

17-β Hydroxysteroid Dehydrogenase 13 Inhibitors are Hepatoprotective and Anti-Inflammatory In a Mouse Model of Autoimmune Hepatitis

Manuel Roqueta-Rivera, Yaohui Nie, Jordan Butts, Mary Chau, Kelsey Garlick, Archie Reyes, Jonathan Lloyd, Joshua Klaene, Sourav Ghorai, Jiang Long, Bin Wang, Scott Mitchell, Yat Sun Or, Bryan Goodwin.
Enanta Pharmaceuticals, Inc. 500 Arsenal Street, Watertown, MA 02472, USA.



BACKGROUND

Genome-wide association studies identified a loss of function gene variant (rs72613567:TA) for 17-β hydroxysteroid dehydrogenase 13 (HSD17B13), a hepatic lipid droplet-associated protein, linked to decreased risk for chronic liver diseases. HSD17B13-deficient TA variant carriers have delayed onset of autoimmune hepatitis (AIH)¹, and presence of the TA variant decreases an AIH polygenic risk score in an allele-dependent manner². HSD17B13 inhibitors, previously shown to be anti-inflammatory *in vivo* with modulation of sphingolipids, were evaluated in a mouse model of AIH for anti-inflammatory and hepatoprotective effects.

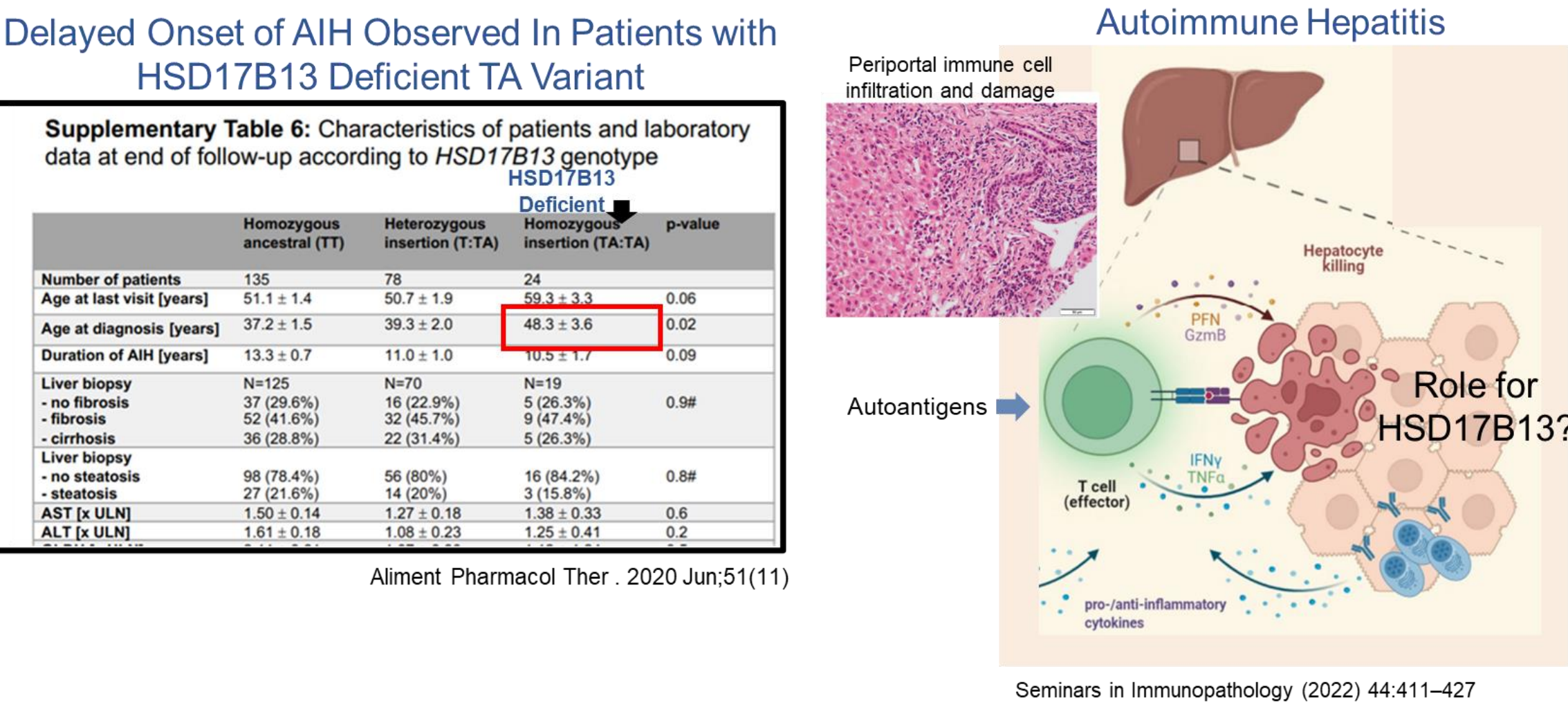


Figure 1. Rationale for targeting HSD17B13 for Autoimmune Hepatitis.

1. Mederacke et al, *Aliment Pharmacol Ther* 2020 Jun 51(11); 1160-1168
2. Zandanel et al, *J Pers Med*. 2023 Mar 17;13(3):540; online
3. Schultheiß et al, *Semin Immunopathol* 2022; 44(4): 411–427.

METHODS

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

In Vitro. HSD17B13 inhibition was monitored by Rapid Fire mass spectrometry in biochemical and cellular assays, which utilized either recombinantly expressed HSD17B13 or HEK293 stably expressing human or mouse HSD17B13, respectively. Leukotriene B4 served as the substrate for biochemical assays, whereas estradiol was used in cellular assays.

In Vivo. 8-week-old male C57BL/6J mice were pretreated with HSD17B13i by oral gavage for 3 days, followed by retro-orbital vein delivery of concanavalin (ConA) one hour after last dose. Liver, spleen, and plasma were collected at 6 hours post-ConA injection. Plasma ALT levels were measured by a colorimetric enzymatic assay. Inflammatory gene markers were evaluated in liver and spleen by qPCR. Plasma cytokines and chemokines were measured by mesoscale immunoassay. The HSD17B13i effect on sphingolipids was evaluated in mouse liver and primary human hepatocytes by mass spectrometry. Primary human hepatocytes deficient for HSD17B13 (rs72613567:TA) were used in rescue studies, 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

HSD17B13 Inhibitors are Potent and Selective

Table 1. Potency and Selectivity of Distinct Chemical Series: EP-036332 and EP-040081.

Assay		Series1: EP-036332	Series2: EP-040081
Biochemical Activity¹ Inhibition of product formation (RF/MS)	Human ¹ , IC ₅₀	14 nM	79 nM
	Mouse ² , IC ₅₀	2.5 nM	74 nM
Cellular Activity² Inhibition of product formation (RF/MS)	HEK293-Human, IC ₅₀	47 nM	34 nM
	HEK293-Mouse, IC ₅₀	55 nM	1083 nM
Selectivity³ IC ₅₀ ratio (HSD17B3/ HSD17B1)	HSD17B1	>7000×	>1265×
	HSD17B2	4357×	322×
	HSD17B4	>7000×	982×
	HSD17B3, B5, B10, B11	>7000×	>1265×
	HSD11B1	>7000×	>630×

¹RF/MS= Rapid Fire Mass Spectrometry with Leukotriene B4;
²RF/MS= Rapid Fire Mass Spectrometry with Estradiol- α ;
³NADH Luminescence Assay

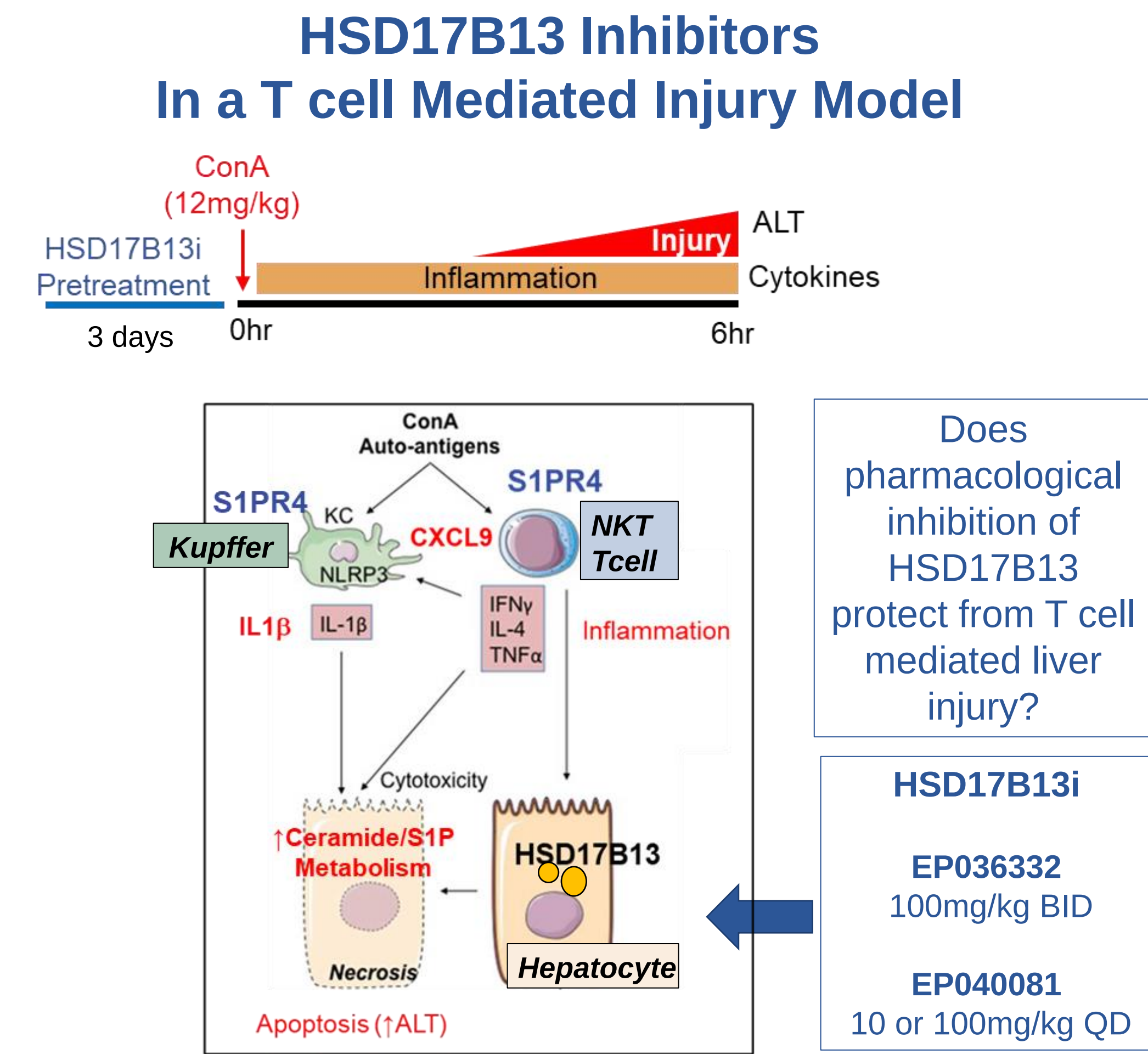


Figure 2. Concanavalin acute liver injury model study design and key end points evaluated +/- HSD17B13 inhibition.

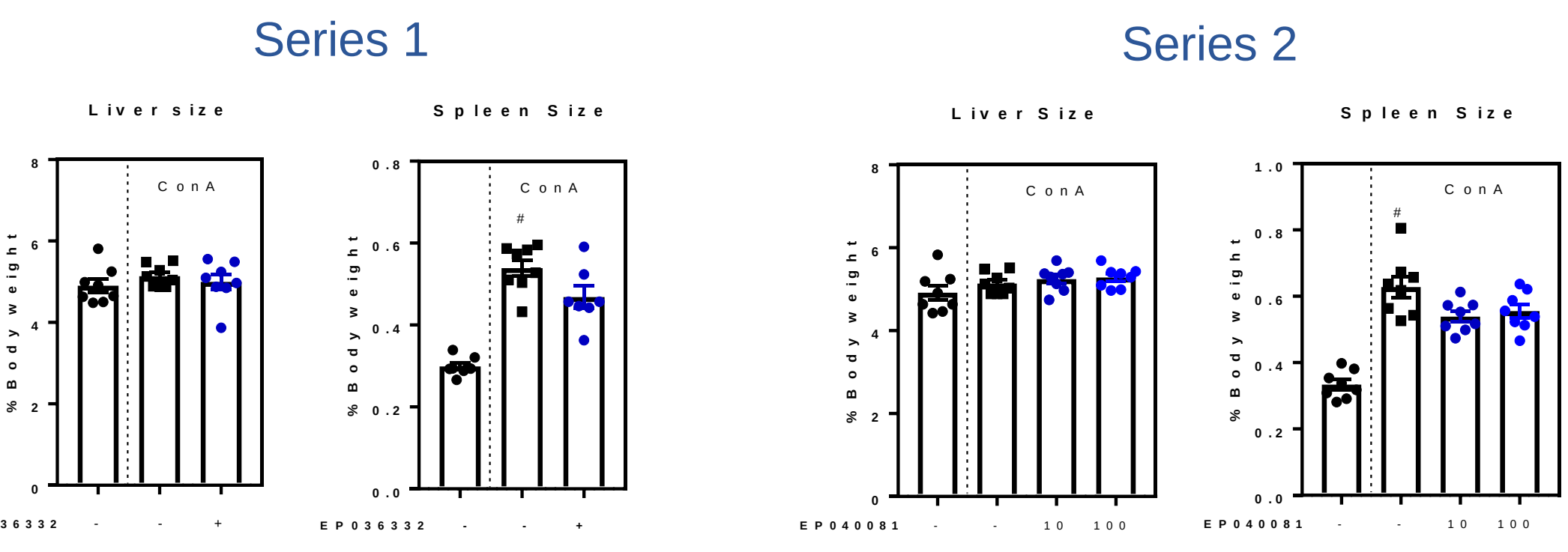


Figure 3. Liver and spleen size were not impacted by HSD17B13 inhibition (mg/kg). #p<0.05 vs uninjured + vehicle. n=8

RESULTS

HSD17B13 Inhibitors are Hepatoprotective and Anti-Inflammatory

HSD17B13i Decreases Plasma ALT and Circulating Inflammatory Cytokines

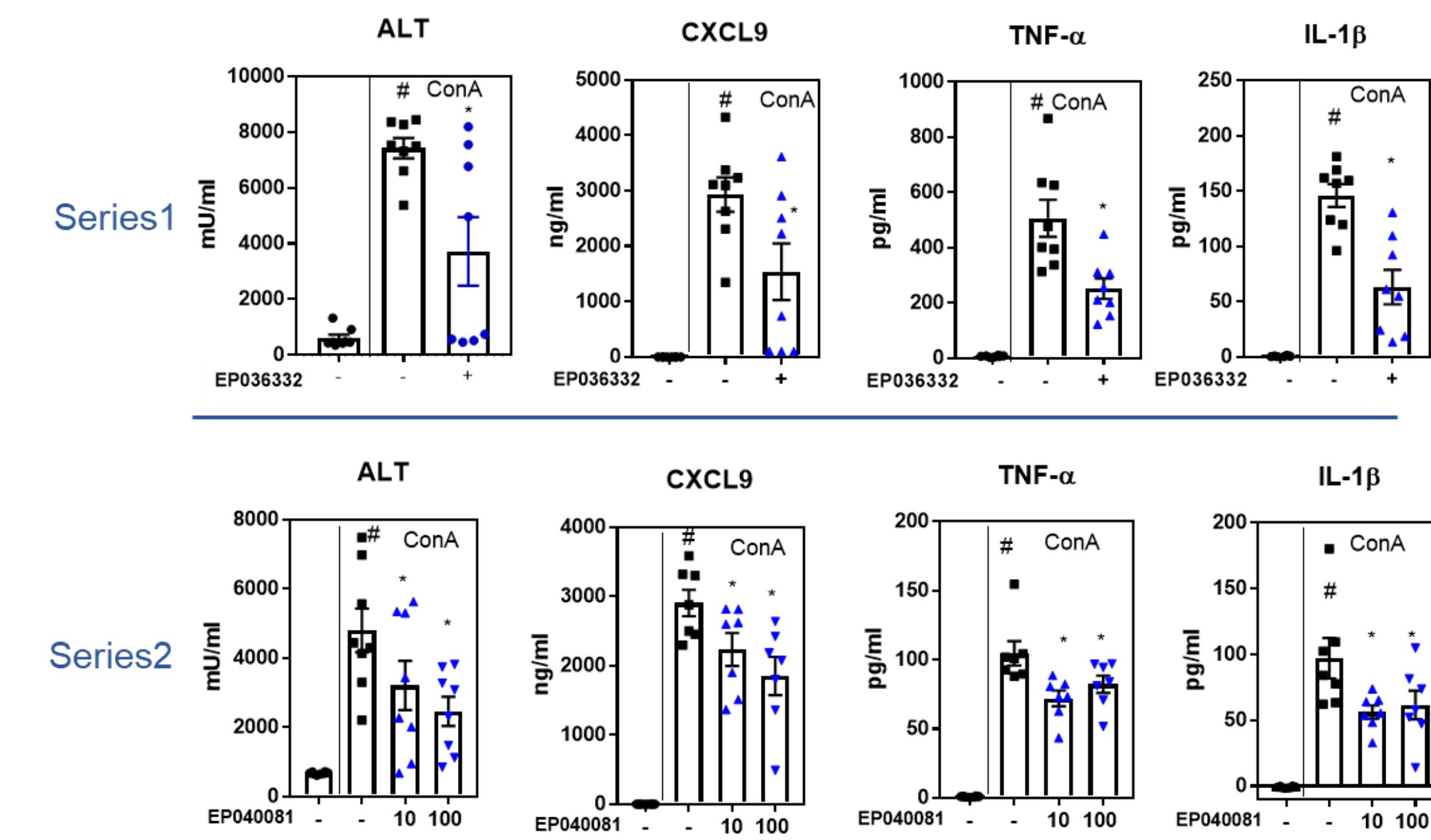


Figure 4. Plasma ALT and cytokines (TNFα, IL1β, CXCL9). *p<0.05 vs ConA + vehicle; #p<0.05 vs uninjured + vehicle. n=8

HSD17B13 Inhibitors Modulate Gene Markers of T cell Activation

HSD17B13i Attenuates Inflammatory Gene Markers In Liver

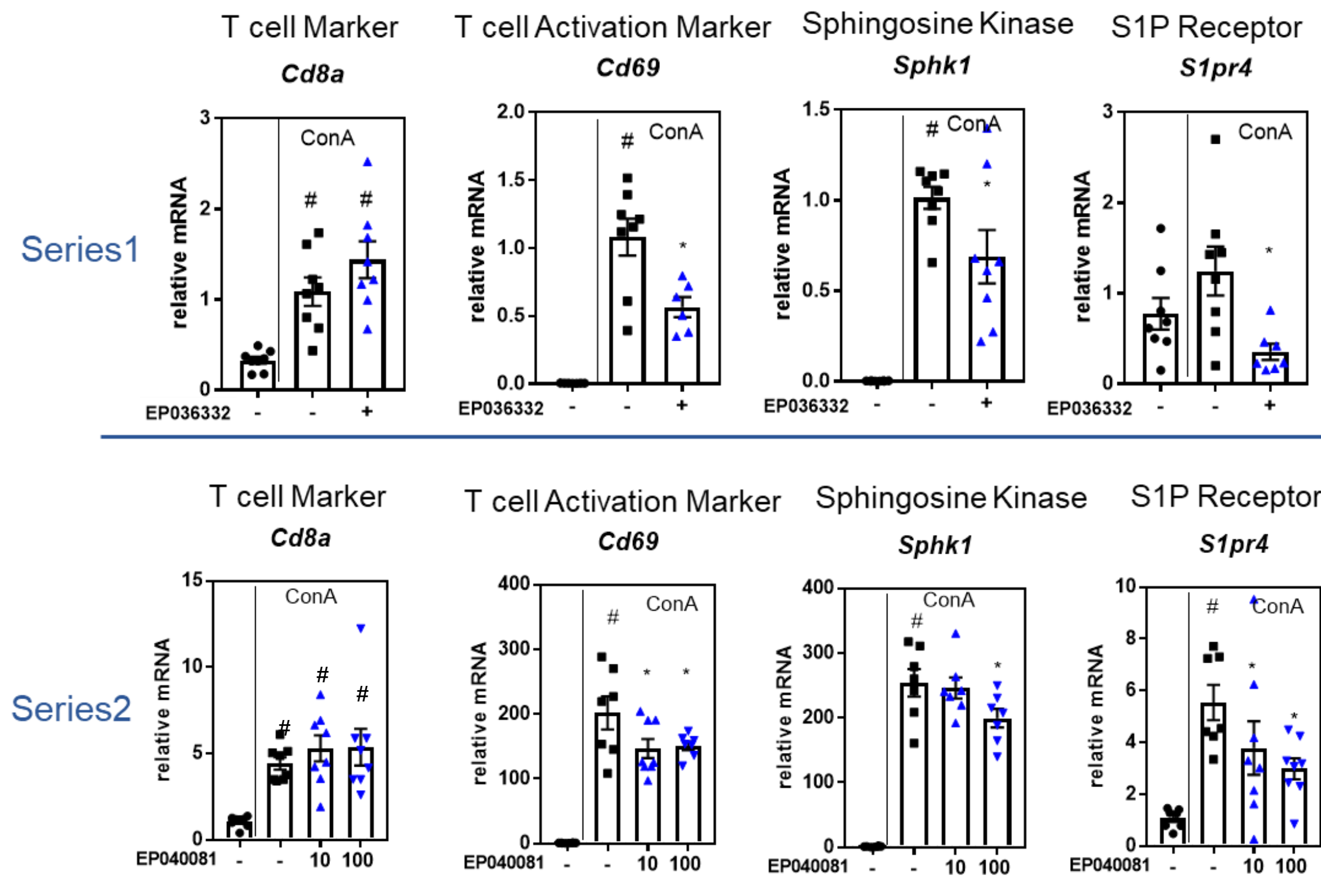


Figure 5. Liver gene expression. T cell gene marker (*Cd8*) was not altered by HSD17B13i in ConA treated livers, however, markers of immune cell activation (*Cd69*, *S1pr4*, *Sphk1*) were decreased. *p<0.05 vs ConA + vehicle; #p<0.05 vs uninjured + vehicle. n=8.

RESULTS

HSD17B13 Inhibitors Decrease Liver Ceramides In T cell Mediated Injury Model

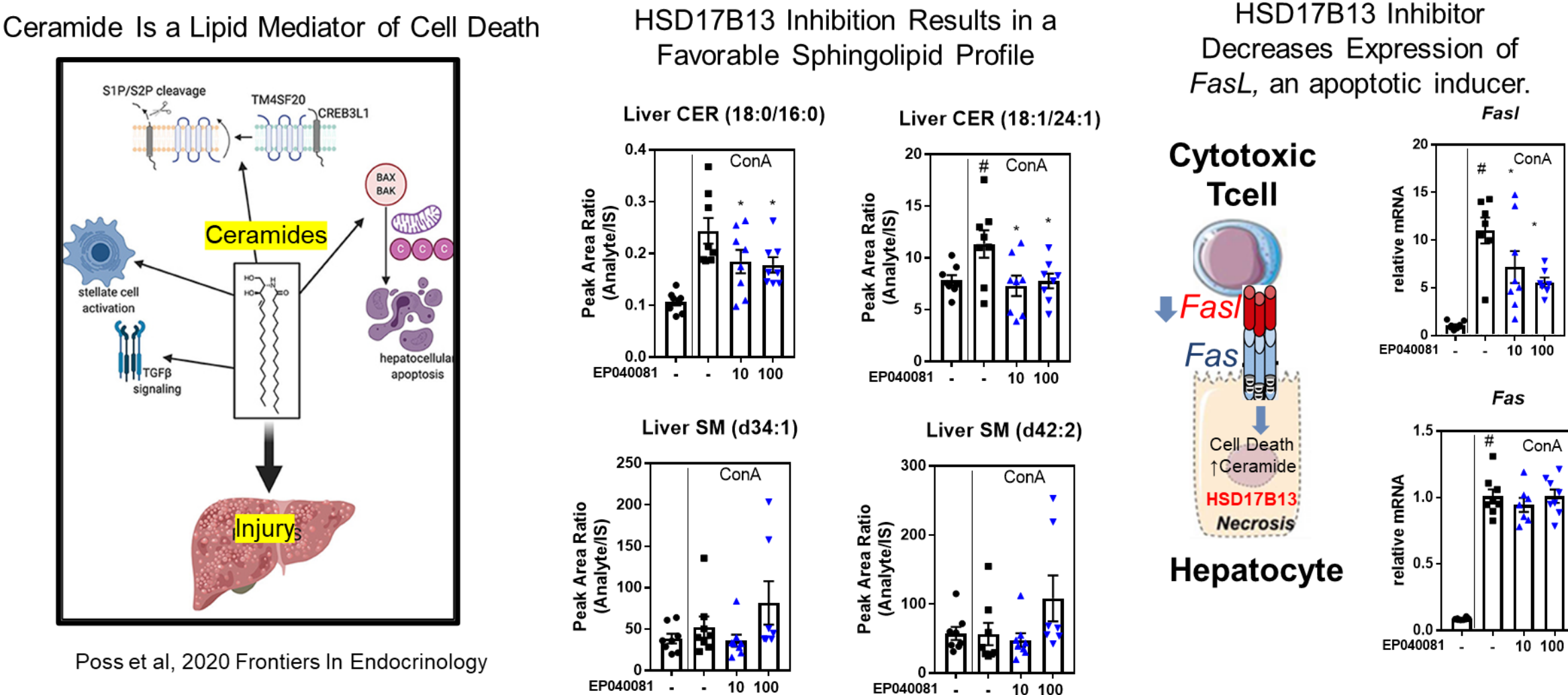
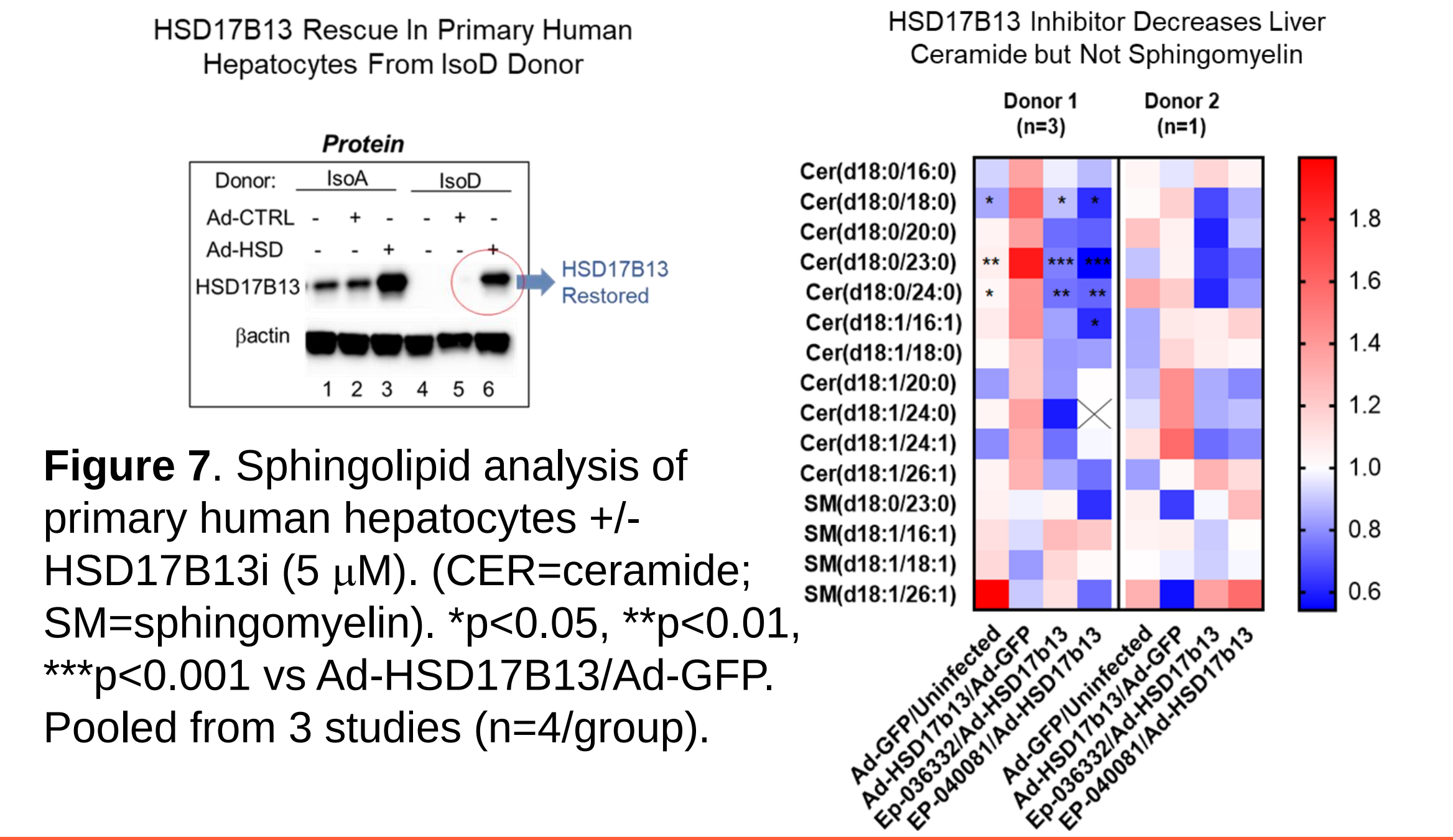


Figure 6. Sphingolipid analysis (CER=ceramide; SM=sphingomyelin) and gene markers of cell death (*FasL*, *Fas*) in mouse livers +/- HSD17B13i. *p<0.05 vs ConA + vehicle; #p<0.05 vs uninjured + vehicle. n=8

Ceramide Levels are Regulated by HSD17B13 Inhibition in Human Hepatocytes



CONCLUSION

Hepatoprotection by HSD17B13 inhibition in a model of autoimmune hepatitis is characterized by a favorable bioactive lipid profile that parallels a decrease in markers of cytotoxic immune cell activation and cell death.

