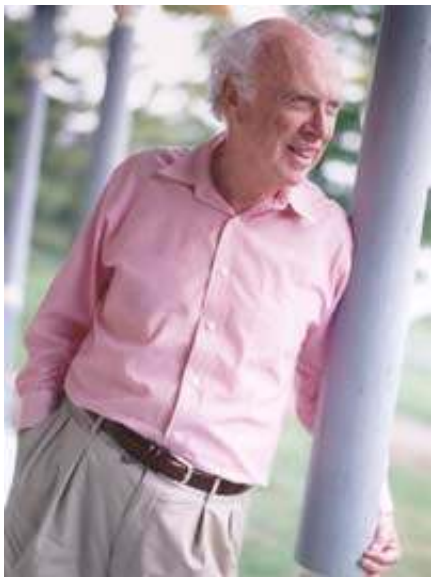
Hepatic Pathophysiology

The pathophysiological role of PRMTs in the onset of Non-Alcoholic Fatty Liver Disease

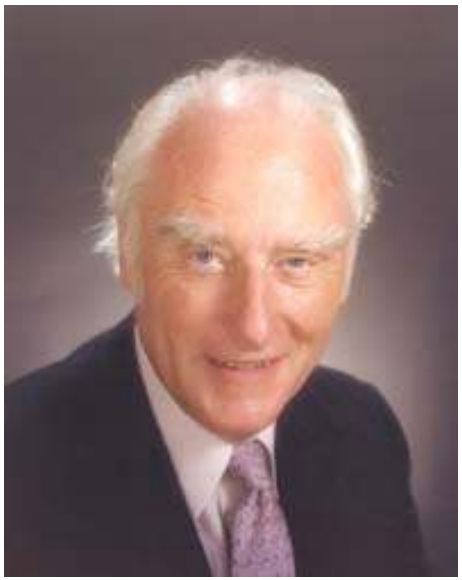
Department of Veterinary Physiology,
College of Veterinary Medicine,
Chonnam National University

Park, Soo-hyun

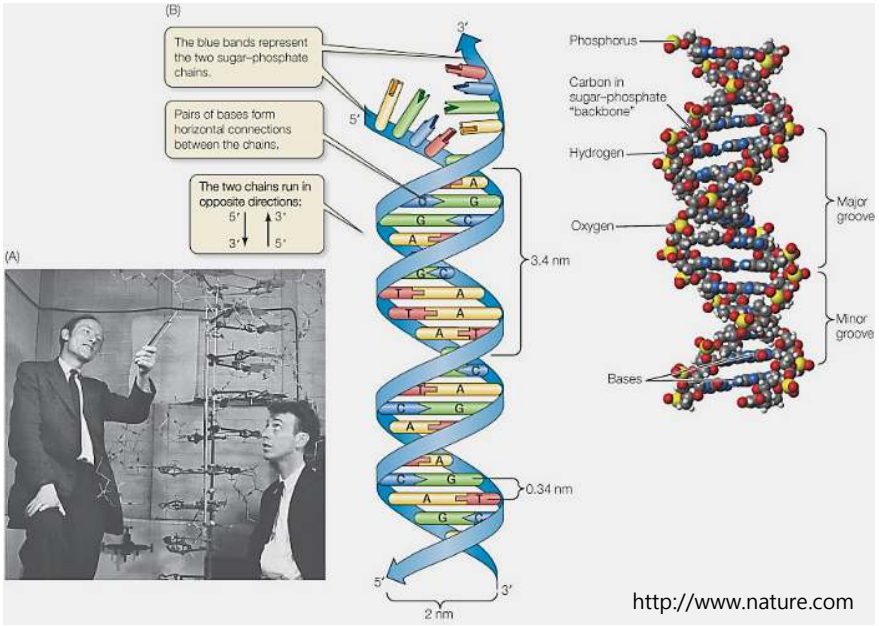
The Watson-Crick DNA Model - 1953



James D. Watson
(1928-)



Francis H. C. Crick
(1916-2004)



- Won Nobel prize in Physiology or Medicine in 1962

http://www.genomenewsnetwork.org/resources/timeline

Discovery of mRNA - 1960

Francois Jacob
(1920-2013; French Biologist)

Jacques Monod
(1910-1976; French Biologist)

- By working with Sydney Brenner and Francis C rick, Jacob and Monod discovered mRNA.

http://www.genomenewsnetwork.org/resources/timeline

Cracking the Genetic Code - 1961

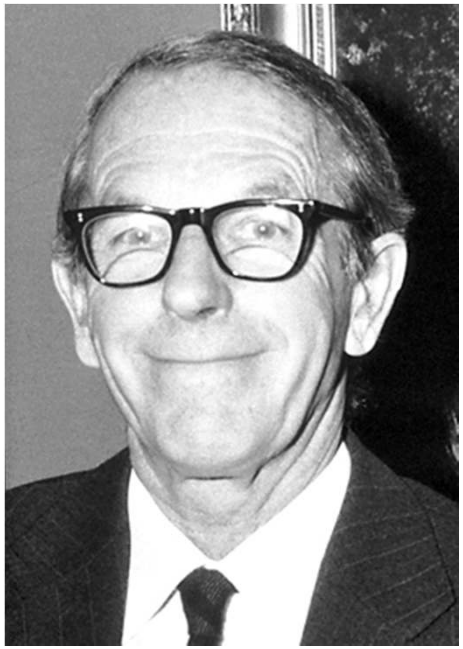
Marshall Nirenberg
(1927-2010; US Biochemist)

- Won Nobel prize with Har G obind Khorana and Robert W. Holley for "breaking the genetic code" and describin g how it operates in protein synthesis in Physiology or M edicine in 1968.

http://www.genomenewsnetwork.org/resources/timeline

DNA Sequencing - 1977

Walter Gilbert
(1932-; US Physicist and Biochemist)



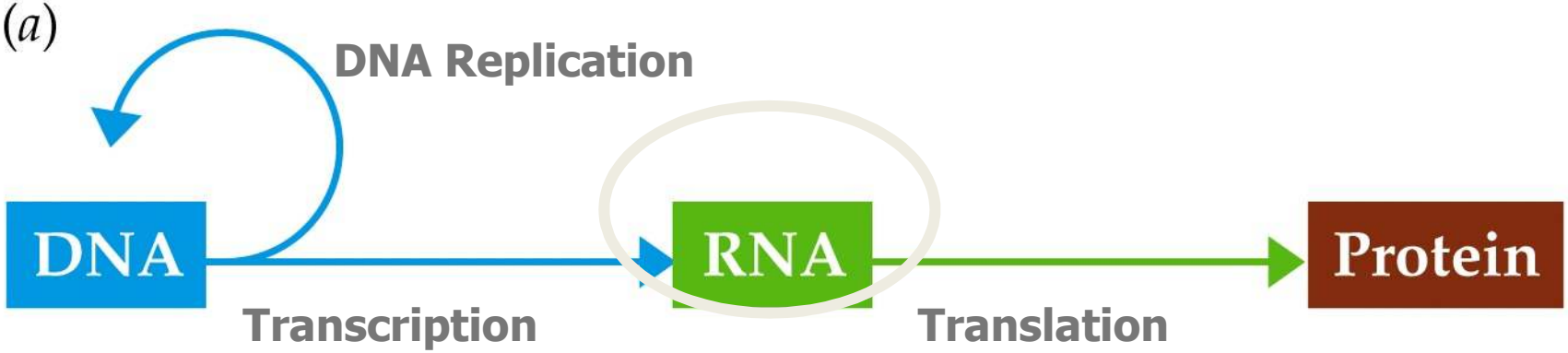
Frederick Sanger
(1918-; UK Biochemist)

- Gilbert and Sanger shared the Nobel Prize i n Chemistry in 1980

http://www.nobelprize.org

The Central Dogma

- The Flow of Information: DNA → RNA → protein



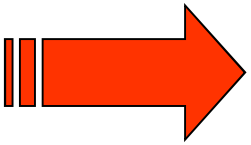
Figure

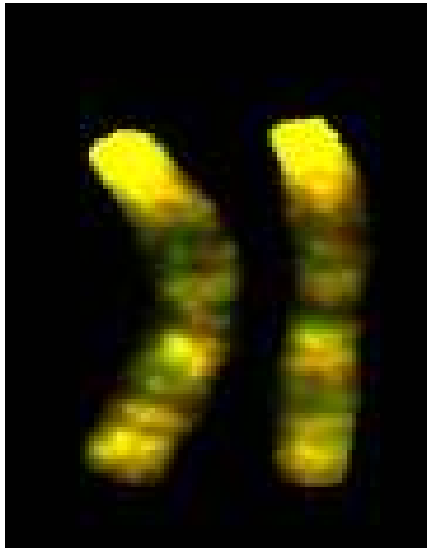
- A gene is expressed in two steps:
  - DNA is transcribed to RNA
  - Then RNA is translated into protein.

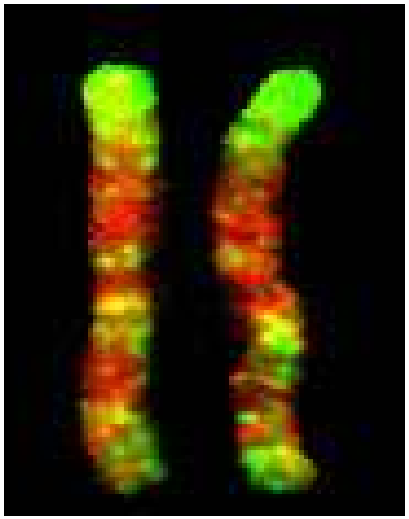
Monozygous twins share a common genotype and are genetically identical

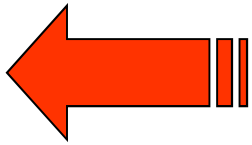
There is significant phenotypic discordance:

- **Mental disorders**
- **Cancer**

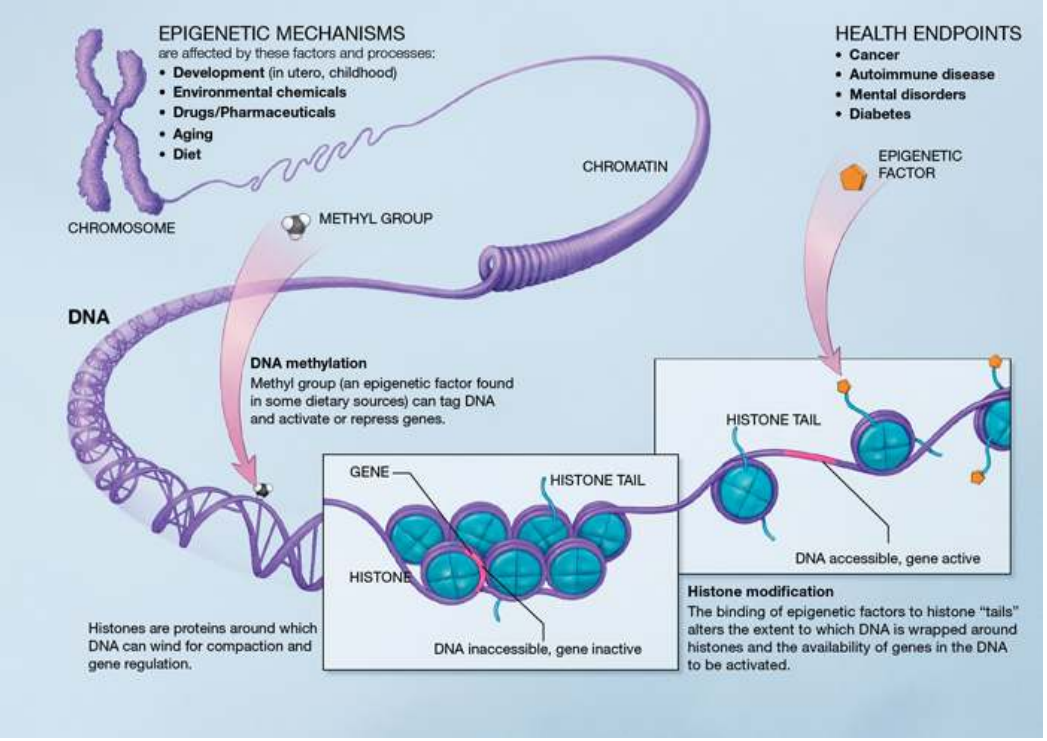








Epigenetics



<Histone modification>

- **Acethylation**
- **Phosphorylation**
- **Ubiquitination**
- **Methylation**

<Histone methylation>

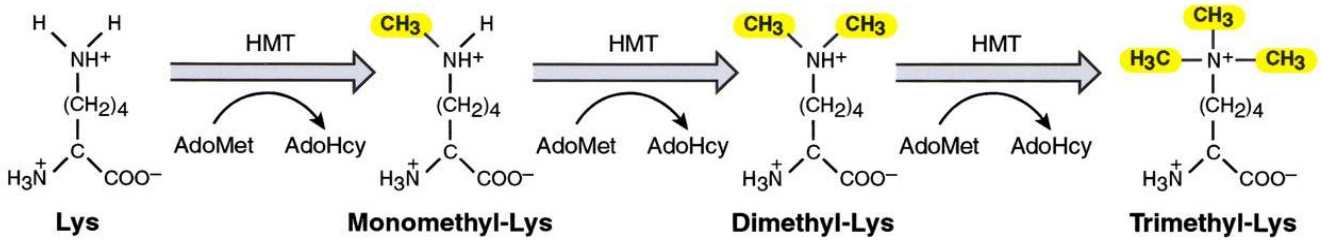
- **Lysine methylaton**
- **Arginine methylation**

Protein Arginine Methyltransferase (PRMT)

- **Epigenetics** is the study of chages in gene expression or cellular phenotype, caused by mechanisms other than changes in the underlying DNA sequence
- **Histone modification :** The N-terminal tails of the core histones targets for posttranslational modifications.

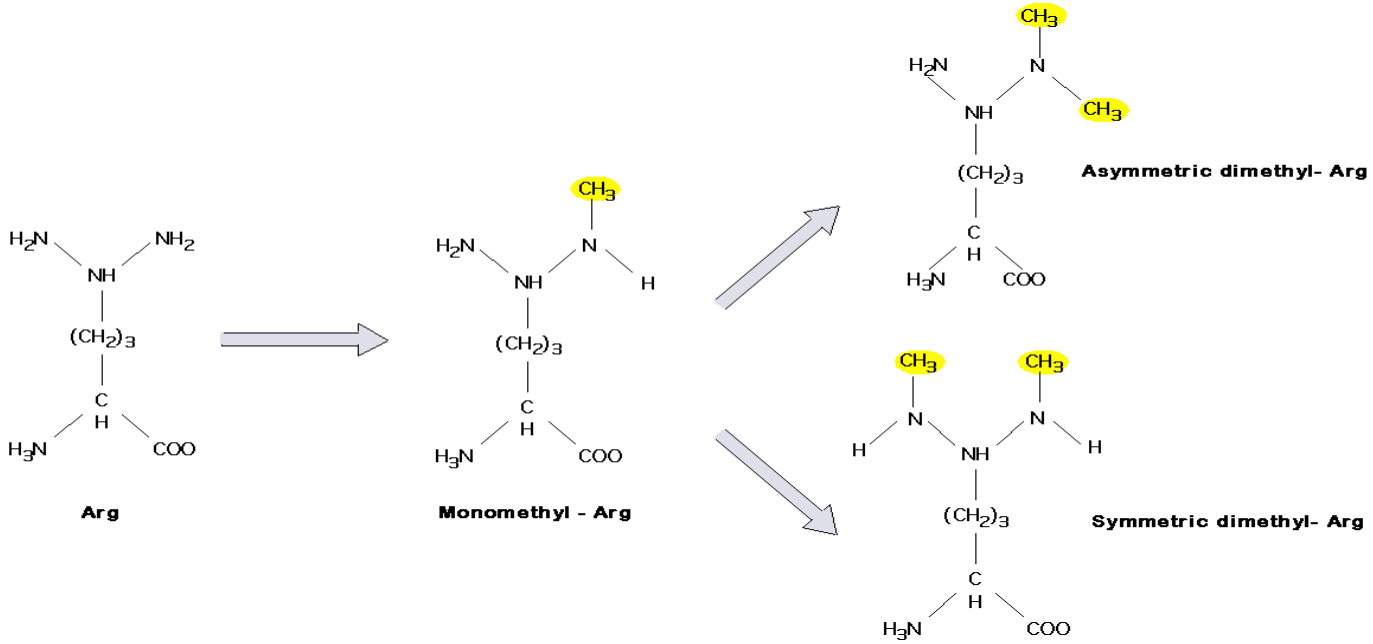
Methyaltion

Lysine methylation



• **Lysine methylation: either repressive or activative**

Arginine methylation



J Cell Sci. 2007; 15;120:4243-6.

Protein Arginine Methyltransferase

There are three structurally defined types of *S*-adenosylmethionine (AdoMet)-dependent methyltransferase

Class I : The largest class has a common seven-stranded -sheet structure.

➡ **Protein Arginine Methyltransferase (PRMT)**

Class II : SET lysine methyltransferases

Class III : membrane associated methyltransferases

Mol Cell Proteomics. 2003; 2(8):525-40.

PRMTs

* The methylation of arginine residues is catalyzed by the protein arginine methyltransferase (PRMT) family of enzymes.

* In the cells, as approximately 0.5 % of all arginine residues are underwent methylation, arginine methylation is a common PTM.

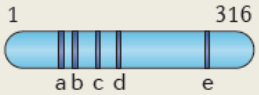
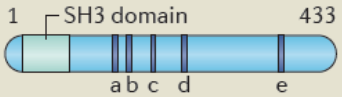
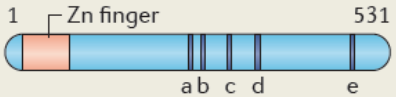
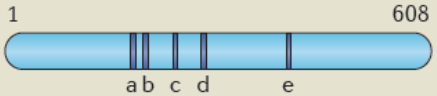
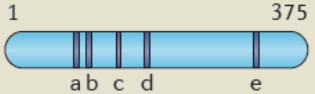
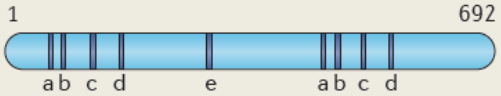
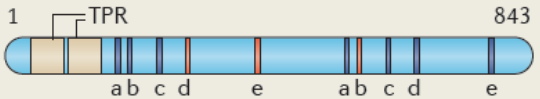
* For methylation event, 12 ATP is required. Despite of such a high energy requirement, arginine mthylation is abundant and well conserved during the evolution.

* Proteins that are arginine methylated are involved in a number of different cellular processes, including cancer, transcriptional regulation, RNA metabolism and DNA damage repair.

* Most PRMTs methylate glycine- and arginine-rich patches (GAR motifs) within their substrates.

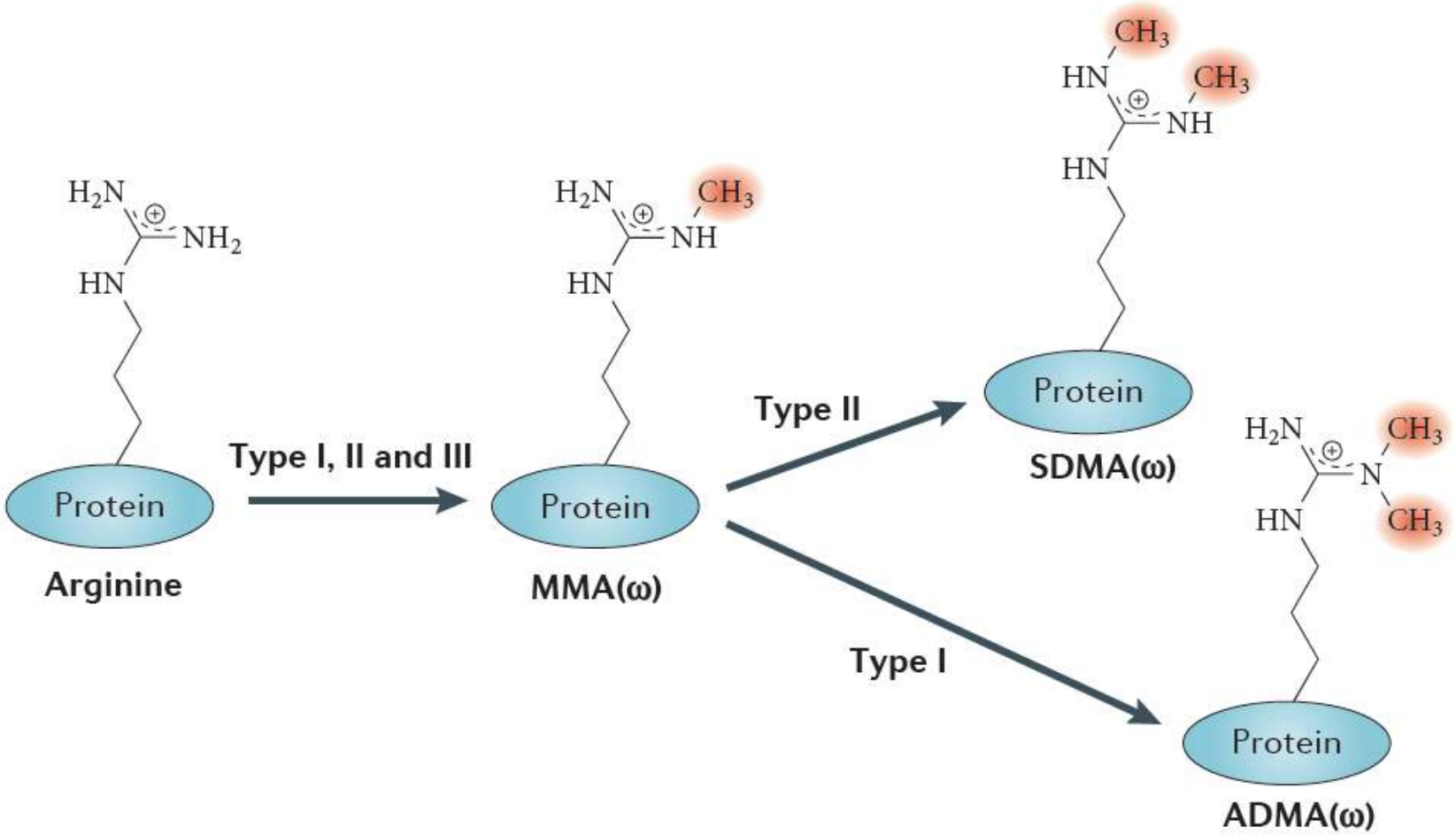
Mol Cell. 2005;18(3):263-72.
Biochim Biophys Acta. 2014;1839(8):702-10

PRMT : at a glance

PRMT (locus)	Domain structures of human enzymes*	Function	Family	Primary substrates‡
PRMT1 (19q13.3)		Transcription activation, signal transduction, RNA splicing and DNA repair	Type I	H4R3, MRE11, 53BP1 and SAM68
PRMT2 (21q22.3)		Transcription regulation	Type I	H3R8
PRMT3 (11p15.1)		Ribosomal homeostasis	Type I	RPS2 and p53
CARM1 (19p13.2)		Transcription activation, RNA splicing, cell cycle progression and DNA repair	Type I	H3R17, AIB1, p300, CBP and RNA Pol II CTD
PRMT5 (14q11.2)		Transcription repression, signal transduction and piRNA pathway	Type II	H3R8, H4R3, E2F1, p53, EGFR and CRAF
PRMT6 (1p13.3)		Transcription regulation	Type I	H3R2 and H2AR29
PRMT7 (16q22.1)		Male germline gene imprinting	Type II and type III	H4R3, H2AR3 and H3R2
PRMT8 (12p13.3)		Brain-specific function	Type I	Unknown
PRMT9 (4q31.23)		Unknown	Not classified	Unknown

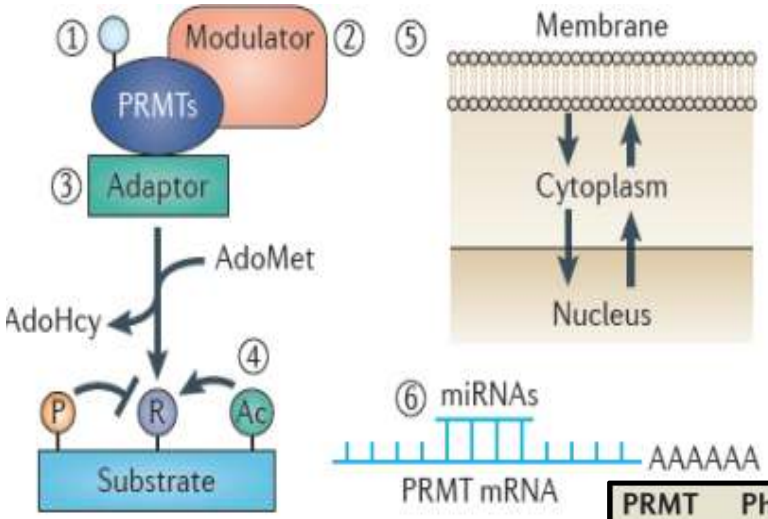
Nat Rev Cancer. 2013; 13(1):37-50

Methylation of Arginine residue



Nat Rev Cancer. 2013; 13(1):37-50

Regulation of PRMTs



Nat Rev Cancer. 2013; 13(1):37-50

Knock-out mice of PRMTs

PRMT	Phenotype of knockout mouse
Prmt1	Embryonic lethal. Trapping mutant generates a hypomorphic allele in which embryonic stem cells can survive, but embryos die shortly after implantation. The complete null is not compatible with cell viability
Prmt2	Mice are viable. MEFs have increased activity of NF-κB transcriptional regulation and are more resistant to apoptosis than wild-type cells
Prmt3	Trapping mutant generates a hypomorphic allele. Mice are viable, but mutant embryos are slightly smaller
Carm1	Newborn knockout mice are small and die shortly after birth. Mutant embryos have defects in the differentiation of T cells, adipose tissue, chondrocytes, muscle and lungs. An enzyme-dead CARM1 mouse model phenocopies the CARM1 null
Prmt5	Early embryonic lethality. Embryos die by E6.5. PRMT5 is required for embryonic epiblast cell differentiation
Prmt6	Null mice are viable. Primary MEFs from these mice undergo rapid senescence

Nat Rev Cancer. 2013; 13(1):37-50

Recent studies about PRMTs and diabetes

Hepatology. 2012 Oct;56(4):1546-56. doi: 10.1002/hep.25809.

Protein arginine methyltransferase 1 regulates hepatic glucose production in a FoxO1-dependent manner.

Choi D[1], Oh KJ, Han HS, Yoon YS, Jung CY, Kim ST, Koo SH.

Sci Signal. 2014 Feb 25;7(314):ra19. doi: 10.1126/scisignal.2004479.

Arginine methylation of CRTC2 is critical in the transcriptional control of hepatic glucose metabolism.

Han HS[1], Jung CY, Yoon YS, Choi S, Choi D, Kang G, Park KG, Kim ST, Koo SH.

Nucleic Acids Res. 2014;42(13):8297-309. doi: 10.1093/nar/gku530. Epub 2014 Jun 17.

A gain-of-function mouse model identifies PRMT6 as a NF-κB coactivator.

Di Lorenzo A[1], Yang Y[1], Macaluso M[1], Bedford MT[2].

Diabetes & Liver disease

Table 1. Liver Disease and Diabetes Mellitus

1. Liver disease occurring as a consequence of diabetes mellitus

- Glycogen deposition
- Steatosis and nonalcoholic steatohepatitis (NASH)
- Fibrosis and cirrhosis
- Biliary disease, cholelithiasis, cholecystitis
- Complications of therapy of diabetes (cholestatic and necroinflammatory)

2 . Diabetes mellitus and abnormalities of glucose homeostasis occurring as a complication of liver disease

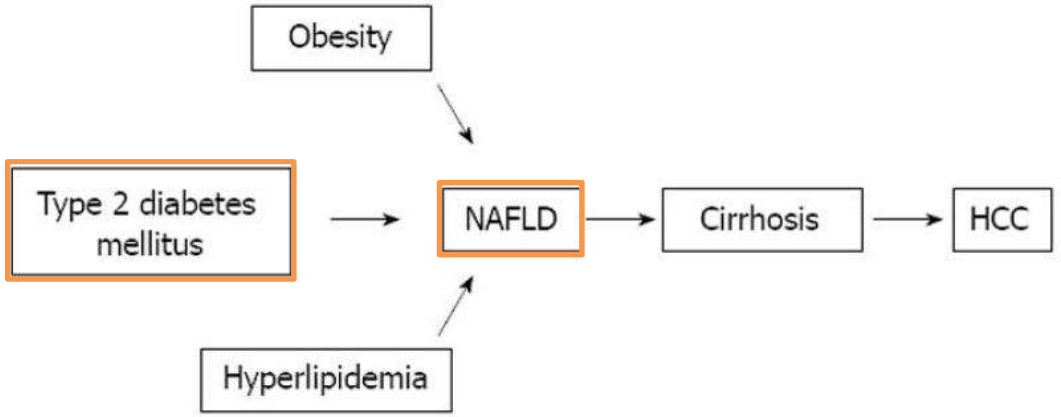
- Hepatitis
- Cirrhosis
- Hepatocellular carcinoma
- Fulminant hepatic failure
- Postorthotopic liver transplantation

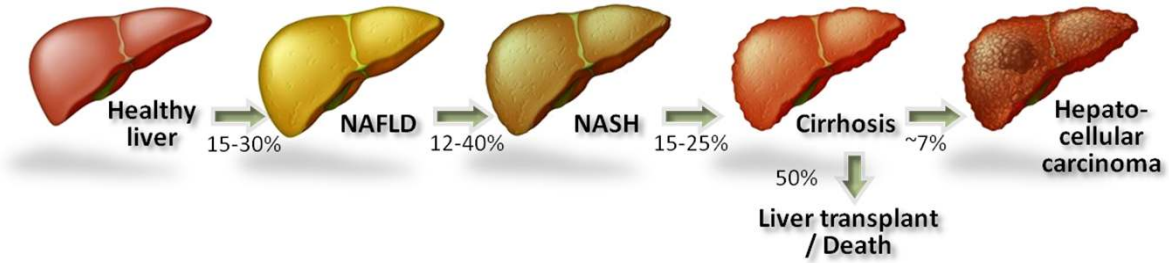
3 . Liver disease occurring coincidentally with diabetes mellitus and abnormalities of glucose homeostasis

- Hemochromatosis
- Glycogen storage diseases
- Autoimmunebiliary disease

(Gavin N, 1999)

Diabetes & Liver disease





Hepatic steatosis & Diabetes

- Type 2 diabetes is strongly associated with nonalcoholic fatty liver disease (NAFLD), a spectrum of liver damage that ranges from relatively benign hepatic steatosis to potentially fatal cirrhosis

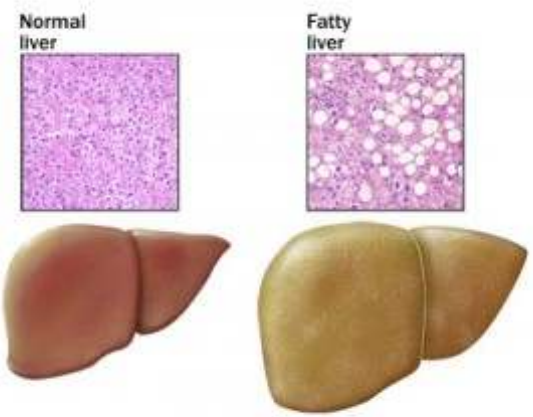
(Clark JM, 2002)

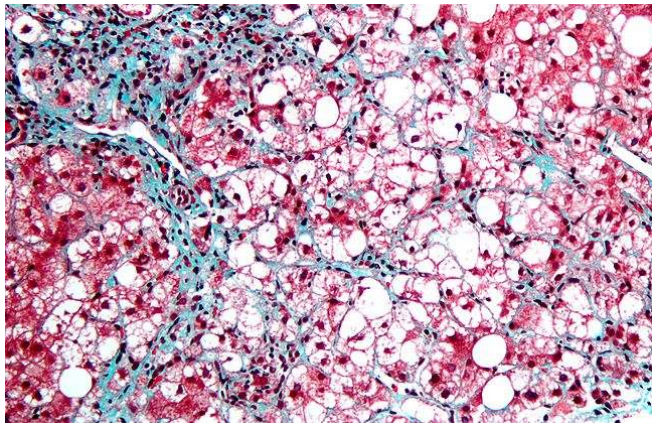
- Elevated HCL (hepatic cellular lipids) levels mainly account for hepatic insulin resistance, which is probably mediated by partitioning of free fatty acids to the liver (fat overflow)

(Roden M, 2006)

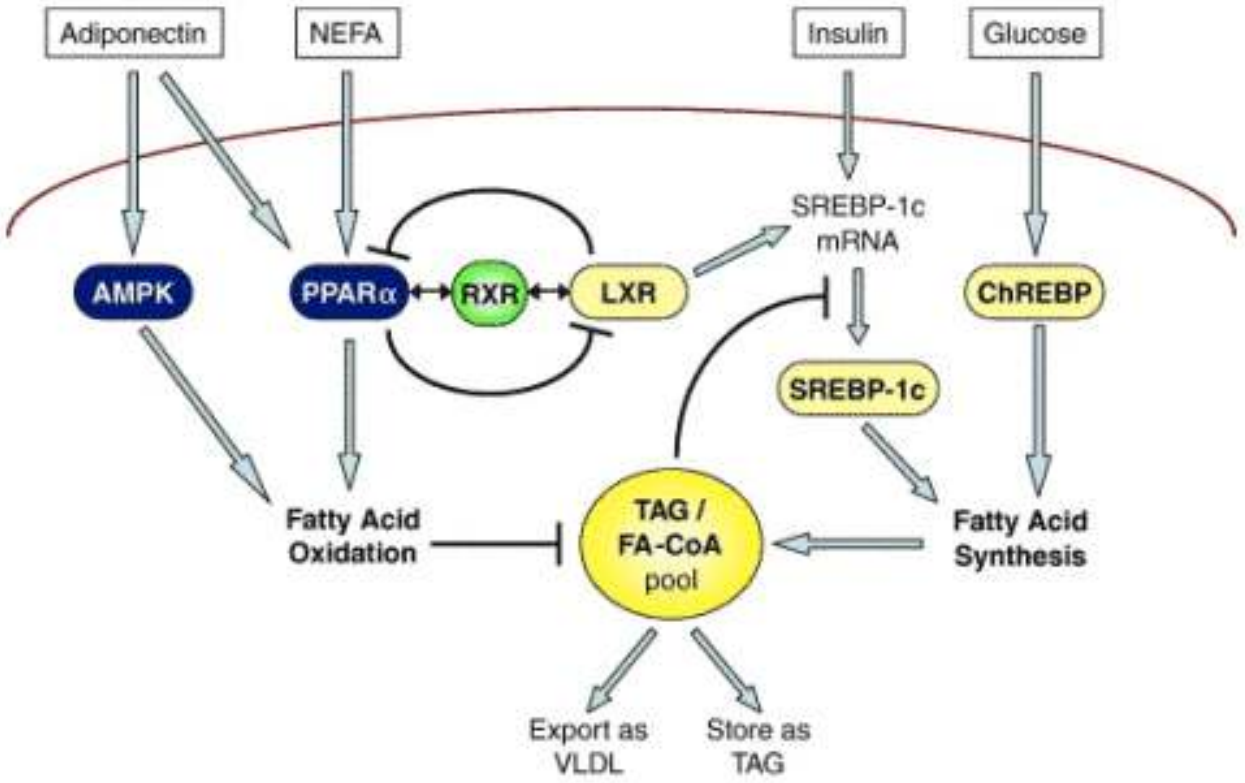
- The disease promote inflammation (steatohepatitis), cell death, and fibrosis, which are the histologic hallmarks of nonalcoholic steatohepatitis (NASH).

(Day, CP, 1998)





Hepatic lipid homeostasis



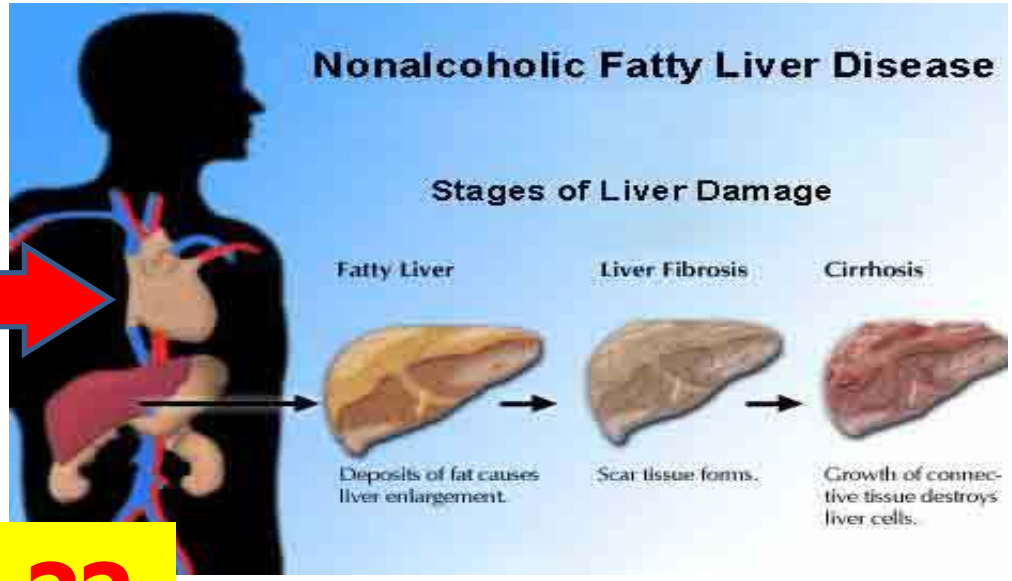
PRMT3 regulates Hepatic Lipogenesis through direct interaciton with LXRα

(Diabetes, 2014 in press)

Introduction for PART 1

Introduction

PART I. PRMT3 & hepatic lipogenesis



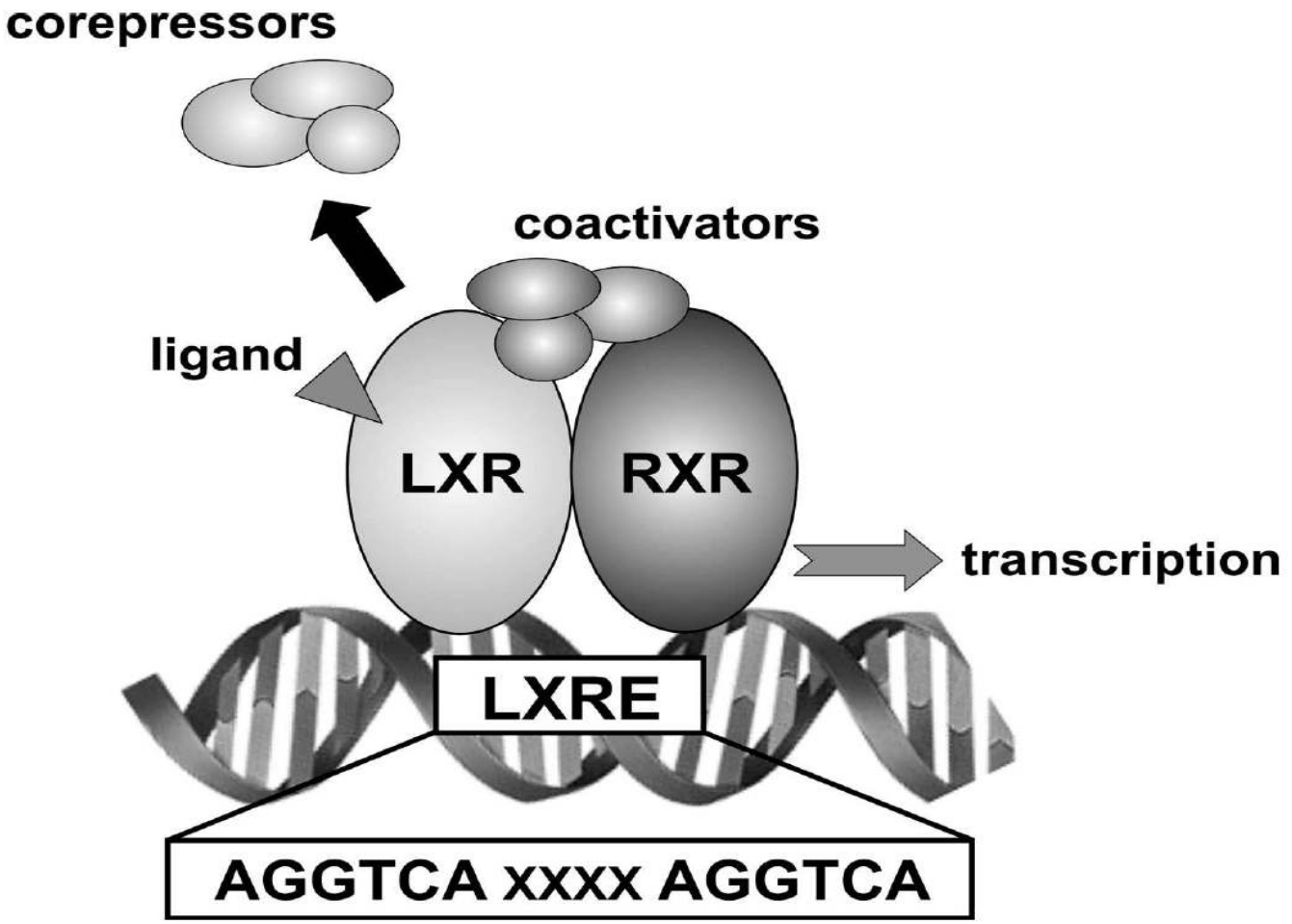
LXRα ??

1. **Diosgenin, the main aglycon of fenugreek, inhibits LXRα activity in HepG2 cells and decreases plasma and hepatic triglycerides in obese diabetic mice.**
   (J Nutr. 2011 Jan;141(1):17-23.)
2. **Piperine, an LXRα antagonist, protects against hepatic steatosis and improves insulin signaling in mice fed a high-fat diet.**
   (Biochem Pharmacol. 2012 Dec 1;84(11):1501-10.)

호로파

Introduction

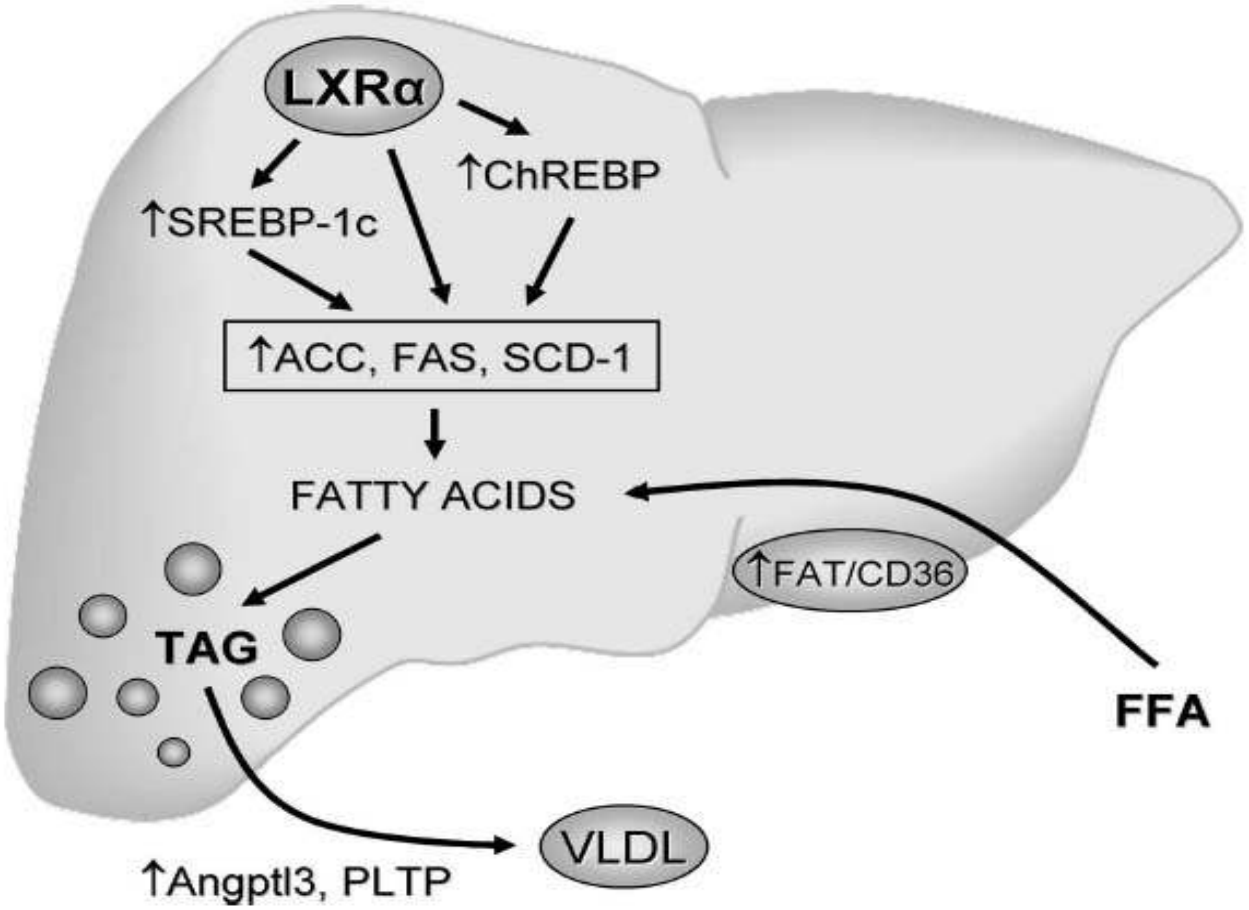
PART I. PRMT3 & hepatic lipogenesis



JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2008: 59, 31–55

Introduction

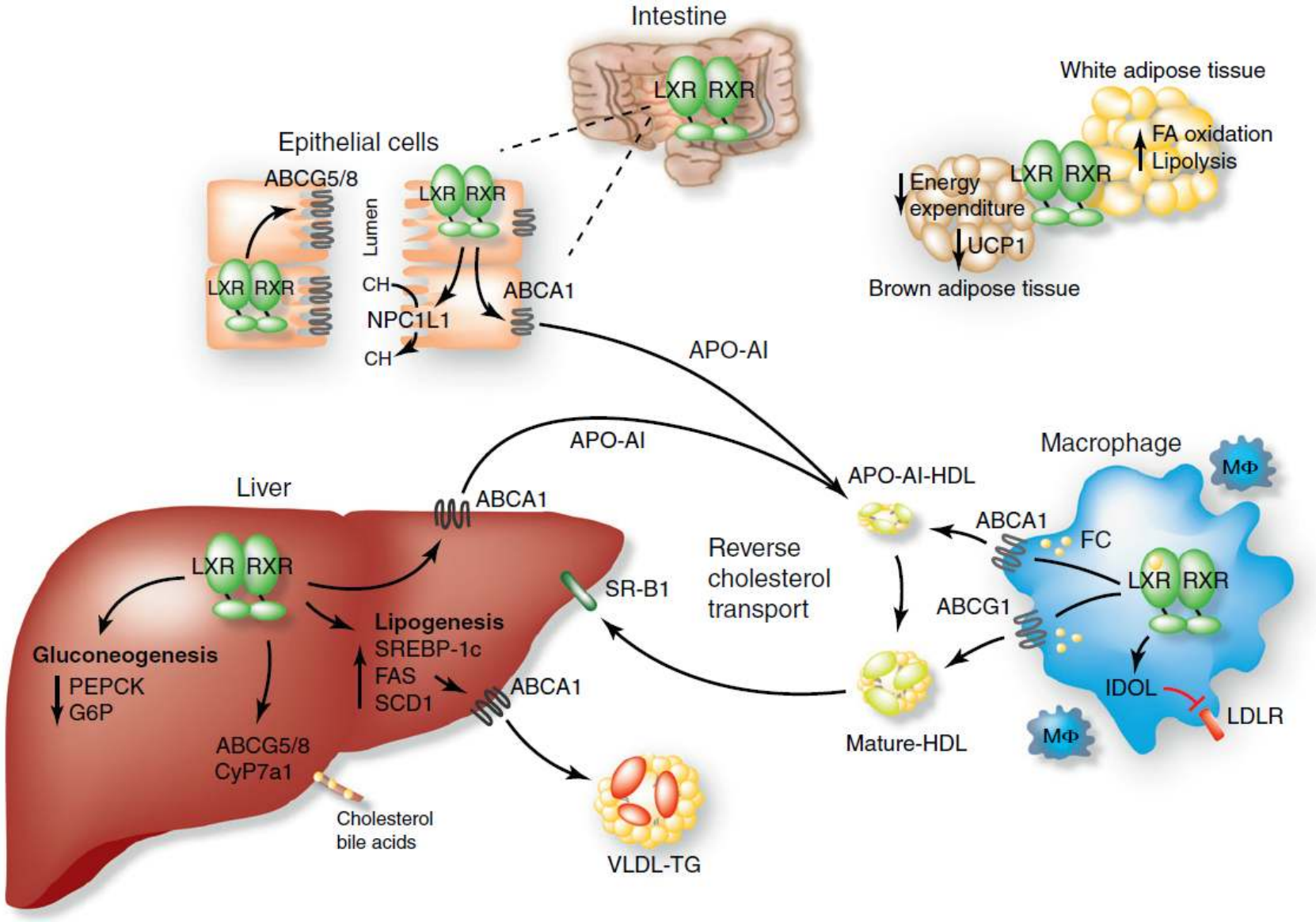
PART I. PRMT3 & hepatic lipogenesis



JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2008: 59, 31–55

Introduction

PART I. PRMT3 & hepatic lipogenesis



Purpose of the Study

In the cells, approximately 0.5 % of all arginine residues are methylated.
For one methylation event, 12 ATP is required.

Arginine methylation is abundant and evolutionally well conserved.

Arginine methylation by PRMTs is biologically important event.

+

However, the role of PRMTs on hepatic lipogenesis is not evaluated.

Find the role of PRMTs on lipogenesis.

PRMT1 regulates hepatic gluconeogenesis.

Protein Arginine Methyltransferase 1 Regulates Hepatic Glucose Production in a FoxO1-Dependent Manner

Dahee Choi,* Kyoung-Jin Oh,* Hye-Sook Han, Young-Sil Yoon, Chang-Yun Jung, Seong-Tae Kim, Seung-Hoi Koo

(Hepatology. 2012;56(4):1546-56)

Methods

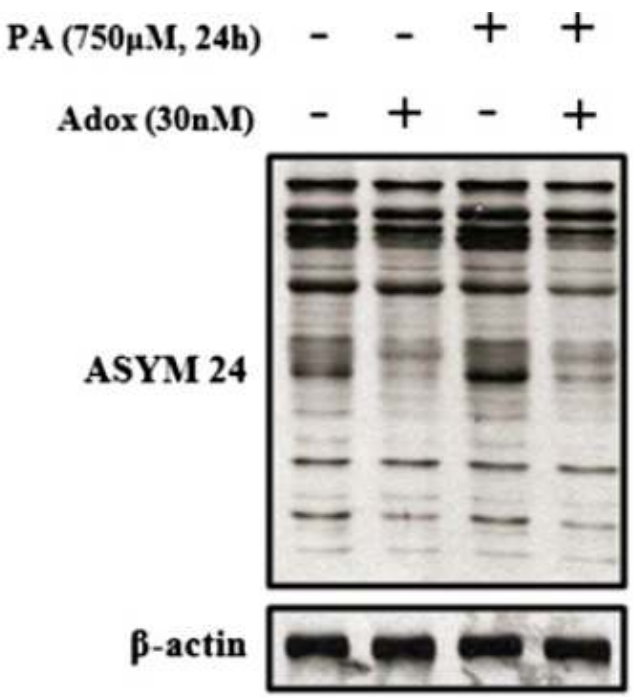
PART I. PRMT3 & hepatic lipogenesis

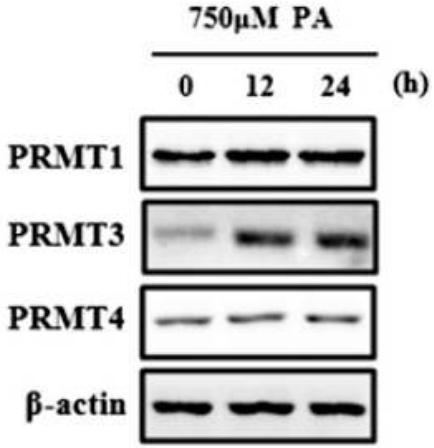
- Cell cultures (HepG2, AML-12, HEK293, PRMT3 WT and KO MEF)
- Cloning and DNA transfection
- siRNA transfection
- FFA, TG and Cholesterol assay
- Protein extraction and Western blotting
- Reporter gene assay
- GST-pull down assay
- Co-immunoprecipitation (Co-IP)
- *in vitro* methylation assay
- Immunoprecipitation
- Chromatin Immunoprecipitation (ChIP)
- Nuclear extraction
- Immunofluorescence with confocal microscope
- RNA isolation and qRT-PCR
- Oil Red O staining
- High Fat Diet and LXRα knockout mice
- Liver isolation and protein extraction
- Human Liver Tissues
- Immunohistochemistry
- Proximity Ligation Assay (PLA)

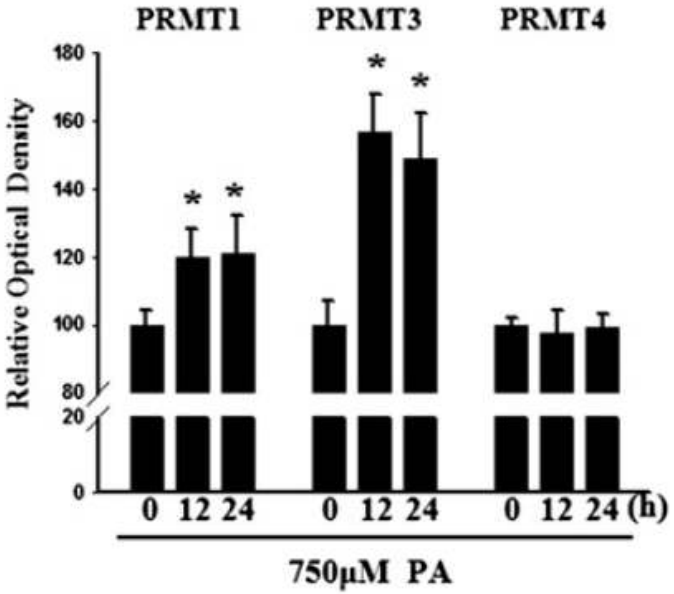
Results

PART I. PRMT3 & hepatic lipogenesis

PA treatment alters arginine asymmetric dimethylation status







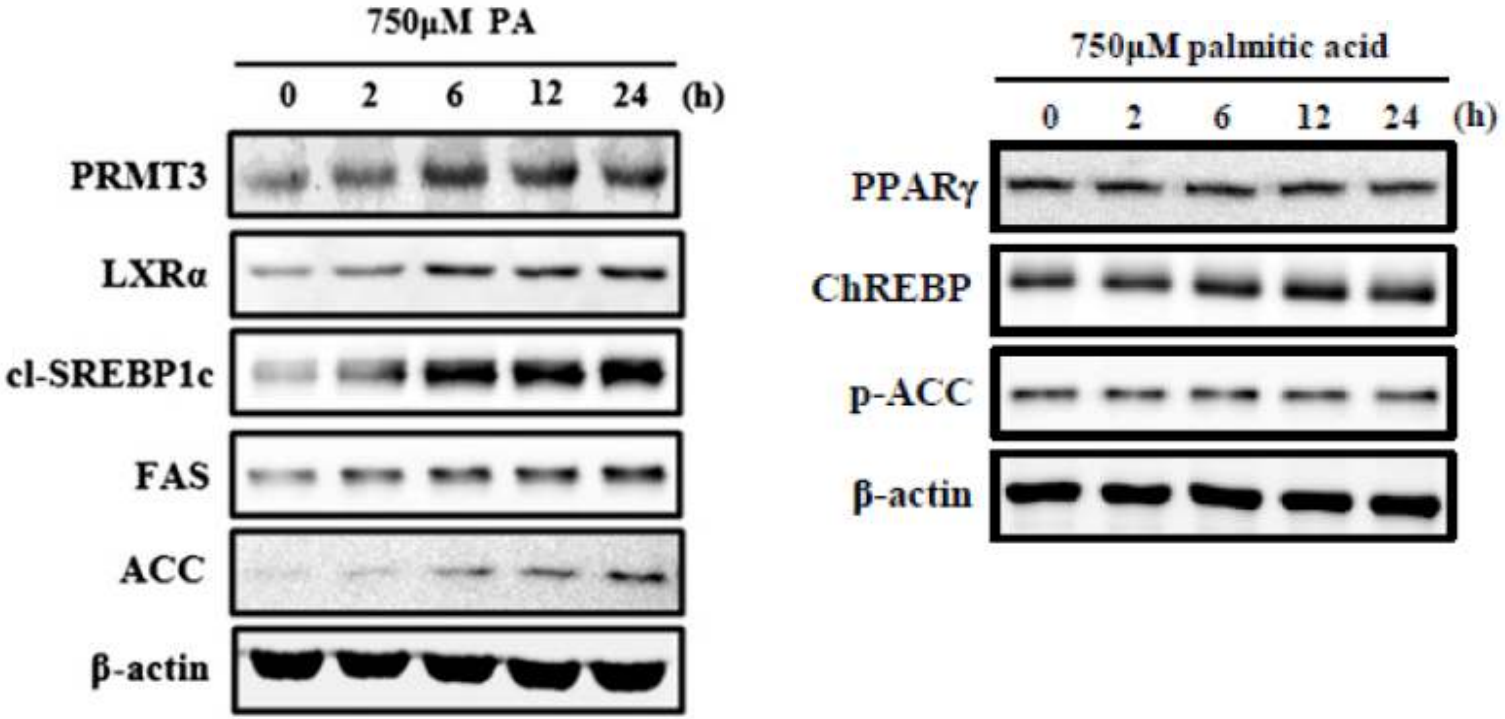
PA treatment increases PRMT3 expression

Adox (adenosine-2',3'-dialdehyde)
- global methylation inhibitor

Results

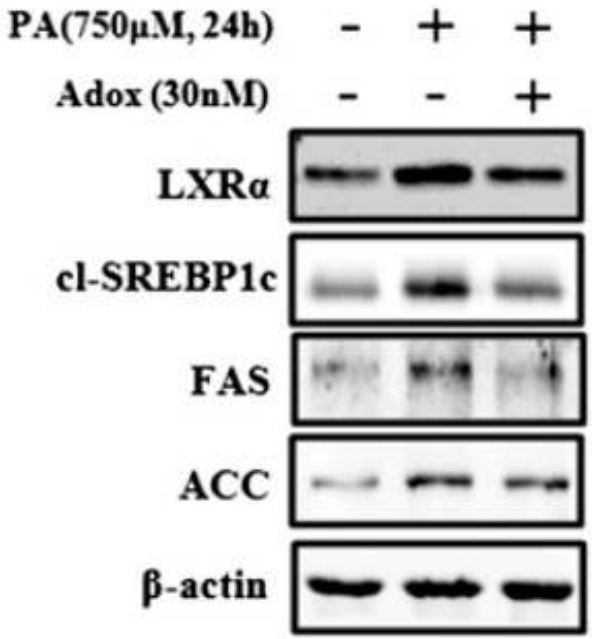
PART I. PRMT3 & hepatic lipogenesis

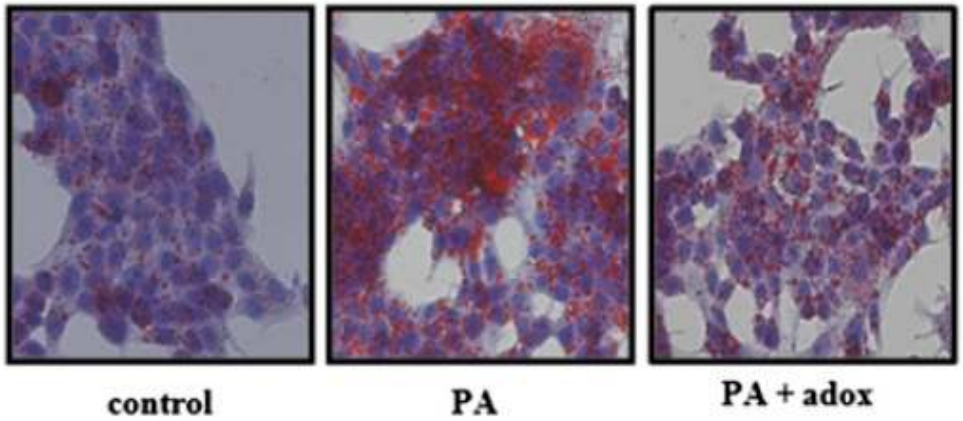
PA treatment increases lipogenic protein expressions



Results

PART I. PRMT3 & hepatic lipogenesis





Adox treatment attenuates PA-induced lipogenesis

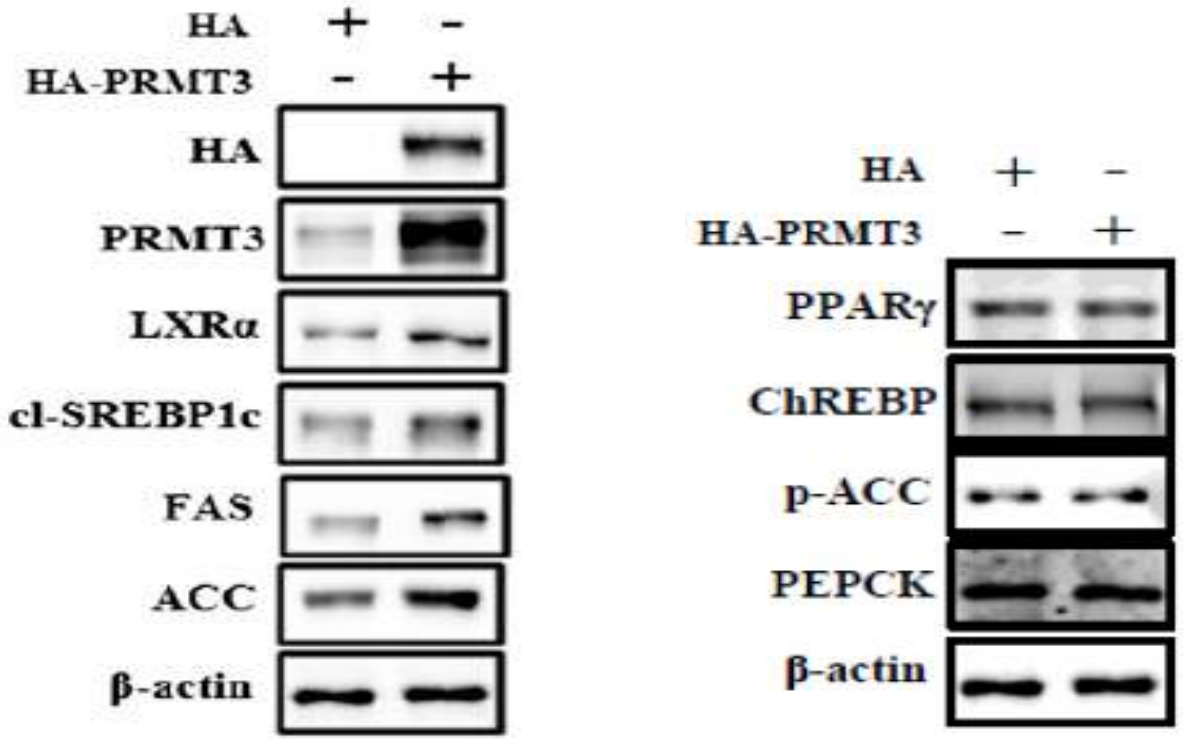
Hypothesis

▶ Hepatic lipogenesis is regulated by PRMT3-mediated arginine methylation??

Results

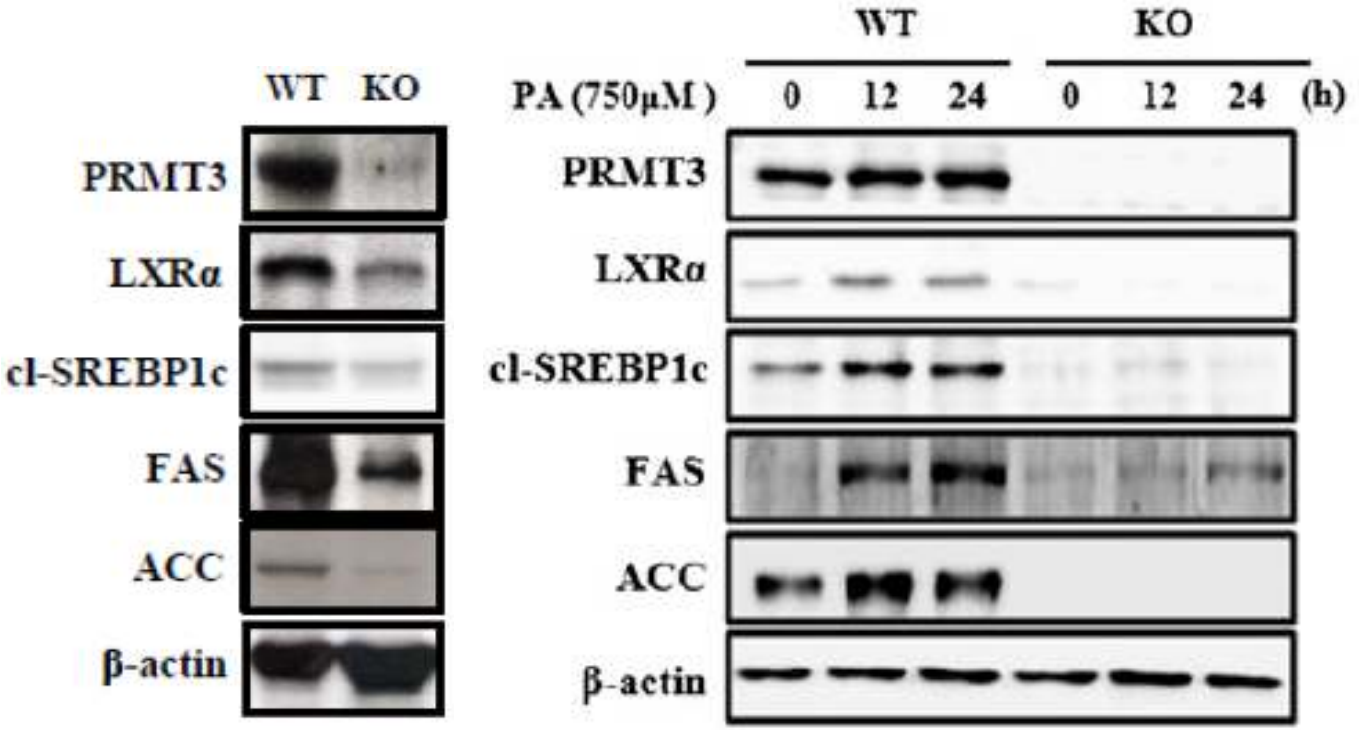
PART I. PRMT3 & hepatic lipogenesis

PRMT3 overexpression increases lipogenic protein expressions in HEK293 cells



Results

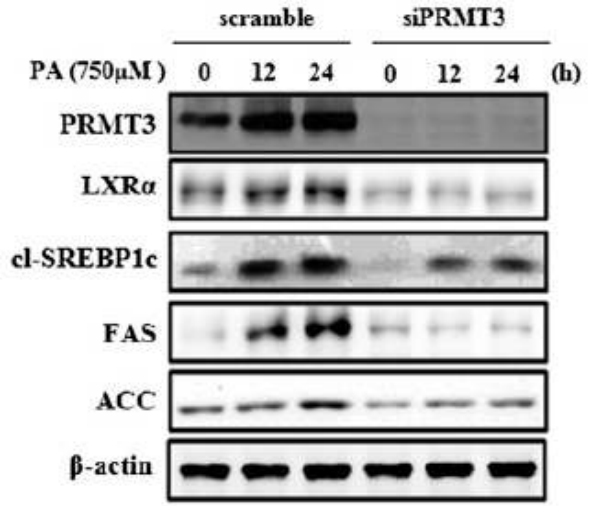
PART I. PRMT3 & hepatic lipogenesis

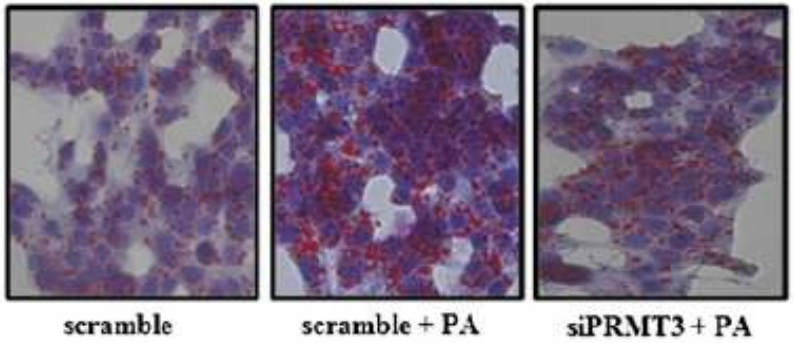


Lipogenic protein expressions are diminished in PRMT3 knockout MEF

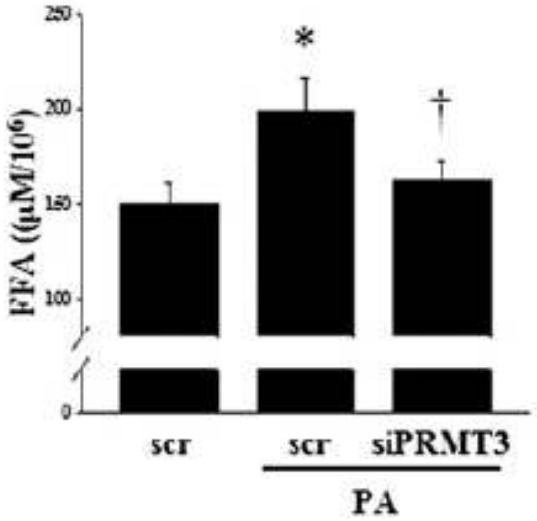
Results

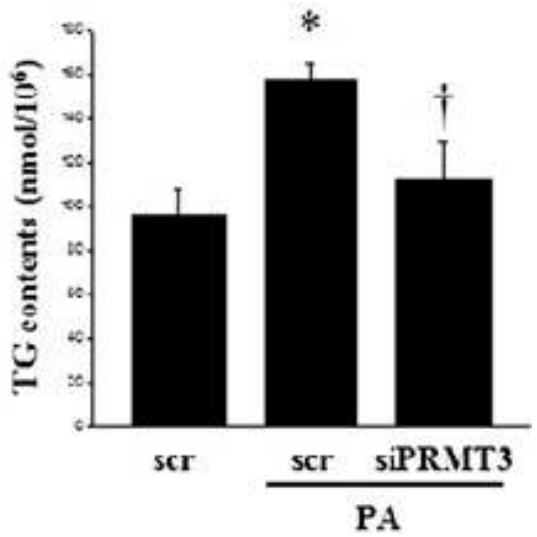
PART I. PRMT3 & hepatic lipogenesis





PRMT3 knockdown attenuates PA-induced lipid accumulation In HepG2 cells

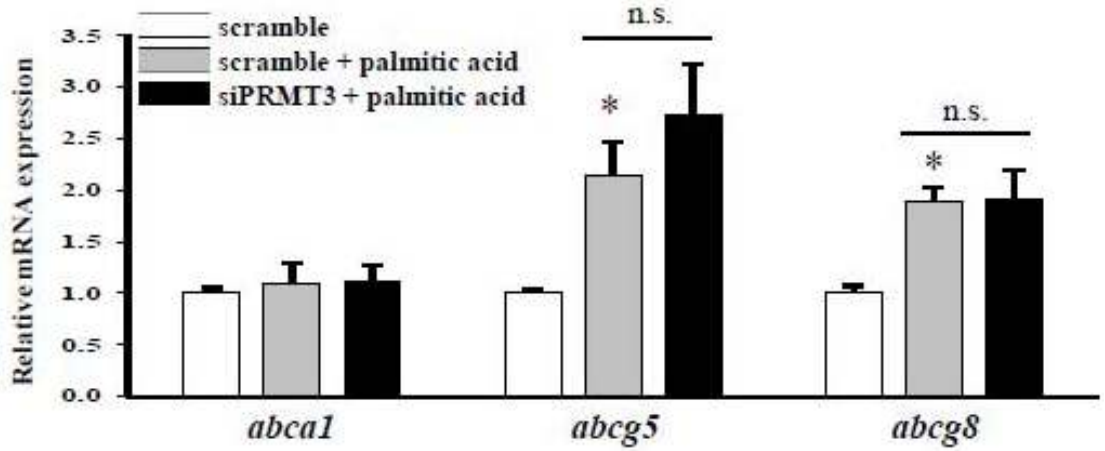


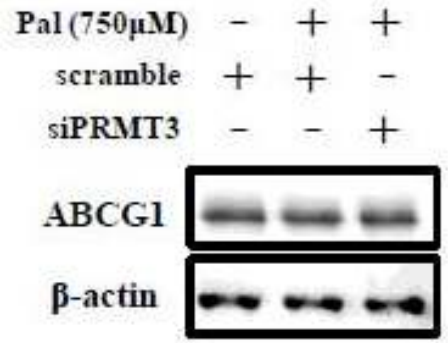


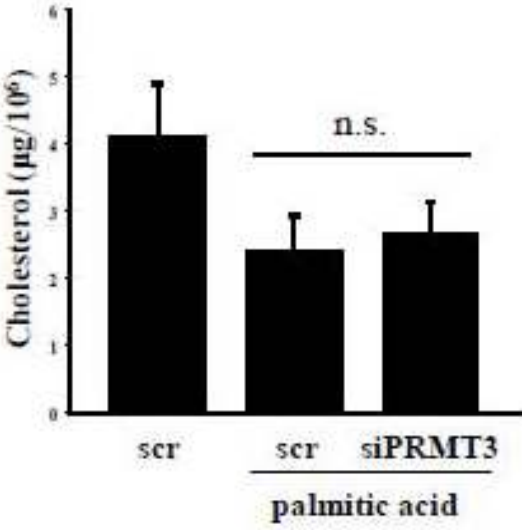
Results

PART I. PRMT3 & hepatic lipogenesis

PRMT3 knockdown does not affect the cholesterol homeostasis.

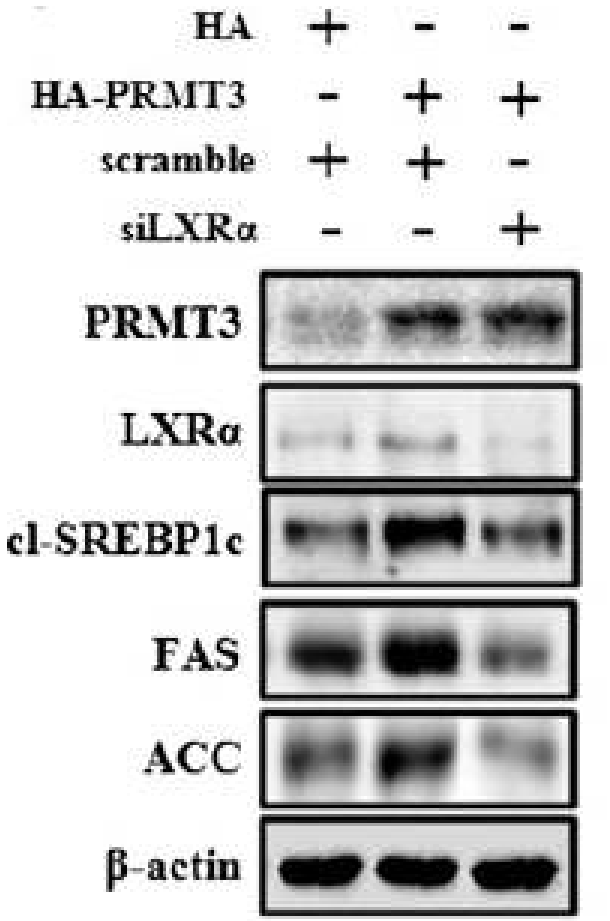




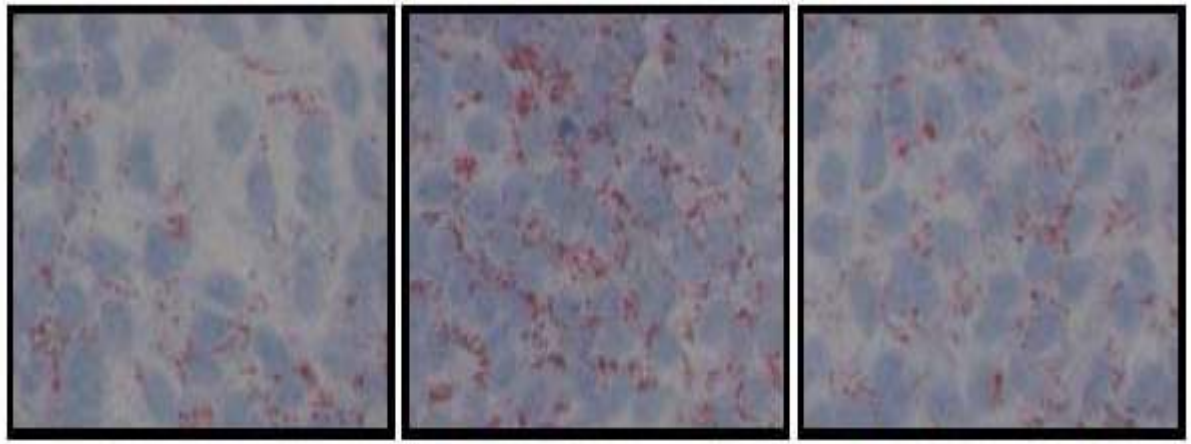


Results

PART I. PRMT3 & hepatic lipogenesis

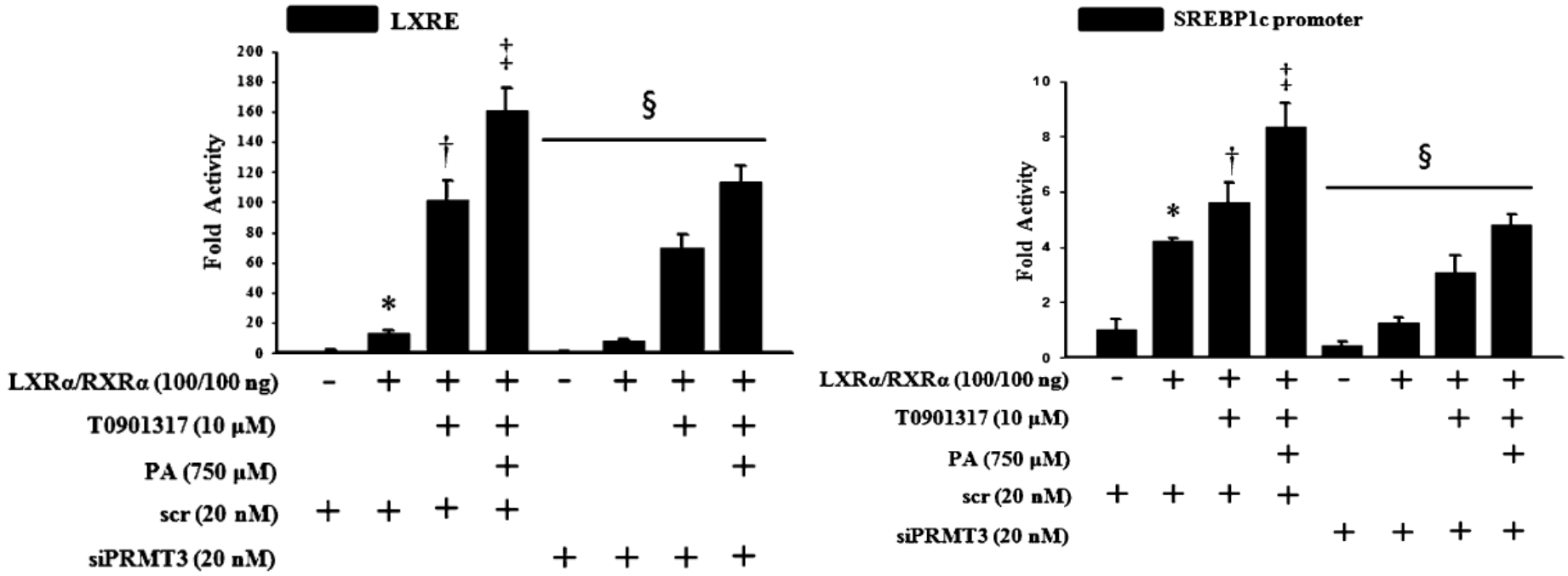


PRMT3 increases hepatic lipogenesis via LXRα dependent manner



Results

PART I. PRMT3 & hepatic lipogenesis

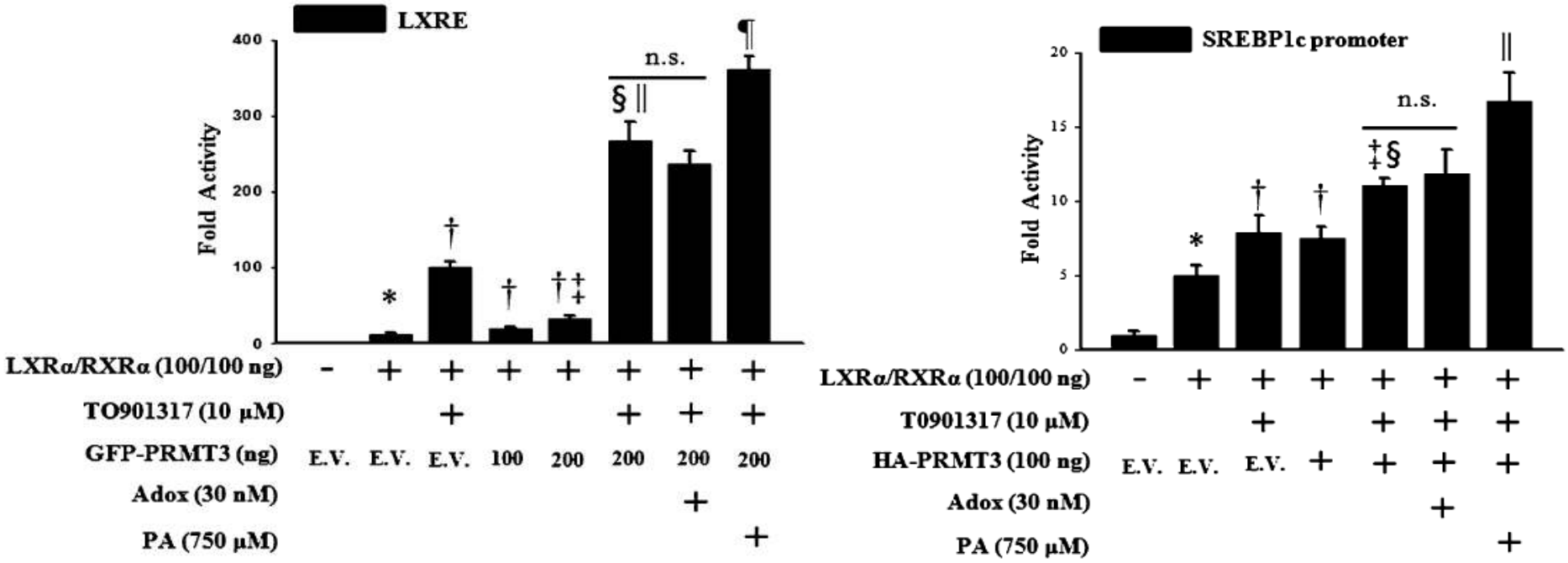


PRMT3 increases the transcriptional activity of LXRα

T0901317
- **LXRα synthetic agonist**

Results

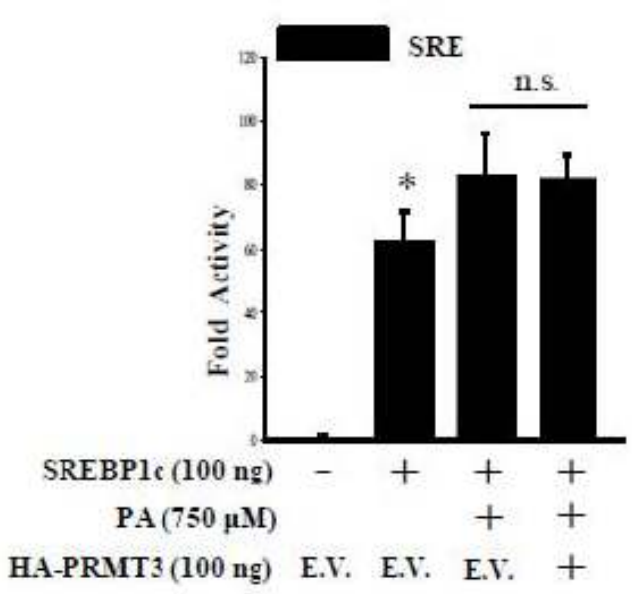
PART I. PRMT3 & hepatic lipogenesis

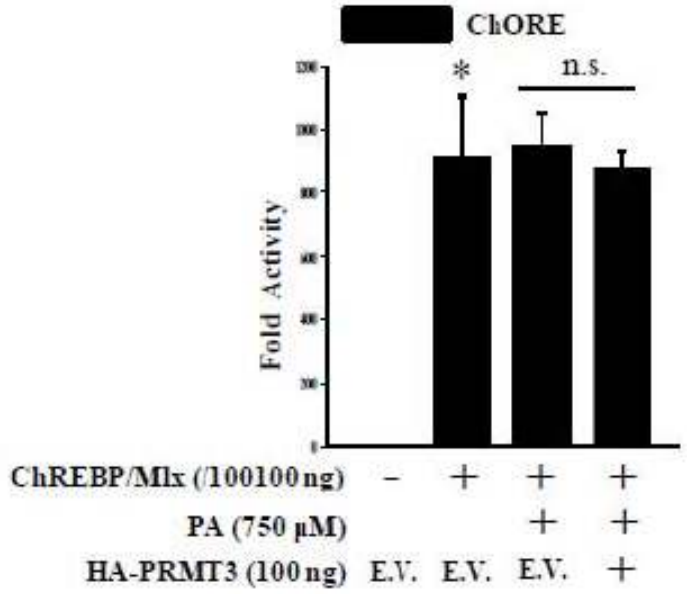


PRMT3 increases the transcriptional activity of LXRα via methylation independent manner

Results

PART I. PRMT3 & hepatic lipogenesis





PRMT3 does not increase the transcriptional activity of SREBP and ChREBP

Hypothesis

▶ PRMT3 binds with LXRα ??

Results

Most studies of PRMT3 have revealed that
it is located exclusively within the cytoplasm.

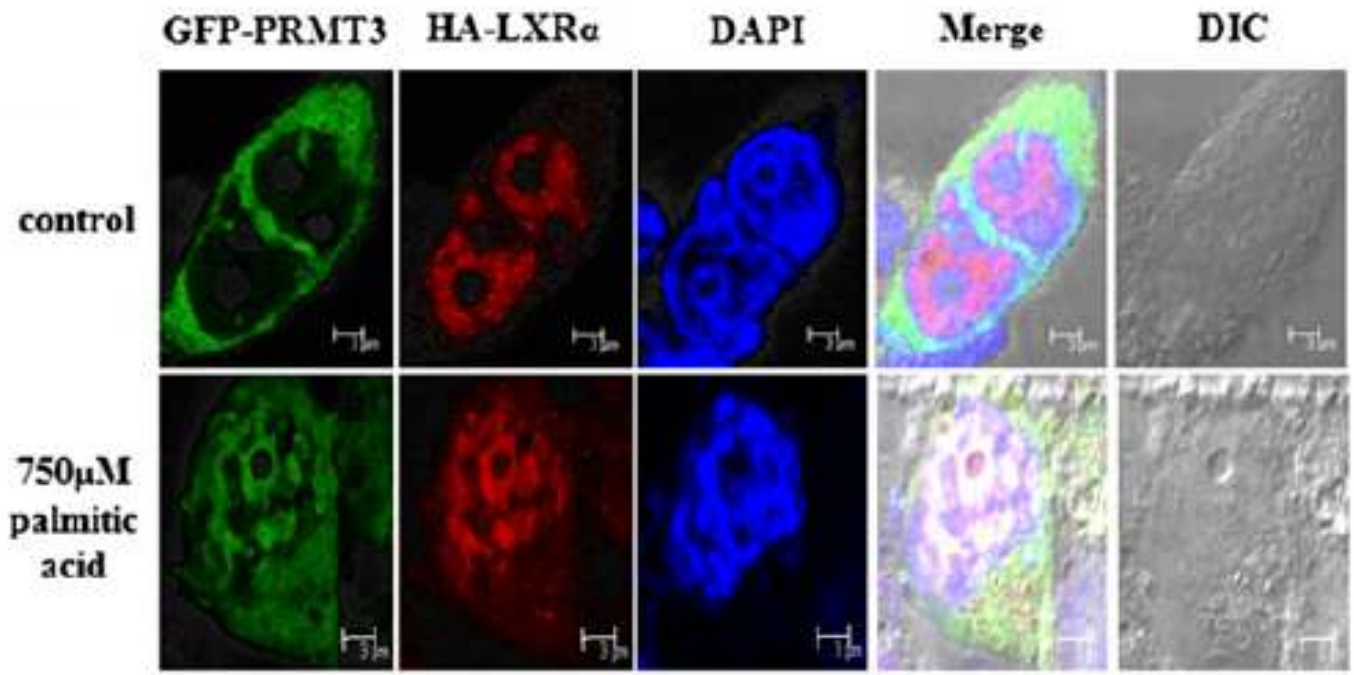
However, PRMT3 might bind with LXRα in the nucleus
because LXRα is a nuclear receptor .

Hypothesis

▶ **Does PRMT3 translocate to the nucleus by PA treatment?**

Results

PART I. PRMT3 & hepatic lipogenesis

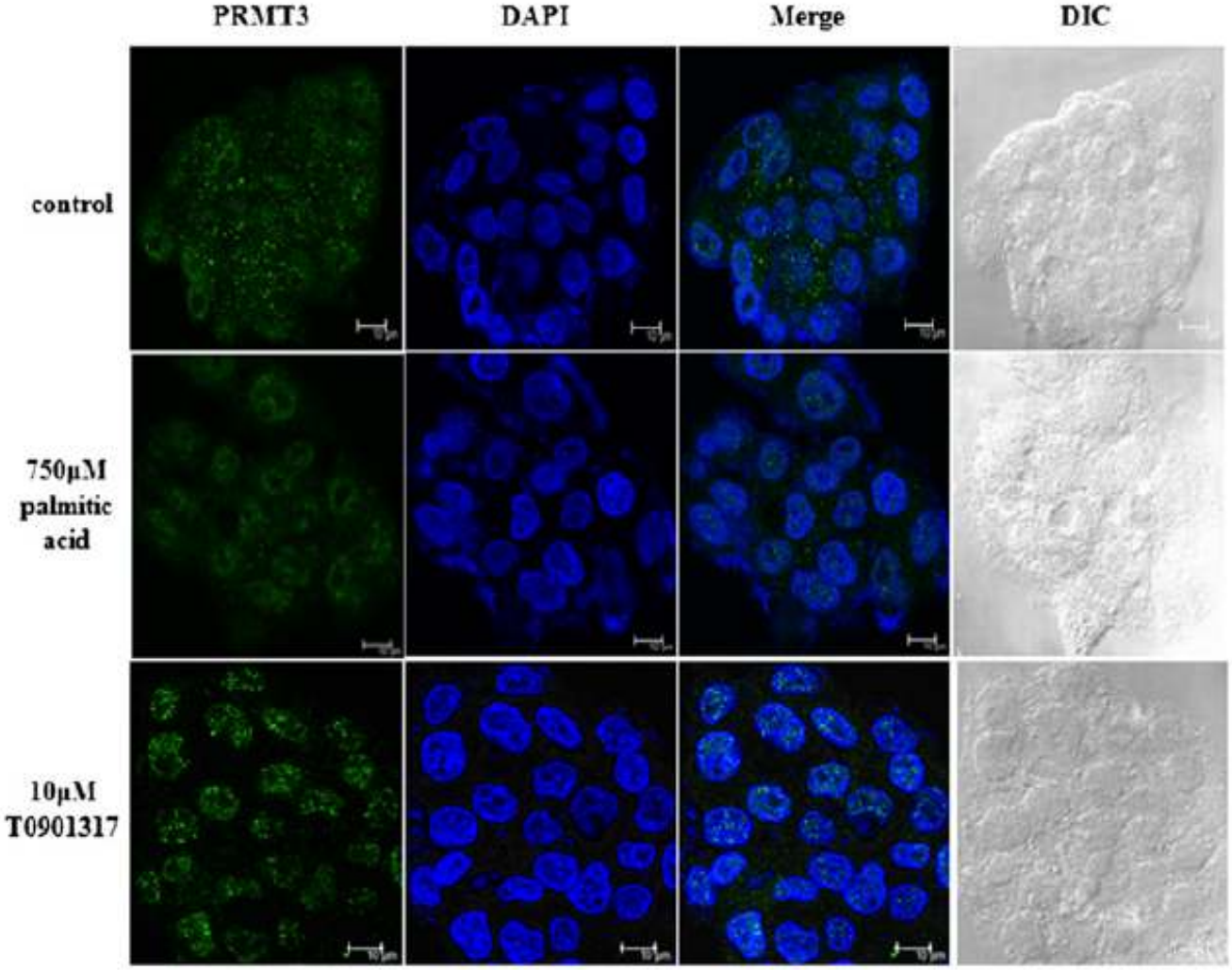


Overexpressed GFP-PRMT3 was located in the cytoplasm.

However, PA treatment increased the nuclear location of PRMT3 and colocalization with LXRα, despite the presence of some cytosolic PRMT3 In HEK293 cells.

Results

PART I. PRMT3 & hepatic lipogenesis



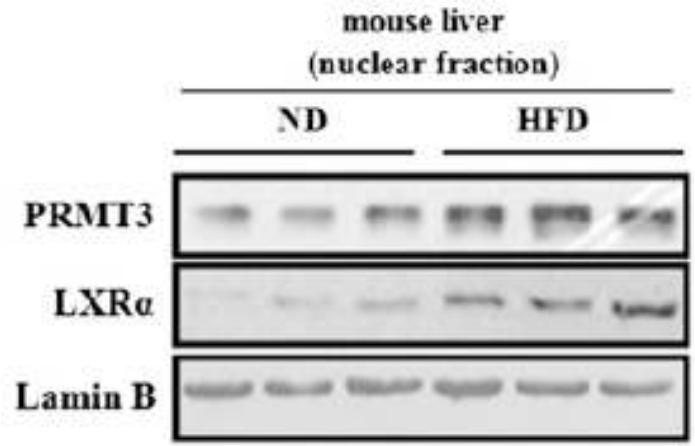
In HepG2 cells, endogenous PRMT3 was located in the cytoplasm and nucleus.

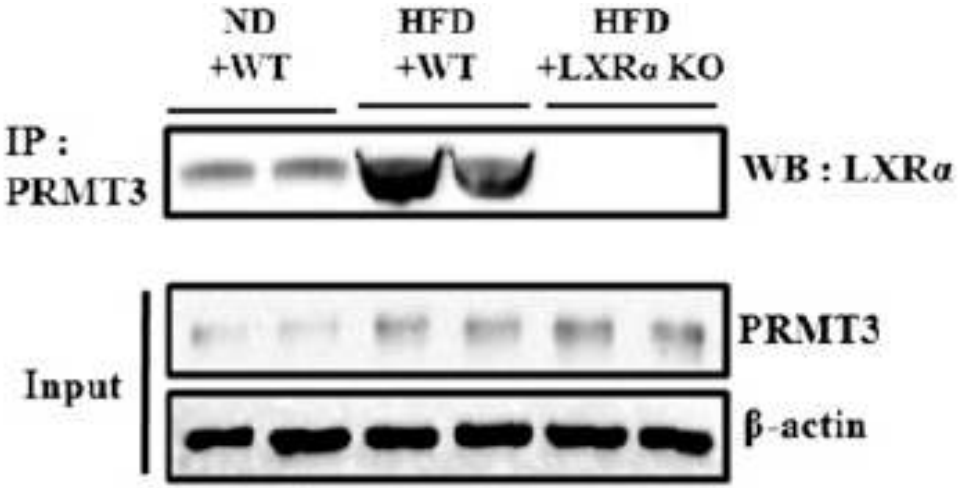
However, PA treatment increased the nuclear accumulation of PRMT3.

Interestingly, T0901317 treatment recruited almost all cytosolic PRMT3 to the nucleus.

Results

PART I. PRMT3 & hepatic lipogenesis





PRMT3 and LXRα are increased in the nuclear fraction of high fat diet mice liver

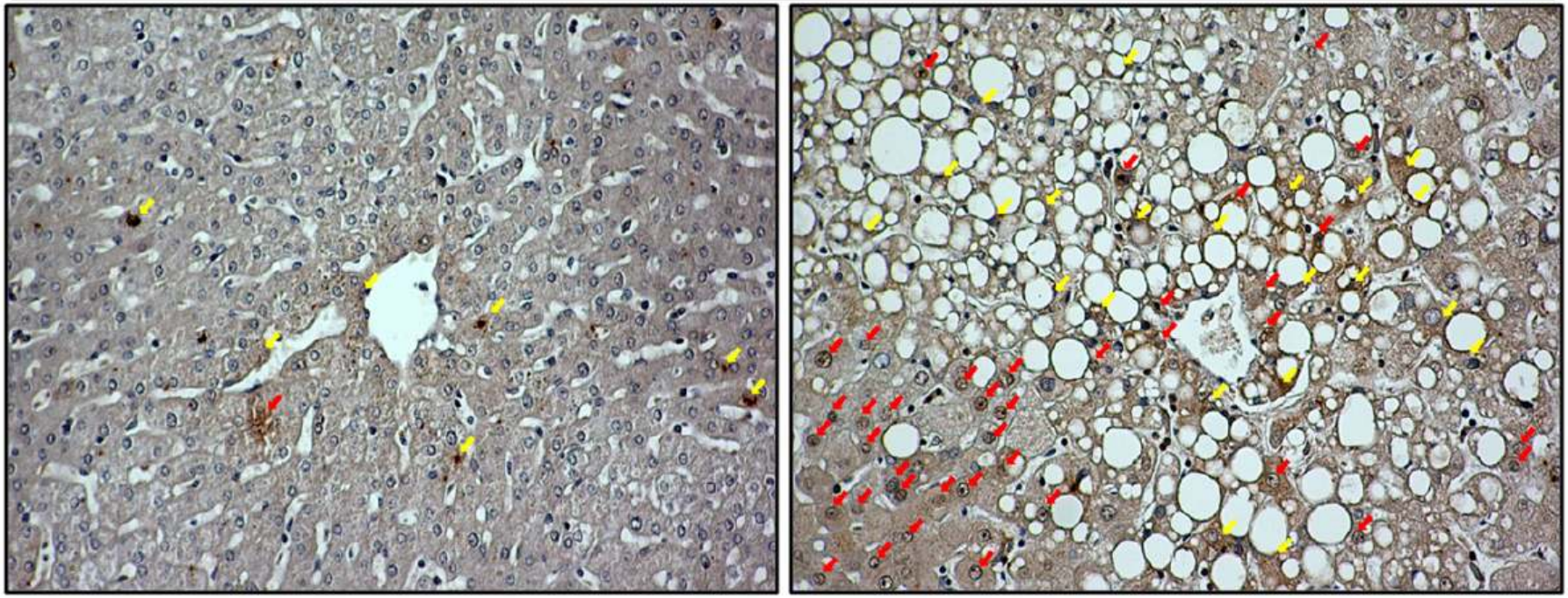
Binding between PRMT3 and LXRα is increased in HFD mice liver.

LXRα-deficiency does not influence HFD-induced PRMT3 expression.

Results

PART I. PRMT3 & hepatic lipogenesis

αPRMT3



non-fatty liver

NAFLD

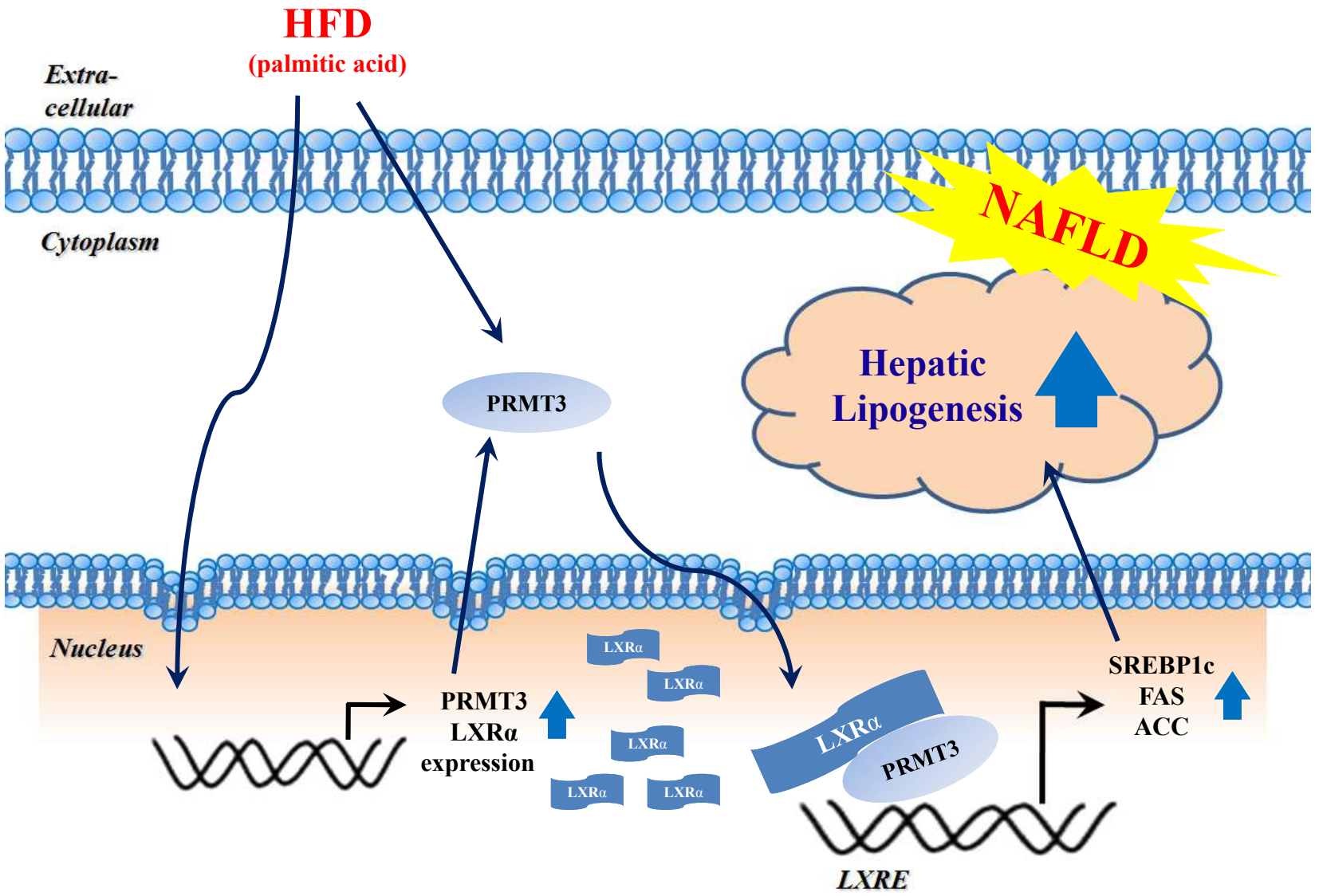
Proximity Ligation Assay (PLA)

Dramatically increased PRMT3 expression is observed in liver from NAFLD patients compared with liver from non-fatty liver patients.

Lots of PRMT3-positive cells are stained in the nucleus.

Results

PART I. PRMT3 & hepatic lipogenesis

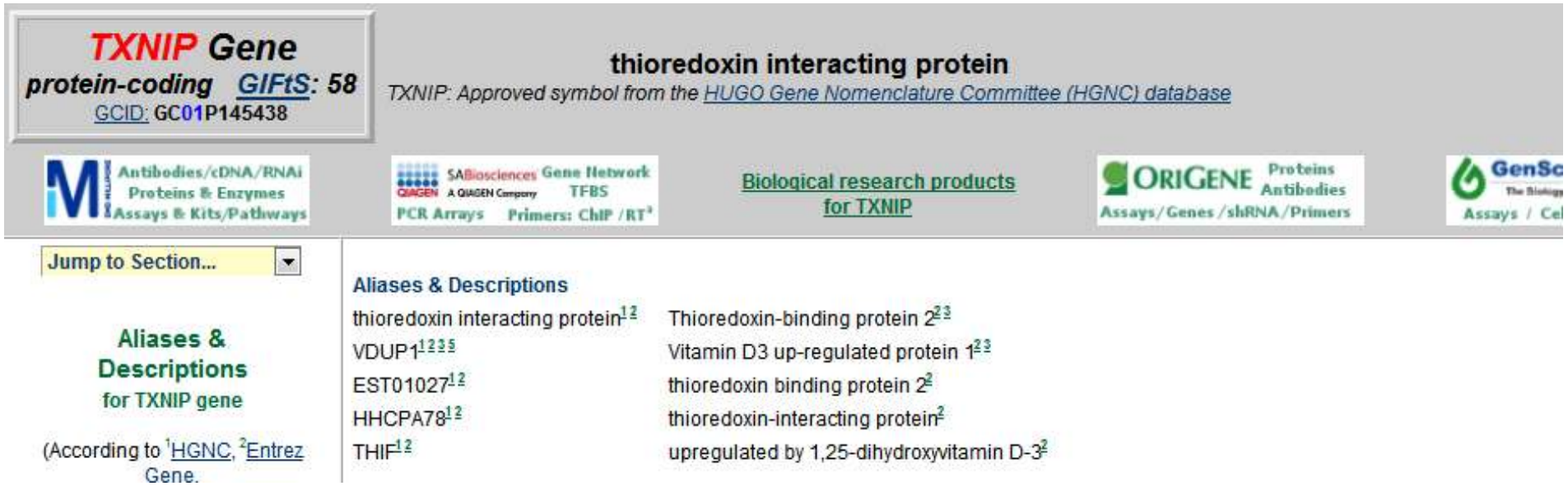


TXNIP mediates hepatic lipogenesis and inflammation via PRMT1 and PGC-1α regulation *in vitro* and *in vivo*

(J. Hepatology, 2014; 61: 1151-1157)

Introduction for PART 2

TXNIP



TXNIP Gene
protein-coding GIFtS: 58
GCID: GC01P145438

thioredoxin interacting protein
TXNIP: Approved symbol from the HUGO Gene Nomenclature Committee (HGNC) database

Antibodies/cDNA/RNAi Proteins & Enzymes Assays & Kits/Pathways

SABiosciences Gene Network TFBS PCR Arrays Primers: ChIP / RT²

Biological research products for TXNIP

ORIGENE Proteins Antibodies Assays/Genes/shRNA/Primers

Jump to Section...

Aliases & Descriptions for TXNIP gene

(According to [1]HGNC, [2]Entrez Gene.

Aliases & Descriptions

thioredoxin interacting protein[1,2]	Thioredoxin-binding protein 2[2,3]
VDUP1[1,2,3,5]	Vitamin D3 up-regulated protein 1[2,3]
EST01027[1,2]	thioredoxin binding protein 2[2]
HHCPA78[1,2]	thioredoxin-interacting protein[2]
THIF[1,2]	upregulated by 1,25-dihydroxyvitamin D-3[2]

- TXNIP
  - a ubiquitously expressed protein
  - binds to and inhibits thioredoxin
  - can modulate the cellular redox state
  - induce oxidative stress

(Junn E, 2patwari 000; Nishiyama A, 2001; Nishiyama A, 1999; Yamanaka H, 2000; Patwari P, 2006)

TXNIP & Diabetes

- **Genetic variation in VDUP1 is associated with hypertriglyceridaemia and blood pressure in diabetes mellitus.**

(van Greevenbroek MM, 2007)

- **Hyperglycemia induced overexpression of TXNIP causes ROS/RNS stress, mitochondrial dysfunction, inflammation and premature cell death in Dibeteic Retinopathy.**

(Singh LP, 2013)

- **Thioredoxin-interacting protein mediates high glucose-induced reactive oxygen species generation by mitochondria and the NADPH oxidase, Nox4, in mesangial cells.**

(Shah A, 2013)

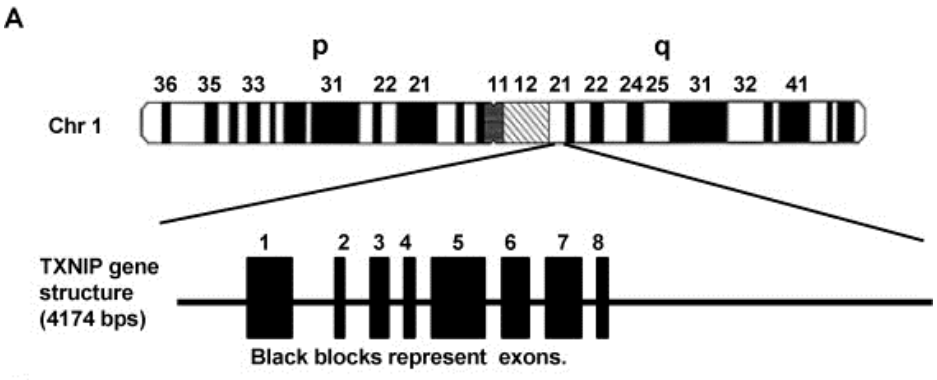
- **Knockdown of thioredoxin-interacting protein ameliorates high glucose-induced epithelial to mesenchymal transition in renal tubular epithelial cells.**

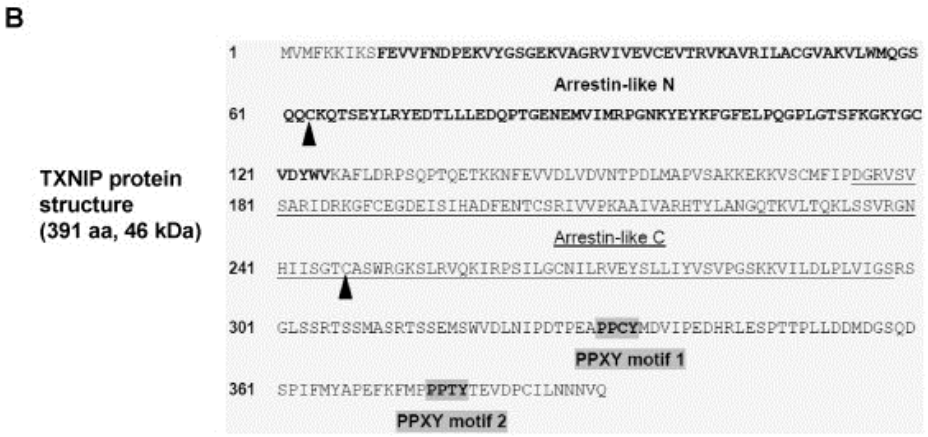
(Wei J, 2013)

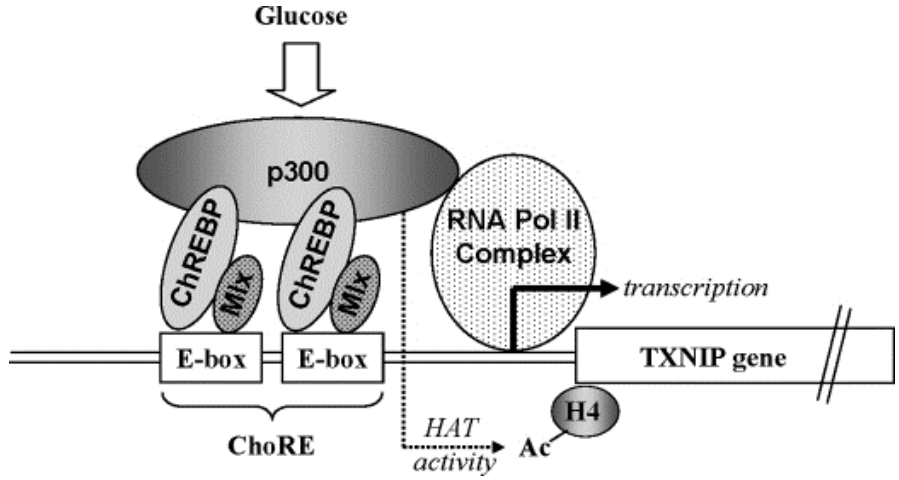
- **Thioredoxin-interacting protein mediates NALP3 inflammasome activation in podocytes during diabetic nephropathy.**

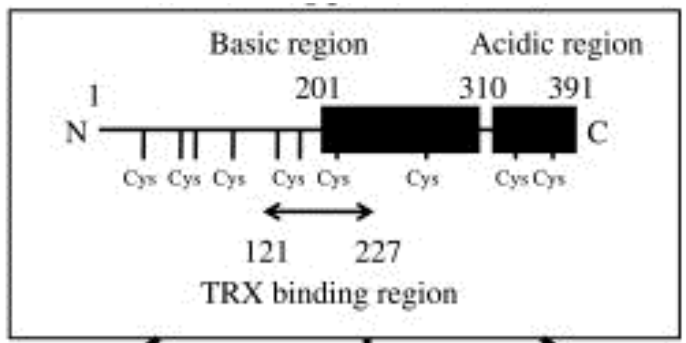
(Gao P, 2014)

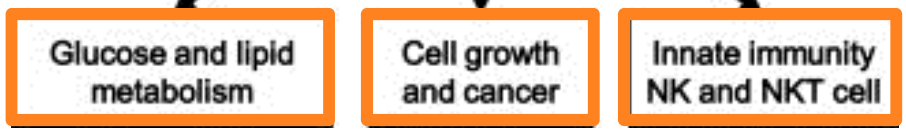
TXNIP





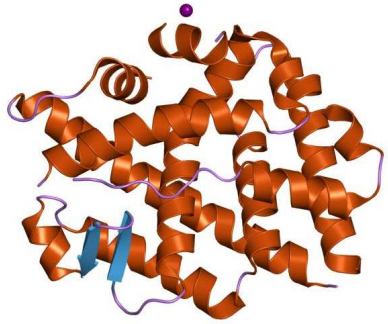






Vet. Physiol. Lab.

PPARγ coactivator 1α (PGC1 α)



- a major regulator of several key metabolic pathways
- PGC1α was initially identified as the key factor driving thermogenesis in brown fat.

(Puigserver P, 1998)

- Numerous studies have since shown a key role for PGC1 α in inducing the expression of genes of oxidative phosphorylation and the tricarboxylic acid (TCA) cycle in various tissues.

(Mootha VK, 2003; Lin J, 2005; Burgess SC, 2006)

- Recent studies show that PGC1 also promotes anabolic pathways such as *de novo* lipogenesis

(Espinoza DO, 2010; Summermatter S, 2011; Bhalla K, 2011)

Hypothesis

: What's the function of TXNIP in liver?

Palmitic acid in Hepatocytes

TXNIP

Lipogenesis & Inflammation

Functions in Pathophysiology of hepatocytes

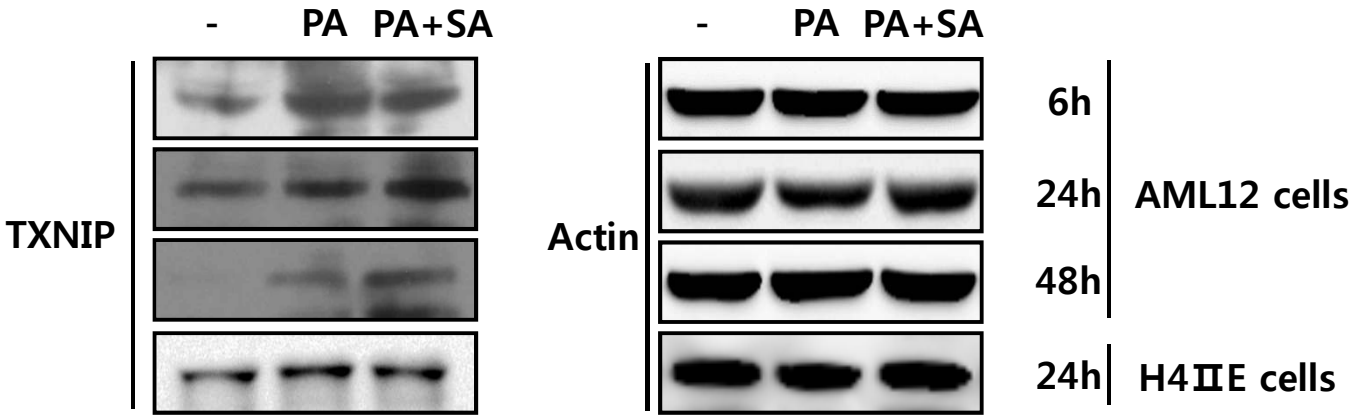
PART 2

Methods

- Cell cultures
  - AML12, hepatocytes from a mouse
  - H4 II E, Hepatoma cell from a rat
- DNA and siRNA transfection
- Whole cell preparation and Western blotting
- Luciferase assay
- Oil Red O Staining
- Animal study
- Liver isolation and whole cell extract preparation

Palmitic acid elevated TXNIP level

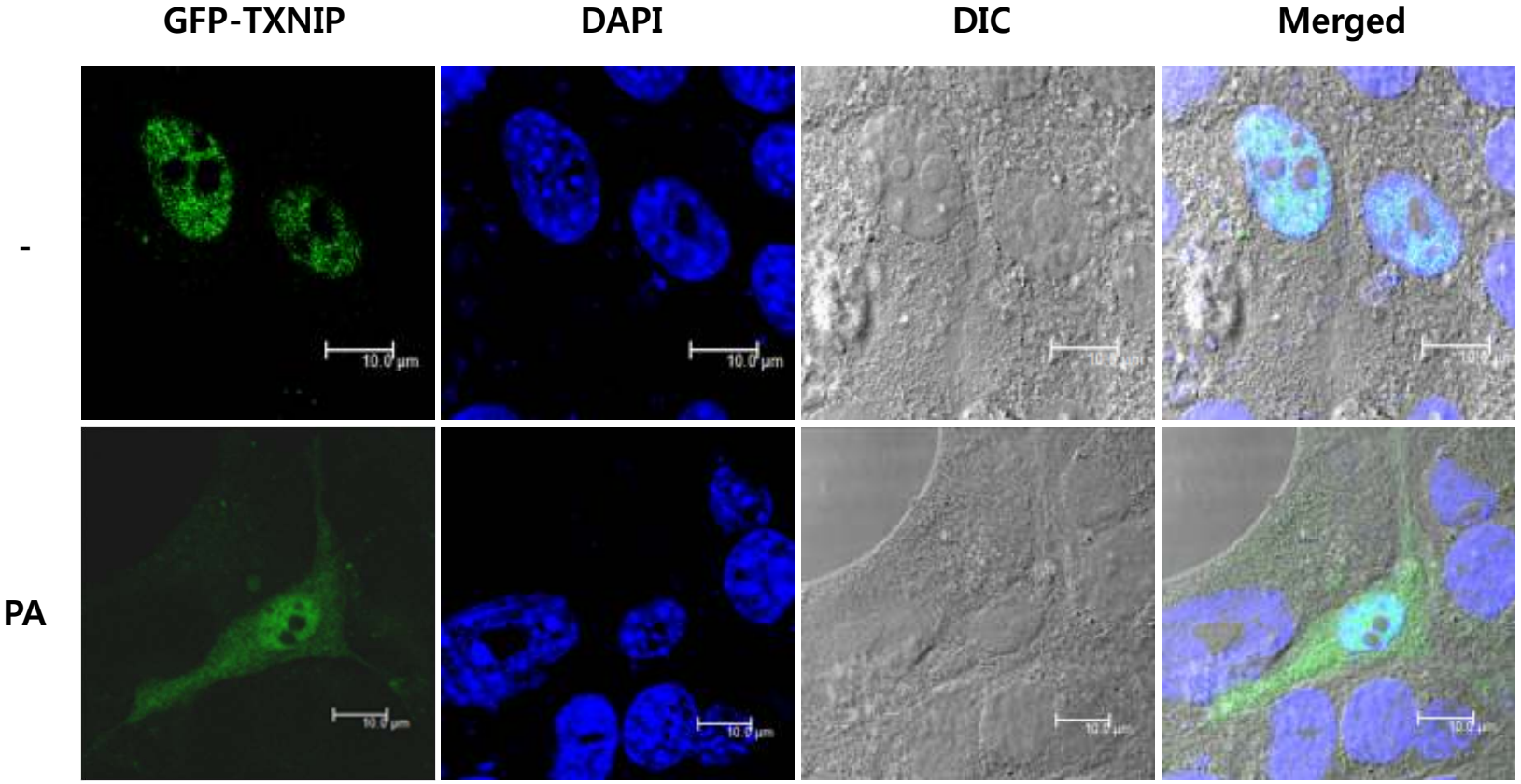
A



Palmitic acid increased TXNIP expression in AML12 cells and H4IIE cells

PA: 350 micromole
SA: 150 micromole

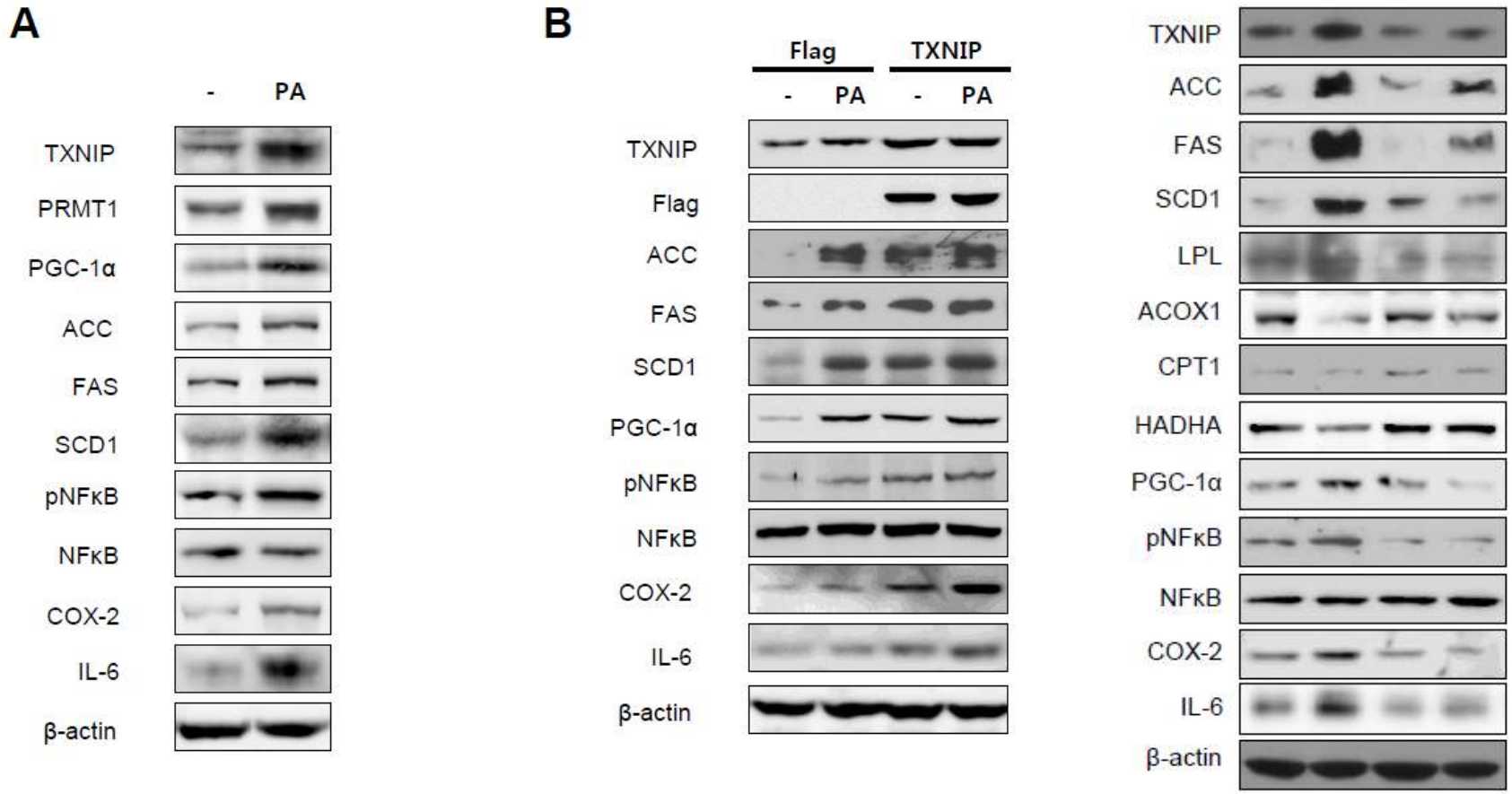
Subcellular localization of TXNIP in AML12 cells



Palmitic acid increased nuclear TXNIP expression in AML12 cells

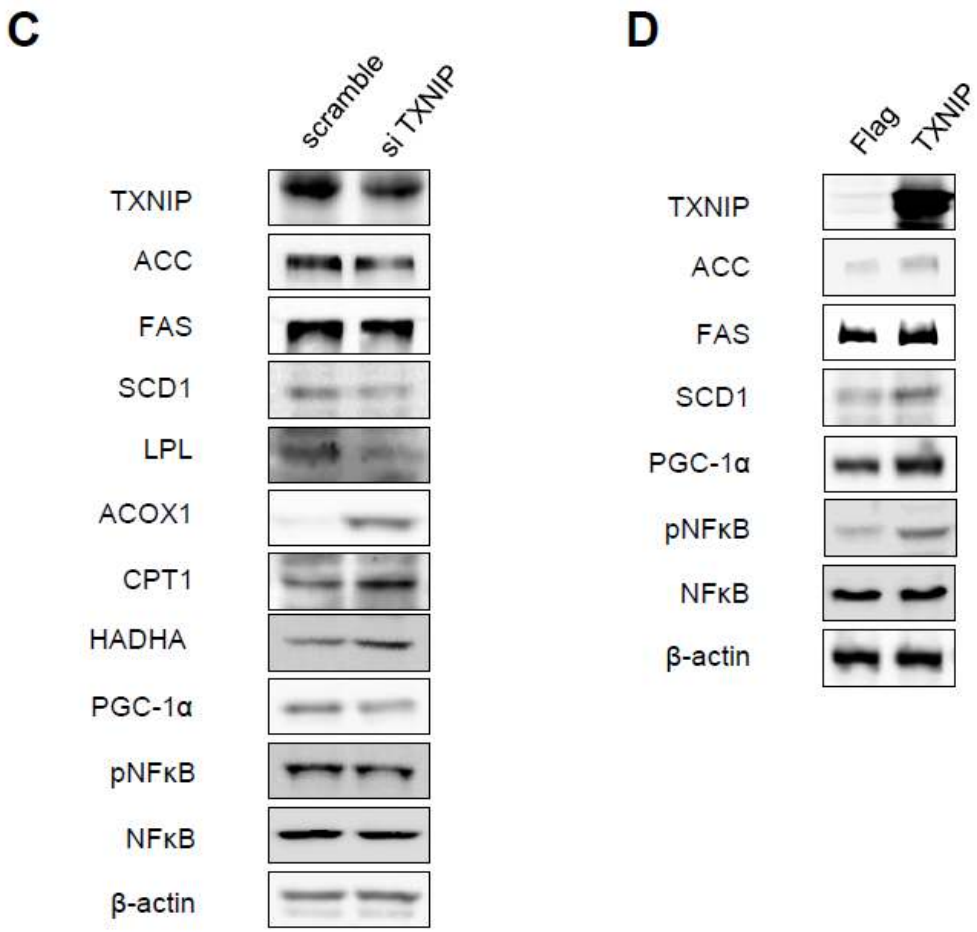
Palmitic acid-induced TXNIP increases expressions of PGC-1α, NFκB and lipogenic proteins in hepatocytes

AML12 cells



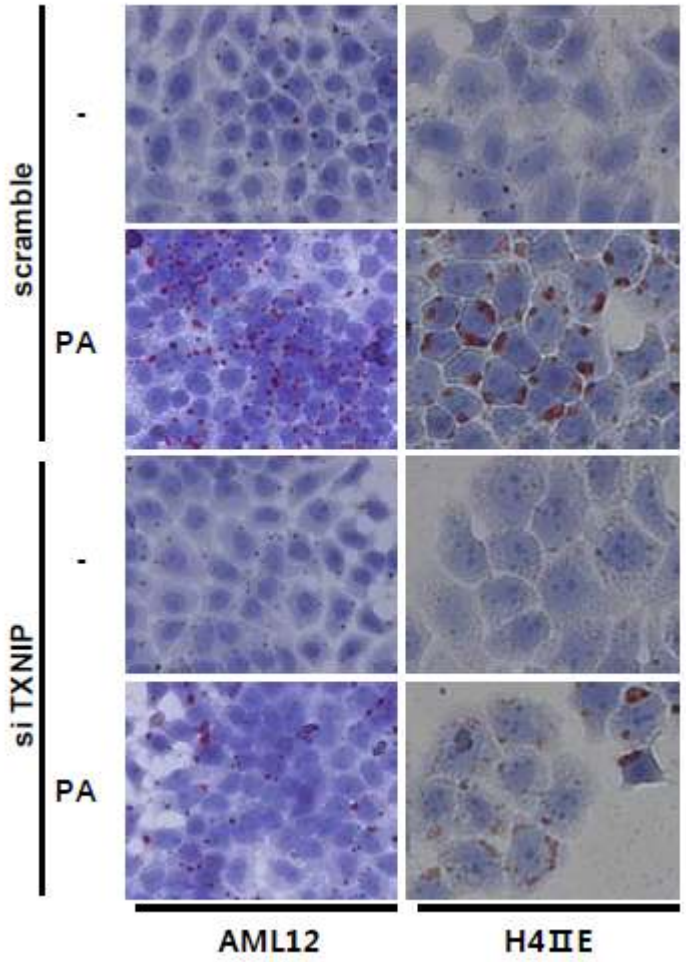
TXNIP expression links with the expressions of PGC1α and NFκB and lipogenic proteins.

H4IIE cells



TXNIP siRNA prevents palmitic acid-induced elevation of PGC1α and lipogenic and inflammatory proteins

A



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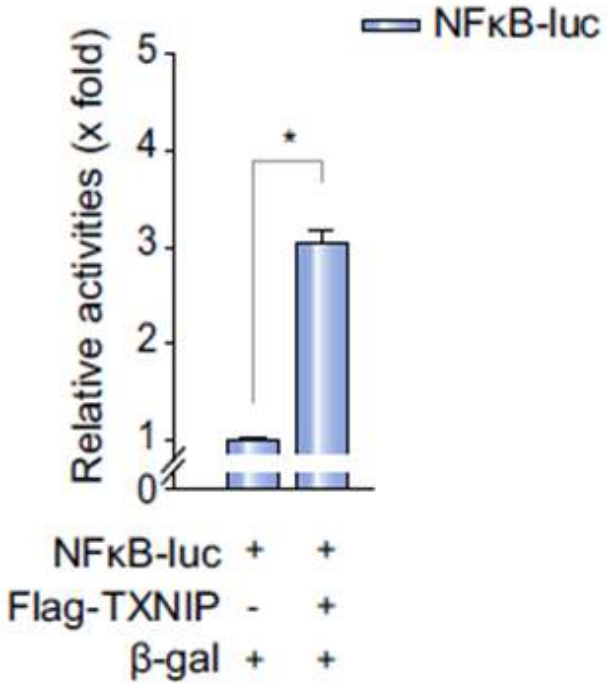
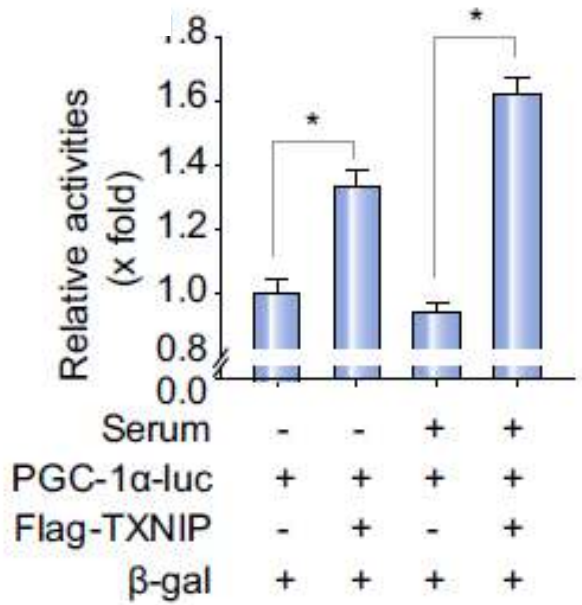


TXNIP increased lipogenesis via PGC1α activity

A



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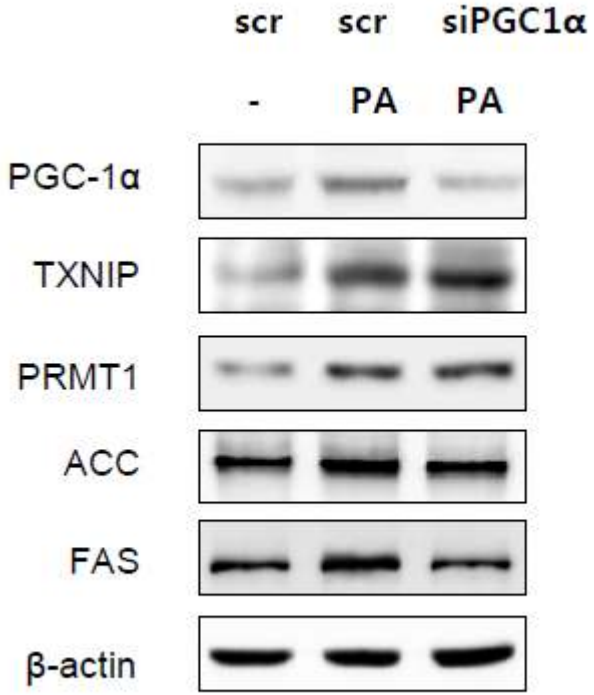
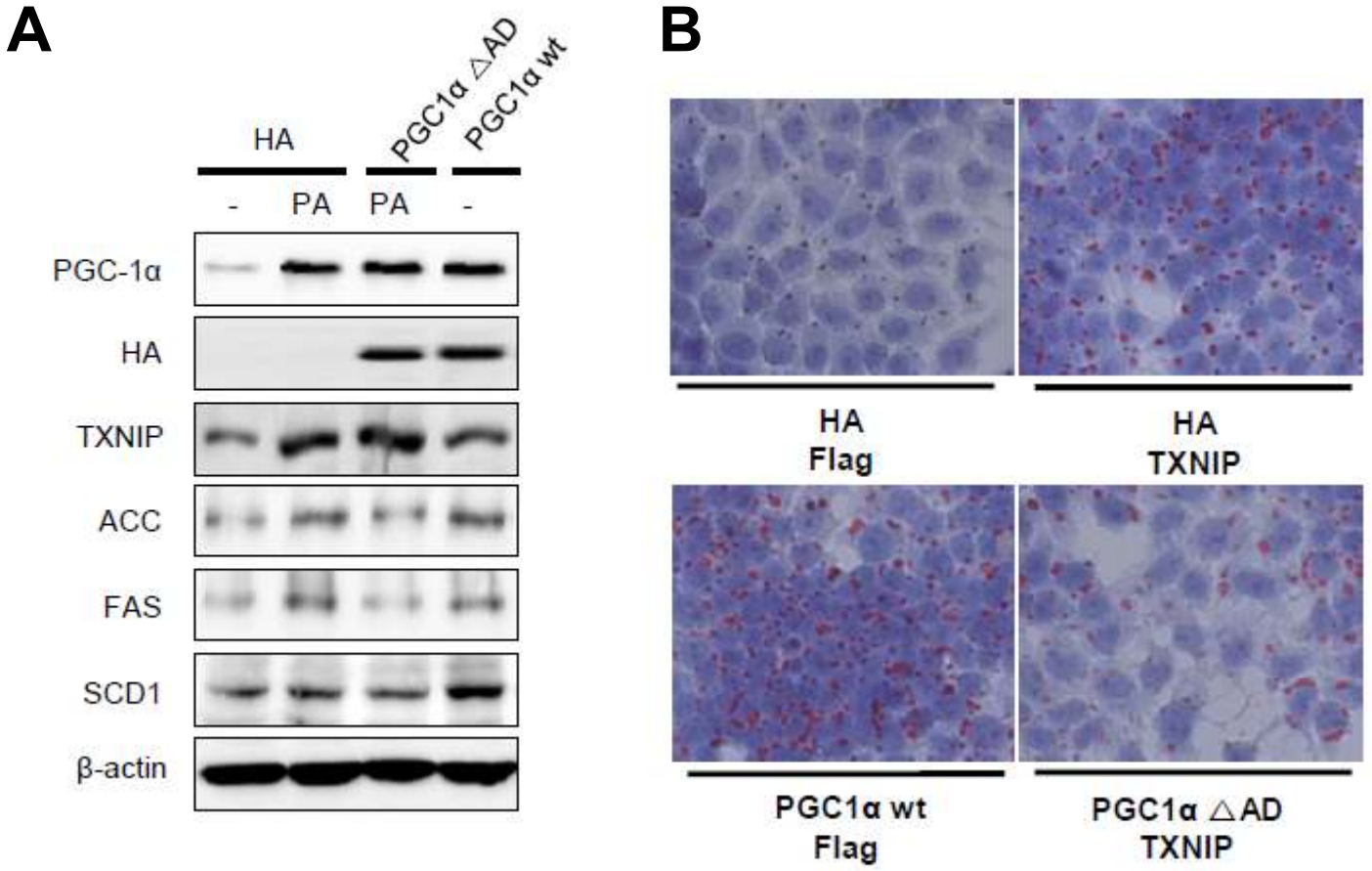


TXNIP increased lipogenesis via PGC1α activity

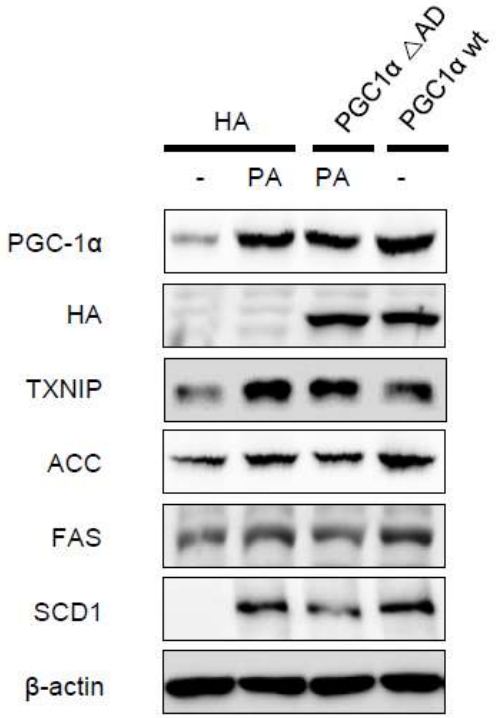
AML12 cells



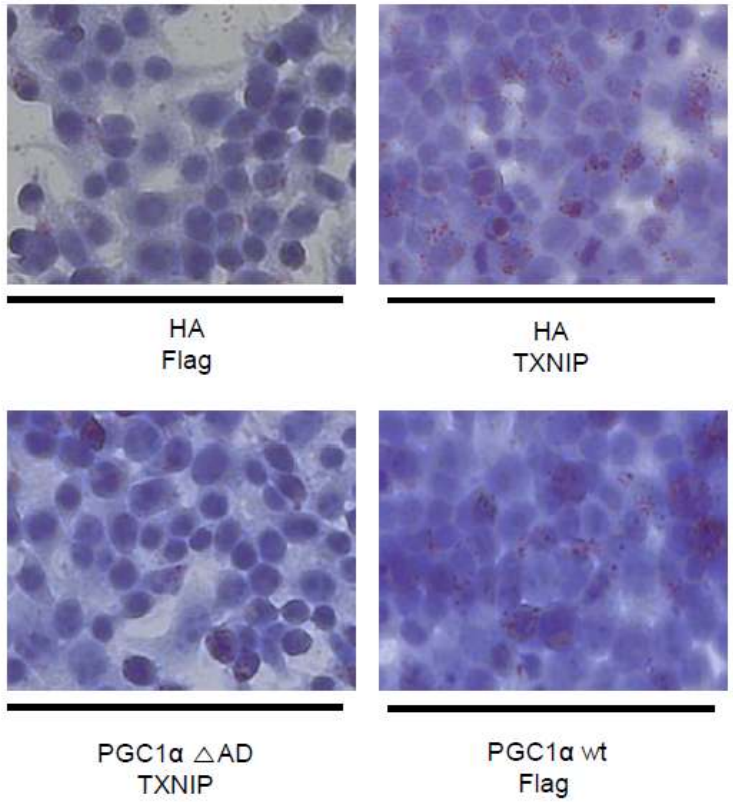
TXNIP increased lipogenesis via PGC1α activity

293T cells

C

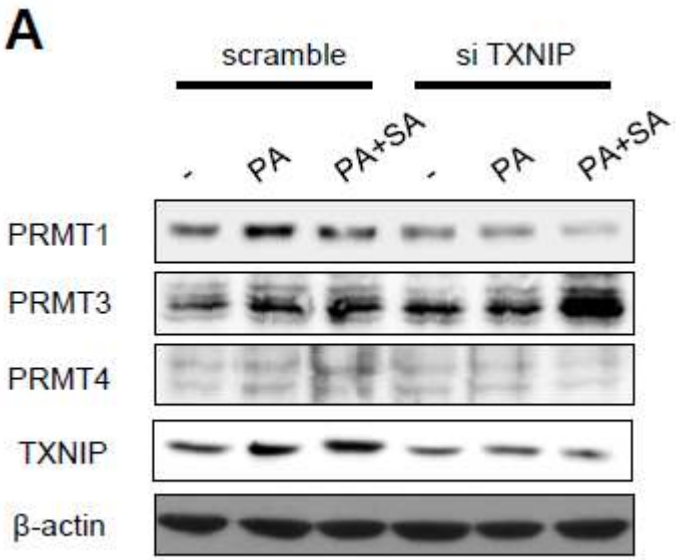


D



PRMT1 mediates TXNIP-induced expression of PGC1α and lipogenic proteins

AML12 cells

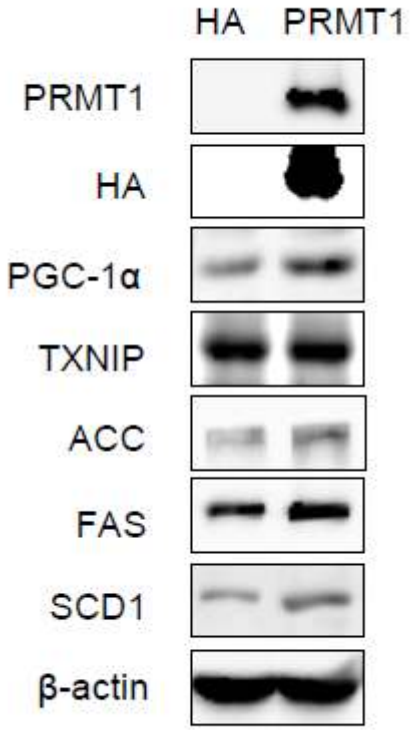


AML12 cells

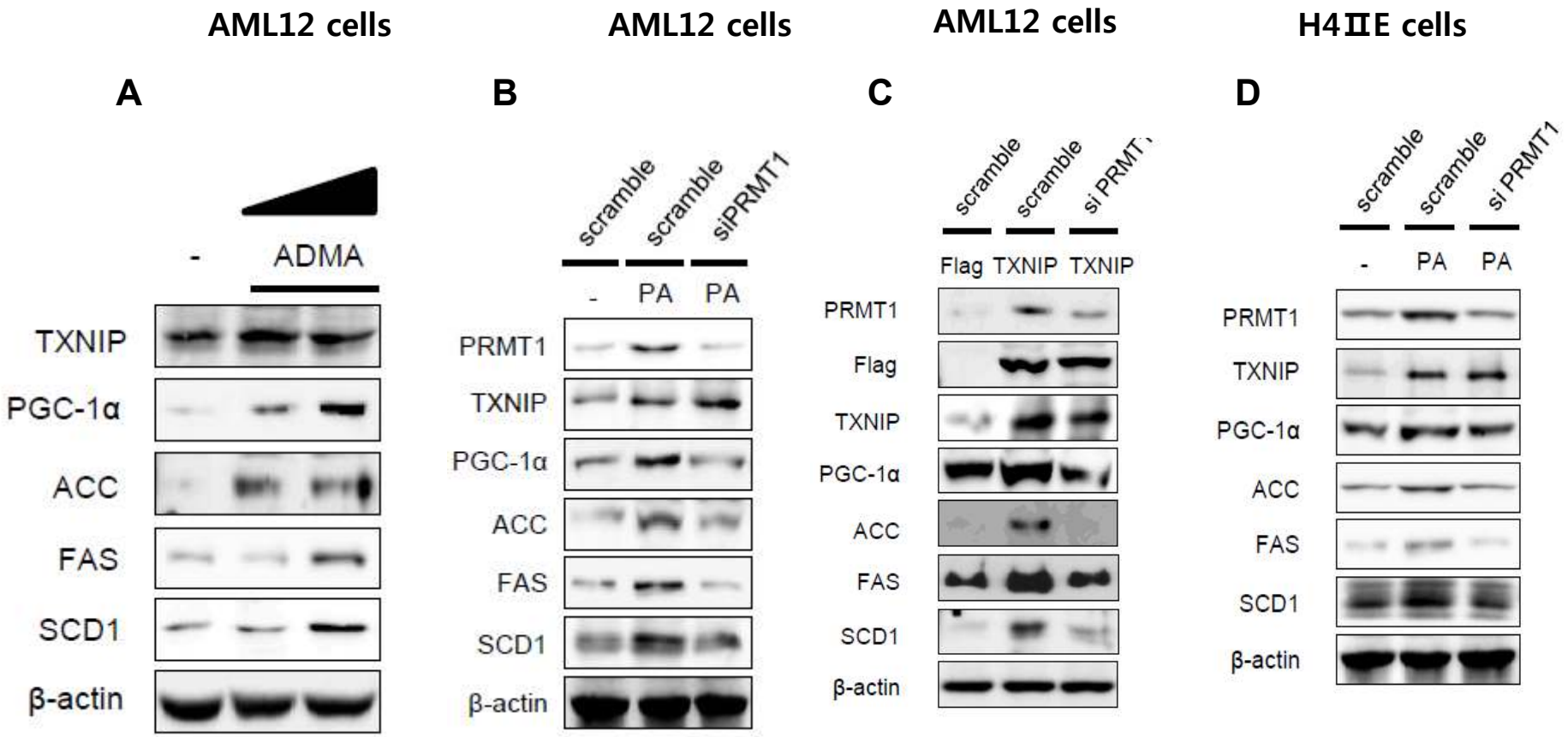
B

293T cells

C

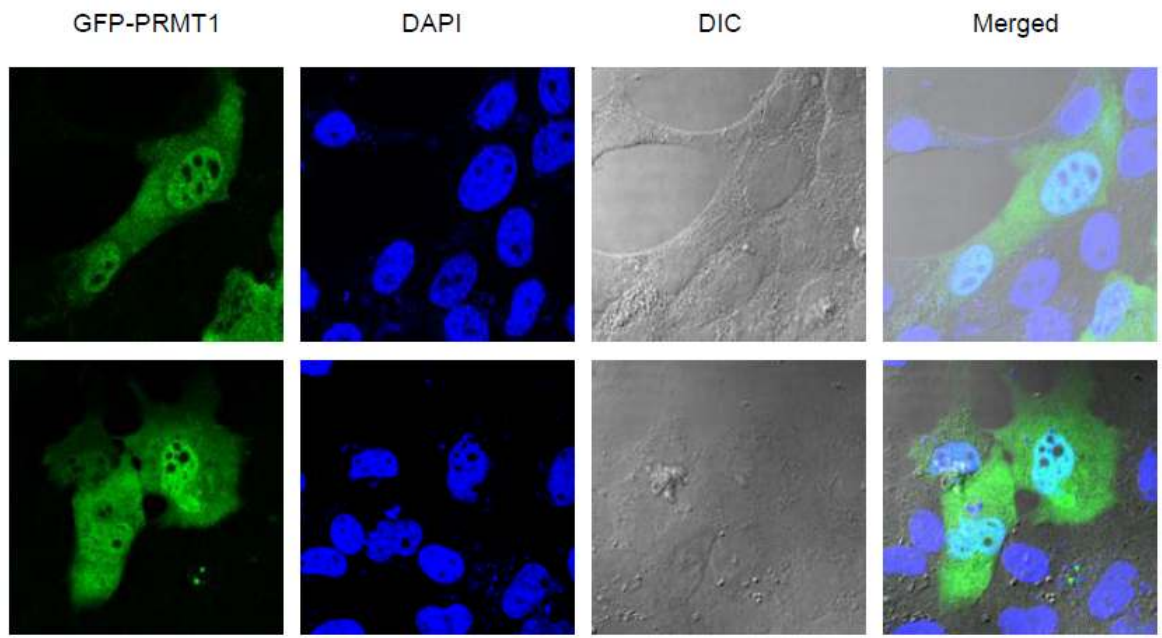


PRMT1 mediates TXNIP-induced expression of PGC1α and lipogenic proteins

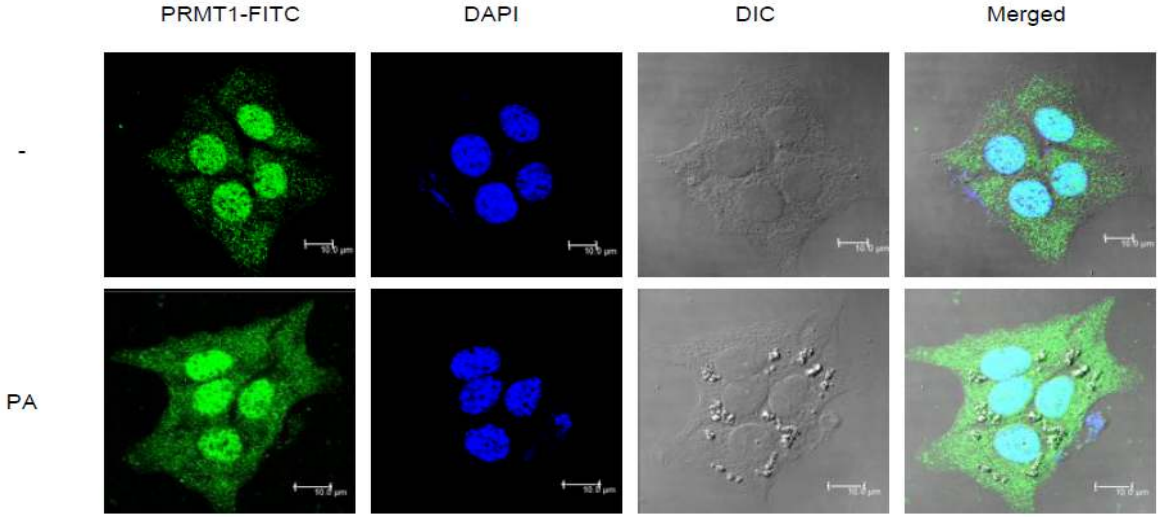


PRMT1 localization in AML12 cells

A Exogenous GFP-PRMT1

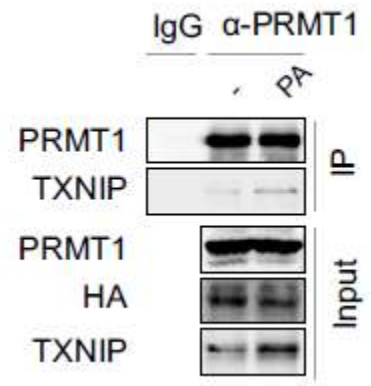


B Endogenous PRMT1

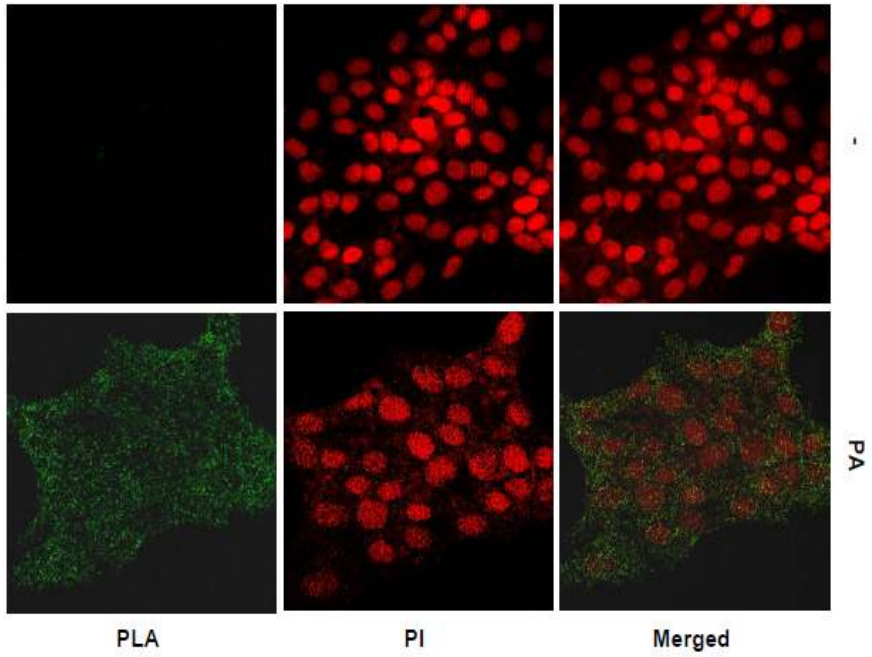


PRMT1 mediates TXNIP-induced expression of PGC1α and lipogenic proteins

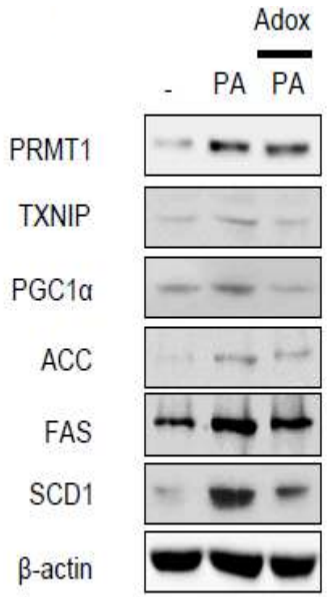
A



B

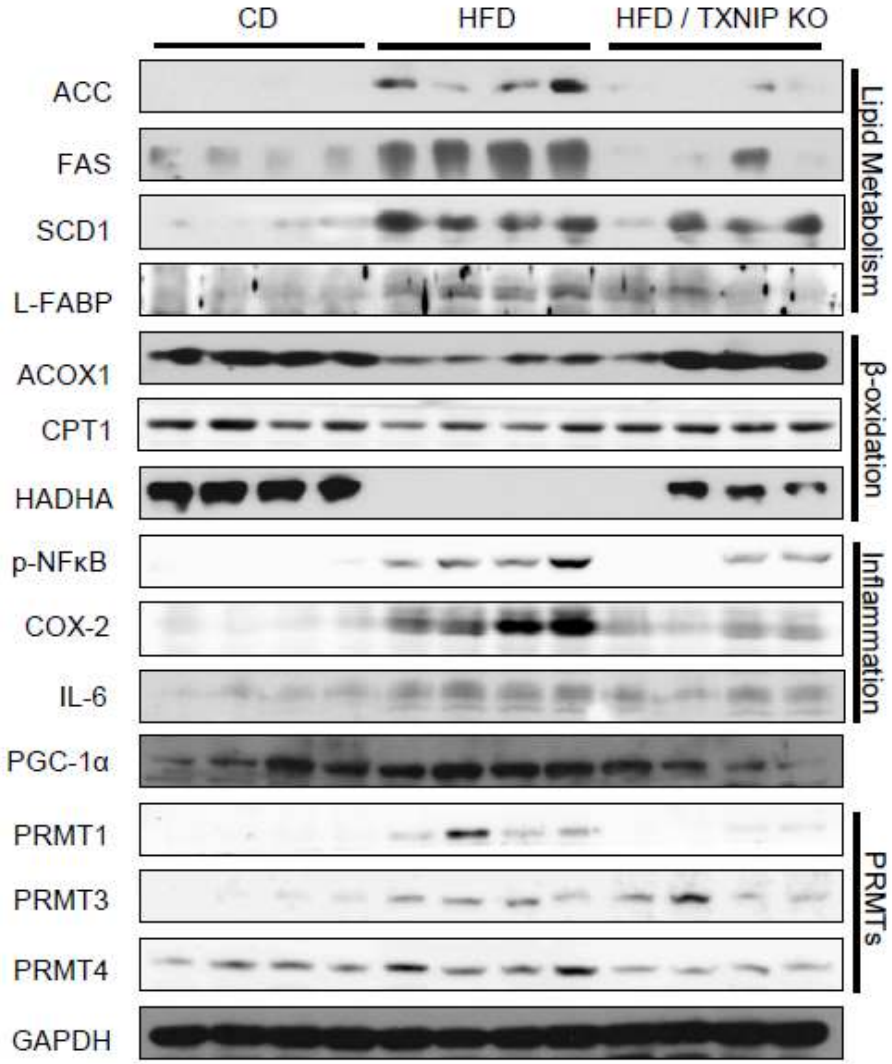


C



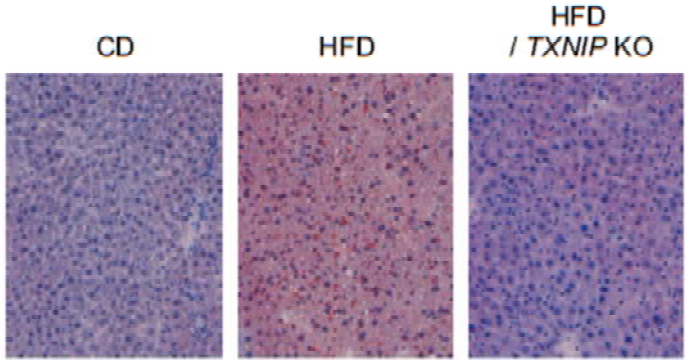
PLA with anti-PRMT1 & anti-TXNIP

TXNIP-deficient mice on a HFD show improved fatty livers mainly via TXNIP-PRMT1-PGC1α pathway



TXNIP-deficient mice on a HFD show improved fatty livers mainly via TXNIP-PRMT1-PGC1α pathway

A



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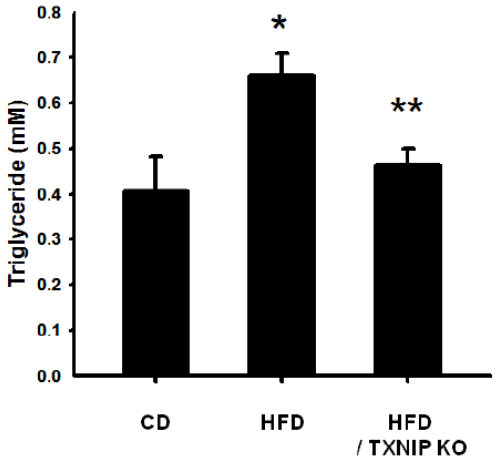

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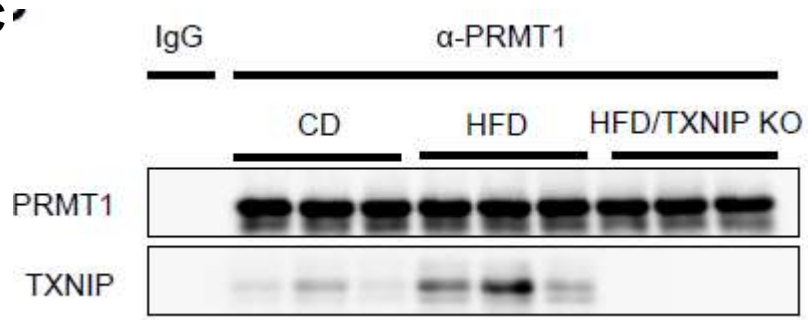
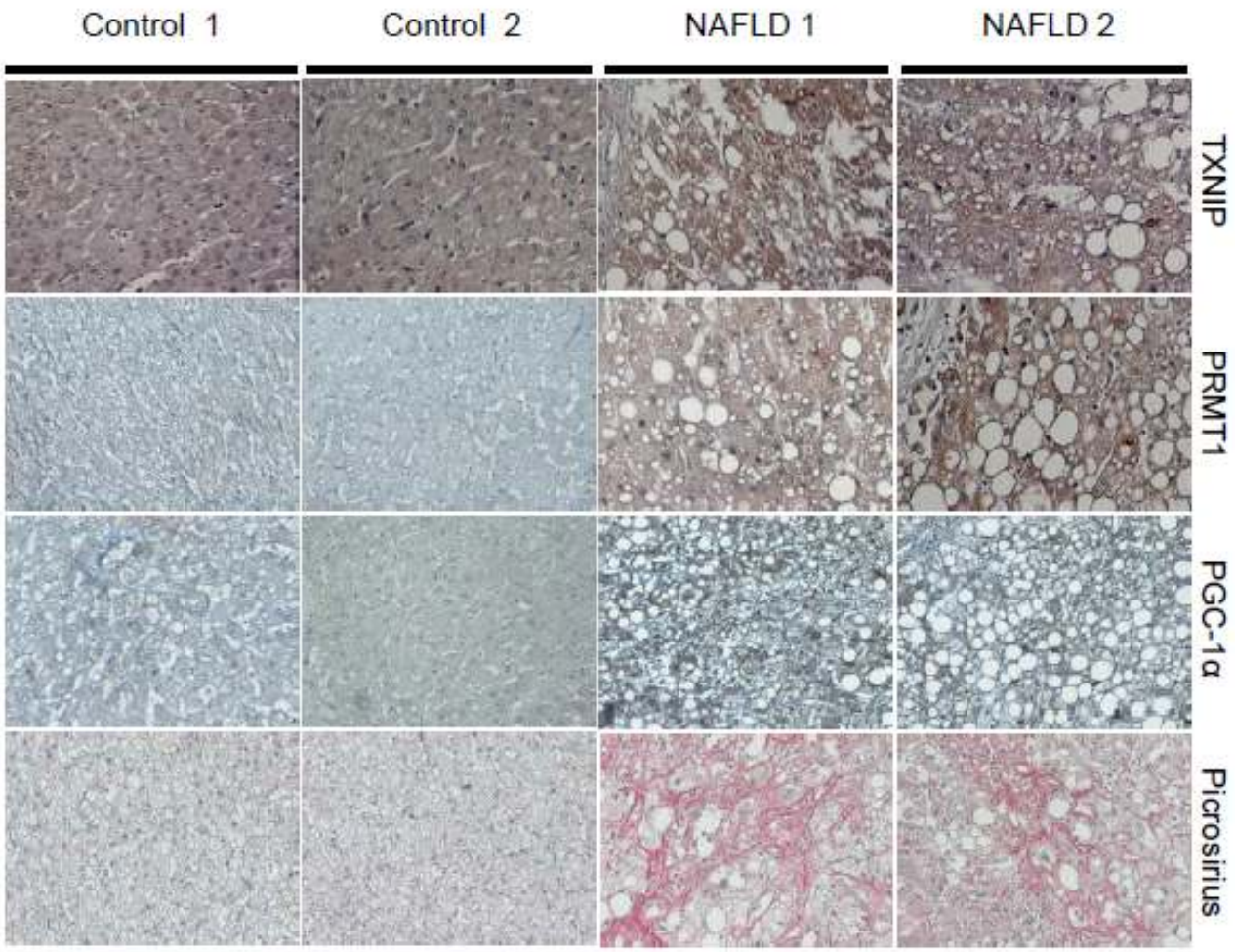
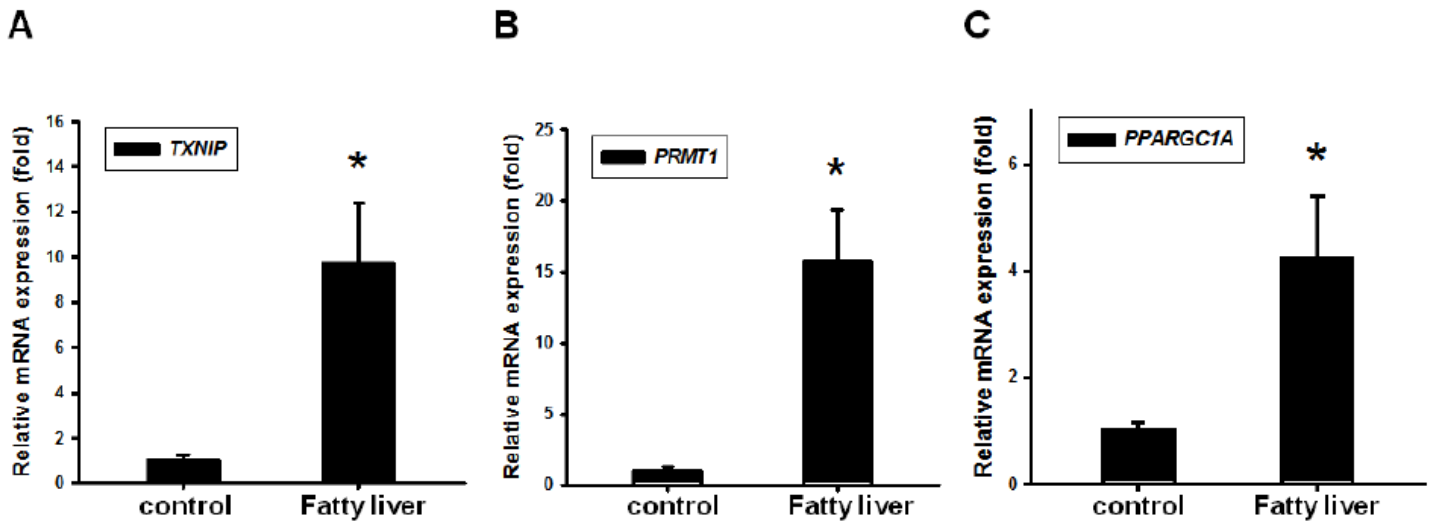


Expression level of TXNIP, PRMT1, and PGC-1a are elevated in the livers of NAFLD patients



Expression level of TXNIP, PRMT1, and PGC-1a are elevated in the livers of NAFLD patients



TXNIP mediates hepatic lipogenesis and inflammation via PRMT1 and PGC-1α regulation *in vitro* and *in vivo*

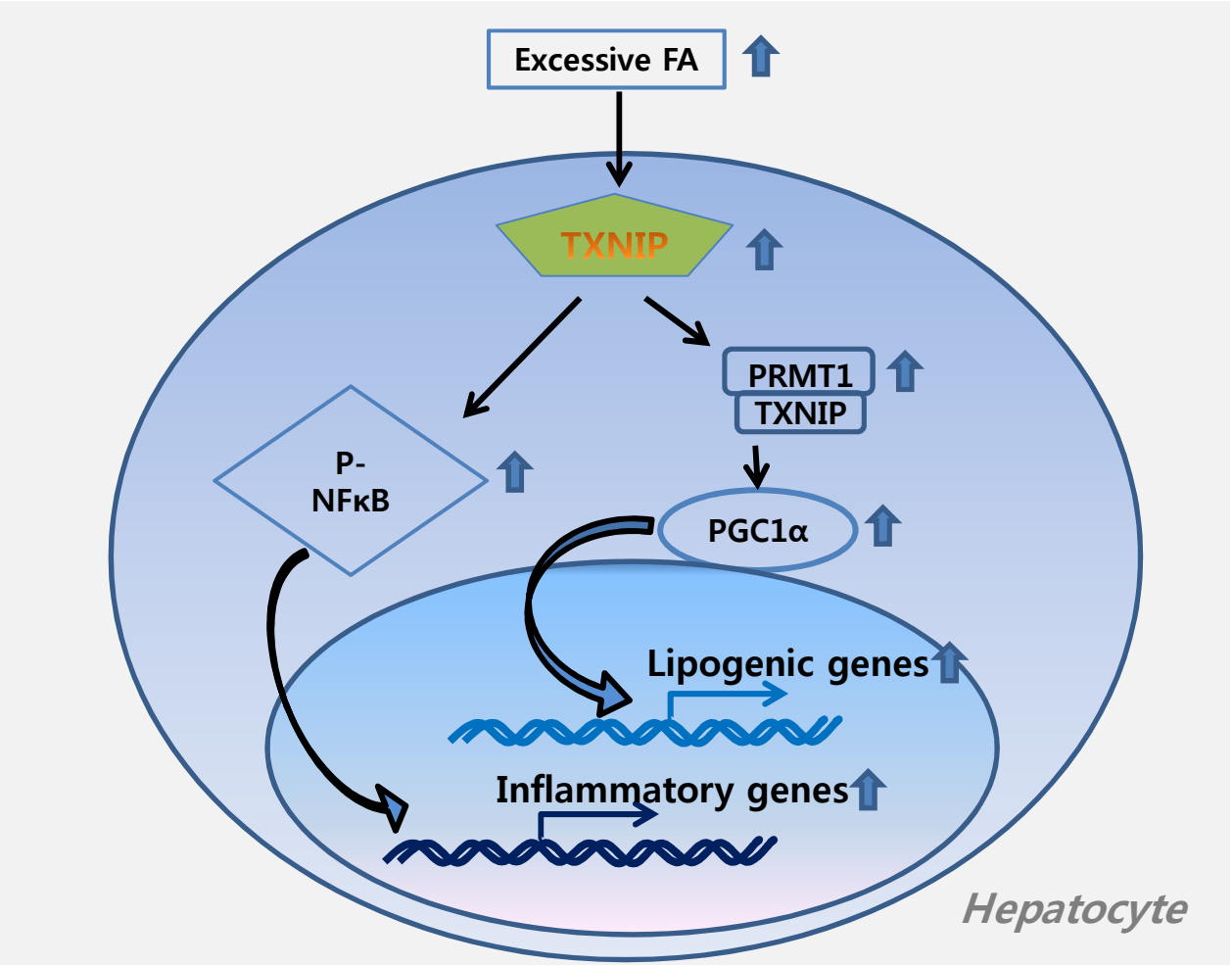
Conclusions:

• TXNIP mediates hepatic lipogenesis via PRMT1 and PGC-1α regulation and inflammation *in vitro* and *in vivo.*

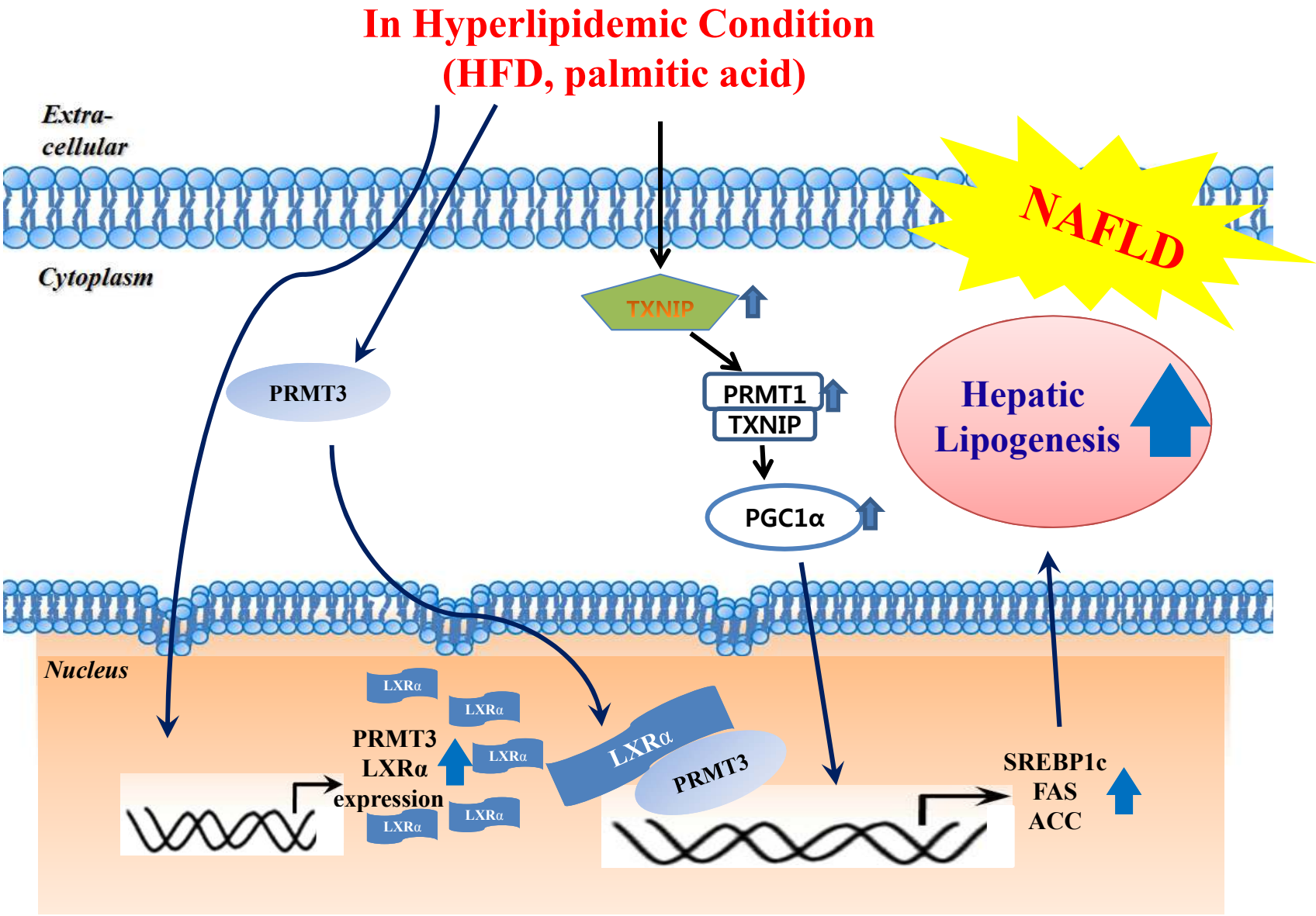
• This implyes that targeting TXNIP and PRMT1 is a potential therapeutic approach for treatment of NAFLD.

Summary

VDUP1 mediates hepatic lipogenesis via PRMT1 and PGC-1α regulation *in vitro* and *in vivo*



Conclusion


In Hyperlipidemic Condition
(HFD, palmitic acid)
Extra-
cellular
Cytoplasm
NAFLD
TXNIP
PRMT3
PRMT1
TXNIP
PGC1α
Hepatic
Lipogenesis
Nucleus
LXRα
LXRα
PRMT3
LXRα
expression
LXRα
LXRα
LXRα
LXRα
PRMT3
SREBP1c
FAS
ACC

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