

Liver mechanosignaling as a natural anti-hepatitis B virus mechanism

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Jianyu Ye^{1,2,5}, Fahong Li^{1,3,5}, Ting Hua^{1,2,5}, Kewei Ma¹, Jinyu Wang^{1,3}, Zixin Zhao¹, Zhongning Yang^{1,2}, Chen Luo^{1,2}, Ruohan Jia^{1,2}, Yaming Li^{1,2}, Menghan Hao^{1,2}, Jian Wu¹, Mengji Lu⁴, Zhenghong Yuan^{1,2}✉, Jiming Zhang^{1,3}✉ & Jieliang Chen^{1,2}✉

The mechanisms underlying the natural control of hepatitis B virus (HBV) infection have long been an intriguing question. Given the wide physiological range of liver stiffness and the growing attention to the role of mechanical microenvironment in homeostasis and diseases, we investigated how physical matrix cues impact HBV replication. High matrix stiffness significantly inhibited HBV replication and activated YAP in primary hepatocyte culture system, a key molecule in mechanosignaling. YAP activation notably suppressed HBV transcription and antigen expression. Several YAP-induced genes exhibited strong anti-HBV effects. Single-cell analysis of liver tissue from male individuals with active HBV replication revealed a strong significant negative correlation between YAP signature activation and HBV transcript levels. Intraperitoneal administration of YAP small molecule agonist potently controls HBV in male mouse models. These findings unveil a mechanism that involves the mechanical environment of hepatocytes and YAP to clear hepatotropic viral infection in the liver, providing new perspectives for HBV cure studies and antiviral development.

Hepatitis viruses have long posed a threat to human health, with hepatitis B virus (HBV) being especially harmful, leading to acute and chronic hepatitis, as well as severe liver diseases such as cirrhosis and hepatocellular carcinoma (HCC). It remains challenging to achieve HBV cure^{1–3}, as the mechanisms for controlling and clearing the virus are not fully understood. Cytotoxic T cells-mediated killing of infected cells is recognized as the primary mechanism for viral clearance. Additionally, “noncytolytic” mechanisms are gaining attention⁴: studies have suggested that inflammatory cytokines, including IL-6 and interferons (IFNs), can reduce HBV replication and covalently closed circularDNA (cccDNA) reservoir maintenance^{5–7}. IFN-stimulated genes (ISGs), such as ISG20, Mx2, and APOBEC3A, are known to reduce HBV

RNA stability, inhibit reverse transcription, and promote cccDNA degradation^{8,9}. However, the efficiency of such HBV control and the regulation of viral restriction genes by factors beyond cytokines remain unclear.

Recent research highlights the impact of the extracellular physical microenvironment on cell function^{10,11}. Matrix stiffness and mechanosignaling play a substantial role in regulating cell function and homeostasis, closely related to immunity and diseases^{12–14}. The liver, with its unique tissue structure, exhibits a wide range of liver stiffness (typically 2.0 to 7.5 kPa). Liver stiffness exceeding 7.5 kPa may indicate the presence of liver tissue fibrosis, while stiffness exceeding 12.5 kPa may suggest cirrhosis. Persistent cirrhosis is associated with severe

¹Key Laboratory of Medical Molecular Virology (MOE/NHC/CAMS), School of Basic Medical Sciences, Huashan Hospital, Shanghai Medical College Fudan University, Shanghai, China. ²Shanghai Frontiers Science Center of Pathogenic Microorganisms and Infection, Shanghai Institute of Infectious Diseases and Biosecurity, Research Unit of Cure of Chronic Hepatitis B Virus Infection (CAMS), Fudan University, Shanghai, China. ³Department of Infectious Diseases, Shanghai Key Laboratory of Infectious Diseases and Biosafety Emergency Response, National Medical Center for Infectious Diseases, Huashan Hospital, Fudan University, Shanghai, China. ⁴Institute for Virology, University Hospital Essen, University of Duisburg-Essen, Essen, Germany. ⁵These authors contributed equally: Jianyu Ye, Fahong Li, Ting Hua. ✉e-mail: zhyuan@shmu.edu.cn; jmzhang@fudan.edu.cn; jieliangchen@fudan.edu.cn

liver diseases, yet little study explores the physiological functions and compensatory significance of stiffness elevation¹⁵. Interestingly, studies show that in acute HBV infection, liver stiffness values increase, even exceeding the values seen in liver fibrosis or cirrhosis, and then returning to normal as liver damage gradually recovers^{16,17}. These evidences suggest a potential role of mechanical forces during natural control of HBV infection.

Therefore, this study investigates how changes in matrix stiffness affect HBV replication, with the goal of elucidating novel natural antiviral mechanisms. Using various cell and mouse models, along with single-cell data from liver tissues of HBV patients, we have found that elevated matrix stiffness significantly inhibits HBV replication. We have

also analyzed the mechanosignaling and effector genes, which we hope to harness for controlling HBV viral infection.

Results

Elevation of matrix stiffness restricts HBV infection

Viral replication levels vary among HBV patients with different liver statuses. We analyzed liver tissue samples from 1056 individuals with active HBV replication (HBeAg-positive) to examine the relationship between liver stages and viral expression. The Scheuer scoring system, which assesses the state of liver fibrosis, correlates well with transient elastography measurements of liver stiffness. It is evident that HBV DNA levels significantly decrease as the Scheuer grades increase (Fig. 1a).

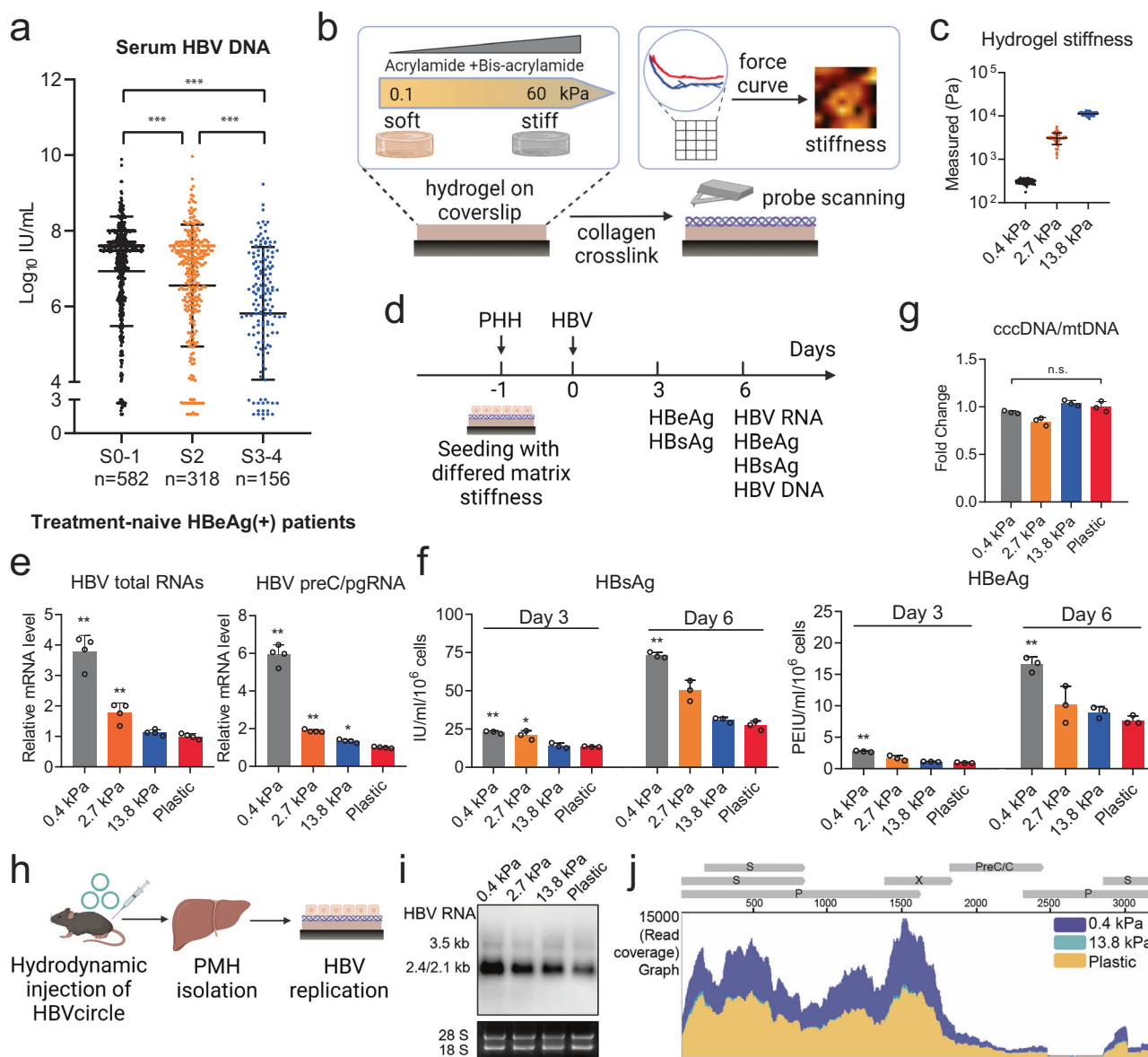


Fig. 1 | Repression of HBV viral replication by high matrix stiffness. **a** Serum HBV DNA level in treatment-naïve HBeAg-positive patients with different fibrosis grades according to Scheuer scoring system ($n = 1056$ individual patients). **b** Schematic representation of hydrogel with tunable matrix stiffness (Figure was created with BioRender). **c** Measurement of hydrogel stiffness using the atomic force microscope. **d** Diagram outlining the HBV infection experiments (Figure was created with BioRender). **e** Quantification of intracellular HBV RNAs using qPCR in HBV-infected PHHs cultured on hydrogels with varying matrix stiffness (four biological replicates). **f** Detection of supernatant HBsAg and HBeAg levels via ELISA (three biological replicates). **g** Examination of cccDNA levels using qPCR (normalized to

mitochondrial DNA) (three biological replicates). **h** Diagram outlining the HBV circle transfection experiments (Figure was created with BioRender). **i** Determination of intracellular HBV RNAs through Northern blot analysis in HBV circle-transfected PMHs cultured on hydrogels with different matrix stiffness. Experiment was repeated three times independently with similar results. **j** Quantification of HBV transcripts by nucleotide mapping in the indicated PMHs analyzed via RNA sequencing. The data are represented as mean $\pm$ SD. P value by two-tailed Student's t -test (**a**, **e**–**g**). Source data are provided as a Source Data file. (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

Beyond the damage to hepatocytes caused by inflammations and their impact on the virus, the impact of changes in the physical matrix cues on the virus remains unclear.

To reveal the effect of matrix stiffness on HBV replication, we established an *in vitro* model using hydrogel-based cell cultures with distinct matrix stiffness¹⁸. Conventional collagen-coated plastic plates were used as a control, and atomic force microscopy (AFM) was used to measure the stiffness (Fig. 1b), demonstrating that the model could mimic the physiological range of liver stiffness (Fig. 1c, Supplementary Fig. 1a). We assessed cell morphology and cell surface area under different matrix stiffness conditions (Supplementary Fig. 1b–d), which were consistent with previous findings using similar models¹⁹. We employed this model to observe HBV replication in primary human hepatocytes (PHHs) infected with HBV (Fig. 1d). The results indicated that, as matrix stiffness increased, there was a significant reduction in intracellular levels of HBV preCore/pgRNA and total HBV RNAs (Fig. 1e, Supplementary Fig. 1e), as well as a decrease in the levels of viral antigens including HBsAg and HBeAg (Fig. 1f). However, there were no statistically significant differences in intracellular HBV cccDNA levels between the groups (Fig. 1g), suggesting that the inhibition of HBV replication primarily occurred at the transcription and post-transcriptional levels, with limited impact on the step of infection establishment. Furthermore, we validated the impact of high matrix stiffness on HBV transcription and antigen expression in a primary mouse hepatocytes (PMHs) model with HBV replication (Fig. 1h, Supplementary Fig. 1f–h). Results obtained through Northern Blot and RNA-seq revealed a global suppression of HBV RNAs under conditions of high matrix stiffness (Fig. 1i–j).

Elevation of matrix stiffness activates YAP, a key molecule in mechanosignaling

In the process of extracellular mechanical signaling transduction into cells, previous reports have highlighted the central role of YAP (Yes-associated protein)¹². Therefore, we assessed the activation status of YAP in our cell models. The results indicated that with an increase in matrix stiffness, there was a decrease in YAP phosphorylation levels in PHHs and PMHs (Fig. 2a, Supplementary Fig. 2a). Furthermore, subcellular localization of YAP translocated from the cytoplasm to the nucleus (Fig. 2b, Supplementary Fig. 2b), and the activity of YAP-responsive elements increased, as indicated by luciferase reporters (Fig. 2c), collectively suggesting YAP activation. We additionally conducted a bioinformatic analysis to explore statistical associations between genes regulated by stiffness and gene signatures indicating the activation of specific signaling pathways. RNA-seq analysis further revealed that YAP-related pathway was the most significantly enriched pathway under high matrix stiffness (Fig. 2d, e). Considering that the Hippo signaling pathway plays a crucial role in YAP activation, we examined the expression of key upstream kinase molecules MST1/MST2 and LATS1, as well as their phosphorylated forms. We found a slight decrease of p-MST1/2 protein levels with increased stiffness. The results indicated a weak inhibition in the Hippo pathway under high matrix stiffness conditions, which might contribute to YAP activation (Supplementary Fig. 2c). Additionally, given that the integrin pathway is involved in mechanical signal transduction independently of Hippo, we examined the expression of downstream kinases such as FAK, Src, as well as other potential kinases ERK and Akt, among which FAK showed some activation under these conditions (Supplementary Fig. 2d, e). Notably, in hepatocellular carcinoma-derived cell lines like Huh7 cells, neither significant YAP activation nor HBV inhibition was observed under high matrix stiffness conditions (Supplementary Fig. 2f). To rule out the possibility that the matrix stiffness-induced inhibition of HBV is mediated by IFNs, we also examined the activation status of the IFN-related pathways and did not observe activation of IFN-related gene signatures (Fig. 2f). In addition, HBV infection per se did not activate

YAP in PHHs cultures (Fig. 2g). The above results indicate that under high matrix stiffness conditions, HBV replication is significantly inhibited, while YAP is markedly activated in primary liver cells, which could be associated with the suppression of the Hippo pathway and the activation of FAK.

Activation of YAP inhibits HBV replication

We next elucidated the inhibitory effect of YAP activation on HBV replication and identified at which steps the inhibition occurred. By introducing siRNA targeting YAP into the cells, YAP expression and the activation of YAP-related reporter genes were significantly downregulated, with no apparent impact on cell viability (Fig. 3a, Supplementary Fig. 3a–c). Under these conditions, using the recombinant HBV circle model, we showed that, with the YAP inactivation, there was a significant increase in HBV RNAs (Fig. 3b, c) and viral antigens (Fig. 3d), while there was no significant effect on the levels of HBVcircle in the cell nucleus (Fig. 3e). These results suggest that the inhibitory effect of YAP activation on HBV primarily occurs at the levels of cccDNA transcription and post-transcription. To investigate whether YAP exerts its antiviral effects through nuclear translocation and binding to TEAD elements, we further compared the antiviral efficacy of wild-type YAP and the TEAD-insensitive S94A mutant in the HBVcircle system (Supplementary Fig. 3d–g). We observed that the S94A mutant exhibited significantly weaker activity in driving YAP-reporter and downstream genes expression compared to the wild type (Supplementary Fig. 3f). Both wild-type YAP and the S94A mutant suppressed HBV RNA and antigen production levels compared to the control group. However, the antiviral effect of the S94A mutant was substantially reduced (Supplementary Fig. 3h–k), suggesting that YAP-mediated inhibition of HBV occurs, to some extent, through TEAD.

We further employed several YAP-related pathway inhibitors to elucidate the aforementioned effects. Among them, TRULI and GA-017 are LATS1/2 inhibitors that indirectly activate YAP, while latrunculin A (Lat. A) is an F-actin inhibitor that indirectly inhibits YAP through a Hippo-independent pathway. We confirmed the regulatory effects of these inhibitors on YAP by observing YAP phosphorylation (Supplementary Fig. 4a), nuclear localization (Supplementary Fig. 4b), and the expression of YAP downstream gene like CTGF (Supplementary Fig. 4c). Additionally, we verified that these inhibitors exhibited no significant cytotoxicity at the working concentrations (Supplementary Fig. 4d). In both the PHH and Huh7 systems, we observed a significant downregulation of HBV RNAs upon YAP activation and an upregulation of HBV RNAs upon YAP suppression (Fig. 3f, Supplementary Fig. 4e). We also tried to use the MST1/2 inhibitor XMU-MP-1 to activate YAP, which showed potent activity of activating YAP and inhibiting HBV (Supplementary Fig. 4f–g). Notably, XMU-MP-1 exhibited some cytotoxicity at high concentrations (Supplementary Fig. 4h).

Regarding cccDNA, we observed that the Lat. A-treated group exhibited a higher HBVcircle level (Fig. 3g). Further investigation revealed that the difference in cccDNA level was primarily attributable to variations in the rate of cell division-associated cccDNA loss, unlike the situation where primary hepatocytes cultured on plates cannot divide. This was observed in both the HBVcircle transfection model (Fig. 3h–i, Supplementary Fig. 5a) and the HepAD38 cell model (Supplementary Fig. 5b–c). We also examined the impact of YAP on the chromatin accessibility of HBVcircle minichromosome using ATAC-seq. The results revealed that YAP activation (TRULI group) led to a significant decrease in the accessibility of HBVcircle, whereas YAP inhibition (Lat. A group) resulted in increased accessibility (Fig. 3j). Together, these findings suggest that YAP activation directly has no significant effect on cccDNA level but exerts inhibitory effects on cccDNA chromatin accessibility and transcriptional activity. Furthermore, YAP can influence the rate of HBV cccDNA loss by affecting cell division and proliferation.

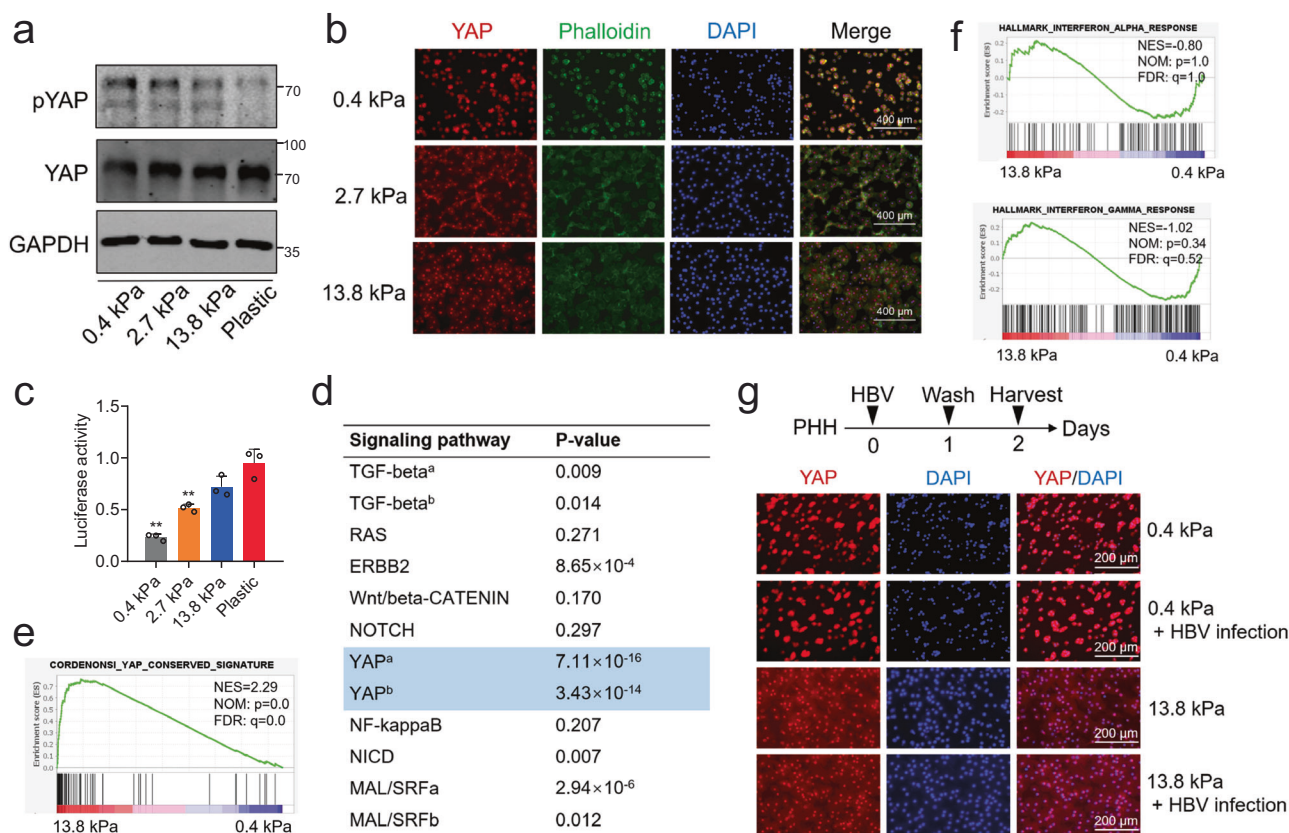


Fig. 2 | Activation of YAP by high matrix stiffness. **a, b** PHHs were plated on collagen-I-coated hydrogels with stiffness ranging from soft to stiff (0.4–13.8 kPa). Western blot showing the amounts of total YAP and p-YAP (inactive). The two YAP bands detected were due to differential splicing (**a**). Immunofluorescence images showing YAP (red) in correlation with actin stress fibers (phalloidin; green) and nuclear DNA (DAPI; blue) (**b**). The experiment was repeated three times independently with similar results. **c** Measurement of YAP activity by luciferase reporter assay (8 $\times$ GT10C-luc) (three biological replicates). **d** Over-representation of YAP target genes among the genes regulated by matrix stiffness using a one-sided

Fisher's exact test. (YAP^a, CORDENONSI YAP CONSERVED SIGNATURE; YAP^b, YAP signature; supplementary Data 1). **e** Gene set enrichment analysis (GSEA) to identify activation states of YAP-related pathways. **f** GSEA enrichment plot between PMH cultured on soft (0.4 kPa) and stiff (13.8 kPa) hydrogel for 2 days. **g** Immunofluorescence images of PHH that were plated on the hydrogel, infected with HBV for 1 day, and then cultured for an additional day. The experiment was repeated three times independently with similar results. The data are represented as mean $\pm$ SD. P value by two-tailed Student's *t*-test (**c**) and Kolmogorov-Smirnov test (**e, f**). Source data are provided as a Source Data file. (**P* < 0.05; ***P* < 0.01).

Genes induced under high matrix stiffness exhibit anti-HBV activity

The intracellular anti-HBV response is typically mediated by antiviral genes. However, under high matrix stiffness, traditional IFN-stimulated genes, which were conventionally believed to play a role in anti-HBV activity, did not show significant changes. Therefore, we conducted further analysis to identify potential new effector genes. We have published gene expression profile data from liver biopsies of individuals with immune-tolerant (IT) and immune-active (IA) phases of HBV infection. In the previous research, we discovered that genes upregulated during the IA phase included a substantial number of novel HBV restriction factors^{20,21}. Interestingly, we found that the differentially expressed genes between IA and IT phases exhibited significant YAP activation signatures (Fig. 4a). We selected genes that exhibited differential expression between IA and IT phases and overlapped with the genes differentially expressed under high matrix stiffness (13.8 kPa vs. 0.4 kPa), resulting in a set of 67 common genes (Fig. 4b–c). After excluding certain challenging-to-express genes, we utilized two HBV replication models, including pHBV1.3 and HBVcircle, to express and screen all these genes for their anti-HBV activity. We identified 11 genes with significant anti-HBV effects, using our previously identified anti-HBV gene, MyD88, as a control. These genes exhibited varying degrees of inhibitory effects on antigen expression (Fig. 4d–e) and HBV RNAs (Fig. 4f). When considering pgRNA/HBVcircle ratio as an index, most of these 11 genes had inhibitory effects on cccDNA at the transcriptional

level, and two genes, FOXM1 and PAQR8, had some effect on the level of HBVcircle pool (Fig. 4g). To further validate that the expression of these genes can be regulated by YAP, we treated Huh-7 cells with the YAP activator TRULI for various durations. The results showed that, under TRULI treatment, these genes exhibited varying degrees of upregulation, with early or late upregulation patterns (Supplementary Fig. 6).

In addition to the gene upregulation induced by YAP activation, a previous study using PMHs has reported that under high matrix stiffness, the liver cell-specific transcription factor HNF4A was downregulated¹⁹. We observed the downregulation of HNF4A expression upon YAP activation (Supplementary Fig. 7a), while the inhibition of YAP activation using LatA effectively restored the intracellular levels of HNF4A (Supplementary Fig. 7b–c). Furthermore, we observed that with increasing matrix stiffness, the expression of hepatic glycogen (Supplementary Fig. 7d), albumin (Supplementary Fig. 7a, e), HNF1A, and TTR (Supplementary Fig. 7f), showed a certain degree of decrease in liver cells. The downregulation of these factors, along with the upregulated antiviral genes, can collectively limit HBV transcription and replication within liver cells.

In addition to HBV, we also examined how changes in YAP activity affect the replication of other hepatotropic virus, such as HCV, as well as the non-hepatotropic virus VSV. Interestingly, similar to the situation with HBV, the replication levels of HCV are significantly influenced by either the activation or inhibition of YAP—decreasing or increasing

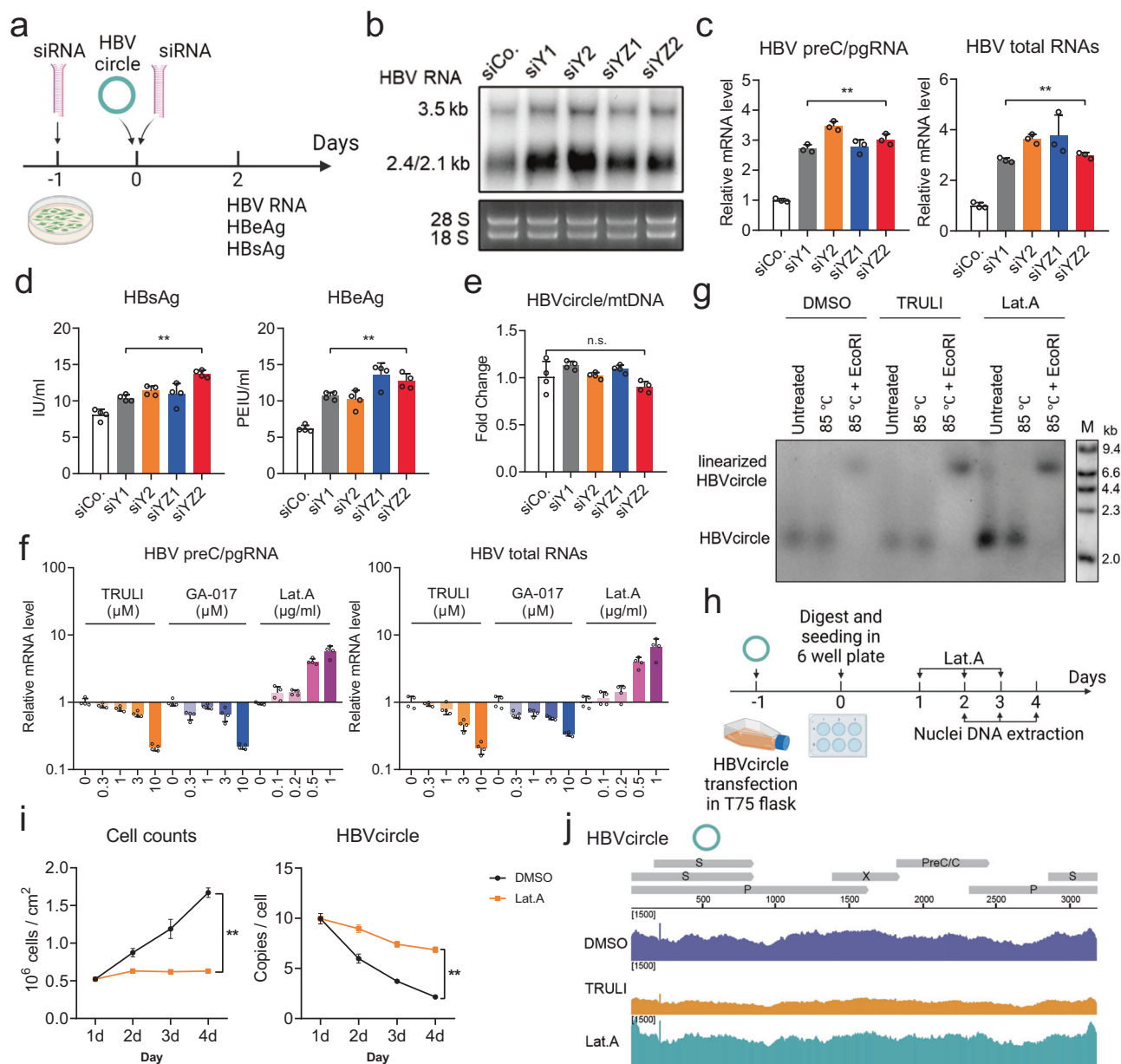


Fig. 3 | Inhibition of HBV replication through YAP activation. **a** Schematic overview of YAP knockdown experiments in the Huh7 cell line (Figure was created with BioRender). Cells were transfected with the indicated siRNAs (control, siCo.; YAP, siY1 and siY2; YAP/TAZ, siYZ1 and siYZ2). **b**, **c** Levels of intracellular HBV RNAs determined by Northern blot and qPCR (three biological replicates). **d** Levels of HBsAg and HBeAg detected by ELISA (four biological replicates). **e** Levels of HBVcircle examined by qPCR (normalized to mitochondrial DNA) (four biological replicates). **f** PHHs infected with HBV, followed by treatment with the LAST1/2 inhibitor TRULI and GA-017, as well as the F-actin inhibitor latrunculin A (Lat.A) for 48 hours. Subsequent qPCR analysis (four biological replicates). **g** Huh-7 cells

transfected with HBVcircle treated with TRULI and Lat.A for 48 hours and detection of intracellular HBVcircle through Southern blot. Experiment was repeated three times independently with similar results. **h** Schematic model of study design. Huh-7 were treated with Lat.A (1 μg/ml) for 3 days (Figure was created with BioRender). **i** Absolute cell counts and qPCR detection of cccDNA level in Huh-7 transfected with HBVcircle in the indicated group. **j** Genome tracks of bulk-cell ATAC-seq for HBVcircle in Huh-7 cells transfected with HBVcircle and treated with TRULI (10 μM) or Lat.A (1 μg) for 48 hours. The data are represented as mean ± SD. P value by two-tailed Student's *t*-test (c–e) and Repeated Measures ANOVA (i). Source data are provided as a Source Data file. (*P < 0.05; **P < 0.01).

respectively (Supplementary Fig. 8a–d). However, the replication of VSV was not inhibited by YAP activation (Supplementary Fig. 8e–h).

YAP activation is significantly associated with HBV repression in patients

We collected liver specimens from HBeAg-positive patients, who exhibited higher viral activity compared to those who are HBeAg negative (Supplementary Fig. 9a) and performed single-cell sequencing analysis (Fig. 5a, Supplementary Fig. 9b–d). In both IT and IA patients, there were high levels of viral DNA and a high proportion of

HBV-positive liver cells (Supplementary Fig. 9e), facilitating subsequent analysis of antiviral response. Next, we performed an unbiased single-cell gene set variation analysis (scGSVA) to assess the correlation between HBV transcript level and various pathways in the two IA patients, using the Human MSigDB hallmark gene sets collection. The results showed a dramatic negative correlation between the activation of YAP-related pathways and HBV transcripts, compared to other pathways analyzed (Fig. 5b). At the single-cell level, we observed a strong negative correlation between HBV transcripts and YAP activation signatures in liver specimens from all four enrolled patients, while

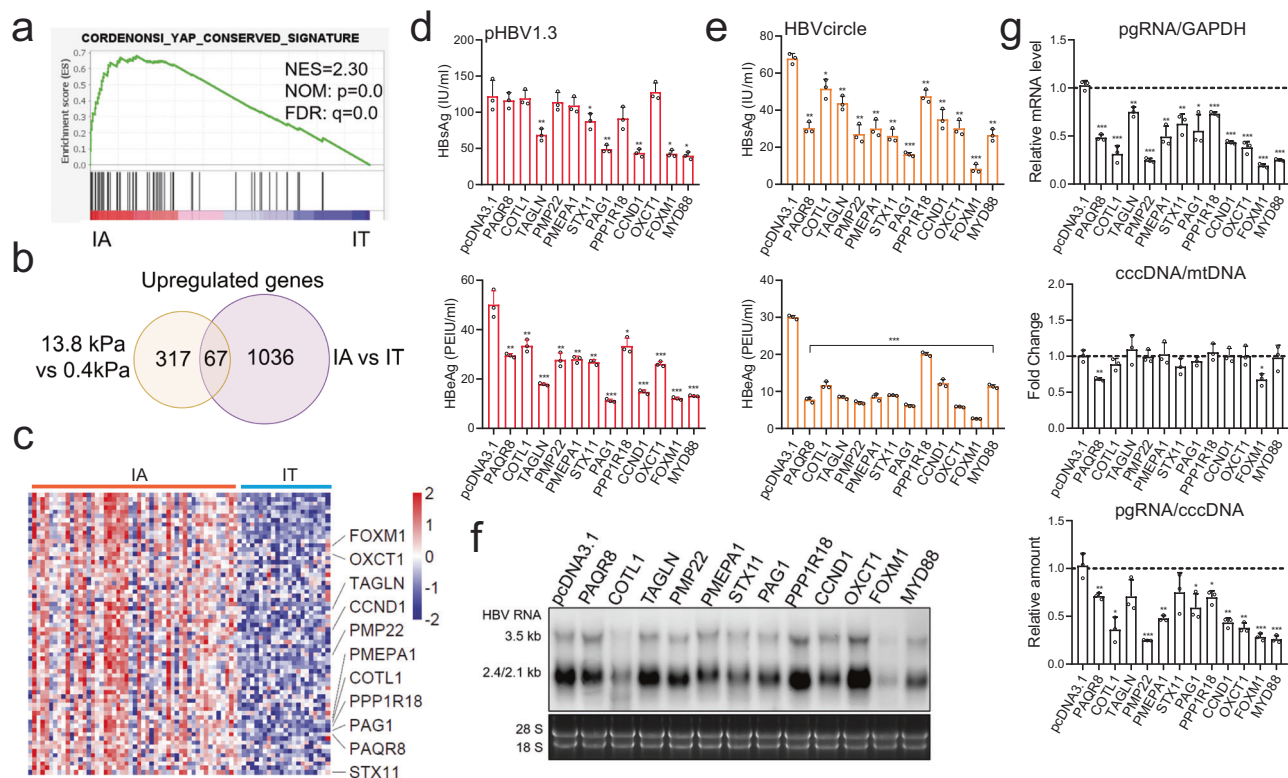


Fig. 4 | Mechanical signaling induces multiple downstream antiviral effectors.

a GSEA enrichment plot of GSE65359 comparing IA and IT states. **b** PMH were cultured on low stiffness (0.4 kPa) or high stiffness (13.8 kPa) substrates for 2 days, followed by RNA extraction and RNA-seq analysis. Venn diagram illustrates common upregulated genes between PMH and the GSE65359 dataset (IA vs. IT). **c** mRNA expression levels of the 67 common upregulated genes identified in (b) within the GSE65359 dataset. **d, e** Among the 67 genes, 11 exhibit antiviral effects: levels of HBsAg and HBeAg detected by ELISA in two HBV replication models (pHBV1.3; HBVcircle) (three biological replicates). **f** In the HBVcircle model, intracellular HBV RNAs were quantified using Northern blot. **g** In the

HBVcircle model, HBV Hirt DNA was extracted and subjected to HBVcircle quantification via qPCR. Relative HBVcircle levels are presented as fold changes compared to the pcDNA3.1 control. Total RNA was extracted and subjected to HBV pgRNA qPCR, with relative pgRNA levels presented as fold changes compared to the pcDNA3.1 control. The relative levels of HBVcircle-based transcription were further determined by normalizing pgRNA levels to HBVcircle levels in each treatment group compared to the pcDNA3.1 control group (three biological replicates). The data are represented as mean $\pm$ SD. P value by Kolmogorov-Smirnov test (**a**) and two-tailed Student's t-test (**d–e, g**). Source data are provided as a Source Data file. (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

a positive correlation was noted with HNF4A (Fig. 6a). Moreover, immunofluorescent staining of the liver biopsies from two IA patients supported the significant negative correlation between YAP activation and HBV expression, with HBsAg serving as the indicator (Fig. 6b). The 11 genes with previously mentioned anti-HBV activity showed a significant increase in expression within the IA phase compared to the IT phase (Supplementary Fig. 9f). A recent study has reported that YAP activation not only reduces HNF4A but also elevates TGF- β ²². Based on our previous data from liver biopsies of HBV-infected individuals²⁰, we observed generally consistent patterns that higher YAP (Fig. 6c), lower HNF4A (Fig. 6d, Supplementary Fig. 9g) and higher TGF- β (Fig. 6e, Supplementary Fig. 10a) expression in the IA phase compared to the IT phase, but the detected expression proportion of TGF- β in liver cells was generally low (less than 10%). Additionally, in contrast to the lack of activation of IFN response in vitro, the in vivo analysis showed some activation characteristics of the IFN pathway during the IA phase (Supplementary Fig. 10b). However, the degree of IFN activation was not significantly correlated with HBV transcription levels at the single cell level (Supplementary Fig. 10a). These results demonstrate that YAP activation is significantly associated with HBV suppression in the physiological setting.

Activating YAP potently controls HBV replication in mouse models

We further validated the impact of YAP activation and high matrix stiffness on HBV replication in mouse models. Hydrodynamic tail vein

injection of a YAP expression vector (prcccdDNA) into mouse livers²³ (Fig. 7a) confirmed the inhibitory effect of YAP activation on HBV RNA transcription (Fig. 7b) and antigen expression (Fig. 7c, Supplementary Fig. 11). In addition, we established a model of transient stiffening with varying liver tissue matrix stiffness by injecting mice with carbon tetrachloride (CCl₄) for different short-term durations²⁴ (Supplementary Fig. 12a–b). And transcriptional profiles were analyzed to confirm YAP activation signature and the induction of downstream genes, CTGF and ANKRD1 (Supplementary Fig. 12c), thus verifying that the model could simulate different levels of matrix stiffness and YAP activation. We collected liver samples at 2, 4, 6 and 8 days after injecting HBV replication plasmids (the BPS stain)²⁵, and at the endpoint, the total HBV plasmid levels in the liver among mice from different groups were similar (Supplementary Fig. 12d). However, the levels of HBV antigens (Supplementary Fig. 12e), as well as DNA (Supplementary Fig. 12f) and RNA (Supplementary Fig. 12g–h), were significantly reduced in the groups with higher liver stiffness compared to the untreated and low stiffness groups. The liver morphology (Supplementary Fig. 12i), tissue section staining (Supplementary Fig. 12j), and serum ALT/AST and albumin level measurements (Supplementary Fig. 12k) indicated that, due to short-term CCl₄ treatment, there were no apparent signs of liver damage in this model, except for a transient increase in ALT/AST within 2–3 days after tail vein injection. Furthermore, the activation of the IFN system was not observed in this model (Supplementary Fig. 12l).

We further tested in mice whether exogenous activation of liver YAP through small molecule compounds could control HBV. After

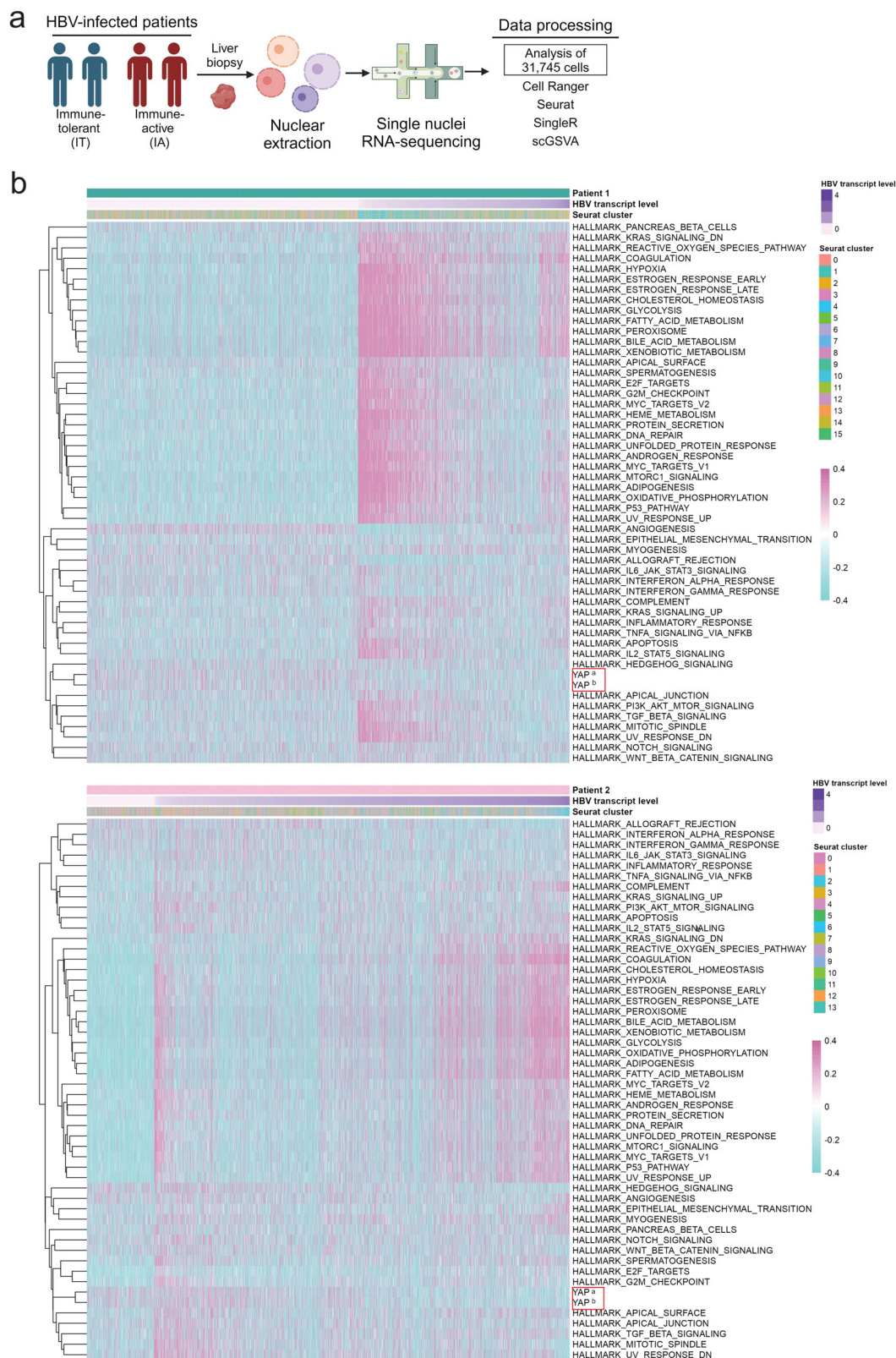


Fig. 5 | Correlation between all common signaling pathways and HBV replication levels at the single-cell level in CHB patients. a Schematic diagram of snRNA-seq study design (Figure was created with BioRender). **b** scGSVA scores of Human

MSigDB hallmark gene sets and 2 YAP related genesets (YAP^a and YAP^b) in all hepatocytes detected in patient 1 and patient 2.

screening several known YAP activators, we identified TDI-011536, an effective inhibitor of Lats kinases²⁶, as capable of promoting YAP activation in mice with low toxicity (Fig. 7d). We thus verified its effect on HBV replication (Fig. 7e). The results showed that TDI-

011536 has a significant anti-HBV effect, reducing levels of HBsAg antigen (Fig. 7f–g) and HBV RNAs (Fig. 7h), but did not have a direct impact on the content of rcccDNA in the liver (Fig. 7i). Additionally, unlike the anti-HBV effects of IFN, which are normally accompanied

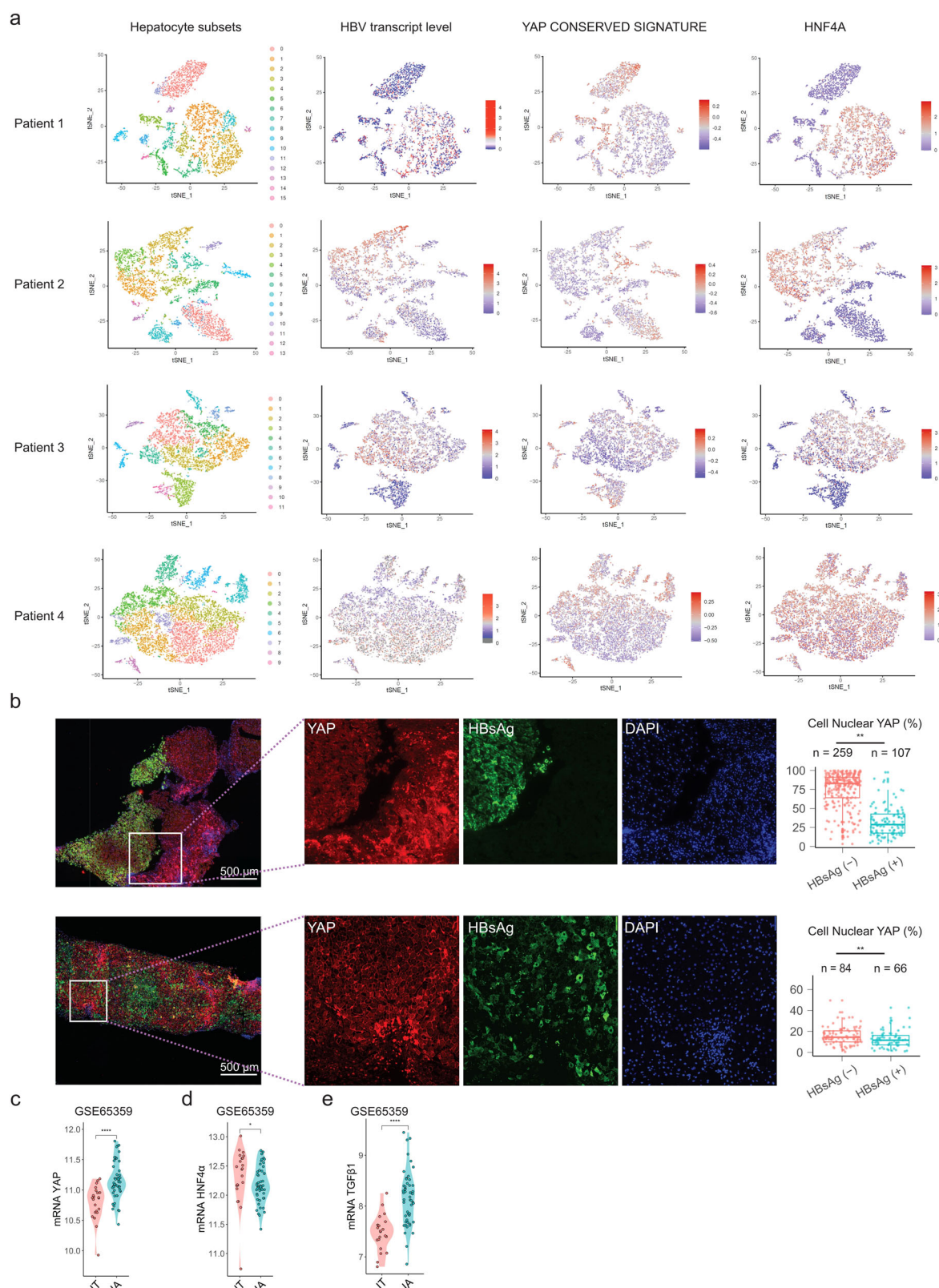


Fig. 6 | Reverse correlation between YAP activation and HBV replication in hepatocytes of CHB patients. **a** snRNA-seq analysis of the hepatocyte population. t-SNE plots showed relative distribution of HBV transcript levels, scGSVA scores of YAP conserved signature, and HNF4A transcript levels in hepatocyte subsets. **b** Immunostaining was performed in liver biopsies obtained from two IA patients. The right panel depicted the proportion of nuclear-localized YAP in each cell under the specified field of view, categorized by HBsAg positivity and

negativity. (n represented the count of individual cells within field of view). Data were represented as box plots (median with interquartile, upper-limit, and lower-limit). **c–e** Comparison of the transcript levels of indicated genes (YAP, HNF4A, and TGFβ1) in GSE65359 between IA and IT patients. The data are represented as mean ± SD. P value by two-tailed Student's t-test (**b–e**). Source data are provided as a Source Data file. (*P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001).

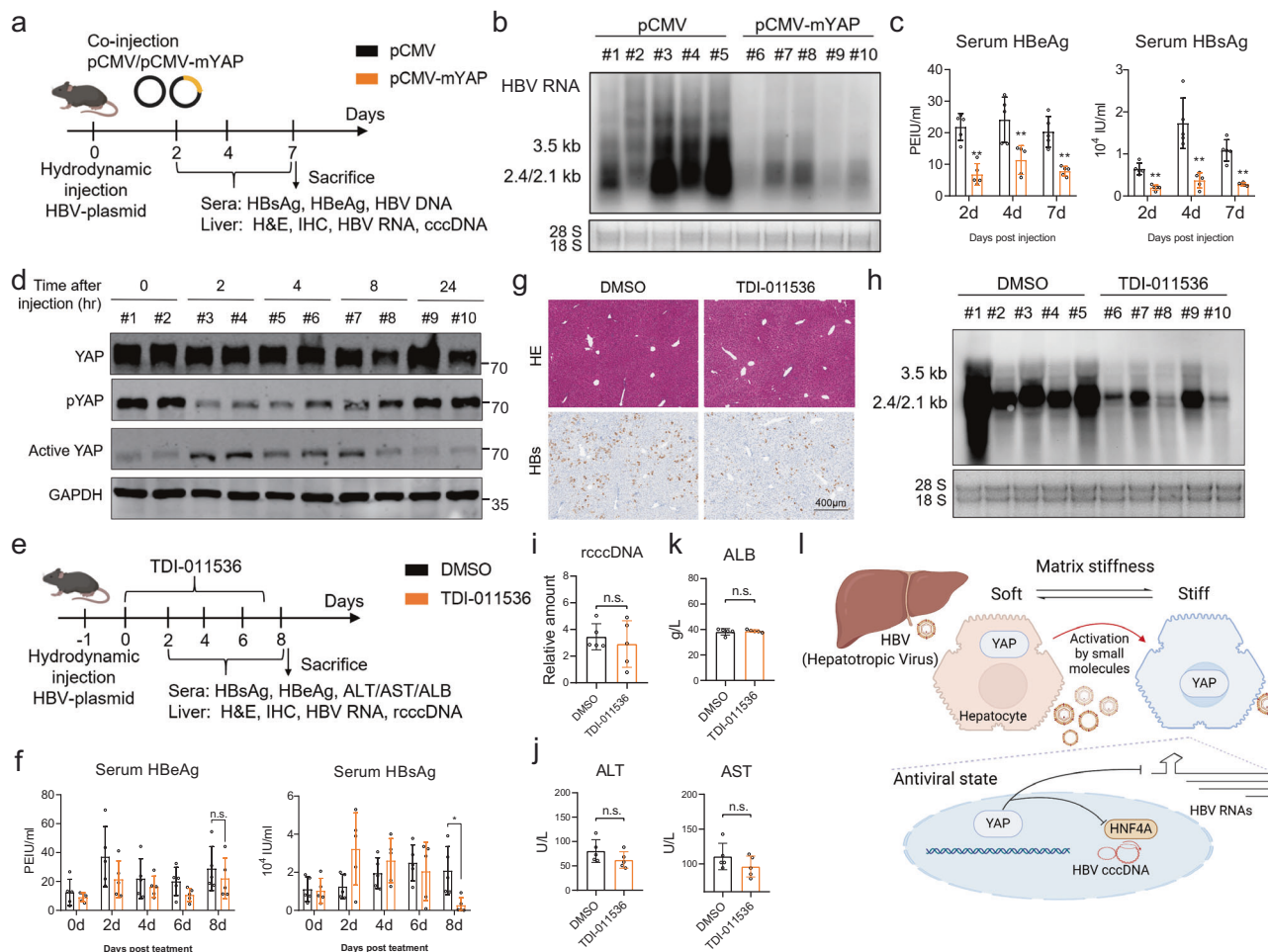


Fig. 7 | Activating YAP potently controls HBV replication in mouse models.

a Schematic representation of the study design (Figure was created with BioRender). The number of mice was $n = 5$ in each experimental group. YAP expressed plasmid were co-injected with HBV-plasmid (prcccDNA). **b** Measurement of total HBV RNAs at the endpoint in five mice from each group using Northern blot. **c** Levels of serum HBsAg and HBeAg in the specified groups. The number of mice was $n = 5$ in each experimental group. **d** Intraperitoneal injection of TDI at 40 mg/kg in mice. YAP activation levels were analyzed by Western blot. Experiment was repeated three times independently with similar results. **e** Illustration of the study design (Figure was created with BioRender). The number of mice was $n = 5$ in each experimental group. **f** Levels of serum HBsAg and HBeAg in the specified groups.

The number of mice was $n = 5$ in each experimental group. **g** Histological analysis and immunohistochemical staining in the specified groups at the endpoint. Representative image from five individual mice in each experimental group. **h** Measurement of viral total RNAs at the endpoint in five mice from each group by Northern blot. Intrahepatic levels rcccDNA (**i**), serum levels of AST ALT (**j**) and ALB (**k**) were quantitated in the specified groups. The number of mice was $n = 5$ in each experimental group. **l** A schematic model of antiviral action through liver mechanosignaling via YAP (Figure was created with BioRender). The data are represented as mean $\pm$ SD. P value by two-tailed Student's t -test (**c**, **f**, **i**–**k**). Source data are provided as a Source Data file. (* $P < 0.05$; ** $P < 0.01$).

by an increase in ALT/AST, TDI-011536-mediated inhibition of HBV did not increase ALT or AST levels (Fig. 7j), nor affect liver ALB expression (Fig. 7k), and liver tissue pathology analysis also revealed no significant liver damage (Fig. 7g). These results conceptually demonstrates that exogenous modulation of YAP-mediated liver mechanosignaling could effectively control HBV (Fig. 7l).

Discussion

During HBV infection, it is generally recognized that the majority of liver cells become infected with HBV²⁷, yet over 95% of adults infected with HBV exhibit acute self-limiting infections without extensive hepatocyte destruction. This suggests the existence of robust natural mechanisms for controlling and non-cytolytic clearance of the virus within the host. While previous research has implicated various cytokines in directly controlling viral replication, their effectiveness has been found to be limited²⁸. Here, we propose a mechanical force-mediated mechanism for HBV control and clearance, highlighting the significant impact of the physical microenvironment on host-viral

interaction. We find that matrix stiffness significantly affects HBV replication and antigen expression. This provides a new perspective on our understanding of natural antiviral mechanisms. Clinical observations have shown that liver stiffness values transiently increase following acute HBV infection¹⁶, supporting a potentially beneficial antiviral significance of such an elevation.

Furthermore, we studied the functional role of the key mechanotransduction molecule YAP in the mechanism^{29,30}. We observed that YAP is significantly activated under high matrix stiffness conditions, and YAP activation leads to a substantial suppression of HBV transcription and replication. This finding stimulates our interest in YAP, as its activation and the expression of its downstream molecules are typically associated with tumorigenesis^{31,32}. Our study presents evidence of its potential role in antiviral defense. While in vivo YAP knockout presents challenges as it is a key factor in liver growth and the maintenance of liver function, we elucidated the role of YAP in the antiviral mechanism using inhibitors and activators. Additionally, we identified a series of YAP-induced molecules with antiviral activities.

YAP activation could also globally alter cell states by negatively regulating HNF4A and positively regulating TGF β . Previous studies have identified HNF4A as a crucial factor in HBV transcription³³, while TGF β exhibits anti-HBV properties³⁴, which strongly supports the working model proposed in this study (Supplementary Fig. 13). More interestingly, we found that in addition to inhibiting HBV, the activation of YAP also suppresses HCV, another hepatotropic virus, but does not inhibit, and even slightly promote, VSV. We speculate that this is because the efficient replication of hepatotropic viruses like HBV and HCV highly depends on factors enriched in the liver, such as HNF4A, and the activation of YAP exert antiviral effects by downregulating factors involved in the replication of these hepatotropic viruses while upregulating restriction factors like TGF β and FOXM1. This antiviral mechanism is distinct from the traditional IFN-mediated response and may possess specificity for both known and unknown liver-tropic viruses.

The elevated matrix stiffness in the liver microenvironment can be attributed to various factors, with some resulting from stimuli from inflammatory processes in the microenvironment. Factors such as cell swelling can also interact with the physical microenvironment to affect cell function³⁵. A very recent study using HBV-integrated HepG2.2.15 cells and HBV-transgenic mice suggested that liver stiffness limits antiviral ISG activation in hepatocytes to promote an increase in HBV infectivity, thereby contributing to end-stage liver disease progression³⁶. However, clinical observations contradict or even oppose these findings: in clinical practice, HBV replication is generally poor in patients with liver cirrhosis and HCC, and circulating HBV DNA levels are significantly lower in patients at higher stages of liver fibrosis pathology. We speculate that this discrepancy may be due to the abnormal response of HepG2.2.15 cells, which are derived from tumor cells, to exogenously increased matrix stiffness at the source. Hence, primary cells were used in this study, with only knockdown or inhibition experiments conducted in hepatoma cell models. Meanwhile, HBV-transgenic mice carry HBV from birth without a normal immune response, and thus may not psychologically respond to liver stiffness. It is essential to emphasize that the liver matrix stiffness proposed and simulated in this study primarily involves transient increases, which differ partially from the conditions underlying pathological liver fibrosis and cirrhosis. However, it should be noted that although CCl₄ can simulate transient increases in liver stiffness in animal models²⁴, its effects on the liver extend beyond stiffness. For instance, it can induce hepatocyte injury and death, and activate the Lox family enzymes to promote protein fiber deposition. These effects might also impact HBV replication, which remain to be considered and further studied.

IFN- α is a key drug in the current clinical treatment of HBV and achieving HBV cure, however, the antiviral response rate to IFN remains limited³⁷. Our study did not find that YAP activation induces the IFN response. Conversely, IFN can cause transient increases in liver stiffness and YAP activation in vivo (our unpublished data), which could play a crucial role in the mechanical communication matters in IFN response and anti-HBV activities. From this perspective, our study provides clues for further research into the relationship between liver stiffness and antiviral treatment response, which requires additional clinical observations to elucidate. Besides affecting virus-infected liver cells, the mechanical properties of the matrix stiffness can also affect immune cell function^{38,39}. Future research will explore how matrix stiffness influences the function of immune cells and whether liver stiffness measurements can be used to assist in assessing a patient's immune response status. Furthermore, investigations into actively modulating the cellular mechanotransduction microenvironment may hold promise for improving HBV treatment outcomes. Our results support the use of drugs that regulate YAP signaling as a potential intervention strategy for controlling HBV infection, beyond traditional antiviral signaling pathways and approaches.

In summary, our findings reveal a mechanism that involves the mechanical environment of liver cells and YAP to control hepatotropic viral infection, offering a new theoretical foundation for understanding HBV control and potential strategic approaches in the development of novel antiviral therapies.

Methods

Cell culture and HBV infection

The hepatoma cell line Huh-7 was obtained from the ATCC. Primary human hepatocyte (PHH, purchased from RIDL Shanghai, China) was cultured and infected with HBV virions as we previously described⁴⁰. Briefly, PHH were cultured in maintenance medium (Life Technologies) containing 2% DMSO and 2% FBS. PHH were infected with HBV in the presence of 4% PEG8000 for 16 hours. Then, PHH were washed five times with PBS and replaced with maintenance medium.

Primary mouse hepatocyte isolation and culture

Primary mouse hepatocyte (PMH) was isolated from wild-type, 6-week-old to 8-week-old C57BL/6 male mice. PMH isolation was performed by two-step perfusion (perfusion and digest), followed by separation with 50% Percoll (GE Healthcare Life Sciences, Pittsburgh, PA) density gradient⁴¹. Purity of PMH was routinely > 90% by trypan blue exclusion. PMH were cultured in 5% fetal bovine serum in William E medium (Gibco) supplemented with L-glutamine, antibiotics, insulin-transferin-selenium, and linoleic acid (Thermo).

Collagen-conjugated polyacrylamide gels

Polyacrylamide (PA) gels were made from 40% acrylamide and 2% bis acrylamide (Sigma) where varying ratios of acrylamide and bis acrylamide were used to create gels of known reproducible stiffness¹⁸. Gels were cast on glutaraldehyde-modified coverslip, and polymerization was activated by 1% ammonium persulfate (Sigma). The PA gel surfaces were then conjugated to Sulfo-SANPAH (Thermo) activated by 365 nm ultraviolet exposure. Rat tail collagen I (Corning) was then added to the surface at 150 μ g/mL to allow coupling to the gel through side-chain primary amines.

Real-time quantitative PCR (RT-qPCR), Immunofluorescence, Luciferase reporter assay, Western blot, Northern blot, Southern blot, Cell counting kit-8 (CCK-8) assay, and Bulk RNA sequencing

They were conducted as described⁴⁰. (For more details see supplementary methods).

HBV cccDNA analysis

For RT-qPCR detection of HBV cccDNA / HBVcircle, the extracted Hirt DNA were heat denatured, followed by T5 exonuclease (NEB) or Plasmid-safe ATP-dependent DNase (as specifically indicated) treatment to remove the deproteinization rcDNA. Then, the pre-cleaned Hirt DNA samples were subjected to qPCR detection of HBV cccDNA / HBVcircle and cellular mitochondrial DNA as described previously⁴².

Biostatistical analysis

The statistical association between genes differentially expressed PMH grown on hydrogel of high/low stiffness (stiffness signature) and belonging to signal transduction pathways is assessed by an over-representation analysis approach using Fisher's exact test. Briefly, considering that there are S single-symbol-annotated genes on the stiffness signature, the over-representation of a pre-defined pathway signature is calculated as the hypergeometric probability of having a gene for a specific pathway in S , under the null hypothesis that they were picked out randomly from the N total genes of the RNA-seq. Over-representation analysis has been conducted using one-sided Fisher's exact test (*phyper* function of R stats package; P-value, 0.05) and

considering all the expressed genes detected by RNA-seq. P-values have been adjusted for false discovery rate (p.adjust function of R stats package; FDR, 5%)¹².

Pathway enrichment analysis

GSEA was used for pathway enrichment of DEGs. For the list of DEGs, online MSigDB tool was used (<http://software.broadinstitute.org/gsea/>). GSEA v4.3.2 desktop software was also used to identify the significantly enriched pathways from the RNA-seq results.

ATAC-seq library preparation

50,000 of cells were subjected to assay for transposase-accessible chromatin using sequencing (ATAC-seq) as described previously⁴³. The pellet of cell nuclei was resuspended in 50 µl of transposition mix (Vazyme Biotech) and incubated at 37 °C for 30 min. Transposed DNA was purified using a column kit (Zymo). Libraries were 150-bp paired end sequenced on a HiSeq X Ten platform (Illumina) with the sequencing read length and dual indexing according to the manufacturer's instructions (Annoroad).

Hydrodynamic tail vein injection

For intrahepatic delivery of the HBV replicon, 6 to 8-week-old male C57BL/6 mice were hydrodynamically injected with 4 µg HBV-plasmid (HBV replicon plasmid of genotype B strain, BPS), or 4 µg pccDNA + 4 µg pCMV-Cre in a volume of phosphate-buffered saline (PBS) equivalent to 8% of the mouse body weight. Injections were finished within 5 to 8 s. For the expression of mYAP, 10 µg pCMV-mYAP or pCMV empty vectors were added to the plasmid mixture. All the mice (GemPharmatech Co., Ltd, China.) were bred and housed under pathogen-free conditions in the animal facility of Fudan University. All the animal studies were approved by the Ethics Committee of School of Basic Medical Sciences, Fudan University (20230301-087).

Atomic force microscopy

Mice livers were immediately dissected and embedded in low melting point agarose (4% in PBS) after sacrificed. A small block of agarose containing the sample was submerged in chilled PBS and cut into 2000 µm thick sections using a vibratome (Leica). Sections were slowly heated to 37 °C in PBS for 30 min prior to AFM measurements. AFM measurements were carried out similarly to the method previously described⁴⁴. Monodisperse polystyrene beads (radius $r = 10 \mu\text{m}$) were glued to tipless silicon cantilevers (spring constants between 0.01 and 0.06 N/m, MLCT-O10, Bruker). The AFM was mounted on an x/y motorized stage of an inverted microscope. Cantilever position relative to the liver sections was monitored via a CCD camera placed on top of the AFM setup. Force-distance curves were taken with a set force of 0.5 nN with an approach speed of 2 µm/s. Apparent elastic moduli K were calculated using the Hertz-fit model using JPK data processing software. Stiffness was measured in maps over defined sample areas, over which multiple force-distance curves were taken at 10 µm steps (each map containing 64 measurements, 2–3 maps per liver). The median measurement stiffness for each map was calculated, and statistical significance between maps was determined using a 2-tailed Student's t test.

Clinical samples

In this study, all the HBV-infected individuals studied were recruited at Huashan Hospital, affiliated to Fudan University (Shanghai, China). Patients refused to take liver biopsy or confirmed co-infection with hepatitis virus C, A, D, and E, or human immunodeficiency virus or complicated by other liver diseases were excluded. CHB patients were classified to IT (HBsAg-positive HBV infection) and IA (HBsAg-positive chronic hepatitis B) phases according to the European Association for the Study of the Liver guideline⁴⁵. Informed written consent was

obtained from each patient and the study was approved by the Institutional Ethics Committee for human studies at Huashan Hospital. All procedures were in accordance with the Declaration of Helsinki. Liver specimens were collected using a 16-gauge Menghini needle. Part of liver sample was fixed in formalin and sent for pathological examination. Partial liver samples were stored in liquid nitrogen within 30 minutes after liver puncture. Samples stored in dry ice were then sent for single cell sequencing in 24 hours.

Single-nucleus RNA-seq data processing and pathway analysis

Raw fastq files were aligned to the human reference genome GRCh38 and the HBV genome (NC_003977). The Cell Ranger (10X Genomics) analysis pipeline was used to generate a digital gene expression matrix from this data. The raw digital gene expression matrix (UMI counts per gene per cell) was filtered, normalized, and clustered using R (4.0.5). Cell and gene filtering was performed as follows: Cells with a very small library size (<1500) or feature counts (<200), and a very high (>0.2) mitochondrial genome transcript ratio were removed. Genes detected (UMI count > 0) in less than three cells were removed. Clustering was performed using standard Seurat package procedures. Cell type was annotated by SingleR (2.0.0). We preserved and further clustered all hepatocytes subsets. For visualization, the dimensionality of each dataset was further reduced using either the Barnes-Hut t -distributed stochastic neighbor embedding with Seurat functions *RunTSNE*. For pathway analysis, the curated gene sets were downloaded from the Molecular Signature Database (<http://software.broadinstitute.org/gsea/>), then ssGSEA was applied, and pathway scores were calculated for each cell using the GSVA (1.46.0).

Statistical analysis

Data were analyzed using Prism 8.0 (GraphPad). For comparison of two datasets, data were analyzed using Student's t test and presented as the mean $\pm$ standard deviation. Correlations between two datasets were determined by linear regression and evaluated by Pearson's r analysis. A P -value < 0.05 was considered to be statistically significant. Data are shown as mean $\pm$ SD.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The Affymetrix RNA Chip data has been deposited in the Sequence Read Archive (SRA) under accession code number [GSE65359](#). Bulk RNA-seq [CRA018643](#) [CRA018604](#), ATAC-seq [HRA008043](#), and Single nuclear RNA sequencing data [HRA008033](#) has been deposited in the China National Center for Bioinformation under accession code number [PRJCA021543](#). Source data are provided with this paper.

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Author contributions

J.C. designed and supervised the research. J.Y., F.L., T.H., K.M., Z.Z., Z.N.Y., C.L., R.J., Y.L., and M.H. performed the experiments. J.Y. developed the key methodology and, with T.H., performed the formal analysis. J.Z., F.L., and J.Y.W. collected the clinical samples. J.C., Z.H.Y., and

J.Z. acquired the funding. J.C. and J.Y. wrote the original draft. J.C., Z.H.Y., J.Z., J.W., and M.L. reviewed and edited the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to Zhenghong Yuan, Jiming Zhang or Jieliang Chen.

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