SUPPLEMENTARY METHODS

Serum biochemistry

Total bilirubin, direct bilirubin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were measured in samples taken before sacrifice. Serum biochemistry was measured by automated testing in the Clinical Chemistry Division, a part of the University of Pittsburgh Department of Pathology's Laboratory Support Services.

Immunohistochemistry

Tissues fixed in 10% formalin were embedded in paraffin, and 4-µm sections cut onto Superfrost Plus glass slides (Thermo Fisher Scientific, Pittsburgh, PA) were used for hematoxylin and eosin (H&E), Sirius Red, or immunohistochemical analysis as described previously(1). For detection of fibrosis, sections were rehydrated and placed in Sirius Red staining solution for 1 h at room temperature. Sections were washed with acidified water, dehydrated, and coverslipped. Apoptosis was determined using terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) staining (ApopTag Peroxidase in Situ Apoptosis Detection Kit, Millipore).

Immunohistochemistry (IHC) on paraffin-embedded sections of mouse livers was performed on 4 µm thick tissue sections, which were deparaffinized and then washed with PBS. Antigen retrieval was performed by microwaving in citrate buffer (pH 6; GS, CD45, MDA, CD10), pressure cooking for 20 minutes in citrate buffer (β-catenin, p21), pressure cooking in Dako Target Retrieval Solution (Dako, S1699; Sox9), or

microwaving in 1% $ZnSO_4$ (PCNA). Tissues were pretreated with 3% H_2O_2 and blocked using SuperBlock (Thermo Fisher) for 10 minutes (β-catenin, PCNA, CD45, MDA, CD10, and Sox9) or 10% goat serum in PBS for 10 minutes (GS, p21). Primary antibodies used for this project include β-catenin (1:100, sc-7199), GS (1:50, sc-9067), PCNA (1:3000; sc-56), p21 (1:25; sc-471), and CD45 (1:100, sc-53665) from Santa Cruz Biotechnology, Dallas, TX; malondialdehyde (MDA; 1:200, AB6463) from Abcam, Cambridge, MA; Sox9 (1:100, AB5535) from Millipore, Temecula, CA; and CD10 (1:100, PA5-29354) from Thermo Fisher. All primary antibodies were incubated for 1 hour in 1% BSA in PBS or 4 overnight. Secondary antibodies were biotinylated horse anti-mouse (Vector Laboratories, Inc., Burlingame, CA) or biotinylated goat anti-rabbit (Chemicon, Temecula, CA), used at 1:500 dilution in 1% BSA in PBS. Immunohistochemistry was performed using the Vectastain ABC Elite kit (Vector Laboratories, Inc., Burlingame, CA) and developed using DAB (Vector Laboratories, Inc., Burlingame, CA). The slides were counterstained with Shandon's hematoxylin (Thermo Fisher).

Quantification of staining

Polarized light images of Sirius red stains were taken with an Olympus Provis microscope. Each image was split into red, green and blue channels using ImageJ Fiji, among which the red channel was chosen since it has the best separation. Next the staining was isolated by using Threshold setting 25 for the upper level and 255 for the lower level. Then the percentage of the stained area to the total image was measured. A total of 5 images per mouse liver ($n \geq 3$ mice) were quantified for each genotype.

Quantification of CD45 staining was done by splitting the image into RGB channels with ImageJ, and then quantifying the green channels, using a threshold of 175 to determine the percentage of the stained area to the total image. A total of 5 images per mouse liver ($n \geq 3$ mice) were quantified for each genotype.

Quantification of PCNA and TUNEL positive cells was performed by counting the number of positive hepatocytes per 100X field. A total of 5 images per mouse liver ($n \geq 3$ mice) were quantified for each genotype.

Protein extraction and Western blot analysis

Approximately 20 mg of liver was dounce homogenized in a buffer containing 25 mM Tris-HCl (pH 7.6), 150 mM NaCl, 1% Nonidet P-40, 1% sodium deoxycholate, 0.1% SDS (RIPA buffer) supplemented with protease inhibitor as described previously(2). Protein assays were performed using bicinchoninic acid protein assay. Equal amounts of protein (10-50 µg) were resolved on Tris-HCl precast gels (Bio-Rad) by SDS-PAGE analysis using the Mini-PROTEAN 3 Electrophoresis Module Assembly (Bio-Rad). The resolved proteins were transferred to polyvinylidene difluoride membranes, followed by immunoblotting and visualization by enhanced chemiluminescence. The primary antibodies used were β-catenin (1:1000, 610154) and E-cadherin (1:2500, 610182) from BD Biosciences, San Jose, CA; non-phospho (active) β-Catenin (Ser33/37/Thr41) (1:1000, 8814) and γ-catenin (1:800, 2309) from Cell Signaling Technology, Los Angeles, CA; GAPDH (1:400, sc-25778) and GS (1:2000; sc-74430) from Santa Cruz; E-cadherin (1:50, ab1416) and γ-catenin (1:1000, ab184919) from Abcam. Horseradish

peroxidase conjugated secondary antibodies were purchased from MilliporeSigma (Burlington, MA).

Densitometry analysis of Western blots was performed using ImageJ software (version 1.44o).

Measurement of liver bile acids

Liver total bile acids were measured using a total bile acids kit from Crystal Chem (Downers Grove, IL), as per the manufacturer's instructions. Briefly, frozen liver tissue was homogenized in 70% ethanol at room temperature and then incubated in tightly capped glass tubes at 50°C for 2 hours. The homogenates were centrifuged at 6,000*g* for 10 minutes to remove debris. For measurement of bile acids in serum, whole frozen serum was diluted 1:5 in H_2O before assaying. Total bile acid levels were measured and concentrations determined using the calibration curve and mean change in absorbance value for each sample.

Transfection of Hep3B cells

The Hep3B human hepatoma cell line, which was obtained from ATCC, Manassas, VA, was transfected as previously described(2). Briefly, Hep3Bs grown in Eagle's Minimal Essential Medium (American Type Culture Collection, Manassas, VA) with 10% FBS (Atlanta Biologicals, Lawrenceville, GA) were seeded onto 6-well plates and transiently transfected with validated human β-catenin (CTNNB1) siRNA or negative control siRNA 1 (Ambion, Inc., Austin, TX) at a final concentration of 25 nM in the presence of Lipofectamine 3000 reagent (Invitrogen, Carlsbad, CA), as per the manufacturer's

instructions. The cells were harvested 48 hours after transfection for RNA extraction, as described below.

RNA isolation and real-time qPCR

RNA was extracted from frozen livers or Hep3B cells using Trizol (Invitrogen). RNA was DNase-treated and equal microgram amounts of RNA from each sample were used to make individual cDNA samples with SuperScript III First –Strand Synthesis System for RT-PCR (Invitrogen). cDNA along with 1x Power SYBR-Green PCR Master Mix (Applied Biosystems) and the appropriate primers (Supplementary Table 1) were used for each real-time PCR reaction. $n \geq 3$ samples per each condition were assayed in duplicate on the Applied Biosystems StepOnePlus Real-Time PCR System with StepOne v2.1 software. The comparative ΔΔCT method was used for analysis of the data and all data is presented normalized to WT at baseline. GAPDH expression was used as the internal control.

Immunofluorescence (IF)

Tissue samples were frozen in OCT compound (Sakura, 4583) on dry ice and stored at - 80°C. Cryopreserved samples were cut into 5 µm sections, washed in PBS, and then fixed in 2% paraformaldehyde for 30 minutes. After washing, slides were washed with PBS and permeabilized with 0.1% Triton X-100 in PBS for 20 minutes at room temperature. Samples were washed three times with PBS and then blocked with 2% goat serum in 0.1% Tween-20 in PBS (PBST) for 30 minutes at room temperature. Antibodies were diluted in 2% goat serum/PBST and incubated at 4°C overnight.

Antibodies used were as follows: β-catenin (1:200; ab32572) from Abcam, and ZO-1 (1:100; 40-2300) from Thermo Fisher. Samples were washed three times in PBS and incubated with the proper fluorescent secondary antibody (AlexaFluor 488/555/647, Invitrogen) diluted 1:800 (β-catenin) or 1:500 (ZO-1) in 2% goat serum/PBST for two hours at room temperature. Samples were washed three times with PBS and incubated with DAPI (Sigma, B2883) for 1 minute. Samples were washed three times with PBS and mounted with fluromount (SouthernBiotech) or ProLongTM Gold antifade reagent (Invitrogen, P10144). Images were taken on a Nikon Eclipse Ti epifluorescence microscope or a Zeiss LSM700 confocal microscope.

For co-localization studies using IF, tissue samples were processed as above and then fixed in 100% methanol at -20ºC for 15 minutes, followed by washing with PBS. Antigen retrieval was performed through pressure cooking for 20 minutes with Dako Target Retrieval Solution (Dako, S1699). After cooling slides were washed with PBS and permeabilized with 0.1% Triton X-100 in PBS for 20 minutes at room temperature. Samples were washed three times with PBS and then blocked using 2% Donkey serum or 2% Goat serum in 0.1% Tween-20 in PBS (antibody diluent) for 1 hour at room temperature. Antibodies were diluted as follows: HNF4a (Santa Cruz sc-6556, 1:50), CK19 (DSHB TROMA-III-s, 29 µg/ml), and p21 (Abcam ab188224, 1:500) in antibody diluent and incubated at 4ºC overnight. Samples were washed three times with PBS and incubated with the proper fluorescent secondary antibody (AlexaFluor 488/555/647, Invitrogen) diluted 1:800 in antibody diluent for two hours at room temperature. Samples were washed three times with and incubated with DAPI (Sigma, B2883) for 1 minute.

Samples were washed three times with PBS and mounted with fluromount (SouthernBiotech). Images were taken on a Nikon Eclipse Ti epifluorescence microscope.

Transmission electron microscopy

Whole liver slices were fixed in Karnovsky's fixative (2% paraformaldehyde, 2.5% glutaraldehyde) for 48 hours. After fixation and dividing into sections, grids were stained with 2% uranyl acetate in 50% methanol for 10 minutes and 1% lead citrate for 7 minutes. Images were taken with a JEM 1011 transmission electron microscope at 80 kV.

Scanning electron microscopy (SEM)

Whole liver was collected from WT, Mdr2 KO, and KO/KD mice for SEM imaging. Liver samples were fixed in 2.5% glutaraldehyde in PBS (pH 7.4) for 10 min. Tissue samples were washed thoroughly in PBS for 15 min. Tissues were then fixed in 1% Osmium tetraoxide (OsO_4) in PBS for 60 min. Samples were dehydrated with different concentration of ethanol (30%, 50%, 70%, 90%) for 15 min and then samples were critical point dried. Samples were visualized using Field Emission Scanning Electron Microscope (JEOL JSM6335F) at the magnification of 10,000X to 30,000X.

Two-photon excitation intravital microscopy

Intravital imaging of liver is described in detail elsewhere(3, 4). Briefly, mice were anesthetized with isoflurane and placed on a heated stage. A catheter was placed into

the carotid artery to enable intravenous delivery of intravascular fluorochromes. Gentle vacuum suction was applied to the abdominal imaging window device to immobilize. Texas Red (TXR) dextran (MW:70,000) was used to visualize the blood flow through liver sinusoids, whereas 6-carboxyfluorescein diacetate (6-CFDA; MW:367.82) was used to visualize biliary canaliculi. After internalization by hepatocytes, CFDA is hydrolyzed into carboxyfluorescein (CF), which emits green fluorescence at wavelength 517. Videos and images were recorded at 1, 3, and 10 minutes post-injection. Microscopy was performed using a Nikon multiphoton excitation microscope at the imaging service of the Pittsburgh Liver Research Center.

Study approval

All animal experiments were performed under the guidelines of the National Institutes of Health and the Institutional Animal Use and Care Committee at the University of Pittsburgh. The studies performed in the current report were approved by the Institutional Animal Use and Care Committee at the University of Pittsburgh (protocol #14013027).

Statistical Analysis

All experiments were performed with three or more animals, and representative data are presented. Quantification of positive cells, serum biochemistry measurements, real-time PCR analysis, Western blot quantification, and others were analyzed for statistical significance by one-way ANOVA with multiple comparisons or student’s t-test using Graphpad Prism 7.0c. P values less than 0.05 were considered significant.

SUPPLEMENTARY METHODS REFERENCES

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2. Nejak-Bowen K, Kikuchi A, Monga SP. Beta-catenin-NF-kappaB interactions in murine hepatocytes: a complex to die for. Hepatology 2013;57:763-774.
3. Bennewitz MF, Watkins SC, Sundd P. Quantitative intravital two-photon excitation microscopy reveals absence of pulmonary vaso-occlusion in unchallenged Sickle Cell Disease mice. Intravital 2014;3:e29748.
4. Pradhan-Sundd T, Zhou L, Vats R, Jiang A, Molina L, Singh S, Poddar M, et al. Dual catenin loss in murine liver causes tight junctional deregulation and progressive intrahepatic cholestasis. Hepatology 2018;67:2320-2337.

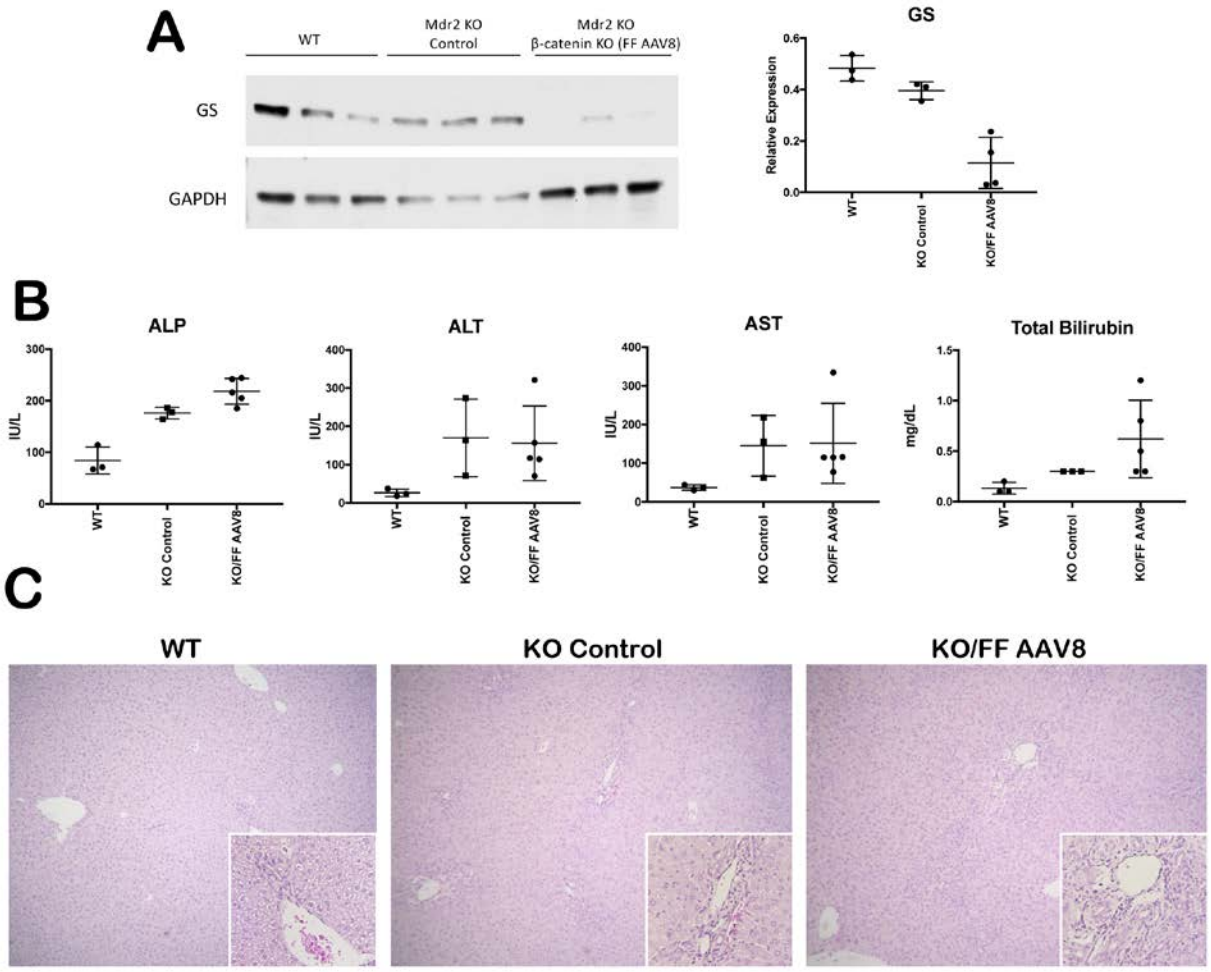
Supplementary Table 1: Primers used for qPCR studies on mouse livers

Gene	Forward Primer	Reverse Primer
Cyp7a1	TGGGCATCTCAAGCAAACAC	TCATTGCTTCAGGGCTCCTG
Cyp8b1	GCCCTTACTCCAAATCCTACCA	TCGCACACATGGCTCGAT
Cyp27	TGCCTGGGTCGGAGGAT	GAGCCAGGGCAATCTCATACTT
Cyp2b10	CAATGGGGAACGTTGGAAGA	TGATGCACTGGAAGAGGAAC
Cyp3a11	CCACCAGTAGCACACTTTCC	TTCCATCTCCATCACAGTATCA
NTCP	CACCATGGAGTTCAGCAAGA	AGCACTGAGGGGCATGATAC
OATP4	GATCCTTCACTTACCTGTTCAA	CCTAAAAACATTCCACTTGCCATA
BSEP	ACACCATTGTATGGATCAACAGC	CACCAACTCCTGCGTAGATGC
MRP2	GCTTCCCATGGTGATCTCTT	ATCATCGCTTCCCAGGTACT
MRP3	TGAGATCGTCATTGATGGGC	AGCTGCGAGCGCAGGTCG
MRP4	TTAGATGGGCCTCTGGTTCT	GCCCACAATTCCAACCTTT
FXR	CTTGATGTGCTACAAAAGCTGTG	ACTCTCCAAGACATCAGCATCTC
SHP	TGGGTCCCAAGGAGTATGC	GCTCCAAGACTTCACACAGTG
PXR	CCCATCAACGTAGAGGAGGA	GGGGGTTGGTAGTTCCAGAT
CAR	GGAGGACCAGATCTCCCTTC	ATTTCATTGCCACTCCCAAG
E-cadherin	CACCTGGAGAGAGGCCATGT	TGGGAAACATGAGCAGCTCT
Claudin-2	TATGTTGGTGCCAGCATTGT	TCATGCCCACCACAGAGATA
Occludin	TTGAAAGTCCACCTCCTTACAGA	CCGGATAAAAAGAGTACGCTGG
ZO-1	CCACCTCTGTCCAGCTCTTC	CACCGGAGTGATGGTTTTCT
Gamma-catenin	ACGCCATTGATGCGGAGGGC	CCCAGGCAGCTGGGTCATGC
Mdr2	ATCCTATGCACTGGCCTTCTGGT	GAAAGCATCAATACAGGGGGCAG
p16	GCTCAACTACGGTGCAGATTC	GCACGATGTCTTGATGTCCC
p21	CGAGAACGGTGGAACTTTGAC	CCAGGGCTCAGGTAGACCTT
TNFα	CATCTTCTCAAAATTCGAGTGACAA	TGGGAGTAGACAAGGTACAACCC
IL-6	GAGGATACCACTCCCAACAGACC	AAGTGCATCATCGTTGTTCATACA
GPX1	GACTGGTGGTGCTCGGTTTC	GTCGGACGTACTTGAGGGAATT
SOD1	GACCTGGGCAATGTGACTGCTG	CACCAGTGTACGGCCAATGATG
GST	CTGTGGCTCCTGGTTCTCTC	TTGACTGGGAAGAGGGTGAG
NQO1	TTCTGTGGCTTCCAGGTCTT	TCCAGACGTTTCTTCCATCC
HO-1	CCTTCCCGAACATCGACAGCC	GCAGCTCCTCAAACAGCTCAA
Claudin-1	CTGGGTTTCATCCTGGCTTC	TTGATGGGGGTCAAGGGGTC
Claudin-3	CCAACTGCGTACAAGACGAG	TCTTGGTGGGTGCATACTTG
Claudin-5	TGGAACGCTCAGATTTCATC	AGGAAGGCAACCCCTCTAAG
Vimentin	CTTGAACGGAAAGTGGAATCCT	GTCAGGCTTGGAAACGTCC
TGFβ1	GTTTAATTGACCCCGAGGAGC	GAGAAGAGAGCGCAGAATCCA
Snail	CCACTGCAACCGTGCTTTT	CACATCCGAGTGGGTTTGG
Slug	CTCACCTCGGGAGCATACAGC	TGAAGTGTCAGAGGAAGGCGGG
β-catenin	GGGTCCTCTGTGAACTTGCTC	TTCTTGTAATCCTGTGGCTTGTCC
GS	CTGAGTGGAACTTTGATGGCT	GGAAGGGGTCTCGAAACATGG
GAPDH	GGAGAAACCTGCCAAGTATGA	TCCTCAGTGTAGCCCAAGA

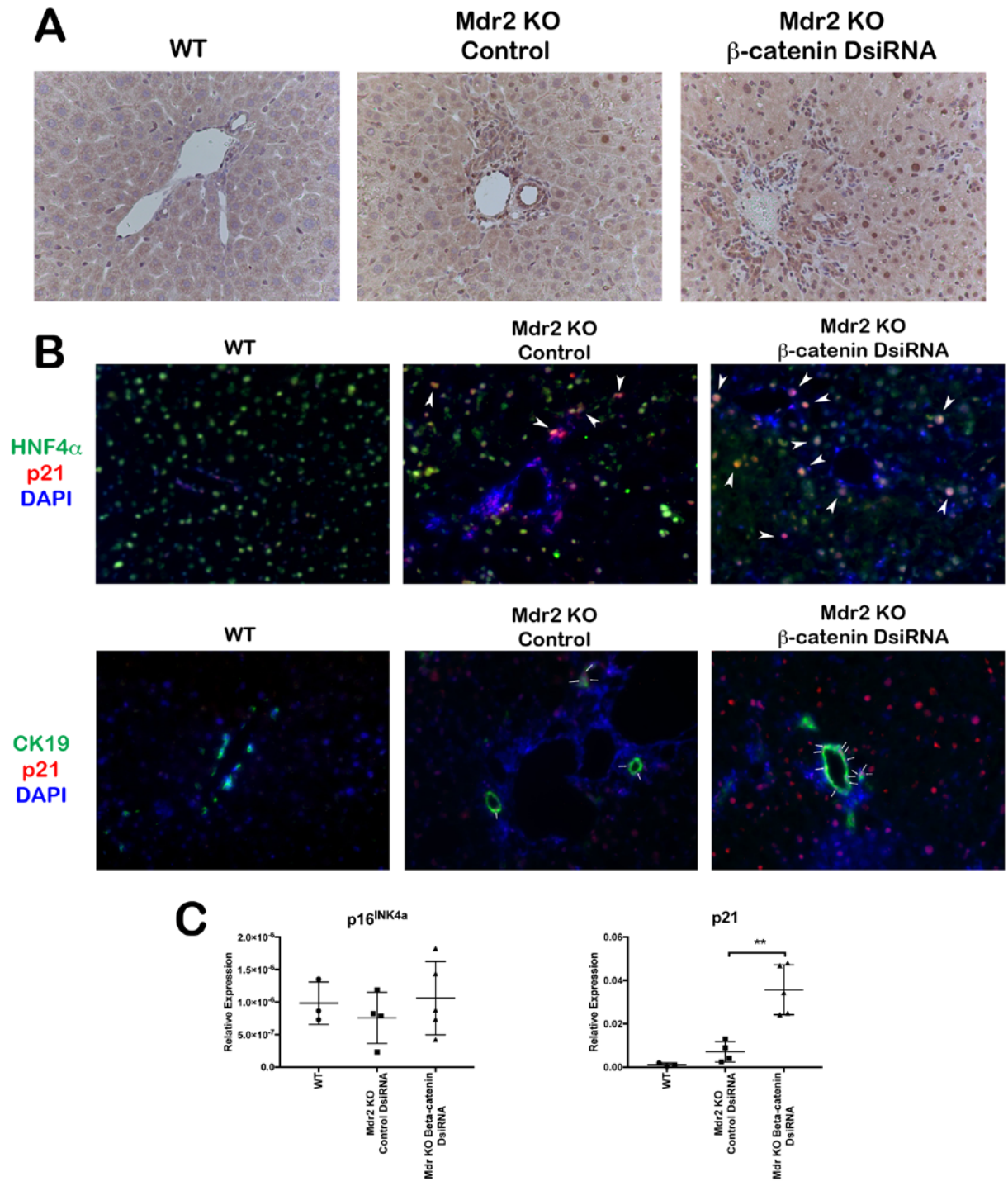
Supplementary Table 2: Primers used for qPCR studies on Hep3B cells

Gene	**Forward Primer**	**Reverse Primer**
β-catenin	GCTTTCAGTTGAGCTGACCA	CAAGTCCAAGATCAGCAGTCTC
GS	AAACGGATAATGGACATGGTGAG	AAGGCCAACCAAAGGGGTG
Cyclin D1*	CAATGACCCCGCACGATTTC	CATGGAGGGCGGATTGGAA
Vimentin	AGTCCACTGAGTACCGGAGAC	CATTTCACGCATCTGGCGTTC
TGFβ1	CTAATGGTGGAAACCCACAACG	TATCGCCAGGAATTGTTGCTG
TNFα	GAGGCCAAGCCCTGGTATG	CGGGCCGATTGATCTCAGC
MRP3	TGGGGTGAAGTTTCGTACTGG	CACGTTTGACTGAGTTGGTGATA
MRP4	AGCTGAGAATGACGCACAGAA	ATATGGGCTGGATTACTTTGGC
GAPDH	TCCAAAATCAAGTGGGGCGA	TGATGACCCTTTTGGCTCCC

*Harvard Primer Bank #77628152c3

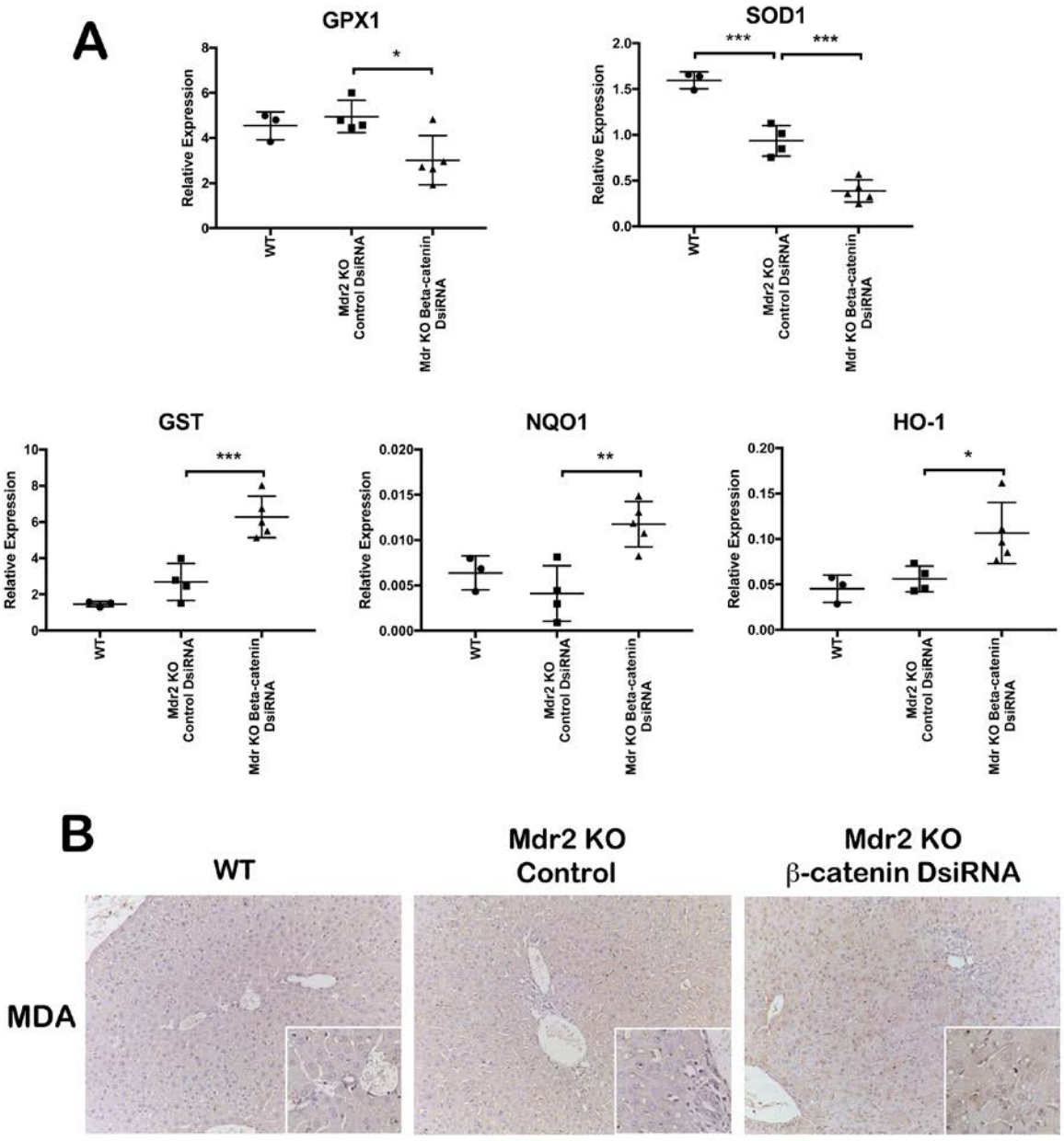


Supplementary Figure 1: Genetic knockout of β-catenin in the background of Mdr2 KO phenocopies many characteristics of KO/KD mice. (A) WB and qPCR confirms significantly decreased expression of GS, a surrogate marker of hepatocyte β-catenin, in double Mdr2/β-catenin KO (designated as KO/FF AAV8). (B) Serum ALP and total bilirubin are elevated in double KO, while ALT and AST levels are similar to Mdr2 KO alone. (C) H&E staining shows increased parenchymal injury in double KO liver compared to WT or Mdr2 KO alone (50X; 100X inset).



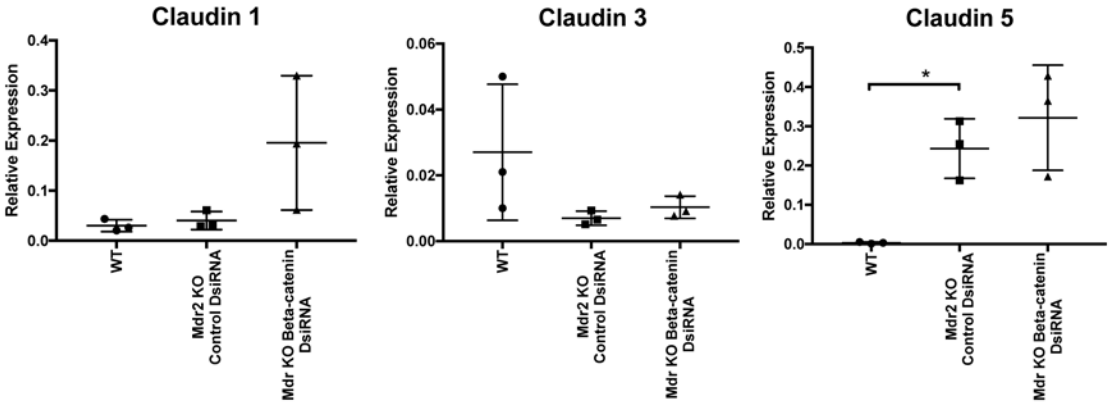
Supplementary Figure 2: Senescence is increased in KO/KD liver. (A) IHC shows increased nuclear p21 in cholangiocytes and a few hepatocytes of Mdr2 KO alone

compared to WT, while KO/KD liver has notably increased nuclear p21 in both cholangiocytes and hepatocytes compared to KO (200X). (B) Co-localization of p21 with hepatocyte (HNF4α) or cholangiocyte (CK19) markers by IF shows more double-positive cells in KO/KD livers compared to Mdr2 KO (200X). Arrowheads point to p21-positive hepatocytes; arrows point to p21-positive cholangiocytes. (C) qPCR for senescence markers shows a significant increase in p21, but not $p16^{INK4a}$, in KO/KD livers. **p<0.01.

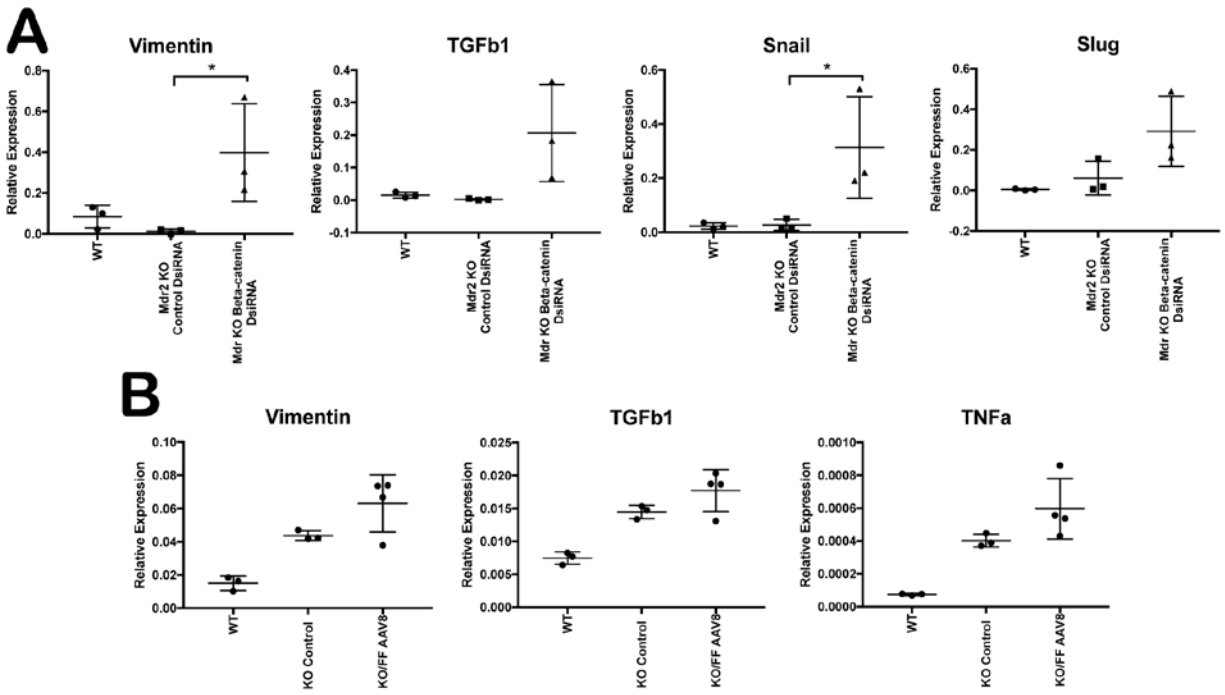


Supplementary Figure 3: Oxidative stress is increased in KO/KD liver. (A) qPCR shows that expression of some genes activated by oxidative stress, including GPX1 and SOD1, are decreased in KO/KD liver, while others, such as GST, NQO1, and HO-1, are increased. (B) IHC for malondialdehyde (MDA), a marker of lipid peroxidation, shows

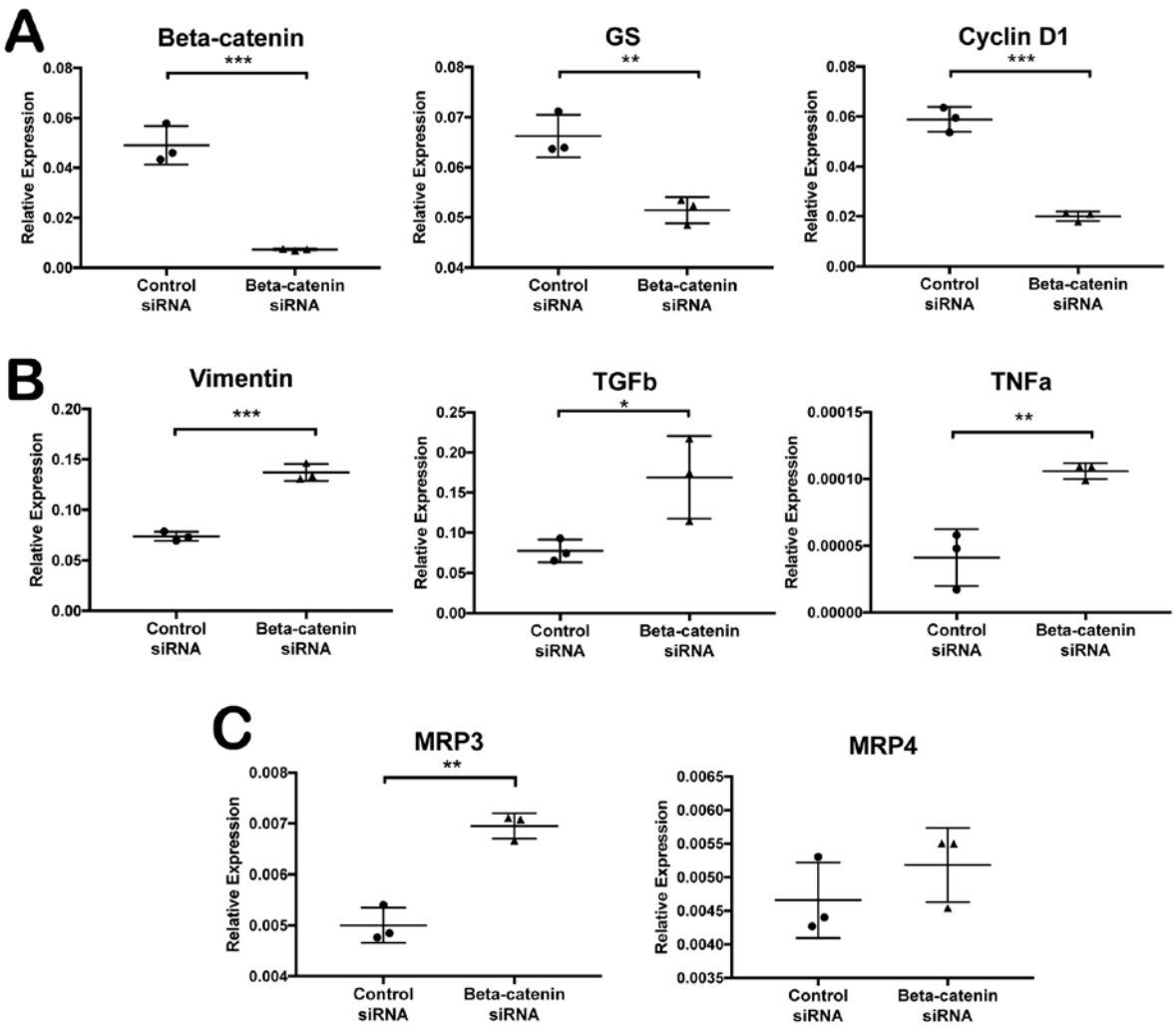
increased staining in KO/KD hepatocytes (50X; 100X inset). *p<0.05; **p<0.01; ***p<0.001.



Supplementary Figure 4: Expression of barrier-forming claudins is equivalent in KO with and without β-catenin inhibition. qPCR shows that expression of Claudin-3 and Claudin-5 are maintained in KO/KD liver compared to KO alone, while expression of Claudin-1, which induces EMT in hepatocytes, is insignificantly increased in KO/KD livers. *$p<0.05$.



Supplementary Figure 5: Loss of β-catenin and Mdr2 induces expression of EMT genes. (A) Expression of genes involved in cell polarity, as well as transcription factors that regulate EMT, are increased in KO/KD compared to WT and Mdr2 KO alone, as assessed by qPCR. (B) Vimentin and TGFβ are also increased in genetic Mdr2/β-catenin double KO, as is TNFα. *p<0.05.



Supplementary Figure 6: Knockout of β-catenin in a hepatocyte-like cell line is associated with phenotypic changes that contribute to loss of polarity, increased inflammation, and dysregulated BA transport. (A) Successful knockdown of β-catenin results in suppression of GS and cyclin D1 expression. (B) Vimentin and TGFβ, which are upregulated during loss of polarity, are increased after β-catenin inhibition; TNFα, an inflammatory cytokine, is also induced by β-catenin loss, as assessed by qPCR. (C) Loss of β-catenin results in increased expression of basolateral efflux transporter MRP3, but not MRP4. *p<0.05; **p<0.01; ***p<0.001.

Supplemental Movie Legends

Movie S1. Visualization of blood and bile trafficking in a wildtype mouse after IV administration of Texas red dextran and CF 1 minute prior to imaging. The sinusoids in wildtype mouse liver visualized by IV injection of Texas red dextran (red). Hepatocytes and biliary canaliculi are enriched in CF (green). Texas Red Dextran and CF localization appeared to be mutually exclusive at this time point. Original acquisition rate.

Movie S2. Time series video exhibiting the flow of Texas red dextran and CF through liver sinusoids and bile canaliculi at 3 min post administration in a wildtype mouse. Wildtype mouse liver imaging 3 minutes after IV injection of CFDA. The sinusoids are visualized by IV injection of Texas red dextran (red). The biliary canaliculi are nicely outlined with CF (green). Texas Red Dextran and CF localization is mutually exclusive at this time. Original acquisition rate.

Movie S3. ***Visualization of blood and bile flow in a wildtype liver through liver sinusoids and bile canaliculi using texas red dextran and CF respectively at 10 minutes post administration.*** Normal liver sinusoids in a wildtypemouse visualized by IV injection of Texas red dextran (red). The bile ducts are visualized with CF (green). CF fluorescence has considerably weakened at this time point as CF-containing bile is emptied from the liver. Original acquisition rate.

Movie S4. ***Visualization of blood and bile trafficking in Mdr2 knockout mouse liver after IV administration of Texas red dextran and CF 1 minutes prior to imaging.*** Mdr2 knockout

mouse liver sinusoids visualized by IV injection of Texas red dextran (red). Increased ductular reaction is visible in CF (green) channel. Sinusoidal blood flow appears normal. The numbers indicate time (1 minutes) after IV injection of CFDA. Original acquisition rate.

Movie S5. ***Visualization of blood and bile trafficking in Mdr2 knockout mouse liver after IV administration of Texas red dextran and CF 1 minutes prior to imaging.*** Mdr2 knockout mouse liver sinusoids visualized by IV injection of Texas red dextran (red). Increased ductular reaction is visible in. CF (green) channel. Sinusoidal blood flow appears normal. The numbers indicate time (3 minutes) after IV injection of CFDA. Original acquisition rate.

Movie S6. ***Visualization of blood and bile trafficking in Mdr2 knockout mouse liver after IV administration of Texas red dextran and CF 1 minutes prior to imaging.*** Mdr2 knockout mouse liver sinusoids visualized by IV injection of Texas red dextran (red). Increased ductular reaction is visible in. CF (green) channel. Sinusoidal blood flow appears normal. The numbers indicate time (10 minutes) after IV injection of CFDA. Original acquisition rate.

Movie S7. ***Visualization of blood and bile flow in Mdr2KO/β-catenin knockdown mouse liver through sinusoids and bile canaliculi at 1 minute post administration.*** Mdr2KO/β-catenin knockdown mouse liver sinusoids visualized by IV injection of Texas red dextran (red). CF (green) extensively co-localizes with Texas red dextran (red). Original acquisition rate.

Movie S8. ***Time series video exhibiting the flow of Texas red dextran and CF through liver sinusoids and bile canaliculi at 3 min post administration in Mdr2KO/β-catenin knockdown***

mouse liver. Mdr2KO/β-catenin knockdown mouse liver sinusoids visualized by intravenous injection of Texas red dextran (red). CF (green) is seen still observed co-localizing with Texas red dextran (red). Original acquisition rate.

Movie S9. ***Visualization of blood and bile trafficking in Mdr2KO/β-catenin knockdown mouse liver after IV administration of Texas red dextran and CF 10 minutes prior to imaging.*** Mdr2KO/β-catenin knockdown mouse liver sinusoids visualized by IV injection of Texas red dextran (red). Substantial co-localization of CF (green) and Texas red dextran (red) is visible in the hepatic sinusoids. CF (green) fluorescence is absent from both hepatocytes and biliary canaliculi. The numbers indicate time (10 minutes) after IV injection of CFDA. Original acquisition rate.