Supplementary Table 1: Primers for human genome AKT and NICD DNA

AKT	Forward: TCAGGATGTGGATCAGCGAG Reverse: GGTGAGCCTGATCGGAAGTC
NICD	Forward: GAGTGGGGAGACGAAGACCT Reverse: GAAGCCATCTGGTCCTCGAA

Supplementary methods:

Determination of maximal tolerable injection parameters of the swine bile duct. A swine liver was removed surgically, and the common bile duct was resected close to duodenum. A 7F biliary extraction balloon (Extractor Pro RX, Boston Scientific, Natick, Mass) was inserted into the common hepatic duct (CHD) of the liver of swine under visual control. The balloon was inflated to 12 mm and connected with an angiographic injector pump (Medrad Mark V, ProVis, Bayer HealthCare LLC, Whippany, NJ). Escalating volumes (10-50 mL) and injection rates (1-4 mL/s) of 1% methylene blue diluted in normal saline were tested until there was evidence of gross rupture of the biliary tree (***Supplementary Figure 1A***).

Anesthesia for in vivo non-survival and survival hydrodynamic injections. Food was withheld beginning 12 hours before the procedure, but water continued to be offered *ad libitum*. On day of procedure, swine were sedated with an intramuscular injection of tiletamine, zolazepam, ketamine, and xylazine (TKX) (Zoetis, Florham Park, NJ) at 1 mL/20 kg. Once sedated swine were intubated and administered maintenance fluids through a marginal ear vein catheter. General anesthesia was maintained with isoflurane (VetOne, Boise, Idaho) and mechanical ventilation was provided. During the procedure, electrocardiogram, blood pressure, heart rate, oxygen saturation, and end tidal CO_2 were monitored by the veterinary technician anesthetist, every five minutes. Given the minimally invasive nature of the procedure, neither intra or post procedural antibiotics nor analgesics were indicated. At predetermined study endpoints, swine were sedated with TKX and euthanized with pentobarbital-based euthanasia solution at 100 mg/kg IV (VetOne, Boise, Idaho).

Plasmids used and their allocation in swine. For the purposes of stable gene expression in target liver of swine, we chose target plasmids combined with Sleeping Beauty-mediated somatic integration. The constructs pT3-EF1a-NICD, pT3-EF1a-AKT, pT3-N90-beta-catenin, and transposon plasmid pCMV-SB were gifts from Dr Xin Chen.[28-30] We choose NICD, AKT and beta-catenin plasmids based on the fact that they contain either myc-tag or HA-tag, allowing detection via Western blot or immunostaining for *in vivo* experiments. All plasmids were purified according to manufactures of GenElute HP Endotoxin-Free Plasmid Maxiprep Kit (cat: NA0410-1KT, St. Louis, Mo). Construct plasmids at the concentration of 100 ug/mL each in total 30 mL mixed together transposon pCMV-SB at the ratio 1:25 to target plasmids diluted in 0.9% NaCl were used for in vivo swine hydrodynamic injection experiments. In total, 12 swine separated into 3 groups (**Table 1**) were used.

Assessment of tissue damage and harvest of liver. To assess for evidence of hepatocellular injury as a result of the intervention, peripheral blood samples were taken immediately before hydrodynamic injection, 7 days after injection and at the time of animal death (day 21, 30 or 60 depending on the swine). Standard complete blood count and liver biochemistry (bilirubin, aspartate transaminase, alanine transaminase, gamma-glutamyl transpeptidase, alkaline phosphatase and albumin) were performed (IDEXX Laboratories, Glen Burnie, Md). An ERCP with a balloon occlusion cholangiogram was performed immediately after animal death to assess the anatomy of the biliary tree. Necropsy was then performed with the entire liver and biliary tree removed en bloc. The swine were examined for the presence of ascites and intra-abdominal organs were macroscopically assessed. The liver was sectioned and representative liver tissue samples (6 per liver

corresponding to proximal and distal areas of each major liver lobe) were collected for analysis.

Genomic DNA PCR. Livers of all killed swine from each group were harvested. Genomic DNA from swine livers were extracted according to manufacturer's protocol (Cat: 69504, Qiagen, Hilden, Germany). Primersfor AKT, NICD, beta-catenin plasmids are shown in ***Supplementary Table 1***. Platinum Taq DNA polymerase (Cat: 10966026, Life Technologies, Carlsbad, Calif) were used for the reaction. The amplification were performed at 95 ℃ for 10 minutes (intimal denaturation), then 35 cycles for 30 minutes at 94 ℃ for 30 sec (denaturation), followed by extension at 59℃ (AKT)/57℃ (NICD)/ 57 ℃ (beta-catenin) for 1 minute, and at 72 ℃ for 1 minute, followed by extension at 72 ℃ for 10 minutes. PCR reaction result were checked on 2% agar gel.

Western blotting. Liver tissue were lysed in RIPA buffer (Cat: no. 9806, Cell Signaling, Danvers, Mass) supplemented with a protease inhibitor complete EDTA-free (Roche, Basel, Switzerland). Pierce BCA Protein Assay kit (Thermo Fisher Scientific, Waltham, Mass) was used to measure protein concentration. Cell lysates (30 µg) mixed with 2X Laemmli sample loading buffer (Cat: 161-0737, Bio-Rad Laboratories, Hercules, CA) were electrophoresed on 4% to 15% polyacrylamide gels (Bio-Rad Laboratories, Hercules, Calif), then transferred to nitrocellulose membrane (Bio-Rad Laboratories, Hercules, Calif). The membranes were blocked with Tris Buffered Saline (TBS: Cat: 46-012-CM, Cellgro, Tewksbury, Mass) containing 5% milk (Bio-Rad: 170-6404) and 0.1% Tween-20 (Cat: 274348, Sigma-Aldrich, St. Louis, Mo), then incubated with the primary antibody at 4 ℃ overnight. Myc tag (cat: no. 2276, Cell Signaling, Danvers, MA) and HA-tag antibodies (cat: no. 3724, Cell Signaling, Danvers, Mass) were purchased from Cell Signaling. The membranes were incubated after washing with anti-rabbit/mice IgF-HRP (Santa Cruz, Dallas, Tex) and analyzed using SuperSignal West Pico Chemiluminescent Substrate (cat: no. 34080,Thermo Fisher, Waltham, Mass).

Immunofluorescence staining. Swine liver tissue was fixed in 4% paraformaldehyde overnight at room temperature, then embedded into paraffin, 6 µM section were used for immunofluorescence staining. Tissue sections were treated via the citrate buffer antigen retrieval protocol, followed by standard immunofluorescence protocol. Section slides were blocked with 5% goat serum, then incubated with the primary antibody HA-tag and Myc-tag (Cat: no. 3724; Cat: no. 2276, Cell Signaling, Danvers, Mass) overnight at 4℃. They were then was hed three times with PBS/Tween 20 and incubated with the secondary antibody for 1 hour in the dark at room temperature, and washed 3 times 5 min with PBS/Tween 20. All cells were incubated with DAPI (Cat: 10236276001 ver.17, Roche, Basel, Switzerland) and washed 3 times with PBS, fixed and checked via fluorescence microscope (Zeiss, Oberkochen, Germany).

Supplementary Figure Legends:

Supplementary Figure 1: Gross picture of and H&E staining of swine liver after hydrodynamic injection from common bile duct ex vivo. A: Ex vivo study performed by inserting a 7F biliary stone extraction balloon into the common hepatic duct, inflating the balloon *(arrow)* and injecting 1% methylene blue diluted in normal saline solution using an angiographic injector pump. Escalating volumes (10-50 mL) and injection rates (1-4 mL/s) were tested. Transient swelling of the entire liver during and immediately after the hydrodynamic injection was observed. The bile duct ruptured when 50 mL of solution was injected at 3 mL/s. **B:** H&E staining shows

normal swine liver architecture 60 days after in vivo hydrodynamic gene delivery. We can see normal liver structure, composed of central vein, portal triads, hepatic sinusoids and liver lobules (orig. mag. x10).

Supplementary Figure 2: Fluoroscopic snap shot of swine bile duct rupture after inject contrast via ERCP. A-F: Rapid sequence fluoroscopic images (every 3 seconds) during injection of 40 mL of contrast medium at 2 mL/s. **D** shows rupture of the proximal CHD during injection, which is represented by contrast extravasation immediately distal to the tip of the balloon catheter just below the hepatic hilum. Therefore, these parameters were too aggressive for hydrodynamic injection.

Supplementary Video 1: **Injecting volumes at ERCP tested in a sequential manner**. Injecting 40 mL at 2 mL/s resulted in rupture of the proximal CHD during the process of injection. Contrast extravasation can be seen immediately distal to the balloon catheter.

Supplementary Video 2: Optimal injection parameters for ERCP directed hydrodynamic injection. Injection volumes greater or equal to 20 mL, regardless of rate, demonstrated acinarization of the liver parenchyma indicating that contrast had exited the biliary tree. Injecting 30 mL at 2 mL/s resulted in acinarization of all liver segments without rupture of the bile duct wall. Therefore, we used these parameters and encountered no rupture or other local adverse events.