Supplemental Methods

Experimental Liver Fibrosis and Pathological Evaluation. Wild-type C57BL/6 mice were either injected intraperitoneally with 0.5μg/g/mouse of carbon tetrachloride (CCl_4) (Sigma, St. Louis, MO) twice weekly for 6 or 24 weeks or given a standard diet containing 1% w/w 5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) (Sigma) for 5 weeks. Untreated C57BL/6 mice were used for comparison as controls. All mice were cared for in accordance to the "Guide for the Care and Use of Laboratory Animals." Mouse livers were harvested and fixed in 4% paraformaldehyde. Paraffin sections, hematoxylin and eosin (H&E), and Sirius Red staining were performed by the UCSF Liver Center Pathology Core in association with the Gladstone Foundation Histology Core (San Francisco, CA) using standard procedures. Descriptive and qualitative assessment of fibrotic disease severity was performed by an expert liver pathologist who was blinded to the identity of the samples.

Quantitative Tissue Fibrillar Collagen Analysis. Frozen sections of liver tissue were stained with 0.1% Sirius Red (Sigma) and counterstained with Weigert's hematoxylin to reveal fibrillar collagen. Sections were imaged using an Olympus IX81 fluorescence microscope (Waltham, MA) fitted with an analyzer (U-ANT) and polarizer (U-POT) oriented parallel and orthogonal to each other. Images were quantified for percent area positive for fibrillar collagen using ImageJ software (Bethesda, MD).

Hepatocyte Cell Size Analysis. Isolated primary hepatocytes were plated at low density (10,000cells/18mm diameter gel) to ensure that single cells could be measured without confounding cell-to-cell contact. Phase contrast photos were taken with a Rebel T3i camera

(Canon, San Jose, CA) adapted to the photoport of an Eclipse TS100 Inverted Microscope (Nikon, Melville, NY). Images were digitally acquired and processed by ImageJ software. Cell size was determined by outlining the cell membrane and calculating the surface area in pixels. An image of a micrometer at the same magnification was used to convert pixel measurements to μm^2.

Hepatocyte Functional Assays. Hepatocytes were plated at a density of 50,000 cells/18mm diameter gel. For quantitative measurement of albumin production, cell culture supernatants were collected at 24h and albumin concentration was determined by a mouse albumin ELISA kit per the manufacturer's instructions (Bethyl, Montgomery, TX). Quantitative determination of glycogen storage was measured using a glycogen fluorometric assay kit per the manufacturer's instructions (Sigma). Cytochrome P450 1A activity was measured quantitatively using the luminescence-based P450-Glo Assay according to the manufacturer's instructions (Promega, Madison, Wisconsin). All functional measurements were corrected for cell number determined by the Cyquant Assay (Life Technologies, Pleasanton, CA). Cyquant was performed following the manufacturer's instructions using a standard curve generated from known numbers of primary mouse hepatocytes to convert DNA content into number of cells.

Quantitative Real-time Reverse Transcription Polymerase Chain Reaction (qRT-PCR). Hepatocytes were plated at a density of 50,000 cells/18mm diameter gel. After 24h of culture, RNA was isolated using RNeasy Mini Kit (QIAGEN, Valencia, CA) according to the manufacturer's protocol. RNA purity was verified by the NanoDrop1000 spectrophotometer (Thermo Scientific, Waltham, MA) and 260/280 absorbance consistently ranged between 2.0 and

2.2. Reverse transcription was carried out with 300ng of RNA for initial gene expression experiments and 100ng of RNA for HNF4α-deficient hepatocytes and inhibitor studies using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) per the manufacturer's instructions. One μl of the resulting cDNA was added to a final 20μl mixture containing 10μl of 2x SYBR Green PCR Master Mix (Affymetrix, Cleveland, OH) and 12 pmol oligonucleotide primers. qPCRs were carried out in a 7300 Real-Time PCR System (Applied Biosystems) using the thermal profile 50^{o}C for 2min, 95^{o}C for 10min, followed by 40 amplification cycles consisting of 95^{o}C for 15s, 63^{o}C for 30s, and 72^{o}C for 30s. Samples were normalized to rRNA 18S internal standard. Relative quantification of gene expression was calculated by using the $2^{\Delta\Delta Ct}$ equation.

Fluorescent Immunohistochemistry. Flash-frozen liver samples were cut to 20μm thick sections. Following thawing and acetone fixation, samples were incubated with primary antibodies phospho-FAK^{Y397} (141-9, 1:200; Life Technologies), activated β1 integrin (553715, 1:100; BD Biosciences, San Jose, CA), HNF4α (C-19; 1:200; Santa Cruz Biotechnology, Dallas, TX), and fibronectin (10, 1:100; BD Biosciences) followed by AlexaFluor-conjugated donkey anti-goat, goat anti-rabbit, goat anti-rat, and goat anti-mouse IgG antibodies secondary antibodies (polyclonal, 1:1000, Life Technologies). Nuclei were counterstained with 1μg/ml DAPI (Sigma). Slides were imaged using an inverted Ti-E Perfect Focus System (Nikon) equipped with a CSU-X1 spinning disk confocal unit (Andor, Concord, MA) and controlled by NIS-Elements software (Nikon).

Immunoblotting. Hepatocytes were plated at a density of 250,000 cells/60mm diameter gel. After 2h, cell lysates were prepared using Cell Extraction Buffer (Life Technologies) supplemented with phenylmethylsulfonyl fluoride and protease inhibitor cocktail (Sigma). Twenty-five μg of total protein was loaded onto 7.5% polyacrylamide gels for electrophoresis and wet transfer performed onto nitrocellulose membranes. Non-specific binding was blocked using 5% dry milk before incubation with primary and secondary antibodies. Antibodies and dilutions used were phospho-FAK^{Y397} (141-9; 1:1000; Life Technologies), total FAK (polyclonal; 1:200; Life Technologies), GAPDH (14C10; 1:5000; Cell Signaling, Danvers, MA), and goat anti-rabbit-IgG conjugated horse radish peroxidase (polyclonal; 1:5000; Thermo Scientific). HNF4α immunoblotting was performed with anti-HNF4α (C-19; 1:1000; Santa Cruz Biotechnology) and donkey anti-goat-IgG conjugated horse radish peroxidase (polyclonal; 1:5000; Jackson ImmunoResearch, West Grove, PA). Chemiluminescent signal was developed using the Pierce ECL2 Western Blotting Substrate (Thermo Scientific). Nitrocellulose membranes were sometimes stripped with Restore Western Blot Stripping Buffer (Thermo Scientific) and probed with another primary antibody. Densitometry calculations were performed using ImageJ software.

Primers. Sequences for primers used in qRT-PCR are listed below. For primer sequences obtained from the Harvard Primer Bank (http://pga.mgh.harvard.edu/primerbank/citation.html), the PrimerBank IDs are also provided. Additional primer sequences were created using the Primer3 Software.

Gene Name	Direction	Sequence 5'->3'	Harvard PrimerBank ID
Baat	Forward	GTCCTCCCTTGGATAGCCTGA	211904119b2
	Reverse	CCGGATGCGGCTTTCCTTTA	
Gys2	Forward	CGCTCCTTGTCGGTGACATC	188035874b1
	Reverse	CATCGGCTGTCGTTTTGGC	
F7	Forward	AGACTTTGAGGGTCGGAACTG	225543549b3
	Reverse	TTGGTCCCTACATGGTCCCTG	
Hnf4a	Forward	GGTTTAGCCGACAATGTGTGG	46575915b3
	Reverse	TCCCGCTCATTTTGGACAGC	
Snai1	Forward	CACACGCTGCCTTGTGTCT	53250a1
	Reverse	GGTCAGCAAAAGCACGGTT	
Vim	Forward	GCTGCGAGAGAAATTGCAGGA	227430362c3
	Reverse	CCACTTTCCGTTCAAGGTCAAG	
18S	Forward	GTGGAGCGATTTGTCTGGTT	
	Reverse	CGCTGAGCCAGTCAGTGTAG	

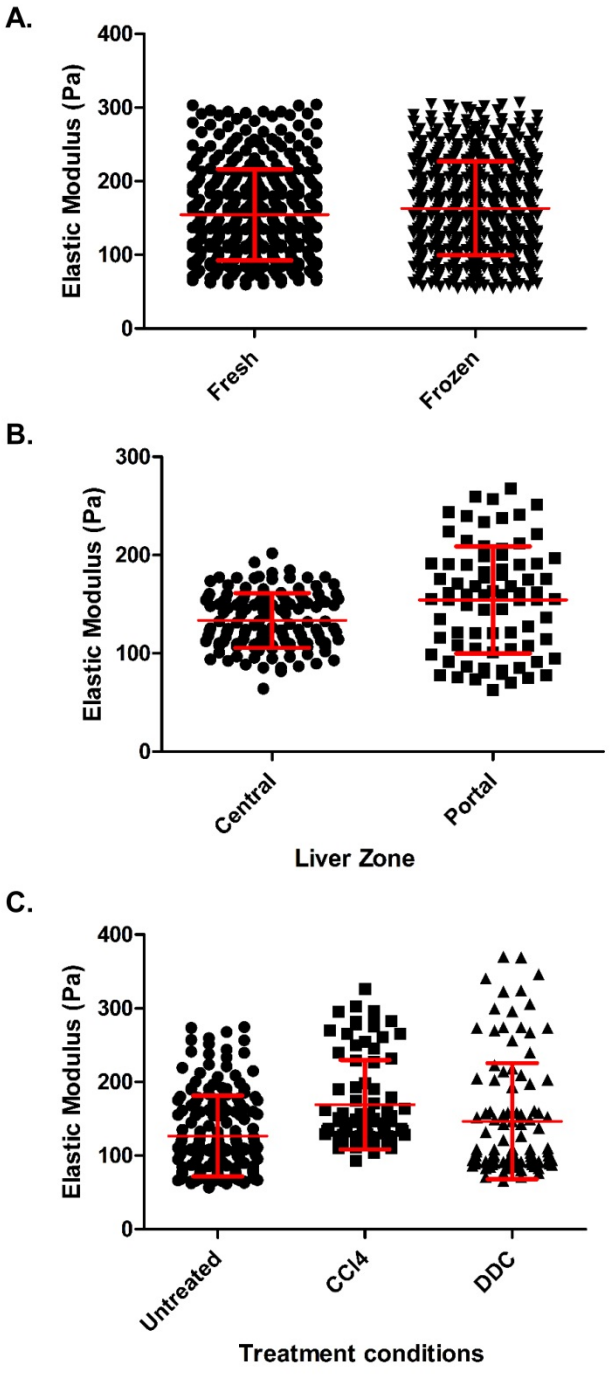
Transient Plasmid Transfections. Two to four hours after plating of isolated primary hepatocytes, adherent hepatocytes were transfected at a 4µg DNA: 8µl Metafectene Pro (Biontex, München, Germany) ratio according to the manufacturer's protocol in a 12-well format. A pAd-CMV/HNF4α/V5 plasmid was used to drive over-expression of mouse HNF4α

and pmaxGFP plasmid (Lonza, Basel, Switzerland) served as a control. Following 24h of culture, RNA was isolated for further analysis.

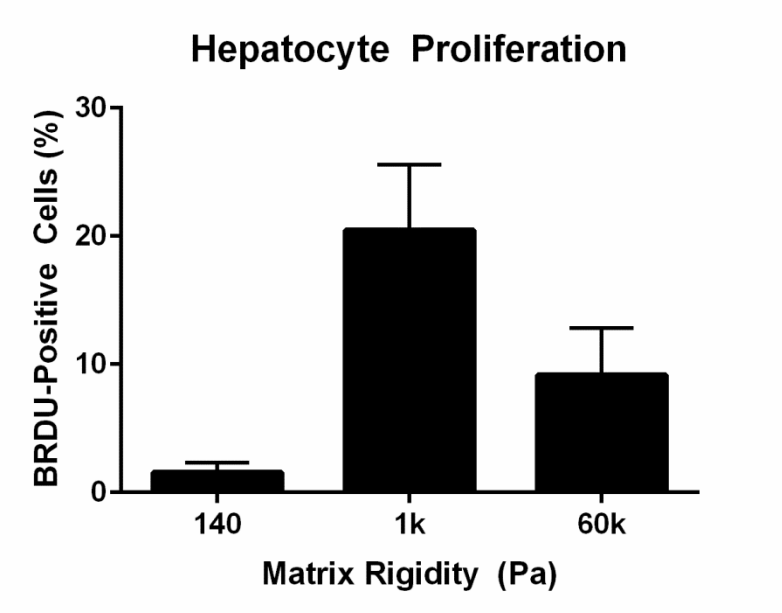
Hepatocyte Proliferation. 5-bromo-2'-deoxyuridine (BrdU) labeling reagent (Life Technologies) was added to hepatocyte cultures at 1:100 dilution. After 3 days, culture media were removed and cells along with collagen matrix were fixed in 4% paraformaldehyde. For staining, cells were permeabilized with 2% triton in phosphate buffered saline and BrdU epitopes revealed by 2.5M HCl treatment. Anti-BrdU-AlexaFluor 488 (1:50; Life Technologies) with DAPI counterstaining was used to identify proliferating cells. Random low-powered epifluorescence micrographs were taken and percentage of $BrdU^+$ cells analyzed using ImageJ. At least 100 total cells were analyzed per condition in each experiment.

Statistical Analysis. Statistical analyses were performed with either Prism v. 5.0 (GraphPad, La Jolla, CA) or SPSS Statistics (IBM, Armonk, NY).

Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure Legends

Supplemental Figure 1. Further analysis of liver tissue matrix stiffness as determined by AFM. (A) AFM measurements were performed on fresh and snap-frozen mouse liver tissues across the liver lobule of normal liver. Each data point represented a single AFM measurement. There were no significant differences in tissue stiffness measured on fresh liver tissue compared to snap-frozen tissue. (B) AFM measurements obtained from pericentral and periportal zones of livers from normal untreated mice were directly compared with each other. Each data point represented a single AFM measurement. Periportal zones showed greater variability in matrix rigidity and trended slightly stiffer than pericentral zones. Overall, there were no statistically significant differences in matrix stiffness between pericentral and periportal areas. (C) AFM measurements of non-fibrotic regions of the liver from untreated, CCl_4-, and DDC-treated mice

were compared. Each data point represented a single AFM measurement. Although there were a few instances in which non-fibrotic regions of CCl_4- and DDC-treated liver had slightly greater matrix stiffness compared to normal liver, there were no statistically significant differences between the 3 groups when considered in aggregate. Statistical analysis was calculated via one-way ANOVA and Tukey's post-hoc for pairwise comparison. Sample size equaled at least 3 mice for each group and 3 readings per mouse. Error bars represent SD.

Supplemental Figure 2. Hepatocytes proliferation as determined by BrdU incorporation on soft and stiff matrices. Primary hepatocytes were on culture on top of 140Pa, 1kPa, and 60kPa matrices. Percentage of BrdU-positive cells was enumerated after 3 days of culture. Data are the average of 4 independent experiments (n=4). At least 100 cells per condition were analyzed in each experiment. Error bars represent SEM.