SUPPLEMENTARY MATERIALS AND METHODS

Histology

Paraffin-embedded liver samples were sectioned, dewaxed and hydrated. Immunohistochemistry was performed as described[1]. Quantitative assessments of IHC staining were performed using FRIDA image analysis software.

Isolation and culture of primary hepatocytes

Primary hepatocytes were isolated from male C57BL6 WT and *Phb1* KO mice via collagenase perfusion as described[1].

In vitro silencing

WT and *Phb1* KO hepatocytes were transfected with 100 nM PHB1 siRNA and 100 nM OPA1 and HDAC4 siRNA (Qiagen) using Jetprime reagent (Polyplus). Controls were transfected with an unrelated siRNA (Qiagen). Protein knockdown was confirmed by Western blotting.

Cell transfection

WT and *Phb1* KO hepatocytes were transfected with two µg of pCMV6-HDAC4 (Origene) using jetPRIME™ reagent (Polyplus). pcDNA3-LacZ (Invitrogen) was used as negative control.

Drug treatments

A detailed description of the drugs used is provided in Supplementary Table I.

Protein isolation & Western blotting

Extraction of total protein from cultured cells was performed as described[1]. Nuclear and cytosolic fractions were obtained using the ProteoExtract Subcellular Proteome Extraction kit (Calbiochem). A description of the antibodies used is provided in Supplementary Table II. Band intensities were quantified using the ImageJ software and normalized to the housekeeping. All experiments were performed at least five times.

Immunoprecipitation experiments

Five hundred ug of total protein extract from hepatocytes were immunoprecipitated with 5ug of IGg2a (BD Pharmigen), anti-PHB1 antibodies and protein A Sepharose beads (Sigma-Aldrich).

RNA Isolation and Quantitative Real-Time Polymerase Chain Reaction (RT-PCR). RNA was isolated with Trizol (Invitrogen), and its concentration and integrity were determined. PCRs were performed using iQ™ SYBR® Green Supermix (Biorad) and the Bio-Rad iCycler thermocycler (Bio- Rad, Hercules, CA). The Ct values were extrapolated to a standard curve, and data was then normalized to the house-keeping expression (GAPDH). The sequences of primers will be provided under request.

Proteasome activity assay

For the *in vitro* assay of 26S proteasome activity, liver samples were collected in lysis buffer (50 mM HEPES, pH 7.5, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100 and 2 mM ATP) without protease inhibitors and the activity was measured following the manufacturers' instructions (Enzo BML-P802-0005).

Isolation of ubiquitylated proteins using Tandem Ubiquitin-Binding Entities Total ubiquitylated proteins were extracted from murine livers using GST TUBEs[2]. Pull-down material was analyzed by Western blot with the indicated antibodies.

Statistical Analysis

Statistical significance was determined by two-way analysis of variance followed by a Student's t test.

REFERENCES

1. Martínez-López, N. *et al.* Hepatoma cells from mice deficient in glycine N-methyltransferase have increased RAS signaling and activation of liver kinase B1. *Gastroenterology* **143,** 787–798.e1–13 (2012).
2. Hjerpe, R. *et al.* Efficient protection and isolation of ubiquitylated proteins using tandem ubiquitin-binding entities. *EMBO Rep.* **10,** 1250–1258 (2009).

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. *Phb1* KO mice are more sensitive to liver injury by BDL. (A) Kaplan Meier curve depicting survival of WT and *Phb1* KO mice after BDL. (B) F4/80, H&E and Sirius red staining on liver sections from WT and *Phb1* KO mice 3 and 7 days after BDL. Graphical representation of the necrotic areas. (C) Western blot analysis of total protein extracts using the indicated antibodies. (D) mRNA expression of the indicated genes in WT and *Phb1* KO livers 3 and 7 days after BDL. (E) mRNA expression of PHB1 and OPA1 in the shPHB1 and shOPA1 animals. (F) Western blot analysis using the indicated antibodies in total protein extracts from WT and *Phb1* KO hepatocytes and WT hepatocytes silencing PHB1 and OPA1 treated with DCA (100mM). (Values are mean ±SEM. *p<0.5, **p<0.01 [BDL vs Control, WT vs *Phb1* KO, shPHB1 and shOPA1 vs Control).

Supplementary Figure 2. PHB1 deficiency sensitizes hepatocytes to bile acids toxicity and induces HDAC4 expression and nuclear localization. (A) Caspase 3 activity in WT, *Phb1* KO, WT silencing PHB1 and OPA1 hepatocytes after DCA (100mM). (B) HDAC4 mRNA expression in shPHB1 animals 14 days after BDL compared to controls (C) Immunofluorescence of HDAC4 on hepatocytes from WT and *Phb1* KO mice. (D) WB analysis of H3 and acetylated H3 in livers from WT and *Phb1* KO mice. (Values are mean ±SEM. *p<0.5, [*Phb1* KO *vs* WT] [siPHB1 and siOPA1 *vs* Control]).

Supplementary Figure 3. The effect of HDAC inhibitors and parthenolide on the apoptosis and gene expression of *Phb1* KO. (A) Caspase 3 activity in WT and *Phb1* KO hepatocytes treated with class I/II HDAC inhibitors after DCA (100mM). (B) Analysis of the indicated genes mRNA expression by qPCR in WT, *Phb1* KO and *Phb1* KO hepatocytes treated with TSA and parthenolide after DCA (100mM). (C) Western blot of HDAC4 in *Phb1* KO hepatocytes treated with parthenolide. (D) 26S proteasome activity and (E) ubiquitin levels in control and parthenolide *Phb1* KO animals after BDL. (F) TUBE enrichment procedure and WB against HDAC4 in WT, *Phb1* KO and *Phb1* KO treated with parthenolide livers after 3 days of BDL. (Values are mean ±SEM. *p<0.5, **p<0.01, ***p<0.001 [*Phb1* KO *vs* WT and BDL *vs* Control], #p<0.5, ##p<0.01, ###p<0.001 [HDAC inhibitors vs *Phb1* KO].

Supplementary Figure 4. Parthenolide delays liver fibrosis in *Phb1* KO mice

(A) Immunohistochemistry using F4/80 and Smad2/3 antibodies on paraffin sections, (B) 26S proteasome activitiy, (C) ubiquitin levels and (D) TUBE procedure and WB against HDAC4 in livers from *Phb1* KO mice and *Phb1* KO mice treated with parthenolide for 2 weeks. Values are mean ± SEM. *p > 0.05, **p<0.01 [Parthenolide *vs Phb1* KO]).

Supplementary Figure 5. Effect of HDAC4 silencing in *Phb1* KO mice. (A) Caspase-3 activity and (B) Western blot analysis using the indicated antibodies in *Phb1* KO hepatocytes silenced with a control (siControl) or HDAC4 siRNA (siHDAC4) after DCA treatment (100mM). (C) mRNA expression of the indicated genes in the siControl and siHDAC4 hepatocytes at basal conditions. (Values are mean SEM. *p<0.5, **p<0.01 [siHDAC4 *vs* siControl]).

Supplementary Figure 6. The absence of PHB1 is linked to and up-regulation and more nuclear localization of HDAC4 promoting cholestatic liver injury. PHB1 expression is markedly reduced in human cholestatic liver diseases and in the experimental mouse model of obstructive cholestasis. These low levels of PHB1 are correlated with an upregulation of HDAC4. Importantly, PHB1 interacts with HDAC4 in the presence of bile acids, and its depletion leads to increased nuclear HDAC4 content and its associated epigenetic changes. HDAC4 regulates the expression of important genes related to bile acid metabolism and cell survival and consequently sensitizes the liver to injury, inflammation and bile duct proliferation.

Supplementary Table I.

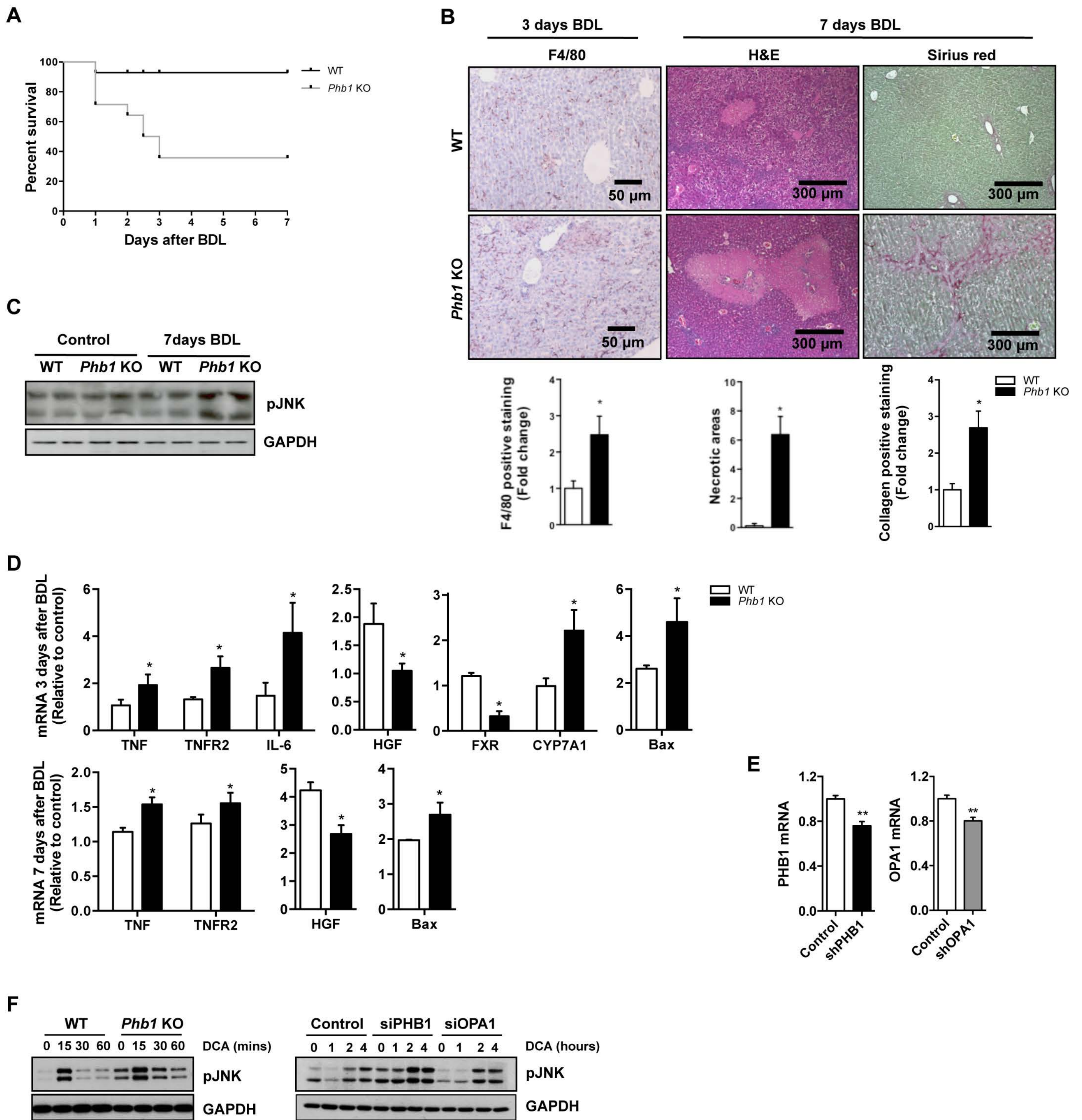
Concentration and Supplier for Each Drug treatment

Apicidin	1nM	Sigma-Aldrich
Deoxycholic acid (DCA)	100mM	Sigma-Aldrich
Mocetinostat	0.15nM	Selleckchem
Parthenolide	2.5mM	Sigma-Aldrich
PCI34051	10nM	Selleckchem
Rocilinostat	50nM	Selleckchem
Trichostatin A (TSA)	3mM	Sigma-Aldrich

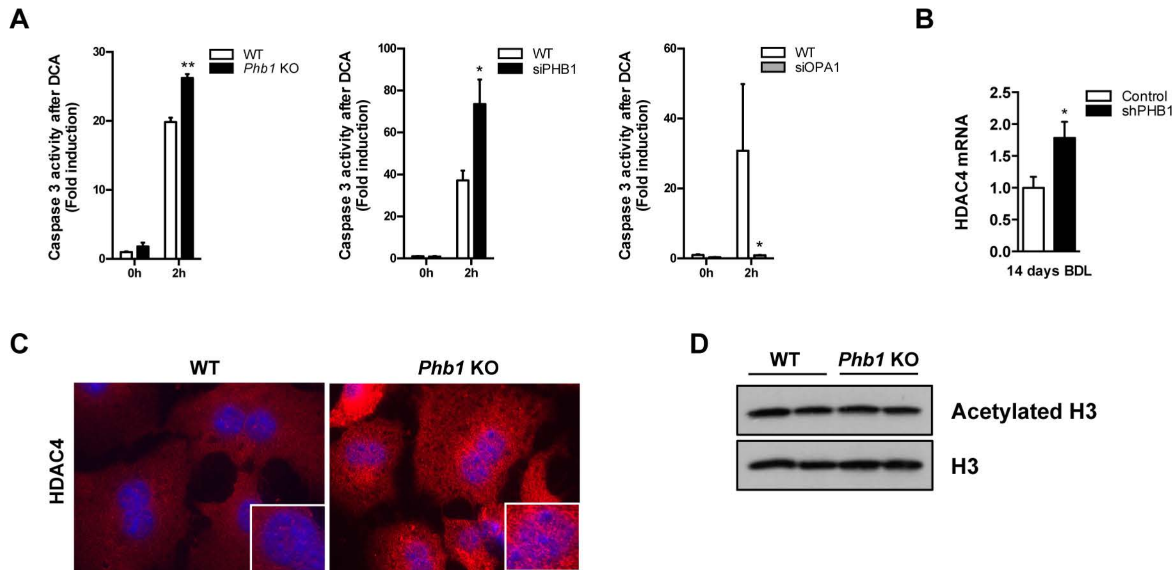
Supplementary Table II.

Optimal Incubation Conditions, Concentration and Supplier for Each Specific Antibody Analyzed by Western Blotting.

β-Actin	Sigma	1/5000	TBS-Tween (0.1%)-milk (5%)
GAPDH	Abcam	1/5000	TBS-Tween (0.1%)-milk (5%)
H3	Cell Signaling Technology	1/1000	TBS-Tween (0.1%)-BSA (5%)
H3 Acetylated	Millipore	1/1000	TBS-Tween (0.1%)-BSA (5%)
HDAC4	Proteintech	1/1000	TBS-Tween (0.1%)-milk (5%)
JNK	Cell Signaling Technology	1/1000	TBS-Tween (0.1%)-milk (5%)
JNK1/2 pT183/Y185	Invitrogen	1/1000	TBS-Tween (0.1%)-milk (5%)
PHB1	Santa Cruz Biotechnology	1/1000	TBS-Tween (0.1%)-milk (5%)
Ubiquitin	Santa Cruz Biotechnology	1/1000	TBS-Tween (0.1%)-milk (5%)



Supplementary Figure 1



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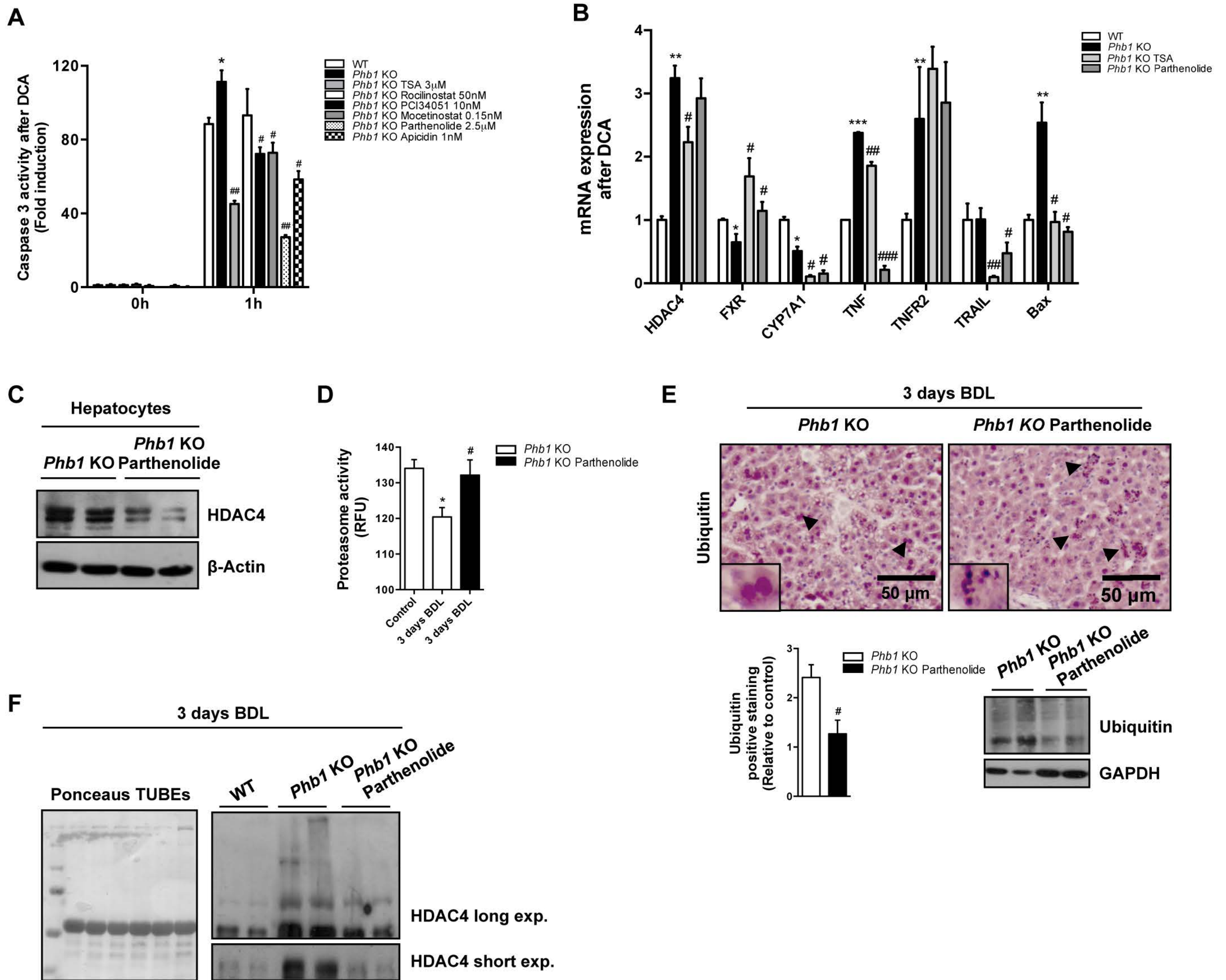

Supplementary Figure 3

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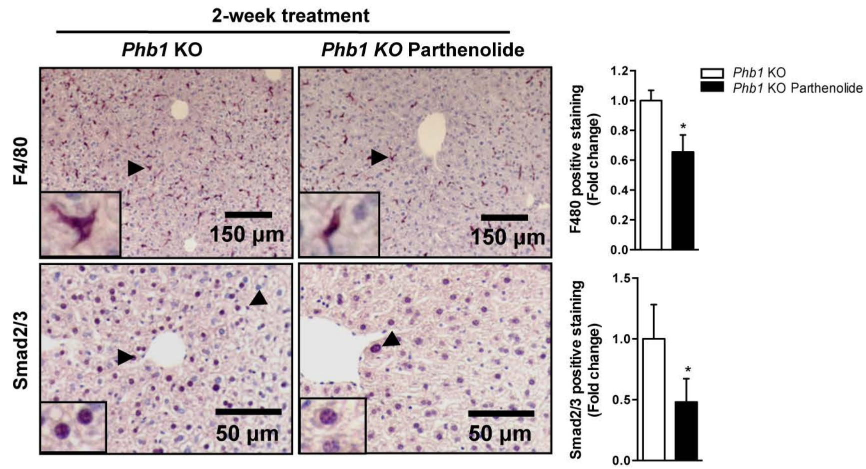

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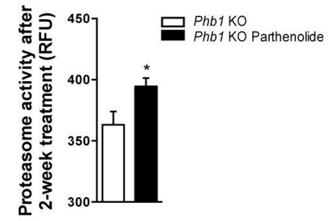

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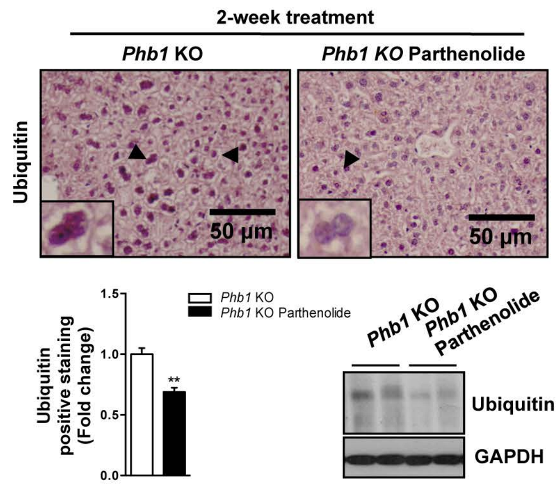

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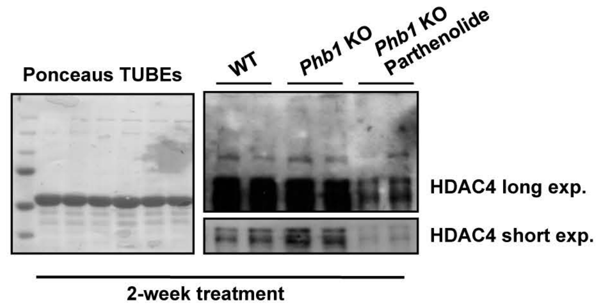

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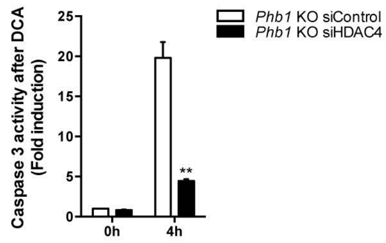

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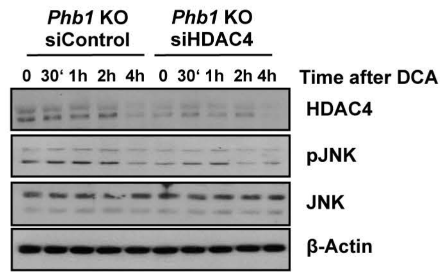

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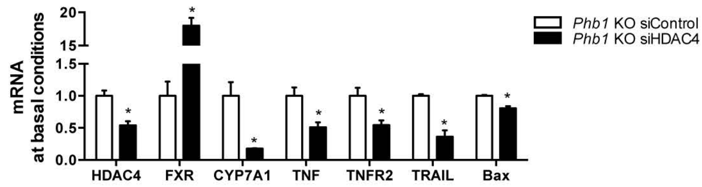
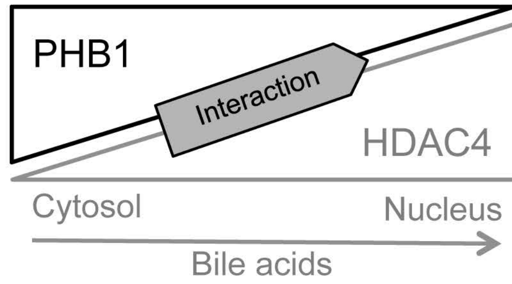


Supplementary Figure 5

Healthy liver



Cholestasic liver disease

- Gene repression
- Liver injury
- Inflammation
- Ductular proliferation

Supplementary Figure 6