

SUPPLEMENTARY METHODS

Animal care and surgeries

Mice were housed in 12 h light-dark cycle with standard chow diet (Teklad Global 18% Protein Rodent Diet) and water provided *ad libitum*. We performed a 2/3rd partial-hepatectomy (PHx) procedure adapted from previously reported protocol (Boyce and Harrison, 2008; Mitchell and Willenbring, 2008). Briefly, a bilateral subcostal abdominal incision was made on the skin of the anesthetized (continuous isoflurane inhalation, 2%) animal to expose the abdominal musculature. 4/0-silk ligatures were then placed across the superior gastric vessels positioned vertically on either side of the xiphoid process to minimize blood loss from the peritoneal wall. Following this, a bilateral incision was made on the abdominal wall to expose the liver. The left lateral and median lobes were ligated and excised. Following this, the peritoneum was closed with a continuous 5/0 silk suture, and the skin was closed using 7 mm reflex clips. The anesthesia was then removed, and the mouse allowed to recover on a pre-warmed heating pad. Sham mice that underwent a similar surgical intervention except for the actual resection of the liver were used as controls when indicated. To minimize post-surgical discomfort, Carprofen (5 mg/kg) was administered subcutaneously as an analgesic immediately after surgery.

Immunofluorescence staining

For cumulative labelling of DNA synthesis in hepatocytes post-2/3rd PHx, EdU solution was replaced every 48h and supplied in feeding bottles protected from light. Harvested liver tissue was fixed in 10% neutral buffered formalin for 24h and embedded in paraffin. 5 µm thick sections were cut, deparaffinized in xylene, rehydrated and antigen retrieved in citrate buffer (10 mM sodium citrate, 0.05% Tween 20, pH 6.0). The sections were

washed with wash buffer (Tris-buffered saline with 0.05% Triton X-100) and blocked for 2h at room temperature in blocking buffer (10% normal goat serum + 1% BSA in TBS). To visualize EdU-labelled DNA, Alexa Fluor 488 was conjugated to EdU using Click-iT EdU Alexa Fluor Kit (Thermo Fisher) as per the manufacturer's instructions. Primary antibodies were then applied to the sections at appropriate dilutions and incubated overnight at 4 °C. Following this, the sections were washed with washing buffer and incubated at room temperature for 1h with Alexa Fluor 588 conjugated secondary antibody. Nuclei were then stained with ToPro3 (1 uM in PBS) for 15 min at room temperature. CC/Mount aqueous mounting media (Sigma-Aldrich) was used to mount and coverslip the sections before imaging on a Zeiss LSM 710 microscope. Primary antibodies used include mouse anti-HNF4A (Abcam, ab41898), rabbit anti-HNF4A (Cell signaling technology, C11F12), mouse anti-CDH1 (BD biosciences, 610181) and mouse anti-GLUL (BD biosciences 610518). A two-step antibody treatment strategy was implemented to combine CDH1 and GLUL staining. Briefly, the liver sections were incubated with PE-Cy5 conjugated anti-GLUL overnight at 4 °C after CDH1 staining and secondary antibody incubation, followed by nuclear staining with Hoechst 33342 (NucBlue reagent, Invitrogen R37605). Mouse anti-GLUL antibody was conjugated with PE-Cy5 fluorophore using the Lightning-Link PE-Cy5 Antibody Labeling Kit (Novus Biologicals 760-0010) according to the manufacturer's instructions.

Liver function tests

Retro-orbital punctures were used to collect whole blood from mice into Capiject gel/clot activator tubes. The serum was isolated by centrifugation at 8500g for 10 min and stored at -80 °C until further analysis. Serum chemistry analysis for ALT and AST levels were

performed using commercial assay kits (Infinity Kits, Thermo Scientific) according to the manufacturer's protocols. GraphPad Prism 6 was used to perform statistical analysis (One-way ANOVA) and prepare graphical representations.

Tissue dissociation and isolation of liver cells

While the animal was anesthetized through continuous inhalation of 2% isoflurane, a 5-cm long incision was made in the abdomen to expose the portal vein and inferior vena cava. To perfuse the liver, the portal vein was cannulated, and ~30 ml of solution I (1x HBBS without $\text{Ca}^{2+}/\text{Mg}^{2+}$ with 1 mM EDTA, 37 °C) was passed followed by ~40-50 ml of solution II (HBSS with 0.54 μM CaCl_2 , 40 $\mu\text{g}/\text{ml}$ Trypsin Inhibitor, 15 mM HEPES PH 7.4 and 6000 units of collagenase IV, 37 °C). After perfusion, the liver was excised and massaged in washing solution (DMEM + Ham's F12 (50:50) with 5% FBS and 100 U/ml Penicillin/Streptomycin, 4 °C) to release cells from the liver capsule. The cell suspension was then passed through 40 μm filter to remove doublets/undigested tissue chunks, pelleted by centrifugation at 350xg for 4 min at 4 °C to remove debris and resuspended in 15 ml wash buffer. Cells were counted with an automated hemocytometer, and ~1-1.5 million cells were processed for library preparation.

Pseudo-temporal trajectory analysis

The CellDataSet class monocle objects were made from log-normalized Seurat object containing the cells under consideration. Dimensionality reduction was performed using the DDRTree algorithm, and the 3000 most significant differentially expressed genes were used as ordering genes to perform pseudotime ordering, to obtain cell trajectories. Genes with expression patterns co-varying with pseudotime were determined by the 'differentialGeneTest()' module, clustered and plotted using the

'plot_pseudotime_heatmap()' module. Expression patterns in clusters were distinguished as upregulated/downregulated along the pseudotime, and GO analysis was performed using DAVID 6.8 (Dennis et al., 2003) to identify biological pathways that are up or downregulated along the pseudotime.

Pathway scoring

Relative scores of biological pathways were assessed with the CellCycleScoring() module in Seurat v3.1. Pathway terms and exhaustive lists of candidate genes for each pathway were obtained from the Rat Genome Database (RGD) (Smith et al., 2020). The summary heatmap was generated using Seurat v3.1, considering all pathways with at least 50 genes. Box plots to demonstrate cell cycle and pathway scores were constructed using ggplot2, and pairwise comparison with reference using a *t*-test was performed with the ggpubr package.

Imputation and Cell-cell communication analysis

ScRNA-seq data often contains dropouts or missing values due to failure in the detection of RNAs. In our dataset, we noticed that NPCs have lower UMIs relative to hepatocytes, and could potentially have dropouts leading to incomplete representations. Hence, Imputation using the MAGIC algorithm (van Dijk et al., 2018) was performed to correct for any missing values in the NPC dataset before interpreting cell-cell interactions. We constructed cell-cell communication networks and performed statistics of interactions using methods previously described in detail by Farbehi et al. (Farbehi et al., 2019). Briefly, we used a directed and weighted network with four layers of nodes, namely, the *source* cell populations expressing the ligands, the *ligands* expressed by the source populations, the *receptors* targeted by the ligands, and the *target* cell populations.

Weights of edges that connect '*source to ligand*' and '*receptors to target*' were computed as \log_2 fold change in expression of ligand/receptor in source/target compared to remaining cells. Ligand-receptor interactions were determined using a mouse-specific ligand-receptor interaction dataset compiled previously ((Farbehi et al., 2019). The sum of weights along the path was used to calculate path weights connecting a source to target through a ligand:receptor interaction. Cells were grouped according to cell types, and hepatocytes were subdivided based on cellular states. We used all ligand:receptor connections with a minimum pathweight of 1.5 and calculated the overall weight, $w_{s,t}$, as the sum of all pathweights between the corresponding source and target. Only edges with Benjamini-Hochberg adjusted P-values, $P_w < 0.01$ were considered significant. Further, we constructed ligand:receptor interaction dot plots using ggplot2.

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