A human liver cell-based system modeling a clinical prognostic liver signature for therapeutic discovery

Chronic liver disease and hepatocellular carcinoma (HCC) are life-threatening diseases with limited treatment options. The lack of clinically relevant/tractable experimental models hampers therapeutic discovery. Here, we develop a simple and robust human liver cell-based system modeling a clinical prognostic liver signature (PLS) predicting long-term liver disease progression toward HCC. Using the PLS as a readout, followed by validation in nonalcoholic steatohepatitis/fibrosis/HCC animal models and patient-derived liver spheroids, we identify nizatidine, a histamine receptor H2 (HRH2) blocker, for treatment of advanced liver disease and HCC chemoprevention. Moreover, perturbation studies combined with single cell RNA-Seq analyses of patient liver tissues uncover hepatocytes and HRH2\(^+\), CLEC5A\(^{\text{high}}\), MARCO\(^{\text{low}}\) liver macrophages as potential nizatidine targets. The PLS model combined with single cell RNA-Seq of patient tissues enables discovery of urgently needed targets and therapeutics for treatment of advanced liver disease and cancer prevention.
Progressive liver fibrosis, caused by viral (hepatitis B and C viruses [HBV and HCV]) or metabolic (alcohol and non-alcoholic steatohepatitis [NASH]) etiologies frequently leads to highly lethal cirrhosis and hepatocellular carcinoma (HCC), a leading cause of cancer death globally. Advanced liver fibrosis has been shown to be the key risk factor for HCC in NASH with no approved treatment options. Moreover, in hepatitis C patients with advanced fibrosis, HCC risk persists despite viral cure. HCC incidence and death rates have sharply increased compared to other forms of cancer. Given the extremely high prevalence of advanced liver fibrosis and cirrhosis, which are estimated to affect ~1–2% of the global population, there is a major unmet medical need for chemoprevention of liver disease progression toward cancer development. While chemoprevention has the potential to significantly impact the prognosis of patients with chronic diseases by reducing lethal complications, the development of effective chemopreventive drugs for advanced liver diseases has been a daunting task as evidenced by the absence of approved therapies.

Previously, we have identified a pan-etiology 186 gene clinical prognostic liver signature (PLS) in diseased liver tissues robustly predicting liver disease progression and carcinogenesis in multiple patient cohorts as well as animal models. While the PLS has been robustly associated with liver disease progression and clinical risk of HCC development, efficient and high-throughput identification of candidate compounds for treatment of advanced liver disease and HCC chemoprevention has been hampered by the absence of tractable model systems and the complex cell circuits of disease biology.

In this work, we describe a simple and robust human cell culture system that models the clinical PLS in an inducible and reversible manner and apply it for drug discovery. By combining our system and validation in rodent and patient-derived models, we discover nizatidine, a histamine receptor H2 (HRH2) blocker, for treatment of advanced liver disease and HCC chemoprevention.

Results

Development of a liver cell-based system recapitulating the clinical PLS in cell culture. To establish a liver cell-based system modeling the clinical PLS, we used the hepatoma-derived Huh7.5.1 cell line differentiated to acquire a hepatocyte-like phenotype (Huh7.5.1\textsuperscript{H2}), as shown by global transcriptome profile by 10 days of culture with dimethyl sulfoxide (DMSO; Supplementary Fig. 1). Metabolism- and coagulation-related pathways, the major functions of mature hepatocytes, were gradually induced in hepatocyte-like cells, indicating a shift of the global transcriptome from a malignant to a hepatocyte-like profile. As chronic hepatitis C is one of the well-established inducers of the PLS in patients, Huh7.5.1\textsuperscript{H2} cells were subsequently infected with HCV strain Jc1 (Fig. 1a, b). Compared to mock-infected cells, persistent viral infection increases the expression poor-prognosis genes and suppresses expression of the good-prognosis genes, leading to the induction of the poor-prognosis pattern of the clinical PLS (Fig. 1c and Supplementary Fig. 2, the full PLS gene list is described in Supplementary Data 1). The global induction or suppression of the PLS signature was then determined by gene set enrichment analysis (GSEA) using mock-infected cells as reference, and results were presented as simplified heatmaps (Fig. 1d). The PLS induction is viral titer- and time-dependent, with full development at day 7 post infection, and virus specific, as shown by infection with highly purified FLAG-tagged HCV (HCV Jc1E2\textsuperscript{FLA2}; Fig. 1d and Supplementary Figs. 2 and 3). Moreover, HCV treatment by interferon alpha-2a (IFNα-2a) or direct-acting antivirals (DAAs) partially reversed the PLS (Fig. 1d), corroborating its causal link with persistent HCV infection. ScRNA-Seq analyses revealed correlation of HCV viral load and the PLS induction in Huh7.5.1\textsuperscript{H2} cells (poor-prognosis genes: $R = 0.5099$, $p = 0.0008$; good-prognosis genes: $R = -0.3751$, $p = 0.0171$, Pearson correlation), further supporting the causality of HCV infection for the induction of the poor-prognosis PLS (Fig. 1e, f).

Along with the induction of the PLS, transcriptome profile of HCV-infected Huh7.5.1\textsuperscript{H2} cells showed that a range of metabolic pathways involved in physiological functions of hepatocytes were suppressed. These results suggest that the PLS induction is accompanied by altered metabolic functions of hepatocytes (Supplementary Fig. 3 and Supplementary Data 2). In contrast, we observed a gradual induction of immune response-related pathways, growth factor signaling pathways and enhanced cell cycle, which are all known to be involved in liver fibrogenesis and carcinogenesis. Taken together, these data indicate that HCV infection of Huh7.5.1\textsuperscript{H2} cells models pathway dysregulation observed in human liver disease.

Infection with hepatitis viruses and metabolic injuries in cell-based models induce the clinical PLS similar to patient cohorts.

Since there are shared mechanisms leading to hepatocarcinogenesis across the main liver disease etiologies, we investigated whether the PLS can be similarly induced in cell-based models by other chronic viral infections or metabolic injuries. To model the PLS for HBV infection, we applied another cell system based on HepG2 cells engineered to overexpress sodium taurocholate cotransporting polypeptide (HepG2-NTCP cells), a cell entry factor for HBV. Persistent HBV infection induced the poor-prognosis PLS (Fig. 1g, and Supplementary Figs. 2 and 4a), however, less robustly than HCV infection. The specificity of the PLS induction in HepG2 cells was confirmed by absent induction of the PLS using other hepatotropic viruses, such as hepatitis D virus (HDV), which requires coinfection of HBV to induce liver cirrhosis/cancer, hepatitis E virus (HEV), and dengue virus (DENV), not related to liver cancer (Fig. 1g, and Supplementary Figs. 2 and 4a, b). Ethanol and free fatty acid (FFA) exposure, which cause alcoholic liver disease and induce NASH, respectively, robustly induced the PLS in HepG2 cells alone or cocultured with LX2 stellate cells. Interestingly, hypoxia, that promotes fibrogenic liver disease, induced expression of the PLS poor-prognosis genes, whereas nutrient starvation did not result in the induction of the PLS (Fig. 1g). The PLS induction appears therefore specific for the two cancer-causing viruses (HBV and HCV) and the two key metabolic injuries (alcohol and dietary FFA) responsible for the fibrogenic/carcinogenic liver diseases in patients.

The clinical relevance of the PLS in the cell-based model (from now referred as cPLS for “cell culture-derived PLS”) was confirmed by similar transcriptomic dysregulation in cell culture and the diseased liver of clinical cohorts, with corresponding liver disease etiologies (Fig. 1h, i). A highly significant similarity was observed for PLS induction between the cells treated with the respective etiological agents and the liver tissue of patients with poor prognosis or severe disease manifestation. These data suggest that upon the etiological insults, transcriptional dysregulation observed in the cell culture models mimics gene expression changes in tissues of patients with advanced liver disease.

Previous studies had already suggested that certain cancer cell-based models share signaling pathways with different cell types (e.g., NIH LINCS program, https://clue.io/). Since we observed the PLS induction in monolayer cell culture lacking multicell-type tissue architecture, we investigated whether the cPLS system models transcriptomic dysregulations not only in liver parenchymal but also non-parenchymal cells (NPCs). Interestingly, an analysis of the expression of the PLS genes in the main cell compartments within patient liver tissues revealed that the PLS poor-prognosis genes are most prominently expressed in NPCs,
whereas the expression of the PLS good-prognosis genes is higher in hepatocytes (Fig. 1j). Next, we analyzed the pathways dysregulated in cirrhotic liver tissues at single-cell level, using recently published scRNA-Seq data from five cirrhotic livers compared to five healthy livers. We compared these pathways with the pathways dysregulated in cPLS model. We observed that the PLS expressing cells share dysregulated pathways with epithelial cells mainly consisting of hepatocytes and with some NPCs, including immune cells (Supplementary Fig. 5a, b). Together, these data demonstrate that the cPLS system captures molecular pathways of epithelial cells, such as hepatocytes, as well as certain pathways shared between hepatocytes and NPCs. ScRNA-Seq and flow cytometry analyses show heterogeneity of the cPLS model on a cellular level (Fig. 1e and Supplementary Fig. 1 Modeling the clinical prognostic liver signature (PLS) in a cell-based system. a Experimental approach. b Analysis of HCV infection by qRT-PCR (log10 of relative RNA quantity normalized to GAPDH mRNA; mean ± SD, n = 3). Immunodetection of HCV E2 protein 10 days post infection. Scale bar: 50 µm. c PLS assay in Huh7.5.1dif cells. Heatmap shows expression of poor- and good-prognosis genes (186 gene signature). Results are representative of one out of three independent experiments performed in triplicate. d Analysis of the PLS after HCV Jc1 infection and antiviral treatment. PLS induction was determined by GSEA analysis using “mock” non-infected cells as reference. Simplified heatmaps show PLS global status and PLS poor- and good-prognosis gene expression. Results from two experiments performed in triplicate are shown. FDR false discovery rate, NES normalized enrichment score. e scRNA-Seq profiling reveals HCV-dependent induction of the PLS in Huh7.5.1dif cells. Heatmaps show: PLS global status and modulation of the poor-prognosis (FDR = 0.013) and good-prognosis genes (FDR = 0.016). White: non-infected cells, n = 17; black: infected cells, n = 23. f Enrichment scores of PLS gene modulation for their correlation with the HCV viral load measured at the single-cell level (Pearson correlation tests). g PLS assays in the cell-based models. Cell-based models were infected with different viruses or exposed to metabolic cues. HBV infection was performed in HepG2-NTCP cells and free fatty acids (FFA) treatment was performed in Huh7.5.1dif cells cocultured with LX2. Results are from three independent experiments. h PLS in clinical liver tissues from patient cohorts. HCV-related cirrhosis (Italy, n = 216 GSE156546; US, n = 145 GSE541027), HBV-related liver cancer (n = 199, GSE14520), alcoholic hepatitis (n = 22, GSE28619), and NASH (n = 72, GSE49541). Induction of the PLS was assessed by comparing diseased tissues with non-diseased tissues. i Induction of the PLS in cell-based systems was compared to liver transcriptome profiles from clinical cohorts using Subclass Mapping. j PLS deconvolution showing mean gene expression of the poor- and the good-prognosis genes in the main cell compartment within the liver. The scRNA-Seq dataset was extracted from GSE115469. Source data are provided as a Source data file.
Fig. 5c–e). To further investigate cell heterogeneity, we assessed the diversity across individual HCV-infected Huh7.5.1dif cells, according to KRT18 expression levels, a marker of hepatocyte. While the sample size was limited, the diverse KRT18 expression across the cells enabled a robust comparative analysis. We observed no biased expression of the poor- and good-prognosis-associated genes, according to the KRT18 expression levels, suggesting that there is no differentiation of the HCV-infected Huh7.5.1dif cells to form either parenchymal- or NPC-like subpopulation with differential expression of the poor- or good-prognosis-associated PLS genes (Supplementary Fig. 5f). Then, we assessed similarity of global transcriptome between each cell and the major liver cell types in human cirrhotic livers. Interestingly, all Huh7.5.1dif cells showed similar magnitude of resemblance to both parenchymal and NPC cell types irrespective of the KRT18 expression levels and the HCV viral load (Supplementary Fig. 5g). These data support that HCV infection, as an example of etiologic agent to induce the PLS, does not result in cellular heterogeneity with regard to induction of the parenchymal- or NPC-associated PLS genes, i.e., the cPLS system at least partially models the transcriptional program of both parenchymal and NPC liver cell types at comparable level across the Huh7.5.1dif cells.

Screening of computationally prioritized compounds in the cell-based model identifies nizatidine as a candidate chemoprevention agent. We next hypothesized that the cPLS system enables high-throughput screening of compounds that reverse the PLS poor-prognosis status to a good-prognosis status, and therefore could halt progression of liver disease. To test this hypothesis, we first performed a transcriptome-based in silico drug screening, using the PLS as a query in the chemogenomic database connectivity map (cmap, www.broadinstitute.org/cmap) and LINCS database (https://clue.io/)

Nizatidine reverses the cPLS by targeting the HRH2/cAMP/CREB signaling pathway. HRH2, the nizatidine target, is a member of the G-protein-coupled receptor family widely expressed in the gastrointestinal tract that mediates its activity through cAMP 

Increase of intracellular cAMP is known to activate the cAMP response element binding (CREB) protein family, which has key functions in cell survival, growth, inflammation, and differentiation. We focused on CREB1 and CREB5, two key members of the CREB family found to be overexpressed in many solid tumors, including HCC. HCV infection resulted in an increase of CREB1 phosphorylation and of CREB5 expression, both reversed by nizatidine (Fig. 3e and Supplementary Fig. 7), suggesting a role of these transcription factors in the induction of the poor-prognosis PLS. Loss-of-function studies using CRISPR/Cas9 revealed that knockout of CREB5 reversed the poor-prognosis cPLS, verifying CREB5 as a key mediator of the cPLS (Fig. 3f and Supplementary Fig. 7). The finding that CREB5 KO results in robust reversal of the PLS is most likely due to the fact that CREB5 is a transcription factor activated by several pathways, which mediate expression of PLS genes. Furthermore,
CREB5 itself is a transcriptional activator for expression of several PLS genes\textsuperscript{5,26}. Collectively, our results demonstrate that the histamine/HRH2/cAMP/CREB signaling pathway is a mediator of the cPLS poor-prognosis status (Fig. 3g).

To validate the key findings in primary cells, we established a 3D multicellular spheroid model derived from patient tissues in which the 186 gene poor-prognosis PLS is robustly, and significantly induced by FFA exposure and reversed by nizatidine treatment similar as in the cPLS cell line model (Fig. 3h and Supplementary Table 3). Interestingly, the induction and reversal of the PLS poor-prognosis status in this model were associated with the level of HRH2 expression in individual patients (Fig. 3i). Moreover, the HRH2 ligand histamine robustly induced the poor-prognosis PLS status, which was reversed by nizatidine (Fig. 3j). Collectively, these data in primary cells of patient-derived liver spheroids confirm that histamine is a mediator of the PLS poor-prognosis status and the effect of PLS reversal in primary tissues is dependent on HRH2 expression—the nizatidine target.

**Nizatidine reduces liver fibrosis and cancer development in two animal models for chronic liver disease and HCC prevention.** Next, we validated the therapeutic effect of nizatidine in a rat model of progressive fibrotic liver disease and HCC based on repeated low-dose injections of diethylnitrosamine (DEN). This model closely mimics global transcriptome dysregulation in human cirrhosis at risk of HCC, including the PLS induction\textsuperscript{8,9}. Treatment with nizatidine had a marked effect on hepatocarcinogenesis: nizatidine-treated animals showed a reduced number of liver tumor nodules (Fig. 4a–c). Moreover, nizatidine tends to decrease expression of the Ki-67 protein in the liver, a marker associated with proliferation of mammalian cells (Fig. 4c). Importantly,
Nizatidine does not induce hepatic toxicity as shown by the measurement of hepatic enzymes (Fig. 4d). Decrease of gamma-glutamyl transferase (γGT) activity and of total bilirubin (TBIL) level showed that nizatidine restores normal liver function assumedly by halting liver fibrosis progression (Fig. 4d). Indeed, nizatidine significantly reduced liver fibrosis measured by collagen proportionate area (CPA), α-smooth muscle actin (αSMA) staining, quantitative analysis of hydroxyproline, and expression of genes mediating fibrogenesis (Acta2, Coll1a1, Tgfb-1, and Timp1; Fig. 4e, f). Taken together, these data demonstrate that nizatidine prevents fibrotic liver disease progression and cancer development in vivo. To better elucidate the anti-fibrotic/anticaner effect of nizatidine, we performed RNA-Seq analysis on rat liver tissues. Nizatidine reversed the poor-prognosis status of the PLS (Fig. 4g, h) as observed in the cell-based model, suppressed several pathways mediating fibrogenesis and carcinogenesis (i.e., TGFβ and KRAS signaling) and improved metabolic pathways required for the physiological liver function (i.e., lipid metabolism; Fig. 4g).

We next investigated the expression of nizatidine targets in rat liver tissues. Immunostaining analysis showed that HRH2 and CREB1 phosphorylation are markedly increased in hepatocytes after DEN-mediated liver injury and suppressed by nizatidine, suggesting that HRH2 expression and CREB1 activation are associated with carcinogenic status of the liver (Fig. 4i, j). Decrease of cAMP levels after nizatidine treatment confirmed target engagement in vivo (Fig. 4j). In addition, RNA-Seq analyses revealed that expression of Prkacb (encoding PKA...
catalytic subunit beta) and Creb5 was increased, following DEN liver injury, with reversal by nizatidine (Fig. 4j). Similar results were found for protein expression as shown by immunoblot studies, corroborating our findings (Fig. 4k and Supplementary Fig. 8).

To address a direct relationship of nizatidine treatment and HRH2 expression, we treated cell lines with nizatidine and quantified HRH2 expression after treatment. As nizatidine had no effect on HRH2 expression in cell lines or 3D spheroids (Fig. 3i and Supplementary Fig. 9), we assume that the observed downregulation of HRH2 in nizatidine-treated animals is likely not due to a direct effect of nizatidine on HRH2 expression, but rather due to the improvement of liver disease.

We then investigated the effect of nizatidine on liver disease progression in a mouse model of NASH/fibrosis/HCC induced by a choline-deficient, L-amino acid-deficient, high-fat diet (CDAHFD) and a single dose DEN injection. This diet-based model closely recapitulates the course of disease development, histological appearance, and dysregulation of fatty acid metabolism in human NASH progressing to fibrosis and HCC. As observed in the rat model, nizatidine markedly reduced liver fibrosis and HCC (Supplementary Fig. 10a–d). An increase of...
HRH2 and CREB5 expression and CREB1 phosphorylation in the liver upon injury, reversed by nizatidine treatment, was further confirmed in liver tissues from treated animals (Supplementary Figs. 10f–h and 11). Finally, similar to the rat model, nizatidine reversed the poor-prognosis PLS in the livers of treated mice (Supplementary Fig. 10i–j).

Genetic loss-of-function studies confirm a functional role of HRH2 in hepatocarcinogenesis. To confirm that the observed inhibitory effects of nizatidine on hepatocarcinogenesis are mediated by its target HRH2, we performed genetic loss-of-function studies in animal and cell-based models. First, we performed in vivo KO studies using hydrodynamic tail vein injection (HTVI) in a mouse model of liver cancer. HTVI enables efficient delivery of plasmid DNA into the liver of adult mice, in particular into hepatocytes. In our model, hepatocarcinogenesis and Hhr2 KO were induced by injection of three plasmids, one expressing the Cas9 endonuclease and sgRNAs (targeting Tp53, Hhr2 or a non-targeting sgRNA control), the transposon plasmid expressing KrasG12D allowing constitutive expression of the Kras mutant, and the third plasmid expressing a transposase that integrates the transposon vector randomly into the genome of transduced cells (Fig. 5a, b). Hhr2 KO robustly and significantly (p < 0.01) improved mouse survival compared to control animals (median survival: CTRL 28d, Hhr2 KO 33.5d; Fig. 5b). Moreover, Hhr2 KO decreased the liver tumor burden (Fig. 5c, d) and significantly decreased cell proliferation (Fig. 5e).

To investigate the functional role of HRH2 on the phenotype of hepatoma cells, we engineered Huh7.5.1 HHR2 KO and CTRL KO cell lines. HHR2 KO hepatoma cells showed decreased cell proliferation (Fig. 5f, g), and increased sensitivity to oxidative stress and apoptosis compared to cells expressing a sgCTRL (Fig. 5h and Supplementary Fig. 12). A decrease of Huh7.5.1 cell proliferation was also observed when HHR2 expression was knocked down by RNAi (Fig. 5i–k). Moreover, HHR2 KO impaired the full induction of the poor-prognosis status of the cPLS compared to CTRL cells (Fig. 5l). Collectively, these genetic loss-of-function studies suggest that HRH2 plays a functional role in hepatocarcinogenesis, and that the biological effects of nizatidine on liver disease progression are likely mediated by HRH2. Nevertheless, our data do not exclude that additional mechanisms or targets are at play.

Nizatidine improves liver inflammation and immune surveillance by targeting liver macrophages. Increasing evidence has shown that the interplay between hepatocytes and the surrounding microenvironment plays an important role in liver disease progression and hepatocarcinogenesis. In particular, the recruitment of inflammatory immune cells in the chronically injured tissue is crucial for driving fibrosis and HCC. The role of histamine and histamine receptors in the regulation of immune responses and inflammation has suggested that targeting HRH2 using nizatidine may modulate liver inflammation and immunity.

First, we assessed the effect of nizatidine on immune cell infiltration in livers from nizatidine-treated animals. We observed a decrease of the Ly6G, CD68, and F4/80 cell markers, indicating a reduced infiltration of neutrophils and monocytes/macrophages in livers from treated animals (Supplementary Fig. 13). Moreover, an increase of CD3- and CD8-specific markers after nizatidine treatment indicated a recruitment of CD8+ T lymphocytes (LT; Supplementary Fig. 13), which could contribute to the anticarcinogenic effect of nizatidine. In the NASH-fibrosis mouse model the reduction of inflammatory immune cell recruitment leads to a decrease in the expression of interleukin 6 (Ile6) and chemokine ligand 2 (Ccl2), two pro-inflammatory cytokines contributing to liver disease progression and HCC development (Supplementary Fig. 13).

To uncover the immune cells targeted specifically by nizatidine, we first analyzed target expression in the healthy human liver using scRNA-Seq. ScRNA-Seq of human liver cell atlas showed that HRH2 is highly expressed in liver macrophages (Fig. 6a and Supplementary Fig. 14a). Immunofluorescence analysis and qRT-PCR performed on isolated macrophages and hepatocytes confirmed that HRH2 is expressed in macrophages, as well as hepatocytes with higher levels in macrophages (Supplementary Fig. 14b–d). Macrophage populations include a wide spectrum of phenotypes from the classically activated pro-inflammatory macrophages (M1) to alternatively activated immunoregulatory macrophages (M2). Characterization of macrophage marker expression revealed that HRH2-expressing macrophages express high level of C-type lectin Domain Family 5 Member A (CLECSA) and low level of Macrophage Receptor with Collagenous Structure (MARCO) and CD163 Molecule Like 1 (CD163L1) markers, indicating that HRH2-expressing liver macrophages are mainly pro-inflammatory (Fig. 6a and Supplementary Fig. 14a, b).

To investigate the functional effects of nizatidine on liver immune cells, we isolated CD45+ leukocytes from liver tissues of patients with advanced liver disease and HCC, and treated the immune cell population with nizatidine or vehicle control (Fig. 6b and Supplementary Fig. 15). We then analyzed the effect of the compound by scRNA-Seq using the SORT-seq technology similar to our previously described liver cell atlas pipeline. Cell clustering is based on similar transcriptomic profiles. The different immune cell types were identified using specific cell markers (Fig. 6c, d). As indicated by their marked shift within the t-SNE map the cell population with highest change in gene expression corresponds to liver macrophages (CD45+ MAF BZIP Transcription Factor B (MAFB) cells; Fig. 6c, d). Interestingly, the majority of nizatidine-responding macrophages have a pro-inflammatory phenotype, as demonstrated by high level of CLEC5A expression and low levels of CD163L1 and MARCO expression (Fig. 6e). Nizatidine treatment decreased CLEC5A expression (Fig. 6e), suggesting that nizatidine modulates the phenotype of inflammatory macrophages. Furthermore, we observed that nizatidine decreases expression of the Receptor Sialic-Acid-Binding Ig-like Lectin 10 (Siglec-10) in macrophages (Fig. 6e), a recently uncovered immune checkpoint shown to inhibit effector functions of immune cells in cancer.

GSEA analysis showed that nizatidine suppresses the pro-inflammatory M1 signature and partially induces the immunoregulatory M2 signature (Fig. 6f). Macrophages exhibit remarkable plasticity and can evolve in different subpopulations with atypical or intermediate profiles sharing characteristics of more than one population. In accordance with this observation, we show that nizatidine suppresses pro-inflammatory and pro-fibrogenic responses (i.e., TNFα, IL2, and IL6 signaling pathways), while augmenting antigen processing and presentation and IFNγ response, suggesting a shift from pro-inflammatory to an “atypical” immunoregulatory profile (Fig. 6g). Moreover, we observed a strong suppression of the c-Myc pathway expression, a major actor involved in the polarization of macrophages towards a typical M2 profile. Gene-level analysis also showed that nizatidine treatment induces a decrease in neutrophil/monocyte chemoattractant cytokine expression (i.e., CXCL5, CCL2, and CCL5), as well as a suppression of pro-fibrotic/tumorogenic soluble factor expression (i.e., IL6, IL1β PDGF, MMP9, and TNFα; Fig. 6h). A direct effect of nizatidine treatment on
Effect of hr proliferative EdU-positive cell from three independent experiments in CTRL and knockdown on cancer cell proliferation. Liver weight body ratio and total number of tumor nodules per mouse are shown (mean ± SD; CTRL mice, Counter). Two representative and independent experiments are shown. (DAPI). Scale bar, 100 μM.

Fig. 5 Genetic loss-of-function studies confirm a functional role of HRH2 in hepatocarcinogenesis. a Validation of sgRNA targeting mouse Hh2 and Tp53. KO efficacy was assessed by TIDE analysis. Plasmid constructs are described in method. b Hh2 (sgHh2) and Tp53 (sgTp53) KO and Kras constitutive expression (KRASG12D) or CTRLs were induced by injecting different plasmids constructs by HTVi in C57BL/6 males. Graph shows survival curves of injected mice. Median survival from injection to death is shown. Log-rank Mantel–Cox test. **p < 0.01. c Pictures of representative livers. Scale bars, 1 cm. d Liver weight body ratio and total number of tumor nodules per mouse are shown (mean ± SD; CTRL mice, n = 5; Hh2 KO mice, n = 6). p > 0.05 (two-tailed Mann–Whitney U test). e Cell proliferation was assessed by the IHC evaluation of Ki-67 in liver tissues (mean ± SD; CTRL mice, n = 5; Hh2 KO mice, n = 6). Scale bar, 300 μM. **p < 0.01 (two-tailed Mann–Whitney). f, g HRH2 KO decreases cancer cell proliferation in cell culture. f HRH2 KO validation at genetic level using T7 endonuclease assay. g Effect of HRH2 KO on cancer cell proliferation. EdU incorporation assay by FACS showing % ±SD of proliferative EdU-positive cell from three independent experiments in CTRL and HRH2 KO cells (n = 8; *p < 0.01, two-tailed Mann–Whitney U test).

h Effect of HRH2 KO on cancer cell apoptosis induced by oxidative stress (H2O2). Cleaved caspase 3 is shown in green. Nuclei were counterstained in blue (DAPI). Scale bar, 100 μM. Graph shows integrated cleaved caspase 3 intensity/total cell number from two independent experiments (n = 12; **p < 0.01, two-tailed Mann–Whitney–U test) measured using Celigo Cytometer. Western blot analysis of cleaved- and total caspase 3 is shown. i-k Effect of HRH2 knockdown on cancer cell proliferation. i siRNA efficacy was assessed by measuring mRNA by qRT-PCR. j EdU incorporation assay by FACS showing % ±SD of proliferative EdU-positive cell from three independent experiments in cell transfected with siCTRL and siHRH2 (n = 6; **p < 0.01, two-tailed Mann–Whitney U test). k Cell proliferation was assessed daily in Hu7.5.1 transfected with a siCTRL or a siHRH2 by cell counting (TC20 Automated Cell Counter). Two representative and independent experiments are shown. I Full cPLS induction is impaired by HRH2 KO. PLS induction was determined by GSEA analysis using “Mock” non-infected cells as reference. Source data are provided as a Source data file.

pro-inflammatory cytokine expression was confirmed in THP1-derived macrophages with similar effects of other HRH2 blockers, as well as patient-derived Kupffer cells and patient-derived tumor associated macrophages, (Supplementary Fig. 16). To address the functional role of HRH2 in macrophages by an additional experimental approach, we generated a HRH2 KO THP1-derived macrophage cell line and assessed their functional phenotype. Interestingly, the HRH2 KO resulted in a similar modulation of pro-inflammatory cytokines as treatment with nizatidine (Supplementary Fig. 16d, e). These data suggest that at least a part of the observed immunomodulatory effect of nitazidine in THP1-derived macrophages is mediated through HRH2 and that macrophage HRH2 likely contributes to the therapeutic effect of nitazidine in vivo.

HRH2 and downstream target expression predicts mortality in patients with chronic liver disease. Next, we sought to validate the clinical relevance of the target pathways in liver disease progression and hepatocarcinogenesis in different clinical cohorts. Transcriptome profiling revealed that HRH2 and CREB5 expression were increased in viral hepatitis and NASH, confirming involvement of the pathway in disease progression across the major etiologies (Fig. 7a, b). Increase of HRH2 expression was further confirmed at protein level in liver tissues from patients with chronic liver diseases and HCC (Supplementary Fig. 17). HRH2 protein was detected both in hepatocyte and liver immune cells, with the highest protein expression levels in immune cells, with the highest expression levels in immune cells.

Moreover, we observed that high HRH2 expression is associated with poorer overall survival in cirrhotic patients at risk for HCC (Fig. 7c). Expression of the downstream effector CREB5 was also associated with poorer overall survival and higher incidence of HCC recurrence after curative tumor resection (Fig. 7d). Collectively, these results support a functional relevance of the HRH2 pathway in pan-etiology liver disease progression and hepatocarcinogenesis in patients.
Nizatidine reverts the PLS poor-prognosis status and decreases HCC cell viability in ex vivo patient-derived models. Finally, we aimed to investigate the potential clinical efficacy of nizatidine in advanced liver disease. First, we performed an organotypic ex vivo culture of diseased, non-tumoral human liver tissues, preserving multicell-type tissue architecture, including liver NPCs. Nizatidine treatment reverted the poor-prognosis PLS (Fig. 8a), suggesting a therapeutic effect on liver disease progression and HCC risk. To assess whether nizatidine may also contribute to a direct effect on emerging HCC, we investigated nizatidine effect in 3D patient-derived HCC tumorspheroids. In contrast to other 3D culture systems, HCC spheroids include the...
Fig. 6 ScRNA-Seq analyses of patient liver tissue uncover pro-inflammatory liver macrophages as nizatidine target. a t-SNE map of single-cell transcriptomes from normal liver tissue of donors without history of chronic liver disease highlighting the main liver cell compartments. Data extracted from ref. 35. Cells sharing similar transcriptome profiles are grouped by clusters and each dot represents one cell. Expression t-SNE map of HRH2, CLEC5A, CD163L1, and MARCO are shown. The color bar indicates log2-normalized expression. b-e Perturbation of gene expression by nizatidine in liver tissue from patient with chronic liver disease and HCC identifies liver macrophages as therapeutic target. b Experimental approach. CD45+ leukocytes from patient liver tissue were enriched by flow cytometry and were treated with nizatidine or vehicle control (DMSO). Single cells were sorted and analyzed as described. c t-SNE map of single-cell transcriptomes showing control (blue) and nizatidine-treated cells (yellow), d the t-SNE map indicating the main cell compartments (MAFB: macrophages, CD8: CD8+ T lymphocytes), e Expression t-SNE map of HRH2, macrophage markers, and Siglec-10 are shown. The color bar indicates log2-normalized expression. f-h GSEA for differentially expressed genes between nizatidine-treated and CTRL macrophages depicted in d. f Normalized enrichment score (NES) of genes related to macrophage activation (classical M1 vs alternative M2), g NES of the pathways significantly enriched after nizatidine treatment (FDR ≤ 0.05), h Expression heatmap of differentially expressed genes in individual nizatidine- and control-treated macrophages. Each row representing a single cell. Markers of inflammation, fibrogenesis/cancer, and antigen presentation are shown. All genes are normalized by row from their own min to max (Log2 fold; p value ≤ 0.05). Source data are provided as a Source data file.

Fig. 7 Expression of the liver HRH2/CREB5 pathway is associated with chronic liver disease progression and hepatocarcinogenesis in patients. a, b HRH2 (a) and CREB5 (b) expression in liver tissues of clinical cohorts with various liver disease etiologies. In box and whisker plots, boxes represent the 75th and 25th percentiles, the whiskers represent the most extreme data points within interquartile range × 1.5, and the horizontal bar represents the median. Open circle indicates actual observation for each sample. Exact p values are indicated for each panel (Wilcoxon's rank-sum test for all panel except for GSE94660, paired Wilcoxon's rank-sum test). GSE84346: normal uninfected, n = 6, HCV infected, n = 40; GSE48452: healthy obesity, n = 27; NASH, n = 18; GSE94660, paired samples, n = 21; GSE48452: healthy obesity, n = 27; NASH, n = 18; GSE49541: mild fibrosis, n = 40, advanced fibrosis, n = 32; GSE14520: non-cirrhosis, n = 14, cirrhosis, n = 185; GSE10143: non-cirrhosis, n = 15, cirrhosis, n = 32. GSE49399: good prognosis, n = 23; poor prognosis, n = 15. c Higher expression of HRH2 in patient liver biopsies is associated with poor-prognosis and decreased probability of overall survival. Exact p values are indicated for each panel (log-rank test for comparisons of Kaplan–Meier survival). d Higher expressions of CREB5 in the adjacent livers were significantly associated with decreased survival and tumor recurrence after curative resection among patients with HCC. Exact p values are indicated for each panel (log-rank test for comparisons of Kaplan–Meier survival). Source data are provided as a Source data file.

tumor microenvironment, allowing establishment of cell–matrix and cell–cell contacts between hepatocytes and NPCs, including macrophages32. While nizatidine had no effect on primary human hepatocyte (PHH) viability in line with its safe clinical profile (Fig. 8b), treatment with the compound resulted in a significant decrease of tumorspheroid viability in four out of six patients, independently of the etiology, including a pronounced effect on tumors that did not respond to sorafenib (Fig. 8c and Supplementary Table 3). Nizatidine-induced alteration of liver macrophage gene expression (Fig. 6) was associated with a robust ex vivo response to the drug in the same patient (Fig. 8c, HCV HCC 2).

Collectively, these functional data suggest that targeting HRH2 may have clinical efficacy in patients with advanced chronic liver disease.

Discussion
There is an unmet medical need for experimental systems modeling human disease-specific gene expression to understand disease biology and enable disease-specific drug discovery. Here, we addressed this need by the development of a simple and robust liver cell-based system that models gene expression of patients with carcinogenic liver diseases. Our findings demonstrate that a clinical prognostic signature can be experimentally modeled in a cell-based model. Overall, the cPLS model offers unique opportunities to discover compounds for chronic liver disease treatment and HCC chemoprevention across the distinct liver cancer etiologies, in a fast-track high-throughput screening format. Moreover, using detailed computational and functional analyses, we demonstrate that the cPLS model system recapitulates global transcriptomic dysregulation in hepatocyte-like and
non-hepatocyte cell types, including macrophages, which supports versatile utility of our system to screen and identify compounds targeting various liver cell types. These findings are in line with studies indicating that modeling of cellular signaling is not necessarily restricted by cell type and tissue lineage\(^8,14,18,43\). Indeed, such cell-based in vitro models (e.g., NIH LINCS program) have revealed that cellular signaling and physiology are often shared across multiple tissues and cell types\(^14\). As any cell culture model, the cPLS platform only partially recapitulates the cell circuits of the major liver cell types in the diseased liver in a simplified manner, and therefore the platform should be used for compound or target screening followed by validation in multicell-type experimental systems, such as patient-derived spheroids, as well as rodent models of advanced liver disease as demonstrated in this study. However, the robust induction of the clinical PLS demonstrates that the model is useful to identify compounds reversing the poor-prognosis status of the signature (Fig. 2) or pathways involved in the generation of the signature (Fig. 3). Induction of the PLS in authentic patient tissue-derived spheroids and animal models and its reversal by the top-scored compound (Fig. 3h) confirms the validity of the cell line-based model. Whereas the spheroid cPLS model is closer to the patient allowing validation of compounds and mechanisms, the limited availability of patient-derived tissues preclude its application for compound screening.

The therapeutic effect of nizatidine was mediated by two complementary mechanisms:

1. Hepatocytes/parenchymal cancer cells: we demonstrate that the HRH2/CREB5 signaling pathway is perturbed in hepatocytes during liver injury. Moreover, we show that HRH2 is involved in cancer cell proliferation (Fig. 5). The clinical impact of these findings was confirmed by an increased expression of HRH2 and CREB5 in HCC patients, and a significant association of HRH2/CREB5 expression with patient overall survival and enhanced HCC risk in several clinical cohorts (Fig. 7). Our discovery is consistent with studies demonstrating that CREB5 overexpression in parenchymal cells is associated with clinical tumor recurrence, metastasis, poor prognosis, and overall survival\(^27,28\). CREB5 is a transcription factor belonging to the CREB protein family that regulates diverse cellular responses, including proliferation, survival, and differentiation. Upregulation of CREB protein can transform normal parenchymal cells into tumor cells through aberrant activation of downstream pathways, such as growth factor receptor (i.e., EGFR) and cytokine/JAK/STAT pathways\(^26\).

2. Liver macrophages: scRNA-Seq analyses in patient tissue uncovered HRH2\(^+\)CLEC5A\(^{high}\)MARCO\(^{low}\) liver macrophages as another candidate target cell for nizatidine (Fig. 6). Clinical and experimental evidences have shown that macrophages play a key role in liver fibrosis progression\(^16\). Furthermore, certain macrophage subpopulations enhance tumor progression by impairing cytotoxic LT CD8\(^+\) immune responses and are emerging as a target in cancer therapy\(^39\). We show that nizatidine enhances pathways mediating antigen processing and presentation in macrophages, which is in line with the observed increase of LT CD8\(^+\) recruitment after nizatidine treatment in vivo (Supplementary Fig. 13). LT CD8\(^+\) responses in human HCC correlate with improved overall survival, longer relapse-free survival, and diminished disease progression\(^34\).
Furthermore, we observed that nizatidine treatment resulted in decreased expression of macrophage Siglec-10 (Fig. 6). Activation of Siglec-10 by its ligands in macrophages induces a “don’t eat me signal”, which blocks phagocytosis of transformed cells and has very recently been shown to contribute to immune evasion\textsuperscript{29,30}. Therefore, it is conceivable that the decrease of Siglec-10 expression by nizatidine may contribute to a restoration of anticancer immunity. The two target cells and mechanisms are most likely influenced by hepatocyte–macrophage cross talk.

While our genetic loss-of-function studies demonstrate a functional role for HRH2 in liver disease cell circuits and hepatic carcinogenesis, and our functional data in hepatocyte and macrophage cell lines suggest a class effect of HRH2 inhibitors, we cannot exclude that nizatidine may exert the observed biological effects also via targets other than HRH2.

More than 80% of HCCs develop in fibrotic or cirrhotic livers, suggesting an important role of liver fibrosis in the carcinogenesis\textsuperscript{1,2}. In patients with chronic viral infection and NASH, the risk of HCC gradually increases as liver fibrosis progresses\textsuperscript{3,4}. Targeting fibrosis-associated carcinogenesis may be relevant to prevent HCC development. By improving liver inflammation, fibrosis, and anticancer surveillance, HRH2-targeting compounds provide a therapeutic candidate approach for patients with fibrotic liver disease at risk for HCC and will guide future optimization of refined HRH2-targeting liver disease therapies. The excellent safety profile combined with robust therapeutic efficacy at doses, which are achieved in patients treated with HRH2 antagonists\textsuperscript{45}, suggest rapid translatability of the approach.

Methods

For reagents, primers, antibodies, and other resources, see Supplementary Table 4

Human subjects. Human liver tissues were obtained from liver disease patients undergoing liver resection with informed consent from all patients for deidentified use at the Center for Digestive and Liver Disease of the Strasbourg University Hospitals University of Strasbourg, France (DC-2016-2616 and RIPH2 LivMod IDRCB 2019-A00738-49, ClinicalTrial NCT04690972) or at Mount Sinai Hospital, New York City, NY (HS13-00159). The protocols were approved by the local Ethics Committee of the University of Strasbourg Hospitals and Mount Sinai Hospital, respectively. All material was collected during a medical procedure strictly performed within the frame of the medical treatment of the patient. Informed consent is provided according to the Declaration of Helsinki. Detailed patient information and informed consent procedures are implemented by the Strasbourg University Hospital Biological Resources Center (HUS CRB). Patients were given an information sheet, which outlines that their leftover biological material (liver resection and blood samples) that was collected during their medical treatment is requested for research purposes. All patients received and signed an informed consent form in order to provide authorization or refuse the use of their biological samples (protocols DC-2016-2616 and RIPH2 LivMod IDRCB 2019-A00738-49 ClinicalTrial NCT04690972). The patients maintain the right to withdraw their consent at any time and to request the destruction of their biological material, which is strictly respected. While there was clinical descriptive data available, the identity of the patients was protected by internal coding. A brief summary of patient characteristics (diagnosis and treatments) is provided in Supplementary Table 3.

Spheroids (Fig. 3) were generated from liver tissue from patient without history of chronic liver disease. Tumorspheroids (Fig. 8) were generated from tumoral tissues from HCC patients. The PLS was analyzed in clinical liver tissues from HCV-related cirrhosis (Italy, n = 216 GSE15654\textsuperscript{5}, ref. 9), US, n = 145 GSE34102\textsuperscript{32}, HBV-related liver cancer (n = 199, GSE14520\textsuperscript{30}), alcoholic hepatitis (alcohol; n = 22, GSE28619\textsuperscript{47}, and NASH (n = 72, GSE49541\textsuperscript{48}) cohorts. Induction of the PLS in cell-based systems was compared to liver transcriptome profiles from published clinical cohorts of HCV-related cirrhosis (n = 145, GSE4102\textsuperscript{32}), HBV-related liver cancer, alcoholic hepatitis, nonalcoholic fatty liver disease (NASH), and NASH (n = 73, GSE48452\textsuperscript{50}, using Subclass Mapping\textsuperscript{50}).

Research experiments on live vertebrates. For rat and mouse models for liver disease and HCC, animals were housed in accordance with the guidelines of the Massachussetts General Hospital Institutional Animal Care and Use Committee (protocol approved numbers 2007N00113 and 2009N00207) and received human care according to the criteria outlined in the “Guide for the Care and Use of Laboratory Animals” of the National Academy of Sciences. Eight-week-old male Wistar rats (Charles River Laboratories, Wilmington, Massachusetts) received weekly intra-peritoneal injections of 50 mg/kg DEN (n = 18) to induce cirrhosis and liver cancer, or PBS (n = 3), once per week over the course of 18 weeks. After 8 weeks, DEN-injured rats were randomly assigned to receive vehicle control (0.5% methylcellulose; n = 9) or 15 mg/kg nizatidine (n = 9) by oral gavage daily for 9 weeks by a blinded technician. Drug doses were estimated based on body surface area assuming a nizatidine dose of 150 mg for a 60 kg human\textsuperscript{31}. Livers were harvested and analyzed in week 18. Male C57BL/6 mice (Charles River Laboratories, Wilmington, MA) received a single dose of 35 mg/kg DEN at day 15. At 6 weeks of age, mice were subjected to either standard chow (n = 5) or CDAHFD consisting of 60 kcal% fat and 0.1% methionine by weight (n = 16) for a total of 24 weeks. Oral gavage of either vehicle control (n = 8) or nizatidine 15 mg/kg (n = 8) was initiated 2 weeks following the onset of CDAHFD. At the time of sacrifice, animals were anesthetized and sedated. A terminal blood collection was performed by cardiac puncture. Livers were weighted, fixed in formalin, and snap-frozen for further analysis.

For HTVi, C57BL/6 male mice were purchased at an age of 8 weeks-old from Janvier, Labs and were used for experiments. All experiments were performed in accordance with the German law and the governmental bodies, and with approval from the the Regierungspräsidium Karlsruhe providing the ethical oversight of the study (approval number G39/18). Mice were housed at the German Cancer Research Center (DKFZ; constant temperature of 20–24 °C and 45–65% humidity with a 12-h light–dark cycle) and maintained under specific pathogen-free conditions. For in vivo CRISP/Cas9 gene editing by HTVi. Groups of six–10- week-old male mice received a mix of 5 μg DNA of transposon vector pt3-EFIa- KRAKS12D-ires-Egfp (KRASG12D), 10 μg of either pX330-sgTp53-Ctrl (sg- Tp53-sgCtrl) or pX330-sgFp53-sghH2a (sgFp53-sghH2a), and a 3:1 ratio of transposase to CMV-SB13 transposase encoding plasmid with ca2+ (250 μg/mL) or NaCl solution and injected in the lateral tail vein, with a total volume corresponding to 10% of body weight in 5–7 s. Once the animals were sacrificed, livers were collected, formalin-fixed and paraffin-embedded, frozen, or embedded in OCT (TissueTek).

Cells. Hep3B.5.1 are a gift of Dr. F. Chisari (The Scripps Research Institute, La Jolla, CA). Hep2G and THP1 were purchased from ATCC. LCX were purchased from Merck. Hep3B.5.1, Hep2G, and human stellate LX2 cells were cultured in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% heat-decomplemented fetal bovine serum (FBS), gentamicin (0.05 mg/mL), and nonessential amino acids (complete DMEM) at 37 °C with 5% CO2. THP1 cells were cultured in RPMI 1640 Medium with Glutamax–1 supplement and HEPES, and supplemented with 10% FBS and gentamicin (0.05 mg/mL). NTCP-overexpressing Hep3B.5.1-NTCP or Hep2G-NTCP cells have been generated by transducing a human NTCP expressing VSV pseudoparticle (GeneCoopedia). After 3 days, the cells were expanded and selected for NTCP expression with 1.8 μg/mL puromycin\textsuperscript{35}. For proliferation arrest and differentiation (Huh7.5.1\textsuperscript{46} cells), Huh7.5.1 cells were cultured in complete DMEM-supplemented 1% DMSO\textsuperscript{10}. All cell lines were certified mycoplasma-free. For perturbation studies in the cell-based system, the dose of nizatidine was chosen according to the nizatidine blood concentration measured in patients. Plasma concentrations reach between 700–1400 μg/L (2–4 µM) after a 150 mg oral dose and between 1400–3600 µg/L (4–11 µM) after a 300 mg oral dose\textsuperscript{47}.

Primary human hepatocyte (PHH) isolation. PHH were isolated as described\textsuperscript{35} from patient-derived liver tissues obtained from surgical resections (Strasbourg University Hospitals, France). Primary human hepatocytes (PHH) were isolated from liver tissues of male mice with calcium-free 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer containing 0.5 mM ethylene glycol tetracetic acid (Fluka), followed by perfusion with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid containing 0.5 mg/mL collagenase (Sigma–Aldrich) and 0.075% CaCl\textsubscript{2} at 37 °C for 15 min. Then the cells were washed with phosphate-buffered saline (PBS) and nonviable cells were removed by Percoll\textsuperscript{48} (Sigma–Aldrich) gradient centrifugation. Part of the isolated cells was further separated into PHH and NPCs fractions by an additional centrifugation step (5 min at 50 g). PHH were seeded in collagen type 1 coated plates (Corning)\textsuperscript{49}.

PLS calculation. The PLS 186 (complete) or 32 gene (reduced, see below) expression profiling was performed using 250–500 ng total RNA by using either nCounter Digital Analyzer system (NanoString) or the HumanHT-12 beadarray (Illumina) for the time-course experiments (Supplementary Figs. 1 and 3). For full PLS gene list, refer to Supplementary Data 1. PLS gene expression was normalized according to six housekeeping gene expression (Supplementary Data 1), using GenePattern genomic analysis toolkits\textsuperscript{52,53}. Detailed PLS gene expression profiles are presented as heatmaps showing the mean expression of the 186 or 32 PLS genes, using Morphors software (z-scores of log2-normalized data). Induction or suppression of the PLS signature was determined as previously reported by using GSEA, imple-mented in the GenePattern genomic analysis toolkits\textsuperscript{54,55}. Gene expression was determined by using CTRL cells, CTRL animals, or CTRL patient-derived tissues as references. Results are presented as simplified heatmaps showing the classification of PLS global status as poor or good prognosis, and the significance of induction/suppression of PLS genes (log10 of false discovery rate (FDR) values). Global status corresponds to the difference between good- and poor-prognosis gene expression.
The statistical tests, normalized enrichment score (NES), and FDR values are provided for each PLS experiment (poor- and good-prognosis genes variation and global status) in the Source data file.

The 32 gene signature is a reduced version of the PLS, comprising gene bioinformatically defined and validated in multiple patient cohorts in previous studies. The gene signature was bioinformatically reduced from 186 genes to 32 genes (Supplementary Data 1), and implemented in an FDA-approved diagnostic assay platform implemented for clinical use (Element 1, Laboratory Developed Test (LDT). The Clinical Laboratory Improvement Amendments (CLIA) lab implementation of the assay is now underway to provide the assay service soon for clinicians worldwide.

Transcriptomic analyses. Molecular pathway deregulations were determined in Molecular Signature Database (MSigDB, ver.4.0) using GSEA 59. Comparison between the cell-based systems and clinical datasets was performed using Subclass Mapping algorithm implemented in GenePattern. Transcription profiling of nitisulid-treated rat liver was performed by RNA-Seq. Sequencing libraries were generated using the TruSeq RNA Sample Prep Kit (Illumina) using 200 ng of total RNA, according to the manufacturer’s protocol and sequenced, using Illumina NextSeq genome sequencer (Illumina). Data preprocessing and transcript abundance calculation (RPKM: reads per kilo bases of exons for per million mapped reads) was performed using TopHat2.1 and Cufflinks 2.2 software, using the rat reference genome, rn4 (ref.60). Rat genes were converted into orthologous human genes using a mapping table obtained from NCBI HomoloGene database (build 68). Induction or suppression of target genes of relevant cellular signaling pathways was assessed by GSEA.

Statistics and reproducibility. Individual experiments were reproduced three times in an independent manner with similar results (except otherwise stated). The precise number (n) of biologically independent samples used to derive statistics is indicated in the figure legends. The in vitro data are presented as the mean ± SD and were analyzed by the unpaired Student’s t test or the two-tailed Mann–Whitney test as indicated in figure legends. Significance after determination of distribution by the Shapiro–Wilk normality test, p < 0.05 (*), p < 0.01 (**) and p < 0.001 (*** *) were considered statistically significant. Significant p values are indicated by asterisks in the individual figures. The exact p values are provided in the Source data file. The data points are presented with the bar charts. Statistical analyses were performed with GraphPad Prism 8 software. No statistical analyses were performed for in vivo experiments. For in vivo experiments, the sample size estimate was based on a power of 0.01 at 90% power assuming a 50% difference in means in tumor burden with 33% standard deviation between control and drug-treated animals. The one-way ANOVA followed by Tukey’s multiple comparison test was used to compare the three groups. p < 0.05 (** or #) and p < 0.01 (** or #) were considered statistically significant. Number of animals are indicated in figure legends. Some analyses were performed only on representative animals from each group, i.e., RNA-Seq, measurement of serum transaminases and hydroxyproline... The exact p values are provided in the Source data file. For the PLS assay, variation of the poor- and the good-prognosis genes was determined by a NES obtained using GSEA analysis. Significance of the data was determined by the FDR values. According to GSEA (https://www.gsea-msigdb.org/gsea/index.jsp), results are significant if FDR < 0.25. Results are expressed as heat map (log 10 of FDR). All the exact p values, NES and FDR are provided in the Source data file. For clinical data presented in Fig. 7, data were extracted from publicly available data banks. The data were analyzed by a paired test, using R 3.1.3. Number of patient and statistics tests are indicated in figure legend. Representative graphs or heatmaps from independent experiments with similar results are indicated in figure legends. Due to the rarity of patient liver tissues, experiments involving patient-derived spheroids/tumorospheroids were performed one time in quadruplicate.

HCV infection. Cell culture-derived HCVcc C1 (ref. 61) and HCVcc C1E2*FLAG62 (genotype 2a/2a) were produced in Huh7.5.1 cells by electroporation of viral RNA. For HCVcc C1, the cell culture supernatants were harvested every 3 days for a total of 12 days and were concentrated ten times using Vivaspin 20, 10,000 MWCO P25 (Sartorius). HCV.C1E2*FLAG was purified using anti-FLAG M2 affinity gel (Sigma-Aldrich) according to the manufacturer’s instructions. Supernatants were collected and concentrated in the same conditions (flock-electroporated cells). HCV infection was determined by calculating the tissue culture infective dose 50 (TCID50). To analyze the PLS induction Huh7.5.1 cells were infected with HCV C1 or HCV C1E2*FLAG (TCID50 100 infectious tissue/cell/mL) for a total of 10 days. Cell culture supernatants from mock-electroporated cells (without viral RNA) or 100 µg/mL of FLAG peptide were used for control experiments. HCV infection was assessed by qRT-PCR of intracellular HCV RNA and immunostaining using HCV C1-specific AP33 antibodies. To assess the effect of IFN-2a on the PLS, Huh7.5.1 cells were infected with HCV C1 for 7 days and were incubated for 3 more days (for a total of 10 days) with either 10 IU/mL IFNα-2a, or a combination of 1 nM dactaquidar and 1 µM sotrovir (DAAs) treatment. PLS induction in virus-infected cells was always determined using mock-infected cells as control references.

Single-cell RNA-Seq profiling of HCV-infected Huh7.5.1 cells. Huh7.5.1 cells were infected with HCV Jc1E2*FLAG (TCID50 = 7 × 10^3/mL). On day 7, single cells were sorted into 96-well plates. Single cell pellets were lysates in 5 µL TCE buffer (Tris, Cation, Ethanol). Cells were supplemented with 1% 2-mercaptoethanol. HCV RNA was co-amplified with cellular mRNA using a SMART-compatible primer (sequence: 5′-Biotin-AAG- CAGTGGTATACACCGAGATTCTGCGGAAAACTGGTGAAGTA-3′). Paired-end 25 bp reads were sequenced for 59 single cells using the SmartSeq2 protocol63. For each cell, reads were aligned to the human hg19 UCSC reference genome, supplemented as the HCV C1 reference using TopHat64,65, and gene expression levels were quantified for 21,948 human genes using CuffQuant from Cufflinks66. The 42 single cells with a read alignment rate of at least 50% and with at least 10 reads aligned were normalized using Chirunnipa67 and set the following conditions, indicating in the median expression level in each sample, thus reporting normalized expression levels (FPKM: fragments per kilo bases of exons for per million mapped reads). Forty out of 42 single cells were submitted for downstream analyses after excluding samples, i.e., one noninfected single cell that was potentially contaminated and one infected single cell with significantly lower liver cell gene detection levels. Induction or repression of PLS poor- and good-prognosis genes, in association with the HCV viral load in scRNA-Seq data was determined using the pranked GSEA module implemented in GenePattern68,69, with Pearson correlation as the rank metric. The FDR values of the pranked GSEA analysis that are based on the Kolmogorov–Smirnov statistic, specifically, FDR = 0.016 for poor-prognosis genes. Enrichment score of poor- and good-prognosis genes with the HCV viral load was determined using Pearson correlation tests and the p value as estimated by the corcoef function in MatLab.

HBV and HDV infection. HBV (genotype D) was purified from the serum of a HBV carrier. Viral particles were concentrated through ultracentrifugation by pelleting over a 30% sucrose cushion with subsequent gradient centrifugation using a 10–45% iodixanol density gradient70. HepG2-NTCP cells were infected with purified HBV or control preparation in the presence of 4% polyethylene glycol and maintained in culture for additional 10 days in 2% DMSO-complemented primary hepatocyte maintenance medium (PMM) and HBV infection was assessed by immunodetection of HBsAg. NTCP-overexpressing Huh7.5.1 cells were infected with recombinant HDV in presence of 4% polyethylene glycol and cultured for 10 days in 2% DMSO-complemented PMM. HDV infection was assessed by immunodetection of HDV Ag using serum-derived anti-HDAg antibodies. PLS induction in virus-infected cells was always determined using mock-infected cells as control references.

HEV infection. HEV viral stocks were produced by electroporation of PLC3 cells (human hepatoma cell line) with pG5 Kevnor C-1 p6 strain (GenBank accession number DQ5538753). Transfected PLC3 cells (3 days) were maintained at 32 °C in medium containing DMEM/M199 (1:1 v:v), 1 mg/mL of lipid-rich albumin (Albumpin), 1% of nonessential amino acids, and 1% of pyruvate sodium (Life Technologies). Supernatant of confluent 775 flask of HEV producing cells was harvested, centrifuged for 10 min at 800 r.p.m., and used to infect Hep7.5.1 cells in 1% DMSO-complemented DMEM. At 10 days post infection, infected Hep7.5.1 cells were either total RNA extracted or used for indirect immunofluorescence. Briefly, cells were fixed with 3% of paraformaldehyde (PFA) for 20 min, washed twice with PBS, and permeabilized for 5 min with cold methanol and then with 0.5% Triton X-100 for 30 min. Cells were incubated with 10% goat serum for 30 min at room temperature, and then with the 1E6 (Millipore, 1/800) anti-ORF2 capsid protein for 30 min at RT followed by secondary antibodies (Jackson Immunoresearch) for 20 min at RT. The nuclei were stained with DAPI. Image acquisition was carried out using an Axiopt 2 microscope (Zeiss, Oberkochen, Germany). PLS induction in virus-infected cells was always determined using mock-infected cells as control references.

DENV. BHK-21 cells (Hamster Kidney Fibroblasts) were cultured in complete glucose MEM medium. Cells were transfected with recombinant DENV2 (strain 16681) RNA using Lipofectamine RNAiMax, according to manufacturer’s instructions. Supernatants were collected and spun down to remove debris. DENV2 was propagated in GS/Δd Aedes albopictus mosquito cells, maintained in Leibovitz L15 medium, by infection with viral supernatants of BHK-21 cells. Huh7.5.1 cells were then infected with DENV2 (MOI = 0.5) for 24 h in 1% DMSO-complemented DMEM. Infection was assessed using flow cytometry by intracellular staining of DENV E protein using mouse anti-DENV E protein mAb (Millipore)68. PLS induction in virus-infected cells was always determined using mock-infected cells as control references.

Free fatty acid (FFA) and ethanol treatment. Huh7.5.1 cells were cocultured with 20% LX2 stellate cells for 3 days in DMEM supplemented with 10% heat-deactivated FBS, gentamycin supplemented, and 1% DMSO. Cells were then incubated with FFA (800 µM oleic acid and 400 µM palmitic acid) for 72 h. For ethanol treatment, Huh7.5.1 cells were incubated in presence or absence of ethanol (20 or 40 mM) for 48 h in a humidified incubator saturated with ethanol vapor. Fresh medium containing ethanol was replenished every day.
PLS induction in FFA- and ethanol-treated cells was always determined using non-treated cells as references.

**Hypoxia treatment and nutrient starvation of Huh7.5.1.** Hypoxia was induced as described with some modifications. Briefly, Huh7.5.1. cells were cultured in a humidified incubator supplied with 3% O2, 5% CO2, and 95% N2, for 72 h. Cells cultured in presence of 20% O2 were analyzed as controls. To induce nutrient deprivation and starvation, Huh7.5.1. cells were incubated in DMEM medium containing low glucose (5.5 mM glucose), 1 mM sodium pyruvate, 25 mM HEPES, and 0.1% heat-decomplemented FBS for 24 h. Cells cultured in medium containing high glucose (25 mM glucose) and 10% FBS were used as controls.

Selection of compounds for the molecular signature-based drug screening. Transcriptome-based in silico drug screening was performed using the PLS as a query in the chemogenomics database connectivity map (cmap, www.broadinstitute.org/cmap) and LINCS database (https://clue.io), as previously described. Cmap-predicted compounds with a negative enrichment score and a p value < 0.05, and LINCS-predicted compounds with connectivity score < -90 were further selected with respect to their approval in clinical trials, according to www.clinicaltrial.gov. Compounds that have been formally licensed for clinical use, compounds currently being evaluated in phases 3–4 clinical trials and compounds with a putative relevance for liver cancer currently being evaluated in phase 2 clinical trials were selected for assessment in the cell-based model. Known carcinogens, antibiotics, and compounds associated with severe liver toxicity according to LiverTox database (http://liver.tox.nih.gov) were discarded. To assess the effect of selected compounds on the noninfected and HCV-infected cells in the scRNA-Seq data, gene signatures comprising the targets of each specific compound were obtained by extracting the top 100 upregulated and top 100 downregulated Affymetrix probes as indicated in the LINCS database (BING rowspace). Enrichment of these signatures in association with the HCV viral load in scRNA-Seq data was tested using the preranked GSEA module in GenePattern with Pearson correlation as the rank metric. Probes were collapsed to unique genes by primary landmark over inferred probes with the probe with the highest absolute z-score in case of multiple probes. Expression heatmaps were visualized using GENE-E (www.broadinstitute.org/GENE-E).

**Cell-based screen of computationally enriched small molecules.** Huh7.5.1. cells were seeded in 96-well plates and maintained in 1% DMSO-complemented medium. Cells were infected with HCV Jc1 (TCID50 = 10^6/mL) or cell culture supernatant from mock-electroporated cells for non-infected control wells, as described previously. For induction as described with some modifications. Briefly, Huh7.5.1. cells were incubated with either brefeldin-a (0.1 µM), captorpril (1 µM), cediranib (0.5 µM), CL-1040 (1 µM), clofibrate citrate (1 µM), dilazep HCl (1 µM), dorzolamide (10 µM), erlotinib (0.1 µM), iso-liquiritigenin (10 µM), MK-2206 (1 µM), nifedipine (1 µM), oxetacaine (10 µM), PDD325901 (10 nM), pimaisertib (0.5 µM), pimozone (1 µM), pioglitazone (1 µM), pralidoxime (10 µM), primidon (10 µM), resveratrol (10 µM), rolipram (10 µM), selumetinib (0.5 µM), TC-101348 (0.1 µM), tivozanib (0.5 µM), tirofiban (10 µM), toremifene (10 µM), vilanterol (10 µM), and ziprasidone (10 µM).

**Generation of plasmids for HDTVI.** For different HRH2-targeting sgRNAs, predicted to have high on target efficiency and no off targets, were selected using Chopchop V3 (https://doi.org/10.1126/science.1231143). For efficiency testing, sgRNAs targeting Hrh2 were cloned into the vector px439, as a dual sgRNA cassette either in combination with a nontargeting control sgRNA (sgCTRL) or in combination with one target-specific sgRNA (sgHRH2). In the case of a mismatch on the template, one 5′-ACT-quantitative real-time PCR using TaqMan Gene Expression Assays (Thermo Fisher Scientific) on Applied Biosystem 7900HT Fast Real-Time PCR system. Expression of human HDC, IL6, IL1B, TNFα, HRH2, and CREB5 was quantified by quantitative real-time PCR using TaqMan Gene Expression Assays (Thermo Fisher Scientific) on the CFX96 Touch Real-Time PCR System (Bio-Rad). Expression of human CCL2 and TGF was analyzed using iTaq® Universal SYBR® Green Supermix (Bio-Rad). The 2−ΔΔCT method was used for relative quantification of mRNA with normalization to either 18S or GAPDH mRNA. The list of the primers is provided in Supplementary Table 4.

**In vitro CRISPR/Cas9 gene editing.** Lentiviruses expressing single guide RNA (sgRNA) were produced in HEK 293T cells by co-transfection with an envelope plasmid (pMD2.G), a packaging plasmid (psPAX2), and a lentiviral vector expressing the sgRNA component (pSPR_BRD1016). The transfection was performed using the CallPhos™ Mamalian Transfection Kit (Clontech Laboratories), according to manufacturer’s instructions. Huh7.5.1 stably expressing Cas9 endonuclease (Huh7.5.1-Cas9) were generated by transduction of a lentiviral vector expressing Cas9 (pSPR_BRD11, Broad Institute). For HRH2 KO, Huh7.5.1 cells were transduced with lentiviruses expressing human RNA CTRL targeting GFP (sgCTRL) or sgRNA targeting HRH2 (sgHRH2) designed by the Broad Institute. After 48 h, transduced cells were selected under hygromycin treatment (125 µg/mL). HRH2 KO was determined at genetic level using T7 endonuclease assay (Alt-R® Genome Editing Detection Kit, IDT™), according to manufacturer’s instructions. For CREB5 KO, Huh7.5.1-Cas9 were transduced with lentiviruses expressing sgCTRL targeting GFP (sgCTRL) or sgRNA targeting CREB5 (sgCREB5). After 48 h, transduced cells were selected under hygromycin treatment (125 µg/mL). The KO efficiency was assessed by western blot analysis.

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**In vitro apoptosis assay.** Apoptosis level in HRH2 KO or CTRL. Huh7.5.1 cells was assessed by detecting cleaved caspase 3 after H2O2 treatment (300 µM, 3 h) using Caspase-3/7 (CellTiter-Glo® Assay) according to manufacturer’s instructions. Immunofluorescence pictures were acquired using Axio Observer Z1 microscope. Quantification of cleaved caspase 3 levels was performed using Celigo Imaging Cytometer. Data were integrated and normalized to total cell number (DAPI staining).

**Histology, immunohistochemistry.** Animal tissues: formalin-fixed samples were embedded in paraffin, cut in 5 µm thick sections, and stained with hematoxylin and eosin or Picrosiris red staining. The CPA was morphometrically quantified on Sirius red stained sections with image processing software (Image J, NIH).
Additional sections in the DEN injury model were stained with antibody specific for lymphocyte antigen 6 complex locus G6D (Ly6G), F4/80, cluster of differentiation 11 (CD11), cluster of differentiation 11b (CD11b), cluster of differentiation 8 (CD8), HRH2, and CREB5. IHC were quantified using Image J IHC ToolBox.

Human tissues: formalin-fixed, paraffin-embedded human tissue samples were provided by the tissue biobank of the University Medicine Mainz in accordance to the ethics committee of the Medical Association of the State of Rhineland Palatinate, Germany (Immunhistochemical analysis of HCC. No. 837.146.17 (10980), April 24th, 2017). Formalin-fixed, paraffin-embedded tissues were cut at a thickness of 1–2 µm to perform immunohistochemistry89. Human tissue sections were incubated in an oven at 60 °C for 4 h. After rehydration in graded ethanol, antigens were retrieved in 0.1 M Tris-HCl containing 5% H2O2 in a steamer at 100 °C for 15 min. Sections were preincubated with 3% H2O2, for 30 min and blocked with the serum of the species, in which the secondary antibody was raised for 30 min, and then with avidin and biotin for 10 min each. Then, polyclonal rabbit anti-HR22-serum (GeneTex) was used at a dilution of 1:100 in Tris-buffered saline at pH 6.1 overnight. After subsequent washes in PBS for 20 min, sections were incubated with the secondary antibody for 30 min, washed with PBS, and incubated with the avidin–biotin complex for 10 min. After washing with PBS, sections were incubated with the peroxidase complex for 2–10 min. Sections were mounted with Eukitt (Riedel de Haen, Seelze, Germany). Images were taken with an Olympus BX45 microscope using Plan x20 objectives with a Gryphos ProPlus camera ( Jenoptik) and Cell Sense software (Micro Optimal, Kirchheim / Teck).

Single-cell RNA-Seq analyses of patient liver tissues. Human cells were dissociated from HCC patient liver tissues undergoing surgical resection using gentAMCS– Octo Dissociator with Heaters and Human tumor dissociation kit (Miltenyi Biotec) and centrifuged 5 min at 50 g to remove hepatocytes. Supernatants were then centrifuged 10 min at 800 g and cells were washed with Hank’s Balanced Salt Solution (Gibco). Erythrocytes were removed using Buffer EL (Quagen) and washing in PBS ± 1% FBS. Leukocyte CD45+ cells were separated from other cell types by flow cytometry using SH800 cell sorter (Sony). (The gating strategy is described in Supplementary Fig. 15). Living cells were selected using Zombie green staining according to manufacturer’s instructions. Data were acquired using the Sony Cell Sorter Software. CD45+ cells were then treated with nizatidine 20 µM or DMSO vehicle control. Two days after treatment, single cells were sorted into 384 well cell capture plates (Single-Cell Discovery, https://www.sciscoviscovery.com) using SH800 cell sorter (Sony). Sorted plates were briefly centrifuged at 4 °C, snap-frozen on dry ice and stored at −80 °C until processed. The plates contain mineral oil and droplets of poly-A primers, containing a cell–specific barcode and a unique molecular identifier, enabling to distinguish the well-specific (and cell-specific) mRNA molecules. scRNA-Seq was performed by Single-Cell Discoveries B.V. using SORT-Seq, a modified CEL-Seq2 protocol38. Reads were aligned to the human hg19 UCSC reference using Hisat2 R version 3.5.3 with package ‘RaceCD’ for clustering, cluster analysis, and DEG calculation, as described29. Dataset were filtered, analyzed, and normalized using RaceCdl3. As quality control, we removed all the cells with >80% of mitochondrial genes. Transcripts correlating to KCNQ1OT1 with a Pearson correlation coefficient of >0.4 were removed. Race3D was run with the following parameters: minotent = 1500, minexpr = 2, minunum = 5, kmin = 10. This step filtered out the cells with <1500 cells. For normalization, the transcript counts in the total cell were normalized to 1 and multiplied by the minimum transcript count across all cells that passed the quality control threshold. Data are available at NCBI Gene Expression Omnibus database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi? acc= GSE173671).

Culture of organotypic ex vivo patient liver slices, patient-derived spheroids, and tumorspheroids. Organotypic liver slices: fresh liver tissue sections (300 µm thick) were made from surgically resected fibrotic livers from liver disease patients, using Krumdieck Tissue Slicer MD6000 (Alabama Research and Development). The tissues were cultured with nizatidine (50 µM) or DMSO vehicle control for 48 h and harvested for gene expression analysis, as described above. Patient-derived spheroids: spheroids were generated from liver tissues from patients without liver disease undergoing liver resection for metastasis of colorectal cancer. Tissues were perfused and dissociated as described for PHH. Total cell population, including hepatocytes and NPCs, was used to generated multicellular spheroids in Corning® 96-well Black/Clear Bottom Low Flange Ultra-Fluoro-Attachment Microplate (Corning). Spheroids were then treated with FFA and or nizatidine (20 µM) for a total of 3 days before GM6000 assessment. DMSO was used as negative control.

Patient-derived tumorspheroids: tumorspheroids were generated from patient HCC liver tissues undergoing surgical resection and dissociated using Human Tumor Dissociation Kit (described above) and a Cell of patient-derived tissues. Total cell populations, including epithelial (i.e., cancer cells/hepatocytes) and NPCs, were used to generated multicellular tumorspheroids in Corning® 96-well Black/Clear Bottom Low Flange Ultra-Fluoro-Attachment Microplate (Corning). After 48 h, HCC-derived spheroids were treated with nizatidine 20 µM, sorafenib 10 µM, or DMSO vehicle control for 4 days. Fresh medium containing DMSO or drugs were added every day. Cell viability was assessed using CellTiter-Glo® (Luminescent Cell Viability Assay), according to manufacturer’s instruction.

Data availability All data generated in this study are provided in the Supplementary Materials, in Source data file or have been deposited at NCBI Gene Expression Omnibus database. For specific questions, contact the corresponding authors and lead contact, Prof. Thomas F. Baumert (thomas.baumert@unistra.fr).

Reporting Summary Further information on research design is available in the Nature Research Reporting Summary linked to this article.
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The University of Strasbourg, Inserm, the IHU Strasbourg and Mount Sinai Hospital have filed a patent application on the clinical gene signature-based human cell culture model and uses thereof with Y.H. and T.F.B. as inventors (WO 2016174130 A1), which has been licensed to Alentis Therapeutics, Basel, Switzerland. The University of Strasbourg and Inserm have filed a patent application on H2 blockers targeting liver macrophages for the prevention and treatment of liver disease and cancer with E.C. and T.F.B. as inventors (PCT/EP2021/072341). A.R. is a co-founder and equity holder of Celsius Therapeutics, an equity holder in Immunitas, and was an SAB member of ThermoFisher Scientific, Syros Pharmaceuticals, Neogene Therapeutics and Asimov until 31 July 2020. Since 1 August 2020, A.R. has been an employee of Genentech. O.R.R. has been an employee of Genentech. T.F.B. is founder, advisor, and equity holder in Alentis Therapeutics. Y.H. and C.S. hold equity in Alentis Therapeutics. The other authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to B.C.F., Y.H. or T.F.B.

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Author contributions

T.F.B. initiated and coordinated the study. Y.H., B.C.F., and T.F.B. designed experiments and analyzed data. E.C., S.B., N.F., S.L., H.E.S., M.F.-V., T.R., X.S., H.H., N.R., C.P., L.H., S.Z., T.Q., A.V., E.R.Y., J.L., M.A.O., F.H.T.D., M.S., R.M., L.W., C.T., S.C.D., V.G.M., D.H., J.H., S.N., A.O., W-M.S., T.H., R.S., A.S., B.S., M.A., L.C., E.S., N.G., A.P.K., M.M., V.D.N., N.B., M.H., and B.C.F. performed experiments, and/or analyzed data. A.K.S, O.R.R., and A.R. contributed to design of scRNA-Seq experiments in cell culture. E.C., F.J., and A.S. analyzed patient single-cell RNA-Seq data. N.P. supervised the computational analyses of scRNA-Seq profiling. T.C. performed computational analyses of scRNA-Seq profiling. X.S., R.K., and C.B.R. performed liver tissue experiments and bioinformatic analyses. B.K.S. and D.S. performed IHC on human liver tissues. M.F.-V. and T.R. performed HTVI experiments, which were supervised by M.H. K.K., M.E.S., R.T.C., G.G., and P.P. provided reagents. E.F. and P.P. provided patient liver tissues. E.C., S.B., Y.H., B.C.F., M.B.Z., C.S., and T.F.B. wrote the manuscript. B.C.F. and K.K.T. supervised animal experiments (sibrosis/NASH/HCC models). Y.H. supervised the bioinformatic analyses and human liver tissue experiments.

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Gastrointestinal Division, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA. 19Institute of Pathology, University Medicine, Johannes Gutenberg University, Mainz, Germany. 20Institute for Translational Immunology and Research Center for Immunotherapy (FZI), Johannes Gutenberg University (JGU) Medical Center, Mainz, Germany. 21Division of Gastroenterology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA. 22University of Lille, CNRS, Inserm, CHU Lille, Pasteur Institute of Lille, U1019-UMR 8204-CiIL- Center for Infection and Immunity of Lille, Lille, France. 23CNRS UPR3572 Immunopathologie et Chimie Thérapeutique, Institut de Biologie Moléculaire et Cellulaire (IBMC), Strasbourg, France. 24Division of Gastroenterology and Hepatology, Geneva University Hospital, Geneva, Switzerland. 25Department of Neurology, Icahn School of Medicine at Mount Sinai, New York City, NY, USA. 26Recanati/Miller Transplantation Institute, Icahn School of Medicine at Mount Sinai, New York City, NY, USA. 27Massachusetts General Hospital Cancer Center; Harvard Medical School, Cambridge St. CPZN 4216, Boston, MA, USA. 28Institute for Medical Engineering Science & Department of Chemistry, MIT, Cambridge, MA, USA. 29Ragon Institute of MGH, MIT and Harvard, Cambridge, MA, USA. 30Department of Biology, Massachusetts Institute of Technology, Cambridge, MA, USA. 31Present address: Genentech, 1 DNA Way, South San Francisco, CA, USA. 32Present address: Cancer Research Center of Lyon (CRCL), UMR Inserm 1052 CNRS 5286 Mixte CLB, Université de Lyon 1 (UCBL1), Lyon, France. 33Present address: Ferring Pharmaceuticals Inc 4245 Sorrento Valley Blvd, San Diego, CA, USA. 34email: Bryan.Fuchs@ferring.com; Yujin.Hoshida@UTSouthwestern.edu; thomas.baumert@unistra.fr