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The Enhancement of Toxin-Induced Liver Fibrosis in Fatty Liver Disease

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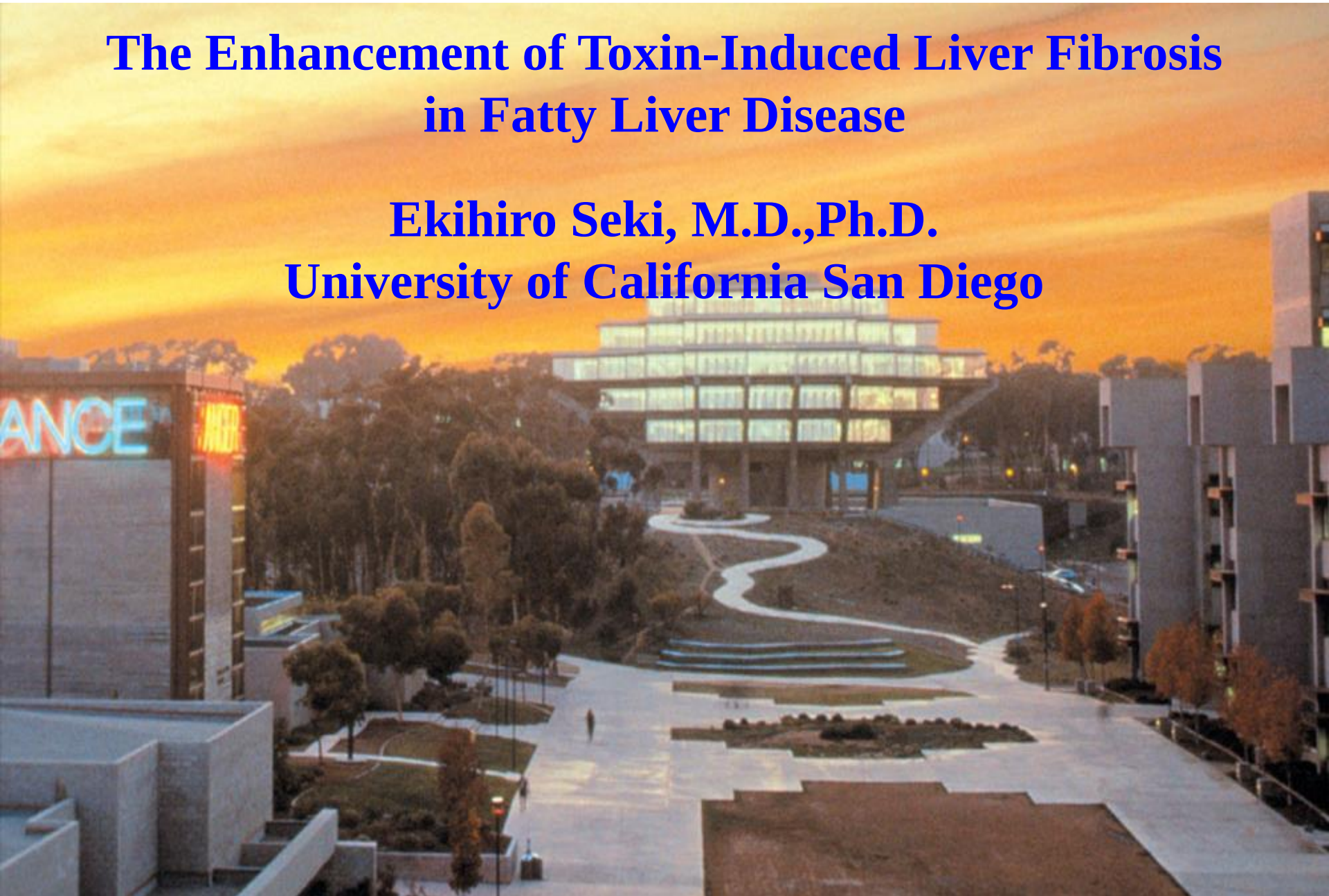


Carbon tetrachloride (CCl_4)

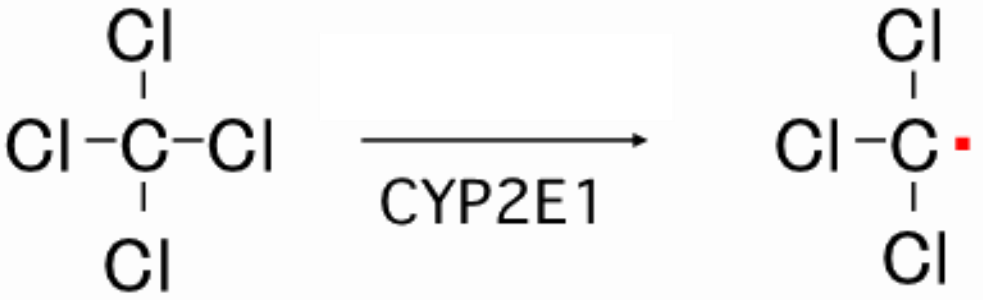
- A manufactured chemical.

- Does not exist naturally.

- Clear liquid with a sweet odor that smells like chloroform.

- Easily vaporizes.

- High levels or continuous low dose exposure influence to liver, and cause hepatocyte injury, liver fibrosis and hepatocarcinogenesis.



Trichloromethyl radical
(Free radical)

Lipid peroxidation
Hepatotoxicity

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Carbon tetrachloride (CCl_4)

- Has been used for dry-cleaning, solvent, reagent in chemical synthesis, fire extinguisher fluid, and refrigerants.

- The use and production of CCl_4 was declined in the mid 1970s, but it has been diffusing into air, water and soil. It takes for several years to break down

- In air of 0.1 ppb are common around the world.

- In water or soil, ranging from 50 to over 1,000 ppb at 22% of the Superfund sites.

- 47 th and 50 th RANK (2007 CERCLA and 2011 ATSDR priority lists)

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NAFLD

- Nonalcoholic fatty liver disease (NAFLD) is one of the leading causes of liver disease in the United States
- The prevalence of obesity and diabetes increases
  - 60-70% of obese adults have NAFLD
- 30-40% of adults in the western world have NAFLD
- 15-20% of NAFLD patients progress to NASH
- According to a 2008 estimation, NAFLD will be the leading cause for liver transplantation by 2020.

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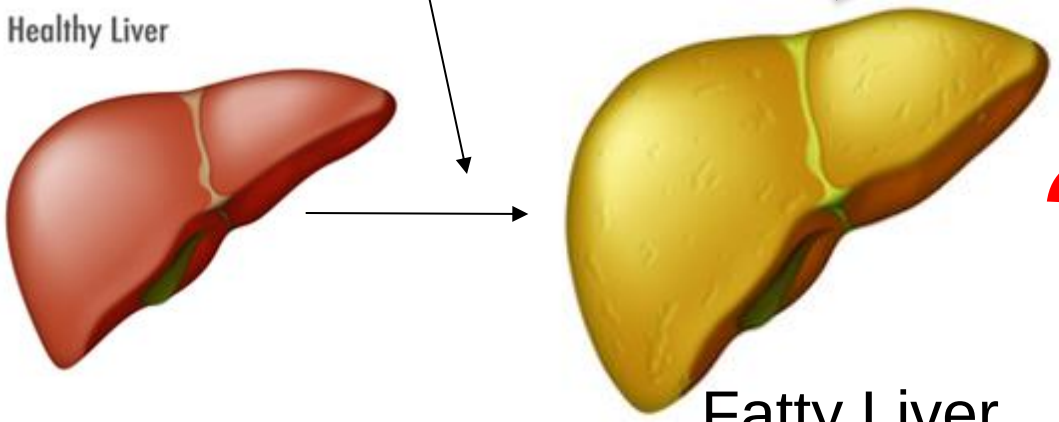
Question: Does Fatty liver disease affects the sensitivity to toxin exposure?

High fat diet

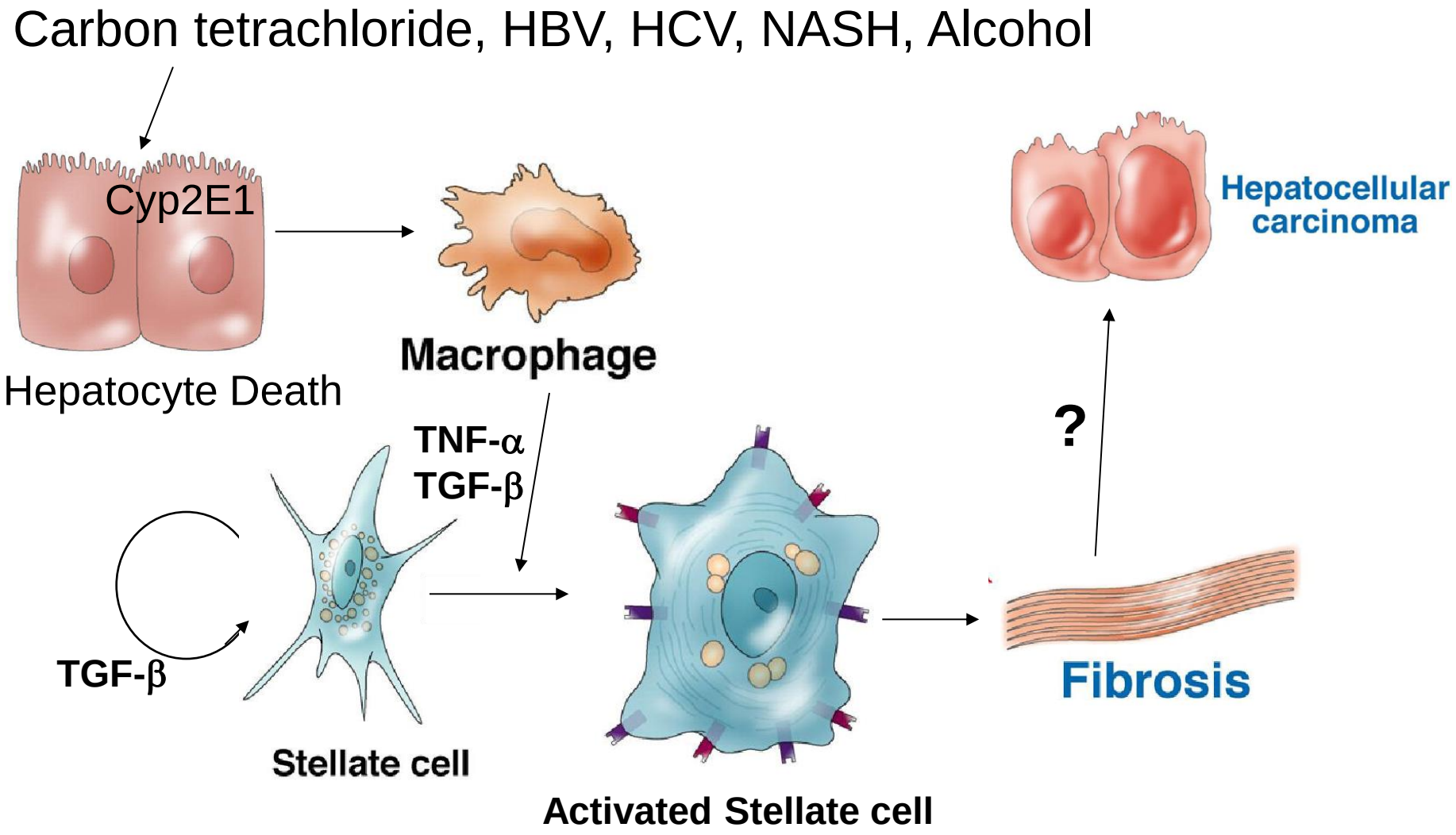
Chronic Industrial Toxin Exposure



?

Fatty Liver

Activation of Stellate cells in Liver Fibrosis



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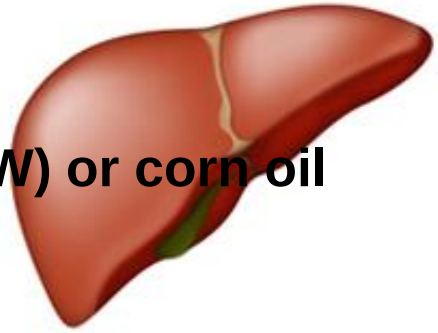
The Effect of Fatty Liver in Toxin-induced Liver Fibrosis



8x CCl_4 (0.25ul/g BW) or corn oil

LFD

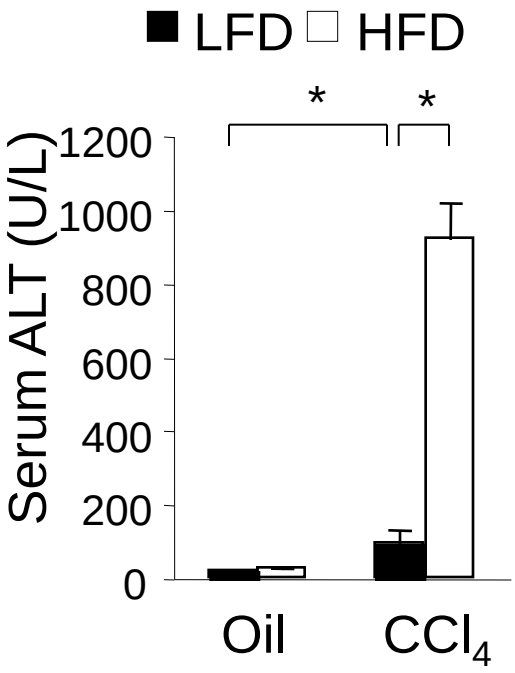
HFD for 2M

HFD

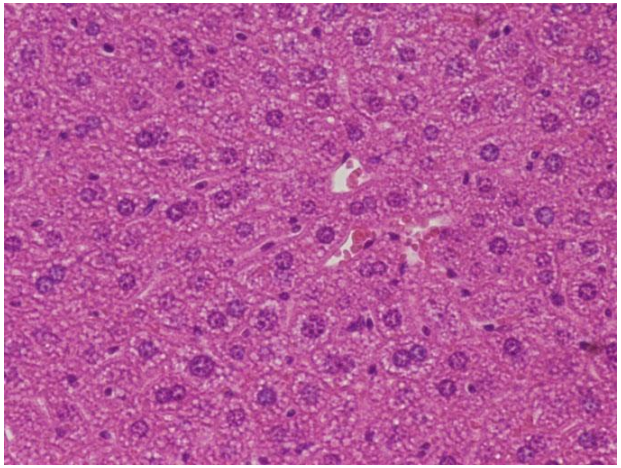
HFD for 1M

8x CCl_4 (0.25ul/g BW) or corn oil

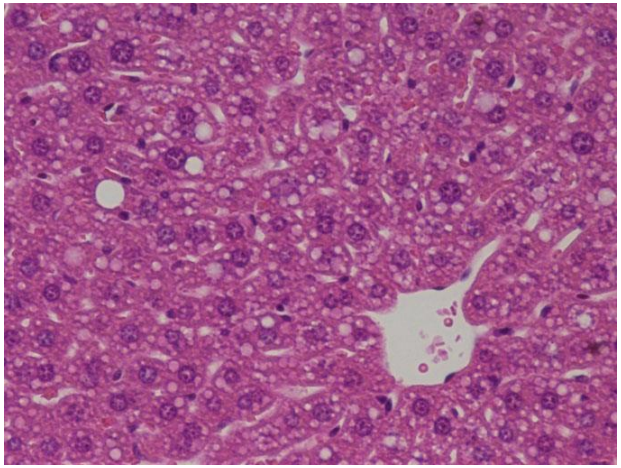
HFD feeding enhances Toxin-induced Liver Injury



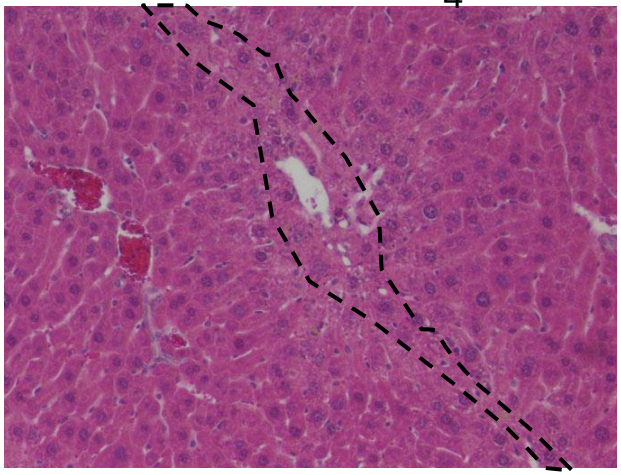
LFD + Oil



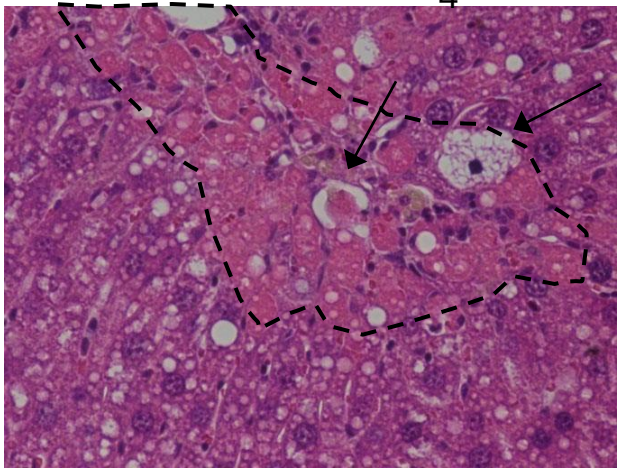
HFD + Oil



LFD + CCl_4

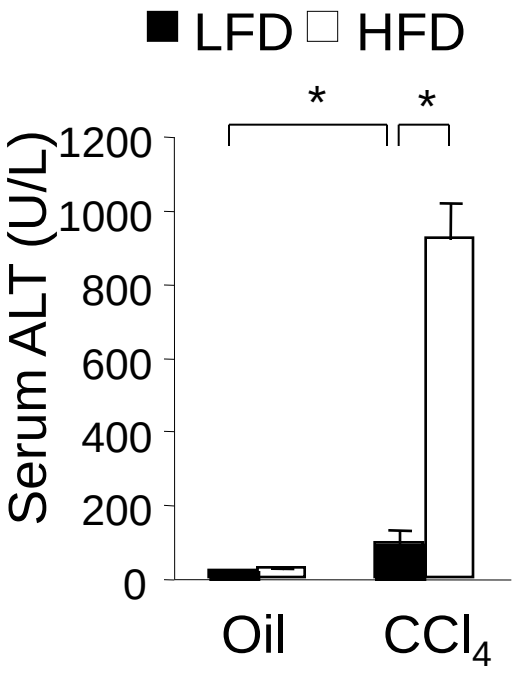


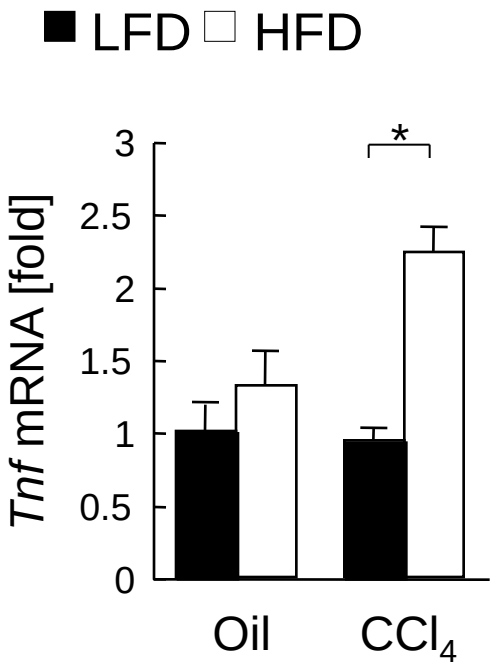
HFD + CCl_4

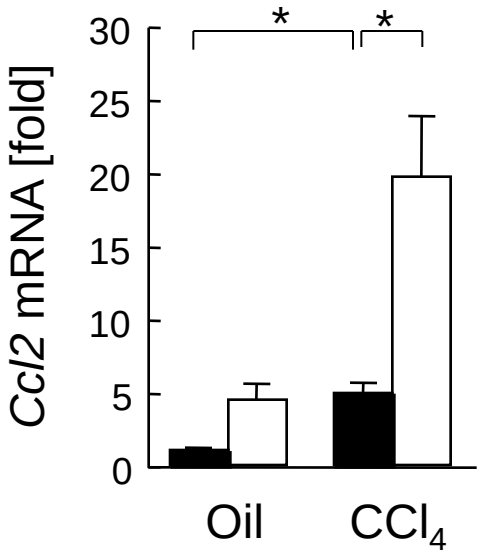


8 x CCl_4 (0.25 ul/g BW)

HFD feeding enhances Toxin-induced Liver Inflammation







8 x CCl_4 (0.25 ul/g BW)

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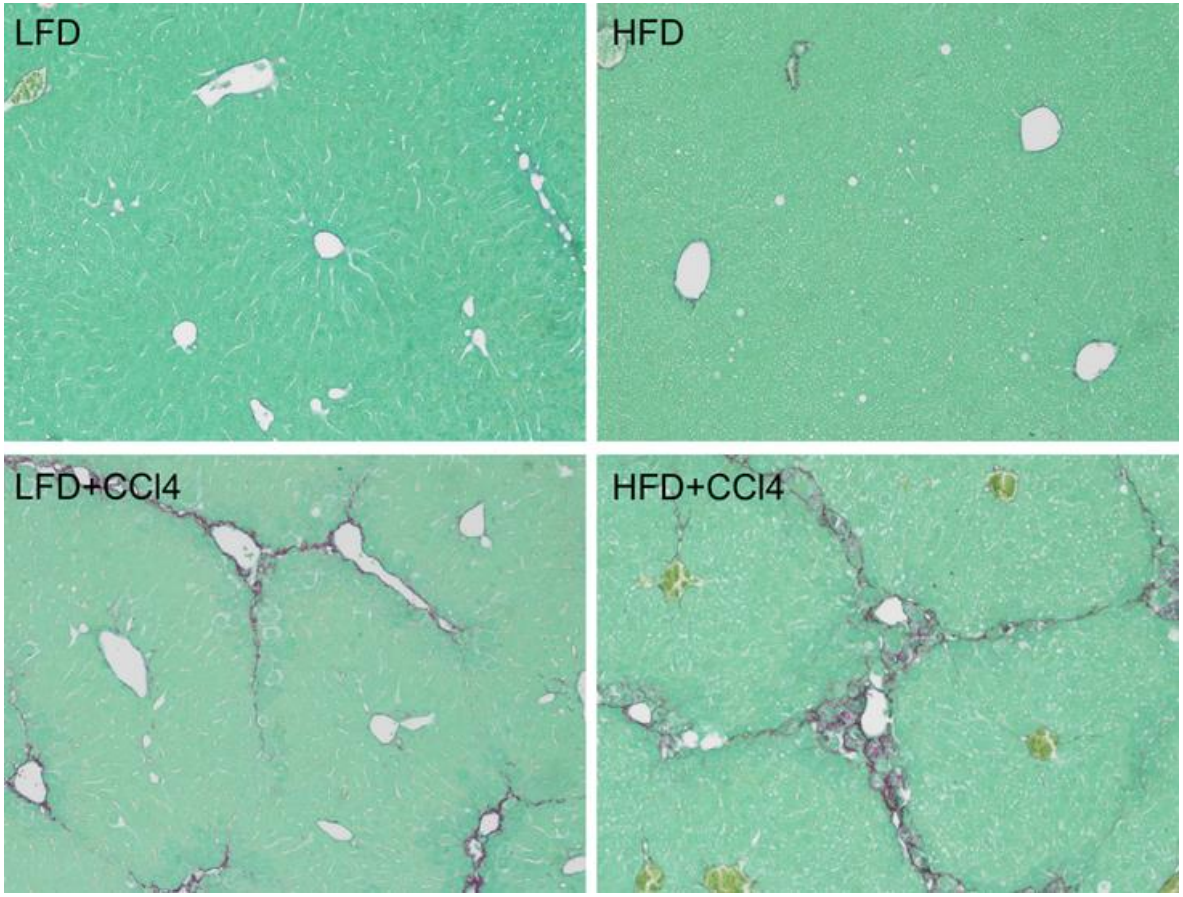
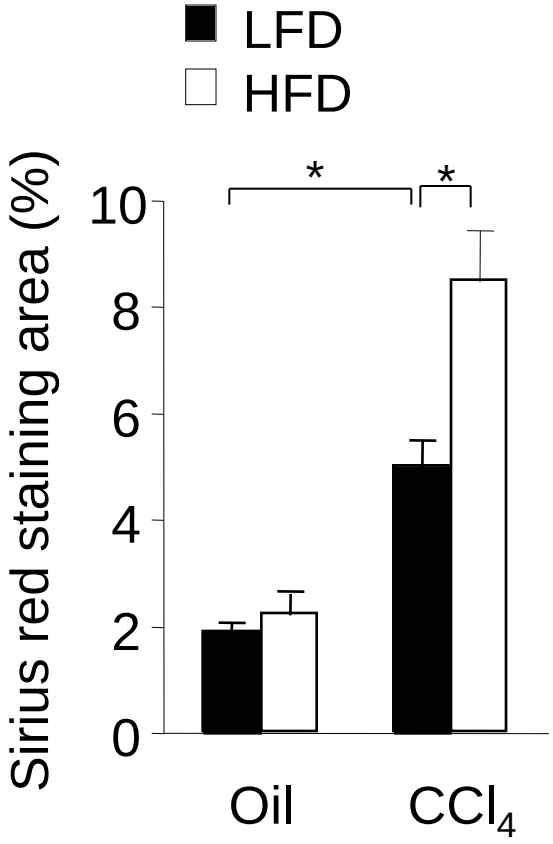
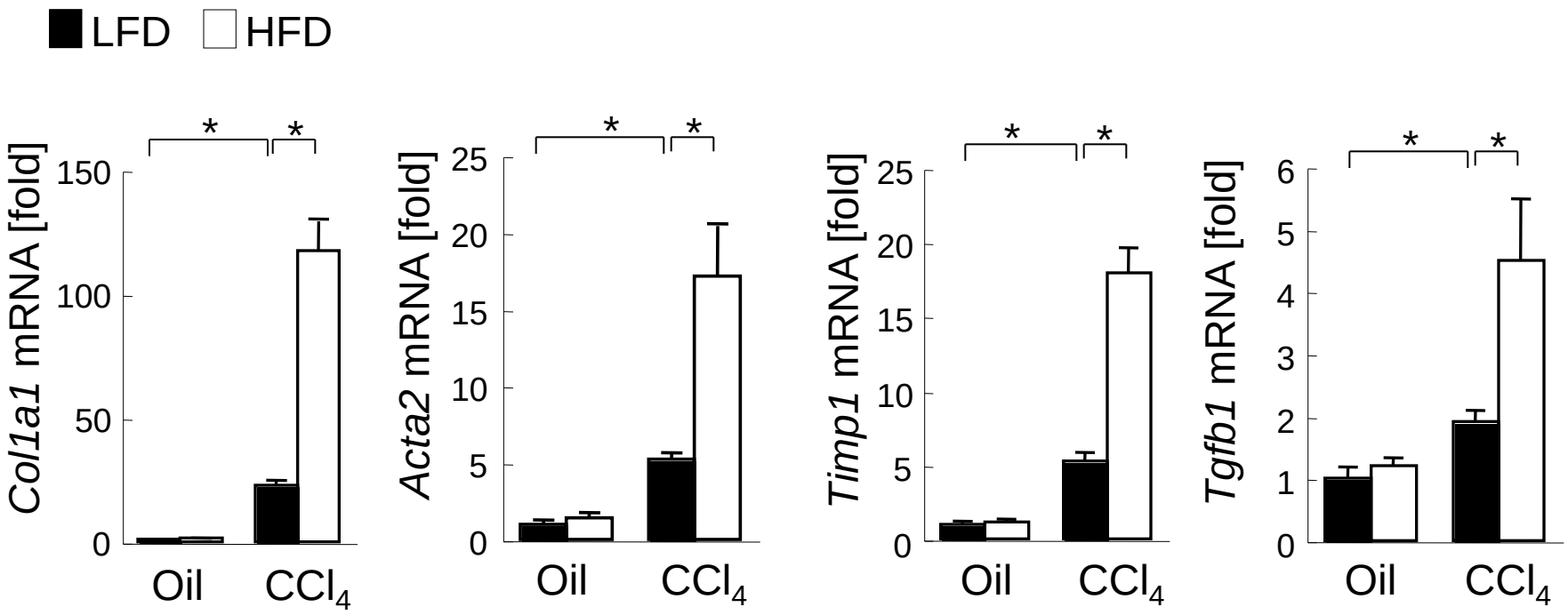
HFD feeding enhances Toxin-induced Liver Fibrosis





HFD feeding enhances Toxin-induced Liver Fibrosis



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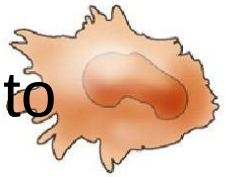
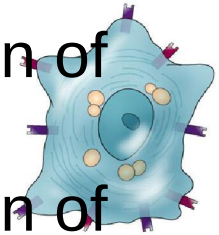
What is the Underlying Mechanism of Increased Sensitivity to CCl_4?

High fat diet

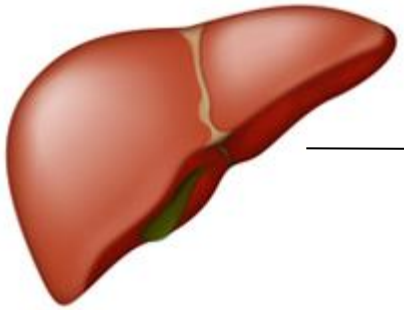
Chronic CCl_4 Exposure

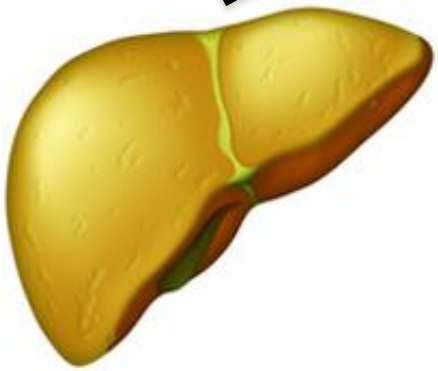
1. Increased sensitivity to hepatocyte death.
2. Increased capacity to produce cytokines.
3. Increased production of extracellular matrix.
4. Increased production of toxic metabolite from CCl_4.





Healthy Liver





Fatty Liver

TGF-β activated kinase 1 (TAK1)

TAK1 is a 78-80 kDa protein, which is encoded by the *MAP3K7* gene.

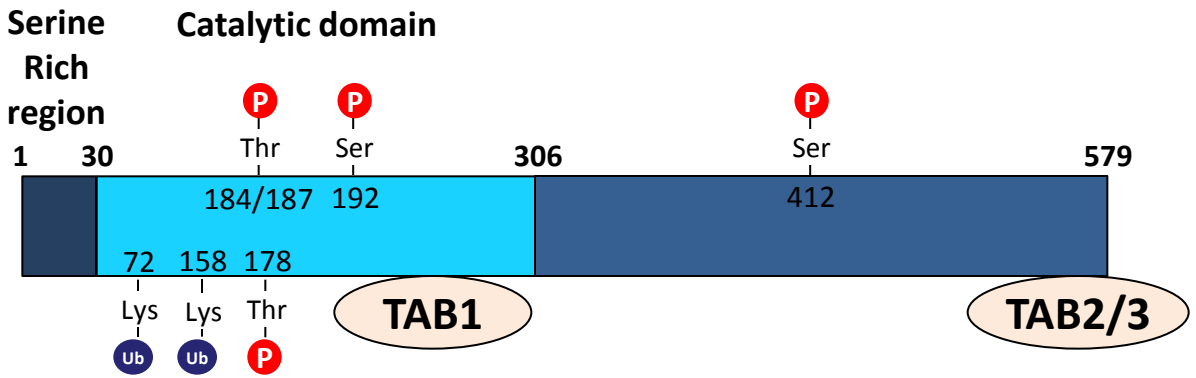
TAK1 is a serine/ threonine kinase, and belong to MAP3K family.

Phosphorylation and ubiquitination of TAK1 are important for activation of TAK1 and its downstream molecules.

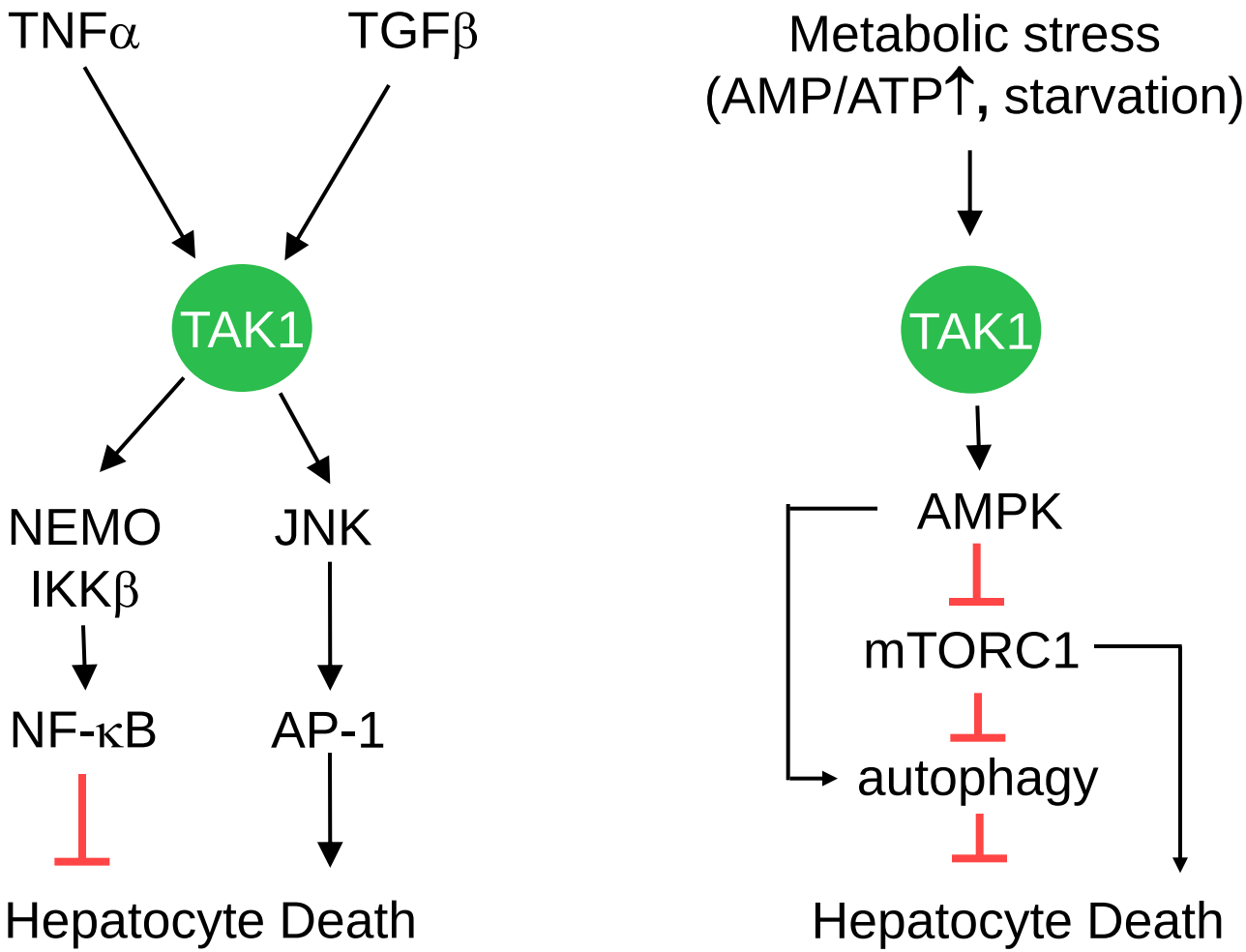
TAK1 interacts with TAB1, TAB2 and TAB3 that regulate TAK1 full activation.

TAK1 is activated in the signaling of TNF, IL-1β, TLRs and TGF-β.

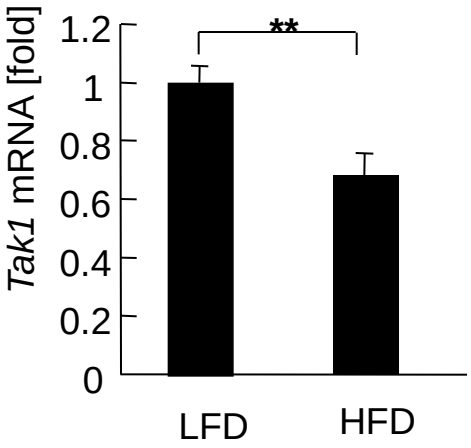
TAK1 regulates activation of NF-κB, JNK, p38, AMPK and NLK.

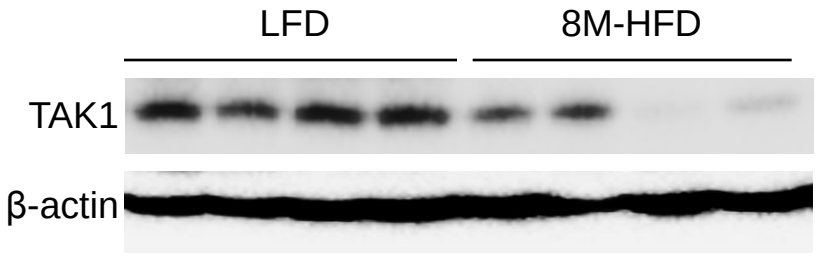


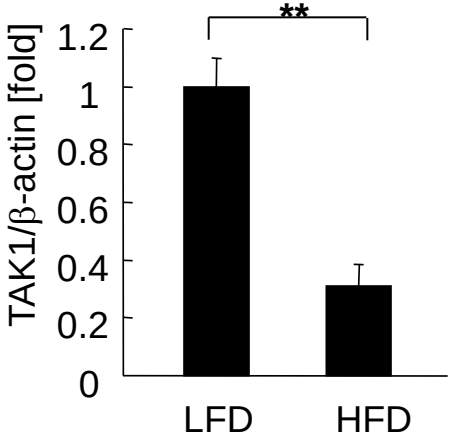
TAK1 Regulates NF-κB and JNK Pathways, and also Regulates AMPK-Autophagy Pathway

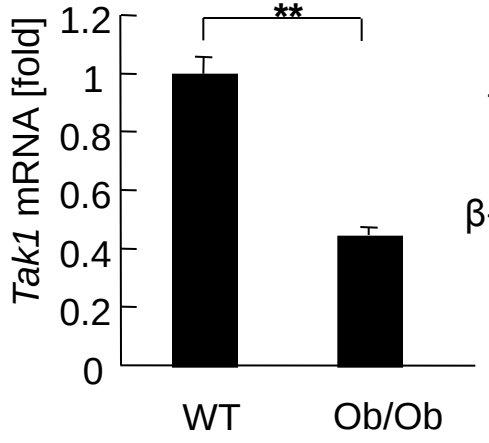


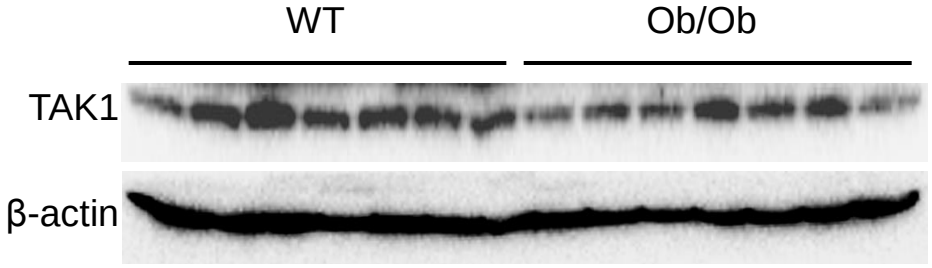
Hepatic TAK1 expression is decreased in advanced fatty liver disease

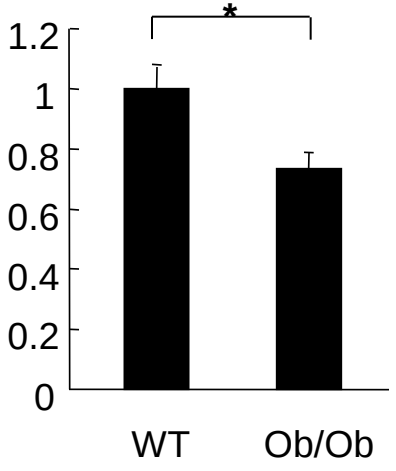




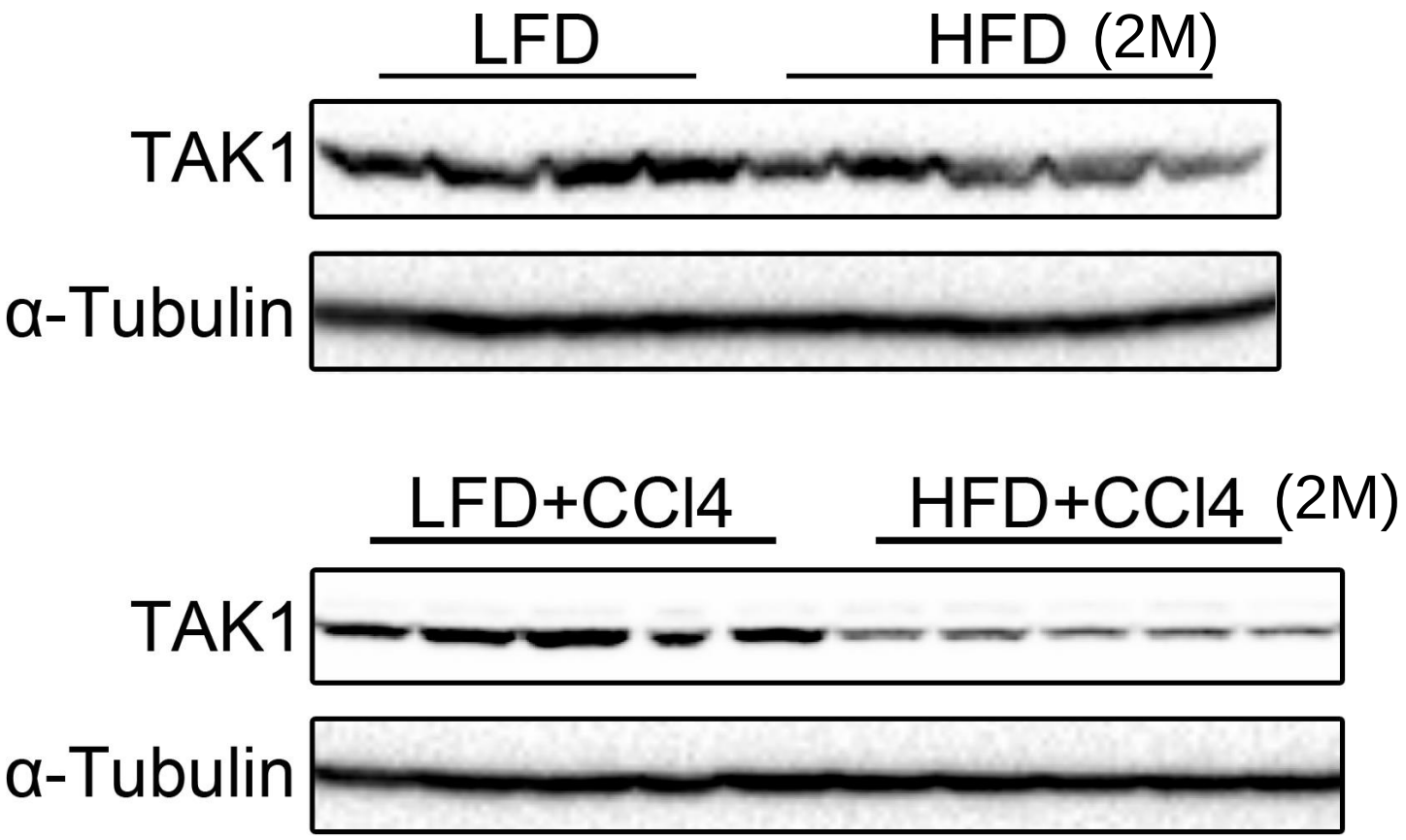








Hepatic TAK1 expression is decreased in fatty liver with toxin exposure

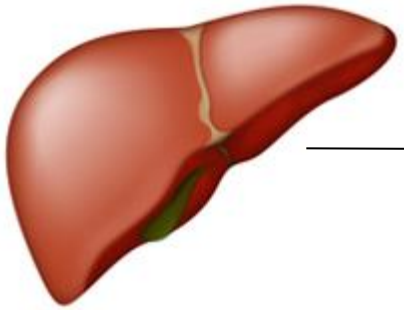


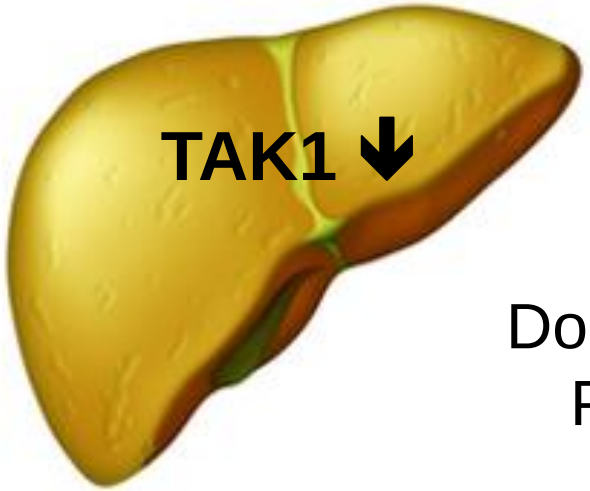
Does Decreased TAK1 Expression Increase Sensitivity to CCl_4?

High fat diet

Chronic CCl_4 Exposure

Healthy Liver

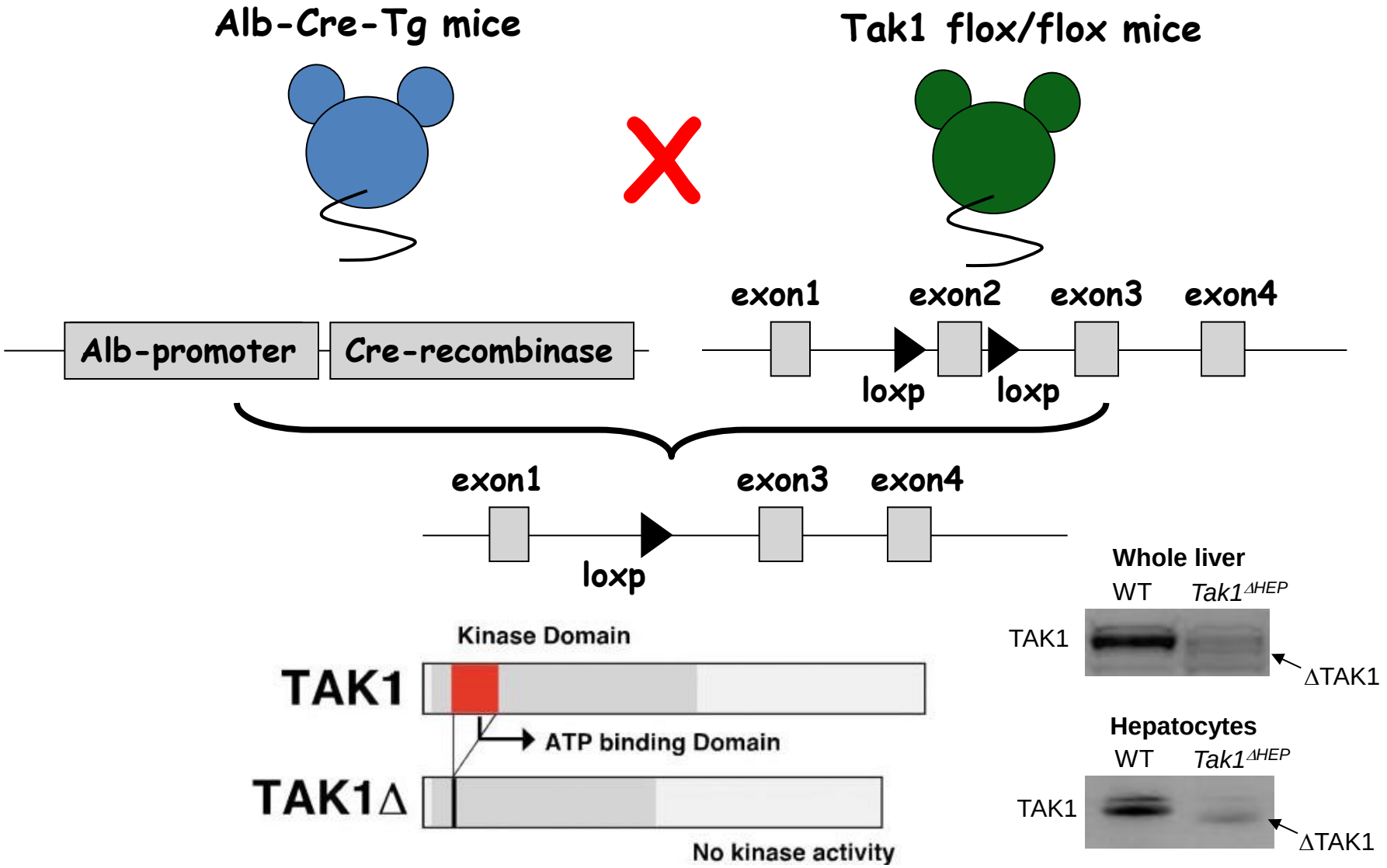




Fatty Liver

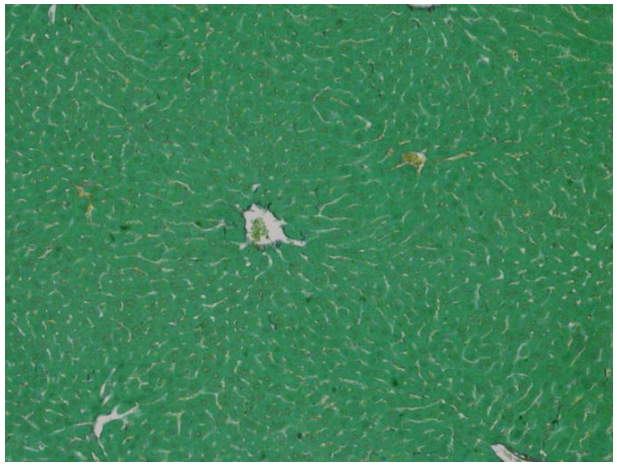
Does Decreased TAK1 Play a Role?

Generation of hepatocyte-specific TAK1-deleted mice

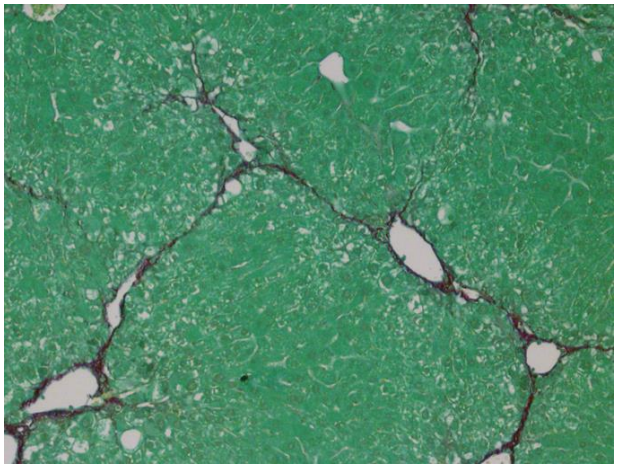


CCl_4 exposure augmentes liver fibrosis in *Tak1$^{\Delta HEP}$* mice

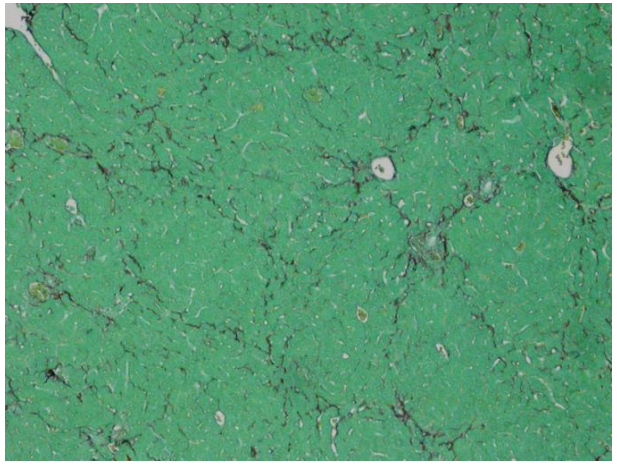
WT-oil



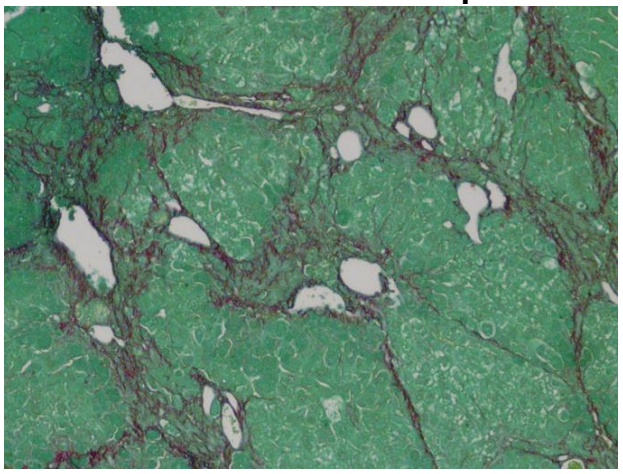
WT-CCl_4

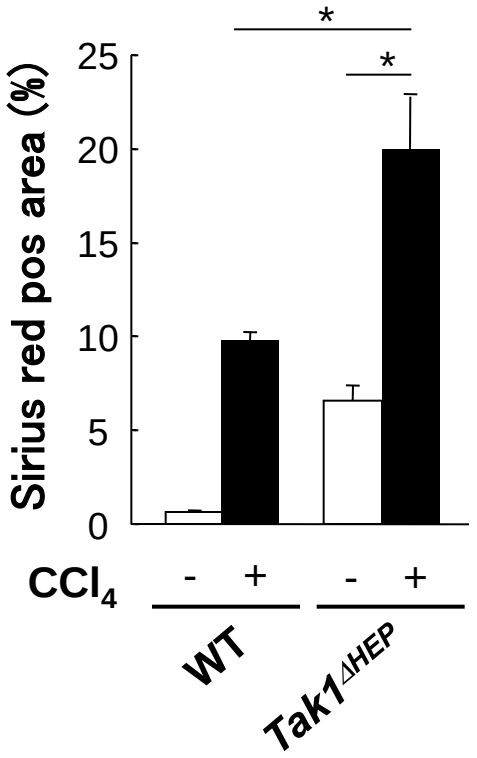


***Tak1$^{\Delta HEP}$*-oil**



***Tak1$^{\Delta HEP}$*-CCl_4**





12 injections
(5-6 month old)

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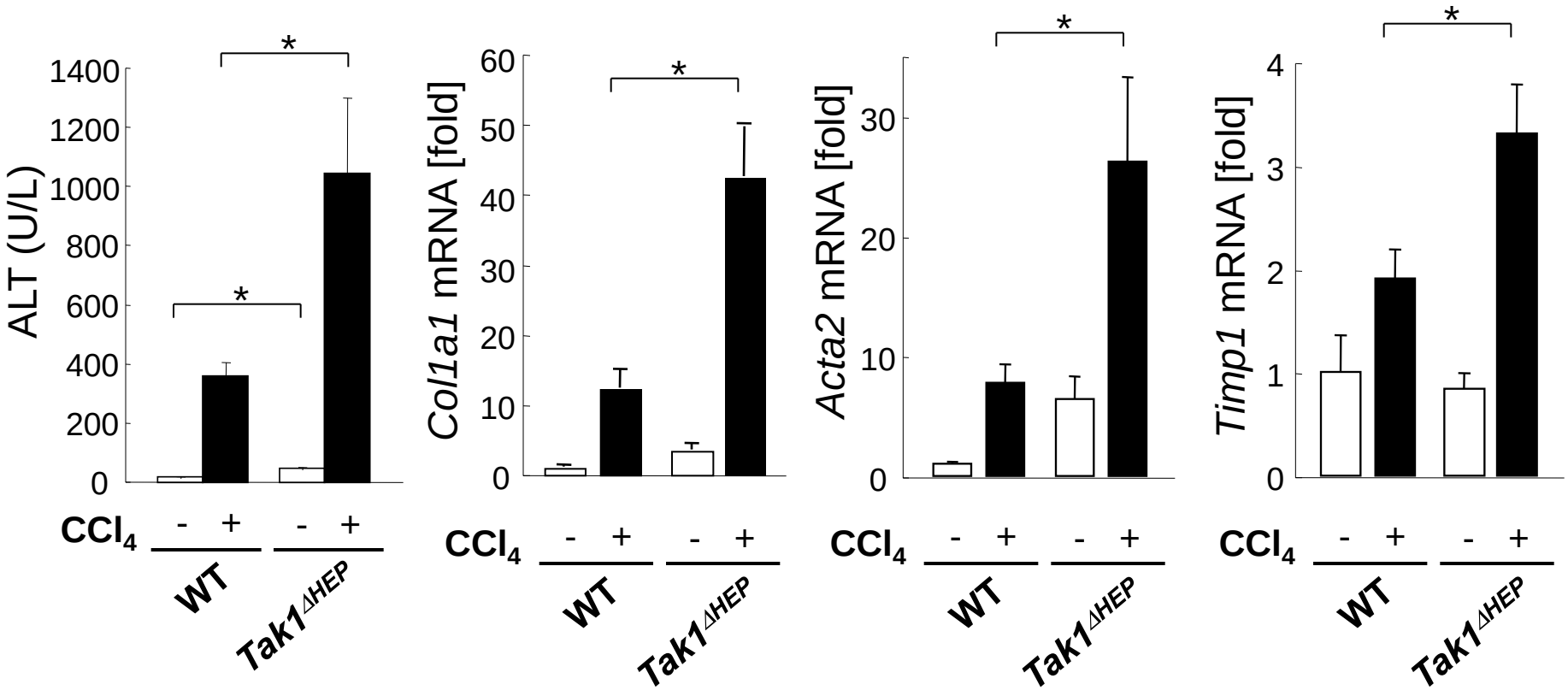
TAK1 deficiency enhanced liver injury and fibrogenic response after chronic exposure to CCl_4



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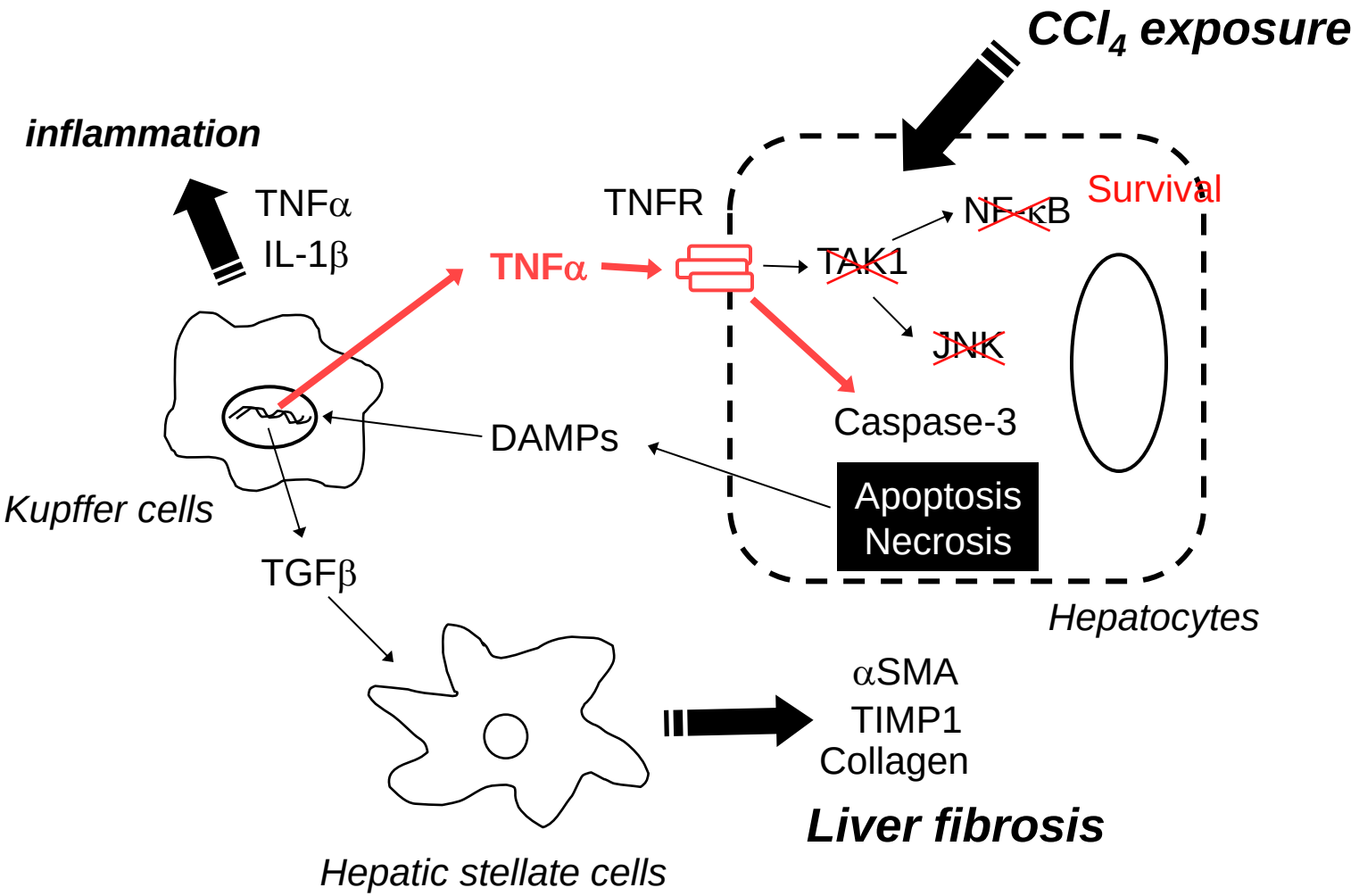
Why Does Decreased TAK1 Expression Increase Sensitivity to CCl_4 Exposure?

1. Sensitivity to TNFα-induced hepatocyte death
2. Sensitivity to TGFβ-induced hepatocyte death
3. Autophagy in hepatocytes.

TAK1$^{-/-}$ hepatocytes lack TNFα-induced NF-κB activation and are susceptible to cell death



(Inokuchi et al 2010, PNAS)

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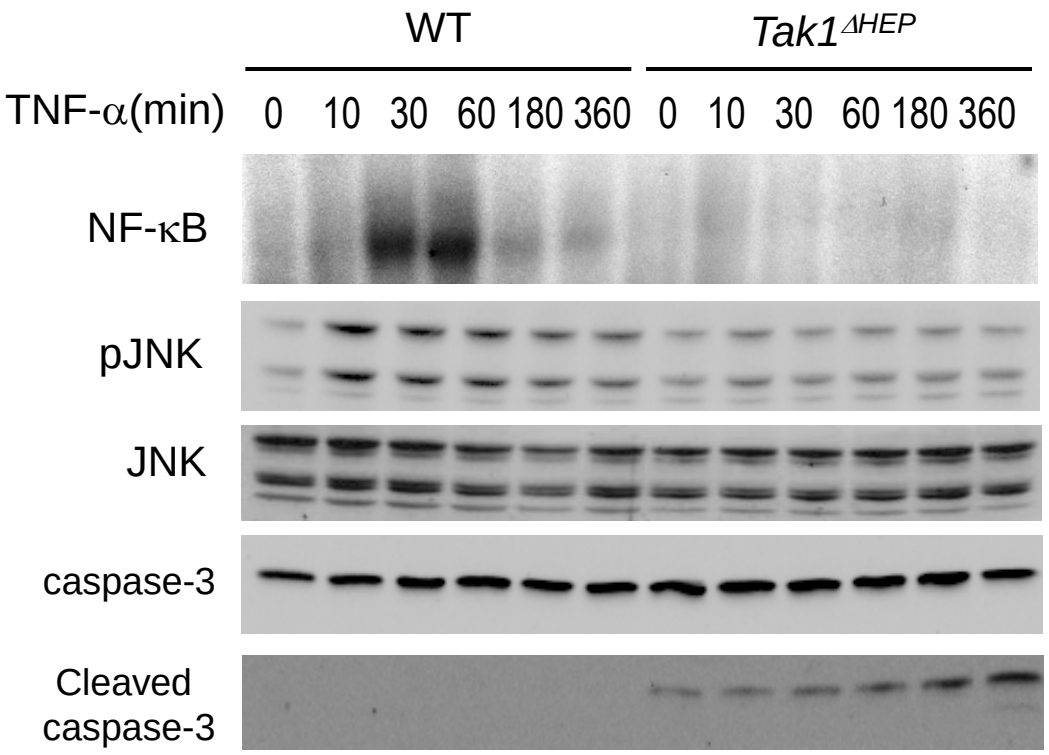
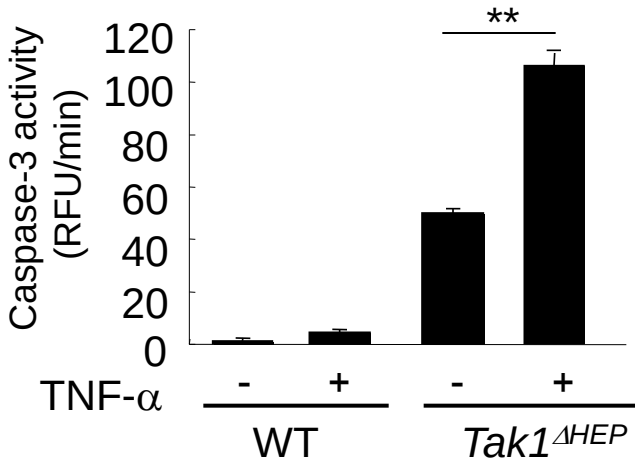
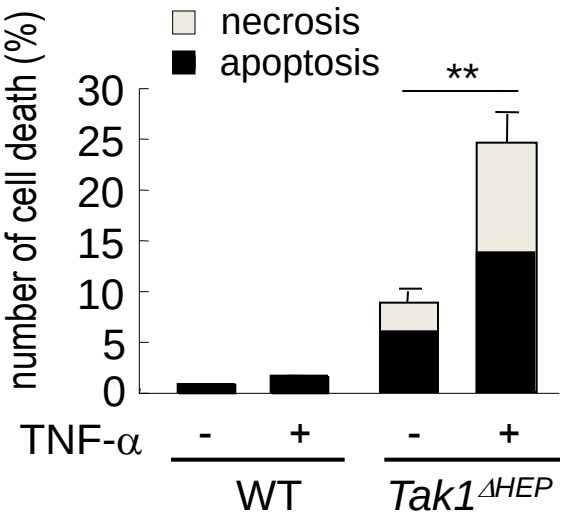
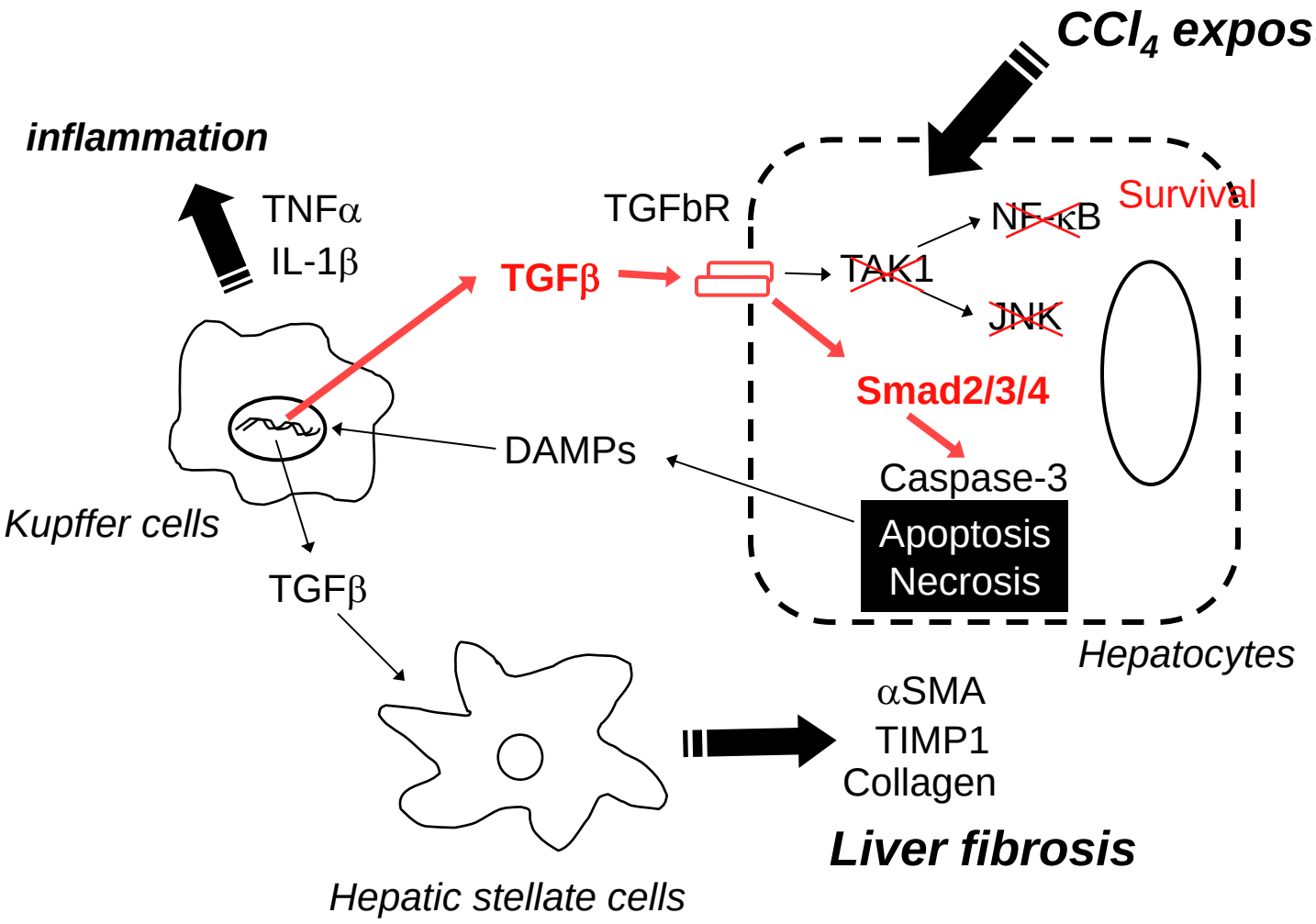






(Inokuchi et al 2010, PNAS)

TAK1$^{-/-}$ hepatocytes augment TGFβ-mediated Smad2/3 activation and are susceptible to cell death



(Yang et al 2013, Gastroenterology)

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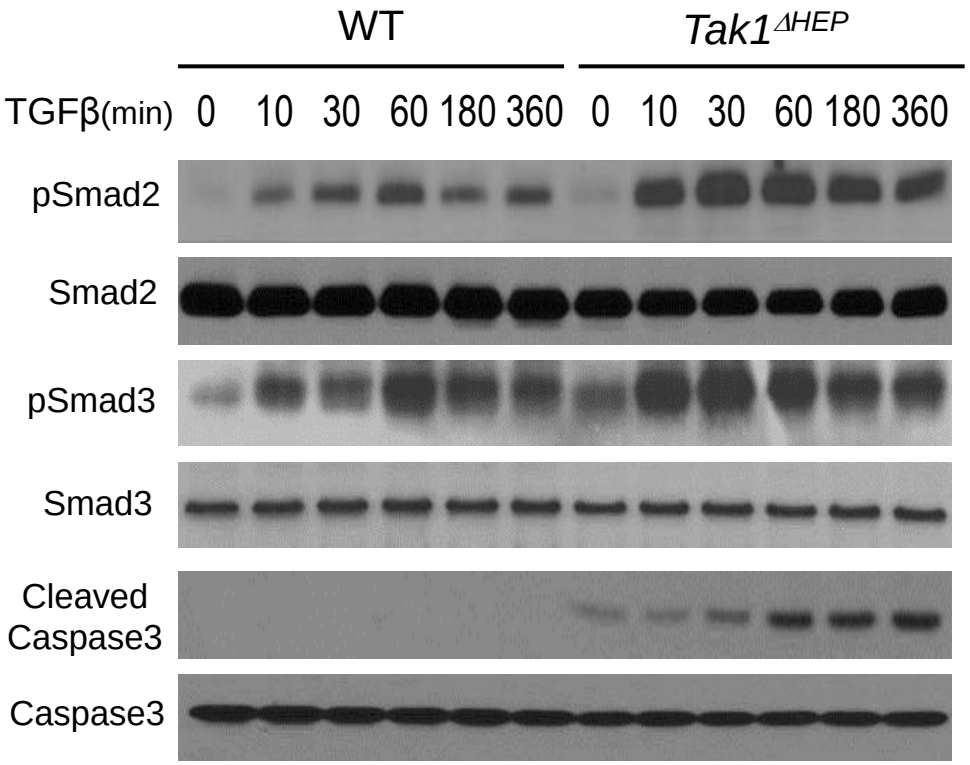
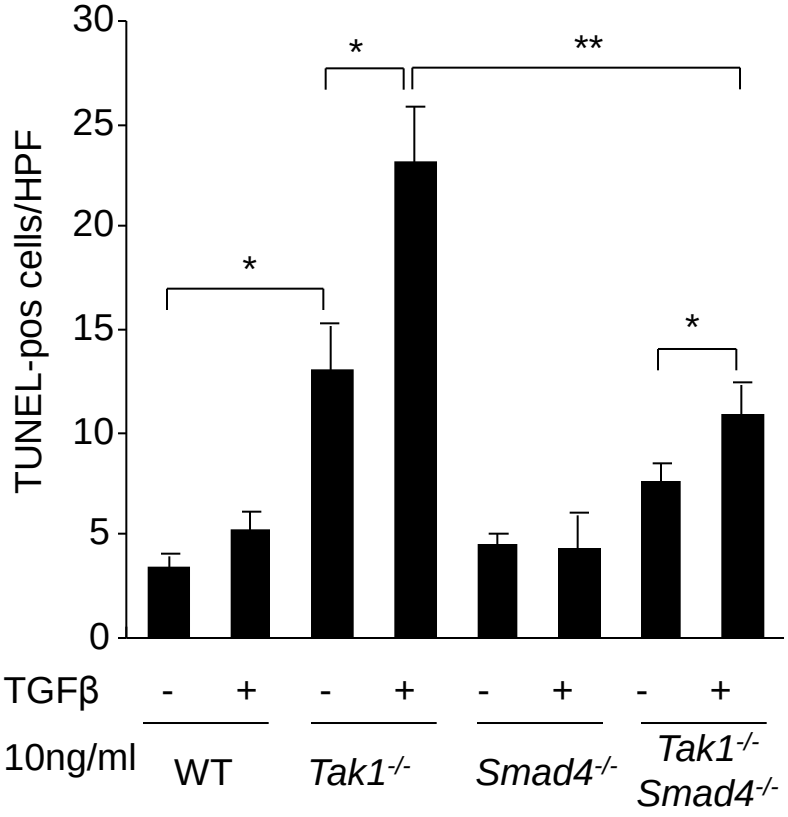
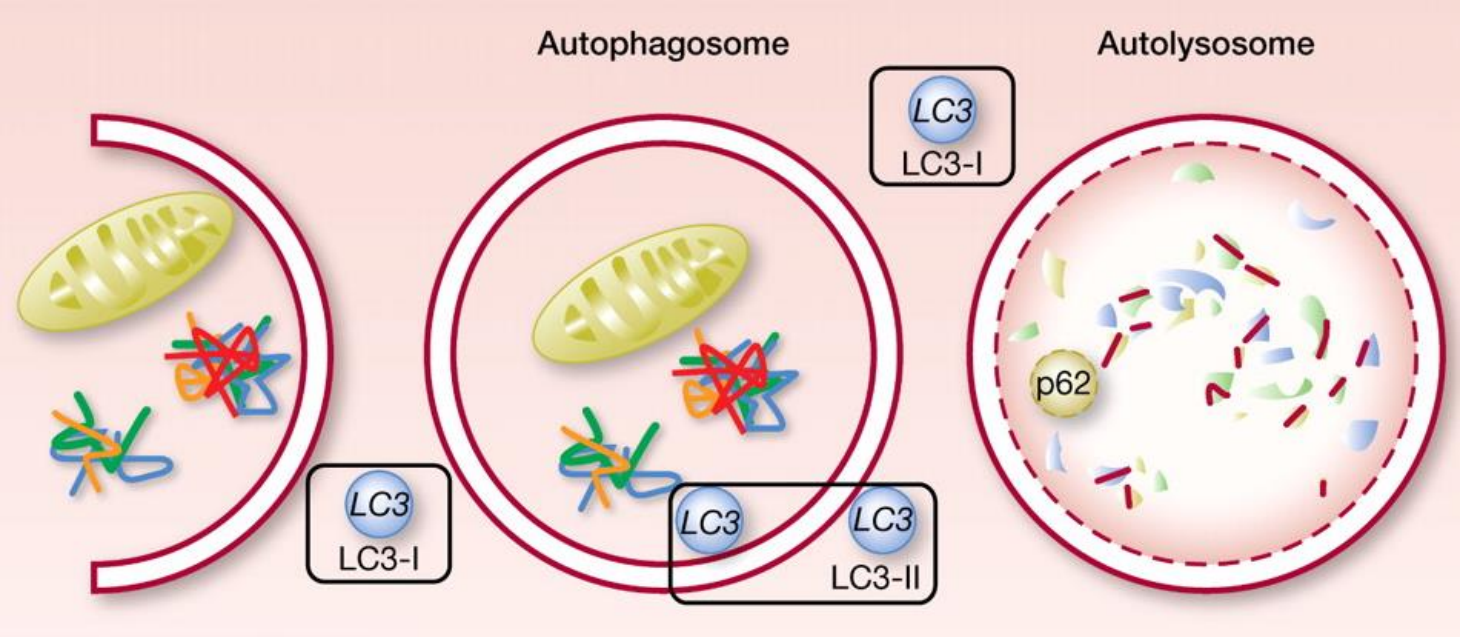




(Yang et al 2013, Gastroenterology)

Autophagy

Autophagy is a process to degrade intracellular components (long-lived or aggregated proteins, lipids, and damaged organelles, such as mitochondria) in lysosomes to supply for energy generation for maintaining cellular homeostasis.



→ Cell death, Cancer, Fatty Liver, Type II Diabetes, Innate Immune Systems, Aging, Infectious Disease, Toxin exposure, Fibrosis.

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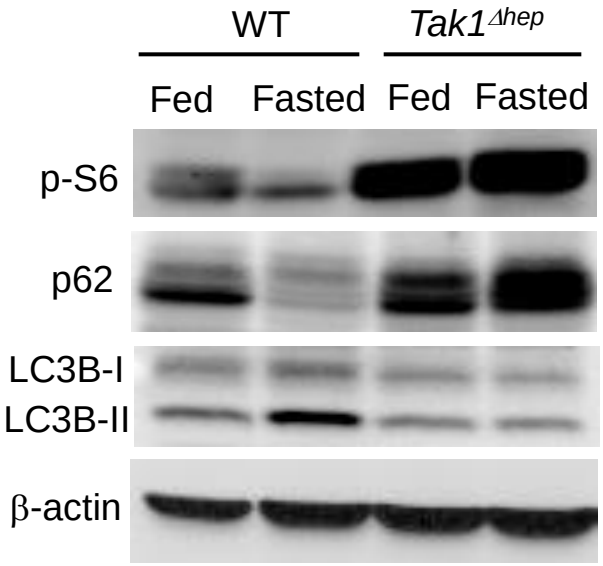
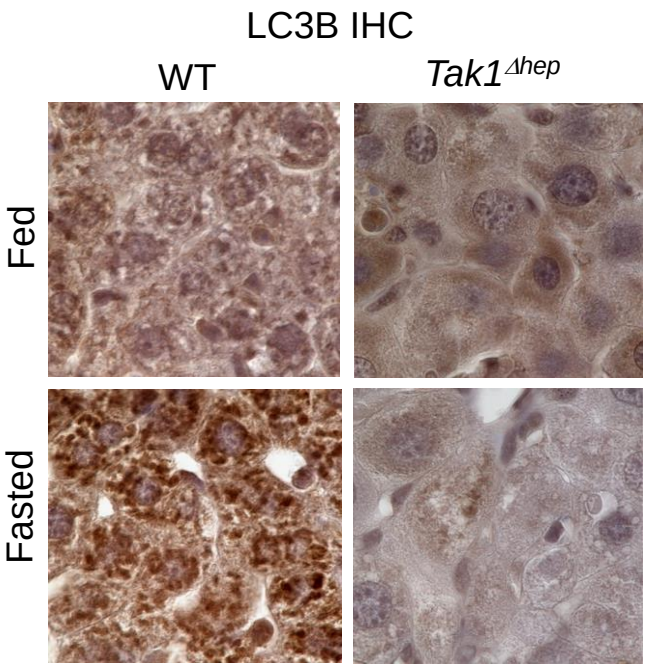
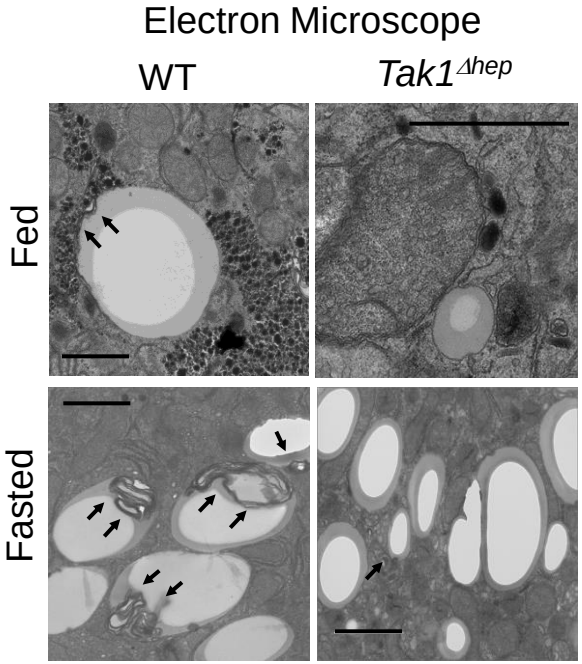
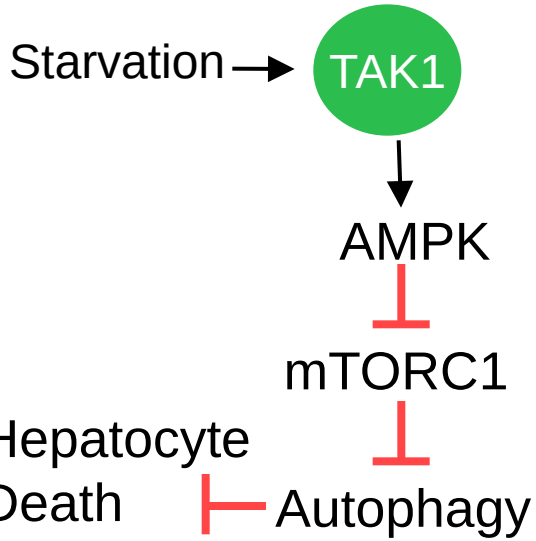
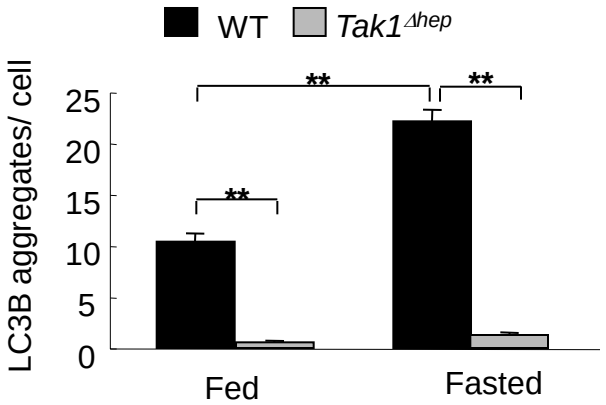
Autophagy is suppressed in *TAK1*$^{\Delta HEP}$ mice

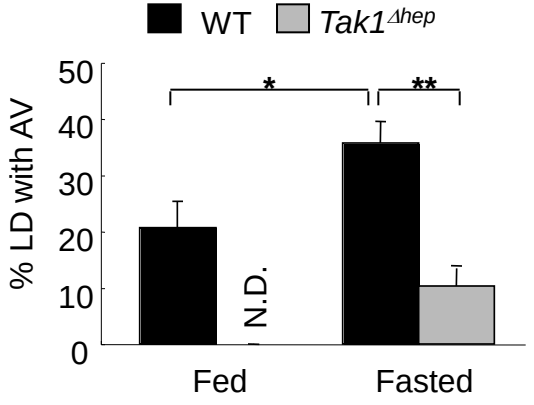






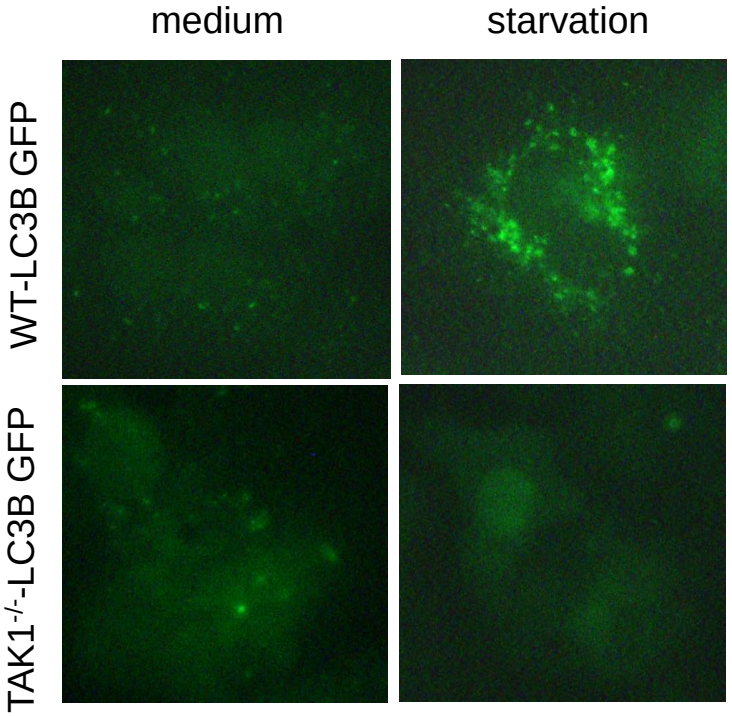


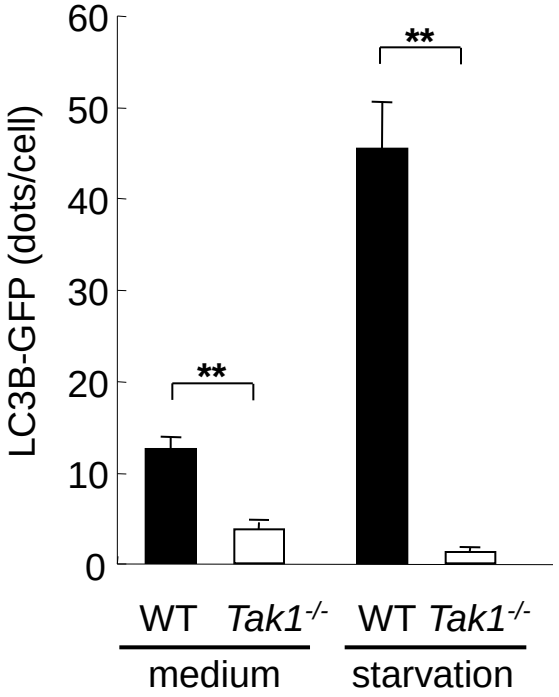


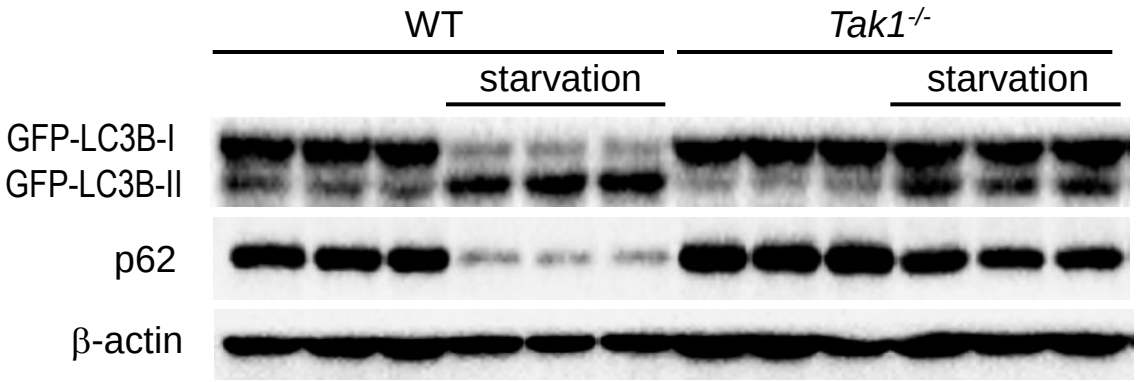


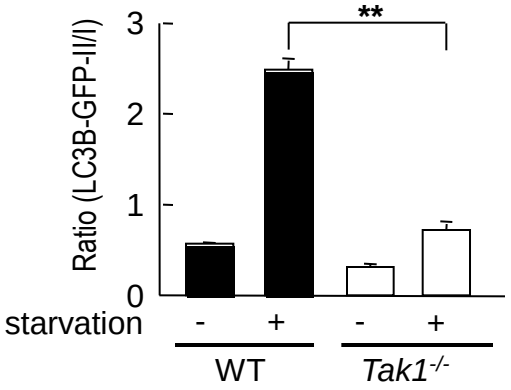
(Inokuchi-Shimizu et al 2014, J Clin Invest)

Autophagic LC3B aggregation is suppressed in TAK1$^{-/-}$ hepatocytes

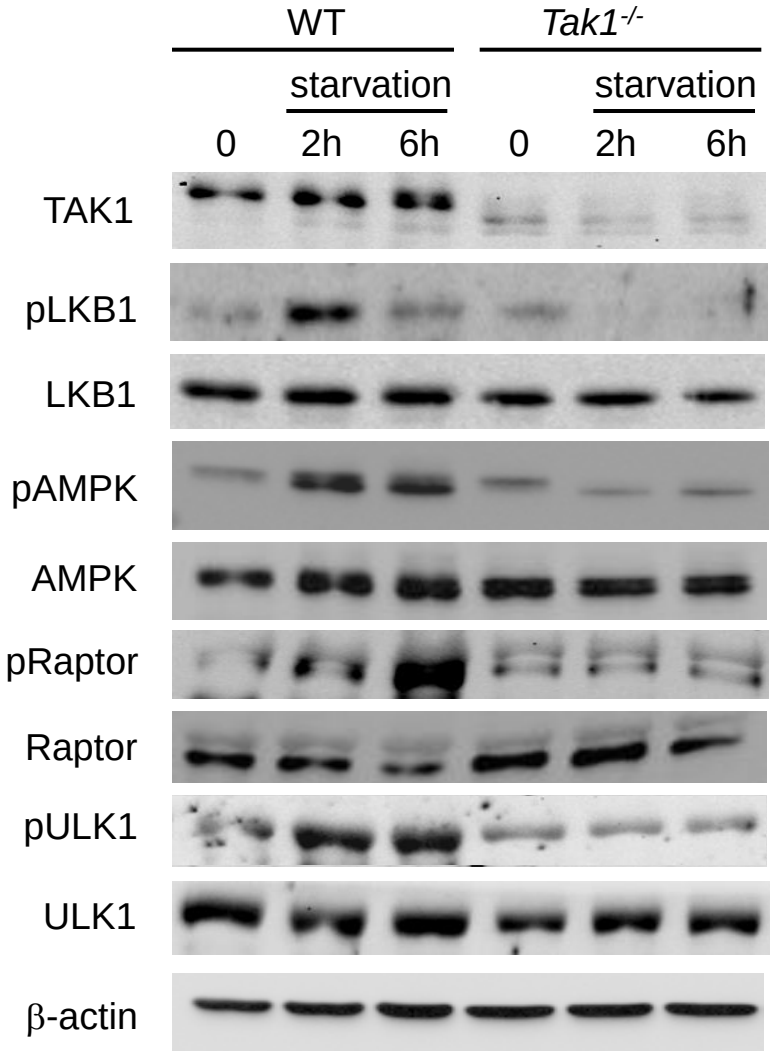


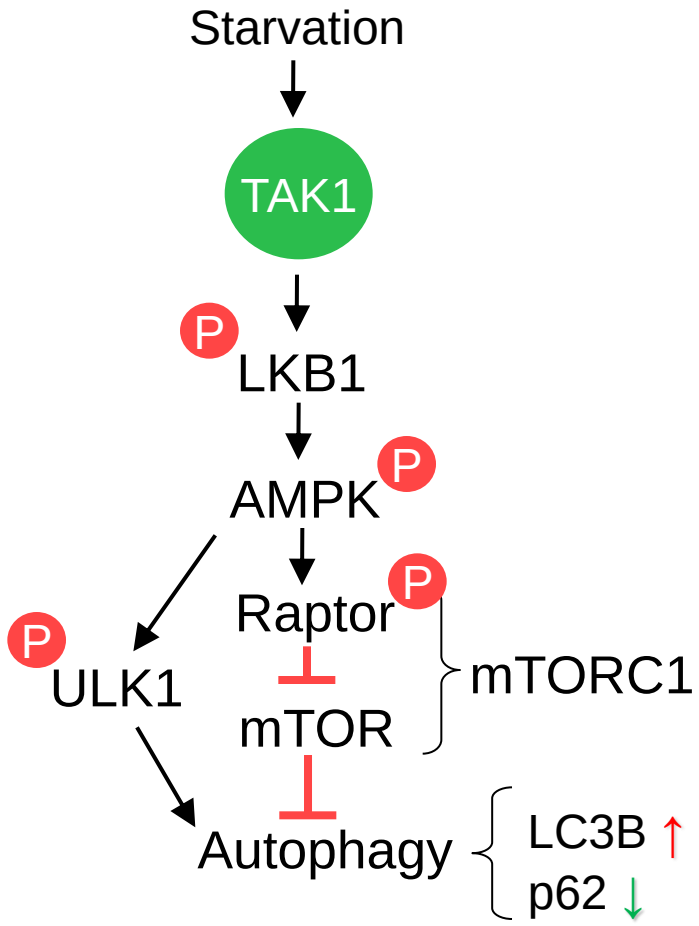




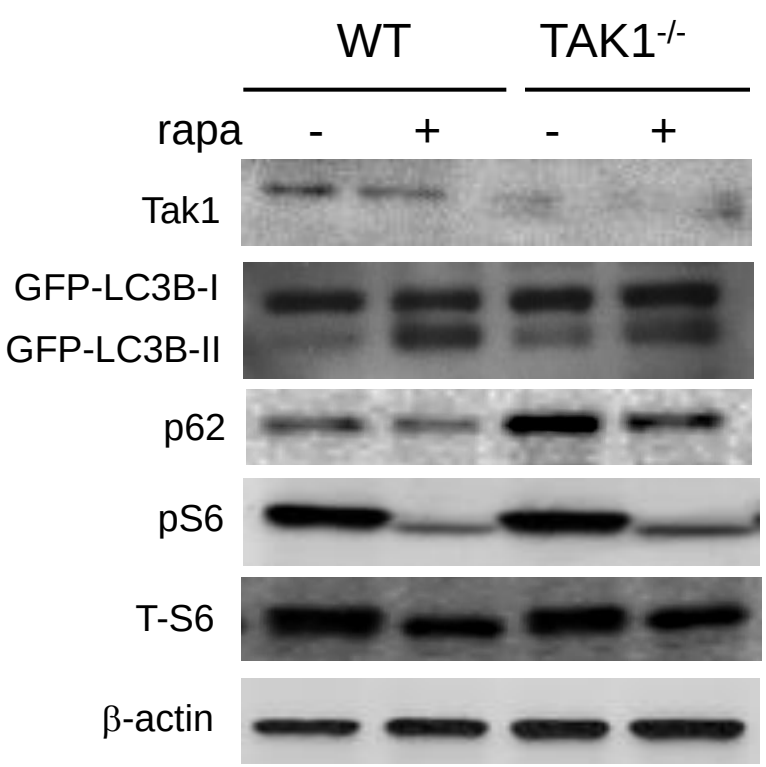


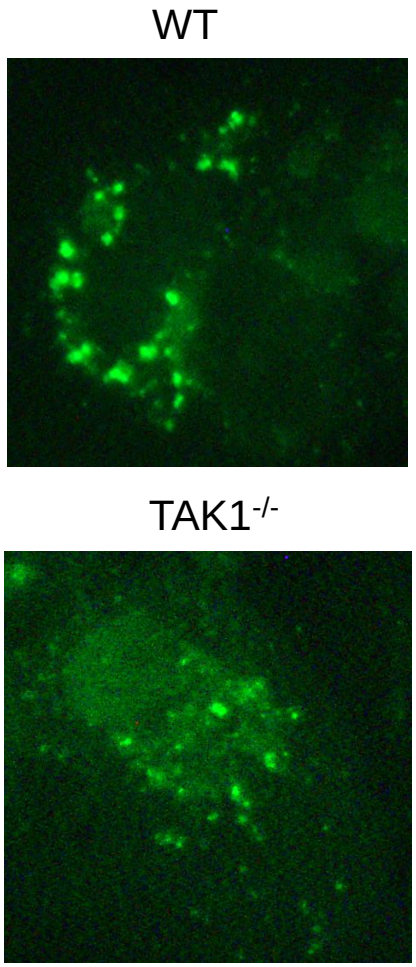
Starvation-induced AMPK activation is inhibited in TAK1$^{-/-}$ hepatocytes

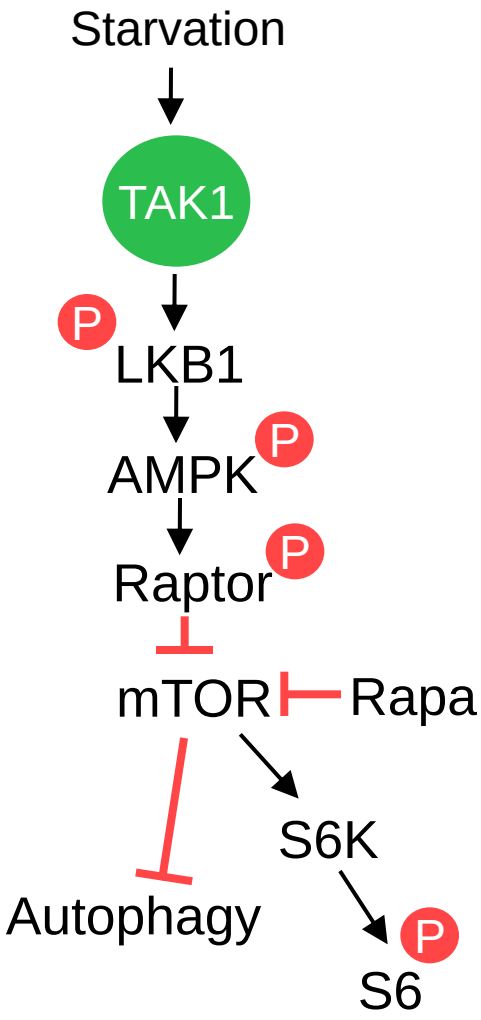




Rapamycin Restores Autophagy in TAK1$^{-/-}$ Hepatocytes

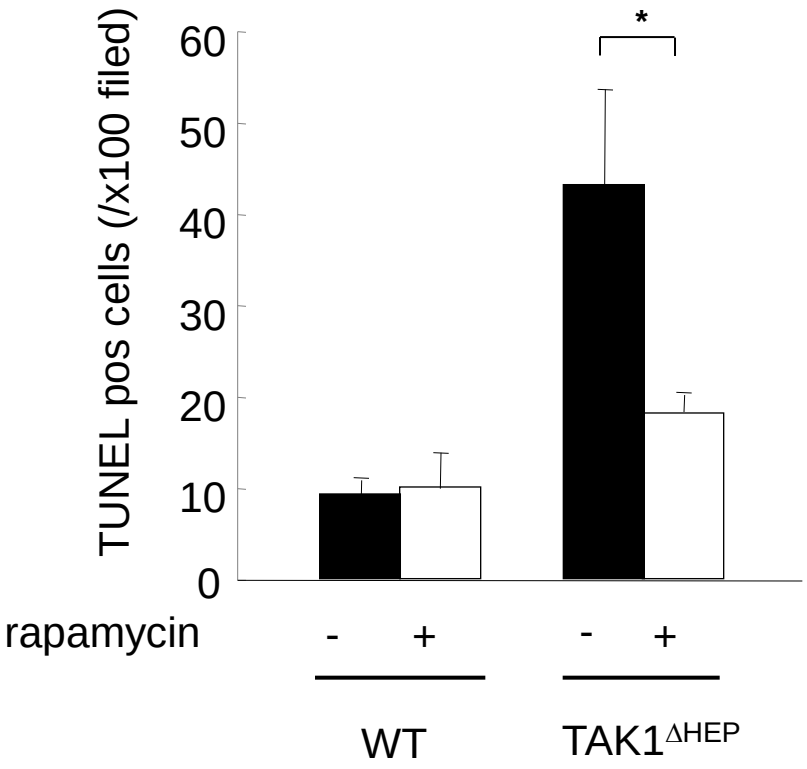


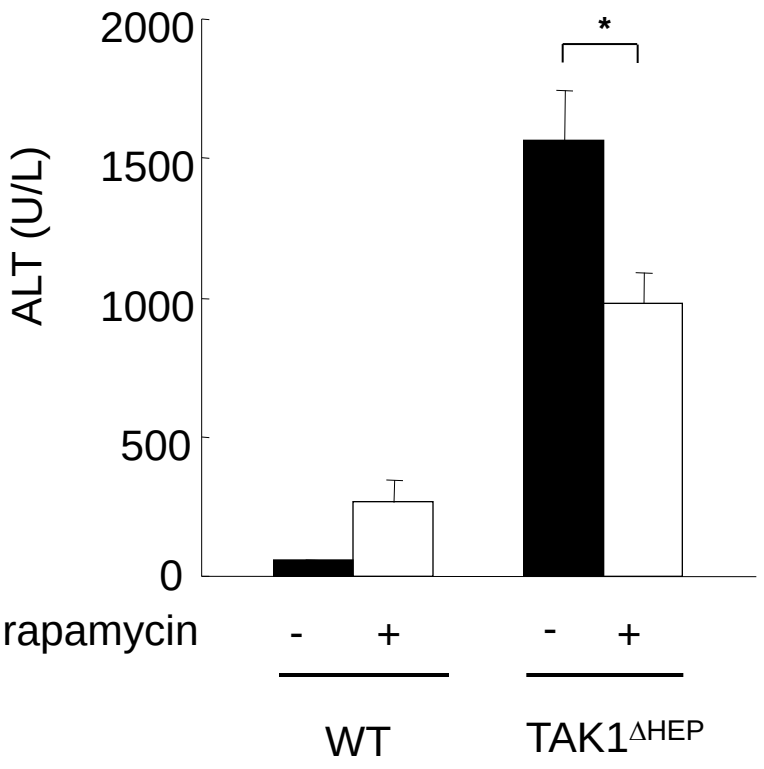




(Inokuchi-Shimizu et al 2014, J Clin Invest)

Rapamycin Suppresses Liver Injury in *Tak1*$^{\Delta HEP}$ mice

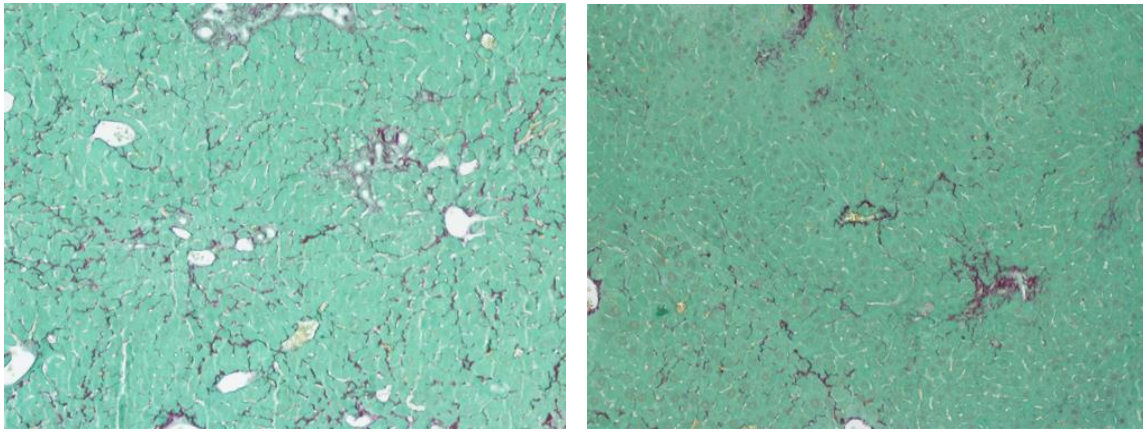




(Inokuchi-Shimizu et al 2014, J Clin Invest)

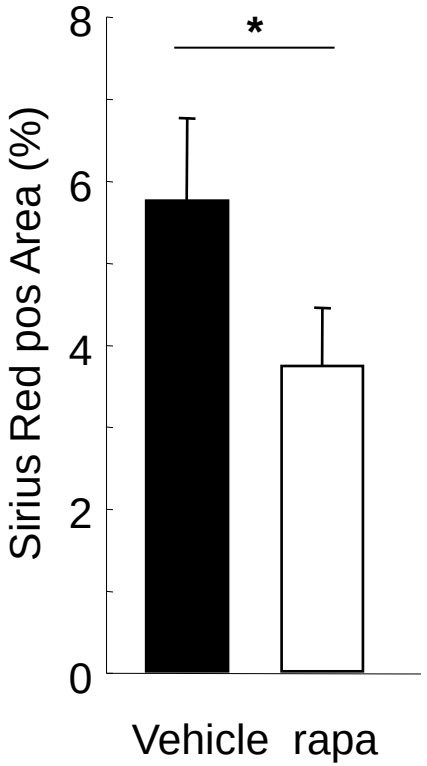
Rapamycin Suppresses Liver Fibrosis in *Tak1*$^{\Delta HEP}$ mice

Tak1$^{\Delta HEP}$ mice



Vehicle rapamycin

Injection of 5mg/kgBW rapamycin 2 times/week from 28 to 36 weeks



(Inokuchi-Shimizu et al 2014, J Clin Invest)

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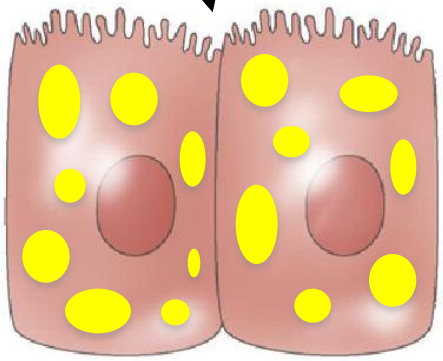
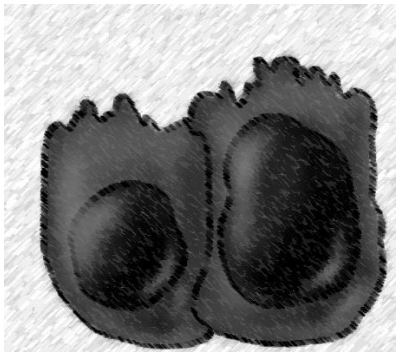
The enhancement of toxin-induced liver fibrosis in fatty liver disease

High fat diet

Carbon Tetrachloride Exposure



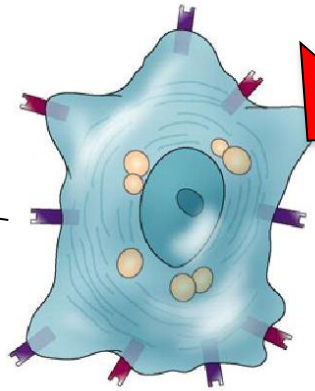
TAK1 ↓
NF-κB ↓
Smad2/3 ↑
Autophagy ↓



Hepatocyte Death

Steatotic Hepatocytes

Fibrosis



Stellate Cell Activation

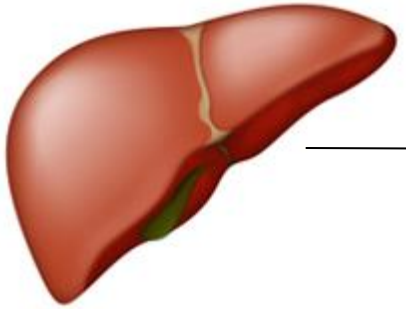
SRC UC SAN DIEGO SUPERFUND RESEARCH CENTER

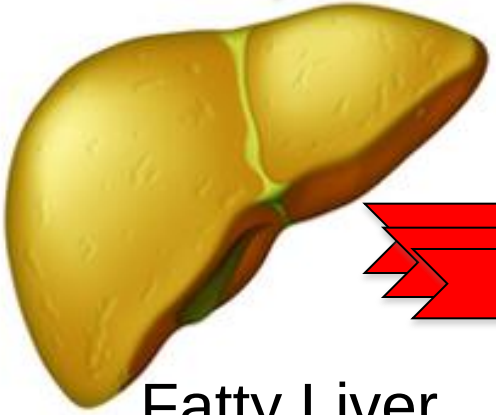
Fatty liver disease changes the sensitivity to toxin exposure that enhances liver fibrosis

High fat diet

Chronic Industrial Toxin Exposure

Healthy Liver





Fatty Liver

Liver Cirrhosis

Acknowledgement

UC San Diego
Superfund Research Center

Ekihiro Seki MD, PhD (Project5)
David A. Brenner MD (Co-leader)

Yoon Seok Roh DVM,PhD
Shuang Liang PhD
Hiroshi Matsushita MD, PhD
Sayaka Inokuchi-Shimuzu MD,PhD
Ling Yang MD,PhD
Bi Zhang
Jingyi Song

Robert Tukey PhD (Director)

Michael Karin PhD (Project 1)
Koji Taniguchi MD,PhD
XieFeng Wu PhD

Mark Ellisman PhD (Core)
Mason Mackey