# Pharmacological Inhibition of HSD17B13 Is Hepatoprotective In Mouse Models of Liver Injury

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## BACKGROUND AND AIM

Genome-wide association studies identified a loss of function variant (rs72613567:TA) in HSD17B13 which confers protection against chronic liver diseases. As a result, HSD17B13 inhibitors may have clinical utility in the management of non-alcoholic steatohepatitis and other liver diseases. Here we describe the identification and characterization of a novel, potent, and selective HSD17B13 inhibitor with hepatoprotective effects in preclinical models of liver injury.

Does pharmacological inhibition of HSD17B13 protect from liver injury? rs72613567:TA (Loss of function)

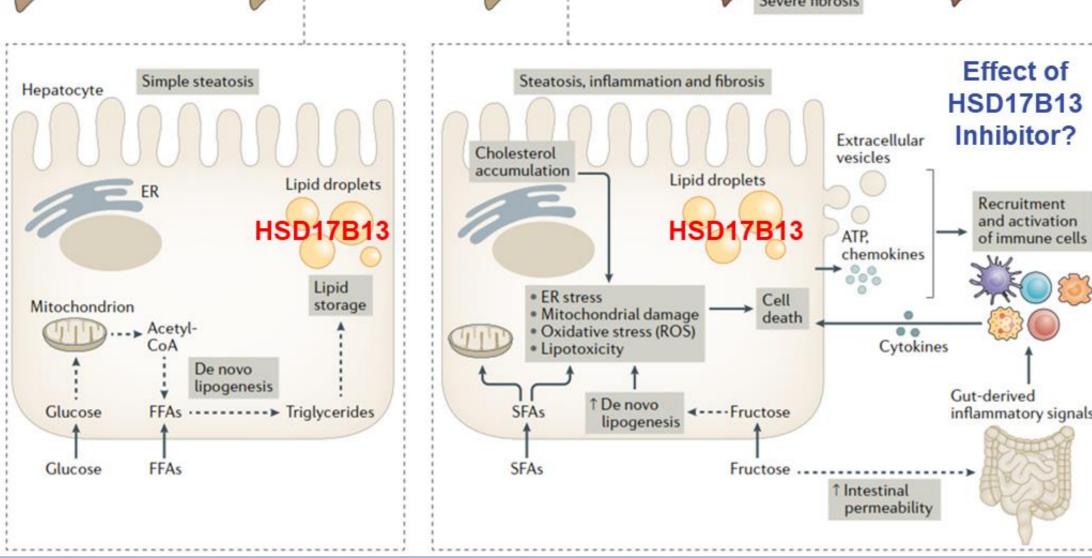


Figure 1. Rationale for targeting HSD17B13 for NASH. Figure modified from Huby et al., Nature Reviews Immunology 2021.

## **METHODS**

Multiple chemical series of HSD17B13 inhibitors (HSD17B13i) were identified and optimized for potency, selectivity, and pharmacokinetic properties.

In Vitro. Mass-spectrometry-monitored HSD17B13 inhibition in biochemical and cellular assays which utilized either recombinantly expressed HSD17B13 or HEK293 stably expressing human or mouse HSD17B13, respectively. Biochemical assays employed leukotriene B4 as substrate whereas cellular assays used estradiol.

In Vivo. A prodrug form (EP-037429) of HSD17B13 inhibitor (EP-036332) was evaluated in a mouse model of acute (adenoviral) and chronic liver injury (choline deficient, L-amino acid defined, high fat diet; CDAAHF; A16092201). Gene and protein markers of inflammation, injury and fibrosis were measured in plasma and liver of mice treated with HSD17B13i or sh-adenoviral-mediated knockdown. Transcriptomics and untargeted lipidomics of HSD17B13 inhibition were compared to HSD17B13 knockdown.

Primary human hepatocytes (PHH) deficient for HSD17B13 (rs72613567:TA) were used in rescue studies for lipidomic experiments where HSD17B13 was restored via infection of an adenoviral construct overexpressing HSD17B13.

Statistical analysis was performed with One Way ANOVA followed by Dunnett's multiple comparison test.

## RESULTS

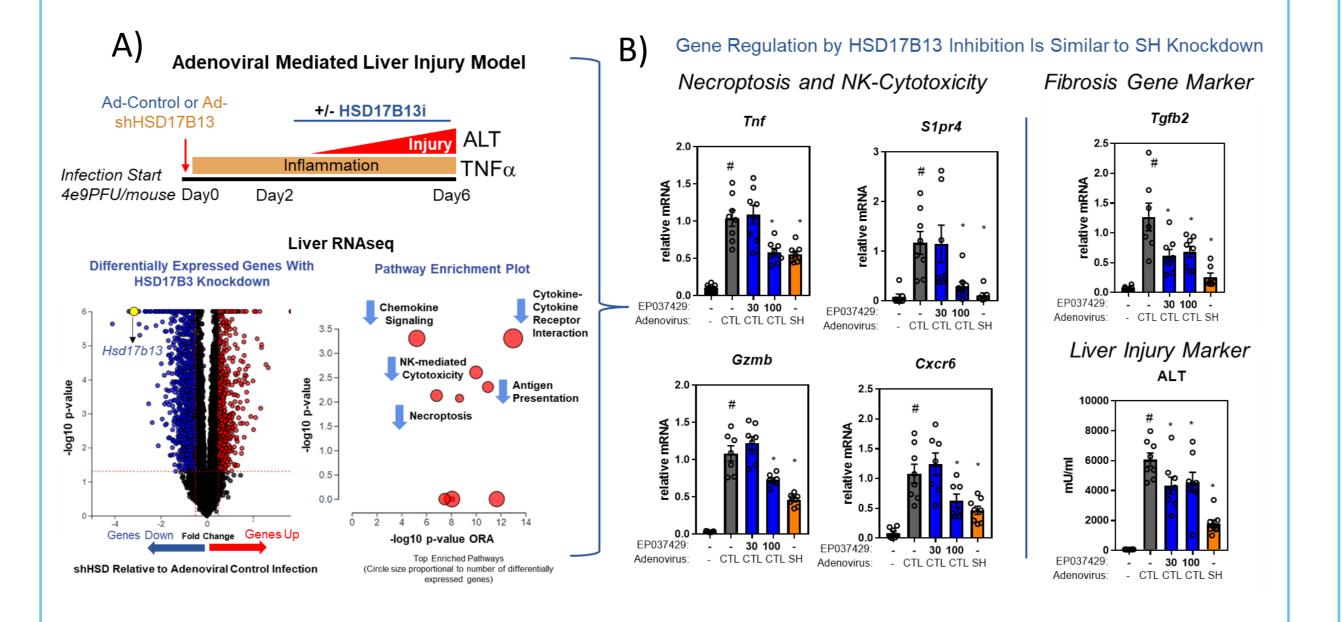
#### **EP-036332** is a Potent and Selective **HSD17B13i**

**Table 1.** Potency and Selectivity of EP-036332.

Assay		EP-036332
Biochemical Activity <sup>1,2</sup> Inhibition of Substrate Conversion (RF/MS)	Human <sup>1</sup> , IC <sub>50</sub>	14 nM
	Mouse <sup>2</sup> , IC <sub>50</sub>	2.5 nM
Cellular Activity <sup>2</sup> Inhibition of E2 to E1 in HEK293(RF/MS)	Human, IC <sub>50</sub>	47 nM
	Mouse, IC <sub>50</sub>	55 nM
Selectivity <sup>3</sup> IC <sub>50</sub> HSD17Bx/ IC <sub>50</sub> HSD17B13	HSD17B1	400 fold
	HSD17B2	650 fold
	HSD17B3, B4, B5, B10, B11	>12500 fold

#### **HSD17B13** Inhibition Is Hepatoprotective In **Adenoviral Injury Model**

RF/MS= Rapid Fire Mass Spectrometry with E2=Estradiol;



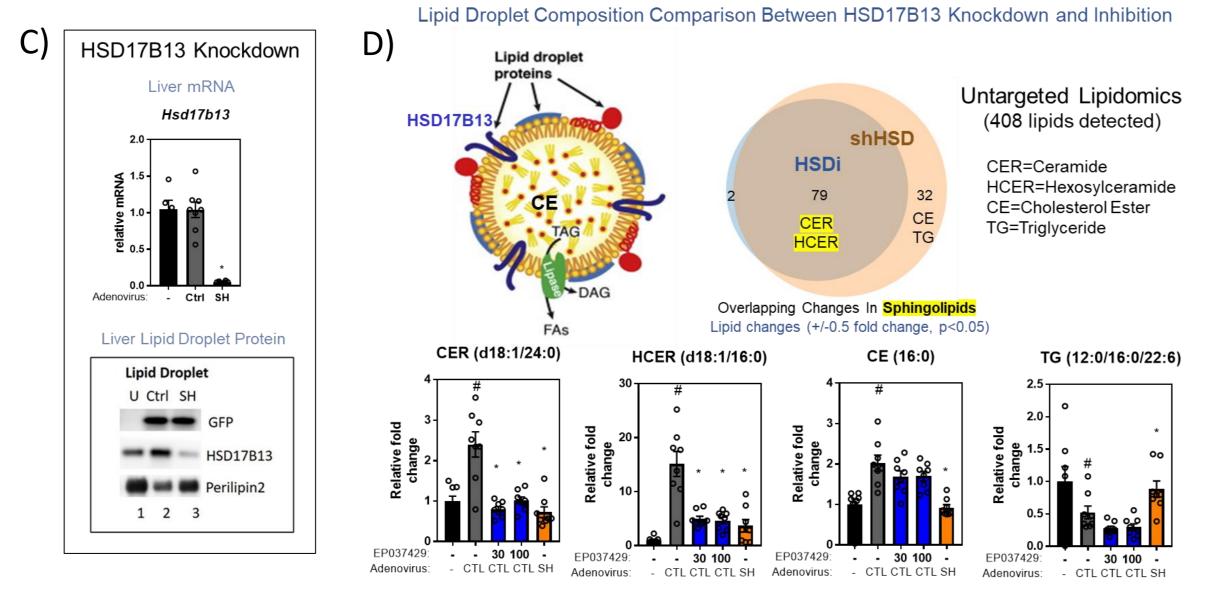


Figure 2. Adenoviral Liver Injury Model with tool compound EP-037429, prodrug form of EP-036332. A) Study Design and Liver RNAseq analysis of HSD17B13 knockdown (shHSD17B13). B) Liver gene expression profile comparison between HSD17B13i and shHSD17b13. C) Lipid droplet protein immunoblot. D) Lipidomics of lipid droplets from mouse livers treated with HSD17B13i or shHSD17B13. \*p<0.05 vs adenoviral infected vehicle; #p<0.05 vs uninfected vehicle. n=8. *Tnf*=tumor necrosis factor; *S1pr4*=S1P receptor 4; *Gzmb*=Granzyme B; *Cxcr6*=CXCmotif chemokine receptor 6; *Tgfb2*=transforming growth factor beta isoform 2.

### RESULTS

**HSD17B13** Inhibition Is Hepatoprotective and Anti-Inflammatory in a 4-Week CDAAHF Diet Model

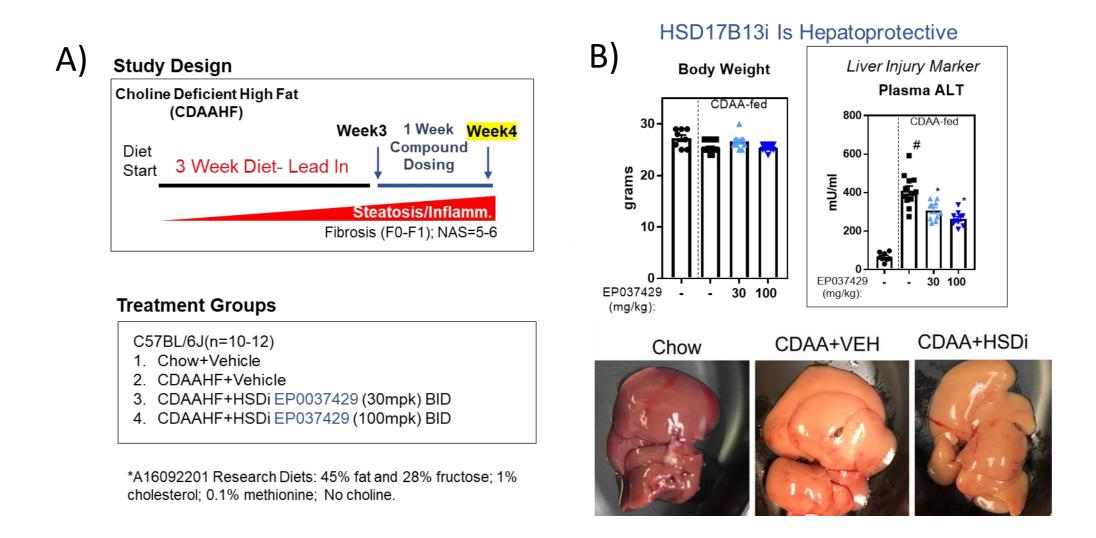


Figure 3. Choline Deficient High Fat Diet (CDAAHF). A) Study Design: 3-Week CDAAHF diet lead-in followed by 1-week CDAAHF+HSD17B13i; B) Body weight and plasma ALT. \*p<0.05 vs CDAA HF vehicle; #p<0.05 vs chow vehicle. n=10-12

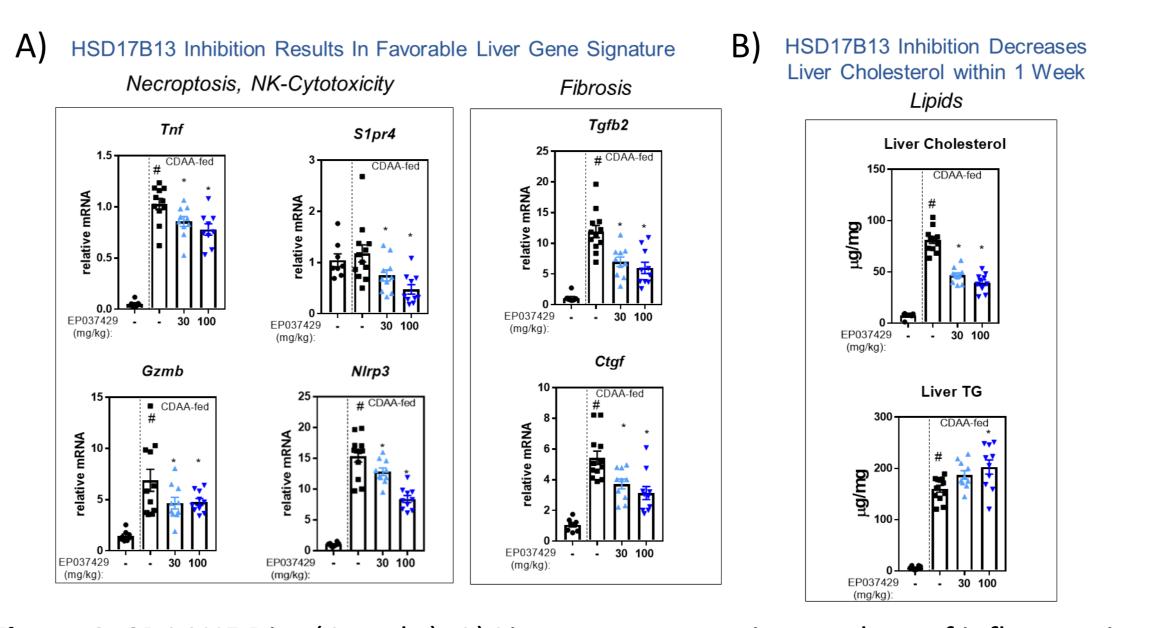


Figure 4. CDAAHF Diet (4weeks). A) Liver gene expression markers of inflammation, injury, and fibrosis in mice treated +/- HSD17B13 inhibitor. Nlrp3=NOD/LRR/pyrin domain-containing protein 3; Ctgf=Connective tissue growth factor. B) Total liver lipids. \*p<0.05 vs CDAAHF vehicle; #p<0.05 vs chow vehicle. n=10-12.

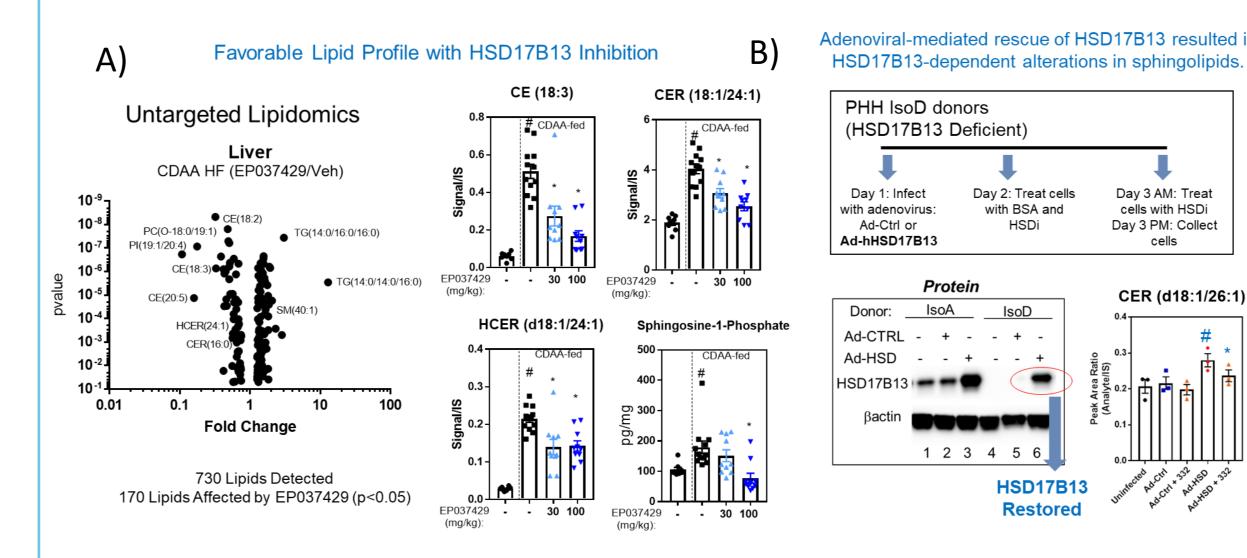
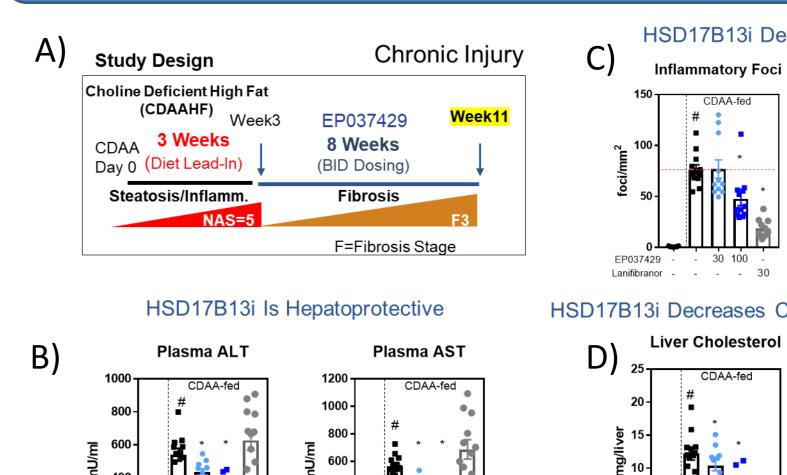


Figure 5. Lipidomics of mouse liver and primary human hepatocytes (PHH). A) Mouse livers from CDAAHF (4weeks). Untargeted Lipidomics of livers treated with HSD17B13 Inhibitor (CE=cholesterol ester, CER=ceramide; HCER=hexosylceramide). \*p<0.05 vs CDAA HF vehicle; #p<0.05 vs chow vehicle. N=10-12. B) PHH, HSD17B13 rescue alters ceramide. \*p<0.05 vs AdHSD; #p<0.05 vs Ad Control (Ctrl);

## RESULTS

#### **HSD17B13 Inhibition Is Anti-Fibrotic** In a Chronic CDAAHF Diet Model



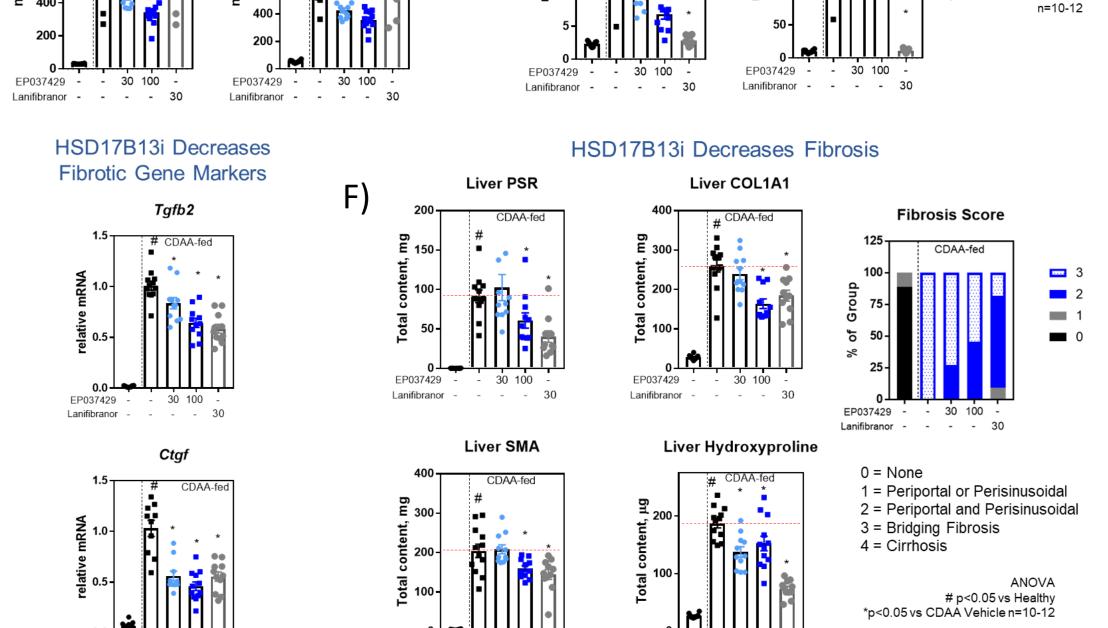
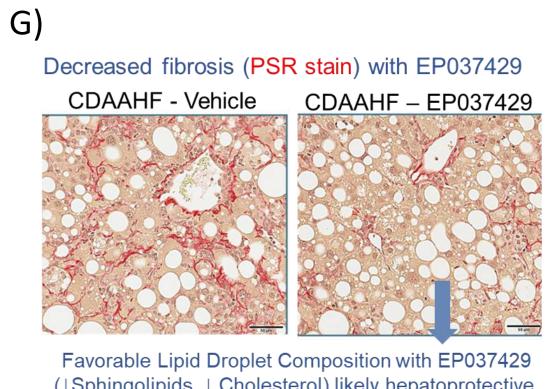


Figure 6. CDAAHF (11weeks). A) Study Design. 3-Week CDAAHF diet lead-in followed by 8-week CDAAHF+HSD17B13i. B) Plasma injury markers; C)Histological inflammatory end points; D) Lipids; E) Fibrotic gene markers; F) Histological fibrosis markers and scoring; G) Picro Sirius Red (PSR) Fibrosis Stain. \*p<0.05 vs CDAAHF vehicle; #p<0.05 vs chow vehicle.



↓Sphingolipids, ↓ Cholesterol) likely hepatoprotective.

# CONCLUSION

Hepatoprotection by HSD17B13 inhibition in rodent injury models is characterized by a favorable bioactive lipid profile that parallels a decreases in markers of cytotoxic immune cell activation, cell death, and

Consistent with *in vivo* observations, adenoviral-mediated rescue of HSD17B13 in human hepatocytes homozygous for the rs72613567:TA allele resulted in HSD17B13-dependent alterations in sphingolipids.

Future studies will explore HSD17B13-dependent biomarkers of target engagement.

Overall, these data pharmacologically validate inhibition of HSD17B13 and support further evaluation of HSD17B13i for NASH.