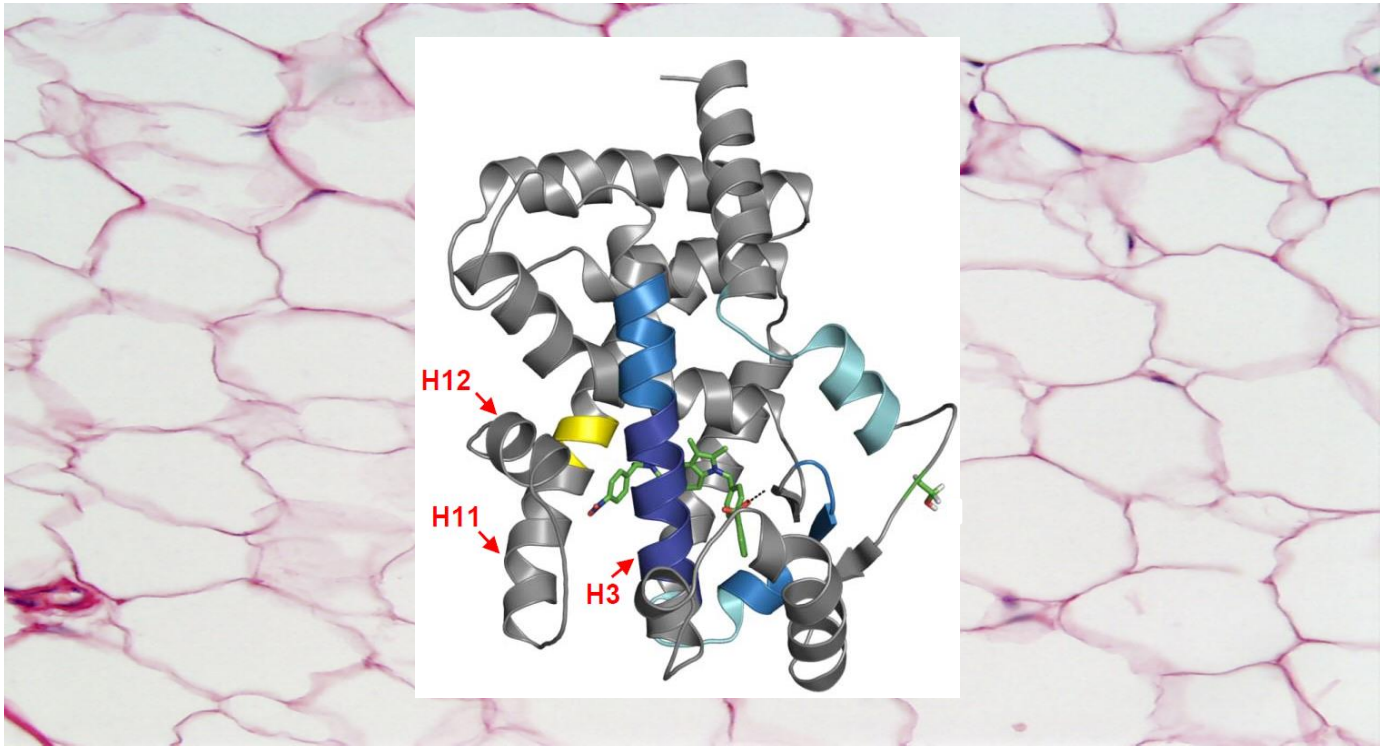
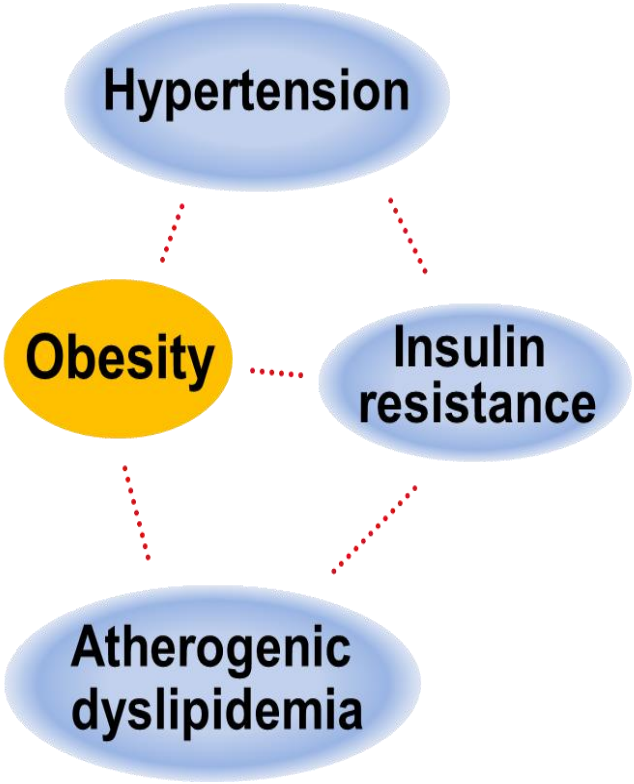
Non-agonist PPARg ligands and energy metabolism

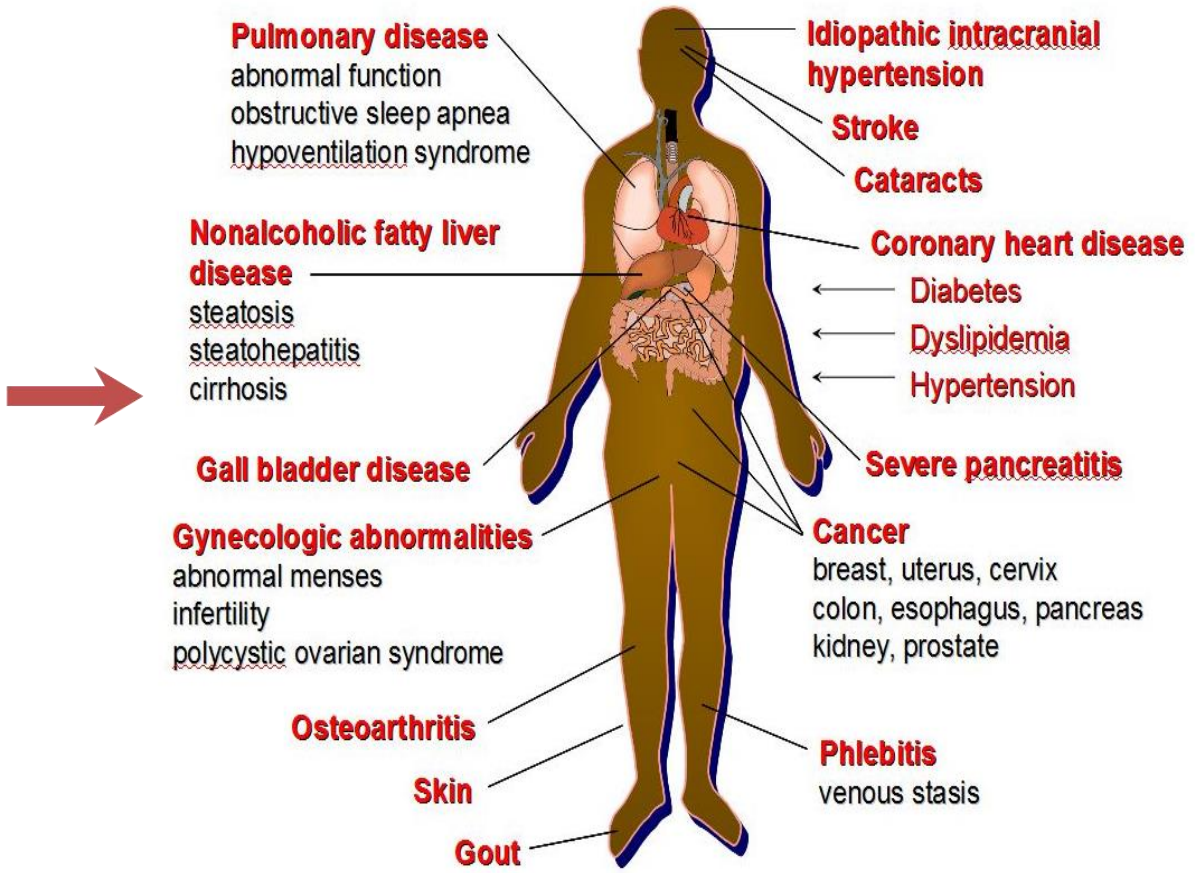


Jang Hyun Choi Ph.D.

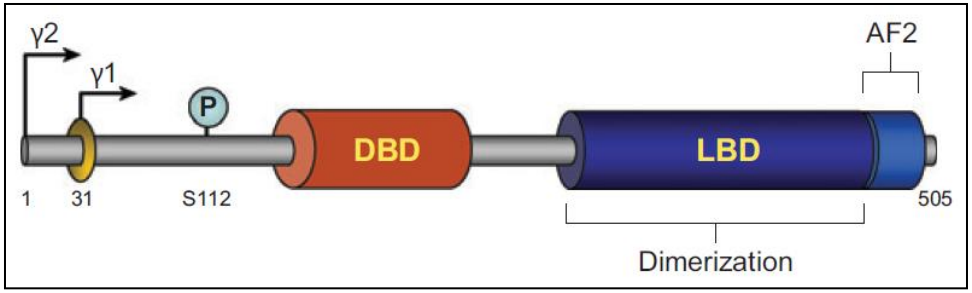
울산과학기술원 (UNIST)

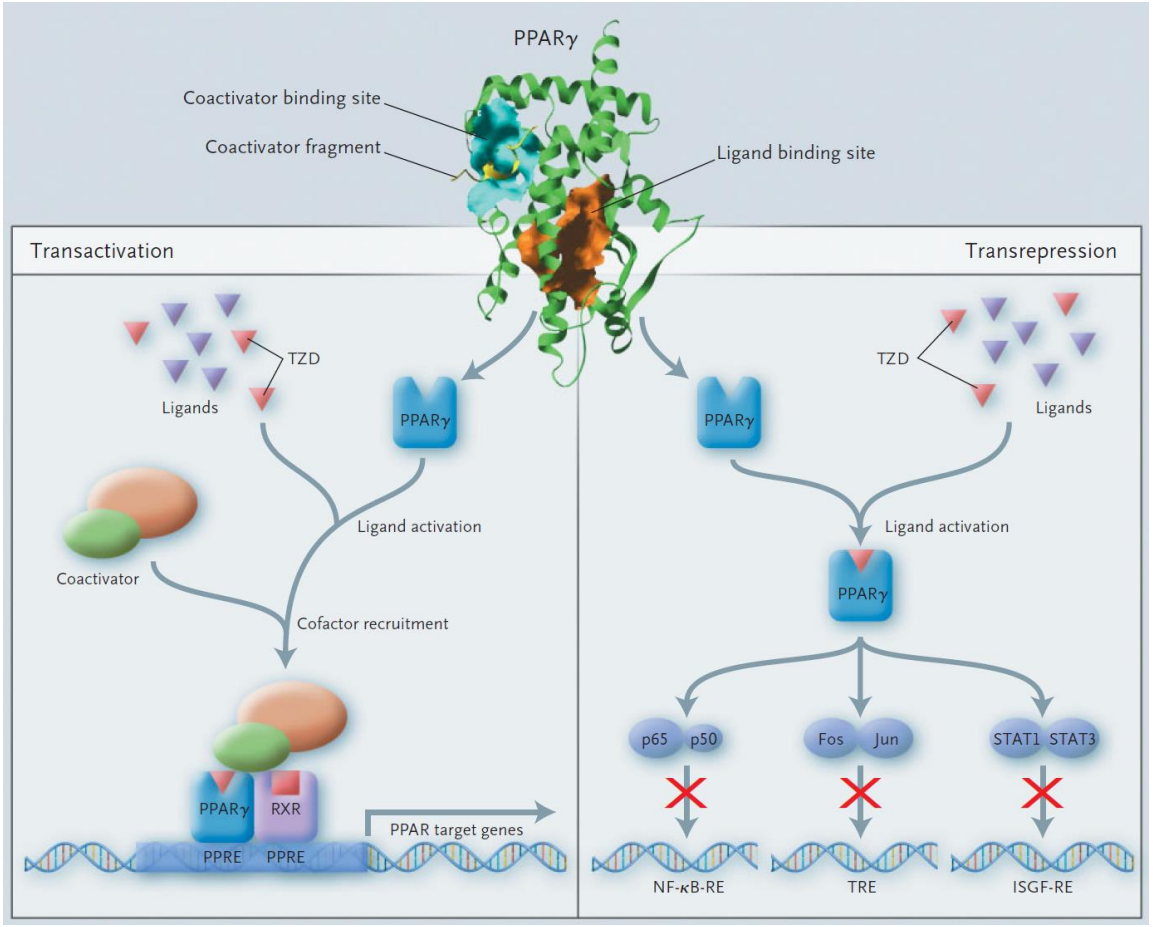
Metabolic Syndrome





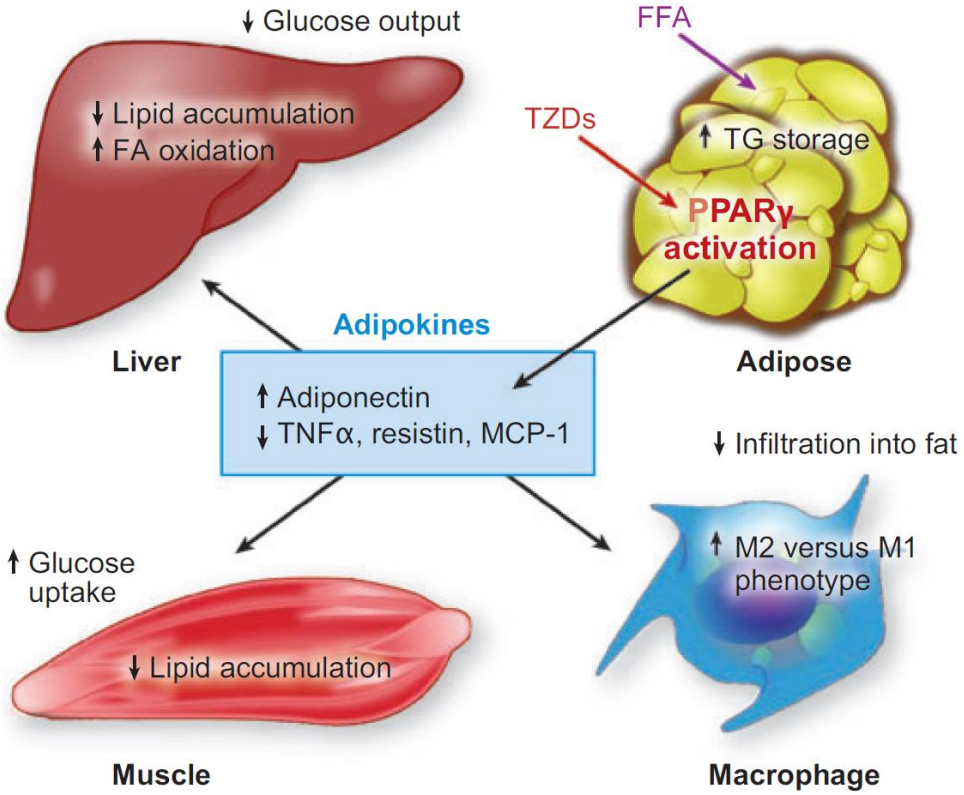
What is PPARγ?

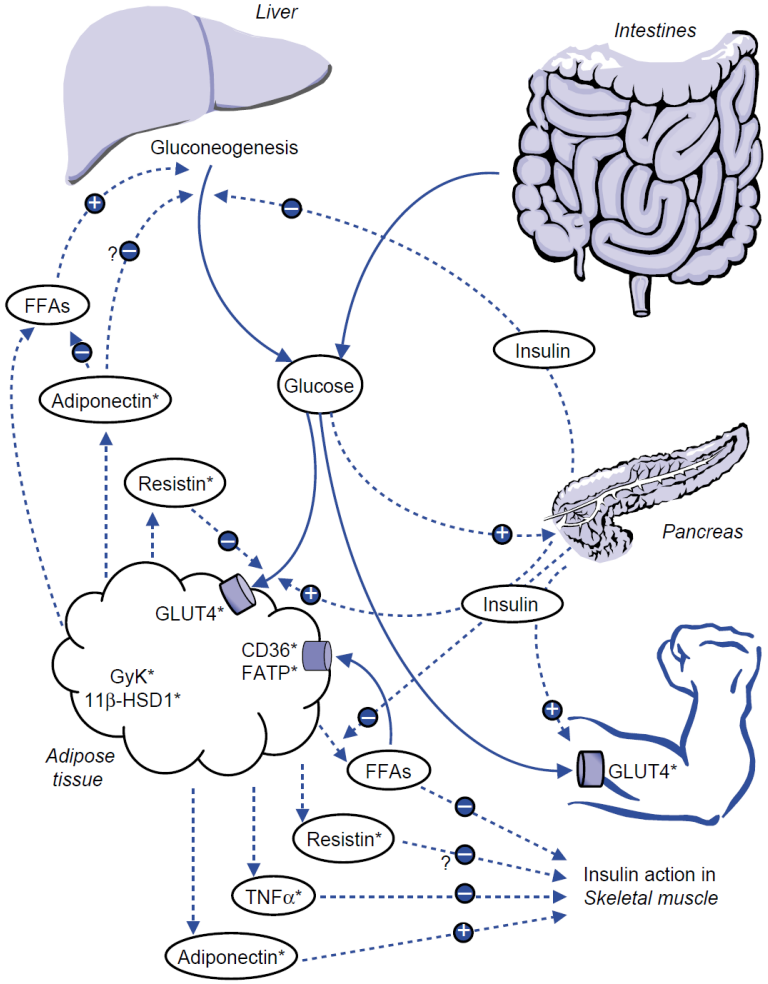




Annu. Rev. Biochem. (2008)

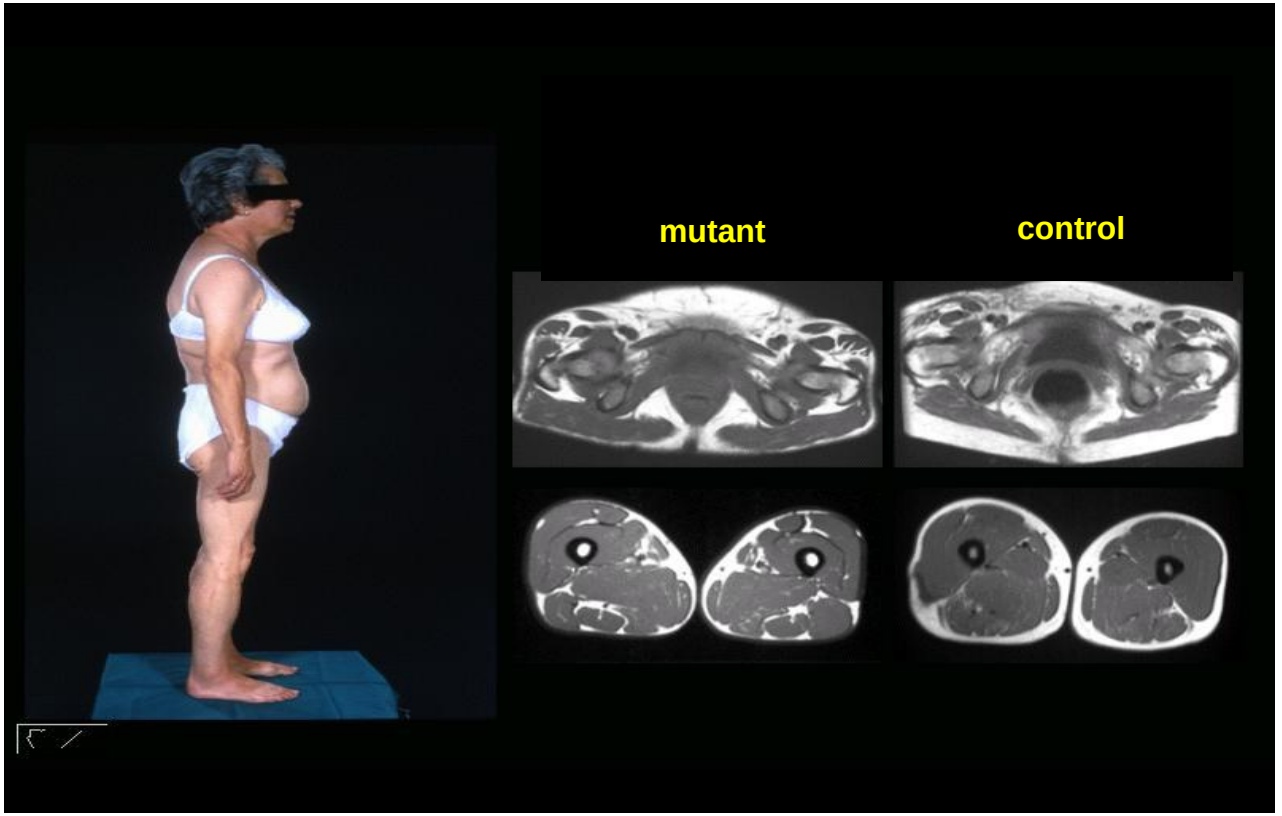
Role of TZDs in PPARγ-mediated glucose metabolism





Annu. Rev. Biochem. (2008)

Dominant-negative PPARγ mutation in human



P467L mutation
MRI images
56-year-old female

Severe Insulin Resistance

Limb and buttock lipodystrophy

Severe dyslipidaemia

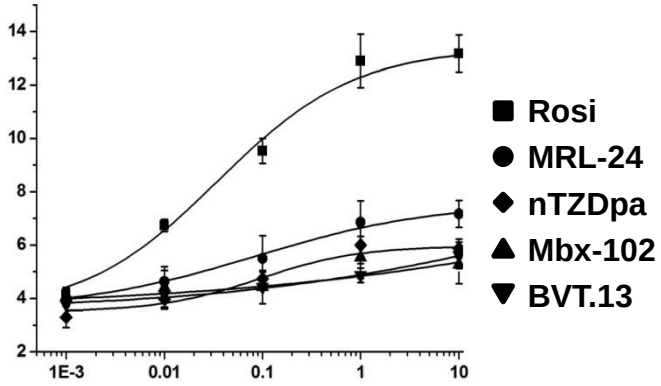
Early Onset Hypertension

Nature (1999)
Diabetes (2003)

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.



Paradoxes regarding PPARγ and insulin-resistance

1. **Partial loss of function mutations in PPARγ in humans unambiguously cause severe insulin resistance.**
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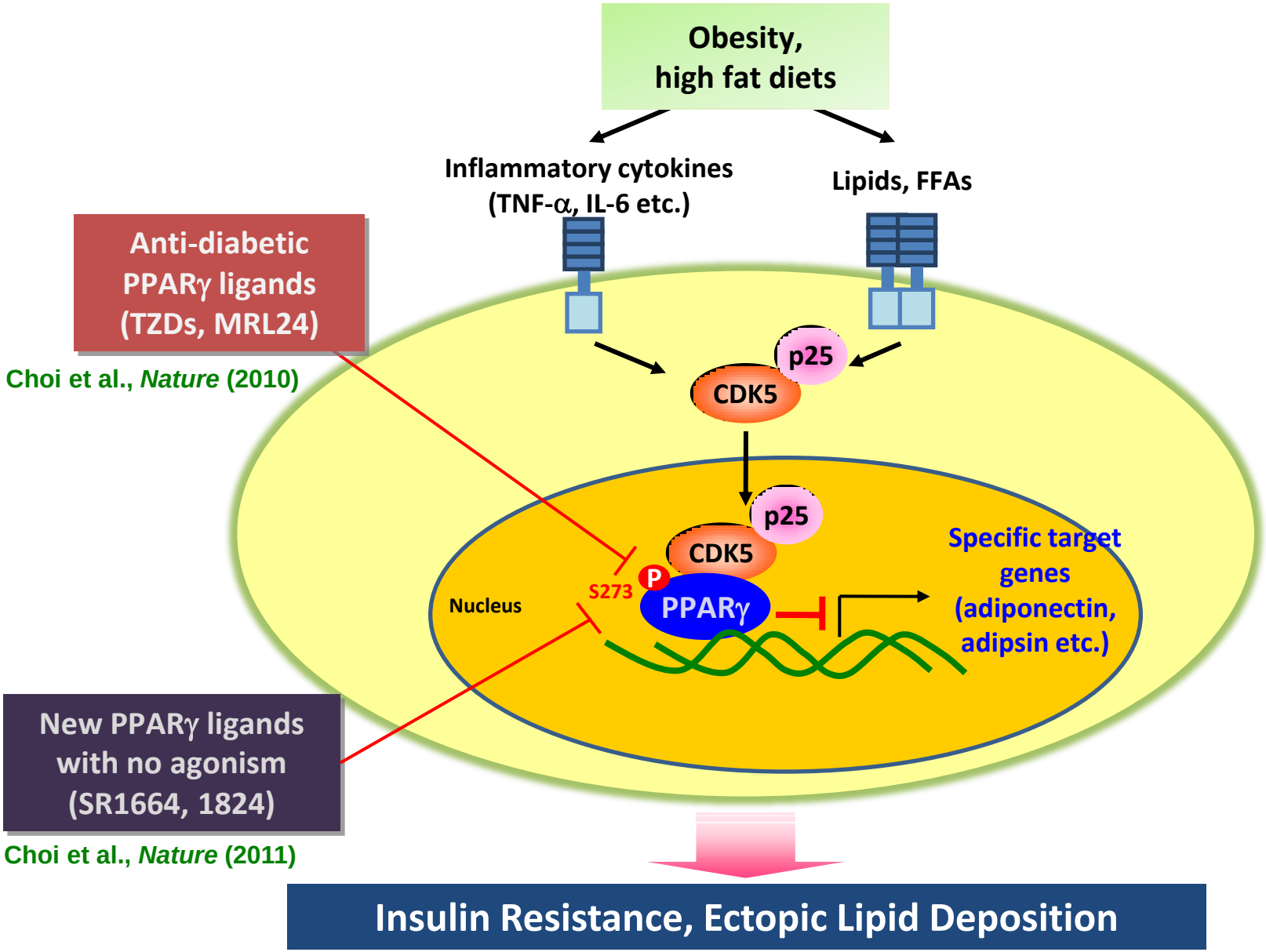
4. **Severe side effects of PPARγ full agonists (TZDs) such as heart failure, weight gain, fluid retention.**
5. **Some PPARγ ligands with poor agonist activity (partial agonists) still have *marked* anti-diabetic actions.**

Can we separate PPARγ agonism from anti-diabetic actions?

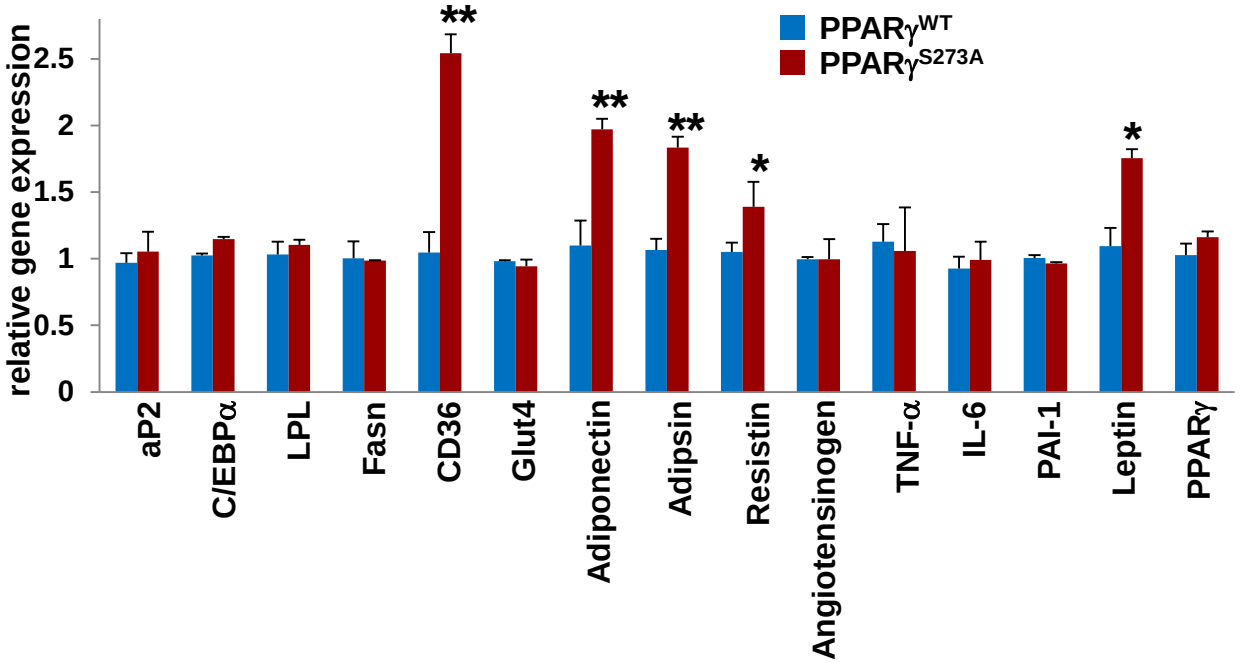
Are there novel mechanisms linking PPARγ to insulin-resistance?

Schematic model of PPARγ phosphorylation and anti-diabetic PPARγ ligands



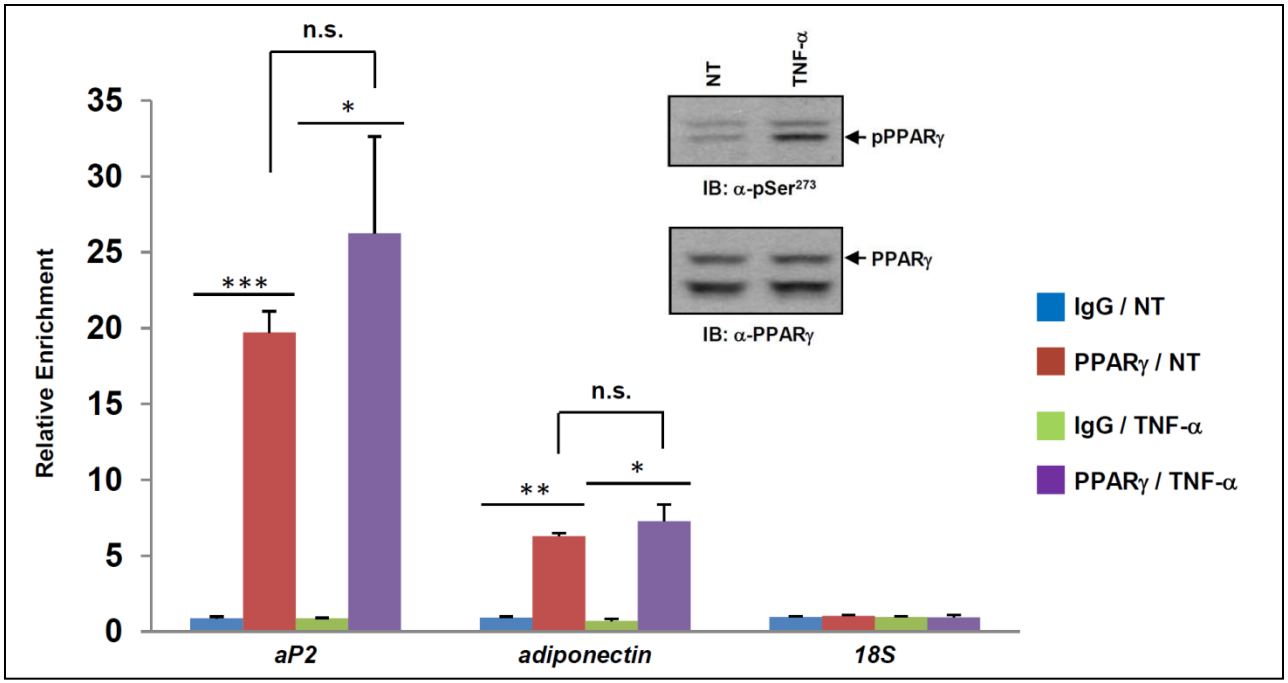
Key questions

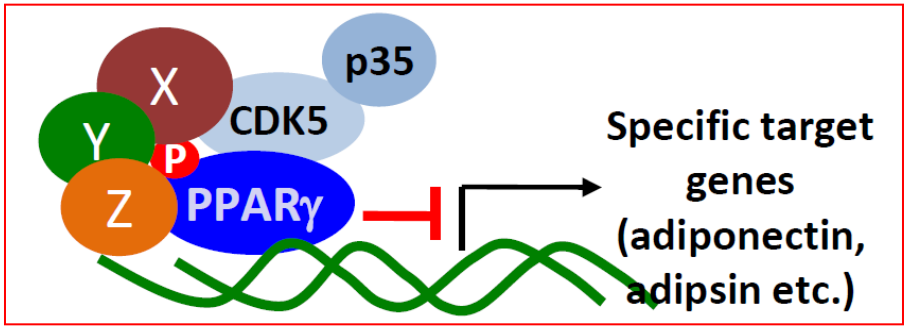
- **What is the molecular mechanism regulating the specific gene expression program controlled by phosphorylation?**
  - ➔ **1. Different DNA occupancy by phosphorylation?**
  - **2. Specific modulators binding to PPARγ?**



Choi et al., *Nature* (2010)

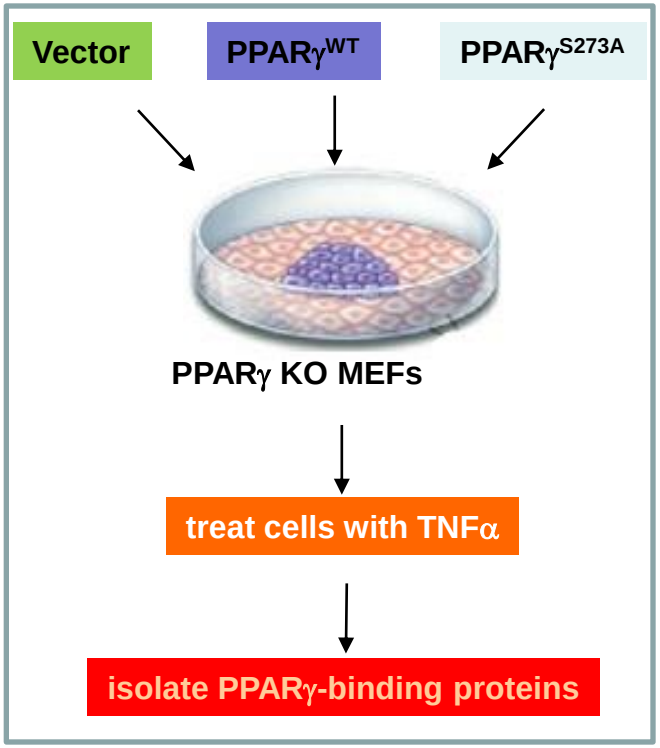
No difference of DNA occupancy by phosphorylation

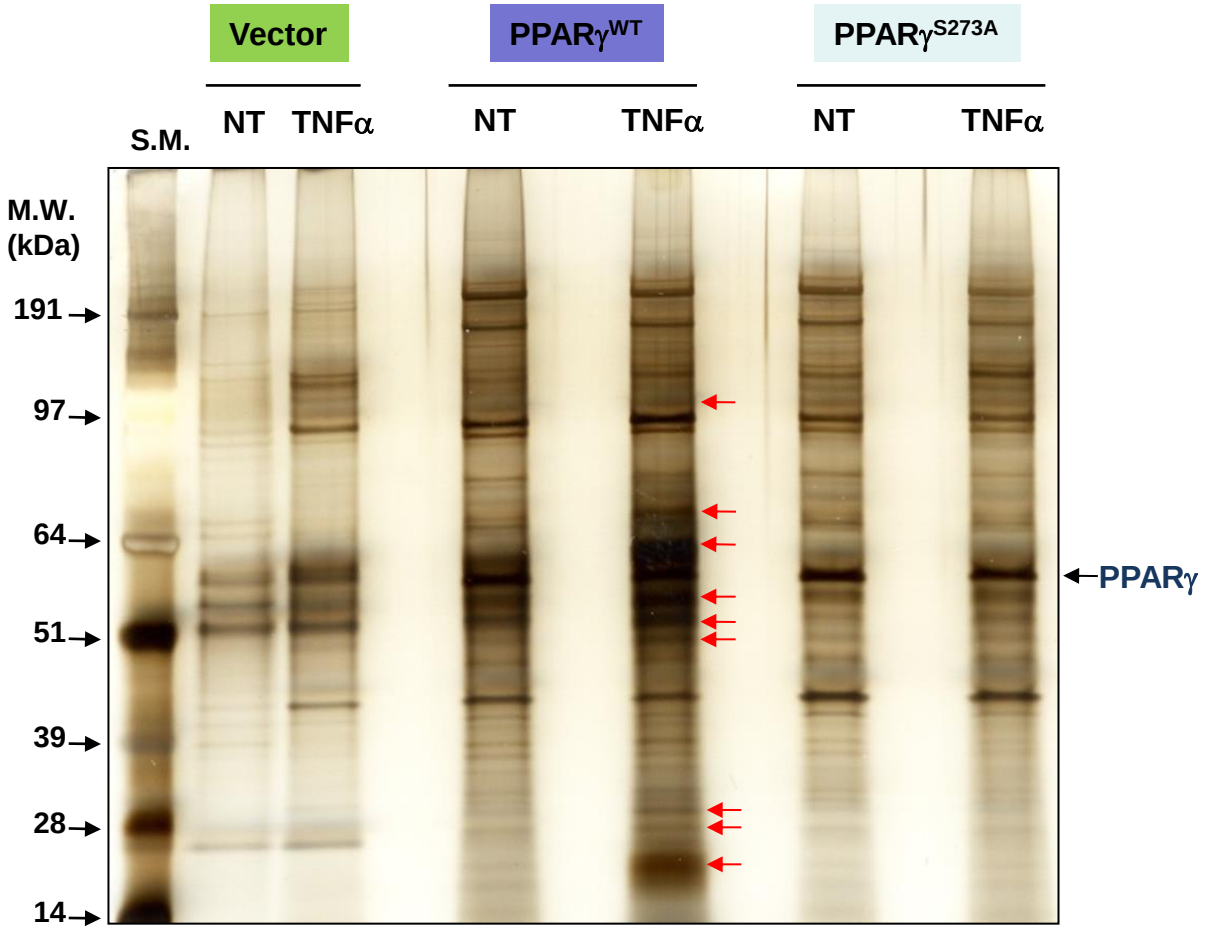




Choi et al., *Nature* (2010)

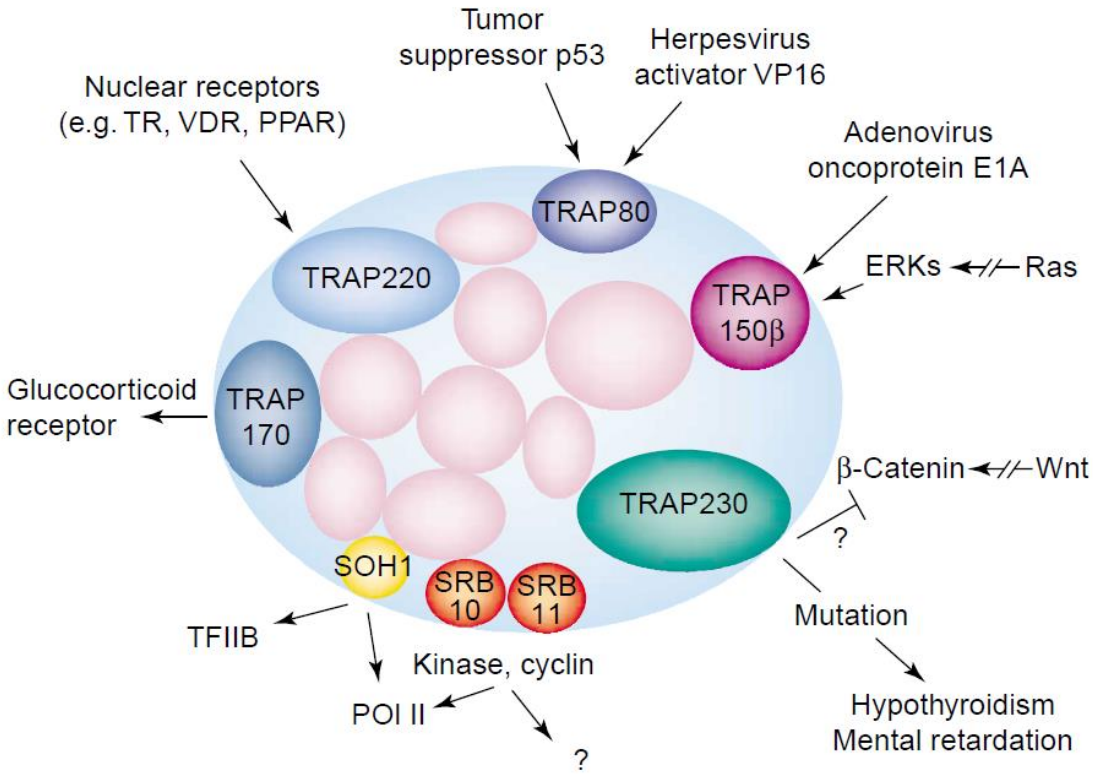
Identifying PPARγ-binding proteins in phosphorylation-dependent manner





Choi et al., *Genes & Development* (2014)

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

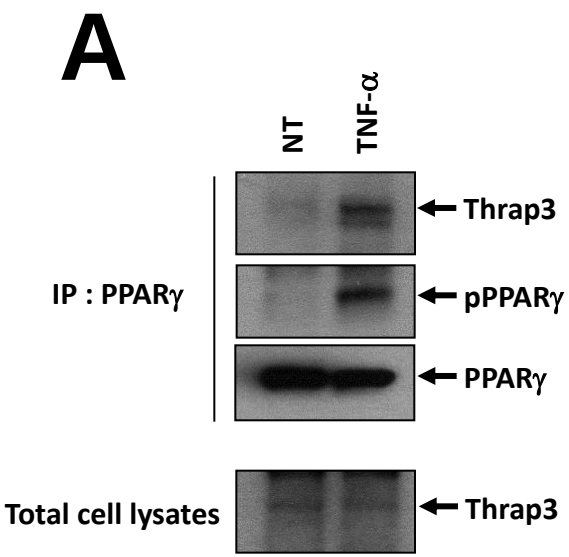
[a]Abbreviations: RNAi, RNA interference; TRAP, thyroid hormone receptor associated protein.
[b]Heterozygotes: dwarfism, pituitary hypothyroidism, transcriptional dysregulation; homozygotes: poor embryonic heart trabeculation, thin neural tube, cell cycle dysregulation and apoptosis, attenuated thyroid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

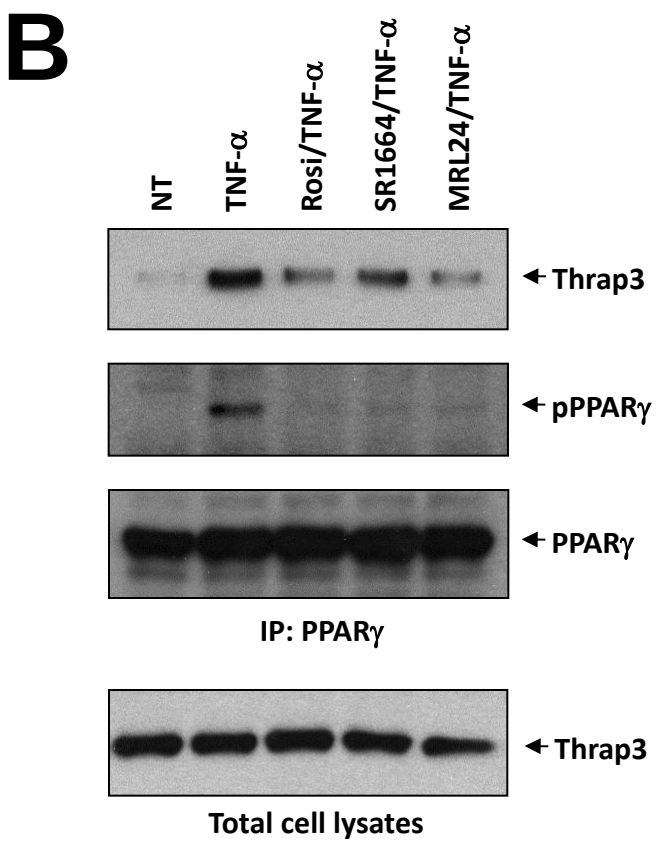
Trends. Endo. & Meta. (2001)

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.**

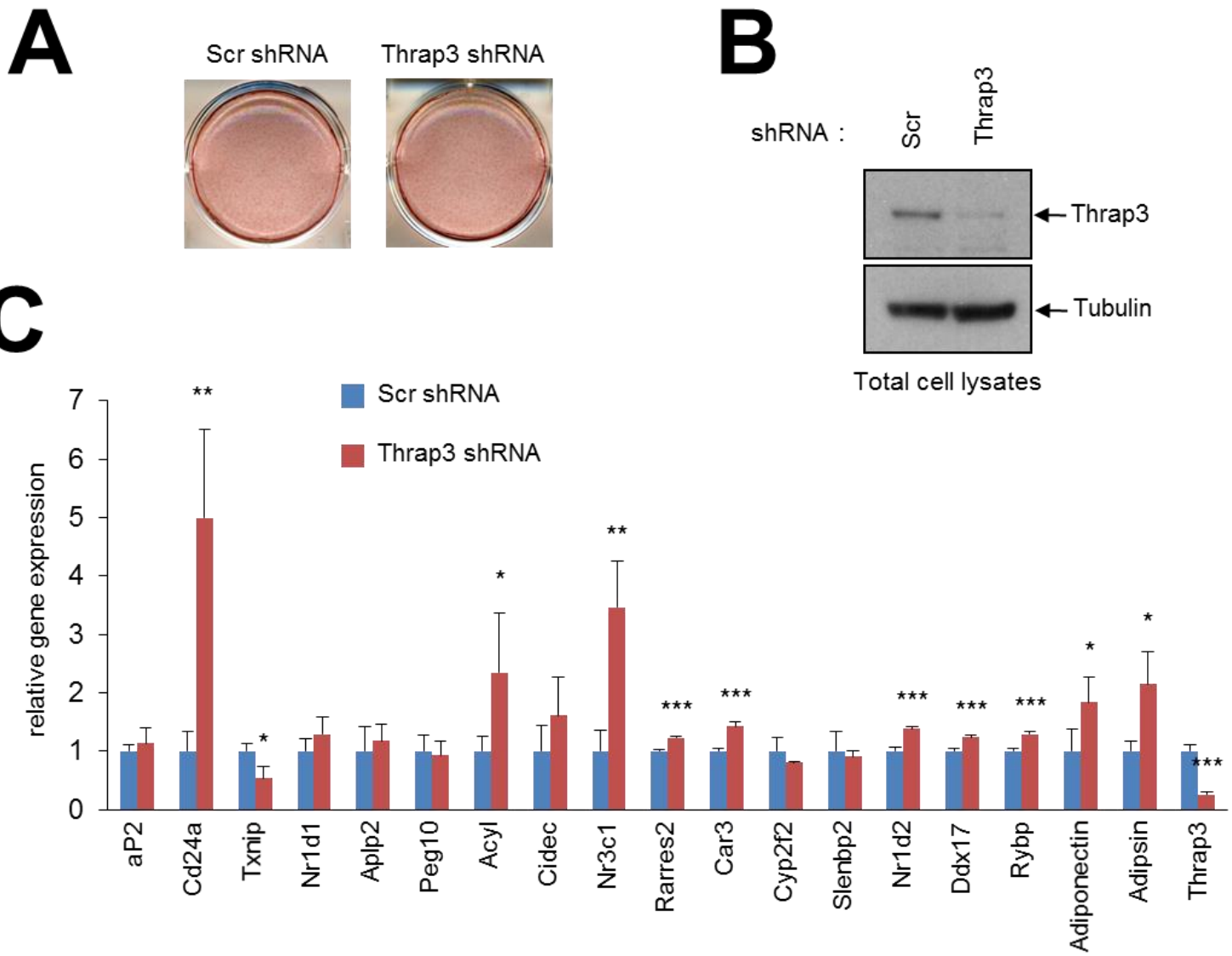
Thrap3 interacts with phosphorylated PPARγ





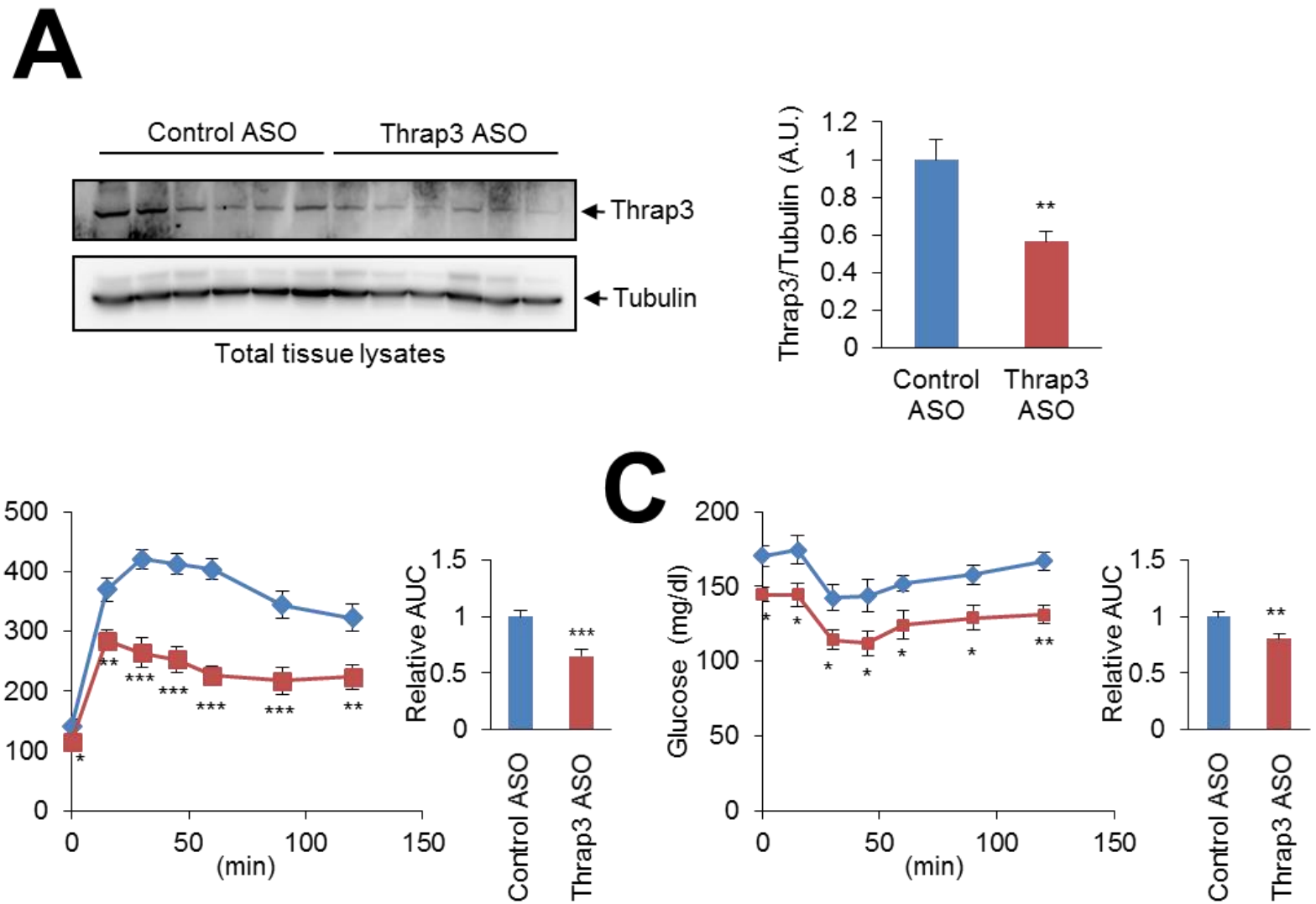
Choi et al., *Genes & Development* (2014)

Specific gene regulation by Thrap3



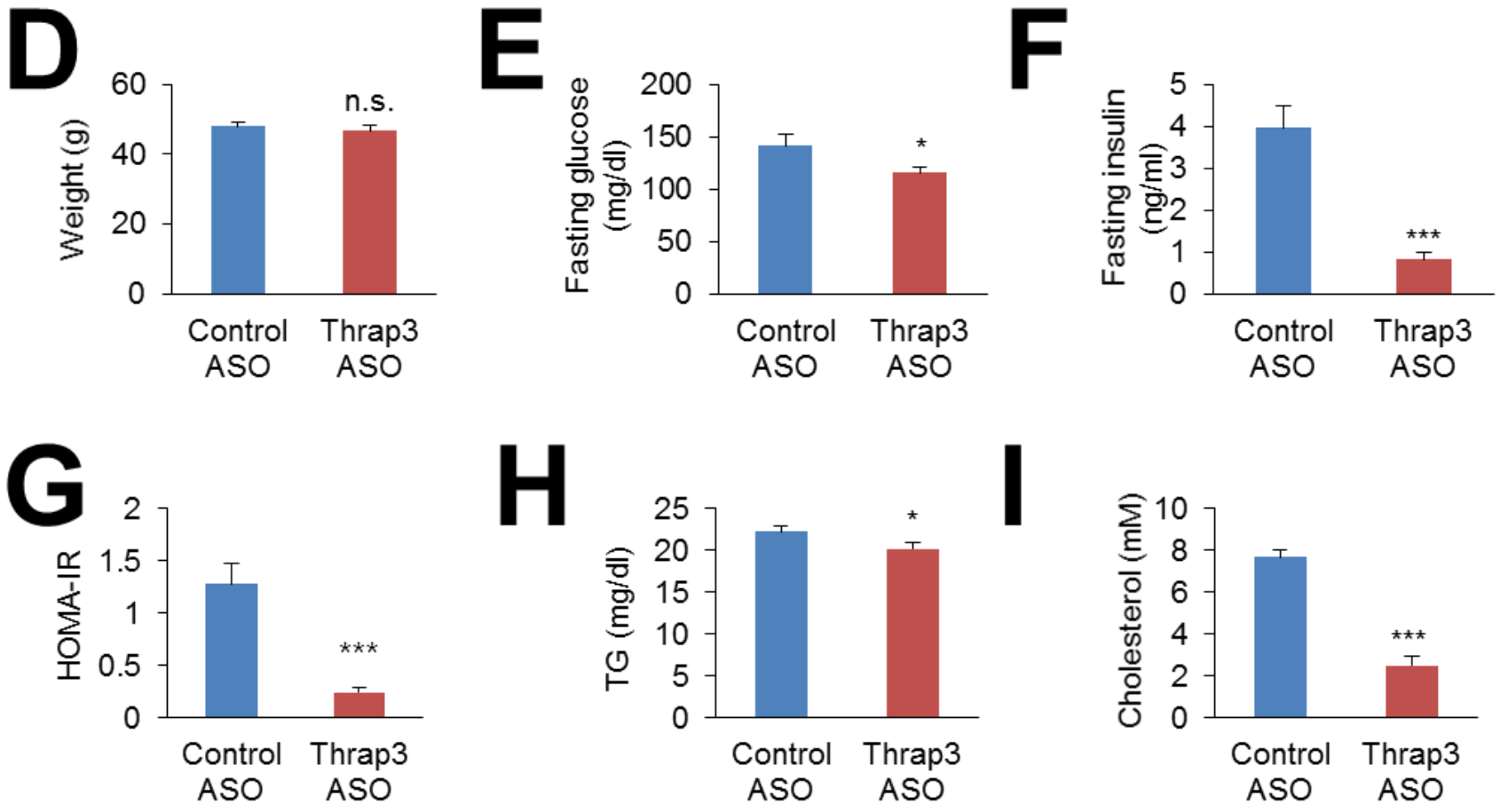
Choi et al., *Genes & Development* (2014)

Improved insulin sensitivity by Thrap3 ASO *in vivo*



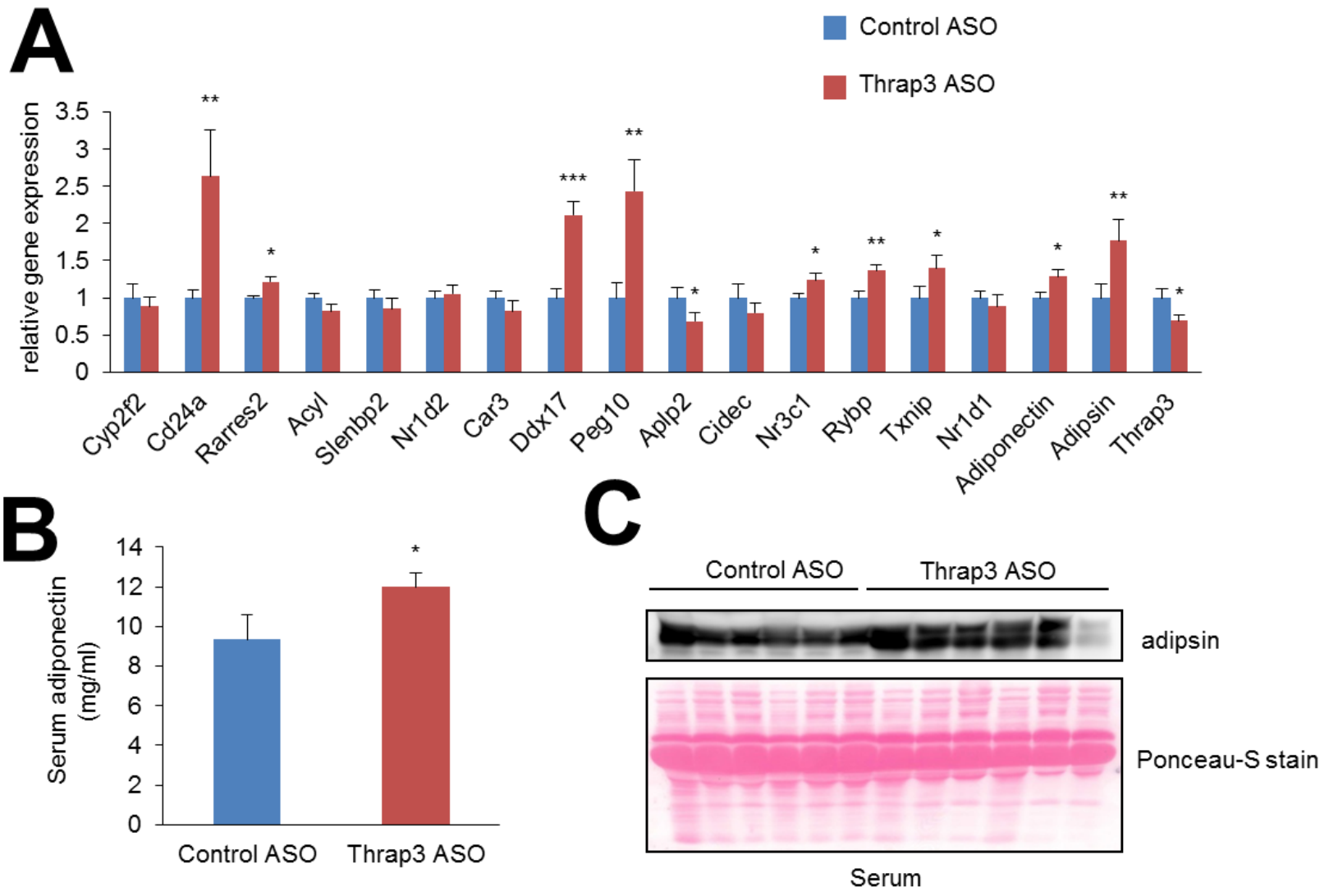
Choi et al., *Genes & Development* (2014)

Improved insulin sensitivity by Thrap3 ASO *in vivo*



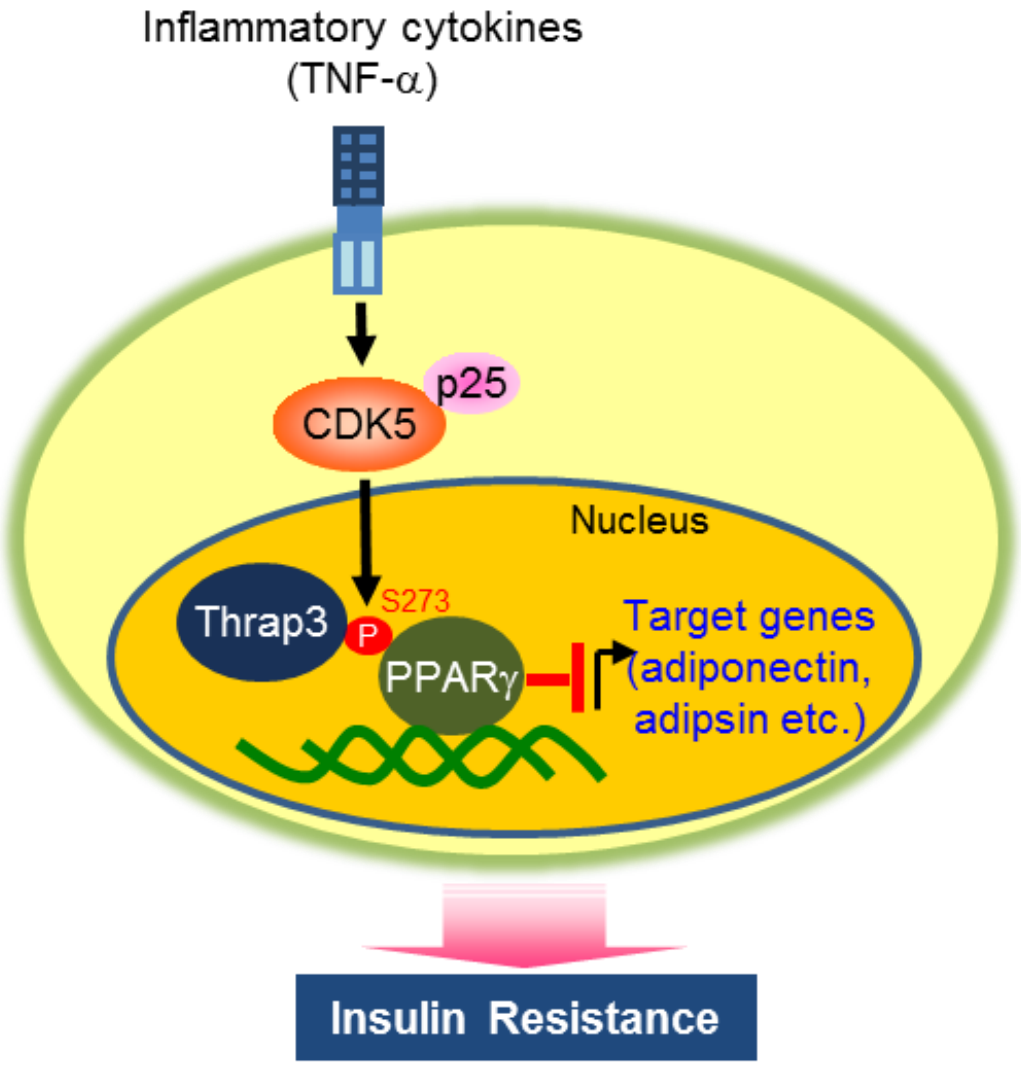
Choi et al., *Genes & Development* (2014)

Improved insulin sensitivity by Thrap3 ASO *in vivo*



Choi et al., *Genes & Development* (2014)

Proposed model of functional interaction between Thrap3 and PPARγ



Choi et al., *Genes & Development* (2014)

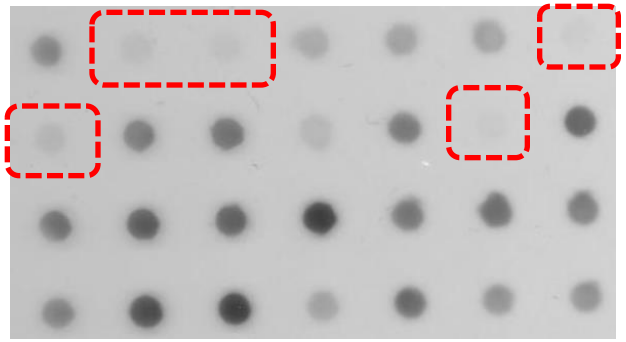
Key Question: Screening strategy to identify non-agonist PPARγ ligands

Synthetic chemical library

In vitro kinase assay

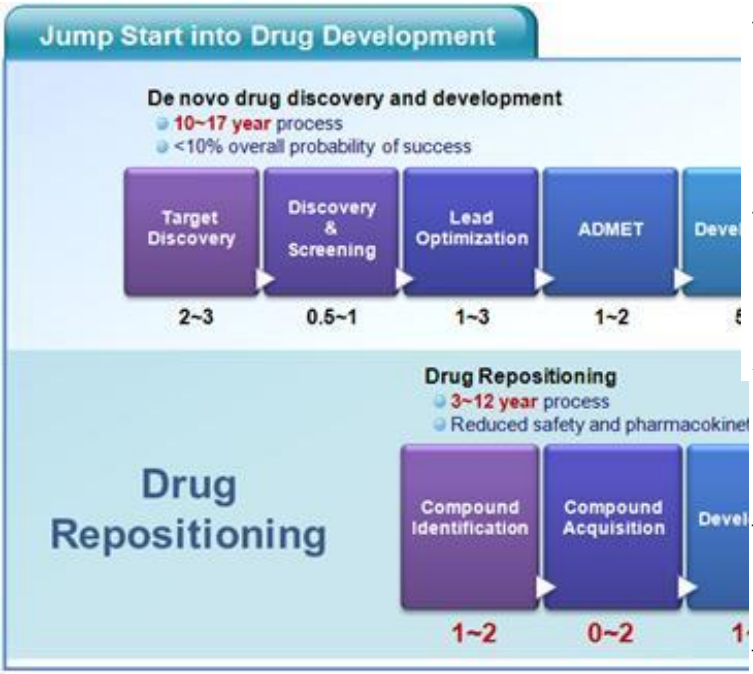
dot blot
(anti-pSer273)

Selection



- **Can we develop the novel non-agonist PPARγ ligands?**
  - ➔ **1. No transcriptional activation**
  - **2. Specific binding to PPARγ**
  - **3. Block CDK5-mediated PPARγ phosphorylation**
  - **4. Improve insulin sensitivity**
  - **5. Lack side effects including weight gain and fluid retention**

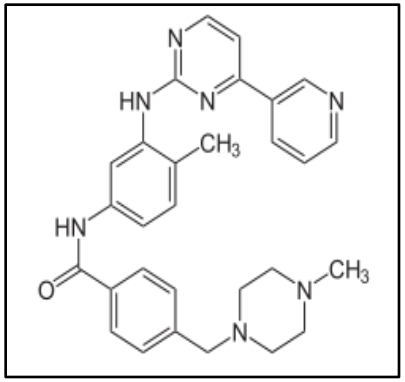
Drug Repositioning

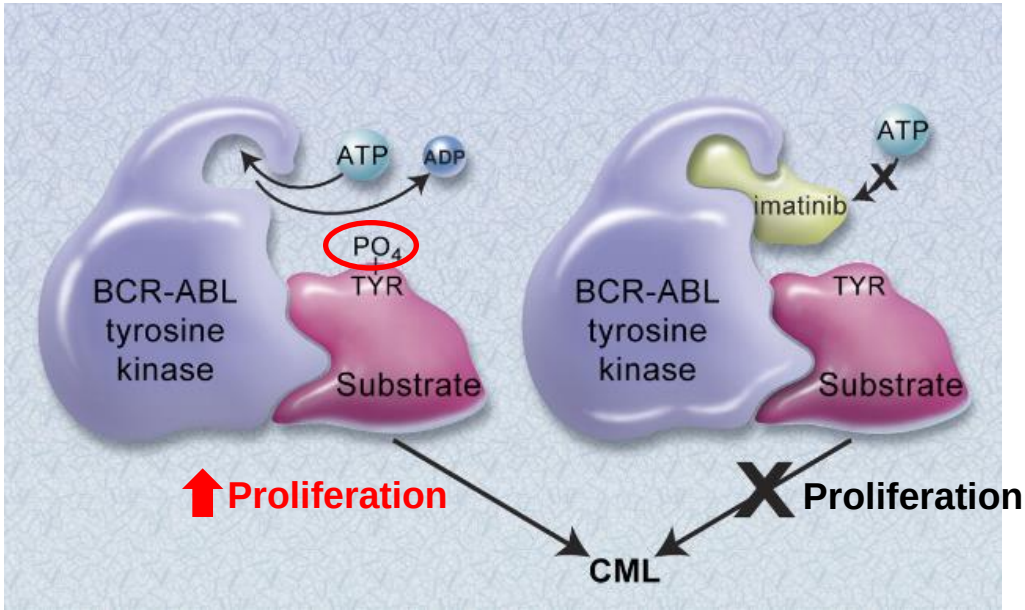


Company	Repositioning Drug	Repositioning Type	Date	Value	Reference
Celgene	Thalidomide (Thalidomid)	Erythema nodosum laprosum (ENL), Multiple myeloma	1998	$224 million in 2003 (US)	Drug Repositioning strategies 2007 by Data monitering(p22)
Forest Laboratories/ Cypress Bioscience (Buyer/Seller)	Milnacipran	Fibromyalgia	2004	US $25 million, Up to US$250 million in milestones	Touch Briefing 2006 Drug Repositioning – Drug Discovery 2006
Pfizer	Sidenafil (Viagra, Revatio)	Erectile dysfuntion, pulmonary arterial hypertension	2007	$2 billion	Drug Repositioning strategies 2007 by Business Insight(p19)
Pfizer	Minoxidil (Rogaine)	Baldness	1998	$162 million in 1995	Drug Repositioning strategies 2007 by Data monitering(p77)
Lilly	Duloxetine (Yentreve)	Stress urinary incontinence (SUI)	2007	$0.8 billion	Drug Repositioning strategies 2007 by Data monitering(p21)
Lilly	Gemcitabine (Gemzar)	Cancer	2007	$1.2 billion	Drug Repositioning strategies 2007 by Business Insight(p19)
Lilly	Raloxifene (Evista)	Osteoporosis	2007	$1 billion	Drug Repositioning strategies 2007 by Business Insight(p19)
GSK	Bupropion (Zyban)	Help smoking cessation	2002	$125 million in 2003	Drug Repositioning strategies 2007 by Business Insight(p114)

Gleevec (Imatinib)

4-[(4-methylpiperazin-1-yl)methyl]-*N*-(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)benzamide



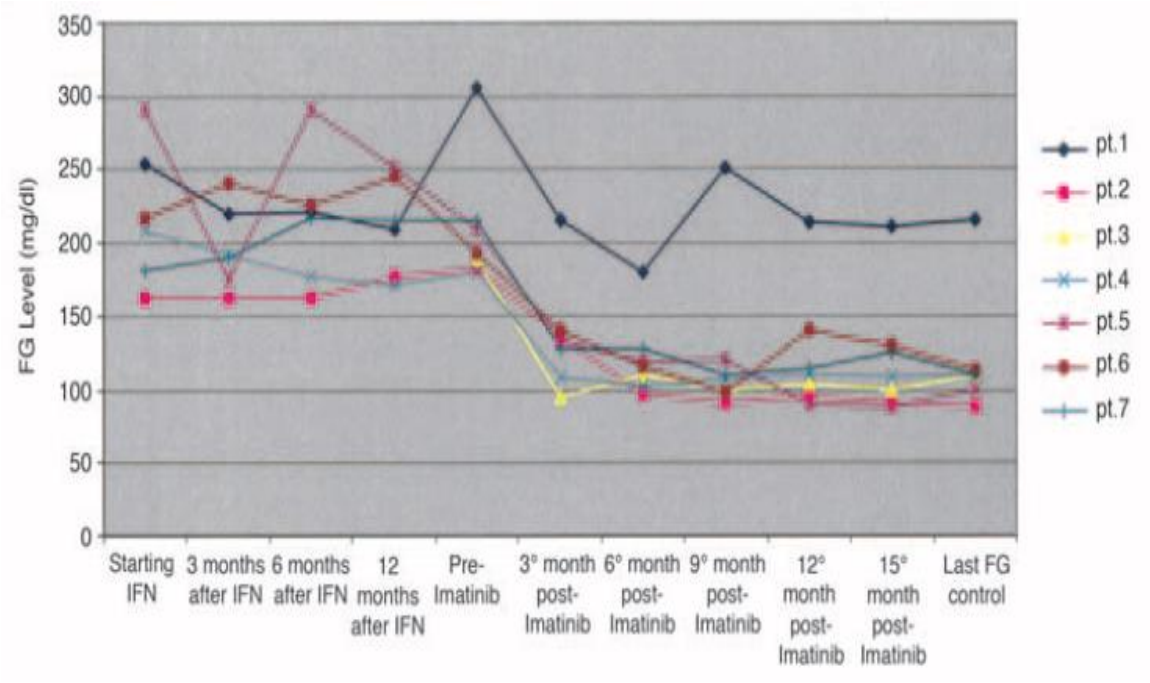


- marketed by Novartis
- **tyrosin-kinase inhibitor**
- works as a **targeted** therapy by preventing **BCR-Abl**, which leads to apotosis.
- **used in** the treatment of multiple cancers, most notably **Philadelphia chromosome-positive chronic myelogenous leukemia (Ph^+CML)**
- inhibits c-kit and PDFG receptor

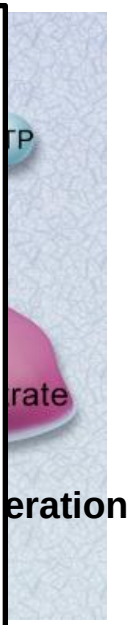
Gleevec improves fasting glucose in patients

Imatinib Mesylate May Improve Fasting Blood Glucose in Diabetic Ph+ Chronic Myelogenous Leukemia Patients

Breccia M, Muscaritoli M, Aversa Z, Mandelli F, Alimena G



J. Clin Oncol. (2004) 15: 653-4655



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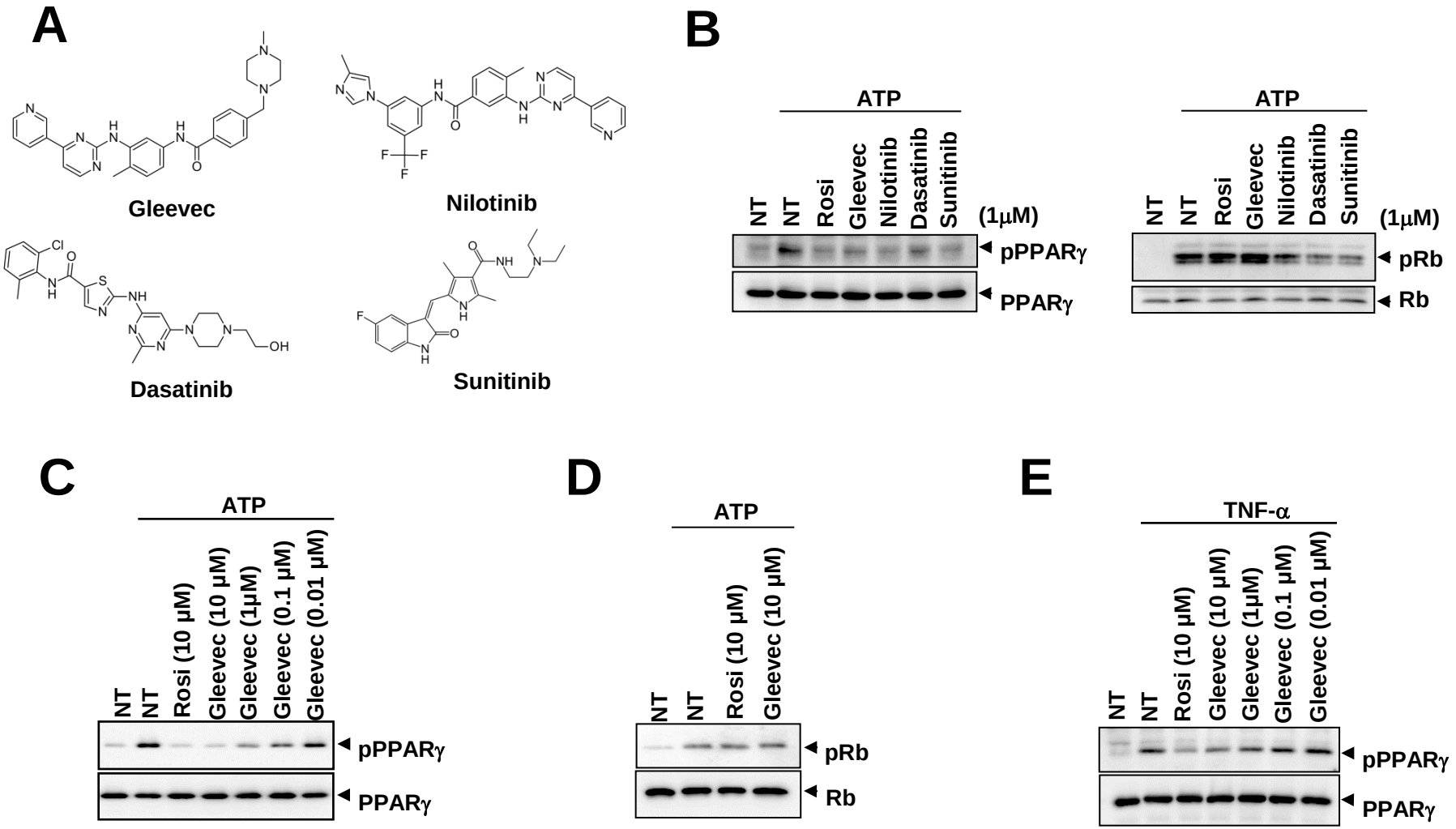
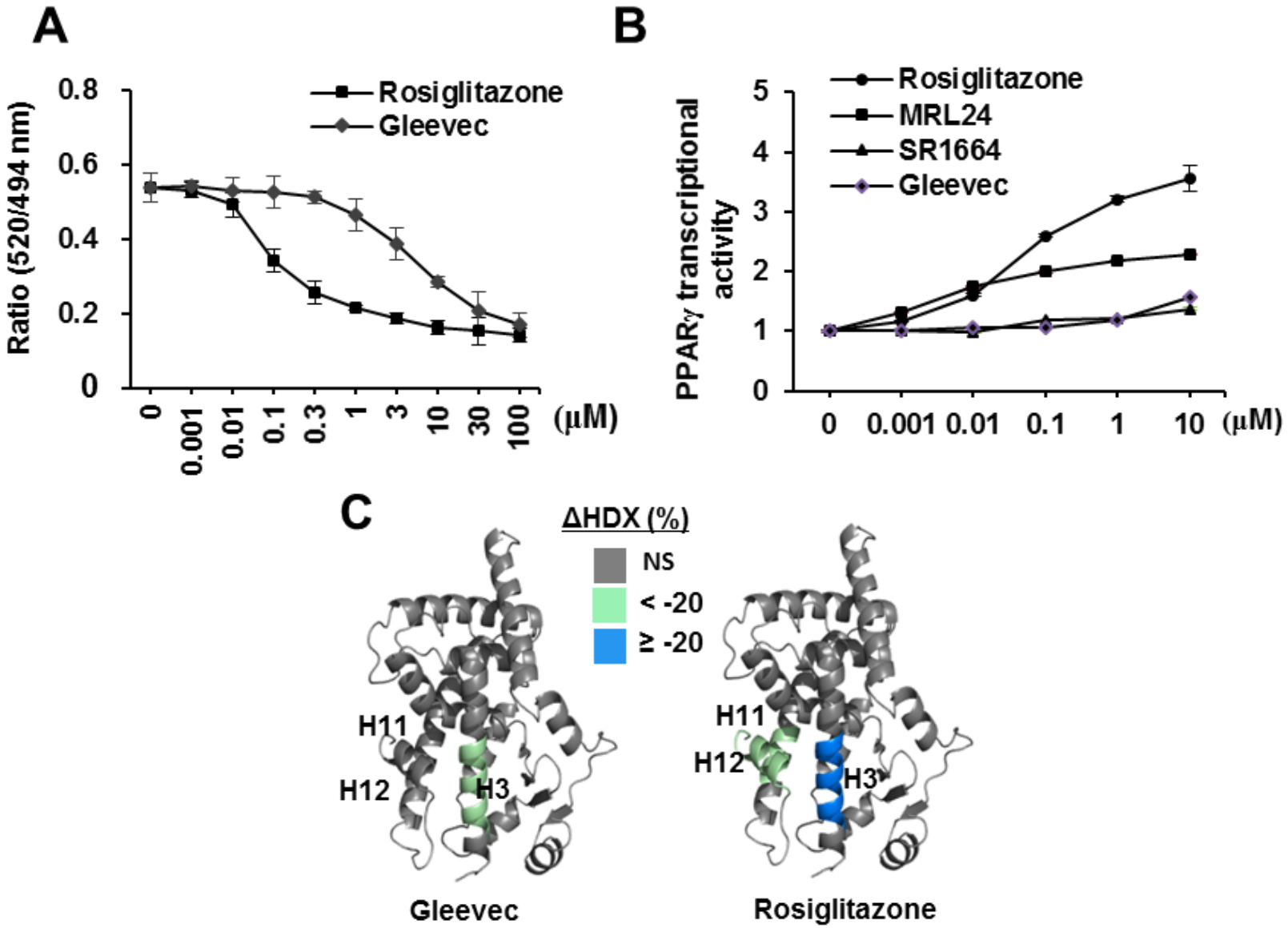
Gleevec blocks CDK5-mediated PPARγ phosphorylation



Choi et al., *Diabetes* (2016)

Gleevec is a non-agonist PPARγ ligand



Choi et al., *Diabetes* (2016)

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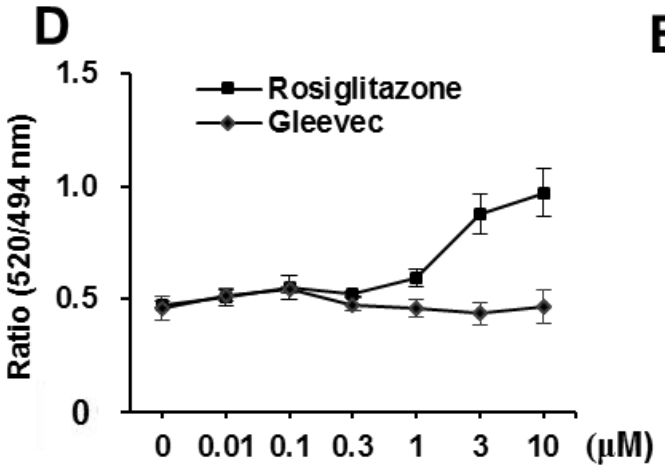
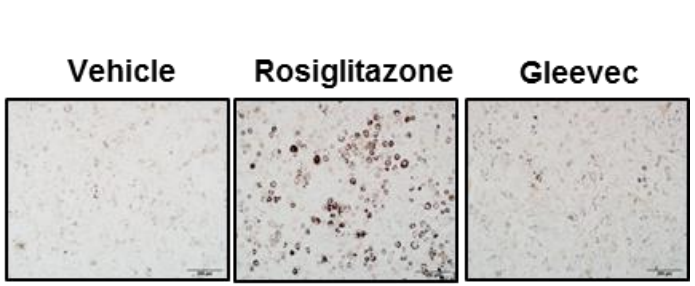
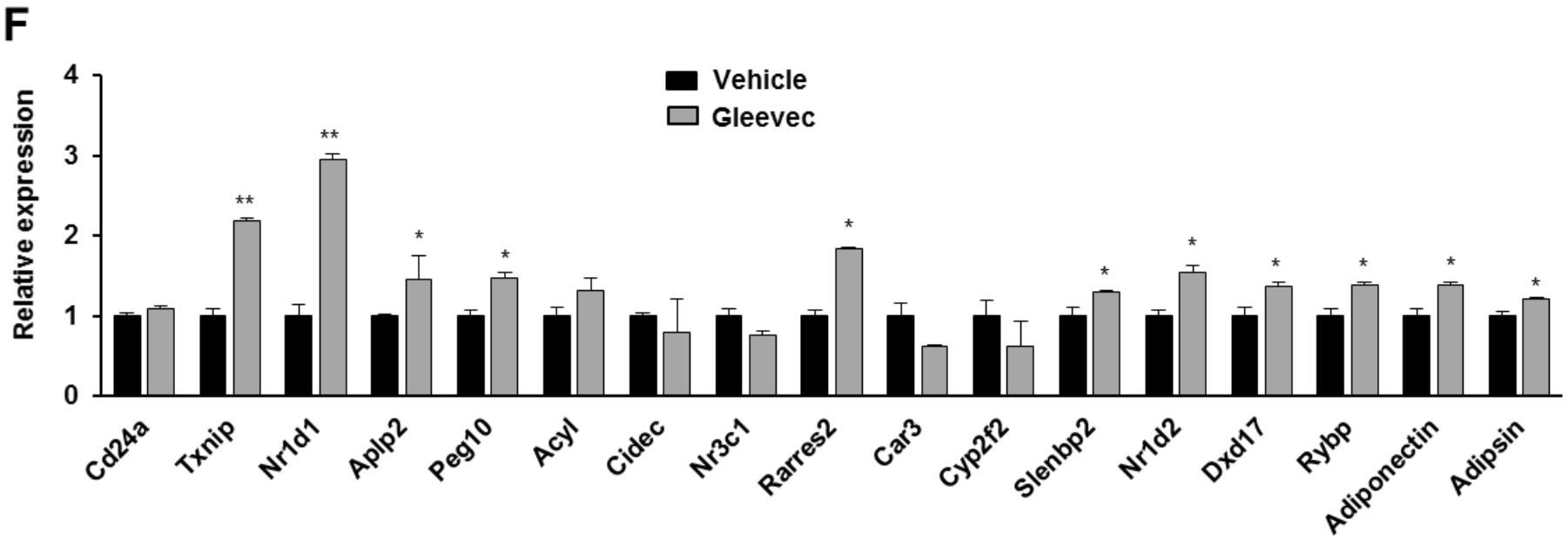
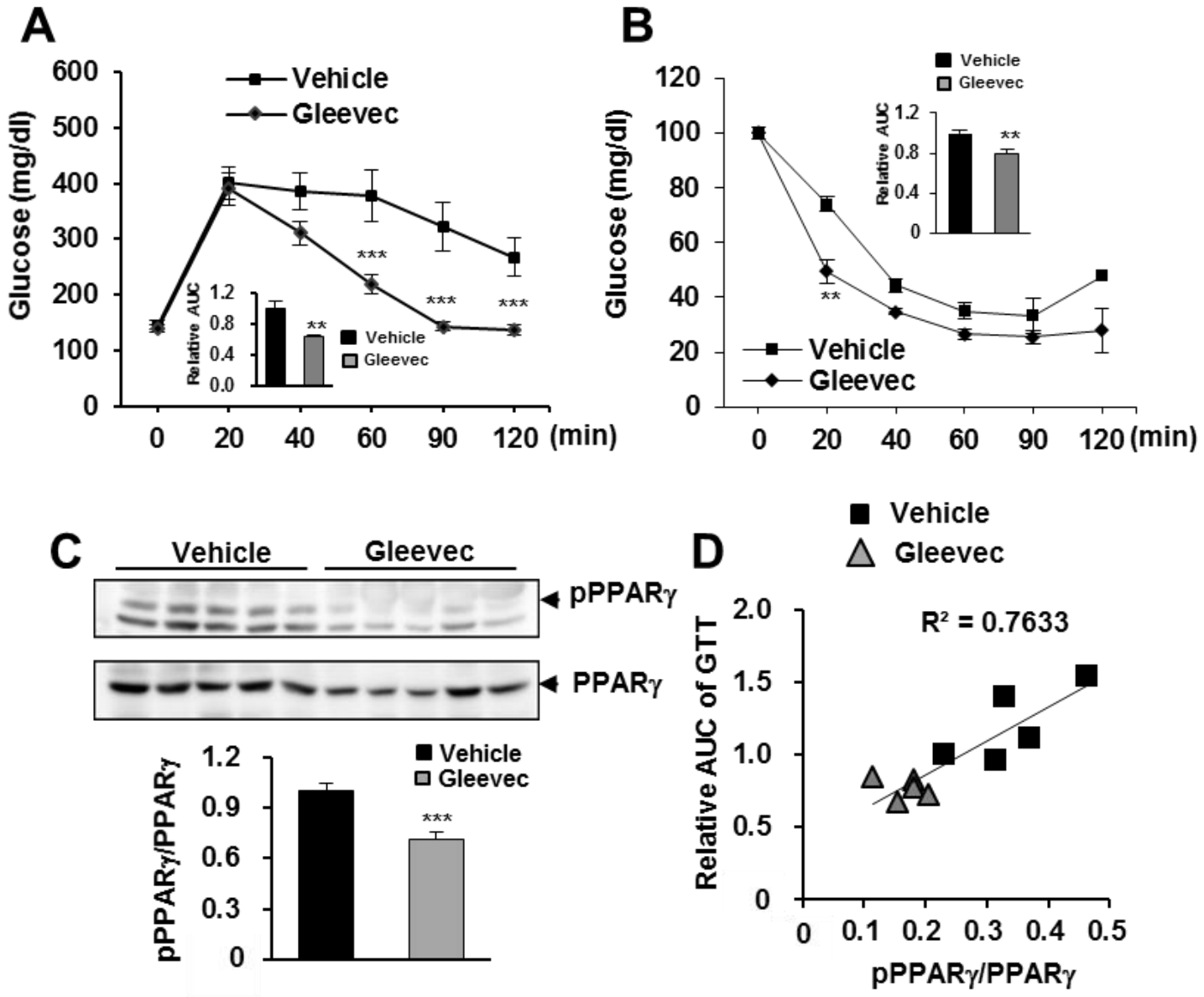






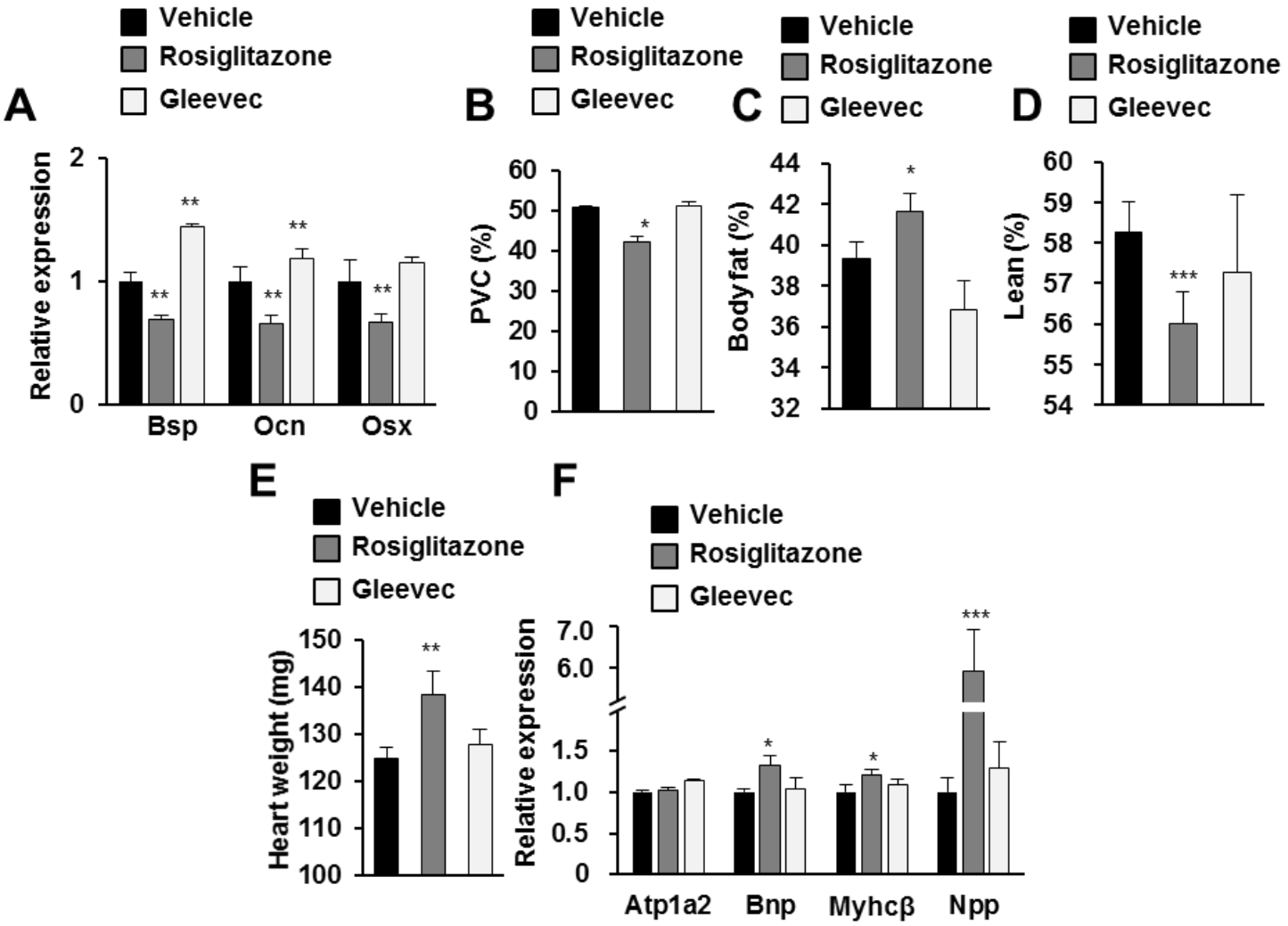
Choi et al., *Diabetes* (2016)

Gleevec improves insulin sensitivity



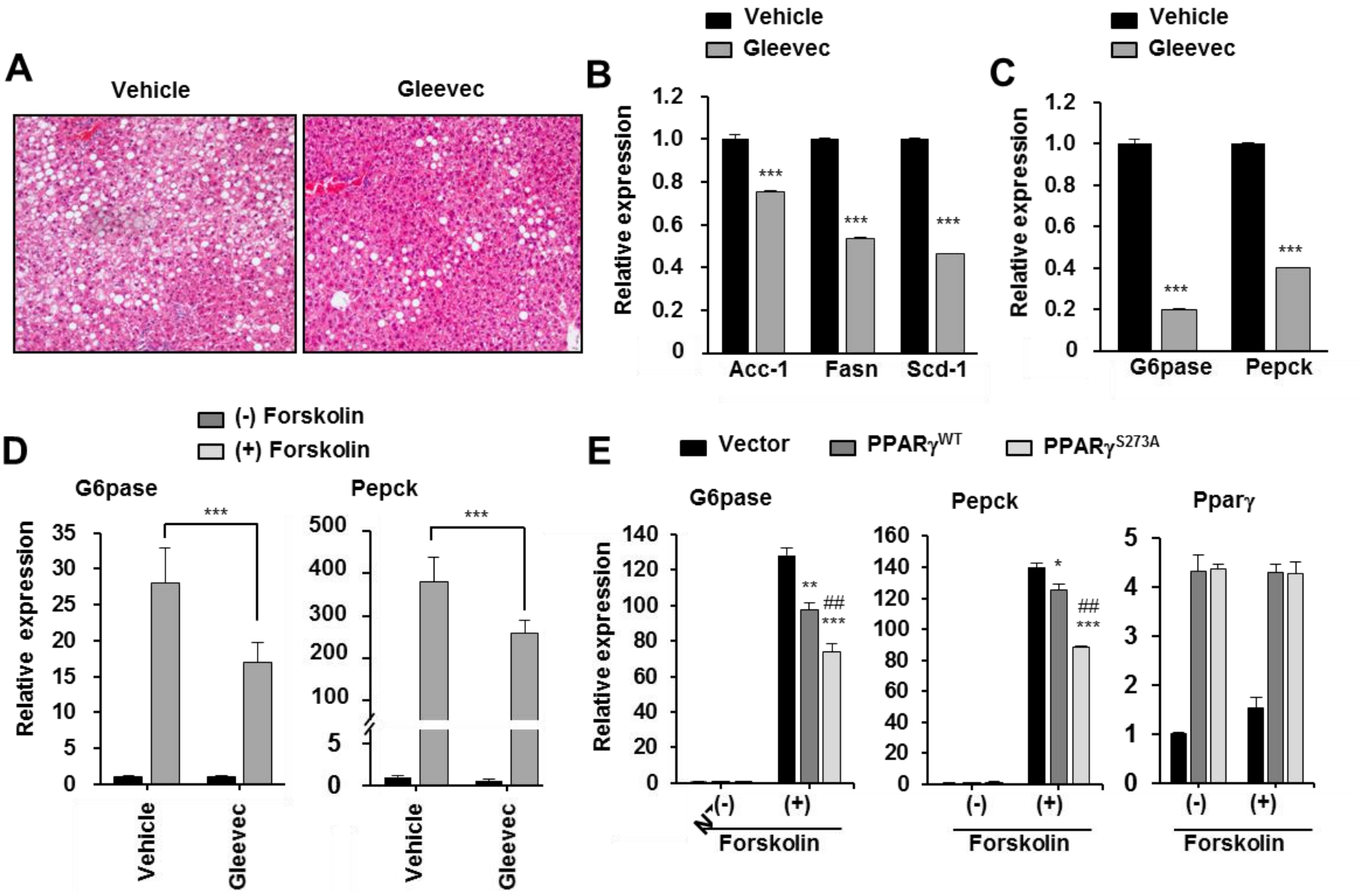
Choi et al., *Diabetes* (2016)

Gleevec does not induce side effects of TZDs



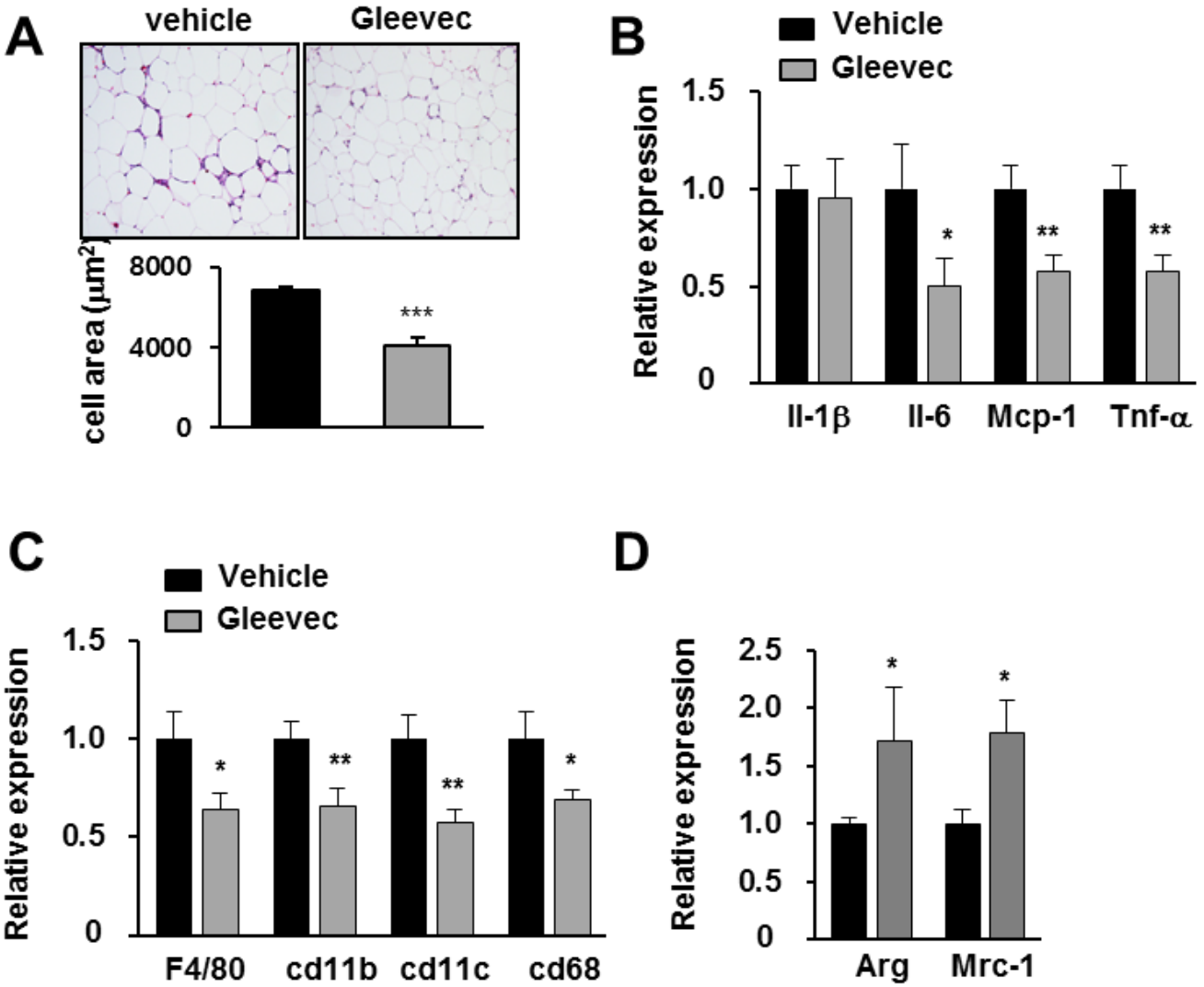
Choi et al., *Diabetes* (2016)

Gleevec ameliorates fatty liver



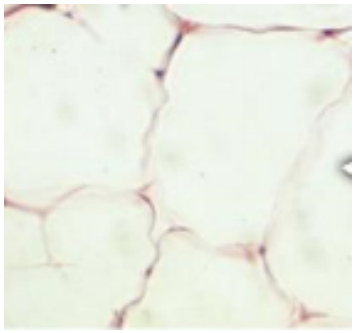
Choi et al., *Diabetes* (2016)

Gleevec ameliorates adipose tissue inflammation



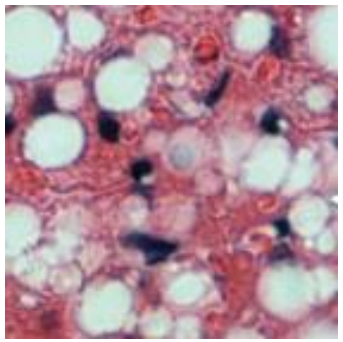
Choi et al., *Diabetes* (2016)

Three types of adipocytes

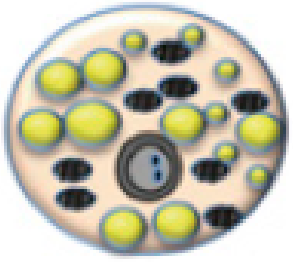


White adipocytes

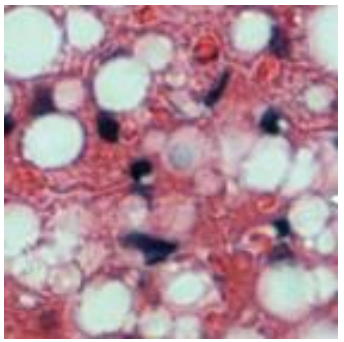
Stores energy
Single large lipid droplet
Adipokine secretion
Pro-inflammatory
Low mitochondria
No UCP-1
In obesity



Beige adiocytes



Dissipates energy
Multiple lipid droplets
high mitochondria
UCP-1
Anti-obesity

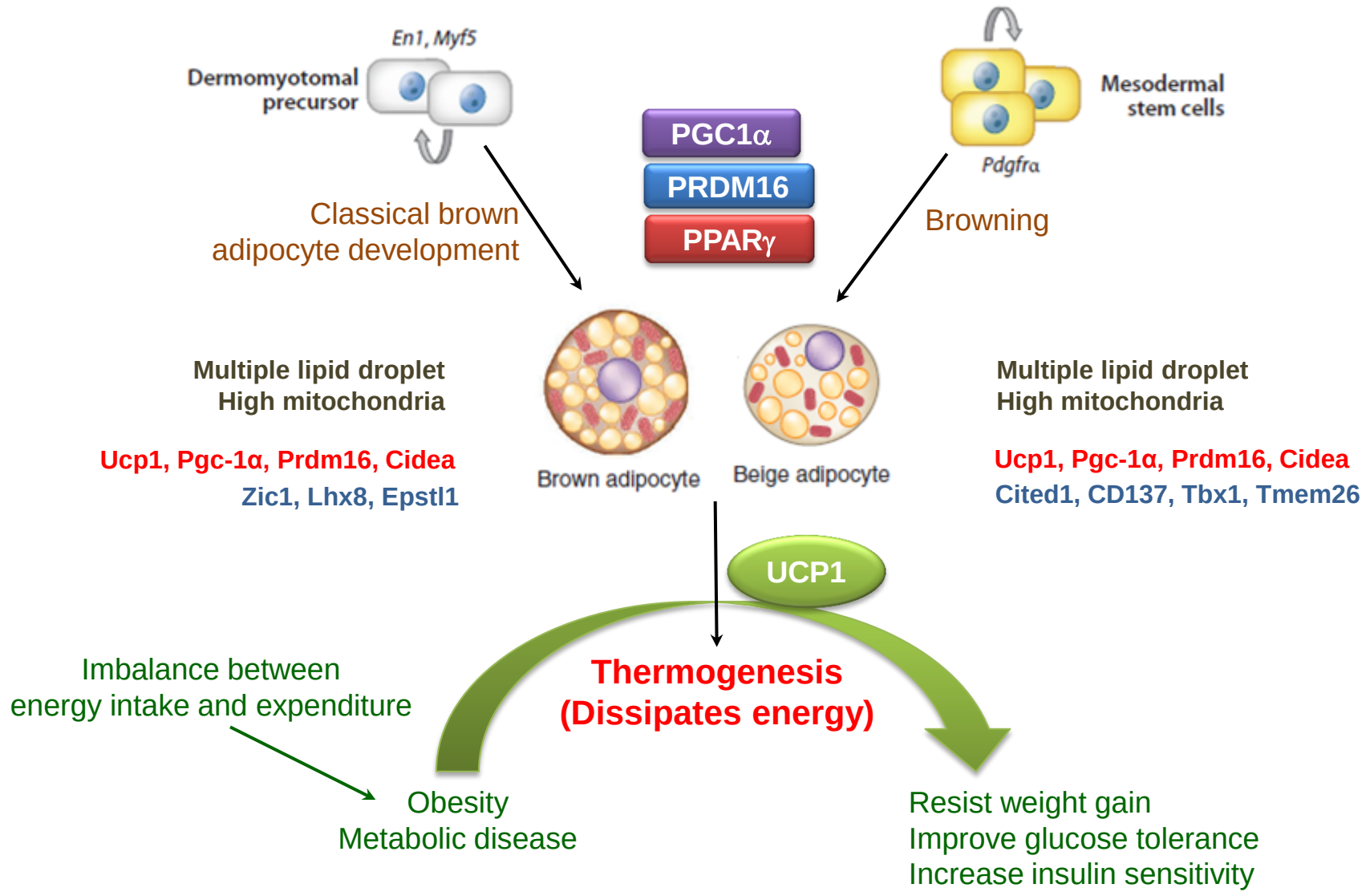


Brown adipocytes

Dissipates energy
Multiple lipid droplets
high mitochondria
UCP-1
Anti-obesity

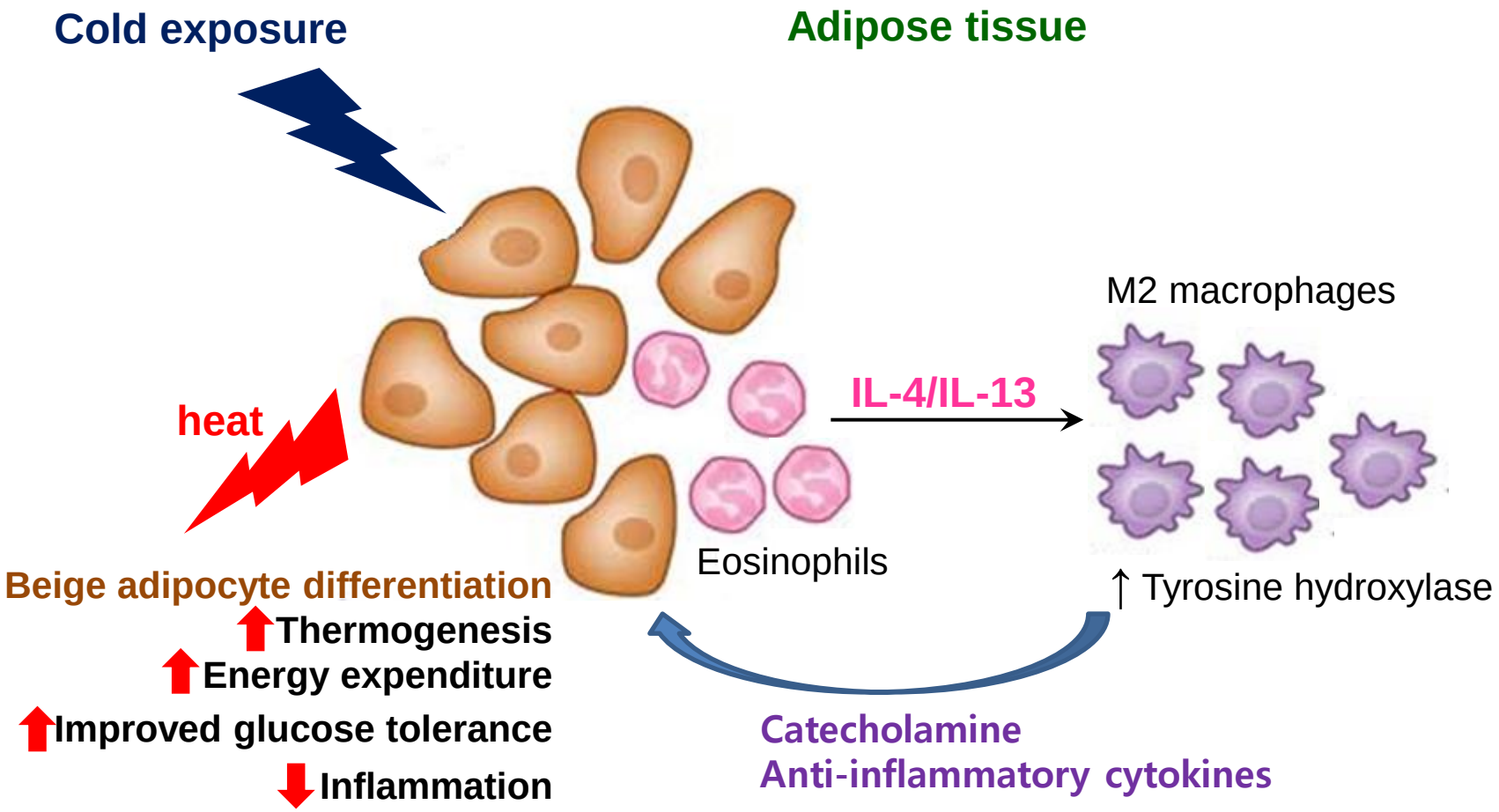
Cell (2012)

Beige/Brown adipocytes and thermogenesis



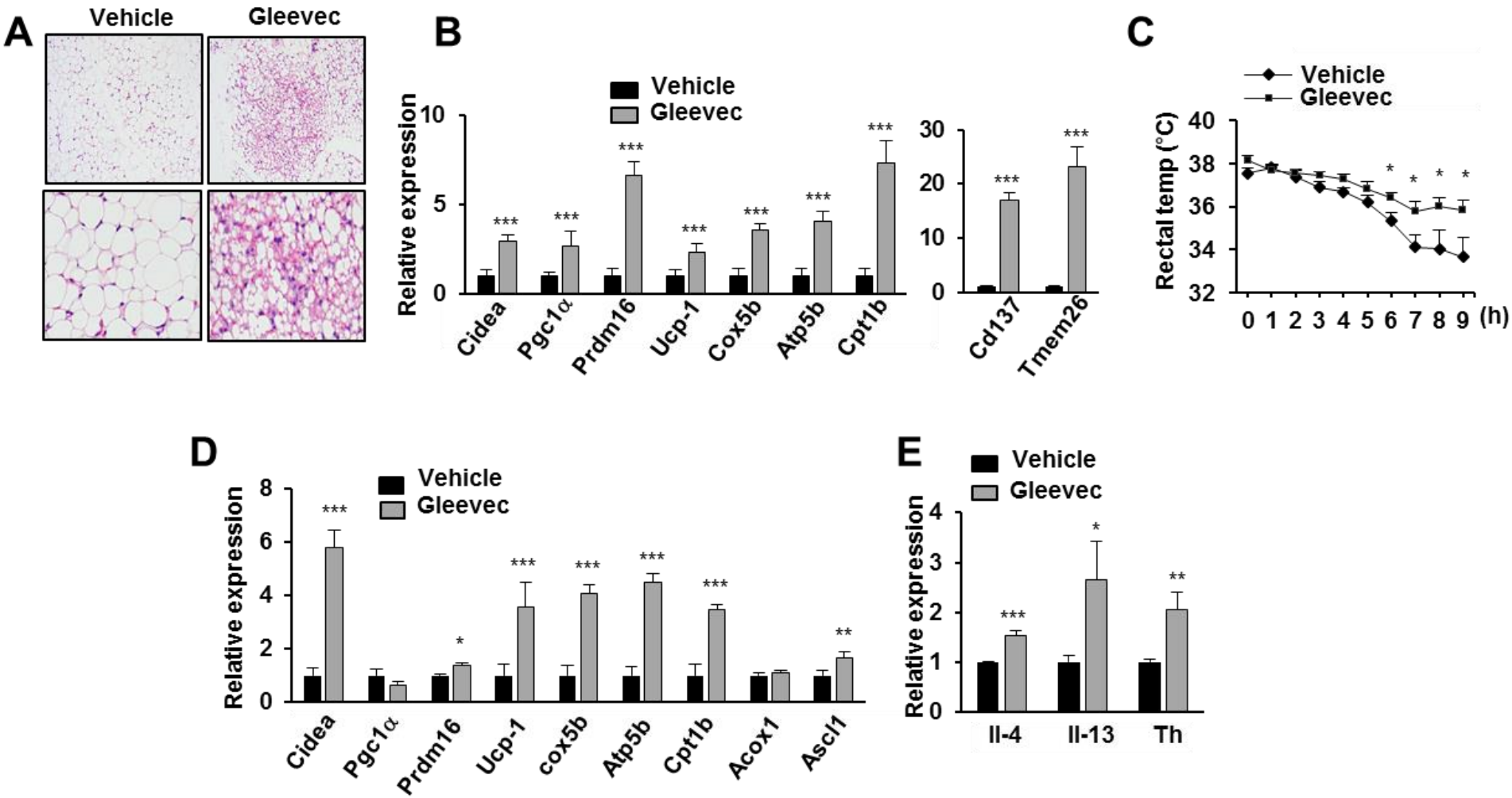
Nature Med. (2013)

Immune responses and browning of white adipose tissue



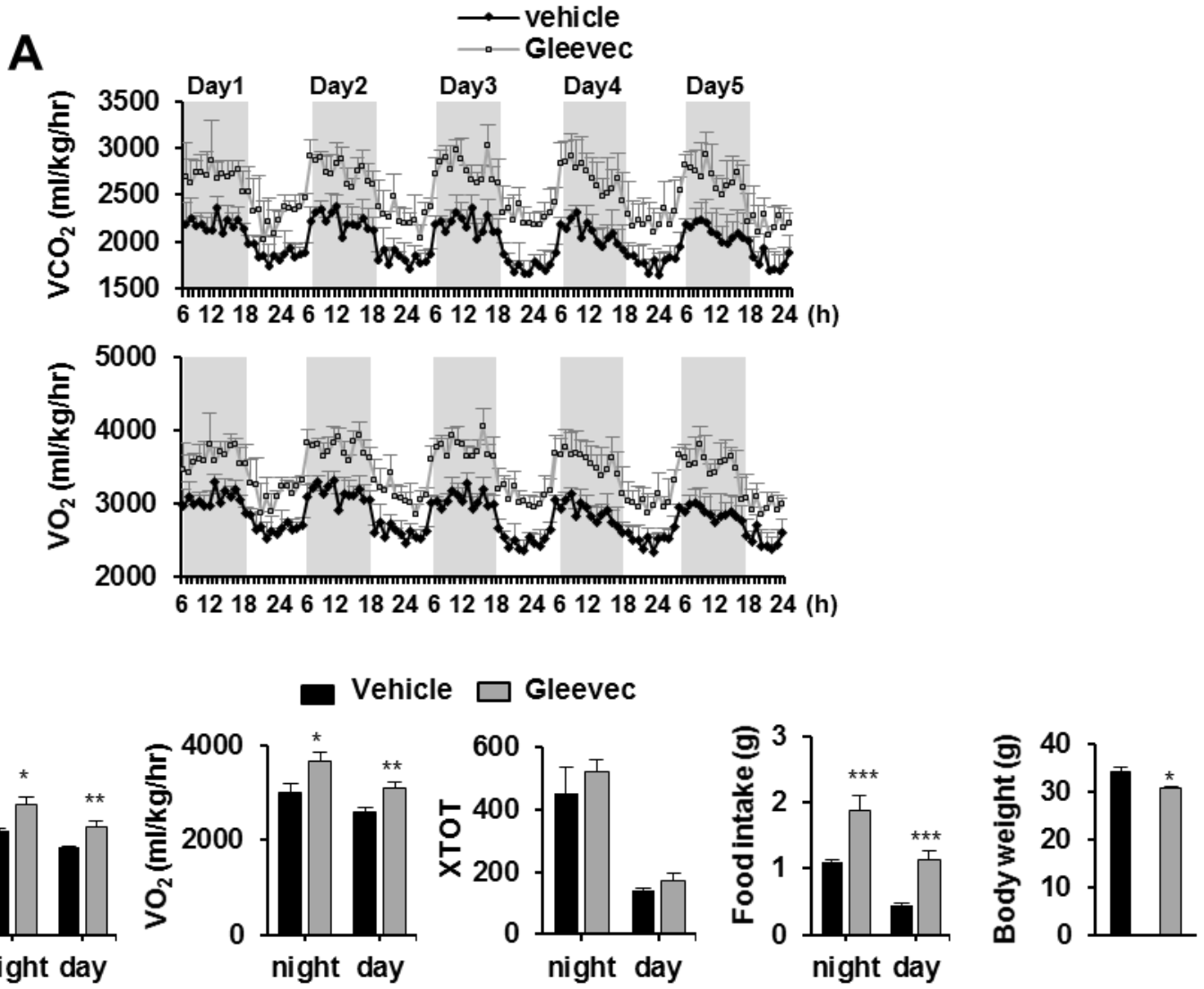
Cell (2014)

Gleevec promotes browning of white adipose tissue



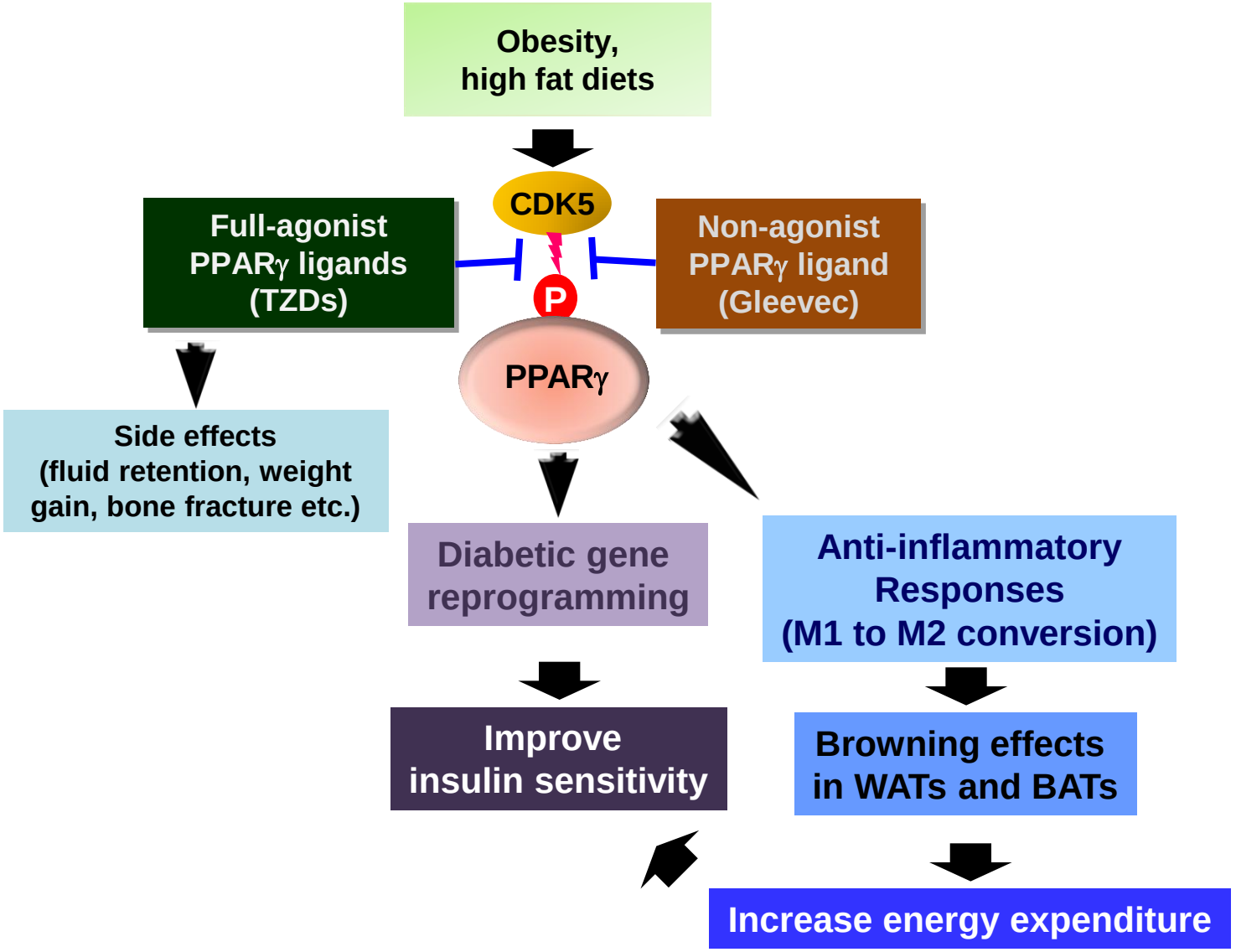
Choi et al., *Diabetes* (2016)

Gleevec increases energy expenditure



Choi et al., *Diabetes* (2016)

Hypothetical model of Gleevec in adipose tissue



Choi et al., *Diabetes* (2016)

Acknoledgements

Choi's Lab

- Dr. Sun-Sil Choi
- Dr. Joo Yong Lee
- Dr. Jung Kwan Kim
- Eun Sun Kim
- Ji Eun Jung
- Hye Jin Eom
- Jae Min Lee

UNIST

- Prof. Pann-Ghill Suh

Hyundai Pharm.

- Dr. In-Hee Lee

SNU

- Prof. Seung Bum Park

Harvard Medical School

- Prof. Bruce Spiegelman

The Scripps in Florida

- Prof. Patrick Griffin