SUPPLEMENTAL MATERIALS:

METHODS:

Mice:

GFAP-Cre mice, ROSA26-Stop$^{f/f\text{-}YFP}$ mice, and Rosa26$^{f/f/\text{-}mT/mG}$ mice [1]were obtained from (Jackson Laboratories [1, 2]). Tamoxifen-inducible CK-19^{CreERT} mice, in which a tamoxifen inducible Cre was knocked into the endogenous cytokeratin-19 locus, were generated as described[3] and crossed to homozygosity to achieve maximal Cre expression. To study EMT, CK-19^{CreERT} mice, were crossed with Rosa26$^{f/f\text{-}YFP}$ reporter mice to generate CK-19^{YFP} mice. Cre-loxP recombination was achieved in these mice by tamoxifen administration (5 mg x 9 times; Sigma T5648-1G). FSP-1^{Cre} mice were obtained from Dr. Nielson [4]. FSP-1^{Cre} mice were crossed to ROSA26$^{f/f\text{-}YFP}$ reporter mice. To study MET, GFAP^{Cre} mice were crossed to ROSA26$^{f/f\text{-}mT/mG}$-reporter mice [1]. Rosa26$^{f/f\text{-}mT/GFP}$ mice ubiquitously express membrane-tagged tomato red, flanked by *floxP* sequences, and upon Cre-loxP recombination upregulate expression of membrane-tagged GFP in the targeted cells. Col2(I)^{Cre} mice [5] were crossed to ROSA26$^{f/f\text{-}YFP}$ reporter mice to generate Col2(I)^{YFP} mice, in which all activated HSCs are labeled by YFP expression. All animal experiments were approved by the UCSD Institutional Animal Care and Use Committee.

Induction of Cre-LoxP recombination by tamoxifen administration:

Tamoxifen (Sigma T5648-1G) was dissolved in ethanol and diluted 1:10 in warm corn oil. To induce Cre-loxP recombination, CK-19^{CreERT} mice were gavaged with tamoxifen (5 mg/100 µl corn oil) for three consecutive days/a week for 3 weeks for BDL-induced liver injury; or for three consecutive days/every two weeks over a period of 12 weeks for CCl_4-liver injury. The repeated stimulation labeled existing and proliferating cholangiocytes. To achieve Cre-loxP recombination in embryos, pregnant CK-19^{CreERT} mice were gavaged with tamoxifen (1 mg /100 µl corn oil) from day 9.5 to 11.5.

Immunofluorescence and immunohistochemistry.

Liver tissues were fixed in 4% buffered formalin and antigens were retrieved in changes of 10% and 30% sucrose in PBS. The tissue then was frozen in O.C.T. (Sakura Finetek) at -80°C for further analysis. For immunofluorescence liver sections were incubated with anti-GFP Ab (Abcam), anti-α-SMA Ab (Abcam), anti-Desmin Ab (Thermo Scientific), anti FSP-1 Ab (gift from Dr. Neilson [6]), anti-GFAP Ab (Abcam), Troma III (DSHB Hibridoma Bank), FITC-conjugated monoclonal anti-α-SMA (Sigma) or the appropriate isotype control. As secondary antibodies Alexa Fluor 488–, Alexa Fluor 594– and Alexa Fluor 633-conjugated secondary antibodies were used. Nuclei were counterstained with DAPI. For immunohistochemistry, tissue was fixed in 10% buffered formalin followed

by 75% ethanol and embedded in paraffin. Immunohistochemistry was performed after antigen retrieval with anti- α-SMA Ab (Abcam) and anti-GFP Ab (Santa Cruz). For antigen detection the HRP labeled polymer (EnVision Dako) and the DAP staining kit (Vector) was used. Counterstaining was performed with Haematoxilin. Hematoxilin/Eosin and Sirius Red staining was performed as previously described [7]. Immunofluorescence and immunohistochemistry images were taken on an Olympus IX71 microscope with an Olympus DP71 camera. Confocal images were obtained by an Olympus FV1000 microscope. Images were processed and merged with NIH software ImageJ [8].

SUPPLEMENTARY FIGURES:

Supplementary Figure 1S. Genetic labeling of cholangiocytes in $K19^{YFP}$ mice in response to BDL.

Tamoxifen induced Cre-loxP recombination in $K19^{Cre}$ mice, as shown by immunohistochemistry for YFP. Representative images are shown at low power magnification to assess morphology. Specific immunostating was observed around the bile ducts (bd) and portal vein (pv) are labeled.

Supplementary Figure 2S. Genetically labeled cholangiocytes co-localize within the proliferating bile ducts in response to BDL in $K19^{YFP}$ mice.

Liver tissues were stained with anti-GFP Ab (to visualize YFP^+ cholangiocytes), following by H&E staining (to assess tissue morphology). The images were taken separately at 40 x and 400 x magnification and overlayed.

Supplementary Figure 3S. Genetically labeled cholangiocytes express Troma III in $K19^{YFP}$ mice in response to BDL.

Serial sections were stained with anti-GFP Ab (to visualize YFP^+ cholangiocytes), with Troma III Ab (to detect $K19^+$ cells), and Pan-CK Ab. Specific immunostainng (arrows) was detected in proliferating bile epithelial cells (bd - bile ducts), but not in portal veins (pv) of portal triade. Representative images are shown at 20, 40 x and 400 x magnification.

Supplementary Figure 4S. Pan-CK positive cells do not express α-SMA in response to liver injury.

Livers from the BDL- or CCl_4-injured wild type mice were co-stained with anti-Pan-CK and FITC-conjugated α-SMA antibody. Expression of Pan-CK was detected using Alexa-Fluor-594-conjugated secondary antibody. The overlap in staining was detected in one

out of 6 fields (shown with arrows), non-specific autofluorescent staining that projected into all filters is indicated (arrow labeled ns, nonspecific).

Supplementary Figure 5S. EMT is detected at E9.5 in $K19^{YFP}$ mice during embryonic development.

Genetic labeling of K19 cells was achieved in E9.5 embryos by tamoxifen administration to the pregnant mice from day 9.5 to 11.5. Embryos were sacrificed at E12.5. Co-localization of α-SMA, desmin and FSP-1 (yellow arrows) in $K19^{YFP}$ in lungs, skin, vasculature in embryos.

SUPPLEMENTARY REFERENCES:

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