Supplemental Methods

Isolation and Study of Primary Liver Cell from Mice

Primary hepatic stellate cells (HSC), liver sinusoidal endothelial cells (LSEC), and hepatocytes were isolated from healthy 12-week-old C57BL/6 wild-type mice. Primary HSC were also isolated from PTN-KO mice and PTPRZ1-KO mice and corresponding strain controls.

LSEC Isolation

Liver sinusoidal endothelial cells were isolated from wild type C567BL/6 mice by collagenase perfusion, iodixanol density gradient centrifugation and centrifugal elutriation, as described.[1] Briefly, yields of LSEC are on average 80 million sinusoidal endothelial cells per 10 g liver with viability of more than 95% and purity greater than 96%, as determined by scanning electron microscopy (SEM), fluorescence-activated cell sorter (FACS) for CD31 and CD105, and positive staining for fluorescent acetylated low-density lipoprotein (Dil-Ac-LDL; Biomedical Technologies, Stoughton, MA) and FITC–FSA. FACS on F4/80 and desmin were used to exclude contamination by macrophage/Kupffer cells and HSC. Cells were cultured on rat-tail collagen-coated plates (400,000 cells/cm^2) in Dulbecco's minimal essential medium (DMEM) low glucose with 10% fetal bovine serum (FBS).

HSC Isolation

Hepatic stellate cells were isolated from mouse livers, as described.[2] Briefly, after in situ perfusion of the liver with pronase (Roche, Indianapolis, IN) and collagenase (Roche), dispersed cell suspensions were layered on a discontinuous density gradient of 15.6% Histodenz (Sigma-Aldrich). The resulting upper layer consisted of >98% HSC. Purity and viability were verified by phase-contrast microscopy examining auto-fluorescence and trypan blue exclusion (50 µg/ml; Roche).

Isolated hepatic stellate cells and liver sinusoidal endothelial cells were cultured for up to seven days in Dulbecco's Modified Eagle Medium containing 4500 mg/L glucose, 10% fetal bovine serum, and 1% penicillin-streptomycin.

Hepatocytes

Hepatocytes were isolated as described by Leffert.[3] Cell viability exceeded 90% in all experiments as determined by Trypan-blue exclusion.

Cell line maintenance

Murine ductular progenitor cell line 603B[4] was cultured in Dulbecco's modified Eagle's medium, supplemented with 10% fetal calf serum, 1 mM L-glutamine, and 100 IU/ml penicillin/streptomycin.

Clonally derived rat MF-HSC line 8B[5] was cultured in RPMI 1640 medium and supplemented with 10% fetal calf serum and 100 IU/ml penicillin/streptomycin. All cells were harvested by trypsinization.

Immunohistochemistry

Liver tissue was fixed in formalin and embedded in paraffin. Immunohistochemical staining was performed using the DAKO Envision System (DAKO Corporation) according to the manufacturer's protocol. Immunostaining were performed, as described.[2] Briefly, formalin-fixed paraffin-embedded liver tissues were cut into 5-μm sections and placed on glass slides. Sections were deparaffinized with xylene, dehydrated with ethanol, and then incubated with 3% hydrogen peroxide to block endogenous peroxidase. Antigen retrieval was performed by heating in 10 mM sodium citrate buffer (pH 6.0) for all antibodies, with the exception of Krt19, which utilized pepsin protease antigen retrieval (00-3009; Invitrogen). Sections were blocked in DAKO protein block (X9090; DAKO), followed by incubation with primary antibodies. Antibodies used are listed in supplemental table 2. DAB reagent (K3466; DAKO) was applied in the detection

procedure. Tissue sections were counterstained with Aqua Hematoxylin-INNOVEX (Innovex Biosciences). Double immunohistochemistry was performed using Vina Green, according to the manufacturer's recommendation (BioCare Medical). Negative controls included liver specimens exposed to 1% bovine serum albumin instead of the respective primary antibodies.

Sirius red staining, as well as αSMA, desmin, and elastin immunohistochemical staining were assessed by morphometry (MetaView software, Universal Imaging Corp.). For morphometric quantification, 10 randomly chosen fields at ×20 magnification per section were evaluated for each mouse. GFP staining was quantified using similar approaches: for each of these parameters, numbers of cells with stained nuclei were counted in 10 randomly chosen ×20 fields per section per mouse.

Phosphotyrosine Immunoprecipitation

Cells were harvested after 24 h of PTN treatment (100 ng/ml), washed once in ice-cold phosphate-buffered saline, and lysed in 0.5 ml of IP buffer (10% [vol/vol] glycerol, 50 mM HEPES [pH 7.5], 150 mM NaCl, 0.5% [vol/vol] Triton X-100, 1.5 mM $MgCl_2$, 1 mM EGTA, 200 μM Na_3VO_4, 10 mM NaF supplemented with PIC III protease inhibitor cocktail [Calbiochem]). For SDS-PAGE, lysates were boiled in SDS-PAGE loading buffer and proteins were analyzed by western blotting was according to standard methods using anti-phosphotyrosine PY20 (1:1,000) (Upstate Laboratories). Immunoreactive proteins were visualized using a horseradish peroxidase-catalyzed chemilluminescence reaction (Amersham-Pharmacia).

To investigate global phosphotyrosine profiles in ±PTN cell states, two independent approaches were utilized to increase coverage of potential downstream PTN dependent targets; a phosphotyrosine protein enrichment approach and a phosphotyrosine peptide enrichment approach. In the first approach, tyrosine phosphorylated proteins were enriched at the intact protein level following cell lysis using anti-phosphotyrosine antibodies (PY20) using

manufacturers recommended protocols (Santa Cruz). Eluents were separated by SDS-PAGE and uniquely enriched proteins determined by Coomassie staining were excised and subjected to in-gel trypsin digestion and TiO2 phosphopeptide enrichments using standardized protocols (http://www.genome.duke.edu/cores/proteomics/sample-preparation/). Phosphopeptide enriched peptides were then subjected to LC-MS/MS analysis using a Waters NanoAcquity UPLC coupled to a Waters Synapt G2 QToF high-resolution mass spectrometer though an electrospray interface. The instrument was operated in a data-dependent mode of acquisition with an MS scan from m/z 50-2000 followed by three MS/MS scans from m/z 50-2000. In the second approach, soluble protein extracts were trypsin digested and then subjected to phosphotyrosine directed immunoprecipitations (IP) using anti-phosphotyrosine (pY1000) antibodies coupled to agarose resin (Cell Signaling Technology). Phosphotyrosine enriched peptides from the IP samples were further enriched using TiO_2 metal oxide resins to reduce non-specific peptide binding to antibodies. pY/TiO_2 enriched phosphopeptides were then subjected to LC-MS/MS analysis using a Waters NanoAcquity UPLC coupled to a Thermo QExactive Plus high resolution orbitrap mass spectrometer though an electrospray interface.. The instrument was set to acquire a full MS scan at res=70,000 followed by ten MS/MS scans at res=17,500 with a 20 sec dynamic exclusion window. Spectra from both LC-MS/MS experiments were submitted to Mascot database searches against a forward/reverse SwissProt_Mouse database allowing for deamidation on Asn and Gln residues, oxidation on Met residues, and phosphorylation modifications on Ser, Thr, and Tyr residues. Spectra were annotated at <1.0 % false discovery rate with Scaffold. To define ambiguity in assignment of modification localization, all spectra were processed through the Ascore algorithim and a percent probability of correct localization was assigned [6 7].

Supplemental Figure Legends

Supplemental Figure 1. PTN deletion has no effect on normal liver histology. Tissue was harvested 14-days post sham surgery and liver sections from mice were stained with H&E (top panels) or Sirius Red/Fast Green (bottom panels); representative images are shown (magnification x4, top, x10, bottom).

Supplemental Figure 2. PTN KO exacerbates and PTPRZ1 KO inhibits cholestatic injury. Wild-type (WT), PTN KO (Top), or PTPRZ1 KO (bottom) mice were sacrificed 14-day after sham surgery or BDL. Liver sections were stained with H&E and examined under 4X magnification. Bile infarcts were quantified in 10 fields/section/mouse (n=4 mice/group) and total infarcts per 40 unique fields are expressed as mean ± SEM relative to respective WT sham control. Photomicrographs from representative mice post-BDL are shown; infarcts are indicated by arrows. **$P < 0.001$; *$P = 0.05$.

Supplemental Figure 3. PTN KO exacerbates, and PTPRZ1 KO inhibits, accumulation of Sox9-expressing cells during the ductular reaction to cholestatic injury. (A) SOX9 protein expression in representative immunostained liver sections from wild-type (WT), PTN KO, or PTPRZ1 KO mice subjected to either sham or 14-day BDL injury (original magnification, ×20). Corresponding quantification of immunostained images and qRT-PCR analysis of *Sox9* mRNA in whole liver RNA is shown in panels to the right. Results are expressed as mean ± SEM relative to WT BDL group. **$P < 0.01$; *$P < 0.05$.

Supplemental Figure 4. PTN does not alter MF-HSC or RDC viability. Vehicle (PBS) or various doses of PTN were added to MF-HSC and RDC for 24 or 48 hours; cell viability was assessed by colorimetric cell counting assay (CCK8). Mean ± SEM data from triplicate cultures are graphed.

Supplemental Figure 5. PTPRZ1 deletion suppresses Hh-regulated gene expression. Changes in expression for the indicated Hh target genes were assessed by qRT-CPR analysis in whole liver total RNA from either WT or PTPRZ1 KO mice subjected to sham or BDL injury. Results are normalized to S9 and expressed as mean ± SEM relative to WT sham-operated mice. *P<0.05; **P<0.01.

Supplemental Figure 6. PTPRZ1 deletion inhibits mesenchymal/migratory phenotypes of cells involved in the ductular reaction. (A) WT or PTPRZ1 mice were subjected to either sham-operation or 14-day BDL. Livers were harvested and representative immunohistochemistry, whole liver morphometry, and whole liver mRNA expression for αSMA is shown for sham (white bars) versus 14-day BDL (black bars). Results are expressed as fold over wild-type sham-operated controls. (B) HSC were isolated and pooled from WT or PTPRZ1 KO mice and treated with either PBS (UT) or PTN for 48 hours under serum free conditions (n=4 animals per group). Total RNA was isolated and analyzed for αSMA expression. Results are expressed as fold over UT control for each marker. *P < 0.05; ***P < 0.01.

Supplemental Figure 7. Differential effects of PTN and PTPRZ1 deletion on macrophage accumulation during cholestatic liver injury. Representative whole liver immunohistochemistry for F4/80 in WT, PTN KO, and PTPRZ1 KO mice either subjected to sham or 14-day BDL injury (magnification x40). Corresponding quantification of immunostained images is shown in panels to the right. Results are expressed as mean ± SEM relative to WT BDL group. **P < 0.01; *P < 0.05.

Supplemental Figure 8. Differential effects of PTN and PTPRZ1 deletion on M2 macrophage polarization after BDL. Representative whole liver immunohistochemistry for the M2 polarization marker, YM1, in WT, PTN KO, and PTPRZ1 KO mice either subjected to sham or

14-day BDL injury (magnification x40). Corresponding quantification of immunostained images is shown in panels to the right. Results are expressed as mean ± SEM relative to WT BDL group. **$P < 0.01$; *$P < 0.05$.

Supplemental Figure 9. PTPRZ1 deletion inhibits induction of IL-4 gene expression after BDL. IL-4 mRNA levels were quantified by qRT-CPR analysis in whole liver total RNA from either WT or PTPRZ1 KO mice subjected to sham or BDL injury. Results are normalized to S9 and expressed as mean ± SEM relative to WT sham-operated mice. *$P<0.05$; **$P<0.01$.

Supplemental Figure 10. PTPRZ1 deletion inhibits liver cell proliferation during cholestatic liver injury. Representative whole liver immunohistochemistry for the proliferation marker, Ki67,in WT, PTN KO, and PTPRZ1 KO mice either subjected to sham or 14-day BDL injury (magnification x40). Corresponding quantification of immunostained images is shown in panels to the right. Results are expressed as mean ± SEM relative to WT BDL group. **$P < 0.01$.

Supplemental Figure 11. PTPRZ1(+) ductular cells accumulate in a murine model of primary sclerosing cholangitis. Representative immunohistochemistry for PTPRZ1 in wild-type (WT) and MDR2 knockout mice is shown. PTPRZ1 is not detectable in normal adult mouse liver (left panel). Mice deficient in the MDR2 gene (which develop a PSC-like disease) demonstrated a marked increase in PTPRZ1 expression, particularly in ductular cells (right panel; arrows).

Supplemental Table 1: Primers used for real time PCR

Gene	Forward sequence	Reverse Sequence
m αSMA	GATGAAGCCCAGAGCAAGAG	CTTTTCCATGTCGTCCCAGT
m IL4	TGAACTGGGCAGCTATGAAGTC	TCCTGCTCTTCTTTCTCG
m Gli1	CCTCCTCCTCTCATTCCACA	CTCCCACAACAATTCCTGCT
m Gli2	CCCCATCACCATTCATAAGC	CTGCTCCTGTGTCAGTCCAA
m Krt19	CATGGTTCTTCTTCAGGTAGGC	GCTGCAGATGACTTCAGAACC
m Ptc	ATGCTCCTTTCCTCCTGAAACC	TGAACTGGGCAGCTATGAAGTC
m PTN	TTTTCATCTTGGCAGCTGTG	CACTCCACTGCCATTCTCCA
m PTPRZ1	CTGTCTAGTGGTTCTTGTTGGT	GGGTGTTGGTGGTGTAGATATT
m/r S9	GACTCCGGAACAAACGTGAGGT	CTTCATCTTGCCCTCGTCCA
m SNAIL	GAGGACAGTGGCAAAAGCTC	TCGGATGTGCATCTTCAGAG
r αSMA	GTGGATCACCAAGCAGGAGGAGT	CATAGCACGATGGTCGATTG
r BMP7	GTGGTCAACCCTCGGCACA	GGCGTCTTGGAGCGATTCTG
r Col1α1	CTGCATACACAATGGCCTAA	GGGTCCCTCGACTCCTA
r E-Cadherin	GCCACCAGATGATGATACCC	GCTGGCTCAAATCAAAGTCC
r Gli2	ATAAGCGGAGCAAGGTCAAG	CAGTGGCAGTTGGTCTCGTA
r Ptc	ATGCTCCTTTCCTCCTGAAACC	TGAACTGGGCAGCTATGAAGTC
r PPARγ	CCCTGGCAAAGCATTTGTAT	ACTGGCACCCTTGAAAAATG
r SNAIL	CATCCCTCCTGCGTCC	CCTAACAAGTGACGGCCATT
r Vimentin	GCCACCTTCGTGAATAACAG	TCCAGCAGCTTCCTGTAGGT

Supplemental Table 2: Antibodies used for IHC analysis

Name	Host / isotype	Source	Catalog No.
Desmin	Rabbit IgG	Abcam	ab15200
αSMA (α-smooth muscle actin)	Rabbit IgG	Abcam	ab32575
Krt7 (Cytokeratin 7)	Rabbit IgG	Bioss	bs-1744R
Krt19 (Cytokeratin 19)	Rat IgG2a	Developmental Studies Hybridoma Bank	TROMA-III-c
Elastin	Rabbit IgG	Cedarlane Laboratories	CL55041AP
PTPRZ1	Rat IgG	R&D Systems	MAB26881
GFP	Chicken IgG	Aves Laboratories	GFP-1020
Anti-Rabbit HRP		DAKO	K400311-2
Anti-Mouse HRP		DAKO	K400111-2
Anti-Chicken HRP		Abcam	Ab6753
Ki67	Rabbit IgG	Abcam	ab15580
F4/80	Rat	ABD Serotech	MCA497GA
YM1	Rabbit	Stem Cell Tech	1404
SOX9	Rabbit IgG	Millipore	AB5535

Supplemental References

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