

SUPPLEMENTARY INFORMATION

Hepatitis B virus-infected hepatocytes promote the secretion of collagen VI to the extracellular matrix

Supplementary methods:

Bioinformatics, statistical analyses. For the database search for proteomic analysis, a Uniprot human database from 2018, supplemented with common contaminants, was included. Corrected log2-transformed reporter ion intensities were normalized by median-MAD. A group-wise differential comparison was conducted by applying two-sample moderated t-statistics using the limma package in R ¹. P-values were adjusted using the Benjamini-Hochberg procedure. Protein expression was represented by the log2 fold change (logFC) between two conditions (HBV vs NI). Values were scaled using a median-MAD normalization by calculating $(x - \text{median}(x, \text{na.rm=T})) / \text{mad}(x, \text{na.rm=T})$. The significance value is represented as adjusted p-value (adj.P), calculated by Benjamini-Hochberg post-test. An adj.P of 0.01 represents a 1% false discovery rate (FDR). The proteomic database was analyzed by GSEA via pre-ranked method ^{2,3} and HALLMARK, REACTOME and KEGG gene sets from the Molecular Signature Database (MSigDB, ver.7.2) ^{4,5} were employed. The following gene sets are shown in Fig. 1e: REACTOME_INTEGRIN_CELL_SURFACE_INTERACTIONS, REACTOME_INTEGRIN_SIGNALING, REACTOME_DEGRADATION_OF_EXTRACELLULAR_MATRIX, KEGG_ADHERENS_JUNCTION, KEGG_FOCAL_ADHESION, REACTOME_ECM_PROTEOGLYCANS. The identified signaling pathways were described using the normalized enrichment score (NES) and FDR<0.05. Morpheus was used for generation of heatmaps (<https://software.broadinstitute.org/morpheus>). Validation and

functional experiments were performed in 3 independent experimental replicates, unless otherwise stated. Statistical analysis was carried out using Prism (version 7.00; GraphPad Software Inc.) and R software (version R 4.3.3).

Reagents, plasmids, antibodies, RNAi. DMSO, polyethylene glycol 8000 (PEG-8000) and 2-(4-Amidinophenyl)-6-indolecarbamidine dihydrochloride (DAPI) were obtained from Sigma. Paraformaldehyde (PFA) was purchased by Euromedex. Presto Blue cell viability assay was purchased by Invitrogen. For HepG2-NTCP, Dulbecco's Modified Eagle Medium 1x (DMEM, Gibco), supplemented with 10% heat-inactivated fetal bovine serum (FBS, Dominique Dutscher, France), 1% Minimum Essential Media Non-Essential Amino Acids Solution 100x (MEM NEAA, Gibco) and 0.5% Gentamicin (10 mg/mL) (Gibco) was used as described⁶. Opti-MEM (Gibco) was used as reduced-serum media. For HepG2-NTCP the medium was additionally supplemented with 0.25% Geneticin selection antibiotic (G418, Invitrogen). For HepaRG cells, William's E medium (Invitrogen), supplemented with 10% of fetal calf serum (FetalClone II, Cytiva), 1.2% GlutaMAX (Gibco) 1.2% Penicilin/Streptomycin (50 U/mL) (Gibco), 0.1% Hydrocortisone hemisuccinate (Sigma), 0.05% Insulin solution human (5 µg/mL) (Sigma). For HepaRG differentiation the medium was additionally supplemented with 1.8% DMSO. Freshly isolated HSCs were cultured for up to two weeks to reach confluence. Stellate Cell medium SteCM (ScienCell, #5301) was reconstituted and replaced every four days for up to two weeks. HSCs were gently passaged using trypsin/DPBS (1:1) and were used for experiments between passages 3 to 5 and seeded in collagen I-coated plates. For HSCs experiments, recombinant human TGF-β1 (PeproTech) and native human collagen VI protein (ab7538) were used at a concentration of 10 ng/mL and 0.5 µg/mL for 4 h, respectively. The treatment was performed after 3 h serum starvation. HBsAg monoclonal (Bio-Techne, clone 1044/329), collagen type VI (Proteintech, 17023-1-AP), Rabbit IgG monoclonal (Abcam, ab172730). Alexa Fluor 647 AffinPure Goat Anti-Rat IgG (H+L) and Alexa Fluor 488 AffiniPure Goat Anti-Rat IgG (H+L) used for flow cytometry analysis were purchased from Jackson ImmunoResearch. Hepatitis B e-antigen (HBeAg) was detected by

chemiluminescence assay (Autobio Diagnostics). The siRNA specific for COL6A1 and non-targeting controls were purchased from Dharmacon (ON-TARGETplus) and used according to manufacturer instructions.

RNA, DNA extraction and gene expression analyses. Total RNA was extracted using ReliaPrep RNA Miniprep Systems (Promega) and quantified using NanoDrop 2000/2000c Spectrophotometer (Thermo Scientific). DNA was extracted using QiaAMP DNA MiniKit protocol (Qiagen). Reverse transcription was performed using Maxima First Strand cDNA Synthesis Kit (Thermo Scientific). Gene expression was measured by qPCR using iTaq Universal SYBR Green Supermix (Bio-Rad) on the QuantStudio 3 instrument (Applied Biosystems). All the qPCR primers were synthesized by Sigma. GAPDH fw: 5'-TGC ACC ACC AAC TGC TTA; GAPDH bw: 5'-GGA TGC AGG GAT GAT GTT C; COL1A1 fw: 5'-GTG CTA AGG GTG ATG CTG GT; COL1A1 bw: 5'-TTT GCC AGG AGA GCC ATC AG; COL6A1 fw: 5'-GAA GAA TGT CAC CGC CCA GA; COL6A1 bw: 5'-GGT GGT GTC AAA GTT GTG GC. ACTA2 fw: 5'-TGA AGA GCA TCC CAC CCT; ACTA2 bw: 5'-ACG AAG GAA TAG CCA CGC.

Protein detection measurement. Flow cytometry experiments were carried out in HepG2-NTCP and HepaRG cells 24 h after seeding. At day 10 post-infection (pi), cells were trypsinized, resuspended in media and washed with DPBS by centrifugation (1000xg, 5 min). Cells were resuspended and fixed in 4% PFA in DPBS for 10 min at room temperature (RT) prior permeabilization at RT for 30 min with permeabilization buffer (DPBS, 0.1% Triton 100X, 1% FBS). Proteins were labeled using primary antibodies diluted in permeabilization buffer for 30 min at RT and dye-coupled secondary antibodies for 30 min at RT after washing with DPBS. Subsequently, cells were washed and resuspended in DPBS. 1.5×10^5 cells were then transferred in each well of a 96 well-plate (V-bottom) for detection using Cytoflex B2R2U0 cytometer (Beckman Coulter, BA47394) and CytExpert 2.3 software (Beckman Coulter) and then analyzed using FlowJo V10.5.3 (Beckman Coulter). Generally, 20 μ g of proteins were loaded for western blotting.

Supplementary figures

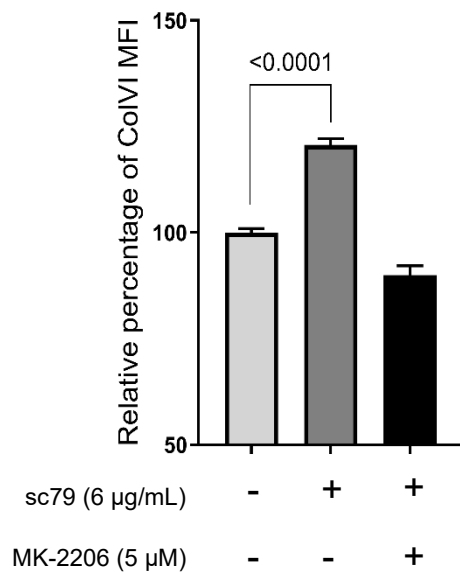


Figure S1. Akt signaling promotes ColVI expression in dHepRG cells. Flow cytometry analysis of dHepRG cells that were pre-treated for 1h with MK-2206 prior 24 h treatment with MK-2206 and or sc79. Relative percentage of ColVI MFI is depicted (Unpaired t test).

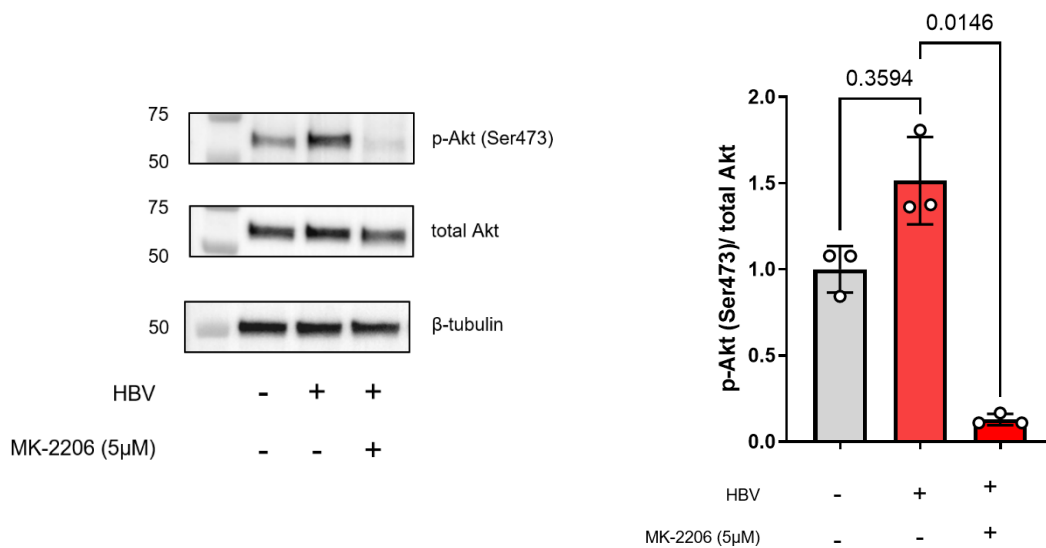


Figure S2. p-AKT is elevated in HBV-infected HepG2-NTCP cells 2 days pi, which is attenuated in presence of MK-2206. Representative Western blot shown from band intensity quantitation of three independent experiments (Kruskal-Wallis test).

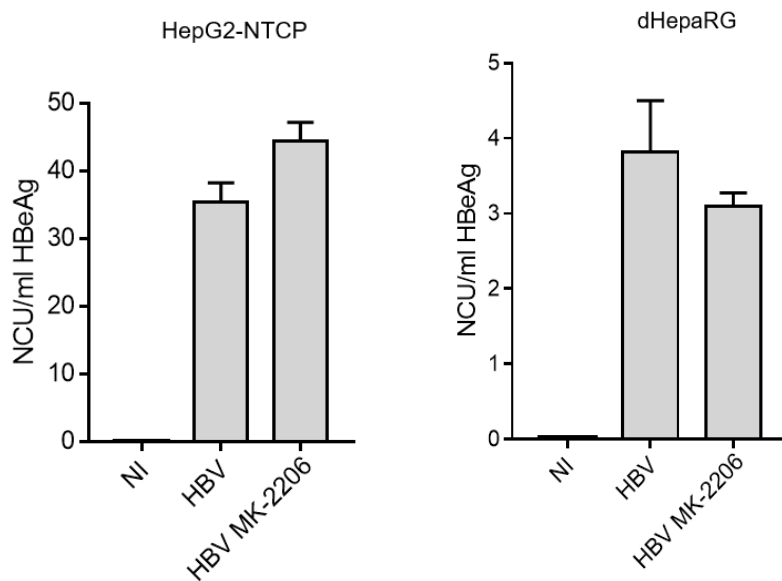


Figure S3. The treatment of HepG2-NTCP and dHepaRG cells with the AKT inhibitor MK-2206 does not perturb HBV replication. ELISA analysis of secreted HBeAg from cell infected for 10 d HBV in the presence or absence of MK-2206 (9 d). Results corresponding to Fig. 3.

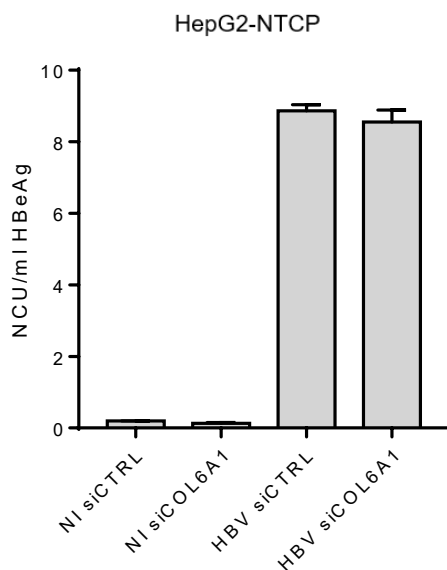


Figure S4. Silencing of *COL6A1* in HepG2-NTCP with established HBV infection (4 d pi) does not impair HBV replication. HBeAg ELISA 3 d post siRNA transfection from cell supernatants shown in Fig. 4e.

Supplementary tables

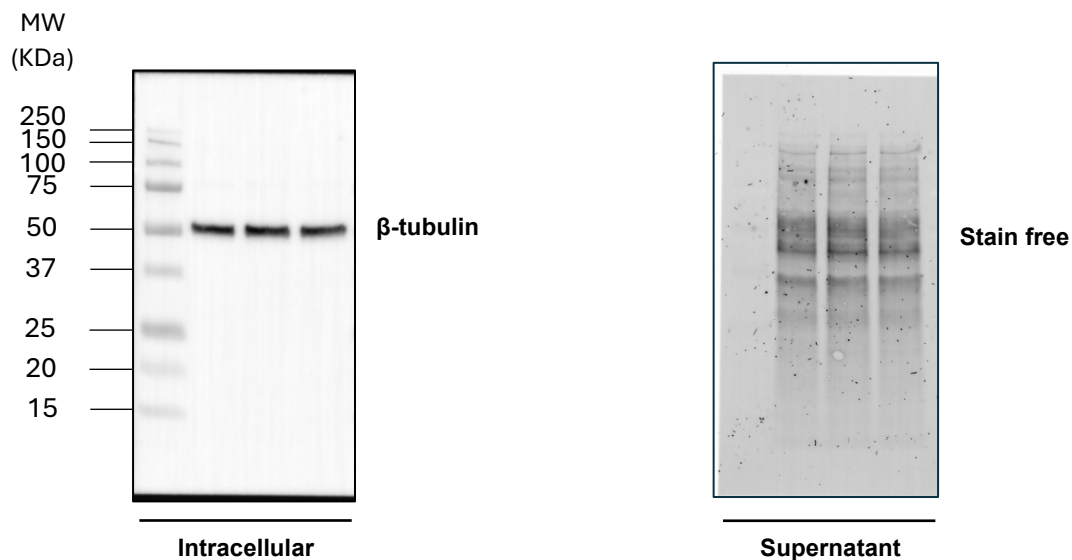
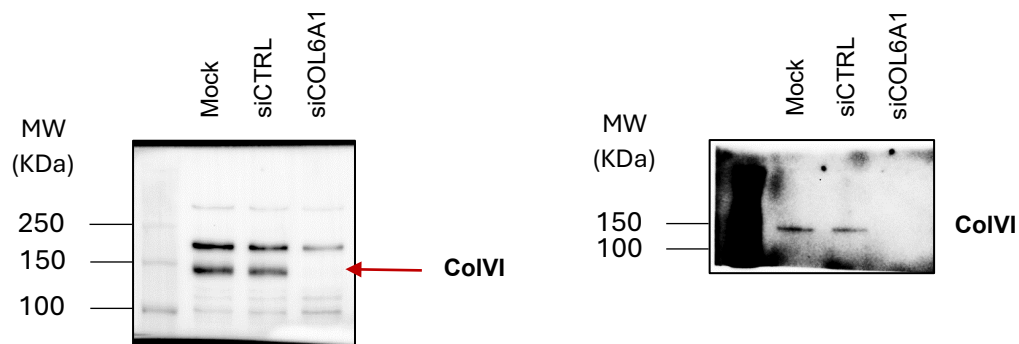
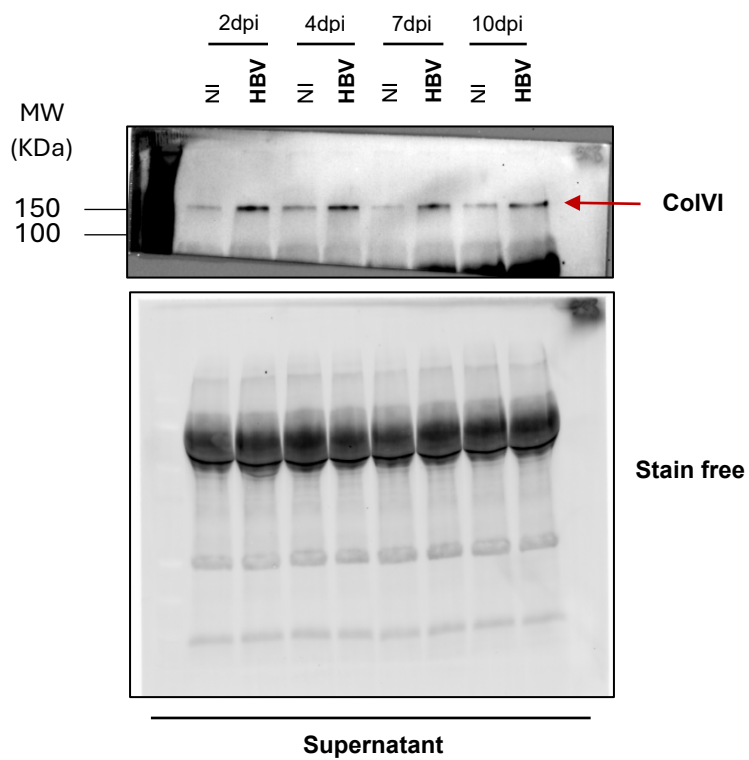
Table S1: proteomics results (day2, day10). Protein expression was represented by the log2 fold change (logFC) between two conditions (HBV vs NI). Proteomic analysis of HepG2- identified a total of 7611 proteins at day 2 and 10 pi. Positive values of logFC indicate an enrichment in HBV condition. Negative values of logFC indicate an enrichment in NI condition. Adj.P<0.01 was used as cutoff for significance for both day 2 and 10. Log Fold Change (logFC).

Table S2: GSEA results (day2). Dysregulated pathways at day 2 pi (HBV on NI). Enrichment Score (ES), Normalized Enrichment Score (NES), False Discovery Rate (FDR), family-wise error rate (FWER).

Table S3: GSEA results (day10). Dysregulated pathways at day 10 pi (HBV on NI). Enrichment Score (ES), Normalized Enrichment Score (NES), False Discovery Rate (FDR), family-wise error rate (FWER).

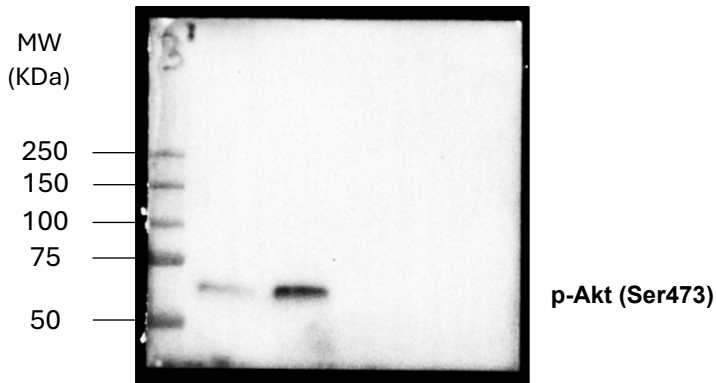
Full length Immunoblots. To confirm the specificity of the antibodies used, full-length gels are provided for each figure. Target protein names indicate the bands shown. Protein analysis was performed on cell lysates from the same experiment using 7% or 12% SDS-PAGE gel electrophoresis. PVDF membranes were probed for the specified target proteins and tubulin (as a loading control for lysates) or analyzed stain-free for supernatants. Molecular weight markers (Precision Plus Protein Standards All Blue, BioRad) are indicated. Due to the proteins' similar molecular weights, analyses were performed on separate gels, however, using same cell lysates from same experiment.

Unprocessed Full length immunoblots (related to figure 2)

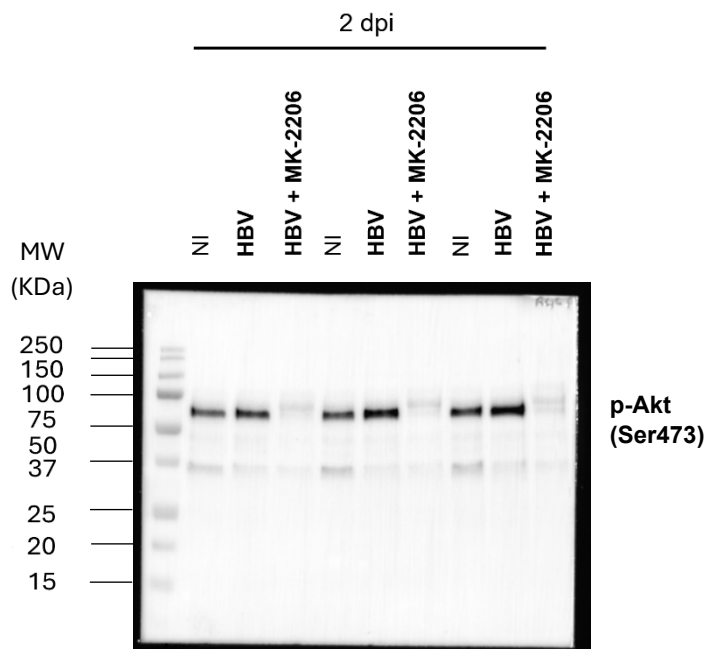
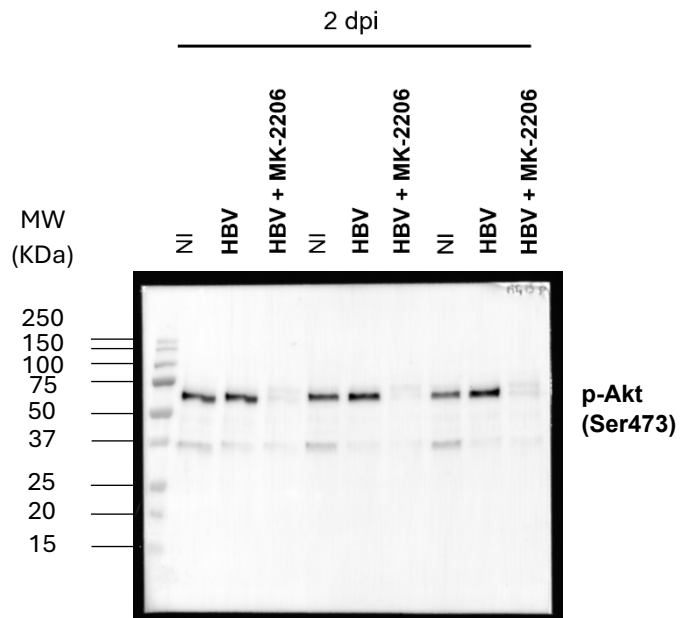
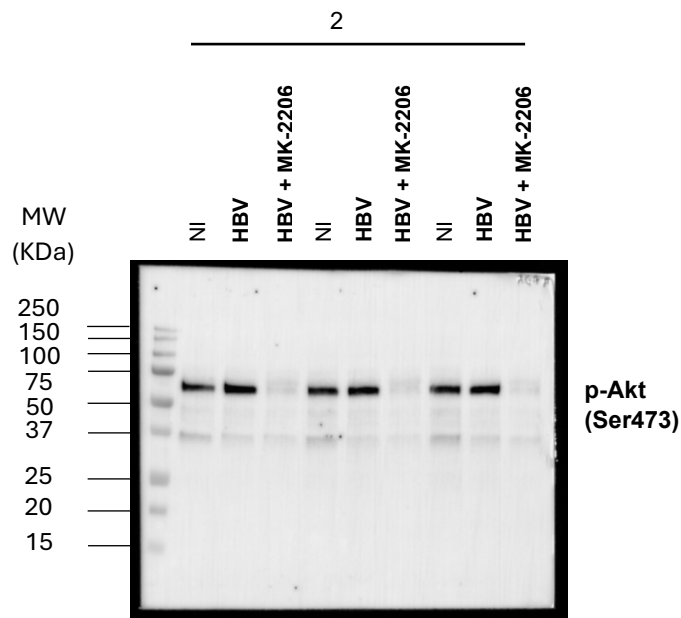
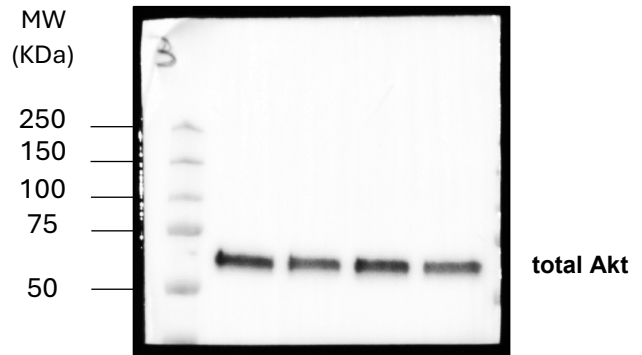


Full length immunoblots (related to figure 3)

sc79 (6µg/mL)	-	+	-	+
MK-2206 (5µM)	-	-	+	+

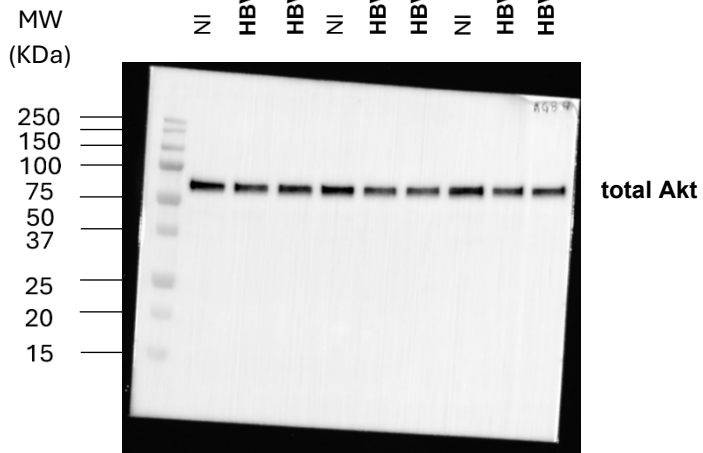


sc79 (6µg/mL)	-	+	-	+
MK-2206 (5µM)	-	-	+	+

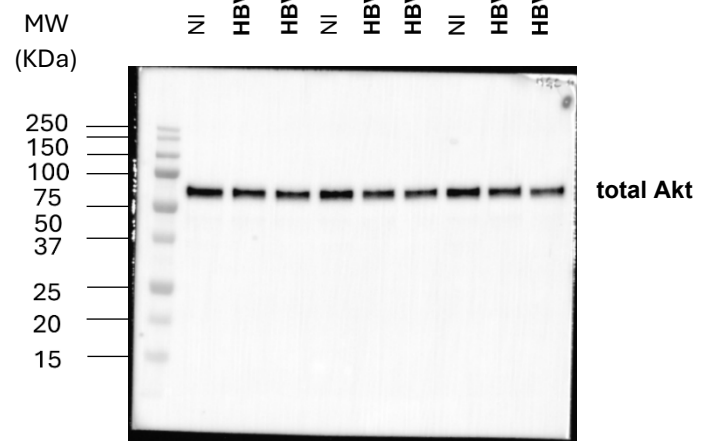


Full length immunoblots (related to figure 3), continued

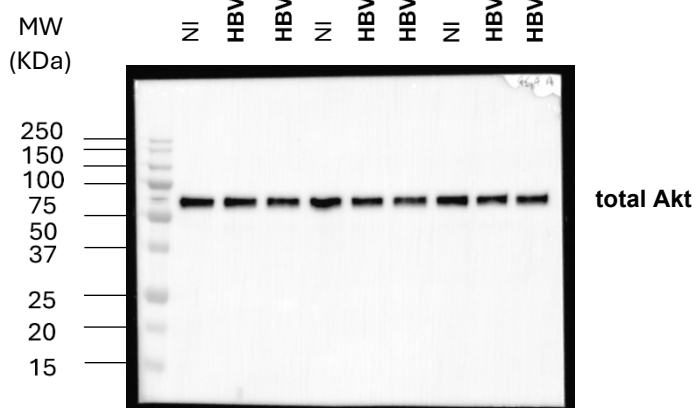
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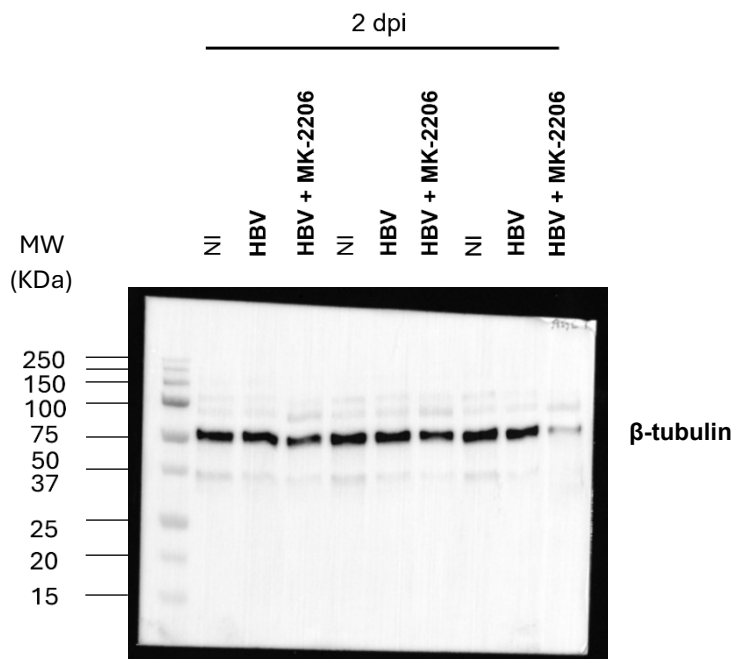
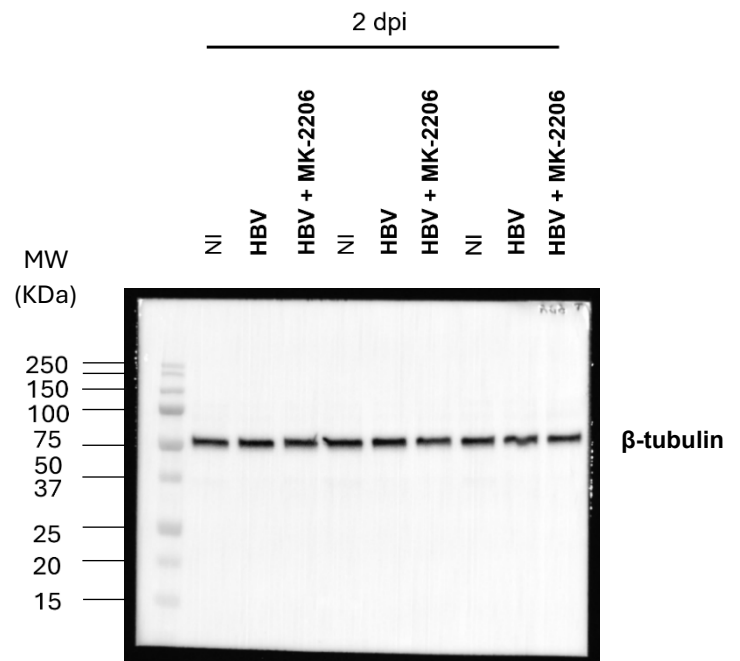
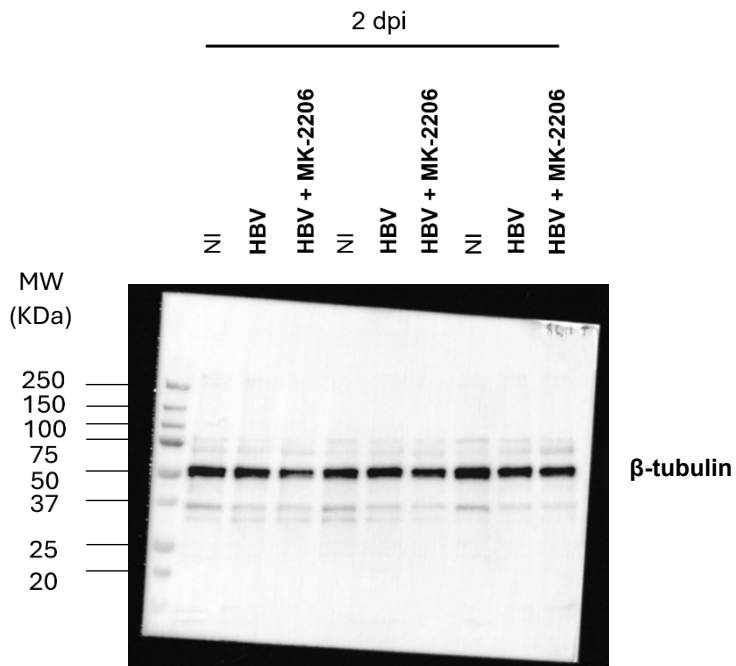
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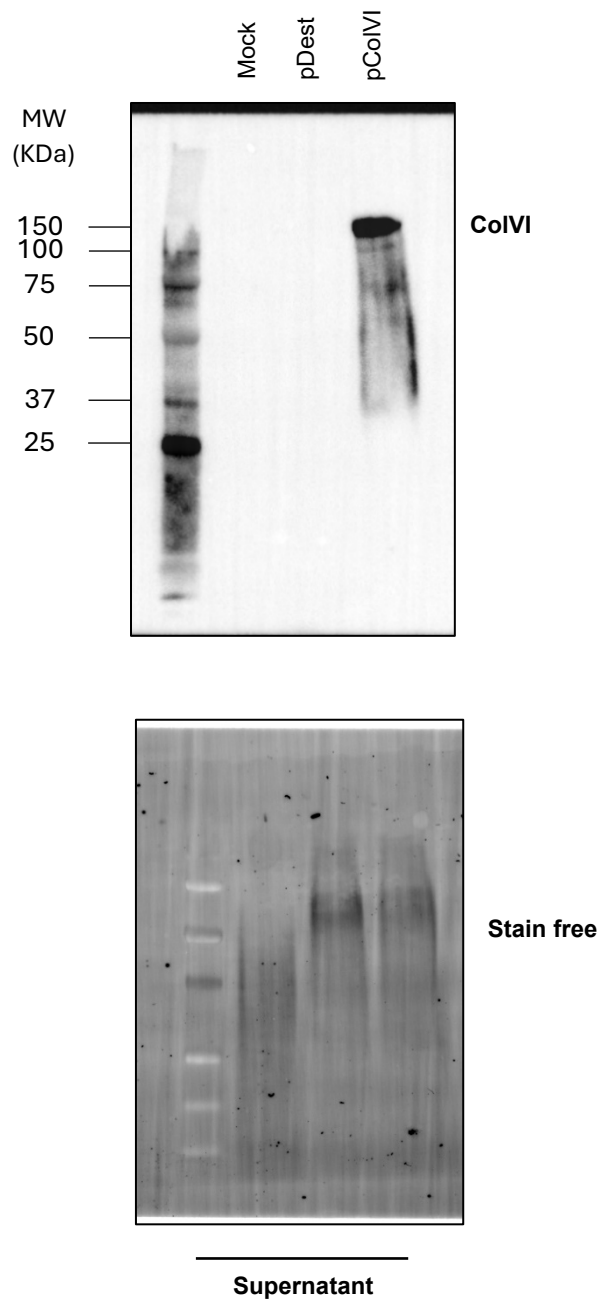
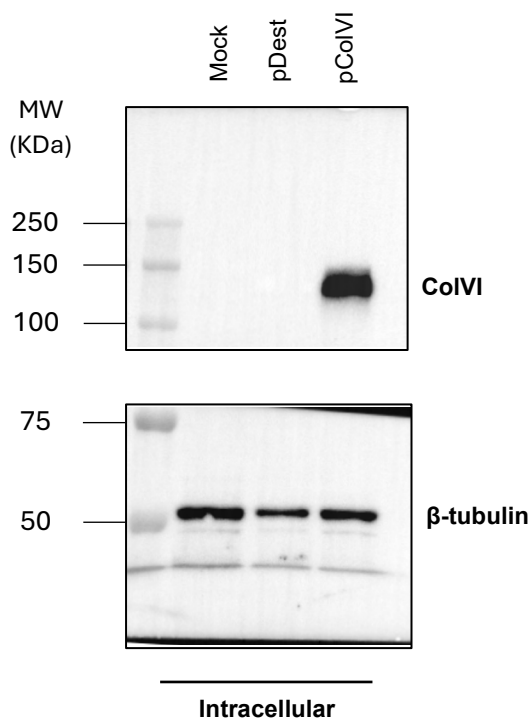
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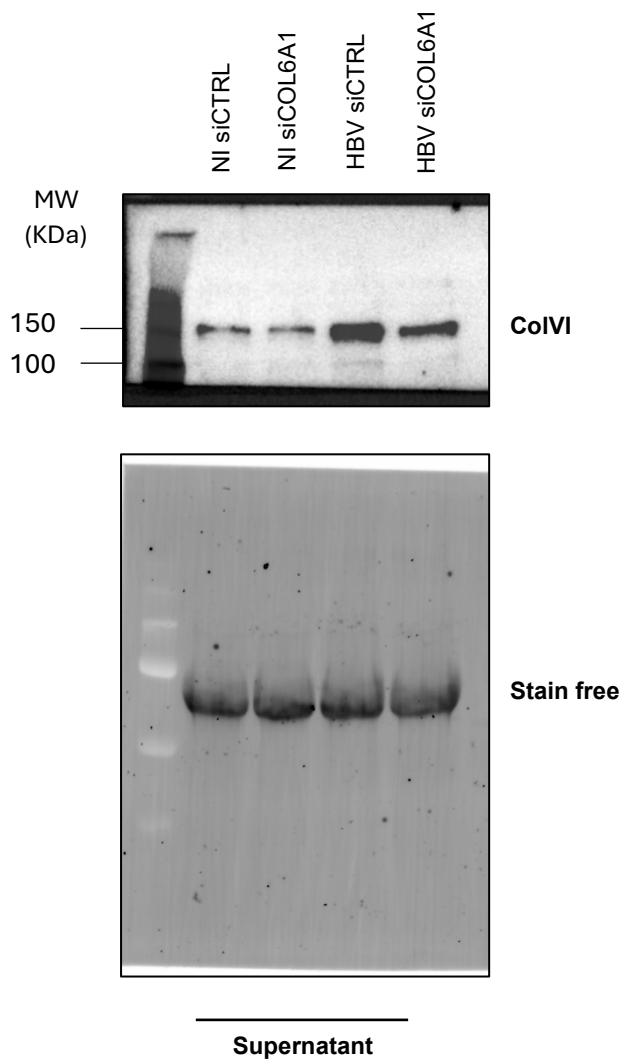
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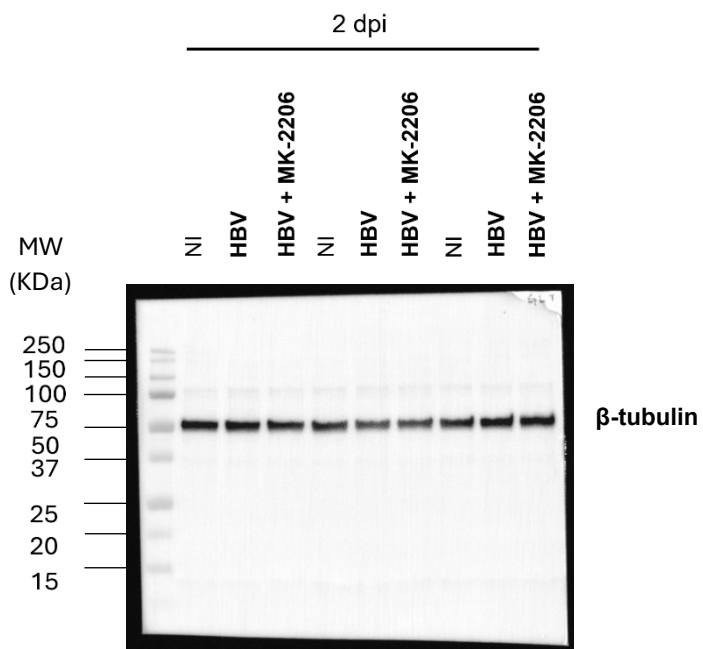
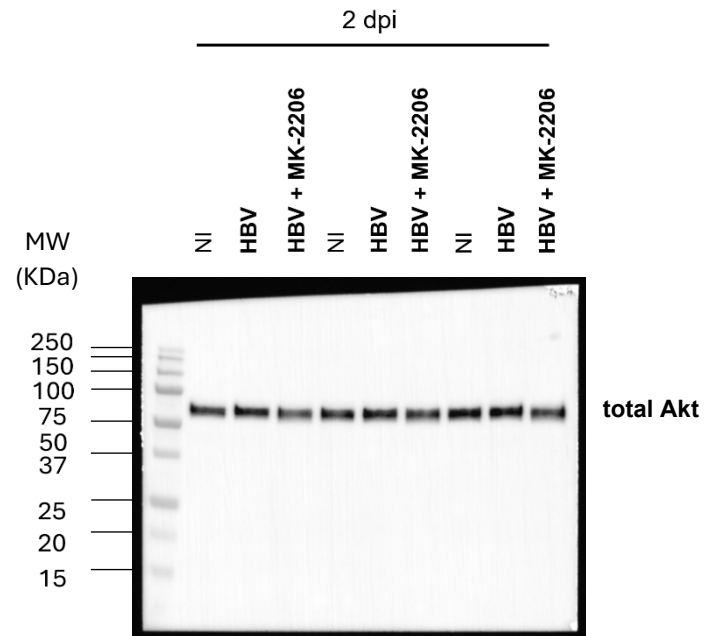
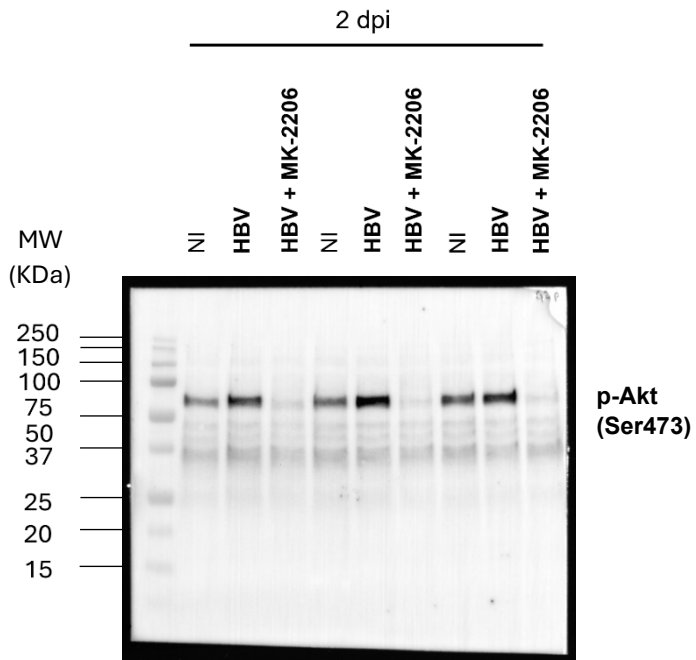
Full length immunoblots (related to figure 4)



Full length immunoblots (related to figure 4)



Full length immunoblots (related to figure S1)



Supplementary references

- 1 Ritchie, M. E. *et al.* limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res* **43**, e47 (2015).
<https://doi.org:10.1093/nar/gkv007>
- 2 Mootha, V. K. *et al.* Integrated analysis of protein composition, tissue diversity, and gene regulation in mouse mitochondria. *Cell* **115**, 629-640 (2003).
- 3 Subramanian, A. *et al.* Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A* **102**, 15545-15550 (2005). <https://doi.org:10.1073/pnas.0506580102>
- 4 Liberzon, A. *et al.* The Molecular Signatures Database (MSigDB) hallmark gene set collection. *Cell Syst* **1**, 417-425 (2015). <https://doi.org:10.1016/j.cels.2015.12.004>
- 5 Liberzon, A. *et al.* Molecular signatures database (MSigDB) 3.0. *Bioinformatics* **27**, 1739-1740 (2011). <https://doi.org:10.1093/bioinformatics/btr260>
- 6 Verrier, E. R. *et al.* Solute Carrier NTCP Regulates Innate Antiviral Immune Responses Targeting Hepatitis C Virus Infection of Hepatocytes. *Cell Rep* **17**, 1357-1368 (2016). <https://doi.org:10.1016/j.celrep.2016.09.084>