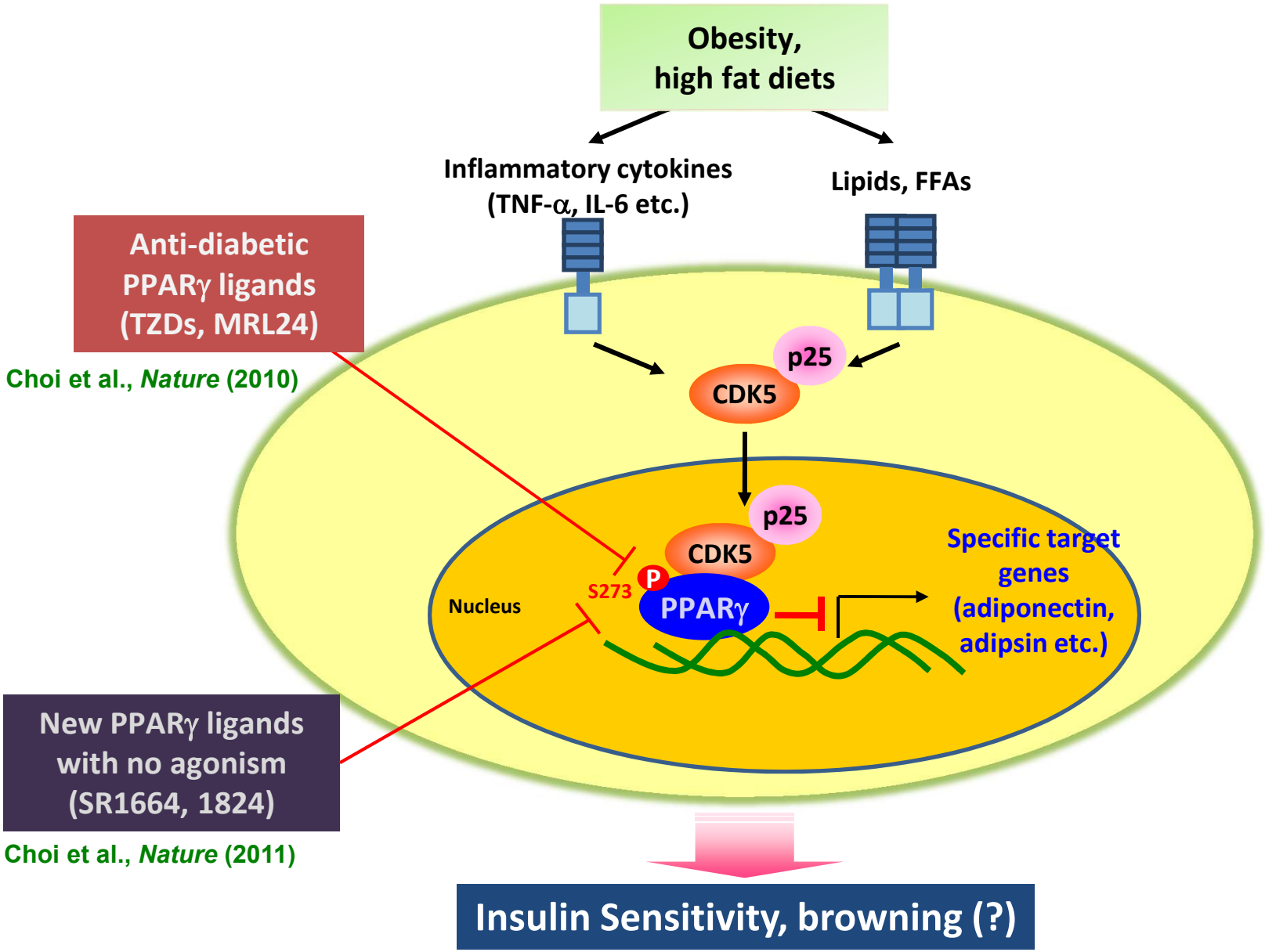
Schematic model of PPARγ and anti-diabetic PPARγ ligands



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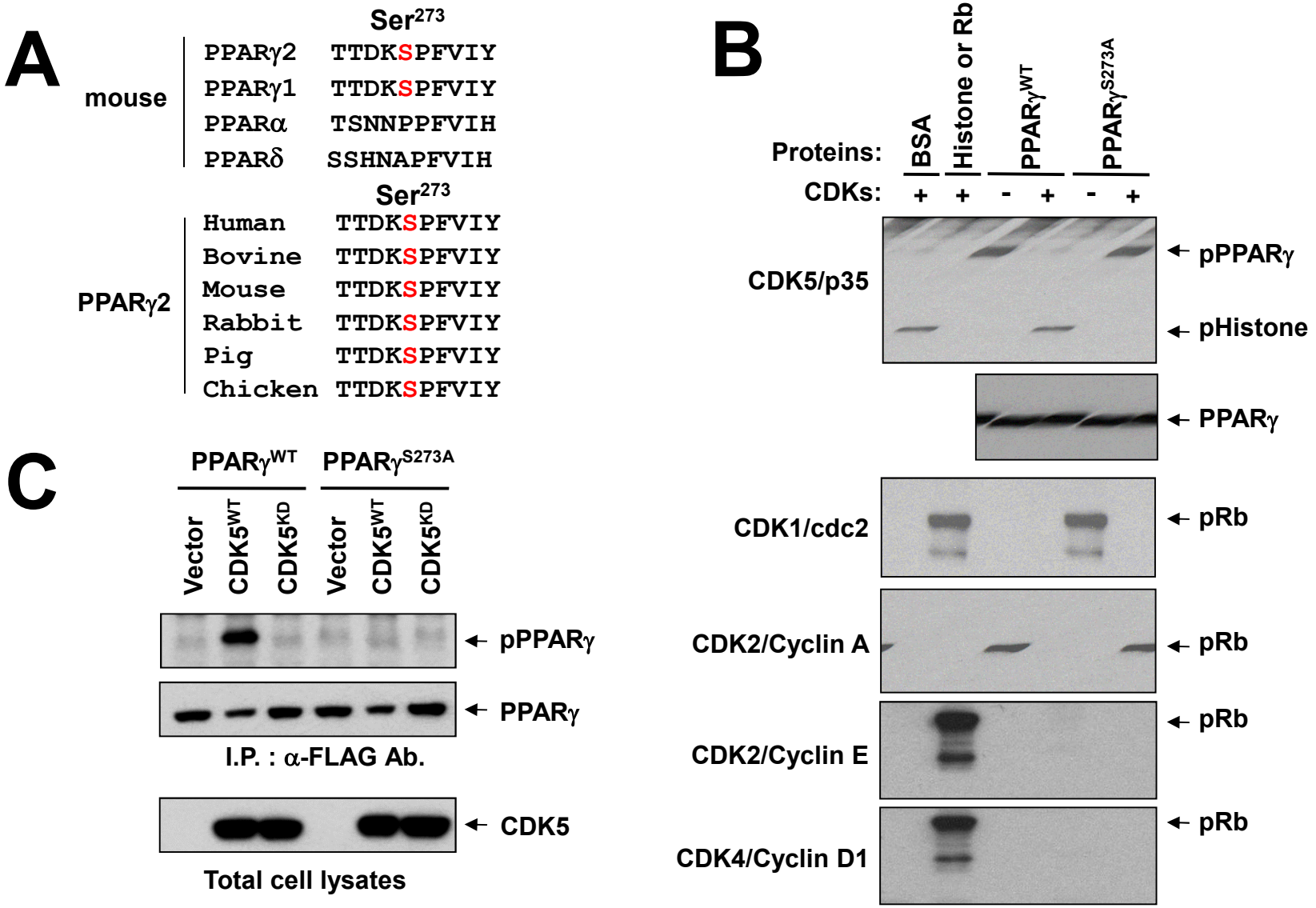
Cdk5 phosphorylates PPARγ



Choi et al., *Nature* (2010)

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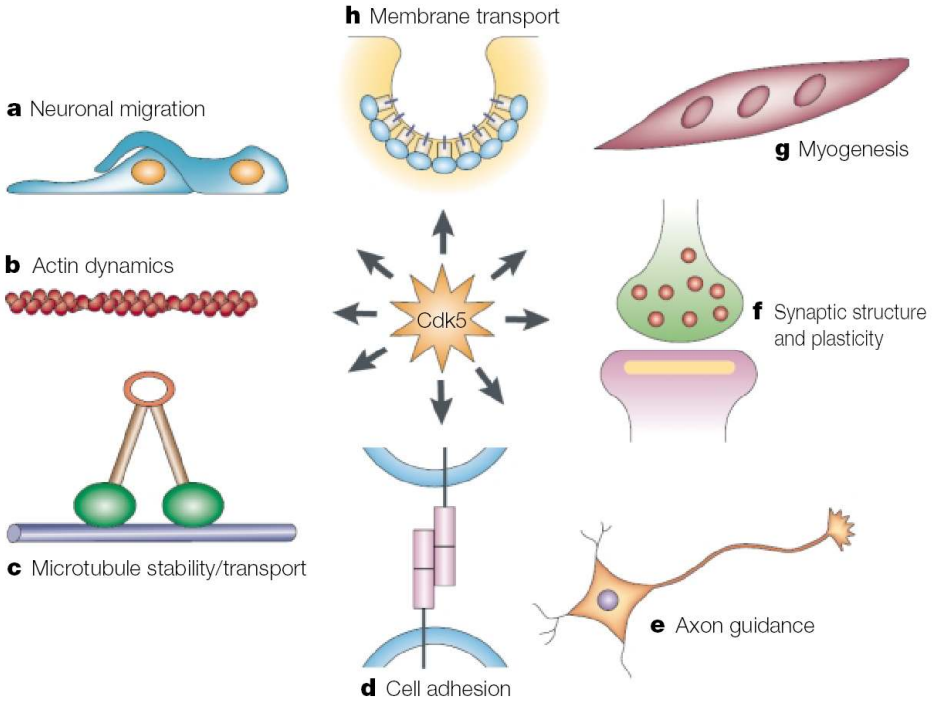
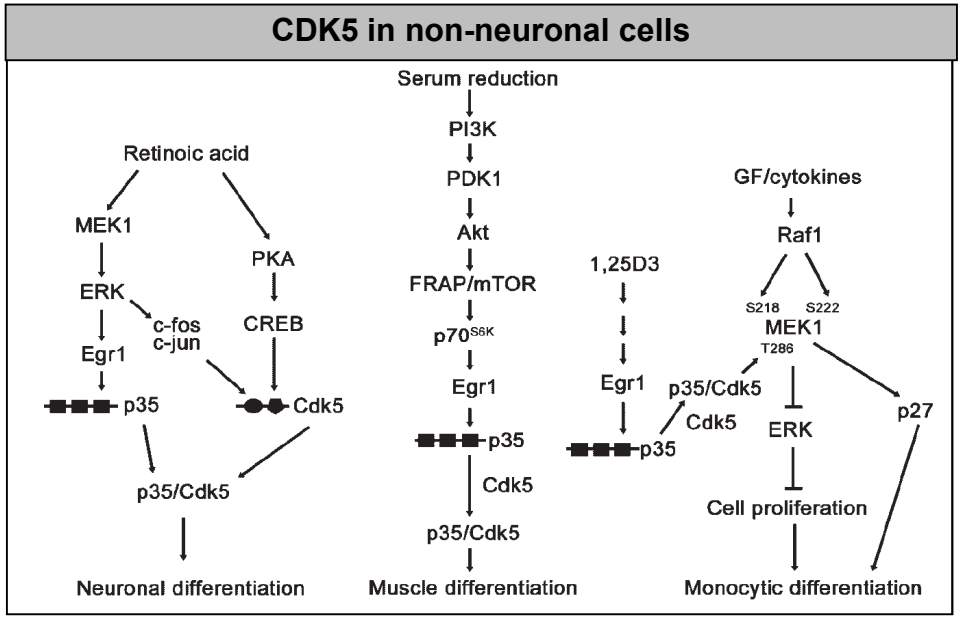
What is Cdk5?





CDK5 KO : perinatal death
Defect on corticogenesis
Cerebellar defolidation

***Bioassay* (2004)**
***Nat. Med.* (2005)**
***Nat. Rev. MCB* (2001)**

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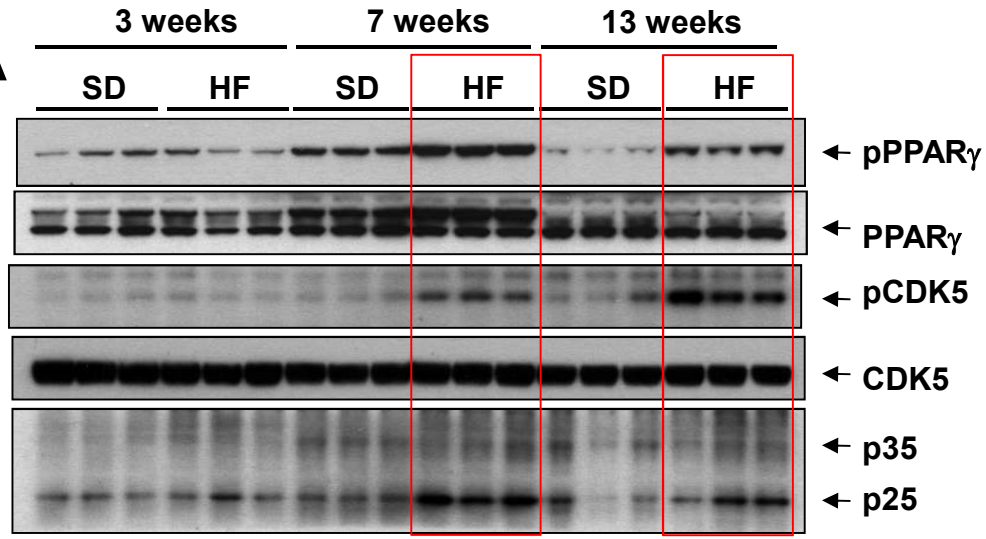
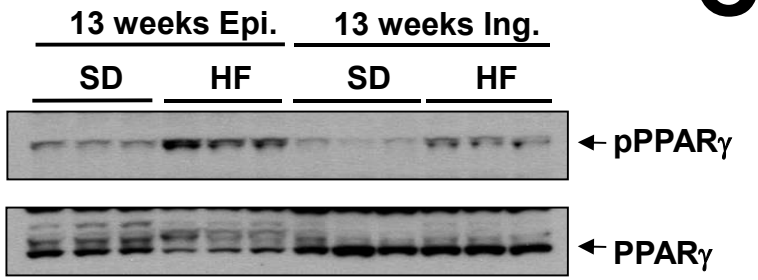
Obesity-induced phoshorylation of PPARγ in fat tissues

A



B



C

	7 weeks Chow	7 weeks HFD	13 weeks Chow	13 weeks HFD
Weight (grams)	26.2 ± 0.3	33.8 ± 1***	29.4 ± 0.6	44.5 ± 1.1***
Glucose (mg/dl)	88.2 ± 6.4	92.6 ± 5.4	115.4 ± 8.9	124.4 ± 6.5
Insulin (ng/ml)	0.1 ± 0.03	0.18 ± 0.06	0.24 ± 0.03	1.07 ± 0.13**

Choi et al., *Nature* (2010)

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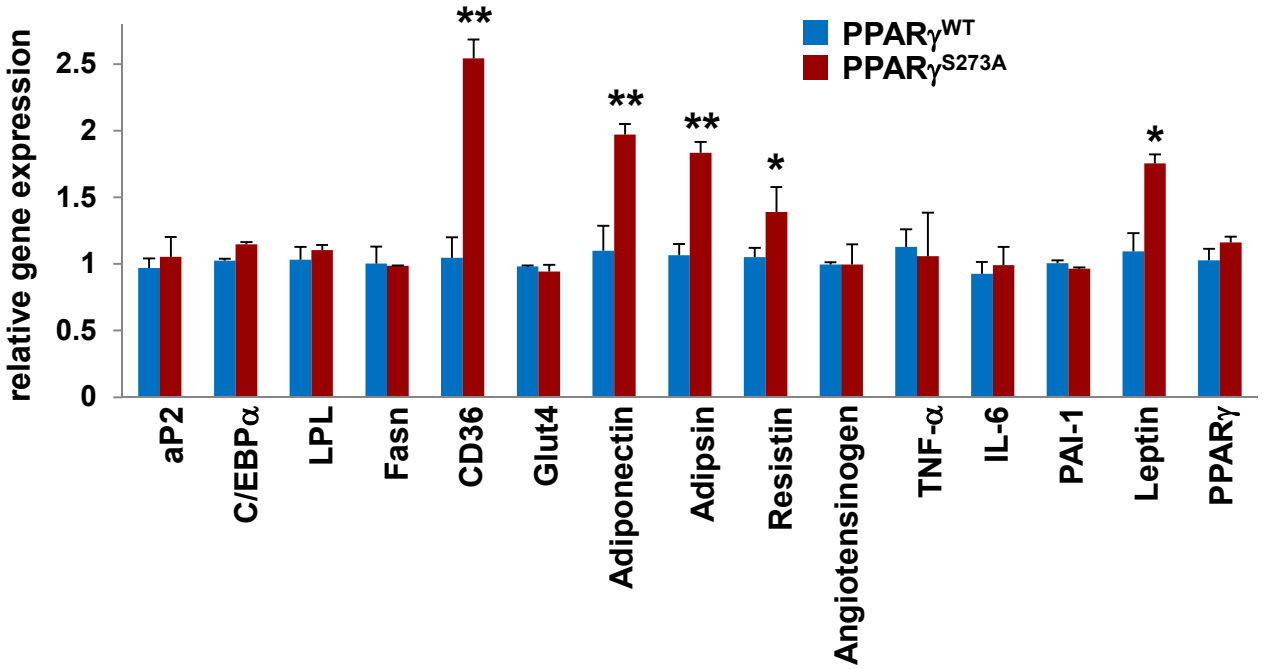
What is the biological role of phosphorylation?

Specific fat cell genes regulated by phosphorylation



Choi et al., *Nature* (2010)

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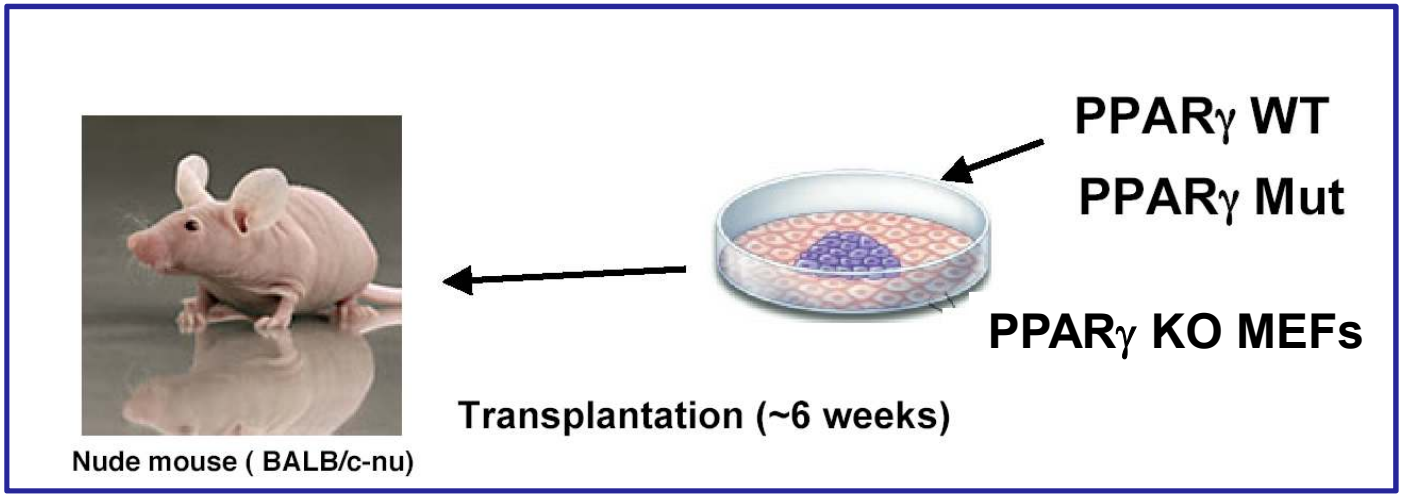
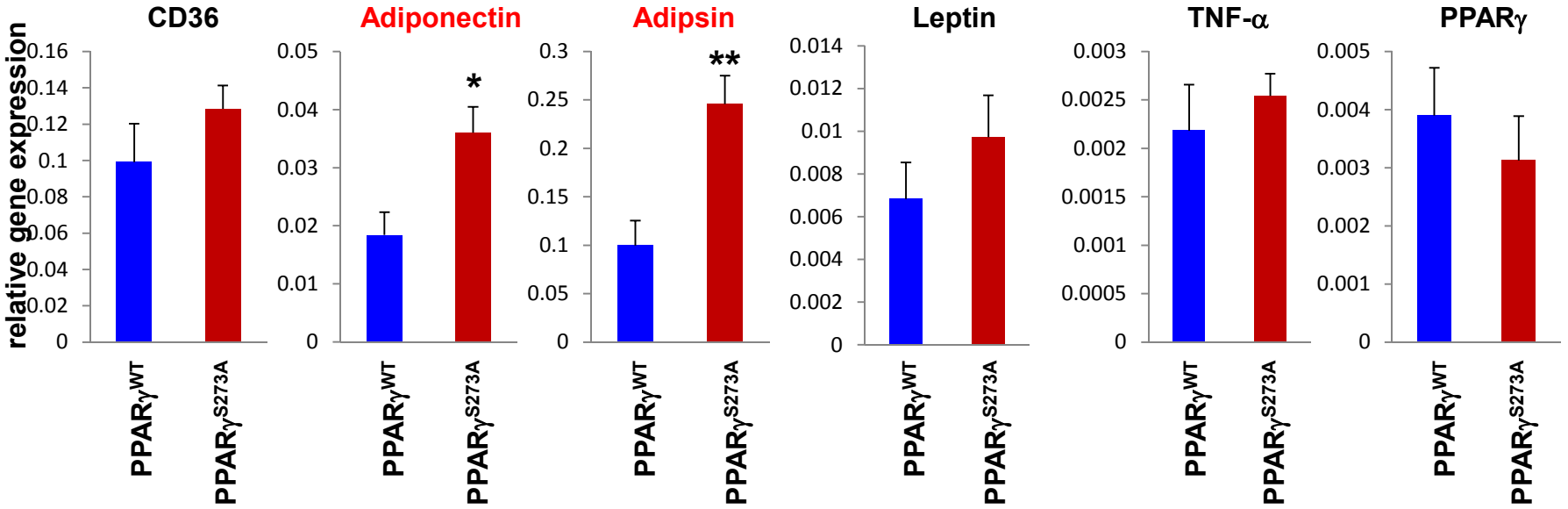
Specific fat cell genes regulated by phosphorylation





Choi et al., *Nature* (2010)

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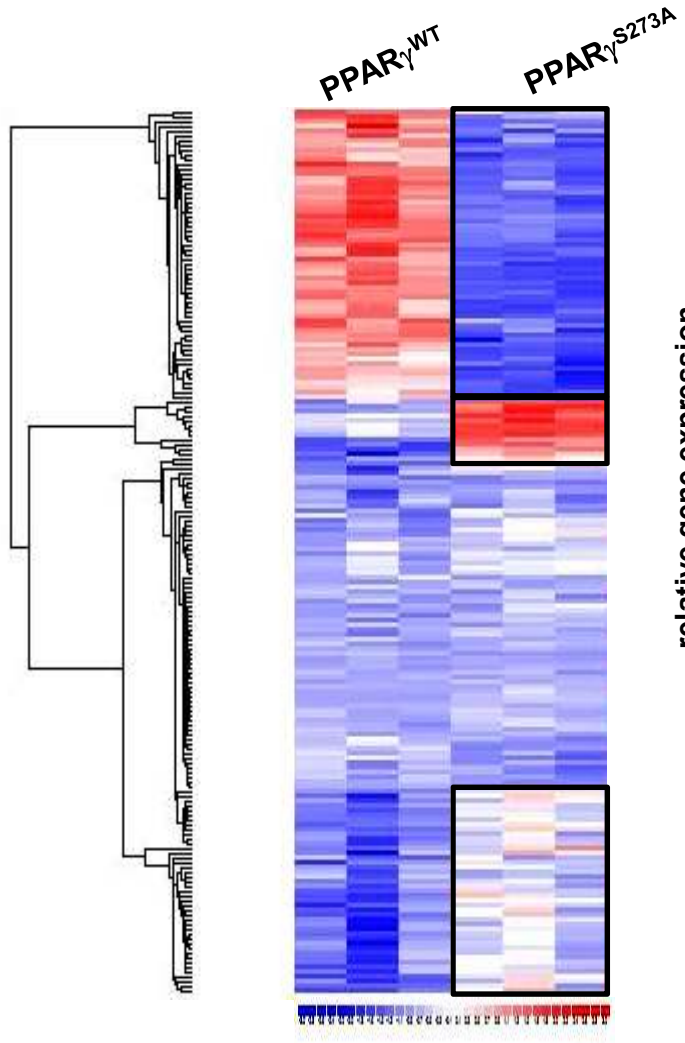
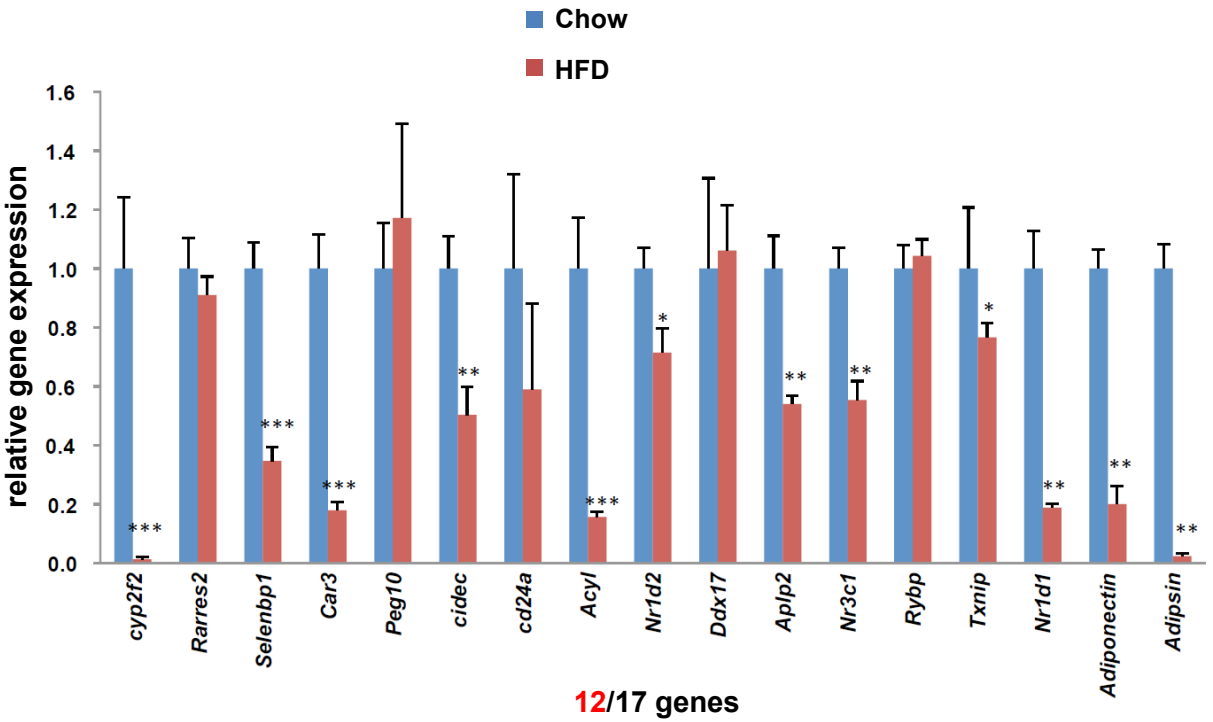
Specific gene sets regulated by phosphorylation





Phosphorylation of PPARγ might play an important role in the context of obesity and diabetes.

Choi et al., *Nature* (2010)

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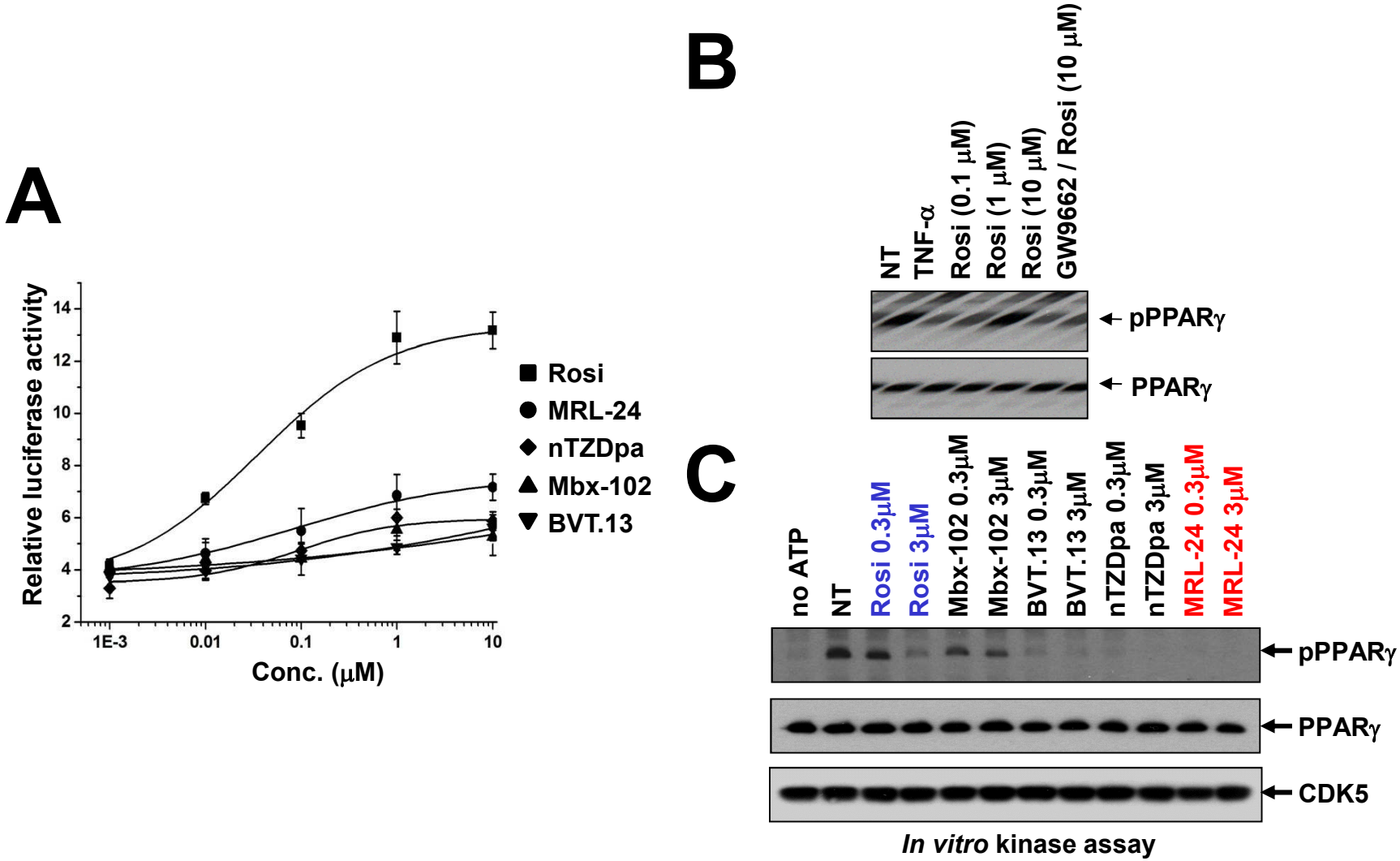
PPARγ ligands block the phosphorylation of PPARγ



Anti-diabetic PPARγ lignads directly block the phosphorylation independent of PPARγ agonism.

Choi et al., *Nature* (2010)

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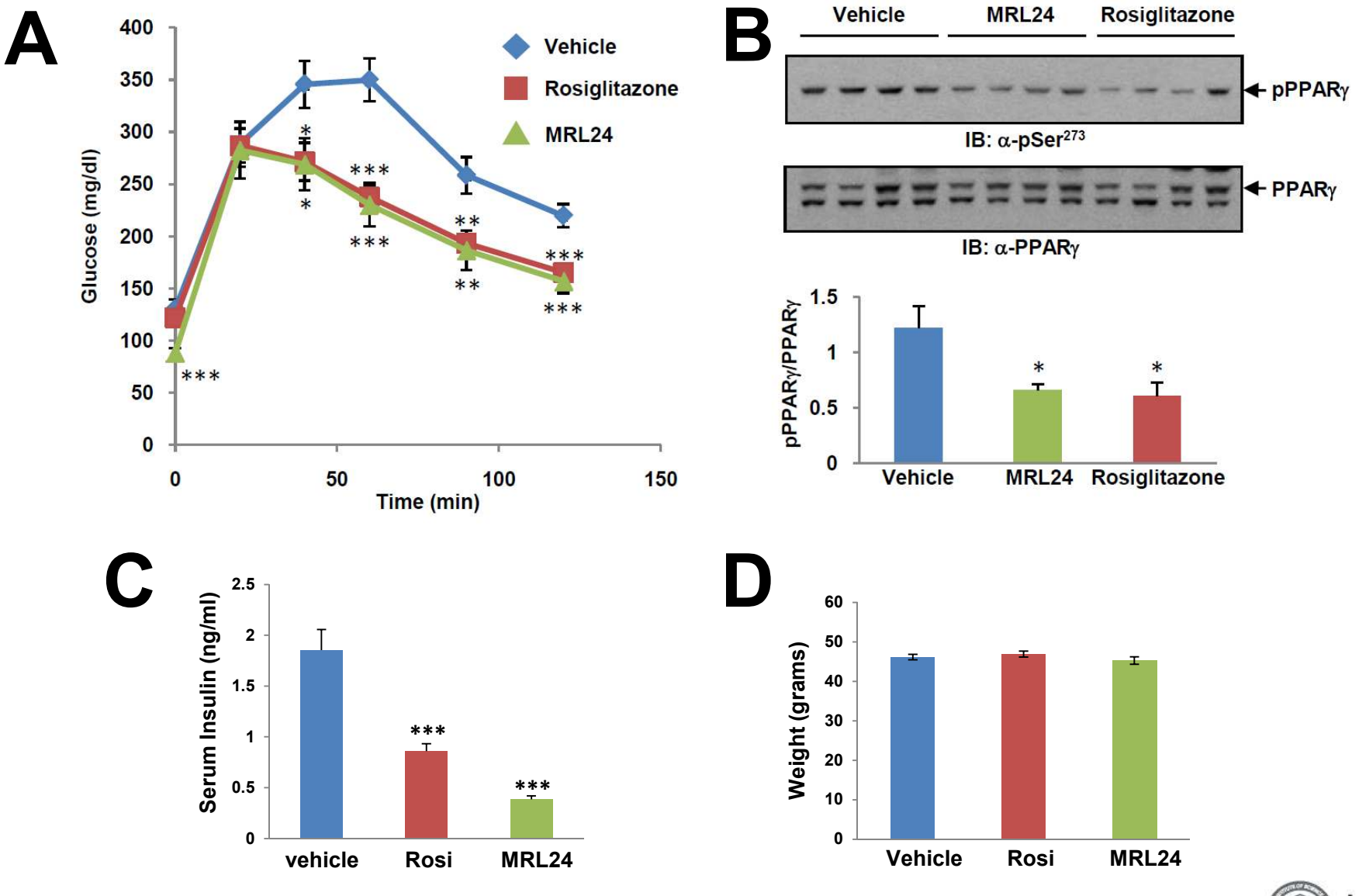
What is the physiological role of phosphorylation *in vivo*?

Anti-diabetic effects of PPARγ ligands in HFD mice



Choi et al., *Nature* (2010)

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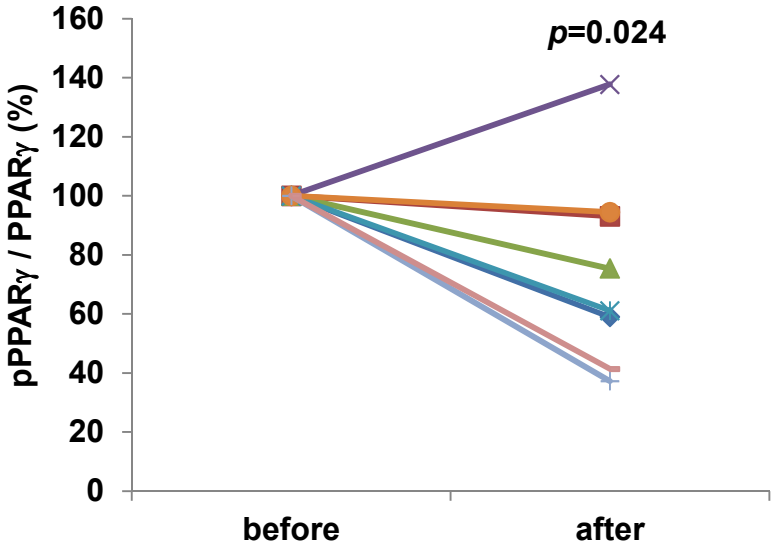
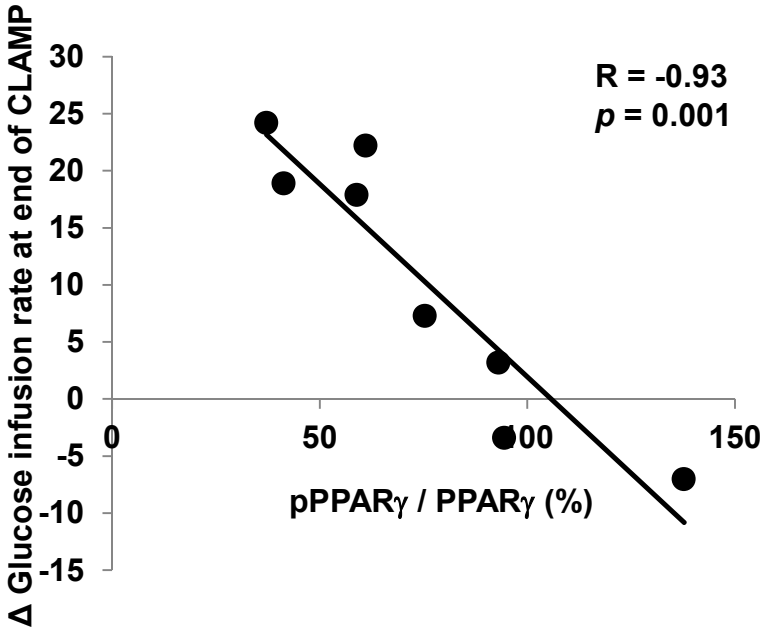
Modulation of PPARγ phosphorylation by rosiglitazone during the therapy of human type 2 diabetes

Newly diagnosed T2D, male, 6 months treatment, 4mg/day





Matthias Blueher, U. Leipzig

Phosphorylation of PPARγ tightly correlates with insulin sensitivity.

Choi et al., *Nature* (2010)

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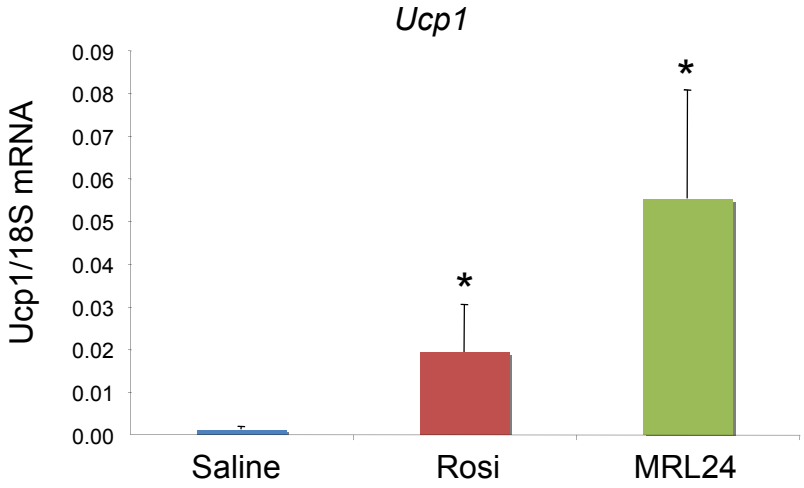
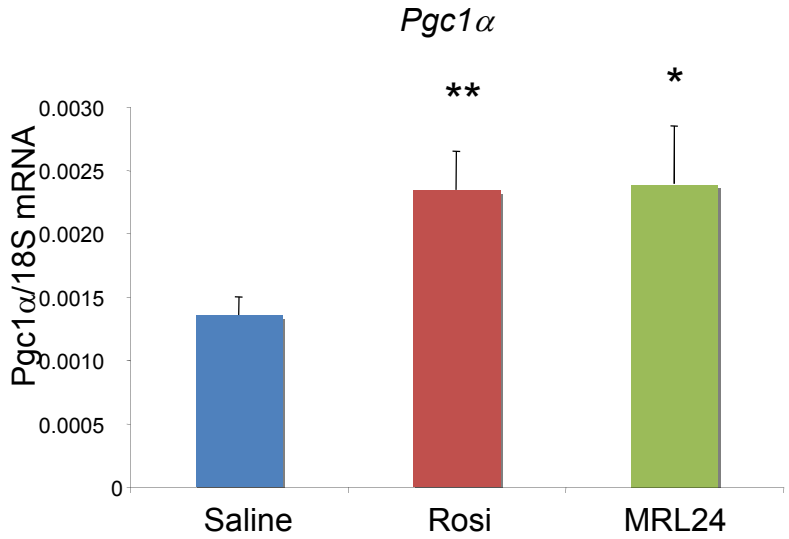
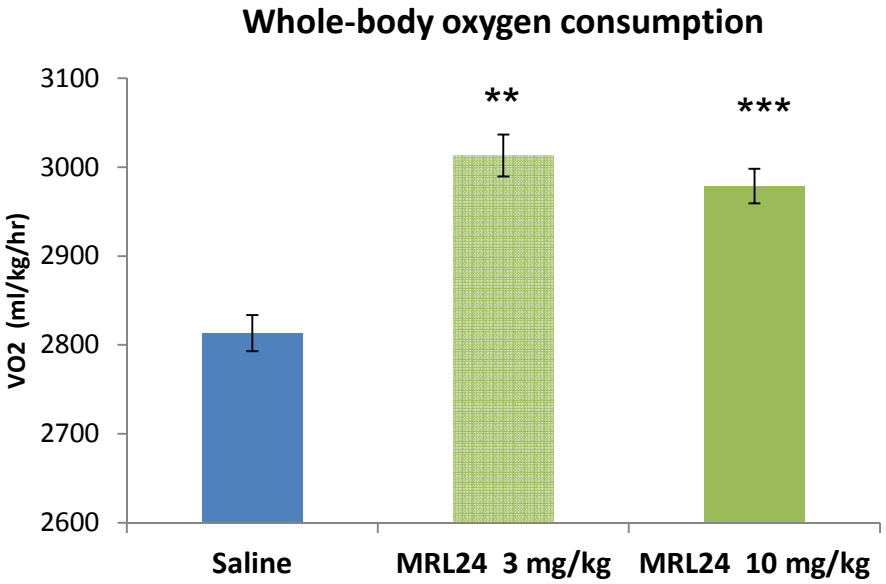
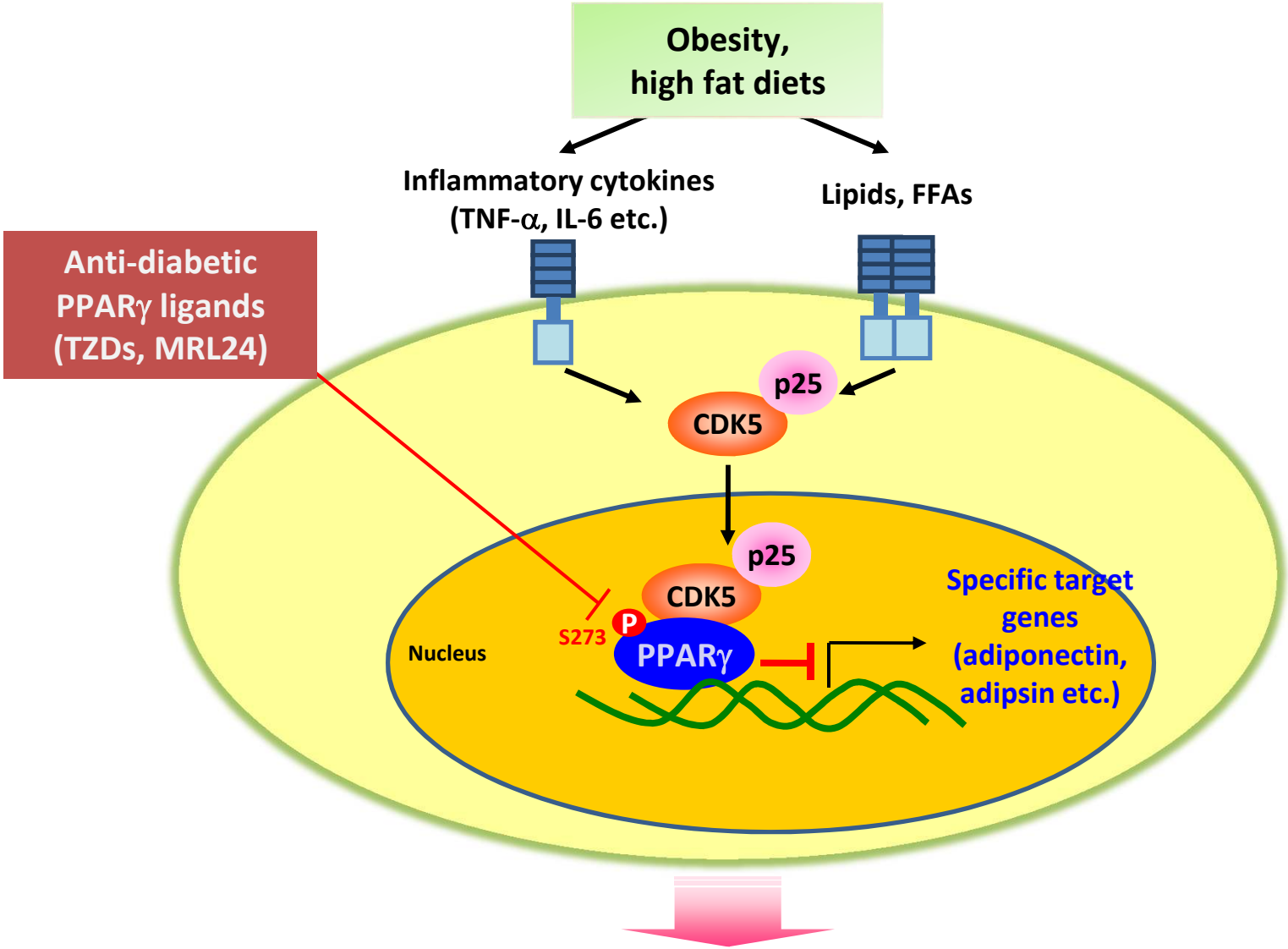
MRL-24 increases metabolic rate and thermogenic gene expression *in vivo*







Proposed model of PPARγ phosphorylation and PPARγ ligands



Choi et al., *Nature* (2010)

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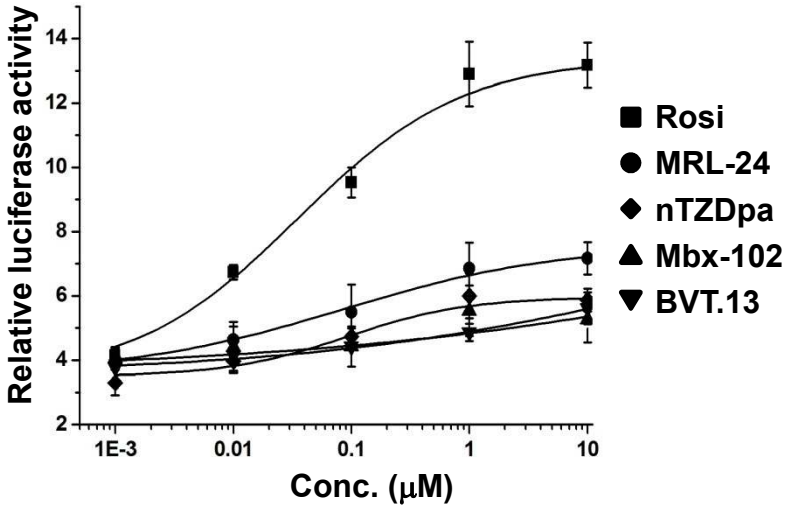
Many unanswered questions?

- The TZD drugs are PPARγ agonists and block Cdk5-mediated phosphorylation. **Can PPARγ ligands be developed with no classical agonism and still block Cdk5-mediated phosphorylation of PPARγ?**
- **Would such compounds have anti-diabetic activity?**
- **Would they have a better therapeutic window than TZDs?**

A



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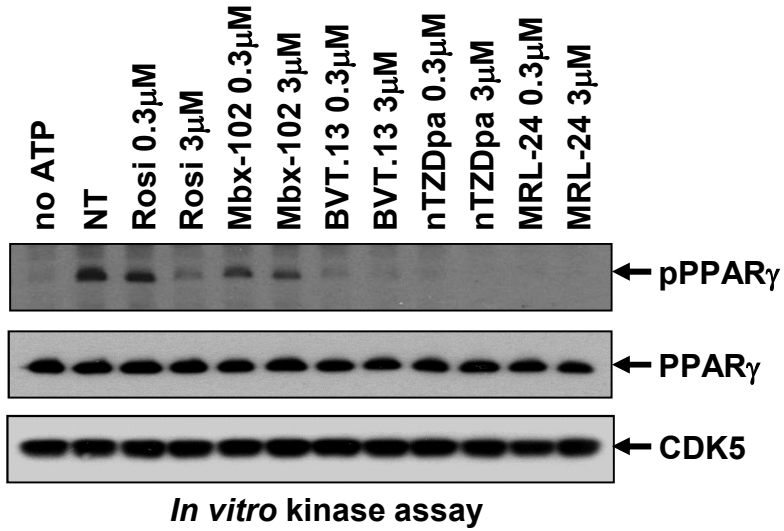

In vitro kinase assay

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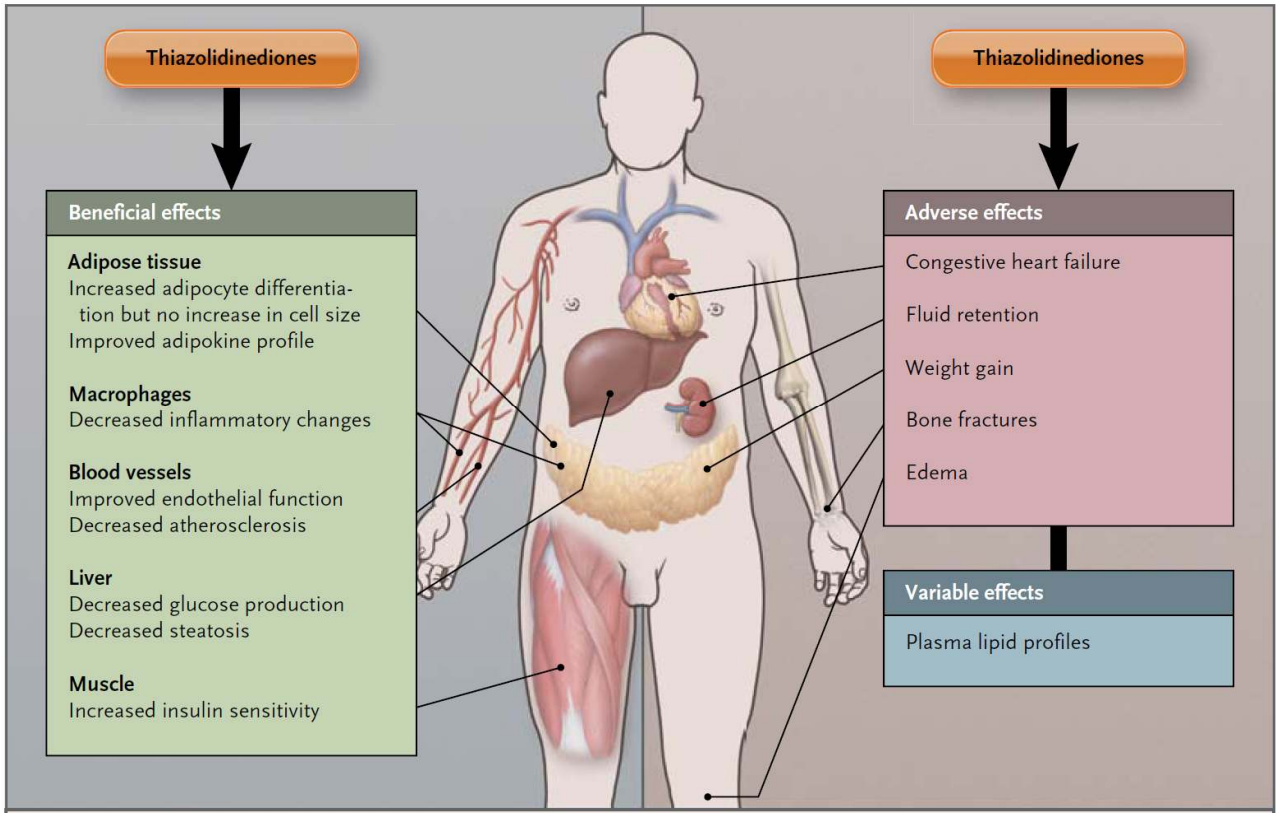

N. Eng. J. Med (2010)

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Withdrawal of Avandia because of its side effects

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Avandia withdrawn in Europe, but remains in US

Published on 23/09/10 at 11:24pm

GSK's diabetes drug Avandia is to be withdrawn from the market in Europe because of high levels of heart attack, heart failure and stroke in patients.

Europe's regulator the EMA has ordered the suspension of the drug, but its US counterpart the FDA has decided to allow it remain on the market, albeit with further restrictions on it use.

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통합 | 뉴스 검색어를 입력하세요. 검색

한국일보 즐겨찾기 지면보기 독자의 소리 2011.3.10.(목)

뉴스 | 핫이슈 | 포토 | TV | 멀티미디어

프리미엄 정보 | 광고문의

- 80kg주부, 48kg족~뺐어요!비법은
- 라식,라섹 가장 많은 부작용은?
- 허리가 찌뿌둥해서 병원 갔더니..
- 팔자주름 한번치료로 90살까지??
- 티안나게 코성형을?? 정말 이가격에?
- 금니가격으로 임플란트를??
- 혈당,중성지방,배변을 비타민으로해결?
- 신용조회없이 확인하는 나의대출한도는?

한국일보 > 뉴스 > 경제

미투데이 | 트위터 | 싸이월드 공감 | +크게 | +작게

경제

당뇨병약 '아반디아' 국내도 사용중지

(서울=연합뉴스) 김세영 기자 =

식품의약품안전청은 24일 심장발작과 뇌졸중 위험을 높이는 것으로 알려진 당뇨병치료제인 글락소스미스클라인의 아반디아(성분명:로시글리타존) 등 15개 품목에 대해 처방·조제 등 국내 사용을 중지시킨다고 밝혔다.

식약청의 아반디아의 사용중지 결정은 건강보험심사평가원에도 통보됐다.

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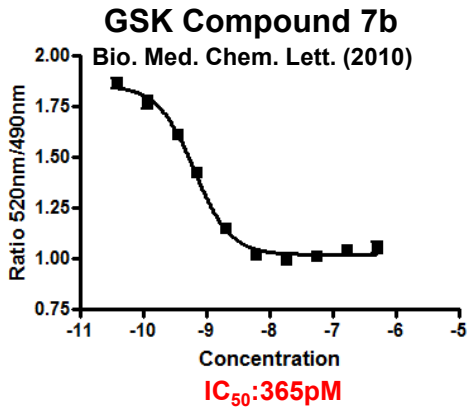
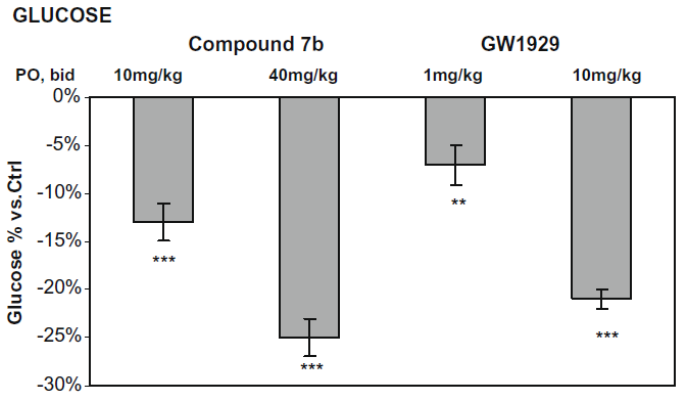
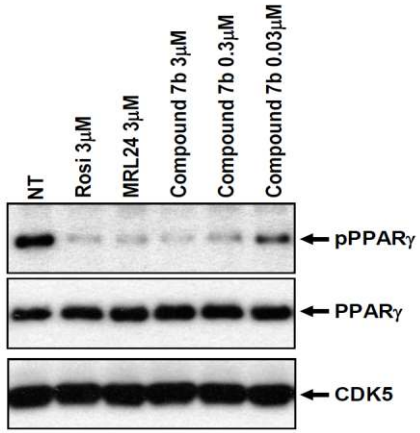
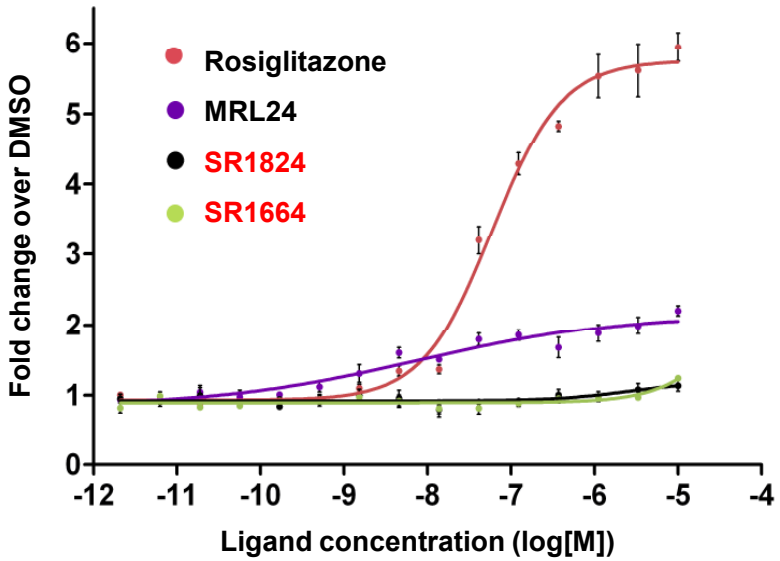
Development of chemical derivatives of Compound 7b







Compound	IC_{50} (binding affinity)	Ki	EC_{50} (PPRE) (%relative to rosiglitazone)
rosiglitazone	18nM	6.45nM	7.4nM (100%)
Compound 7b	370pM	132.61pM	540nM (15%)
SR1663	2nM	716pM	20nM (23%)
SR1664	80nM	28.67nM	Not active (0%)
SR1665	466nM	167.02nM	3μM (7%)
SR1666	76nM	27.24nM	300nM (26%)
SR1701	13nM	4.65nM	2.7μM (20%)
SR1706	>1000nM		Not tested
SR1707	No binding		Not tested
SR1708	10nM	3.58nM	Not active (0%)
SR1713	No binding		Not tested
SR1714	17nM	6.09nM	>1μM (9%)
SR1717	4nM	1.43nM	Not active (0%)
SR1824	28nM	10.03nM	Not active (0%)



Choi et al., *Nature* (2011)

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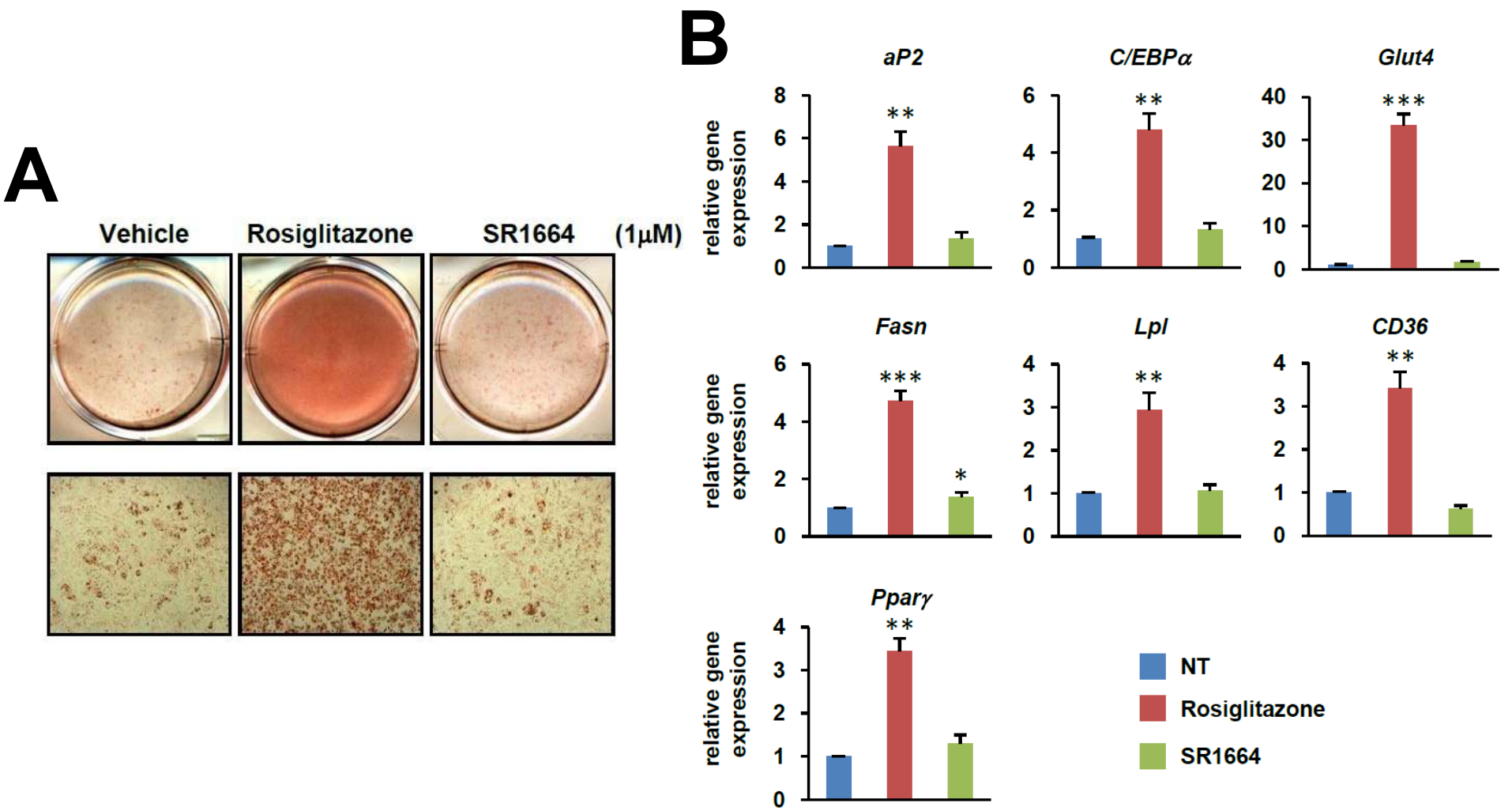
SR1664 has no effect on adipogenesis



Choi et al., *Nature* (2011)

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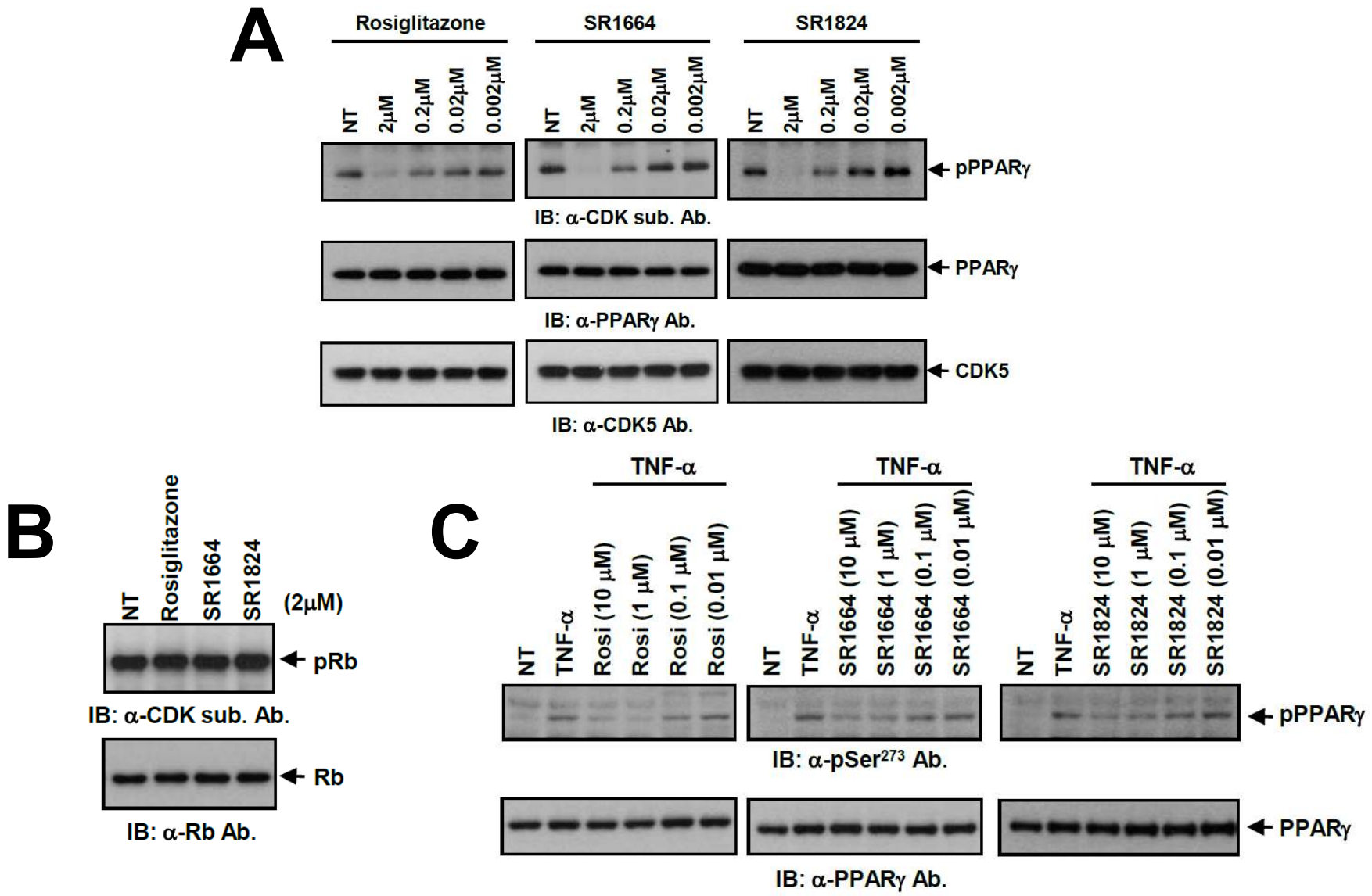
SR1664 & SR1824 block PPARγ phosphorylation



Choi et al., *Nature* (2011)

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What is the physiological role of SR1664 in whole body metabolism?

1.DIO mouse model
2.Ob/ob mouse model

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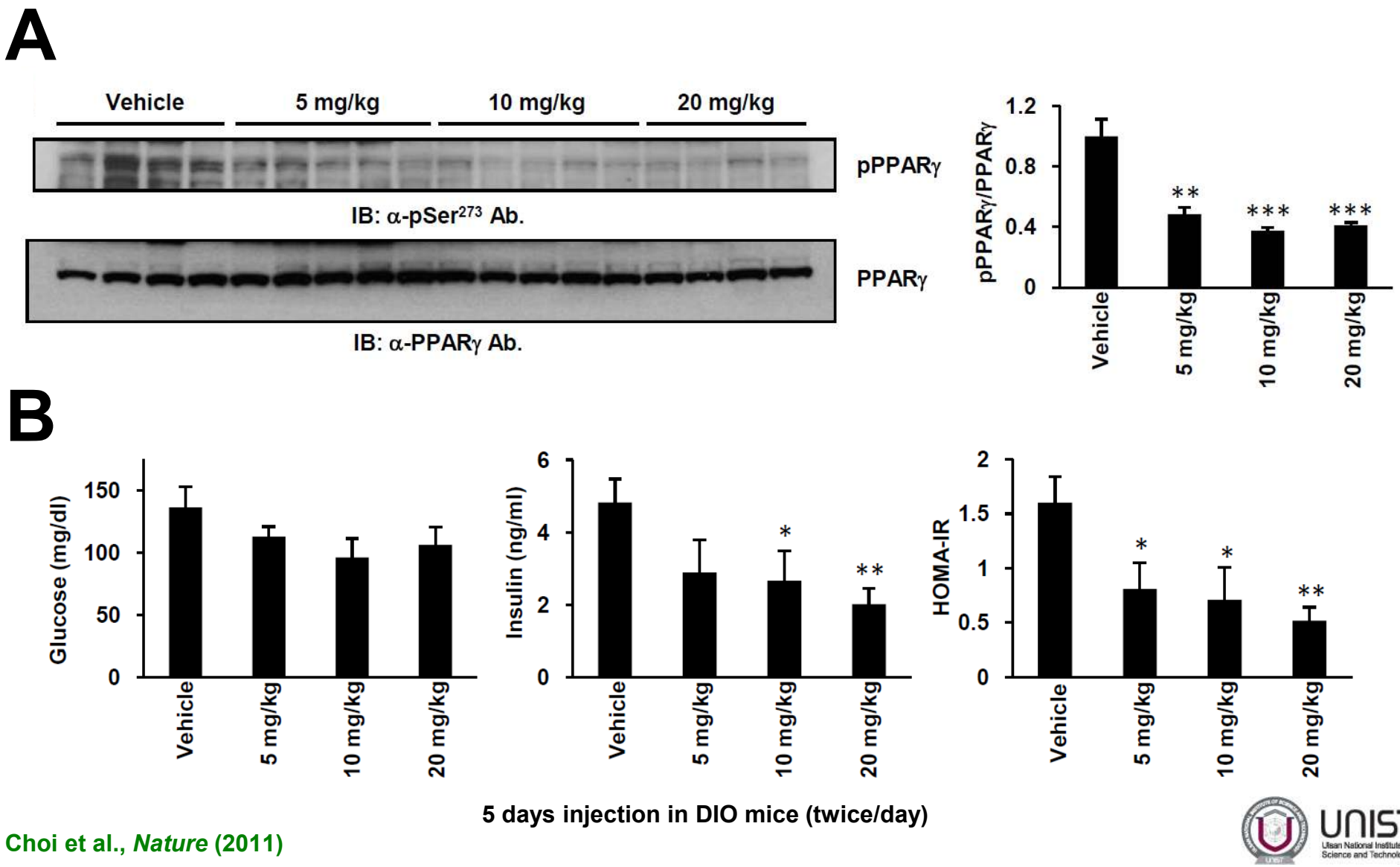
SR1664 improves glucose tolerance in DIO mice



Choi et al., *Nature* (2011)

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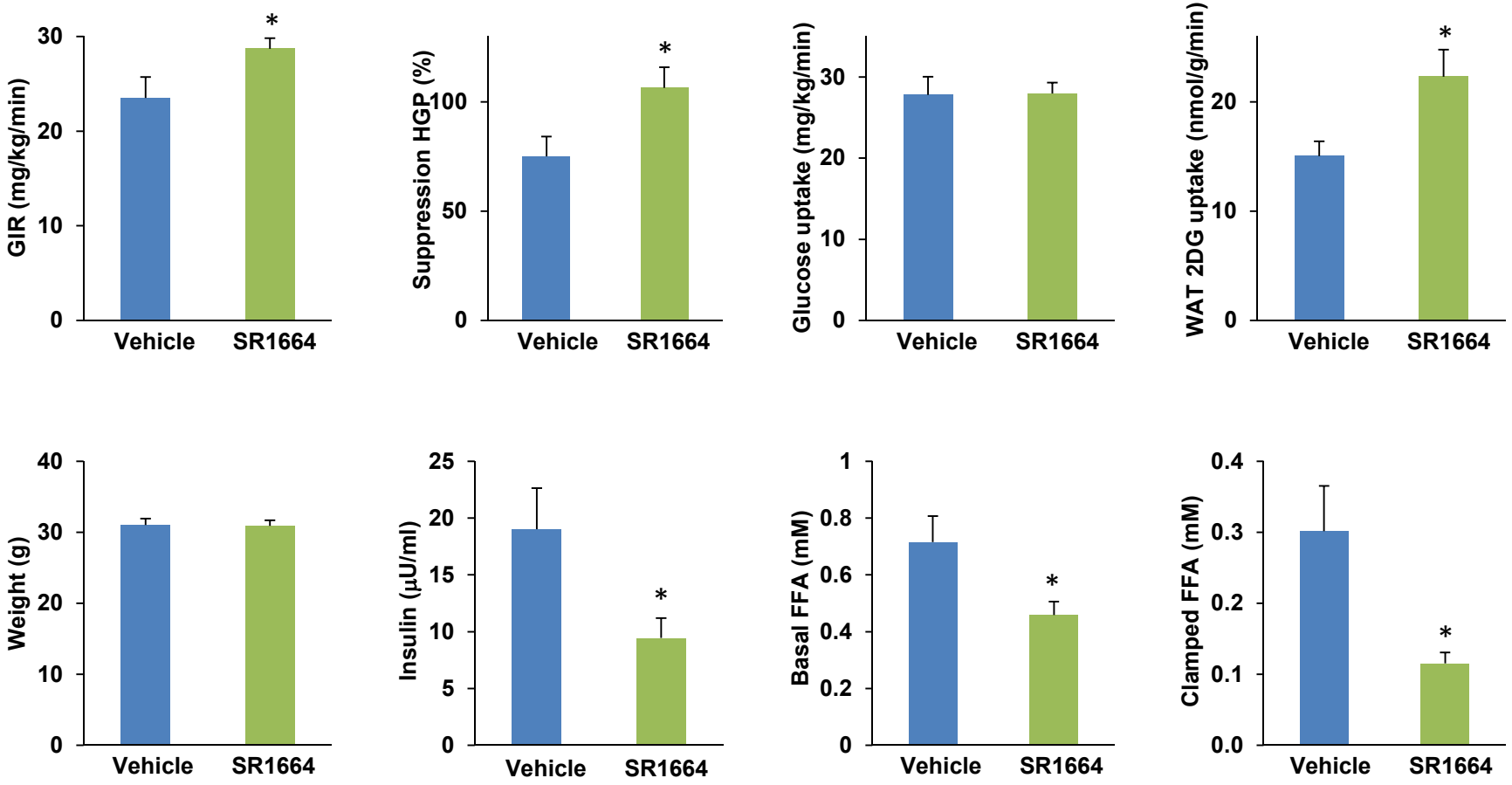
SR1664 improves glucose tolerance in DIO mice (CLAMP)



5 days injection in DIO mice (twice/day)
Dr. Gerald Shulman, Yale Univ.

Choi et al., *Nature* (2011)

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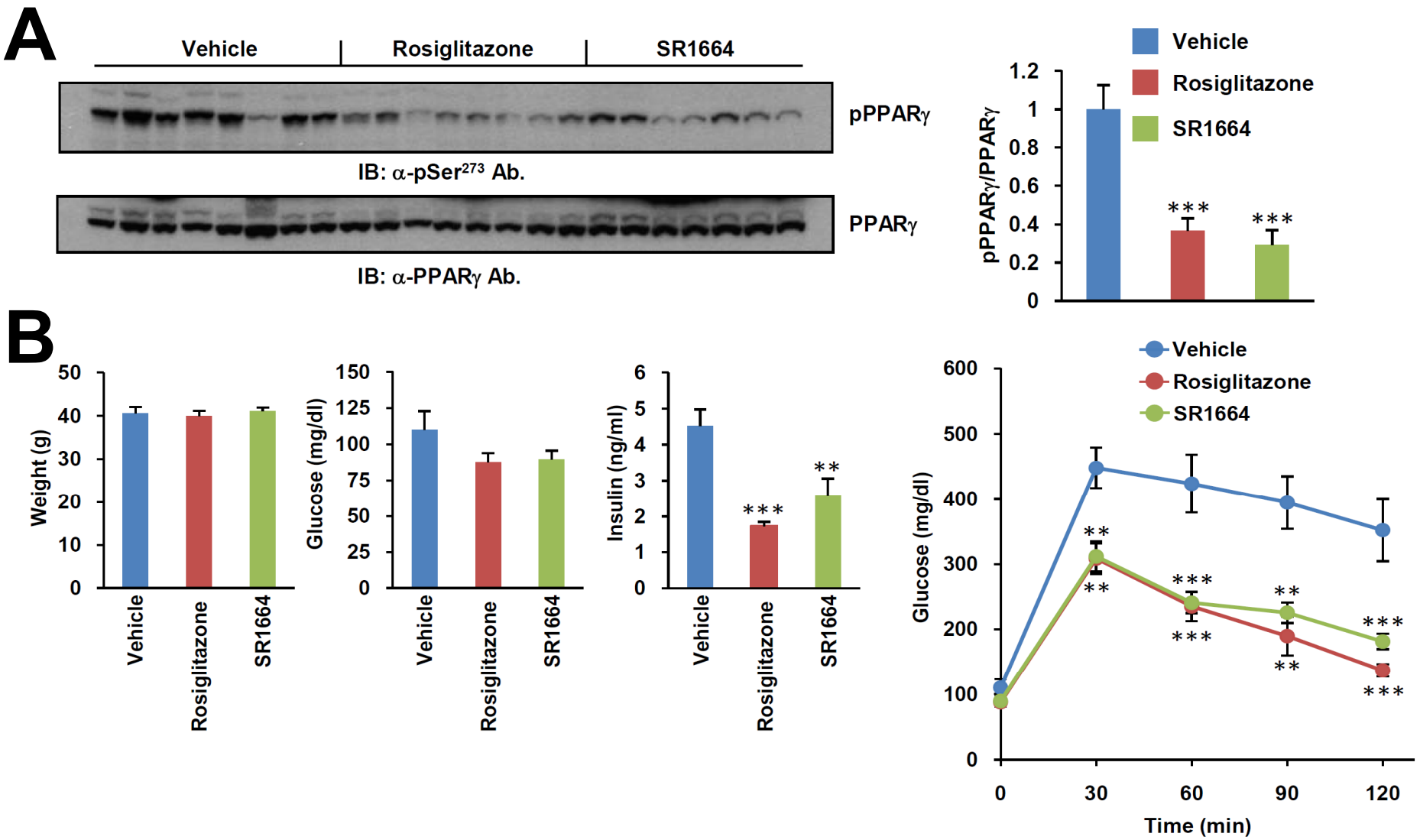
SR1664 improves glucose tolerance in ob/ob mice



6 days 40 mg/kg (BID), ob/ob mice

Choi et al., *Nature* (2011)

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Would SR1664 have a better therapeutic window than TZDs (less side effects)?

1. Bone mineralization
2. Weight gain, Fat % change
3. Hemodilution

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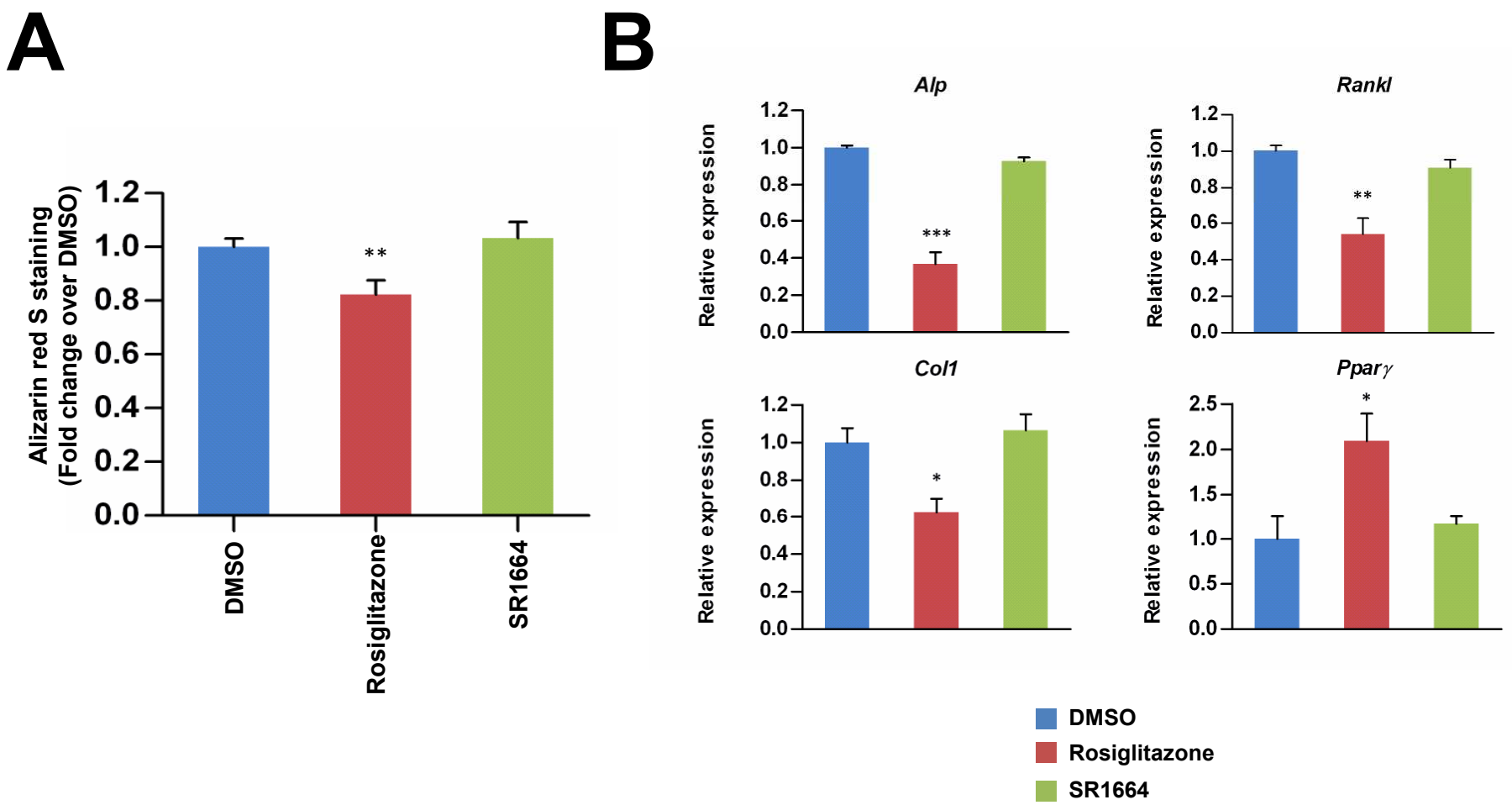
No changes in bone mineralization by SR1664



Choi et al., *Nature* (2011)

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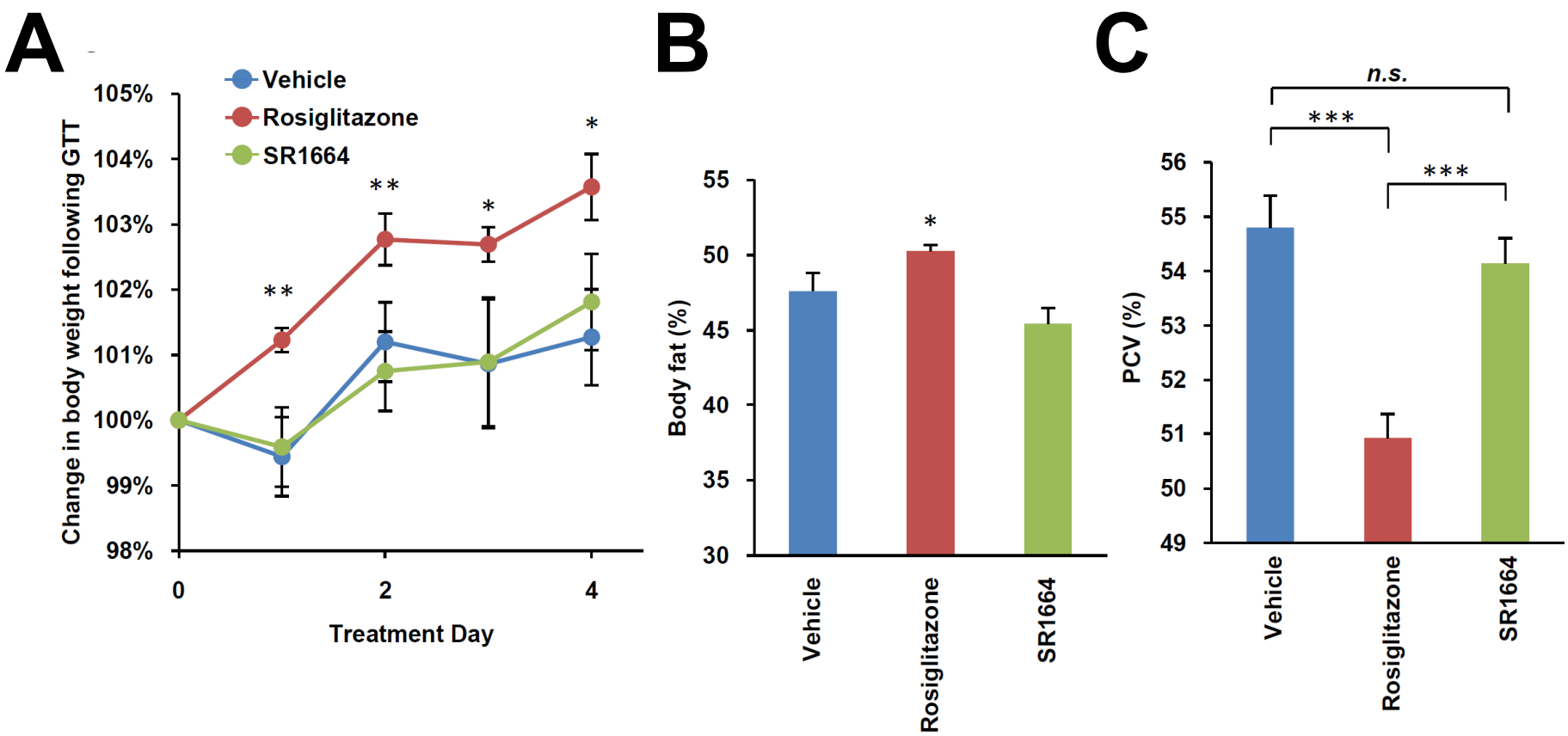
No changes in weight, fat % and hemodilution by SR1664



11 days 40 mg/kg (BID), ob/ob mice

Choi et al., *Nature* (2011)

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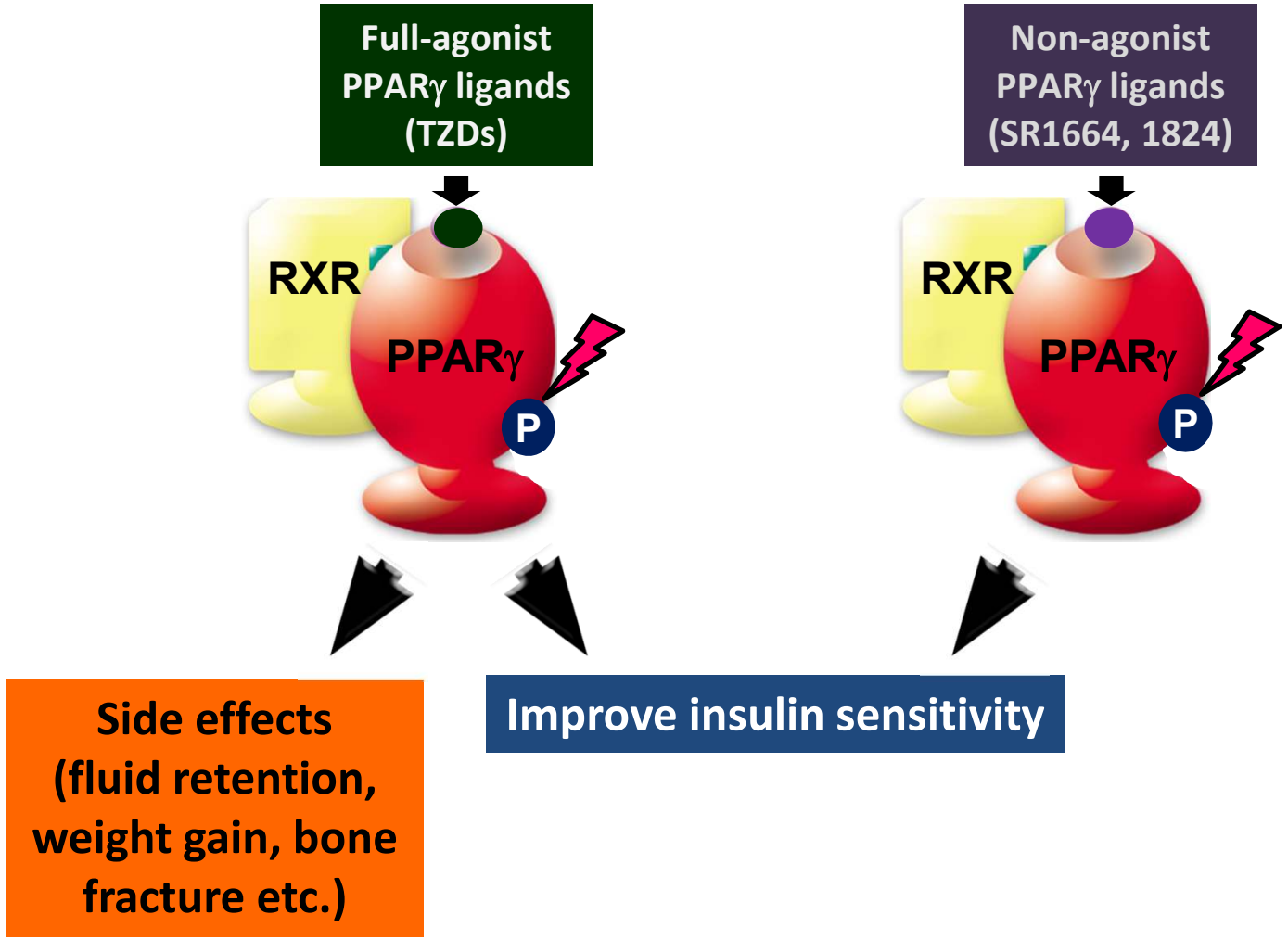
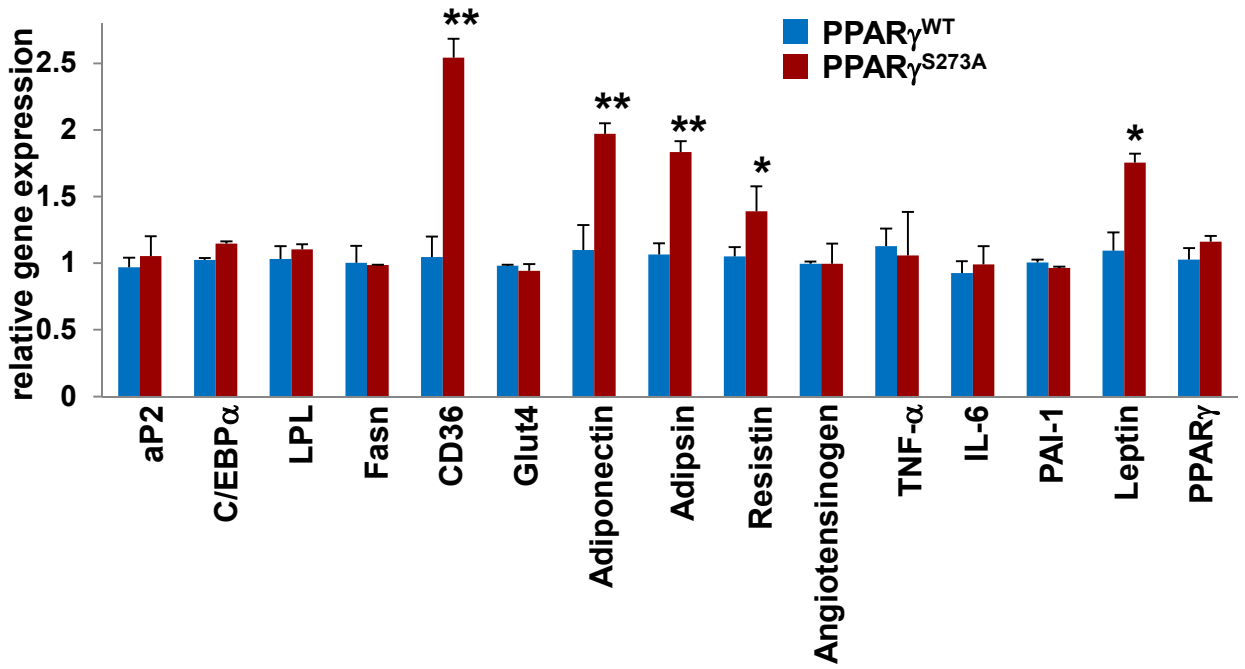
Proposed model of full- or non-agonist PPARγ ligands



Choi et al., *Nature* (2011)

Burning key questions

- What is the molecular mechanism regulating the specific gene expression program controlled by phosphorylation?
  - ➔ 1. Different DNA occupancy by phosphorylation?
  - 2. Specific modulators binding to PPARγ?



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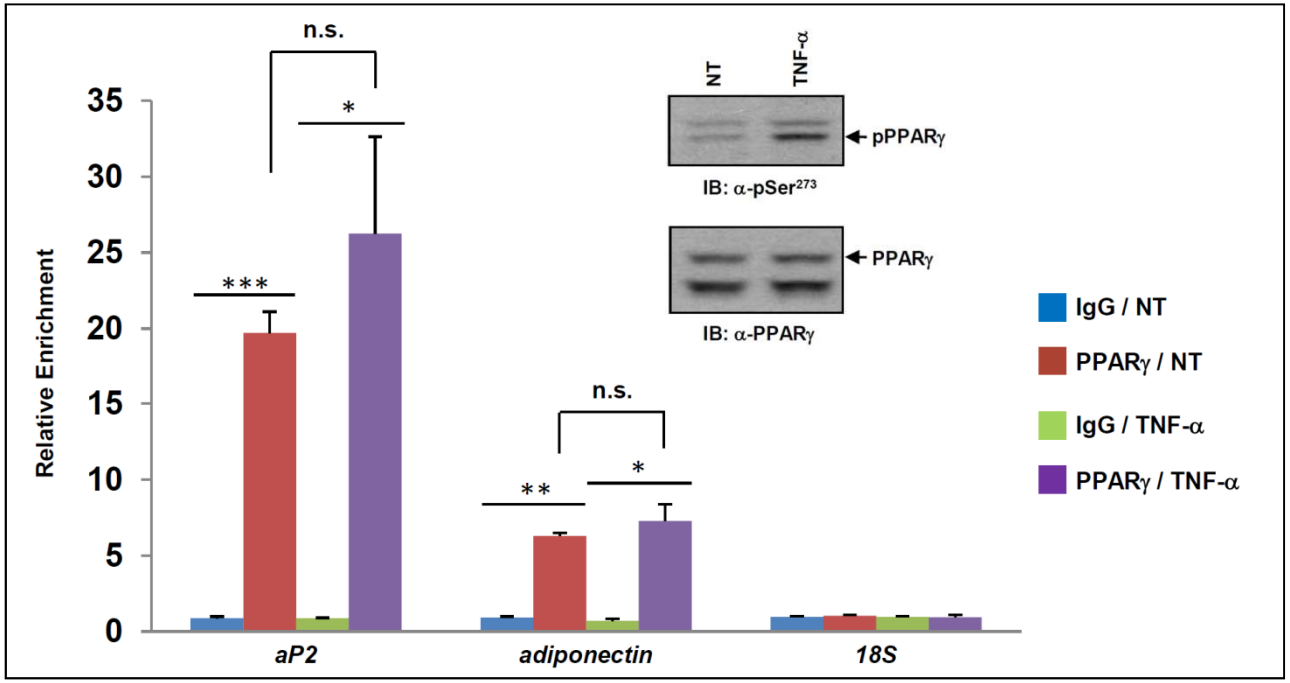
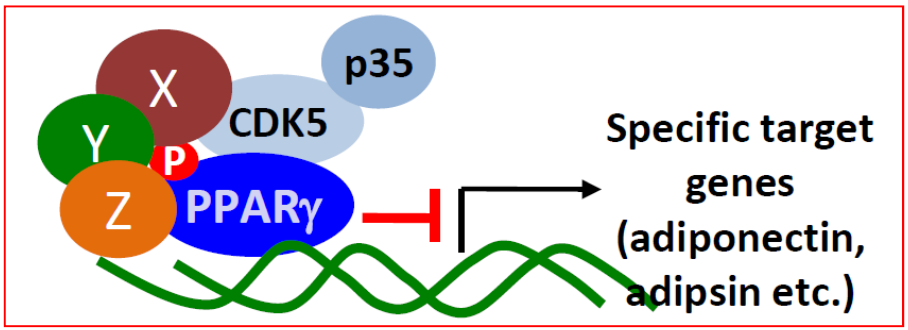
No difference of DNA occupancy by phosphorylation





Choi et al., *Nature* (2010)

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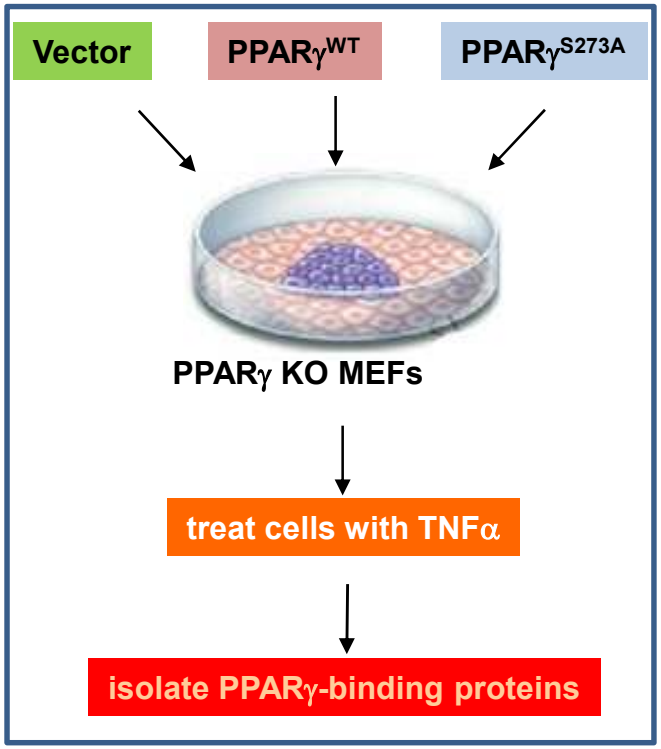
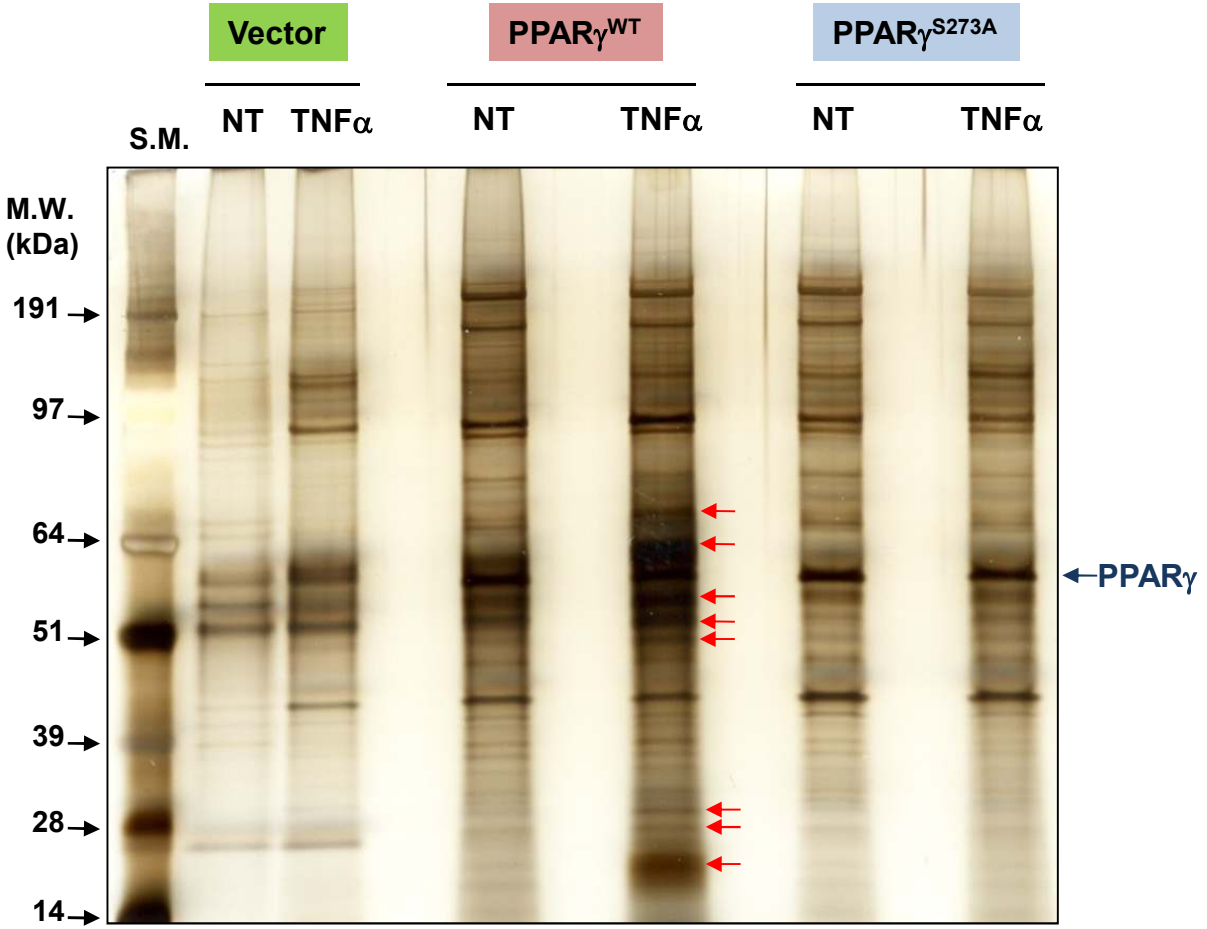
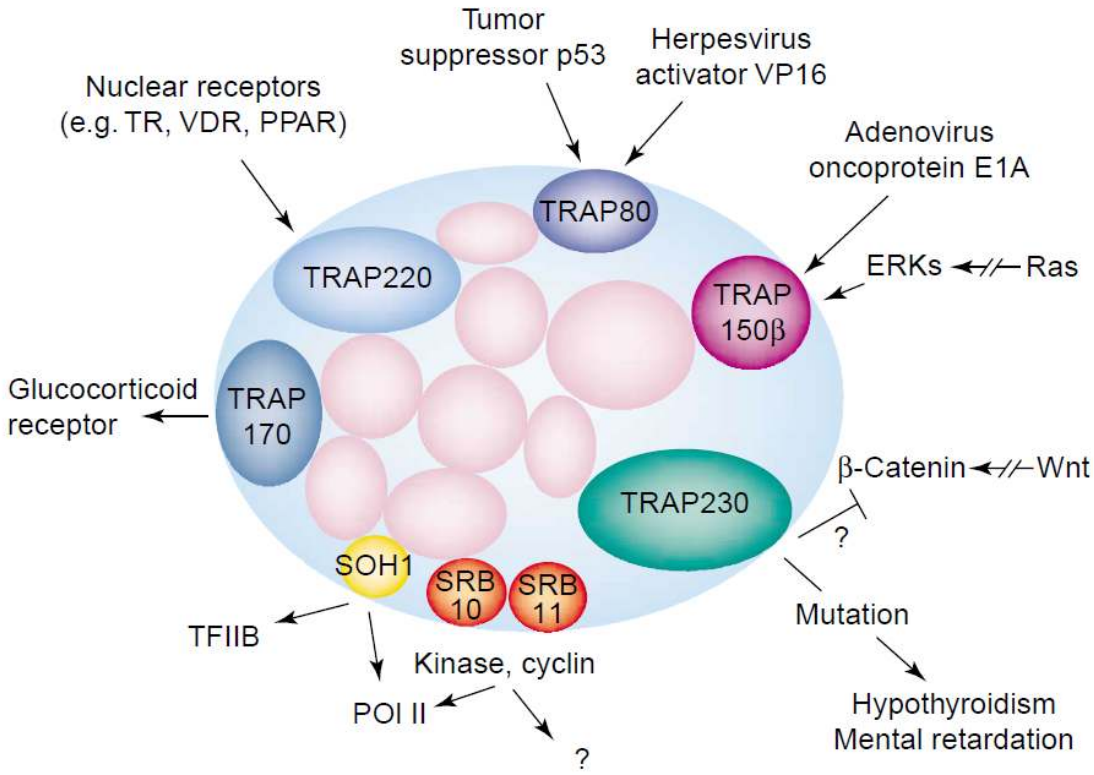
Identifying PPARγ-binding proteins in phosphorylation-dependent manner





Thyroid hormone receptor-associated proteins (TRAP/MED)



Subunit	Organism	Method	Phenotype	Refs
MED6	*C. elegans*	RNAi	Embryonic lethal	37
MED7	*C. elegans*	RNAi	Embryonic lethal	37
MED10/NUT2	*C. elegans*	RNAi	Embryonic lethal	37
TRAP150β/SUR-2	*C. elegans*	Chemical mutagenesis	Larval lethal	38
TRAP80	*D. melanogaster*	P insertion	Recessive lethal	39
SRB7	Mouse	Homologous recombination	Recessive, embryonic lethal	40
TRAP220	Mouse	Homologous recombination	Recessive, embryonic lethal[b]	41,42
TRAP230	Human	Spontaneous mutation	Male dementia; hypothyroidism	61

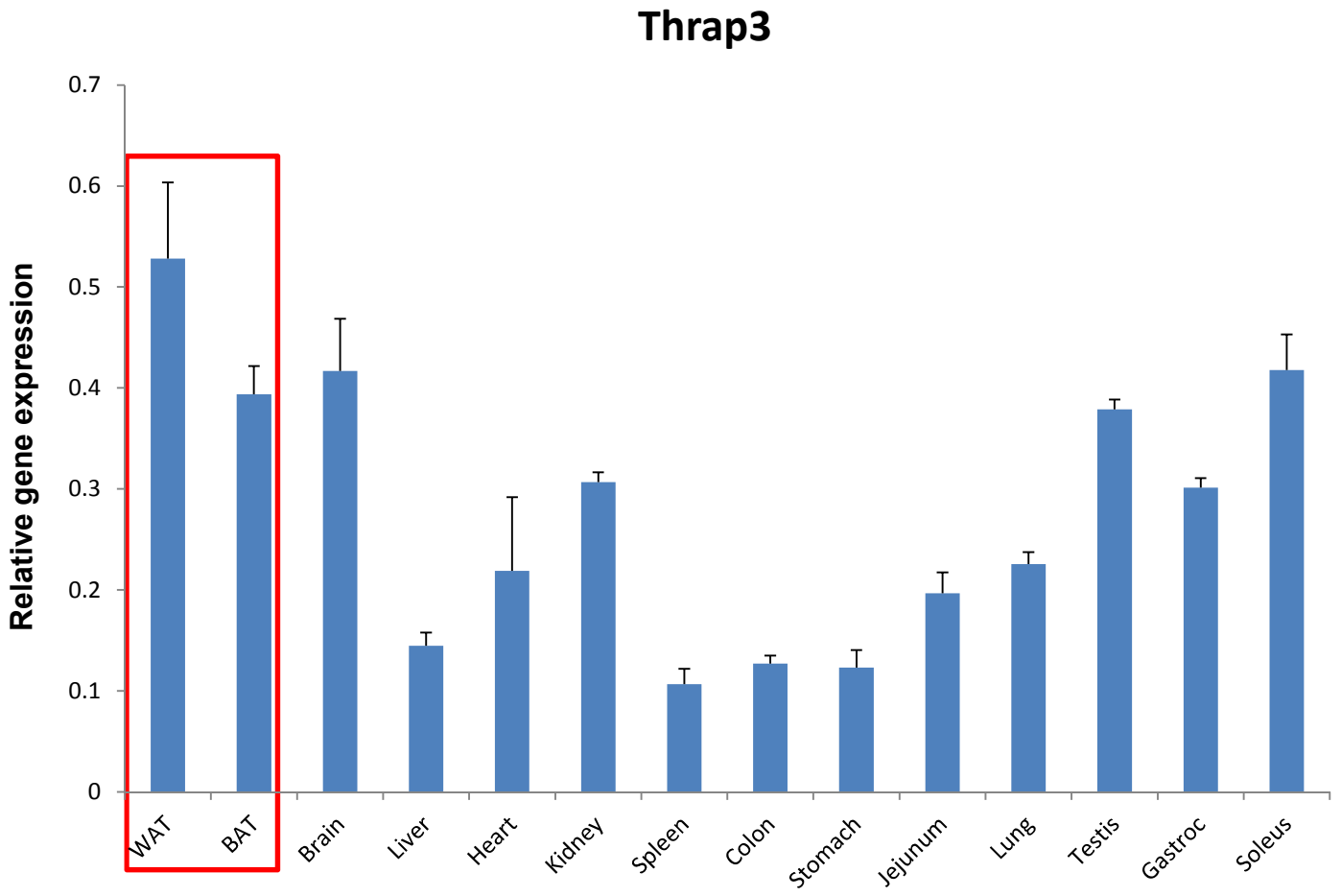
[a]Abbreviations: RNAi, RNA interference; TRAP, thyroid hormone receptor associated protein.
[b]Heterozygotes: dwarfism, pituitary hypothyroidism, transcriptional dysregulation; homozygotes: poor embryonic heart trabeculation, thin neural tube, cell cycle dysregulation and apoptosis, attenuated thyroid hormone receptor function.

Trends. Endo. & Meta. (2001)

Thrap3 (thyroid hormone receptor-associated protein 3) TRAP150

- A subunit of the transcription regulatory complex TRAP/Mediator
  (no LXXLL motif)
- A component of the spliceosome
- It activates pre-mRNA splicing and promotes nuclear mRNA degradation
  (R/S-rich domain)
- Thrap3 is a real subunit of TRAP/Mediator????
- **The exact function of Thrap3 remains unclear.**

Tissue distribution of Thrap3



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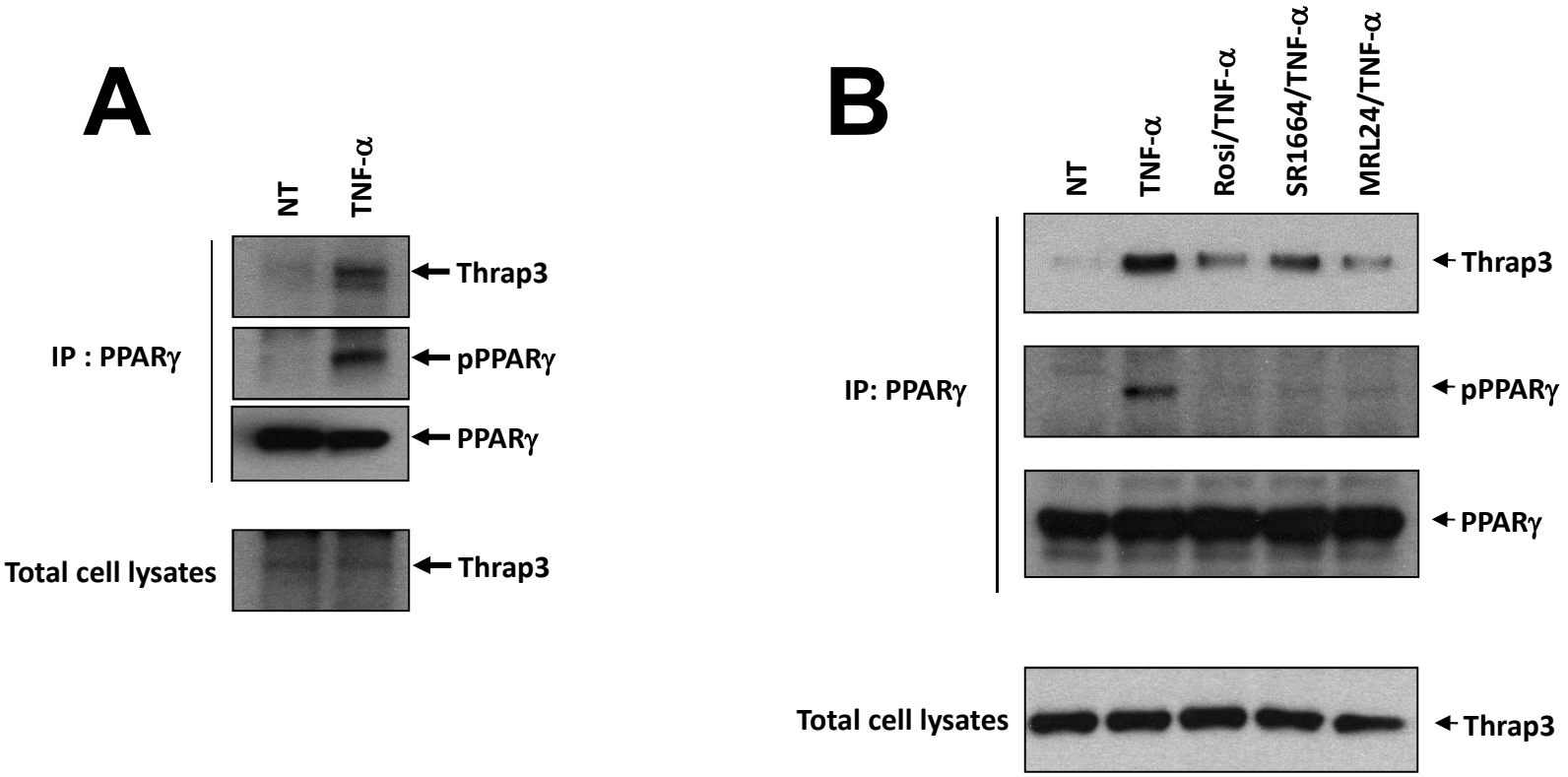
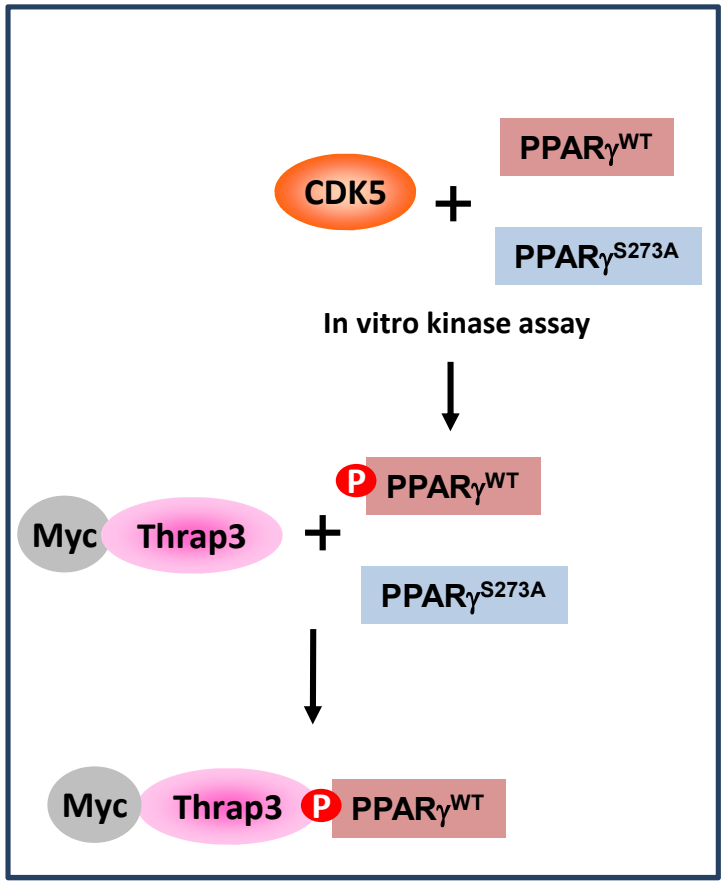
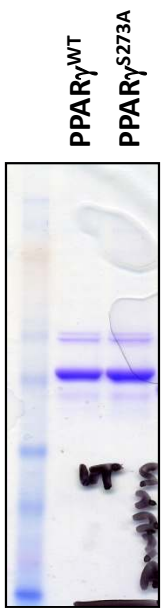
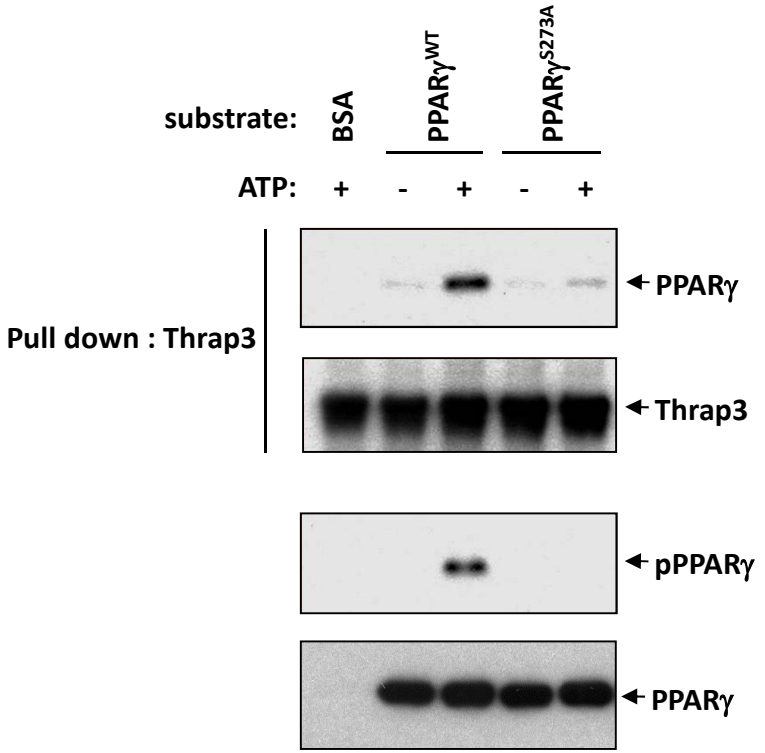
Thrap3 interacts with phosphorylated PPARγ



Direct interaction between phosphorylated PPARγ and Thrap3







Thrap3 binds to phosphorylated PPARγ at Ser273.

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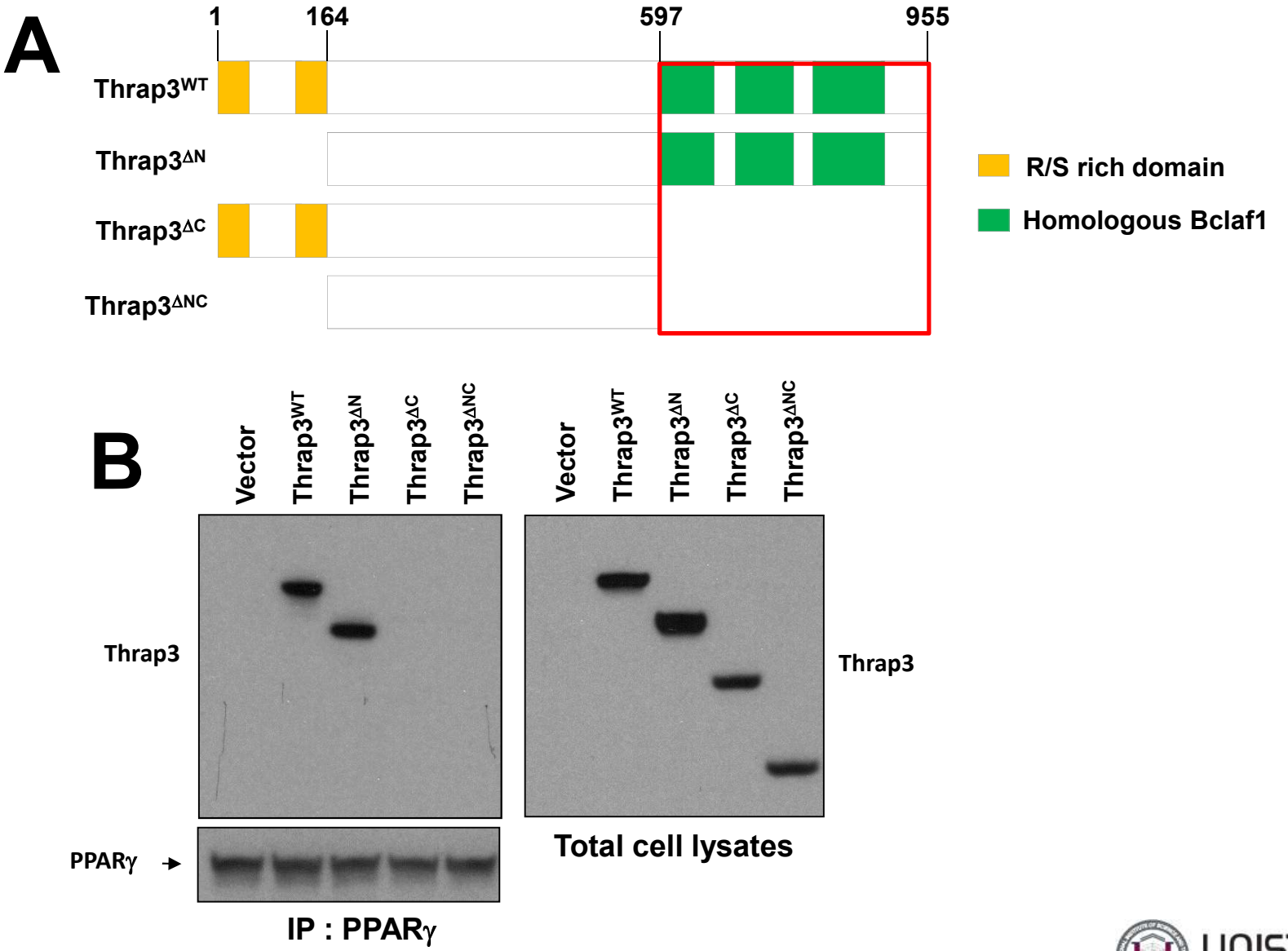
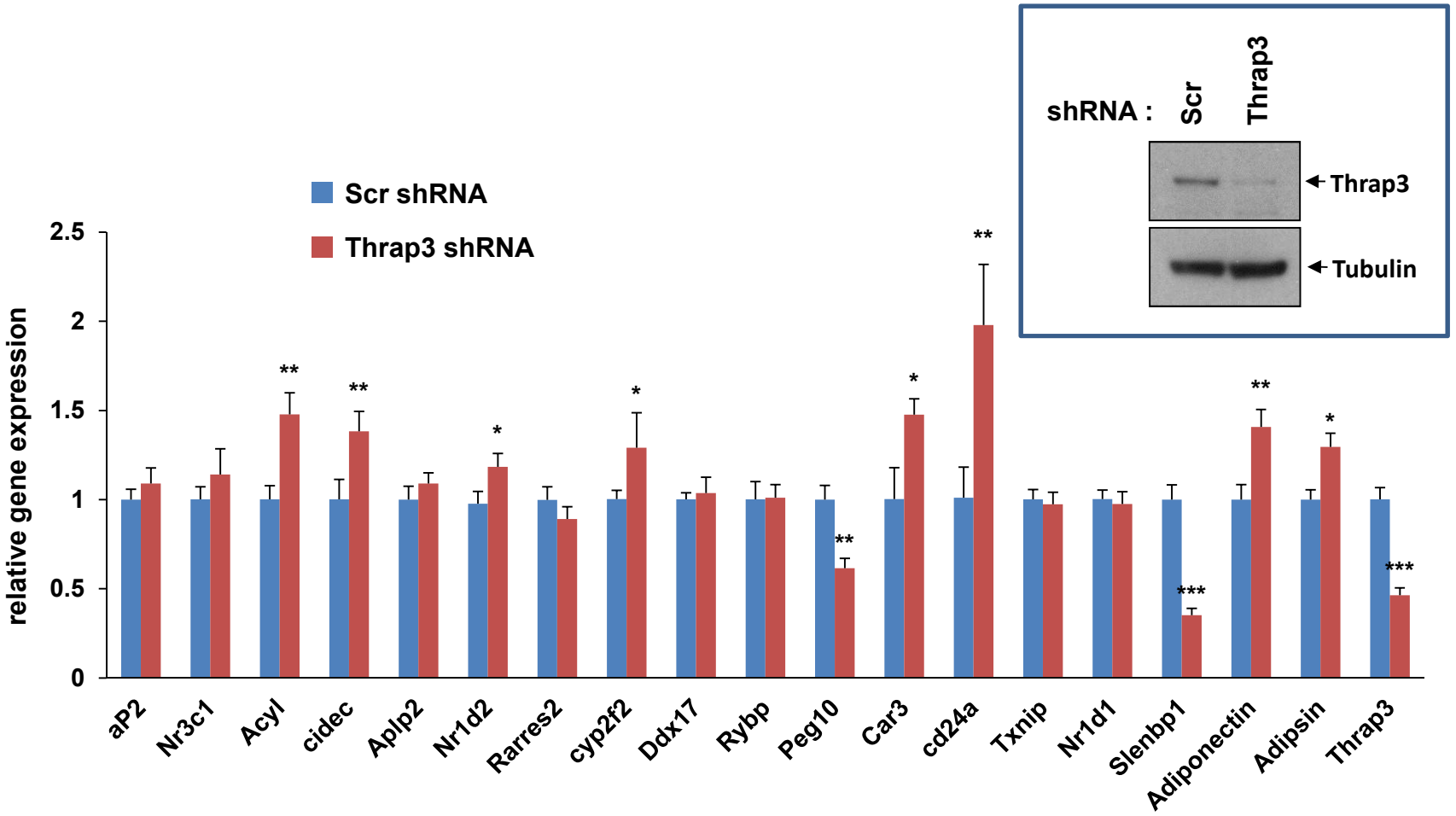
Domain mapping of Thrap3



Specific gene regulation by Thrap3



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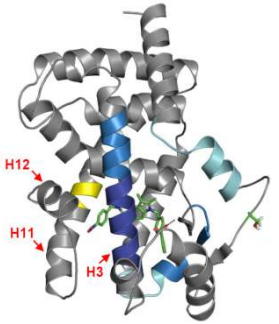
Conclusion



- Cdk5 is activated in obesity and modifies PPARγ at serine 273
- Cdk5 phosphorylation of PPARγ is sufficient to cause dysregulation of several fat cell genes, including some known to be aberrantly regulated in obesity, like adiponectin.
- Anti-diabetic PPARγ ligands block this Cdk5-mediated phosphorylation directly, ***an activity completely separate from classical agonism***.
- These data now indicate that anti-diabetic PPARγ ligands work therapeutically, at least in part, by inhibition of the Cdk5-mediated phosphorylation of PPARγ.
- The development of entirely new classes of PPARγ-targeted drugs with no classical agonism is feasible
- Thrap3 may regulate Cdk5/PPARγ-specific gene reprogramming

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