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Supplementary Materials

Role of HNF4α-cMyc interaction in liver regeneration and recovery after acetaminophen-induced acute liver injury

Manasi Kotulkar, Diego Paine-Cabrera, Sarah Abernathy, Dakota R. Robarts, Wendena S. Parkes, Kristi Lin- Rahardja, September Numata, Margitta Lebofsky, Hartmut Jaeschke and Udayan Apte

Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, KS

Material and Methods:

Animals, Treatment and Tissue Harvesting Continued

Mice were treated with either 300 or 600 mg/kg APAP, and euthanized at 0, 1, 3, 6, 12, 24, 72, and 96 h following APAP treatment. Wild type (WT) and hepatocyte specific HNF4α -KO were generated as described before by injecting AAV8-TBG-eGFP or AAV8-TBG-CRE (Vector Biolabs), respectively to HNF4α -floxed mice [1]. To study the effect of both HNF4α and cMyc deletion on liver regeneration, HNF4α-cMyc double floxed mice were injected i.p. with AAV8-TBG-eGFP or AAV8-TBG-CRE, resulting in WT and hepatocyte specific HNF4α-cMyc double KO (DKO) animals, respectively. All knockout mouse experiments were performed one week after AAV8 injections. Mice were fasted overnight, treated with 300 mg/kg of APAP, and euthanized at 0, 1, 3, 6, 12, 24, and 72 h following APAP treatment to obtain blood and livers. Parts of liver tissue were processed separately to obtain paraffin sections, frozen sections, RNA samples, and nuclear, cytoplasmic, and total protein extracts, as described previously [2].

Western blotting and qPCR

Protein estimation and Western blot analysis were performed using pooled protein extracts, as described before [3]. Imaging for Western blots was done using Li-Cor Odyssey FC, and the densitometry was done using Image Studio software. The antibodies used in this study are listed in Table 1. RNA isolation and conversion to cDNA

were performed as previously described [3]. qPCR was performed to measure the mRNA expression of various genes using 100 ng of cDNA per reaction and using the manufacturer's protocol (ThermoFisher). qPCR analysis was done on the BioRad CFX384. The 18s gene expression in the same samples was used to normalize the ct values as described previously [4]. Post-APAP treatment time points were compared with 0 h control of each respective group to determine mRNA levels. Primers used for real time PCR are listed in Table 2.

Serum ALT

Serum alanine aminotransferase (ALT) was measured using Pointe Scientific ALT Assay kit by Fisher Scientific, according to the manufacturer's protocol.

Staining Procedures

Paraffin-embedded liver sections (5 μm thick) were used for immunohistochemical staining of HNF4α (Perseus Proteomics PP-H1415-00, 1:500) and Ki67, (Cell signaling technology 12202S,1:400), as previously described [3]. Liver necrosis was determined with hematoxylin and eosin (H&E) staining.

Measurement of hepatic glutathione (GSH) and APAP protein adducts

Total hepatic GSH levels were measured in liver homogenates using a modified method of Tietze as described previously [5].

Isolation of Microsomes

Microsomes were isolated from fresh liver sections as described previously to analyze CYP2E1 expression [6].

Nuclear and Cytoplasmic fractions

Nuclear, and cytoplasmic fractions were generated using the NE-PER™ Nuclear and Cytoplasmic Extraction kit, according to the manufacturing protocol (Thermo Fisher cat # 78835).

Immunoprecipitation (IP)

Nrf2 IP was performed using Dynabeads G (10003D) according to the manufacturer's protocol. A total 1 mg of protein from whole liver lysate and 1 µg of Nrf2 antibody was used to precipitate the protein.

Chromatin Immunoprecipitation Assay (ChIP)

The ChIP assay was performed using Abcam high-sensitivity ChIP kit (ab 185913), according to the manufacturer's protocol. qPCR was performed on the BioRad CFX384 to calculate the fold enrichment after ChIP. Ct values of IP were normalized to respective IgG values.

1. Huck, I., et al., *Hepatocyte Nuclear Factor 4 Alpha Activation Is Essential for Termination of Liver Regeneration in Mice.* Hepatology, 2019. **70**(2): p. 666-681.
2. Robarts, D.R., et al., *Regulation of Liver Regeneration by Hepatocyte O-GlcNAcylation in Mice.* Cell Mol Gastroenterol Hepatol, 2022. **13**(5): p. 1510-1529.
3. Borude, P., et al., *Hepatocyte-specific deletion of farnesoid X receptor delays but does not inhibit liver regeneration after partial hepatectomy in mice.* Hepatology, 2012. **56**(6): p. 2344-52.
4. Livak, K.J. and T.D. Schmittgen, *Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method.* Methods, 2001. **25**(4): p. 402-8.
5. Xie, Y., et al., *Inhibitor of apoptosis signal-regulating kinase 1 protects against acetaminophen-induced liver injury.* Toxicol Appl Pharmacol, 2015. **286**(1): p. 1-9.
6. Natarajan, S.K., et al., *Oxidative stress in experimental liver microvesicular steatosis: role of mitochondria and peroxisomes.* J Gastroenterol Hepatol, 2006. **21**(8): p. 1240-9.

Supplementary Figure 1:

(A) Densitometry analysis of HNF4α Western blot using nuclear and cytoplasmic extracts after 300 mg/kg dose of APAP and (B) 600 mg/kg dose of APAP normalized to the loading control.

Supplementary Figure 2:

(A) Quantification of necrotic area in the liver sections of WT and HNF4α-KO mice treated with 300 mg/kg APAP. (B) Densitometry analysis of phosphor- RIP1 and (C) phosphor GSK3β Western blot performed using liver lysates of WT and HNF4α-KO mice treated with APAP 300 mg/kg normalized to the loading control.

Supplementary Figure 3:

(A) HNF4α and cMyc genotyping data for DKO mice. (B) Quantification of necrotic area in the liver sections of WT and DKO mice treated with 300 mg/kg APAP.

Supplementary Figure 4:

(A) Serum ALT levels of WT, HNF4α-KO and DKO mice treated with APAP 300 mg/kg dose at various time points. (B) Representative photomicrographs of H&E-stained liver sections. Dotted lines demark area of necrosis. CV, central vein; Original magnification, 200X; * Indicates significant difference at *=P<0.05, **=P<0.01, ***=P<0.001, ****=P<0.0001

Supplementary Figure 5:

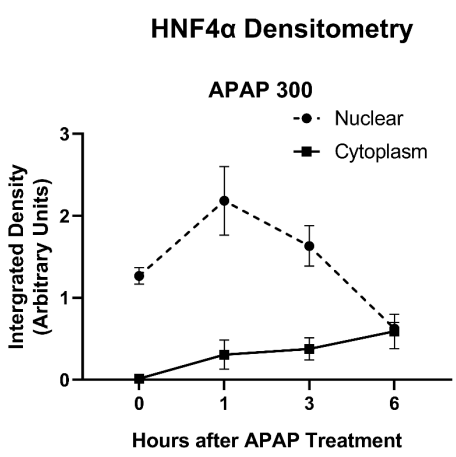
(A) Quantification of Ki67 positive cells in WT and DKO mice at 0, 6, 24, 48 h after APAP overdose.

Supplementary Figure 6:

(A) IP pull down of Nrf2 from liver tissues of WT mice. Western blot analysis was performed on Nrf2 to show successful pulldown and HNF4α and cMyc.

Supplementary Figure 1:

A



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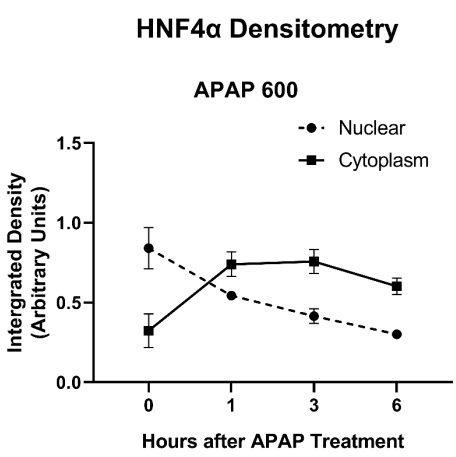
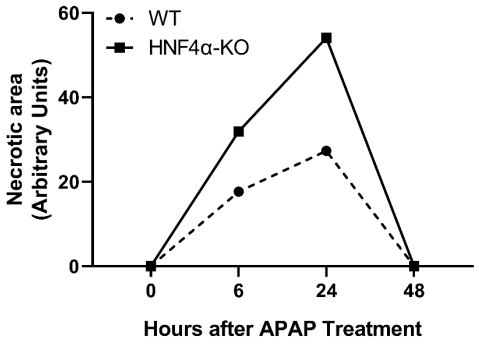


Supplementary Figure 2:

A



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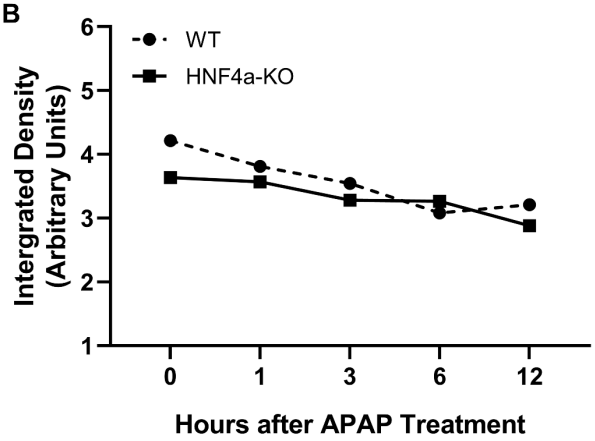

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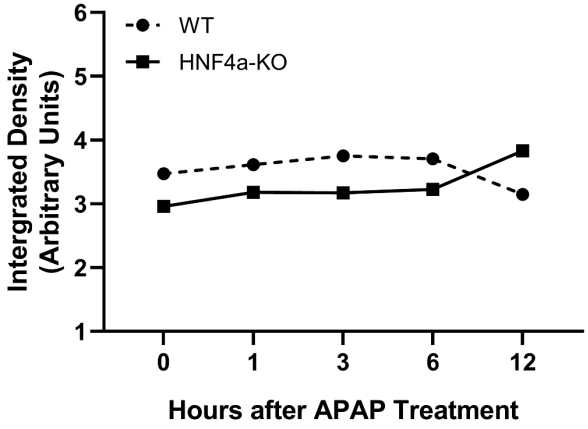
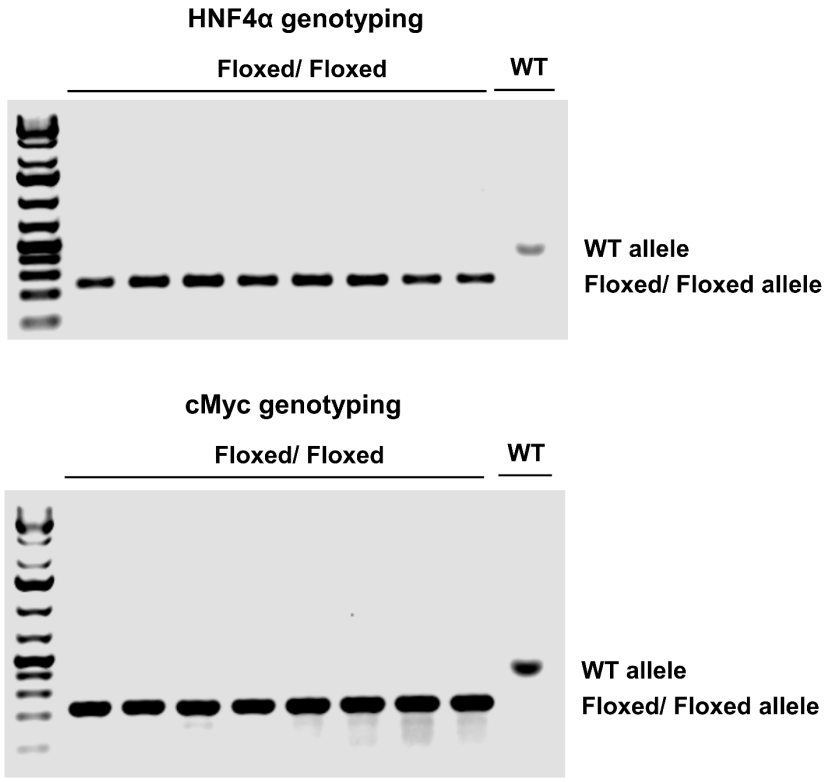


Supplementary Figure 3:

A



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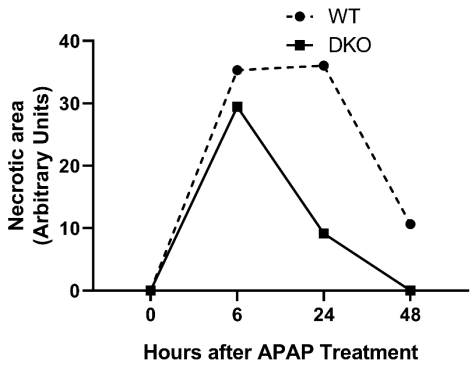
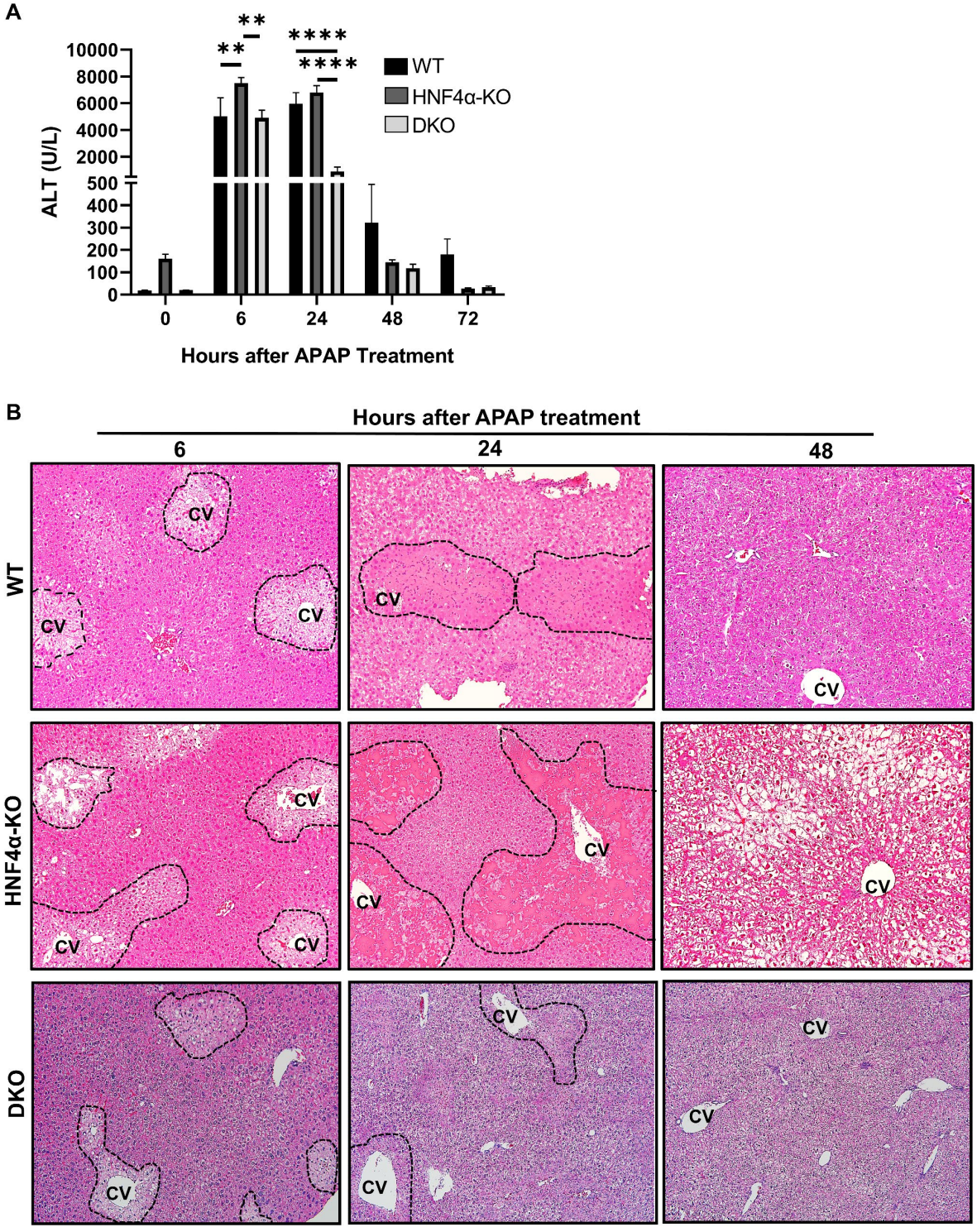


Supplementary Figure 4:



Supplementary Figure 5:

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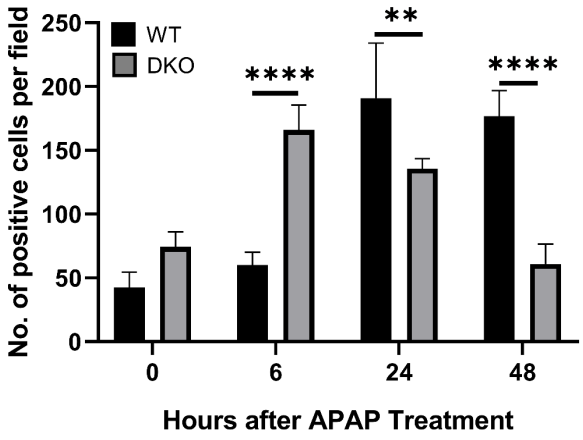

Supplementary Figure 6: