



National Institute of
Diabetes and Digestive
and Kidney Diseases

Pathobiology of HSD17B13

Yaron Rotman

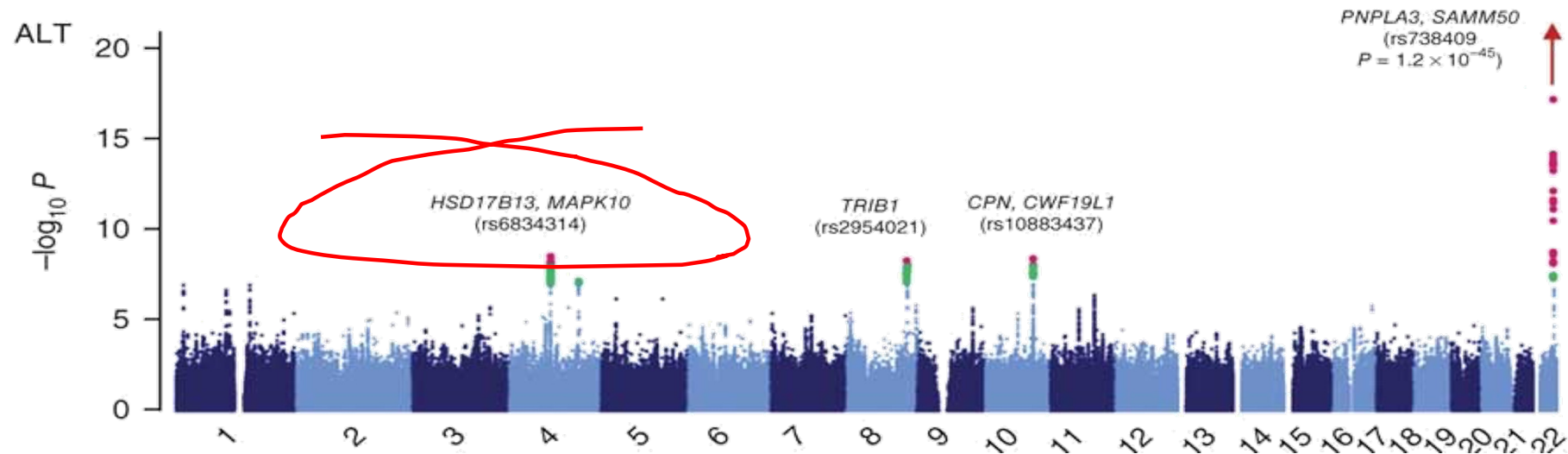
Liver & Energy Metabolism Section

Liver Diseases Branch, NIDDK, NIH, USA

Disclosure

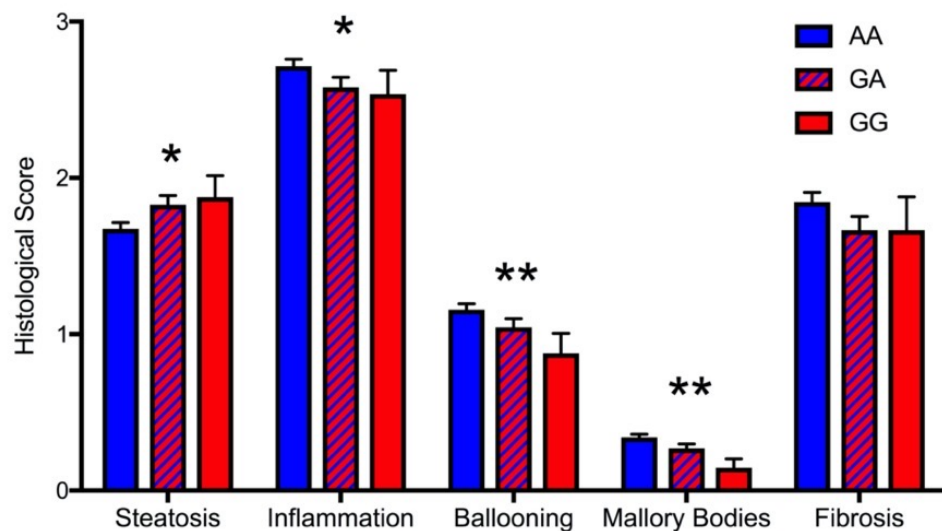
- Research funded by:
 - ✧ Intramural Research Program of NIDDK, NIH
 - ✧ Gilead Sciences
 - Employee of US Federal Government
 - Opinions are own
 - Spouse employed by AstraZeneca
-

Genetic Determinants of Population Plasma Liver Enzymes

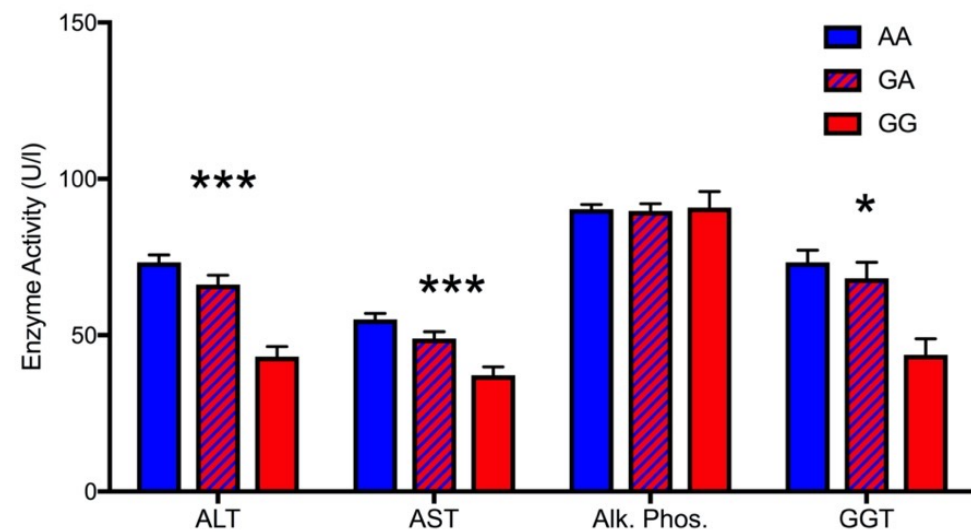


rs6834314 Associated with NAFLD

Histology

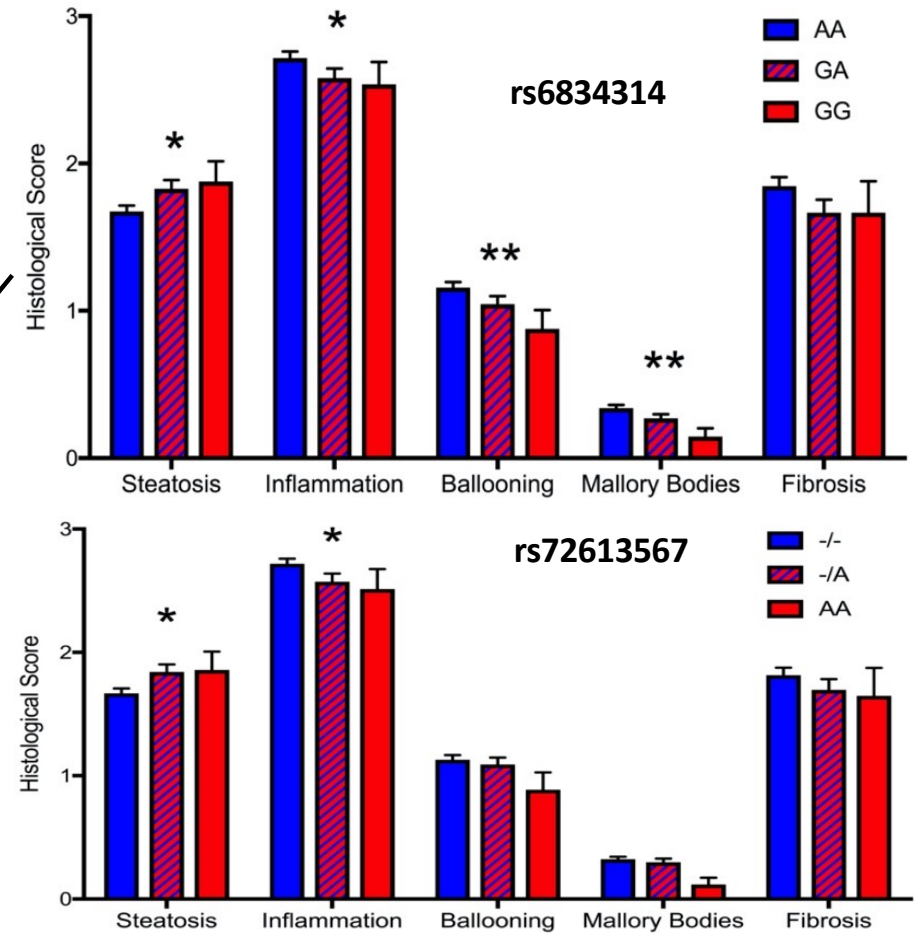
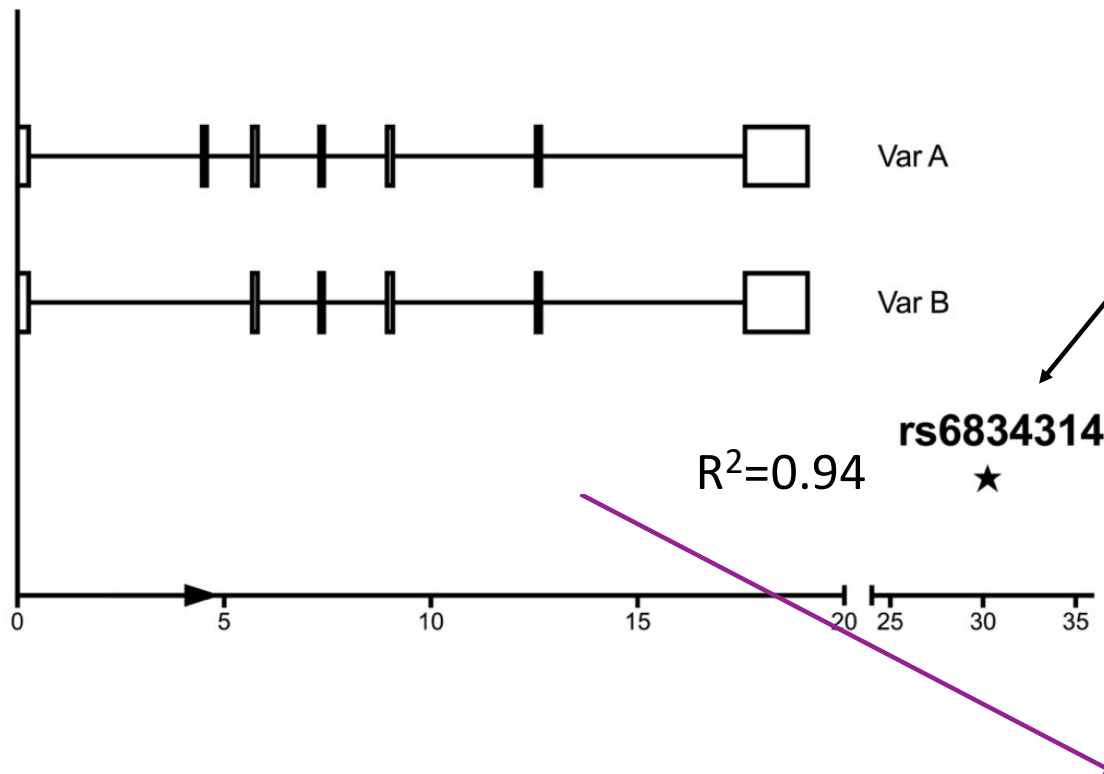


Liver Enzymes

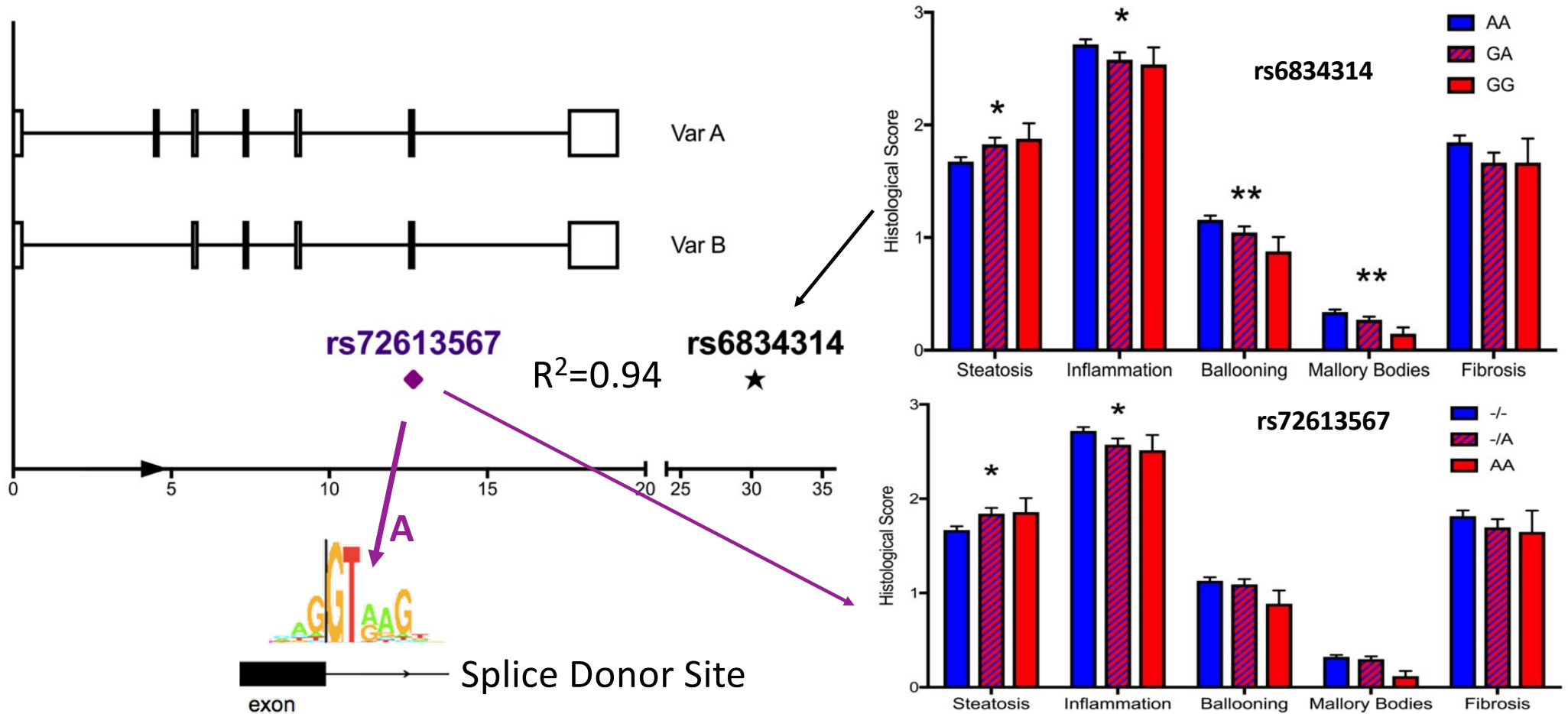


*768 adult Caucasians with histologically-proven NAFLD.
Adjusted for age, gender, BMI.*

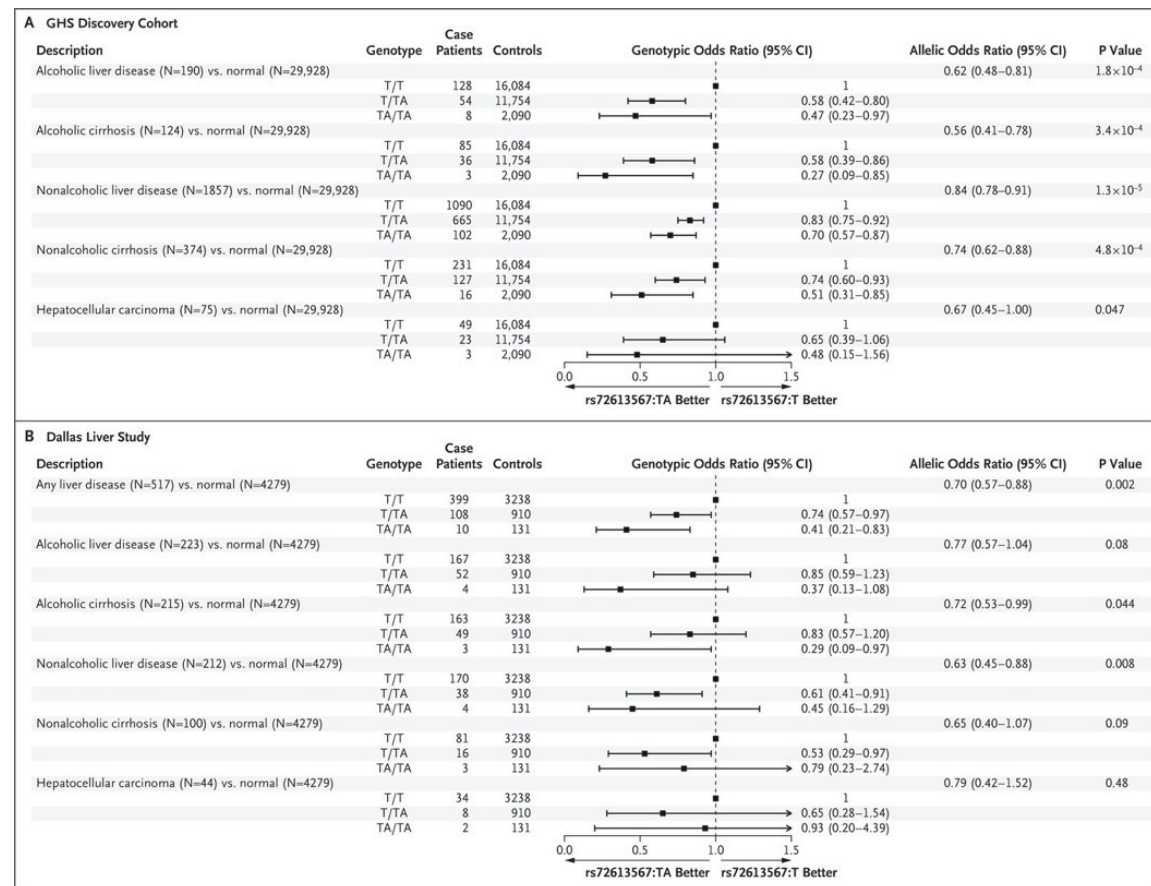
Tag SNP Associated With Splice Site SNP



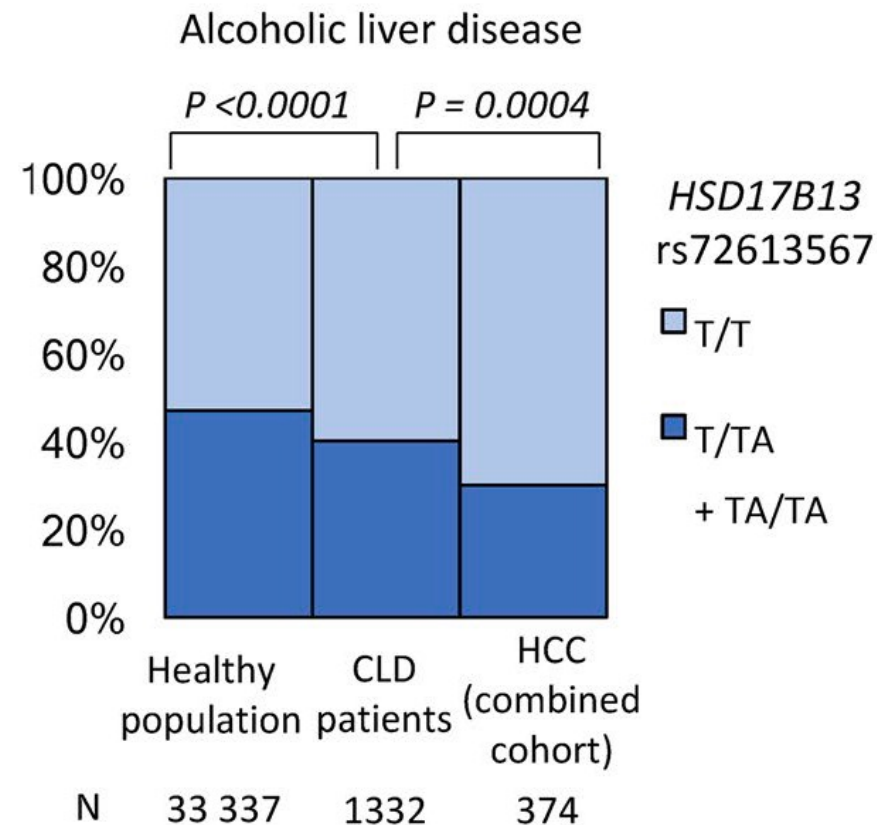
Tag SNP Associated With Splice Site SNP



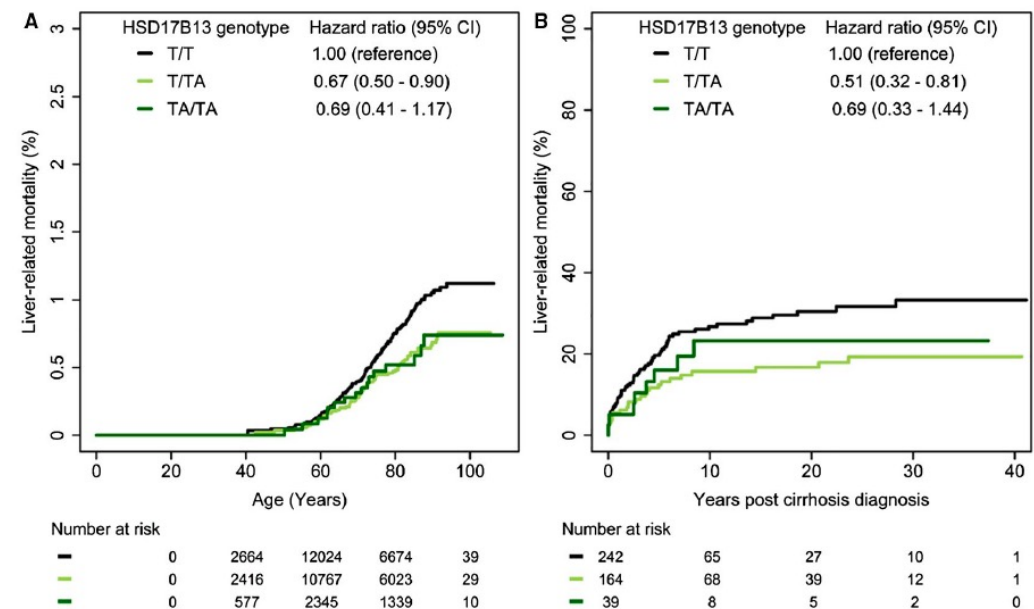
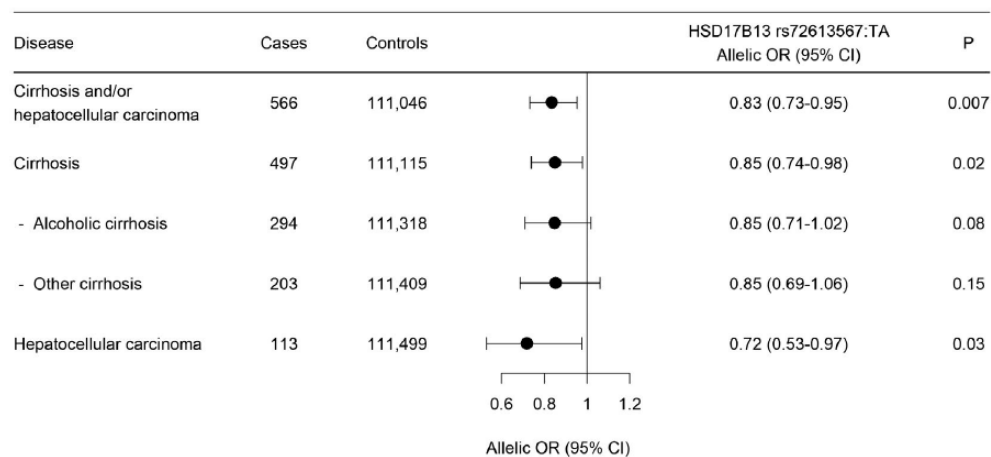
Splice Variant in GHS and Dallas Heart Study



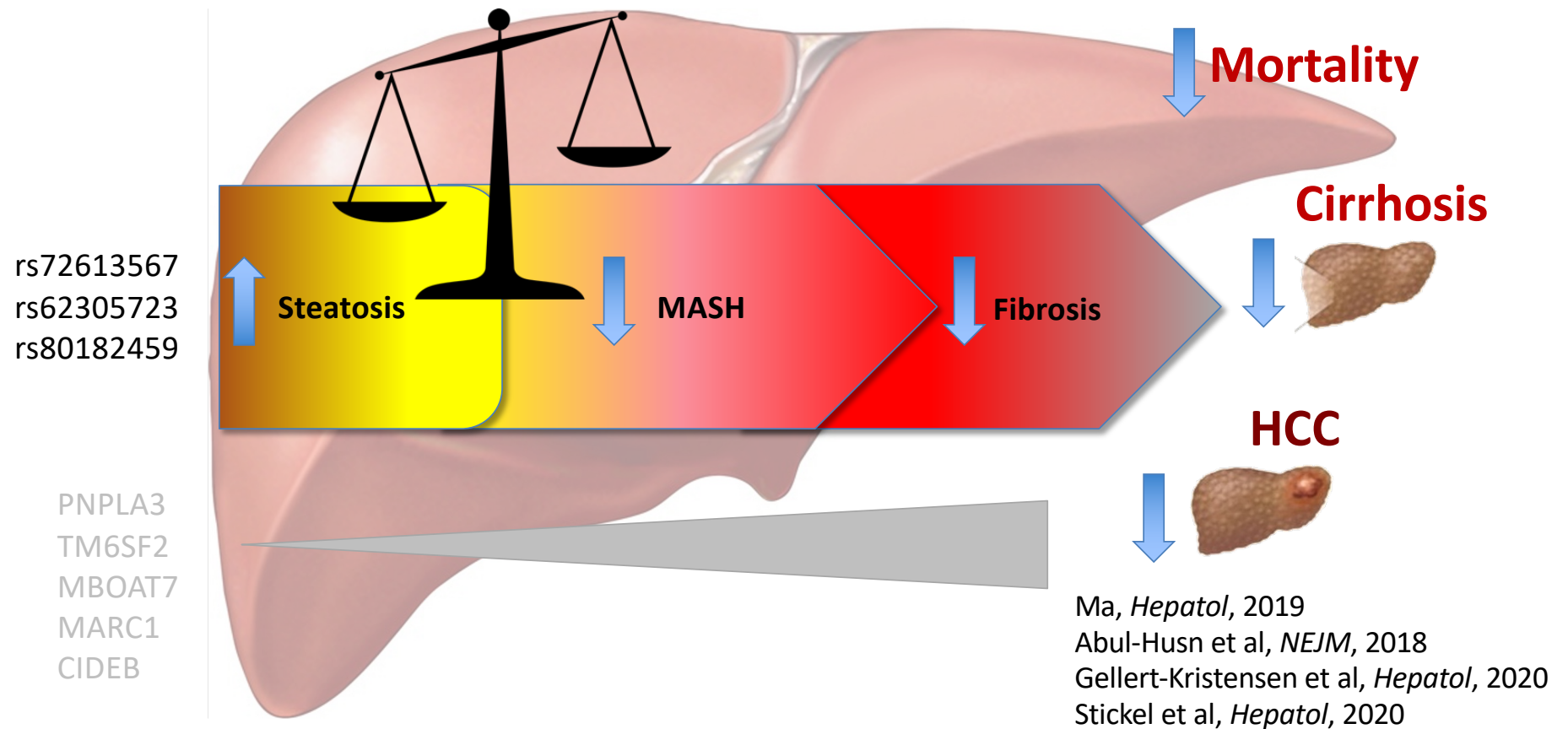
HSD17B13 Variant Protects from Alcohol-Associated HCC



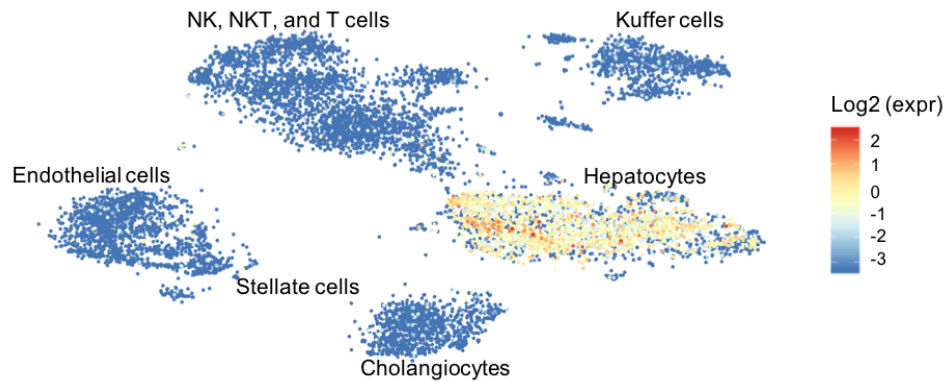
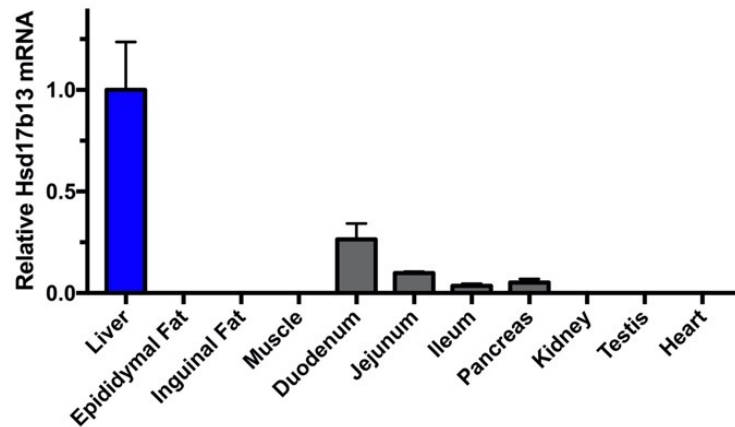
HSD17B13 Variants Protect from Liver-Related Mortality and HCC



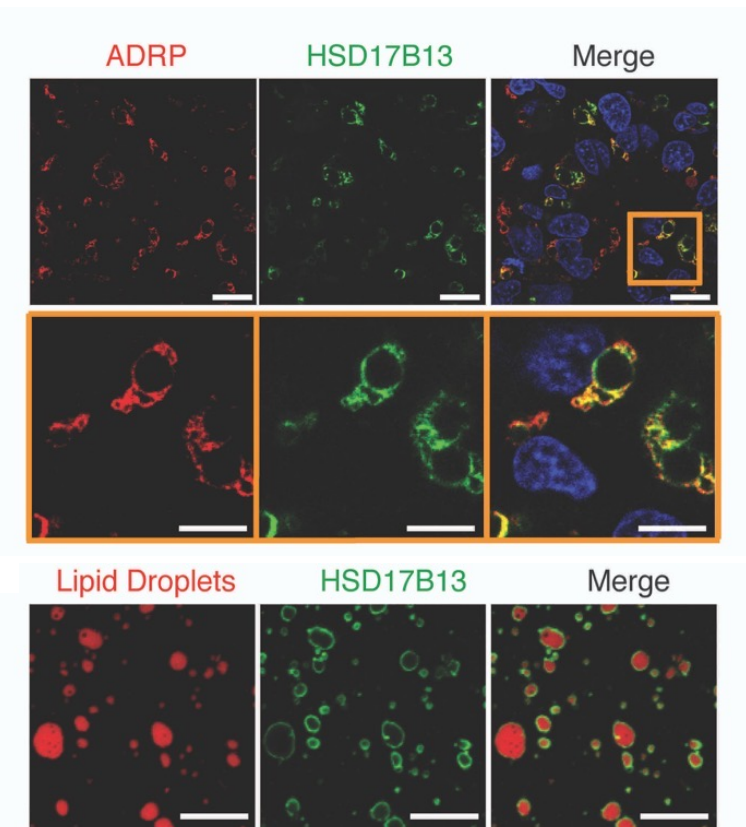
Protective Effects of *HSD17B13* Variants



HSD17B13 is a Hepatocyte Lipid Droplet Protein



<http://human-liver-cell-atlas.ie-freiburg.mpg.de>

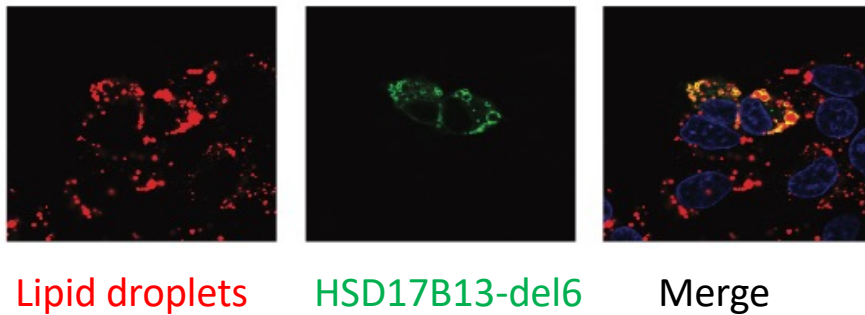


Yanling Ma

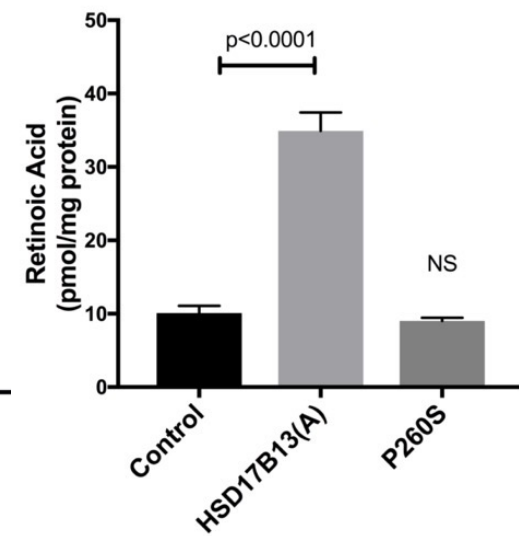
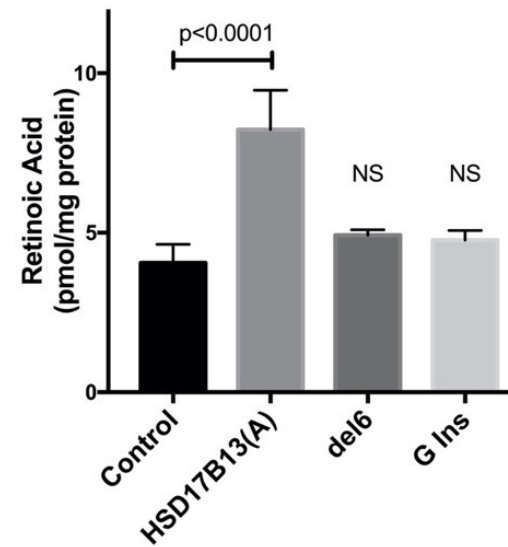
Ma, *Hepatology*, 2019

Variants Cause Loss of Function

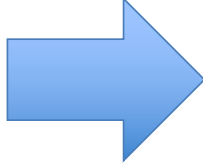
Variants Localize to Lipid Droplets



Retinoic Acid Synthesis



HSD17B13 as Therapeutic Target

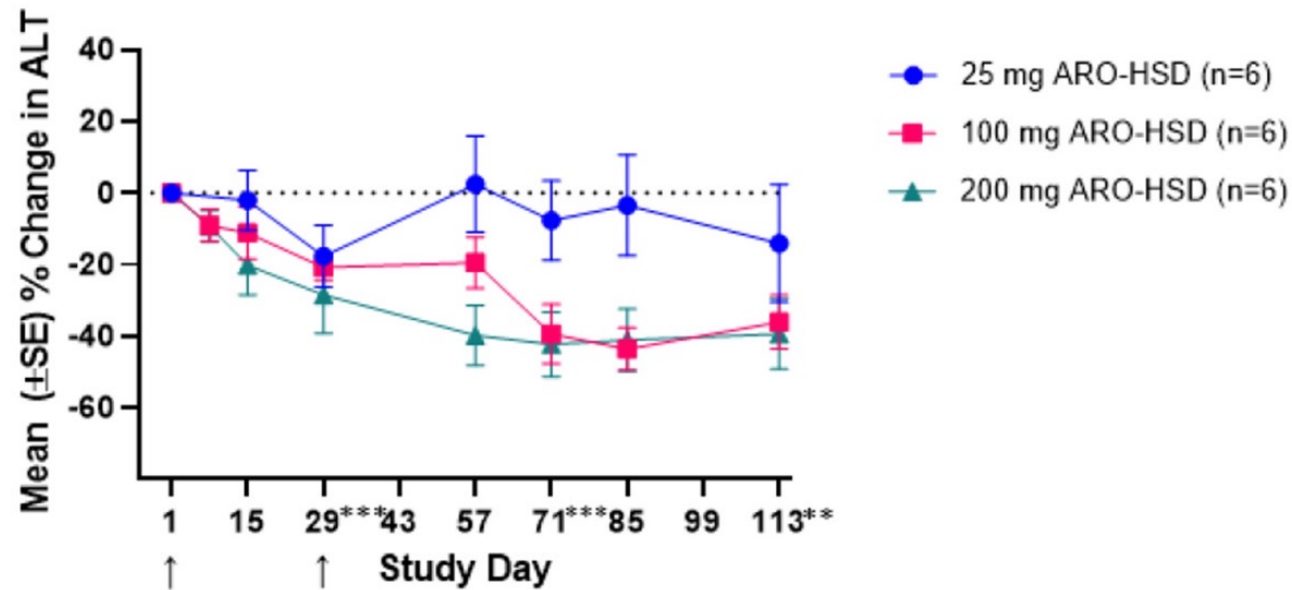
- Liver & hepatocyte specific lipid droplet enzyme
 - Loss of function variants associated with protection from MASH fibrosis
- 
- Lower risk for off-target effects
 - Inhibition/knock-down as strategy
-

Targeting HSD17B13 as Therapy for NASH

ClinicalTrials.gov Search Results 09/04/2023

	Title	Status	Study Results	Interventions
1	A Study of INI-822 in Healthy Volunteers and Participants With Non-alcoholic Steatohepatitis (NASH) or Presumed NASH	Not yet recruiting	No Results Available	•Drug: INI-822 (A) •Other: Placebo (B)
2	Phase 2b Study of GSK4532990 in Adults With NASH	Recruiting	No Results Available	•Drug: GSK4532990 •Drug: Placebo
3	Knockdown of HSD17B13 mRNA, Pharmacokinetics, Safety, and Tolerability, of AZD7503 in Non-Alcoholic Fatty Liver Disease	Recruiting	No Results Available	•Drug: AZD7503 Intervention
4	A Study to Evaluate the Efficacy and Safety of ALN-HSD in Adult Participants With Non-alcoholic Steatohepatitis (NASH) With Fibrosis With Genetic Risk Factors	Recruiting	No Results Available	•Drug: ALN-HSD •Drug: Placebo
5	A Study of ALN-HSD in Healthy Adult Subjects and Adult Patients With Nonalcoholic Steatohepatitis (NASH)	Recruiting	No Results Available	•Drug: ALN-HSD •Drug: Placebo
6	Study of ARO-HSD in Healthy Volunteers and Patients With Non-Alcoholic Steatohepatitis (NASH) or Suspected NASH	Completed	No Results Available	•Drug: ARO-HSD Injection •Drug: sterile normal saline (0.9% NaCl)

Anti-HSD17B13 Treatment in Humans

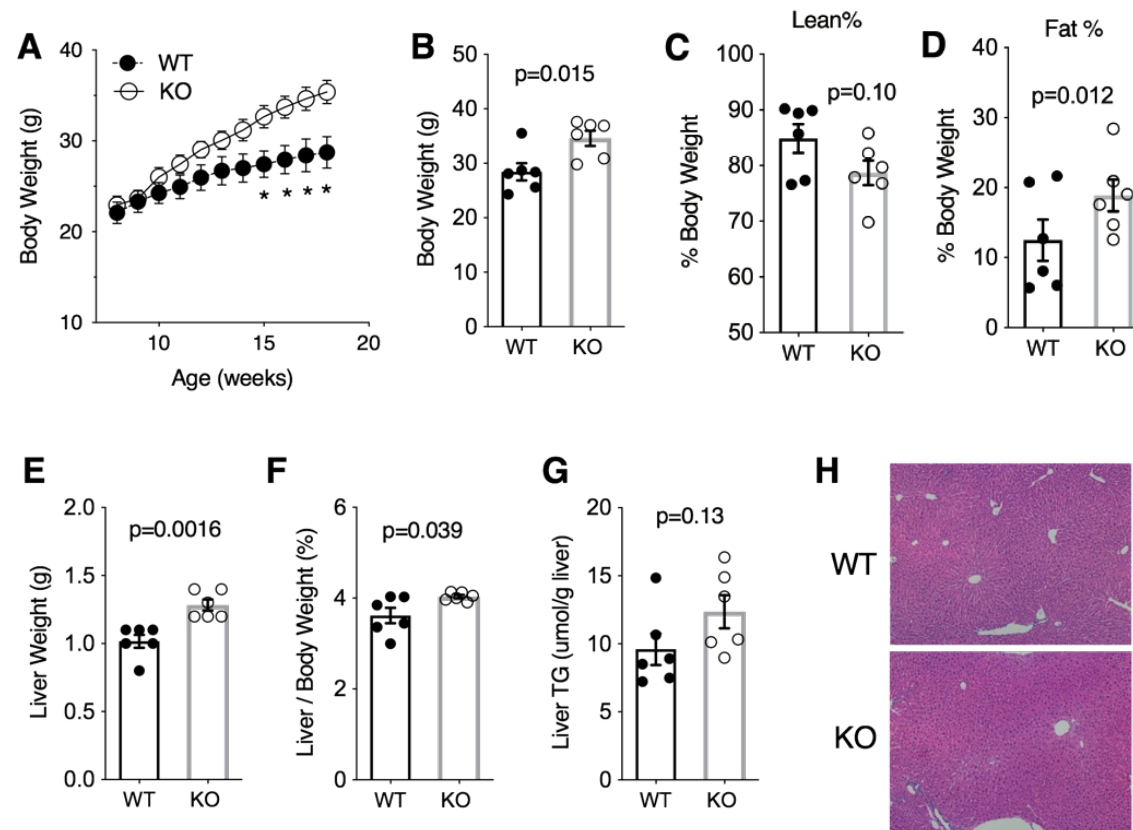


Phase I/II Study of ARO-HSD

Unknown

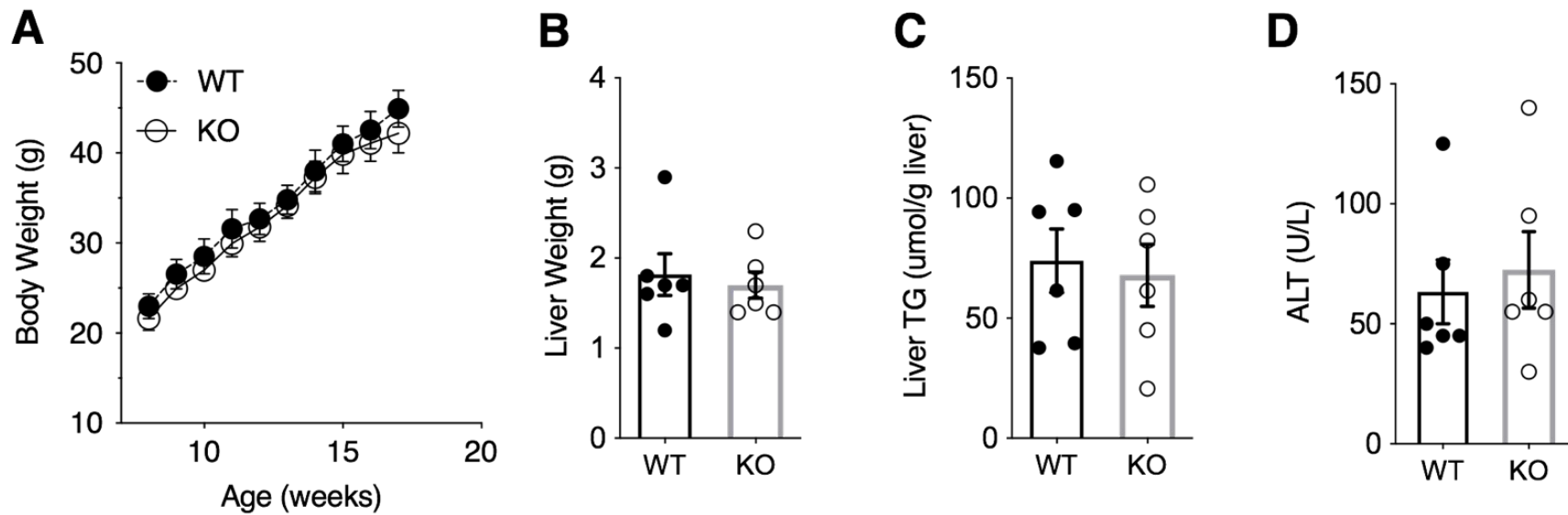
- What is the physiological role of HSD17B13?
 - How does loss-of-function protect from MASH and fibrosis?
-

Hsd17b13 KO – Weight Gain but No Liver Phenotype



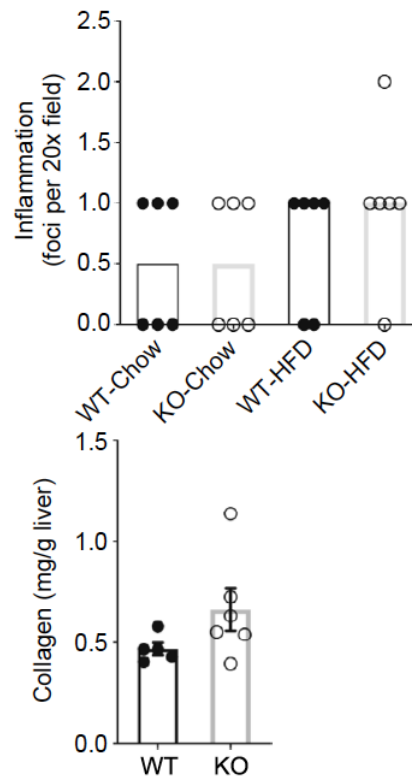
Hsd17b13 KO mice (backcrossed to C57BL/6J and littermate controls Normal chow until age 20 weeks (n=6/group)

High-Fat Diet Effects in Hsd17b13 KO

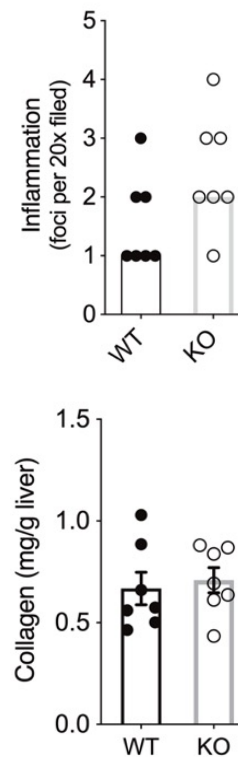


*Hsd17b13 KO mice and littermate controls
High-fat (60%) for 12 weeks (n=6/group)*

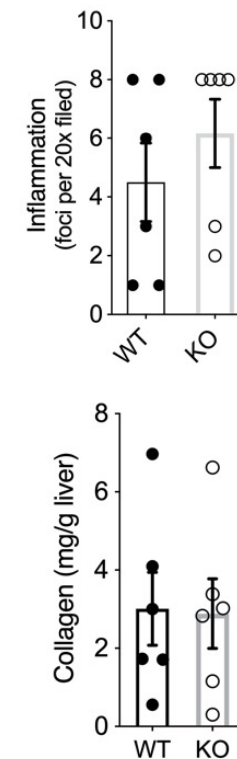
No Protection by Hsd17b13-KO in Obesogenic Diets



High-fat (60%) for 12 weeks

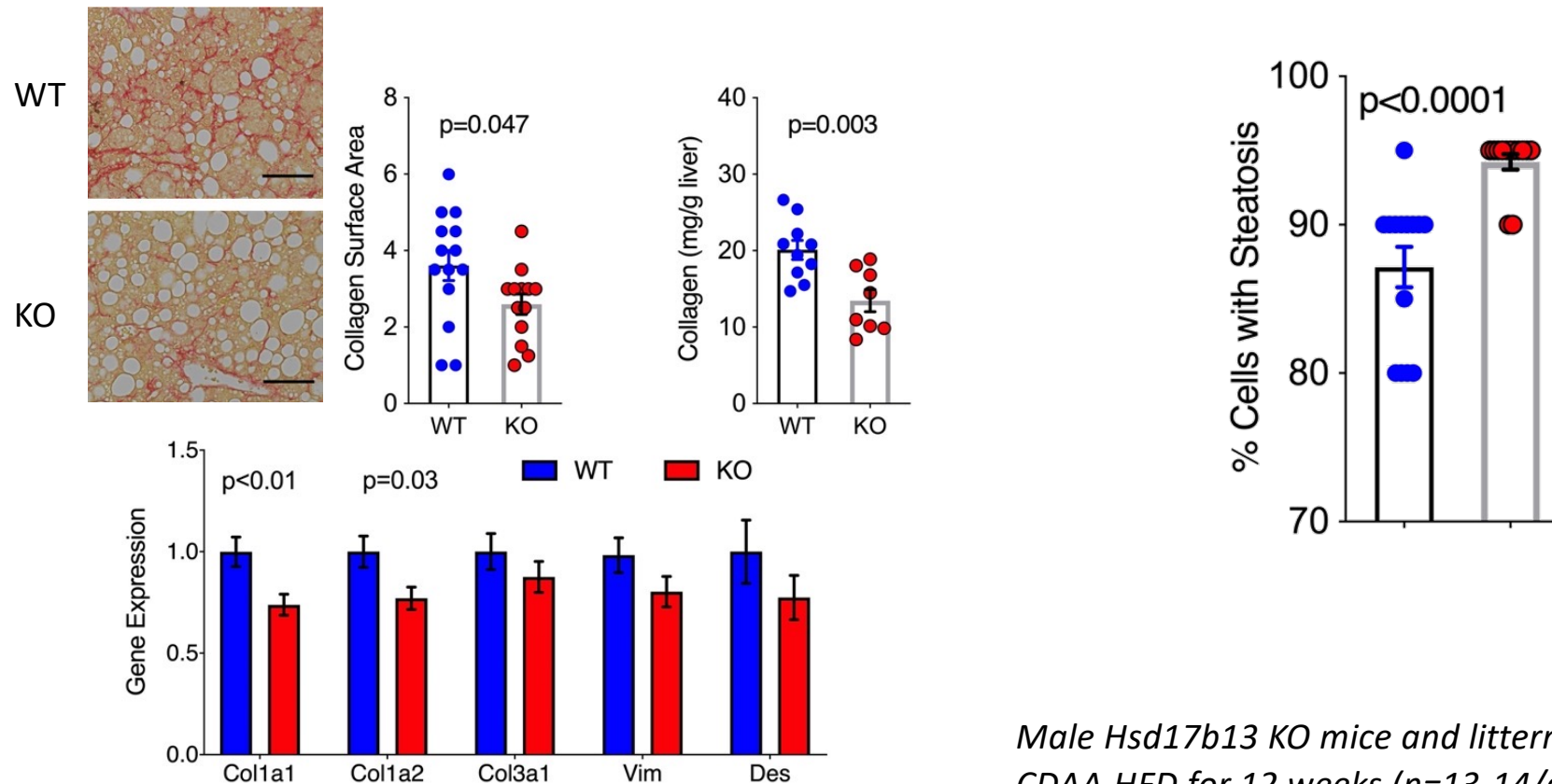


Western Diet (20% fat, 35% sucrose, 0.15% cholesterol) for 16 weeks



Western Diet for 10 months

Hsd17B13 KO Protects from CDAA-HFD Fibrosis



Male *Hsd17b13* KO mice and littermate controls
CDAA-HFD for 12 weeks (n=13-14/group)

Ma, Unpublished Data

Mechanism of Protection

- Hsd17b13-KO **protects** mice from CDAA-HFD induced fibrosis

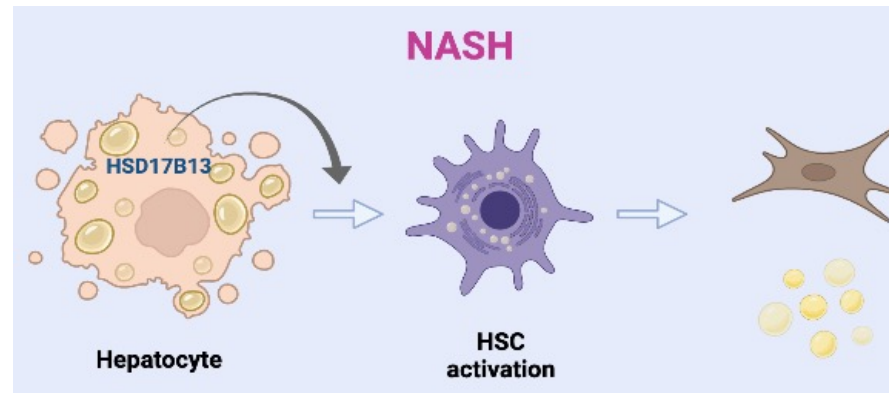


How Does Hsd17b13-KO Protect from Fibrosis?

- HSD17B13 expressed in hepatocytes
- No expression in hepatic stellate cells

Non-specific

- Hsd17b13-KO protects the hepatocyte
- Canonical hepatocyte->HSC signaling pathways

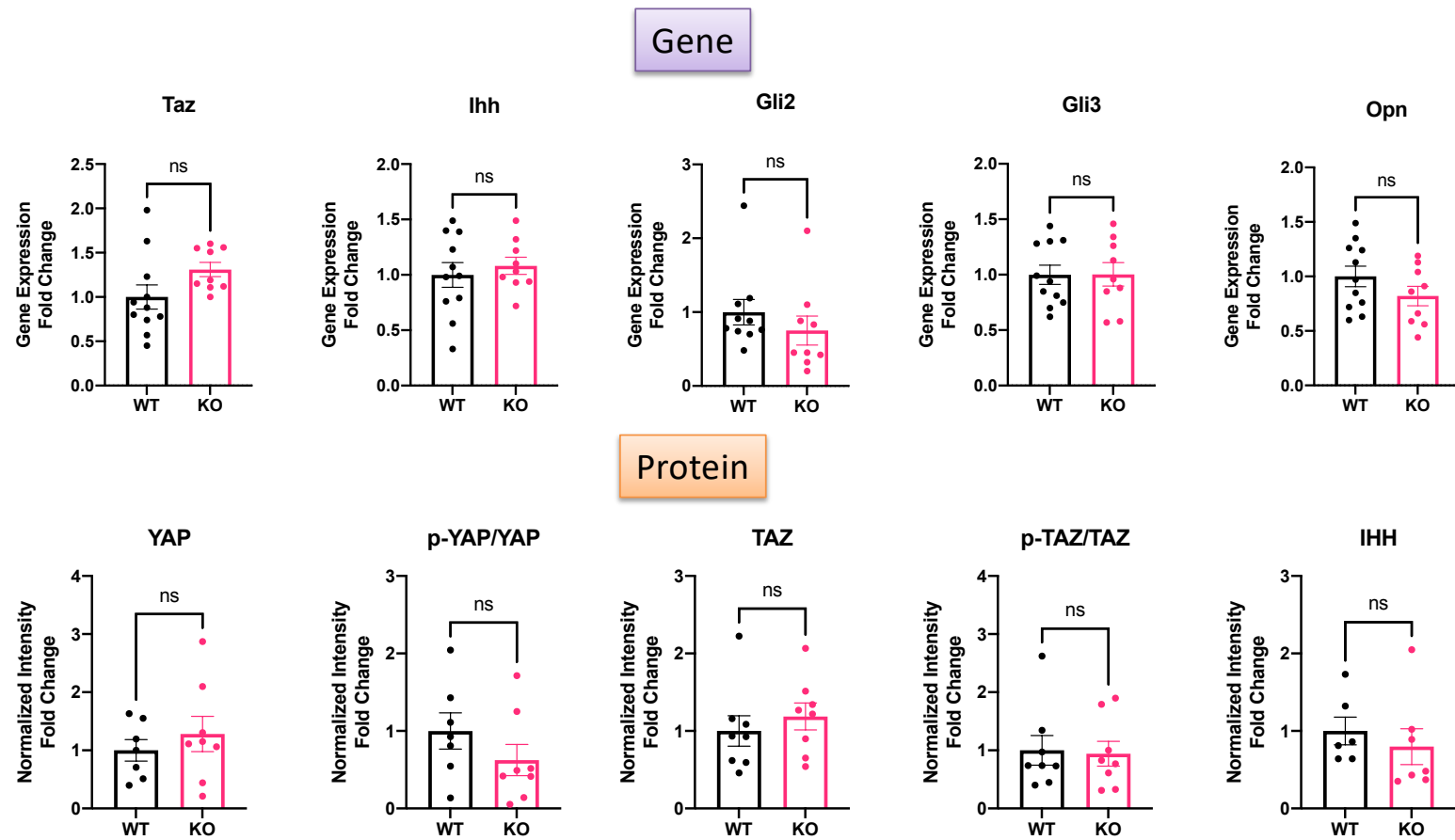


Specific

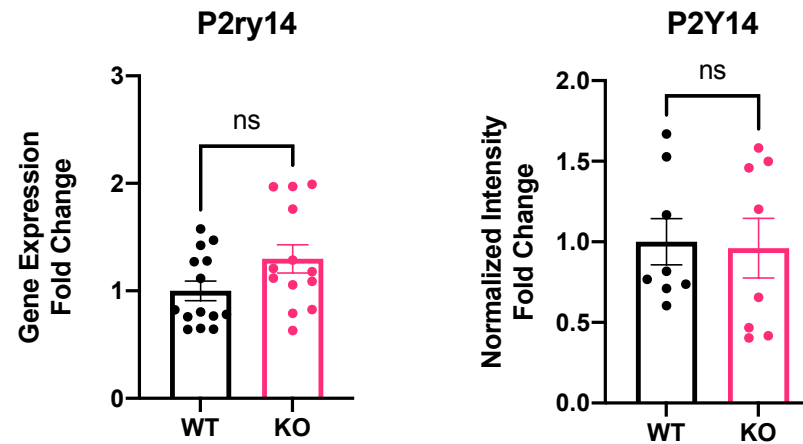
- Hsd17b13-KO modulates a specific signal to HSC



No Impact on Taz-Ihh Pathway



No Impact on P2Y14 Pathway



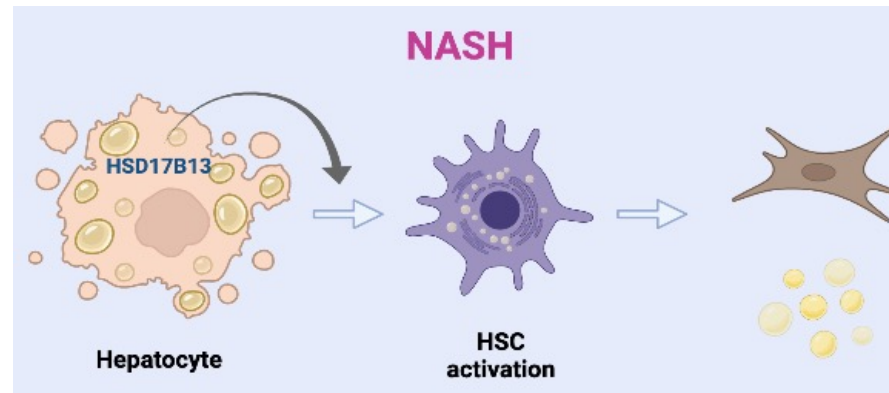
Male *Hsd17b13*-
KO mice and
littermate controls
CDAA-HFD (12 wk)

How Does Hsd17b13-KO Protect from Fibrosis?

- HSD17B13 expressed predominantly in hepatocytes
- No expression in hepatic stellate cells

Non-specific

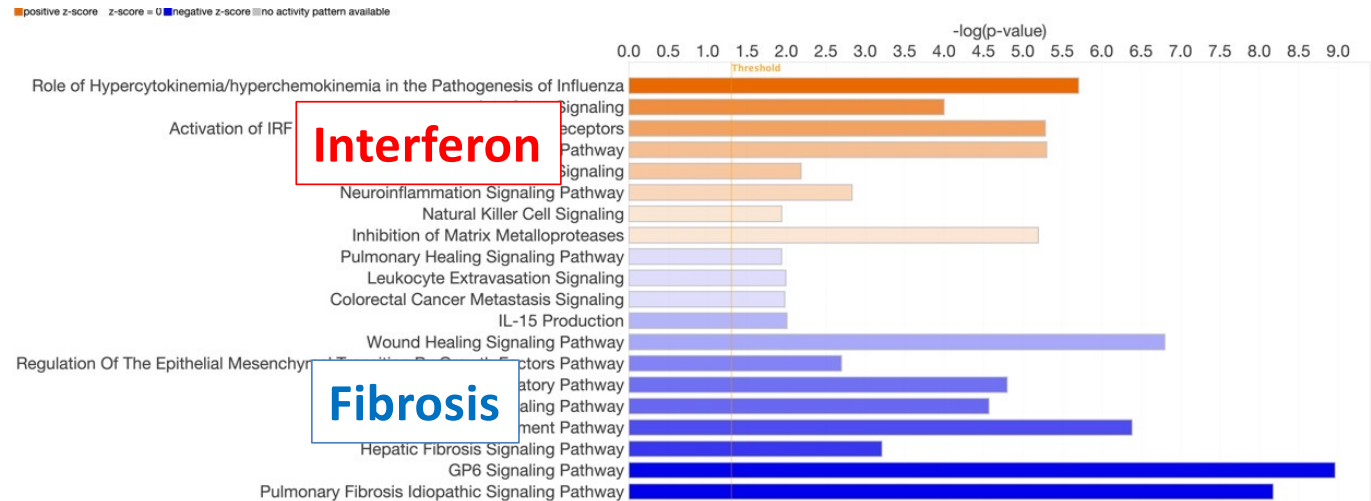
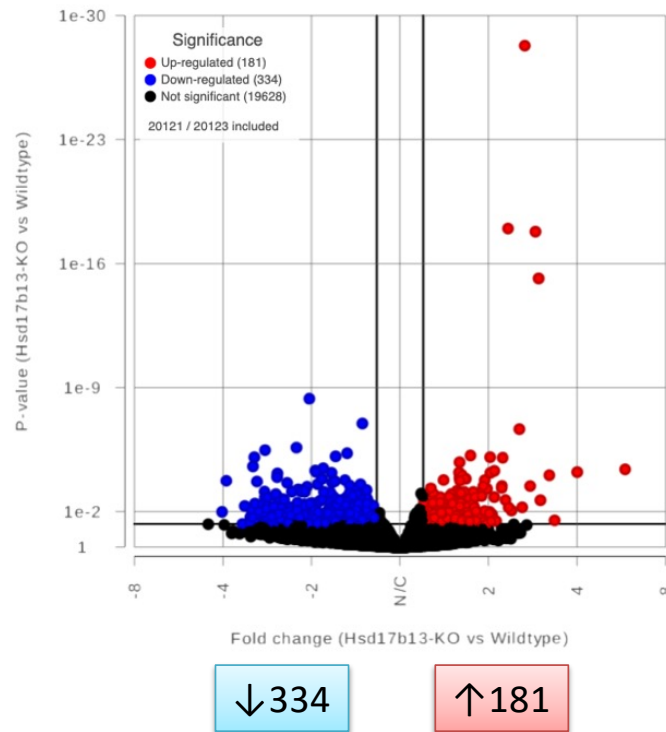
- Hsd17b13-KO protects the hepatocyte
- ~~• Canonical hepatocyte->HSC signaling pathways~~



Specific

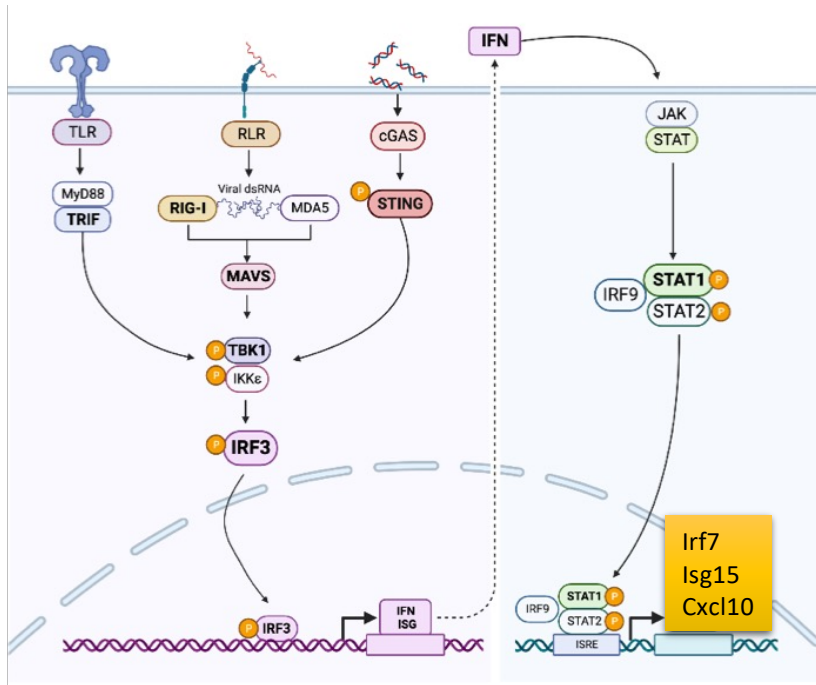
- Hsd17b13-KO modulates a specific signal to HSC

Altered Pathways on Transcriptomic Analysis

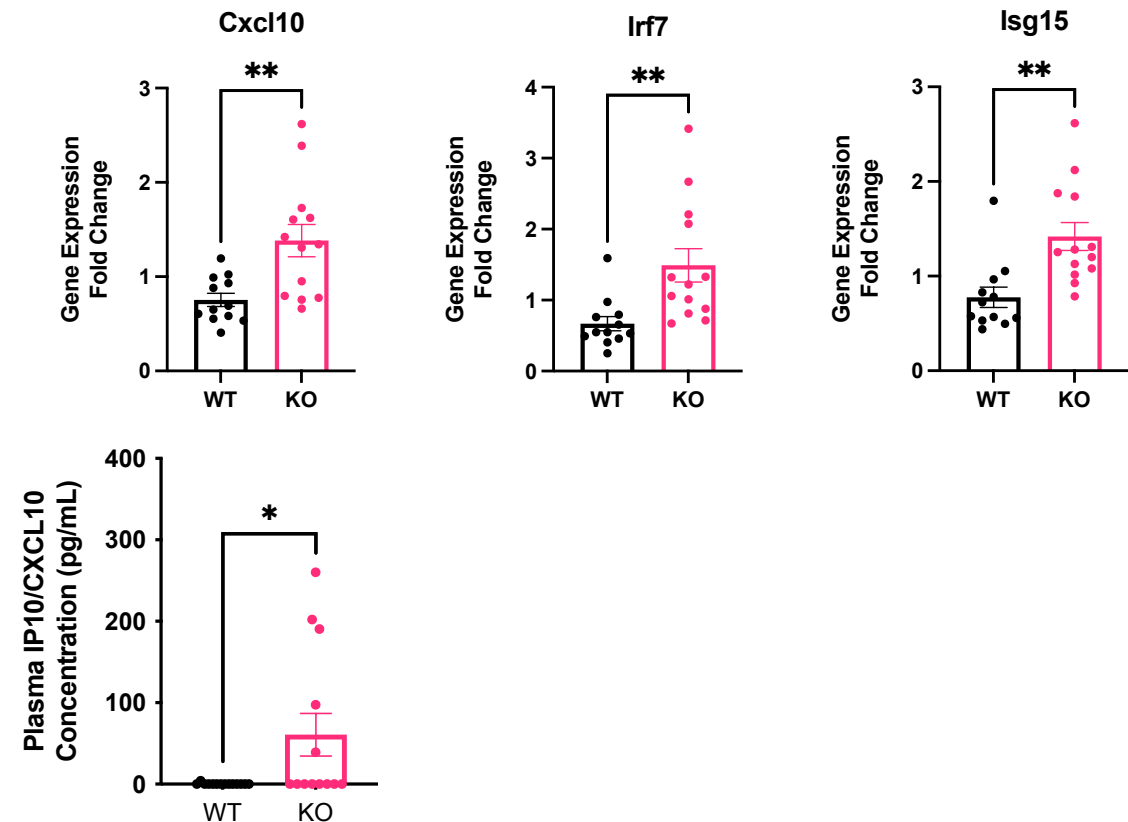


Male Hsd17b13-KO mice and littermate controls CDA-HFD (12 wk) Bulk RNAseq

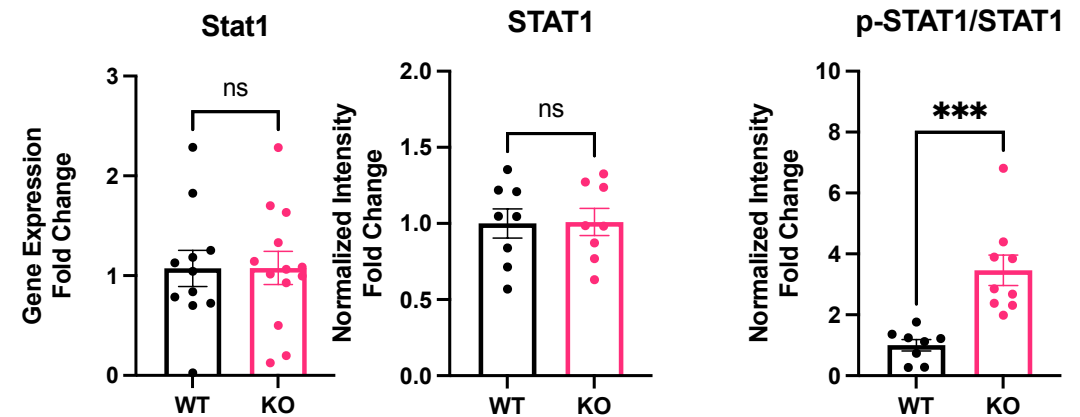
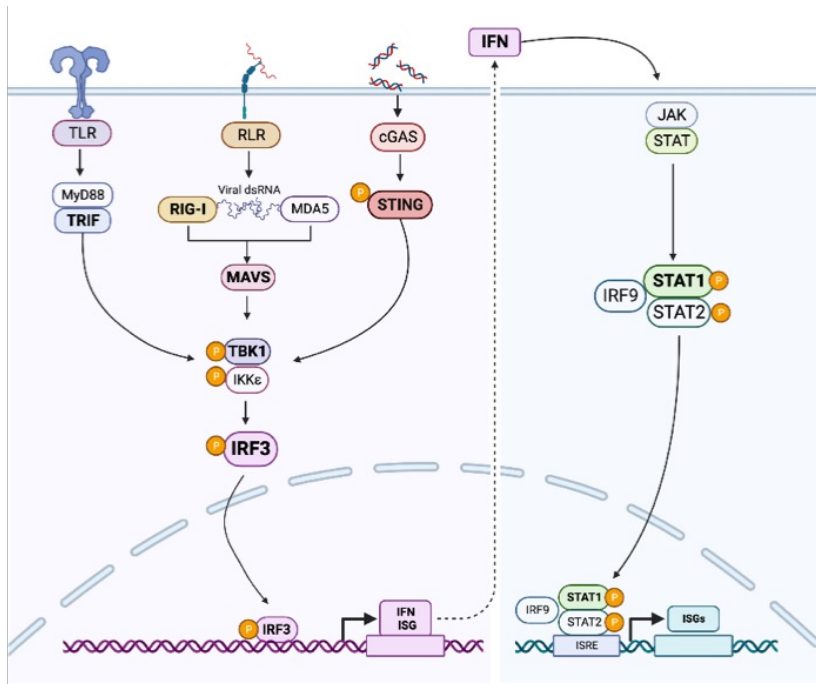
Hsd17b13-KO Upregulates IFN Signaling



Male *Hsd17b13*-
KO mice and
littermate controls
CDAH-HFD (12 wk)

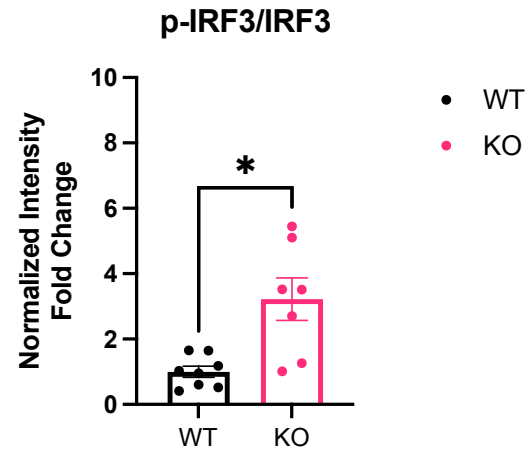
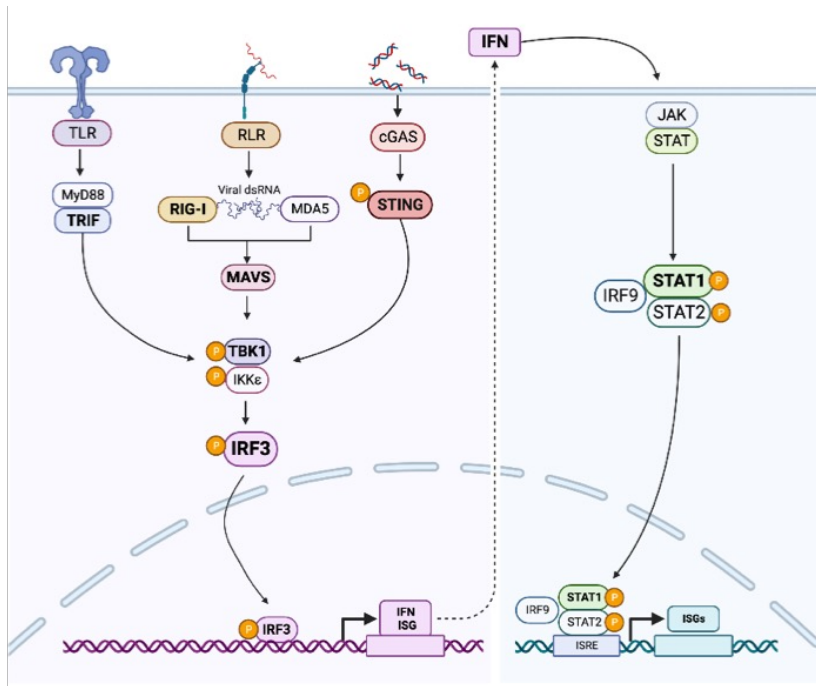


Hsd17b13-KO Upregulates IFN Signaling



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Hsd17b13-KO Upregulates IFN Signaling

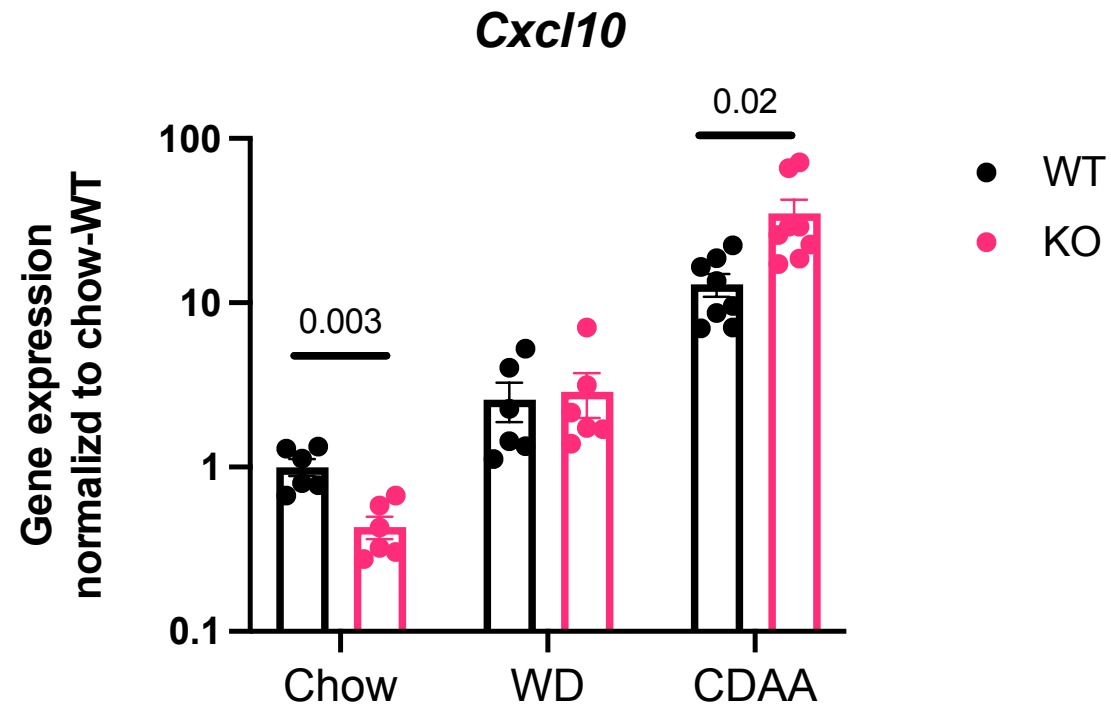


Male *Hsd17b13*-
KO mice and
littermate controls
CDAH-HFD (12 wk)

Mechanism of Protection

- Hsd17b13-KO **protects** mice from CDAA-HFD induced fibrosis
 - Type 1 interferon signaling **upregulated** in Hsd17b13-KO under CDAA-HFD
 - Is IFN signaling upregulated in other MASLD/MASH models?
-

IFN Signaling Upregulation is Limited to CDAA-HFD

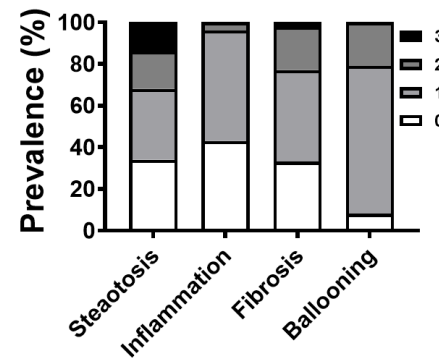


Mechanism of Protection

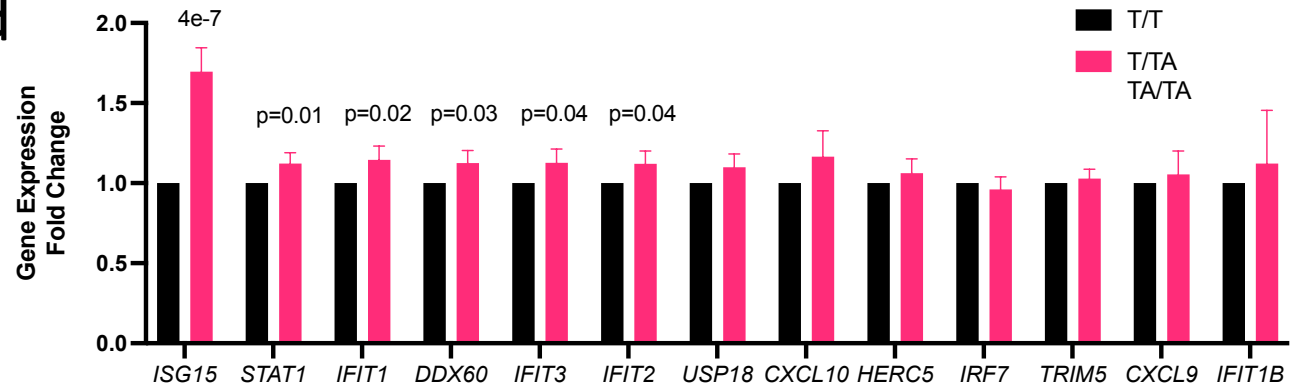
- Hsd17b13-KO **protects** mice from CDAA-HFD induced fibrosis
 - Type 1 interferon signaling **upregulated** in Hsd17b13-KO under CDAA-HFD
 - Upregulation of IFN signaling seen **only** in conditions where Hsd17b13-KO protects from fibrosis
 - Is it relevant to human MASH?
-

Upregulated ISGs in Human HSD17B13-LOF

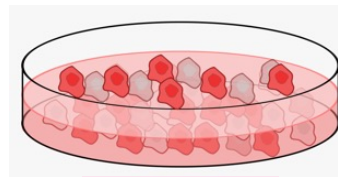
- Bariatric surgery cohort (n=211)
- *HSD17B13* genotype
- Liver histology and gene expression



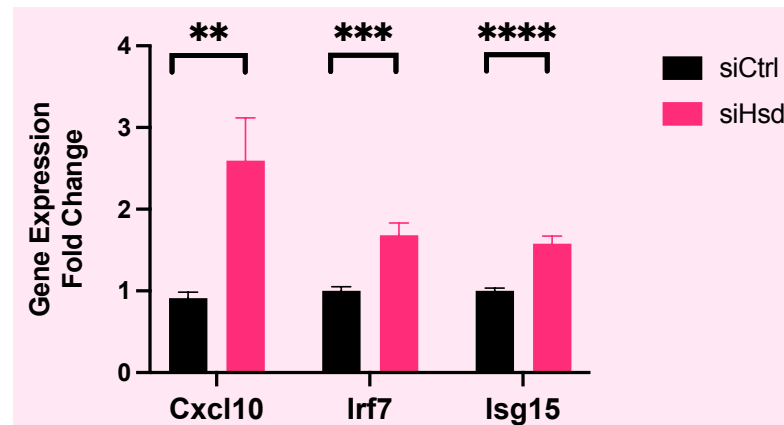
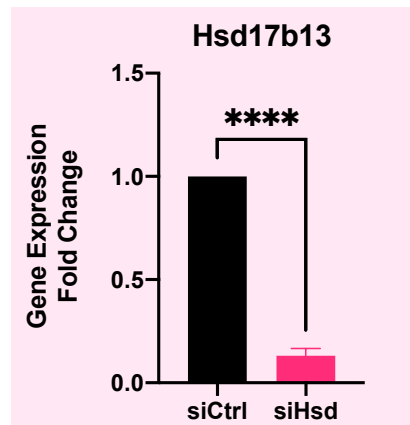
HSD17B13 rs72613567
T/T n=131
T/A n=69
A/A n=11



Hsd17b13-KD Induces IFN Signaling *in vitro*



AML12
siCtrl / siHsd



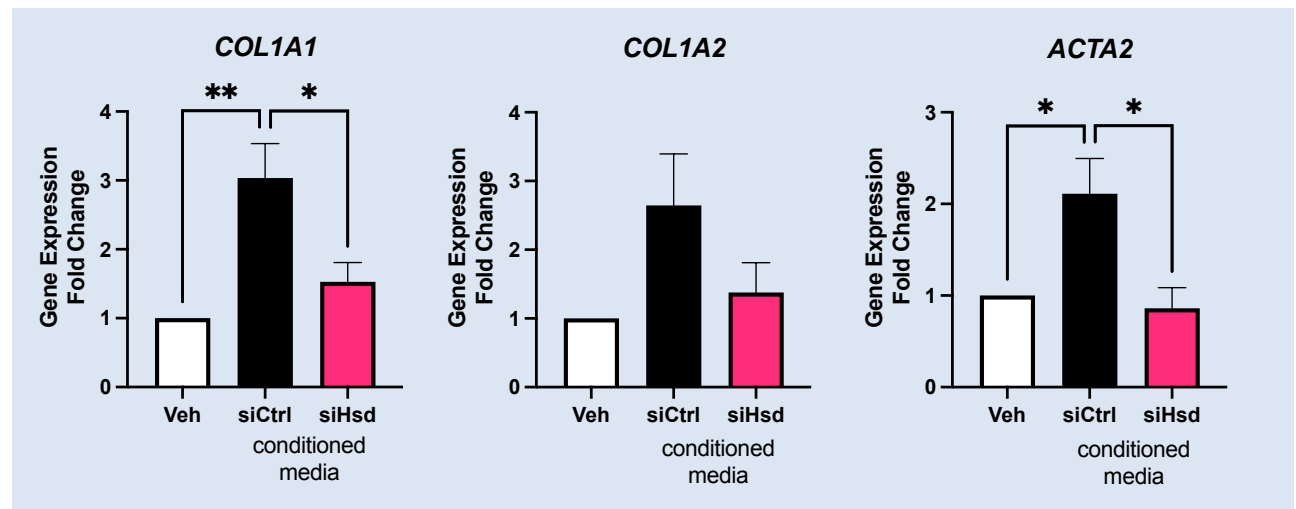
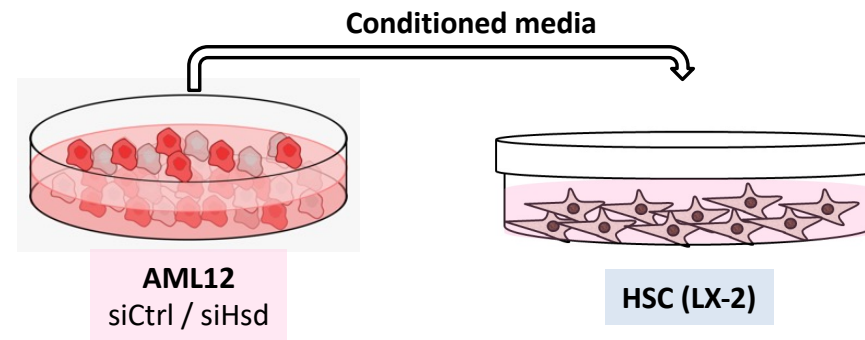
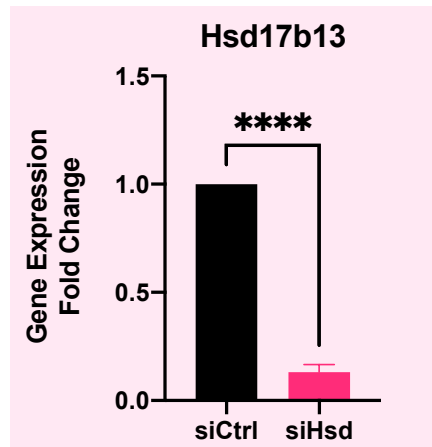
Mechanism of Protection

- Hsd17b13-KO **protects** mice from CDAA-HFD induced fibrosis
 - Type 1 interferon signaling **upregulated** in Hsd17b13-KO under CDAA-HFD
 - Upregulation of IFN signaling seen only in conditions where Hsd17b13-KO protects from fibrosis
 - Upregulated interferon signaling is seen with HSD17B13 deficiency in **humans** and mice
 - HSD17B13 deficiency is **sufficient** to induce hepatocyte IFN signaling
-

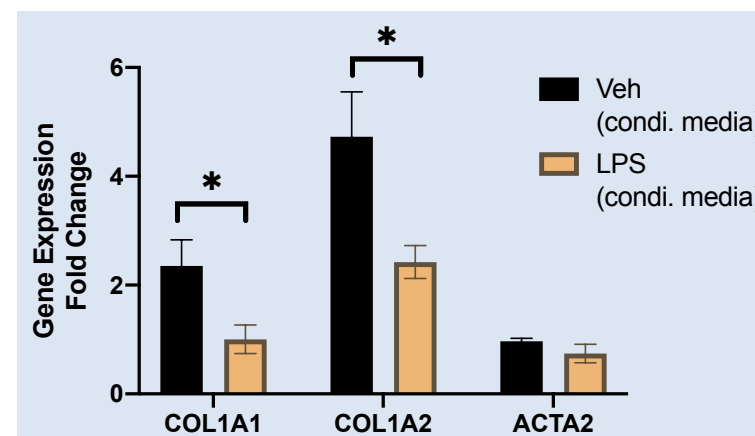
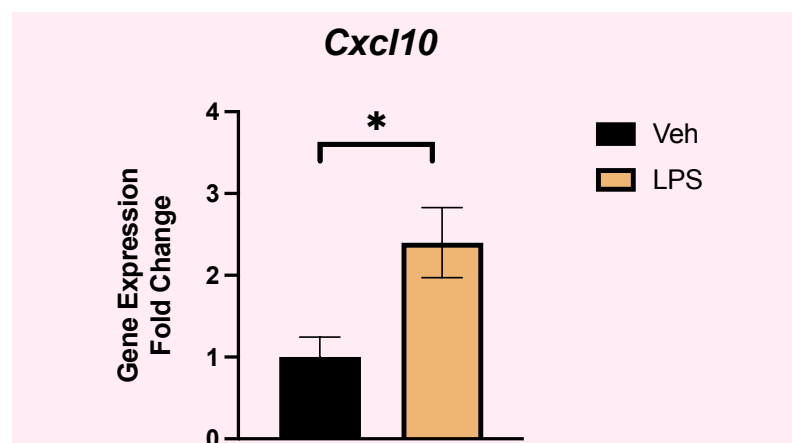
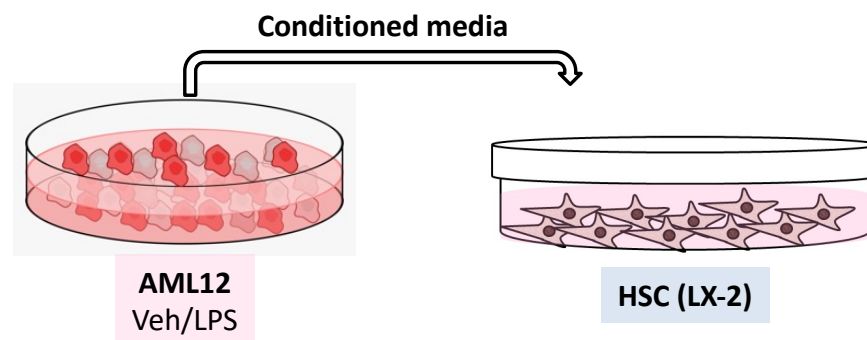
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- Hsd17b13-KO **protects** mice from CDAA-HFD induced fibrosis
 - Type 1 interferon signaling **upregulated** in Hsd17b13-KO under CDAA-HFD
 - Upregulation of IFN signaling seen only in conditions where Hsd17b13-KO protects from fibrosis
 - Upregulated interferon signaling is seen with HSD17B13 deficiency in humans and mice
 - HSD17B13 deficiency is sufficient to induce hepatocyte IFN signaling
 - Can hepatocyte IFN signaling regulate HSC activation?
-

In vitro Effect of Hsd17b13-KD on HSC activation



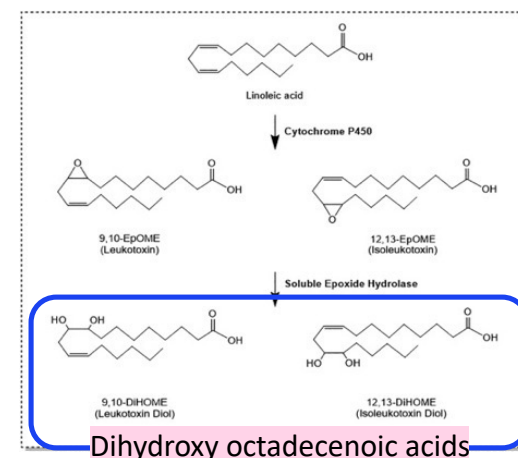
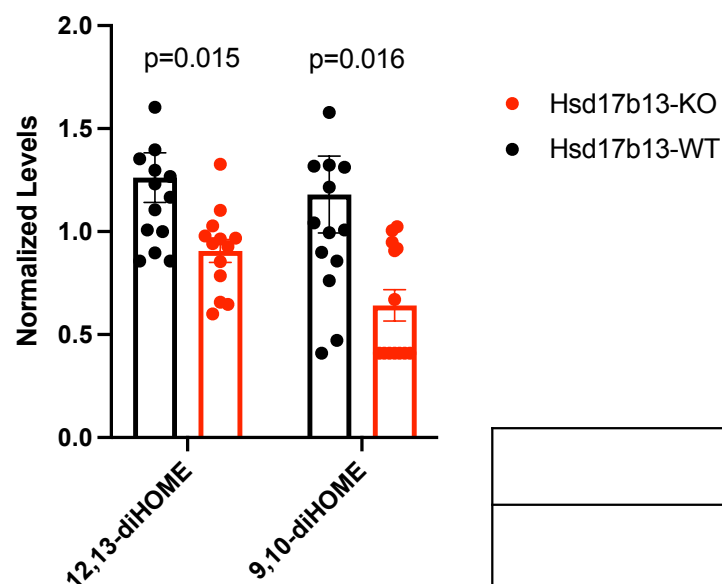
Activation of Hepatocyte IFN Decreases HSC Activation



Mechanism of Protection

- Hsd17b13-KO **protects** mice from CDAA-HFD induced fibrosis
 - Type 1 interferon signaling **upregulated** in Hsd17b13-KO under CDAA-HFD
 - Upregulation of IFN signaling seen only in conditions where Hsd17b13-KO protects from fibrosis
 - Upregulated interferon signaling is seen with HSD17B13 deficiency in **humans** and mice
 - HSD17B13 deficiency is **sufficient** to induce hepatocyte IFN signaling
 - Activation of hepatocyte IFN signaling can **decrease** HSC activation
-

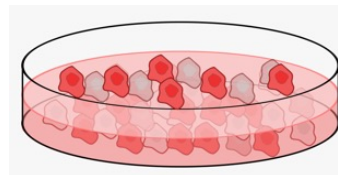
Hepatic diHOMEs Downregulated in Hsd17b13-KO



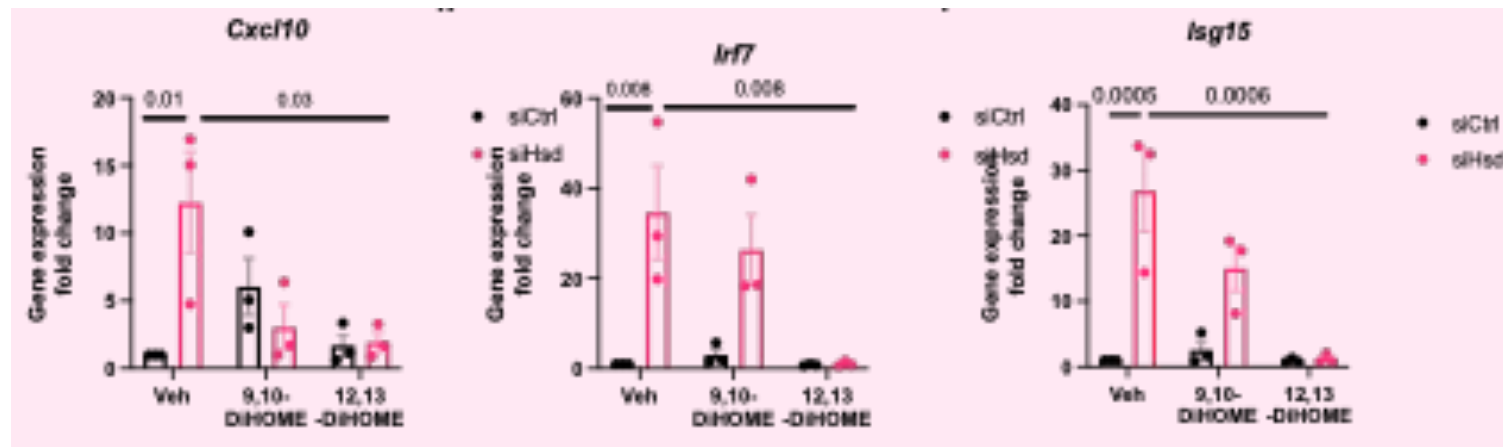
Male Hsd17b13-KO mice and littermate controls
CDAA-HFD (12 wk)
Liver Metabolomics (Metabolon)

Effect of Hsd17b13-KO			
	Chow Diet	High-Fat/Western	CDAA-HFD
12,13-diHOME	↑	↑	↓
ISGs	↓	↓	↑
Fibrosis	Unaffected	Unaffected	↓

12,13-DiHOME Counteracts Hsd17b13-KD Activation of IFN signaling



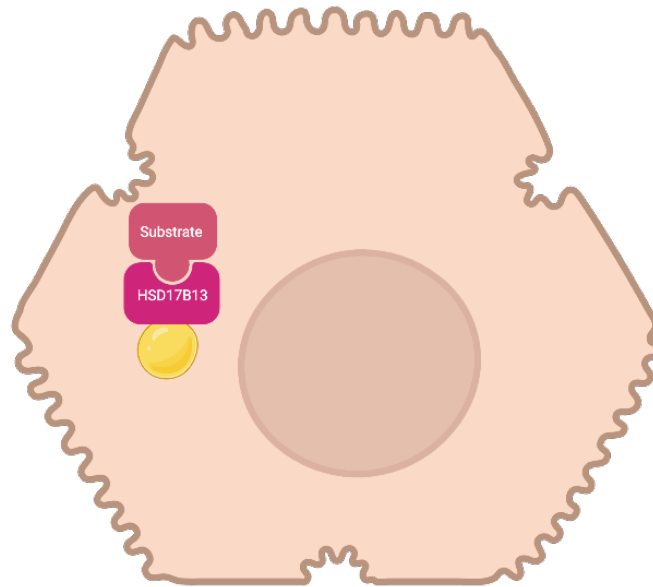
AML12
siCtrl / siHsd
Vehicle/diHOME



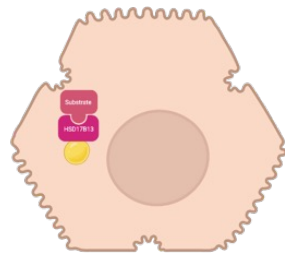
Mechanism of Protection

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 - Type 1 interferon signaling **upregulated** in Hsd17b13-KO under CDAA-HFD
 - Upregulation of IFN signaling seen only in conditions where Hsd17b13-KO protects from fibrosis
 - Upregulated interferon signaling is seen with HSD17B13 deficiency in **humans** and mice
 - HSD17B13 deficiency is **sufficient** to induce hepatocyte IFN signaling
 - Activation of hepatocyte IFN signaling can decrease HSC activation
 - 12,13-diHOME is decreased by Hsd17b13-KO under specific conditions
 - 12,13-diHOME is an **inhibitor** of hepatocyte IFN signaling
-

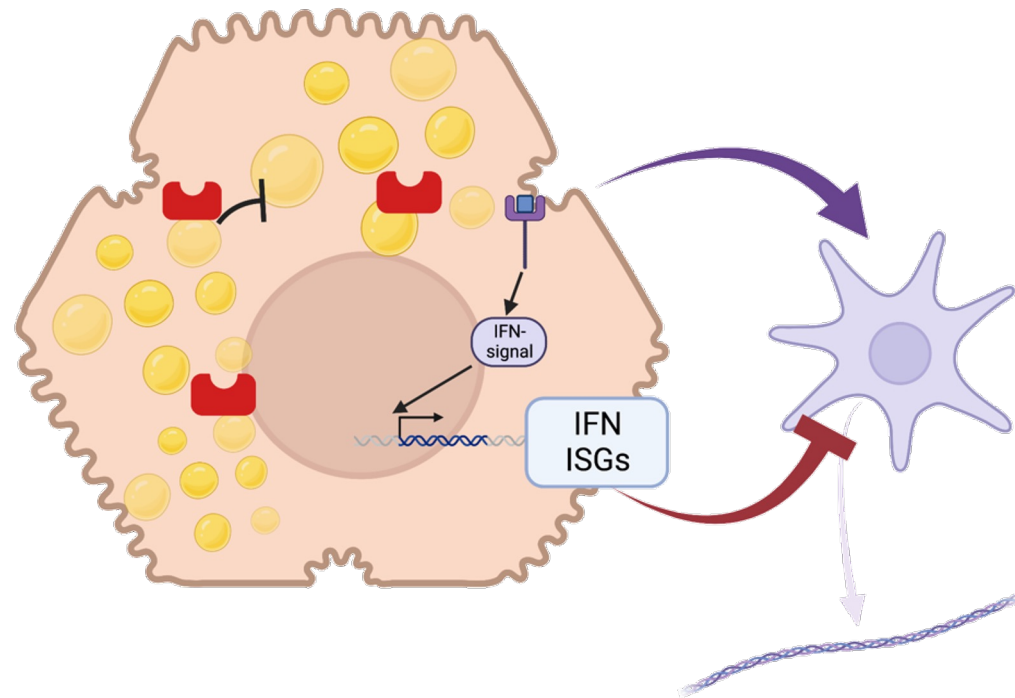
Normal chow/weight

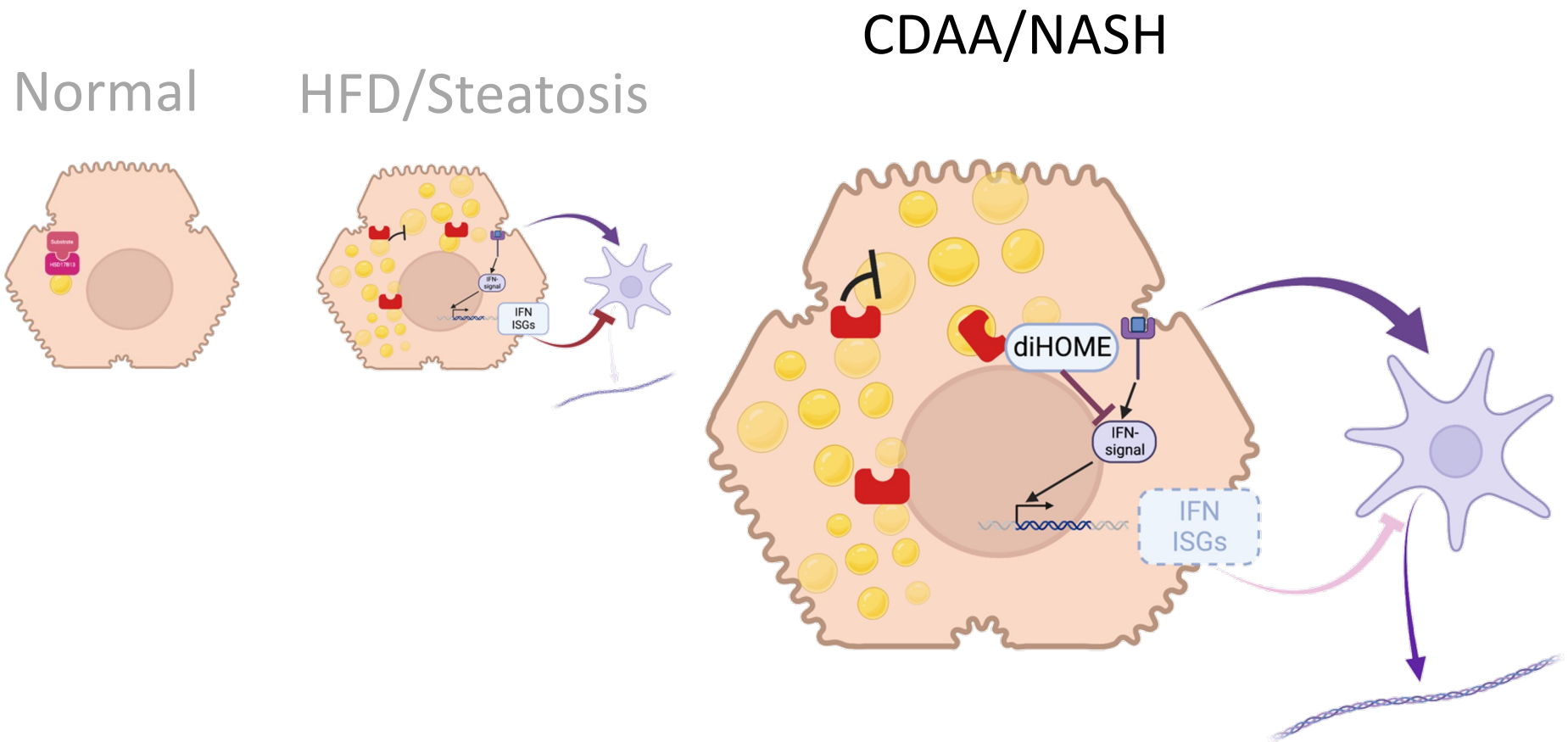


Normal



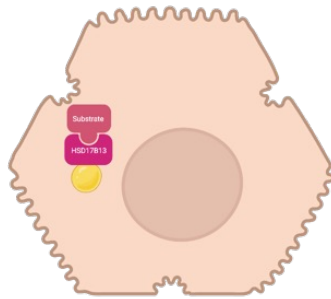
HFD/Steatosis



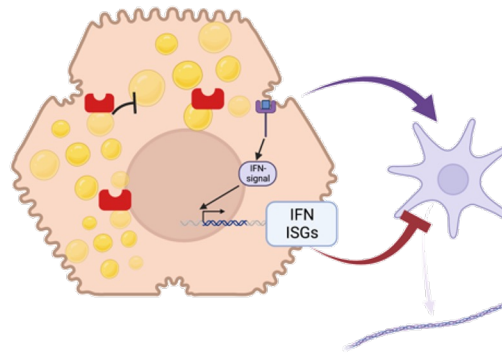


Normal

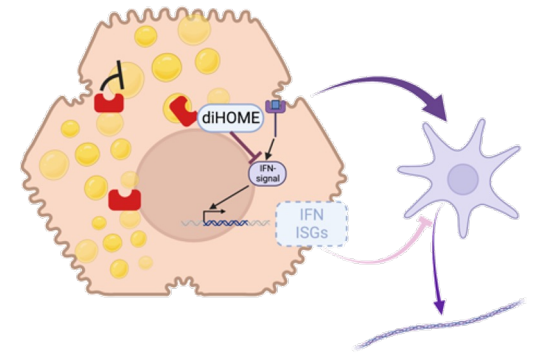
HSD17B13
Present



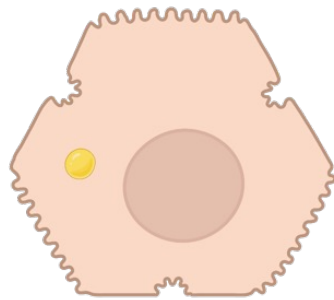
HFD/Steatosis



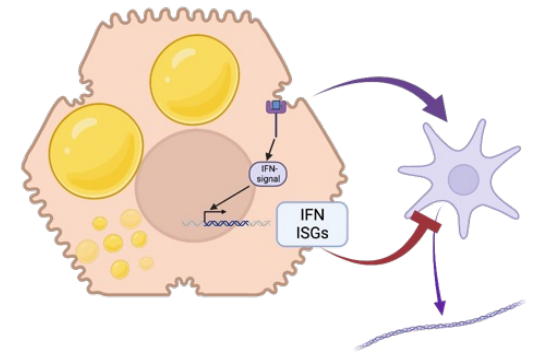
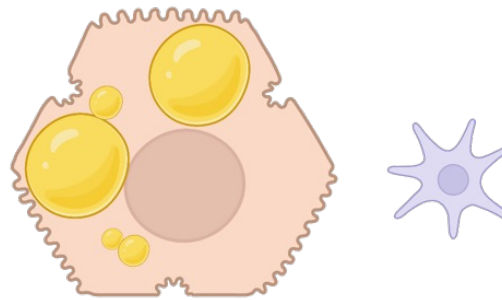
CDAA/NASH



HSD17B13
Deficiency



Weight gain

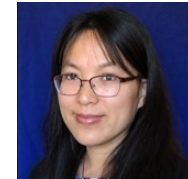


HSD17B13 Mechanism & Implication for Therapy

- HSD17B13 loss-of-function (human) and knockout (mice) protect from NASH fibrosis
 - HSD17B13 (through DiHOMEs) inhibits the protective type 1 interferon pathway
 - Anti-HSD therapies may be effective
 - Targeting DiHOME synthesis or induction of IFN may be a useful antifibrotic therapy
-

Acknowledgements

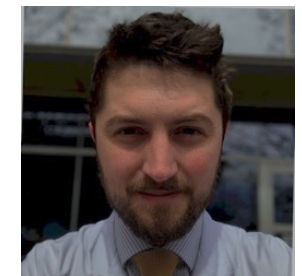
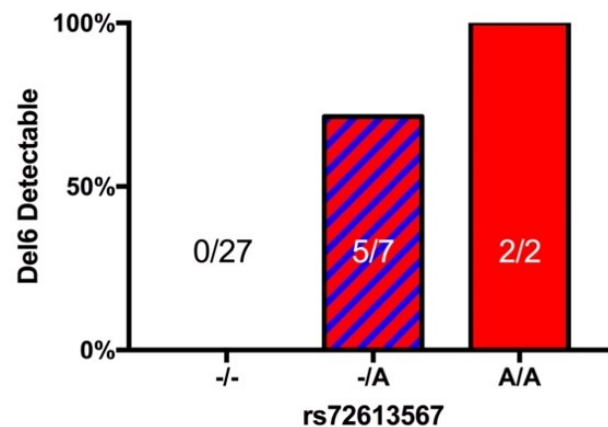
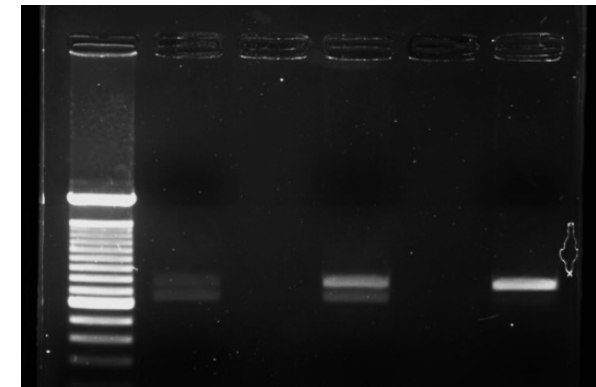
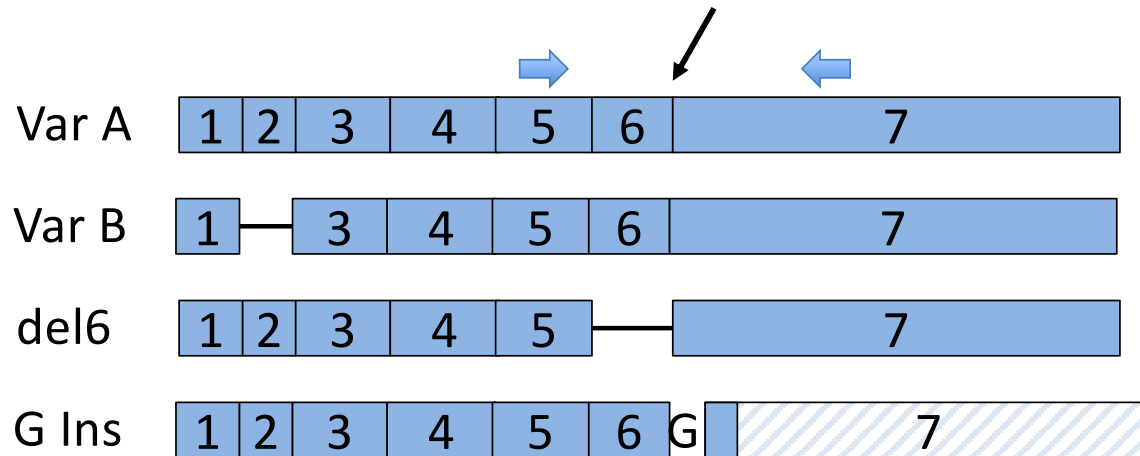
- **Wenqi Cui**
- **Yanling Ma**
- **Lila Gonzalez Hodar**
- Allison Wing
- Bowoo Lee
- Lakshmi Mahajan
- Dennis Lin
- Phil Brown
- Maren Podszun
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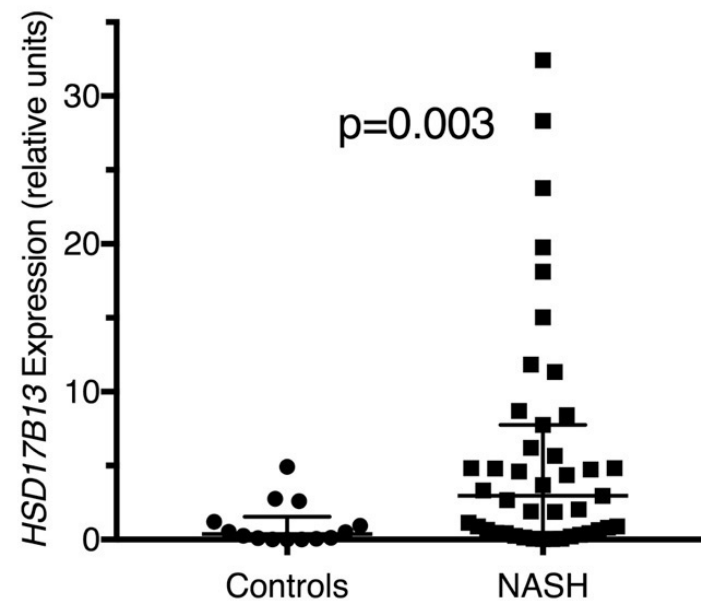
SNP Generates Novel Splice Variants



mRNA & DNA from human liver samples (LTCDS)

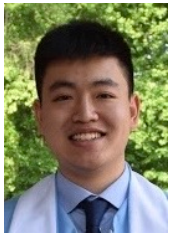
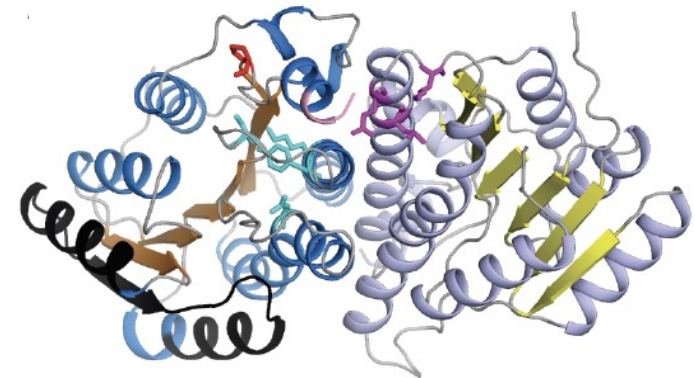
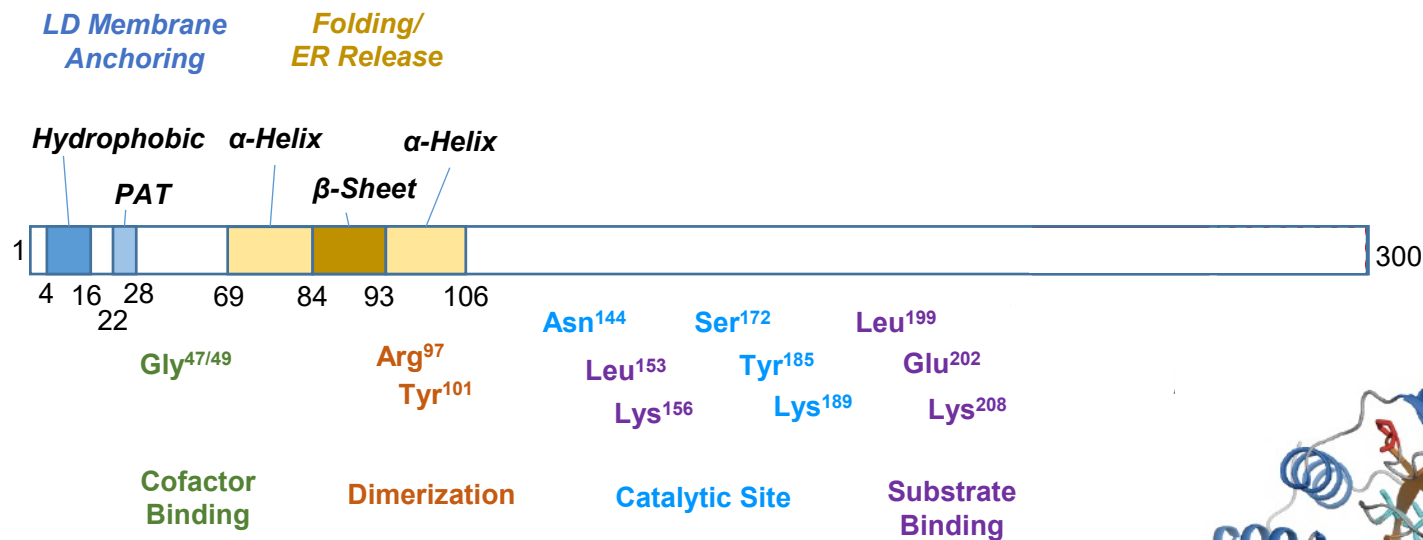
Ma et al, *Hepatol*, 2019; Ma, Brown, et al, *Hepatol*, 2020

Hepatic *HSD17B13* is Upregulated in NASH



Expression levels of HSD17B13 by qPCR in liver samples from healthy controls (n=14) and NASH patients (n=43)

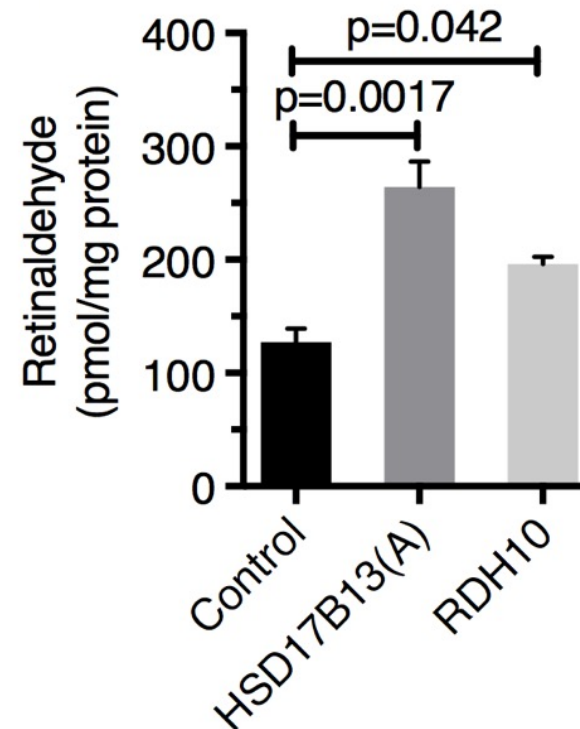
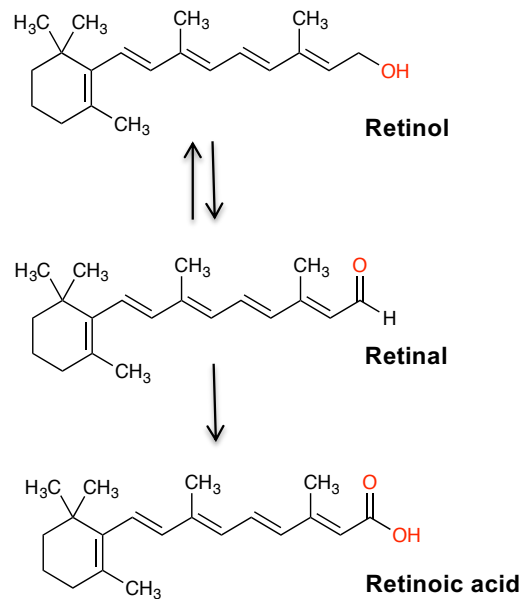
Essential Domains in HSD17B13



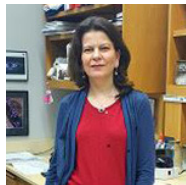
Dennis Lin

Predicted structure based on HSD17B11 and SDR16C

HSD17B13 has Retinol Dehydrogenase Activity *in vitro*



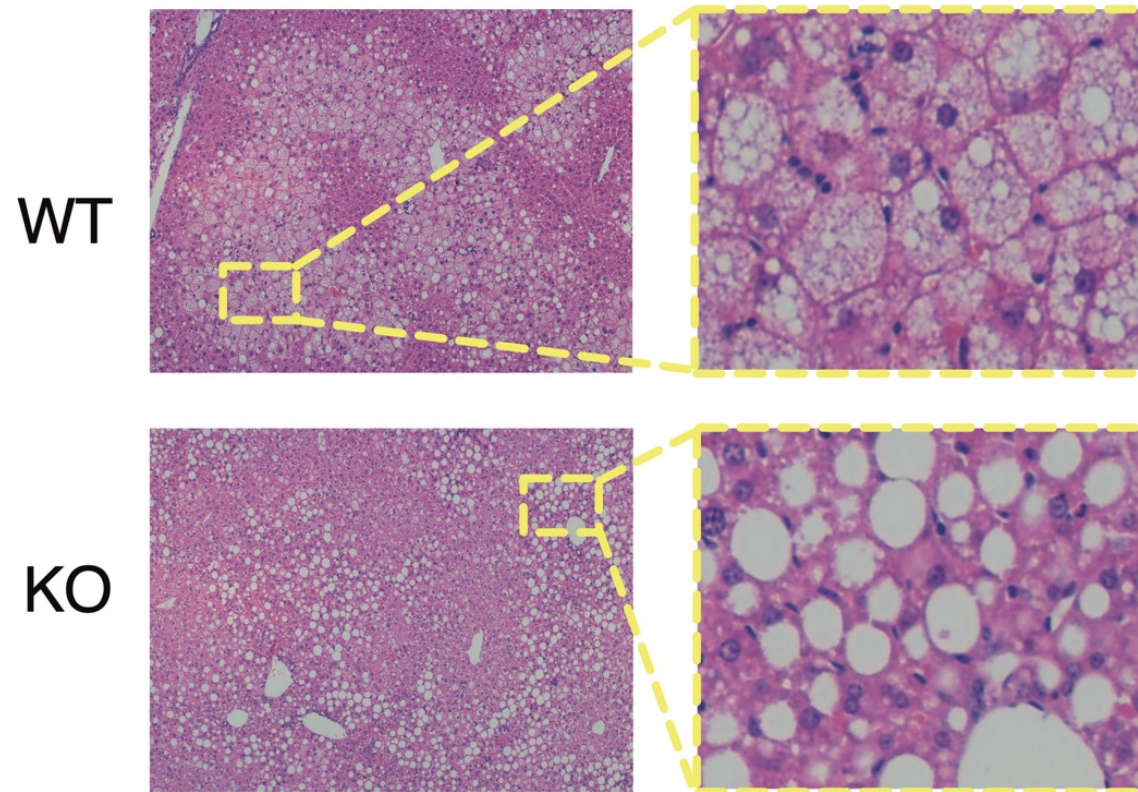
N. Kedishvili



O. Beliaeva

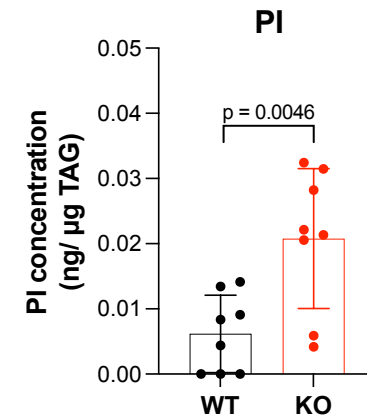
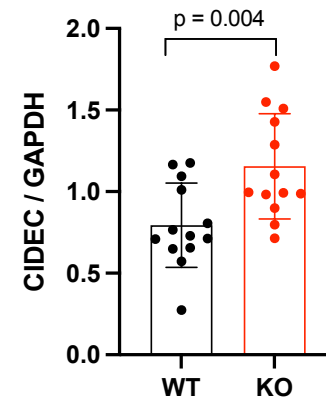
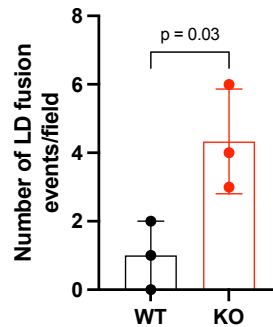
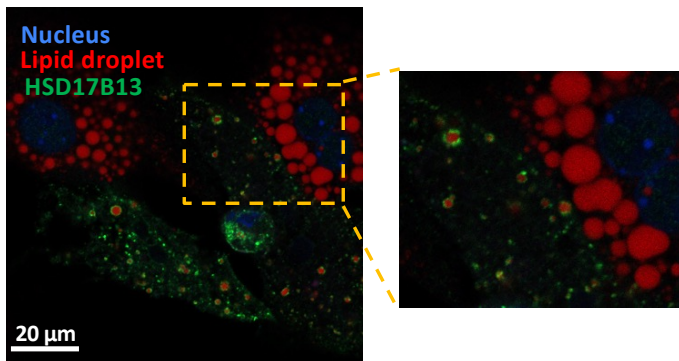
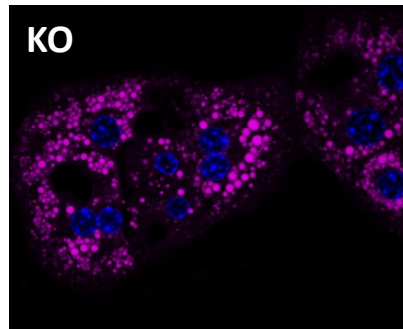
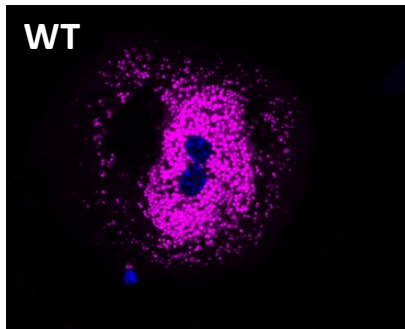
*HSD17B13(A) overexpressed in HEK293 cells
8 hour incubation with retinol; HPLC (Kedishvili)*

Hsd17b13-KO Modulates Lipid Vacuole Size



*Hsd17b13 KO mice and littermate controls
High-fat (60%) for 12 weeks (n=6/group)*

Hsd17b13 Inhibits Hepatic LD Fusion



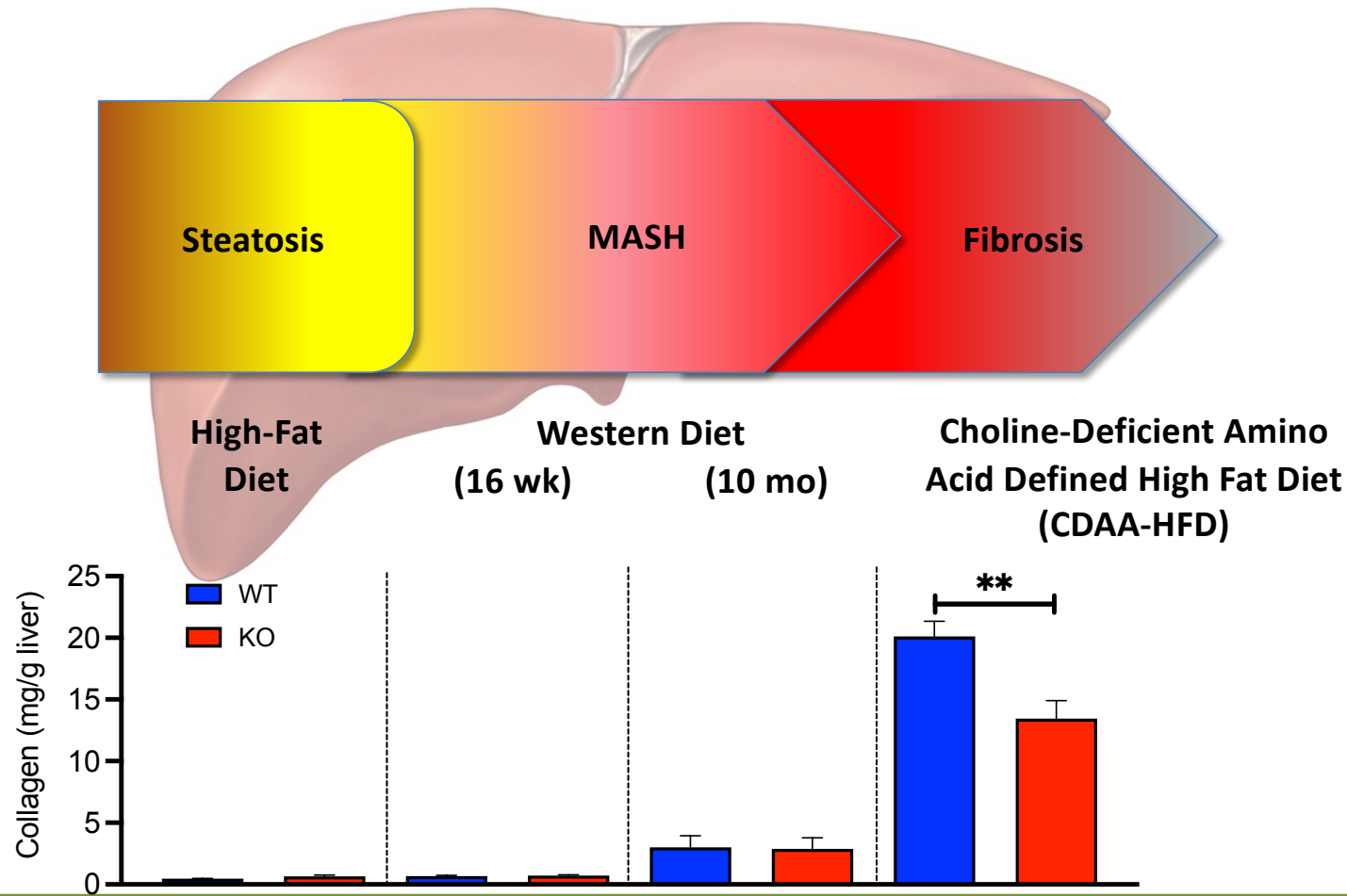
Primary mouse hepatocytes
Palmitate:oleate (2:1)

Male *Hsd17b13*-KO mice and
littermate controls
HFD for 8 weeks



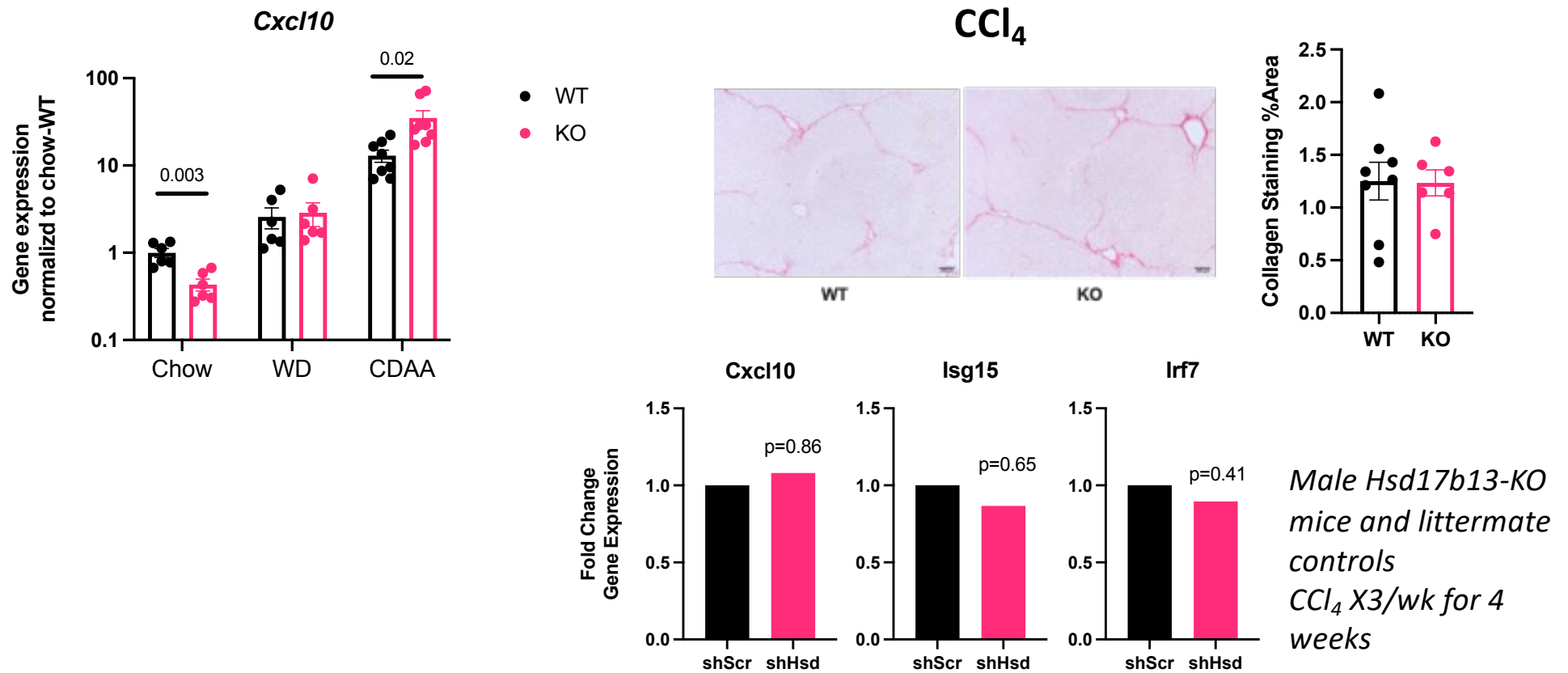
Dr. Lila Gonzalez Hodar, Unpublished

Hsd17b13 KO In Mouse Dietary Models

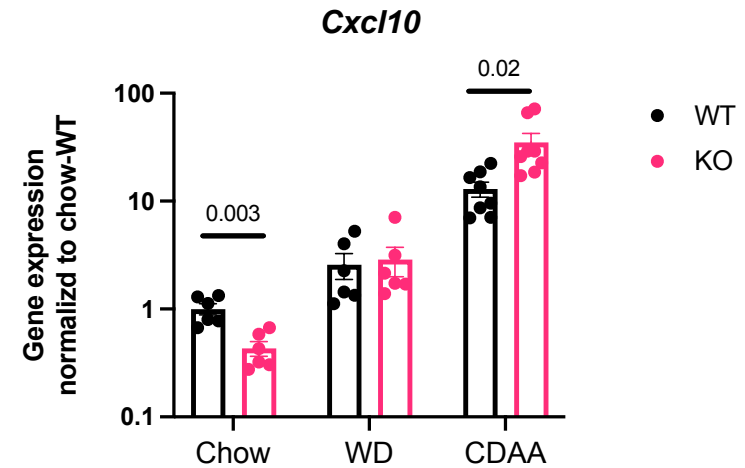
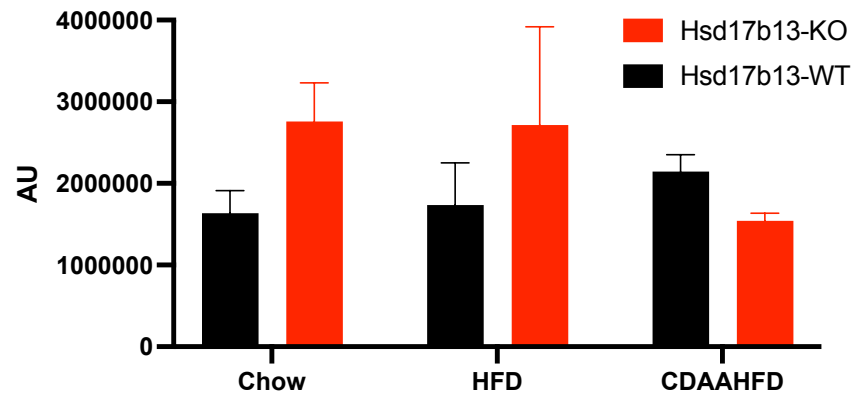


Ma, *Hepatology*, 2021; Unpublished Data

IFN Signaling Upregulation is Limited to CDAA-HFD



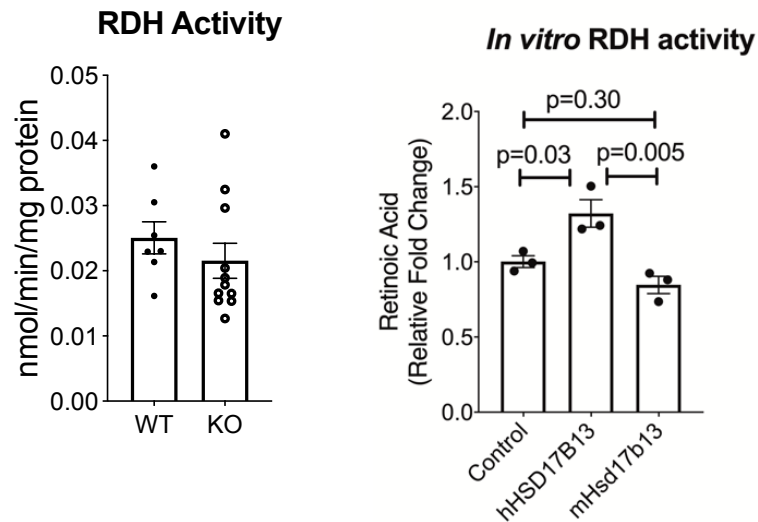
12,13-diHOME Across Models



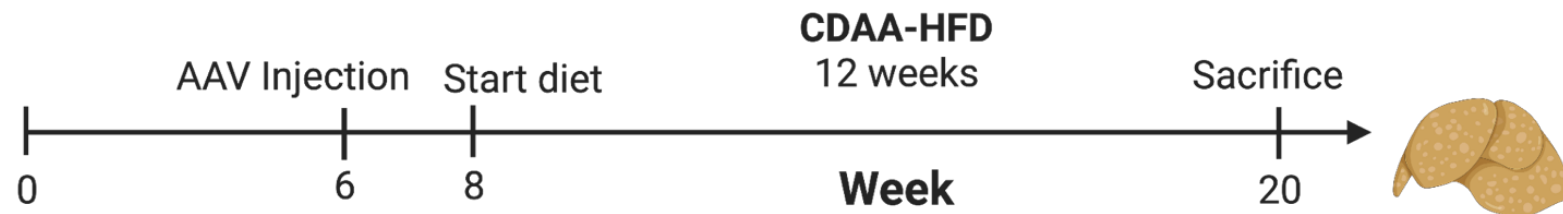
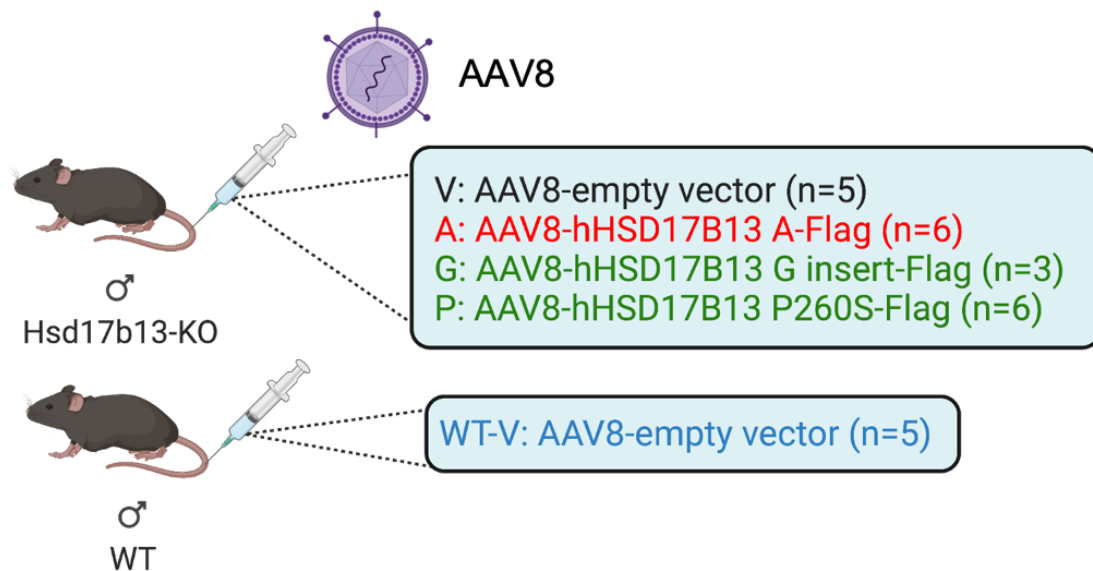
Effect of Hsd17b13-KO			
	Chow Diet	High-Fat/Western	CDAA-HFD
12,13-diHOME	↑	↑	↓
ISGs	↓	↓	↑
Fibrosis	Unaffected	Unaffected	↓

Is the Mouse a Relevant Model?

- Mouse vs. Human - Different effects on liver lipidome
- Mouse vs. Human – Different retinol dehydrogenase activity

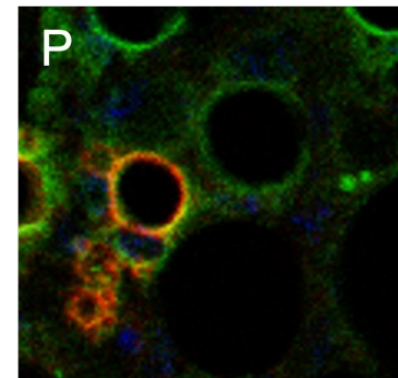
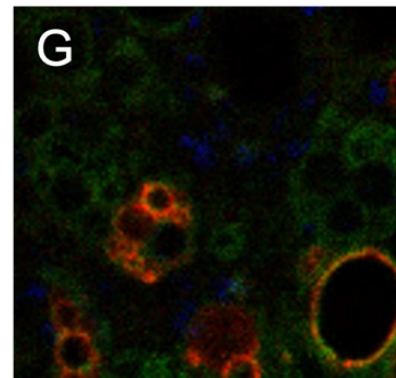
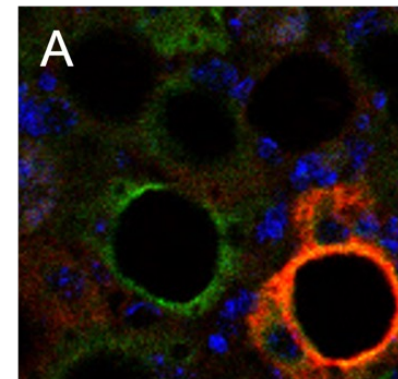
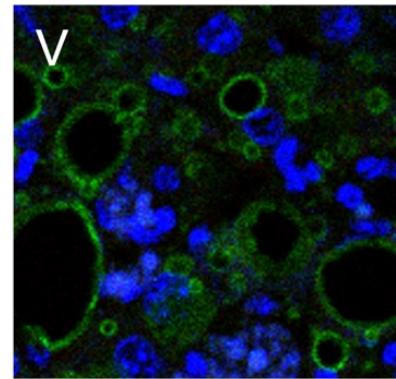
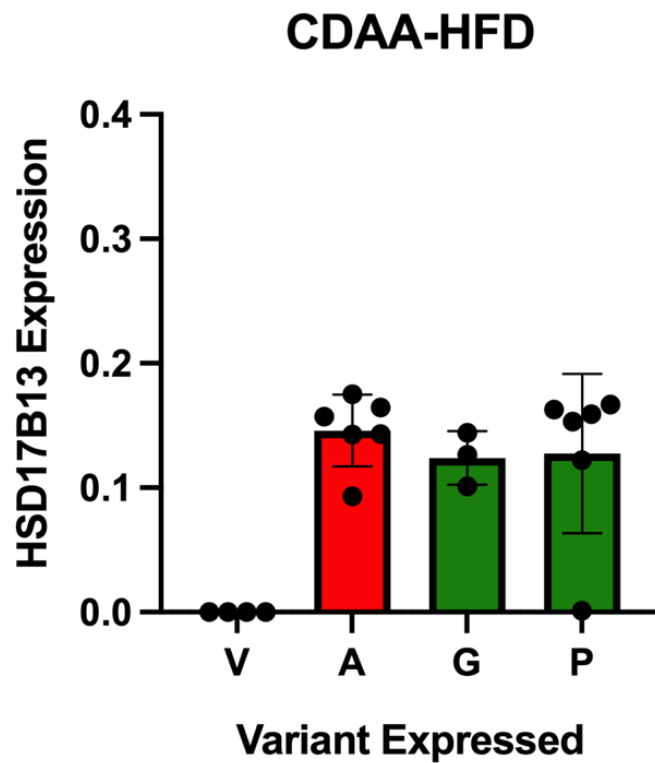


Expressing hHSD17B13 in Mouse Liver



Bowoo Lee

Expression of hHSD17B13 in Mouse Liver



Red: Flag
Blue: DAPI
Green: Perilipin-2