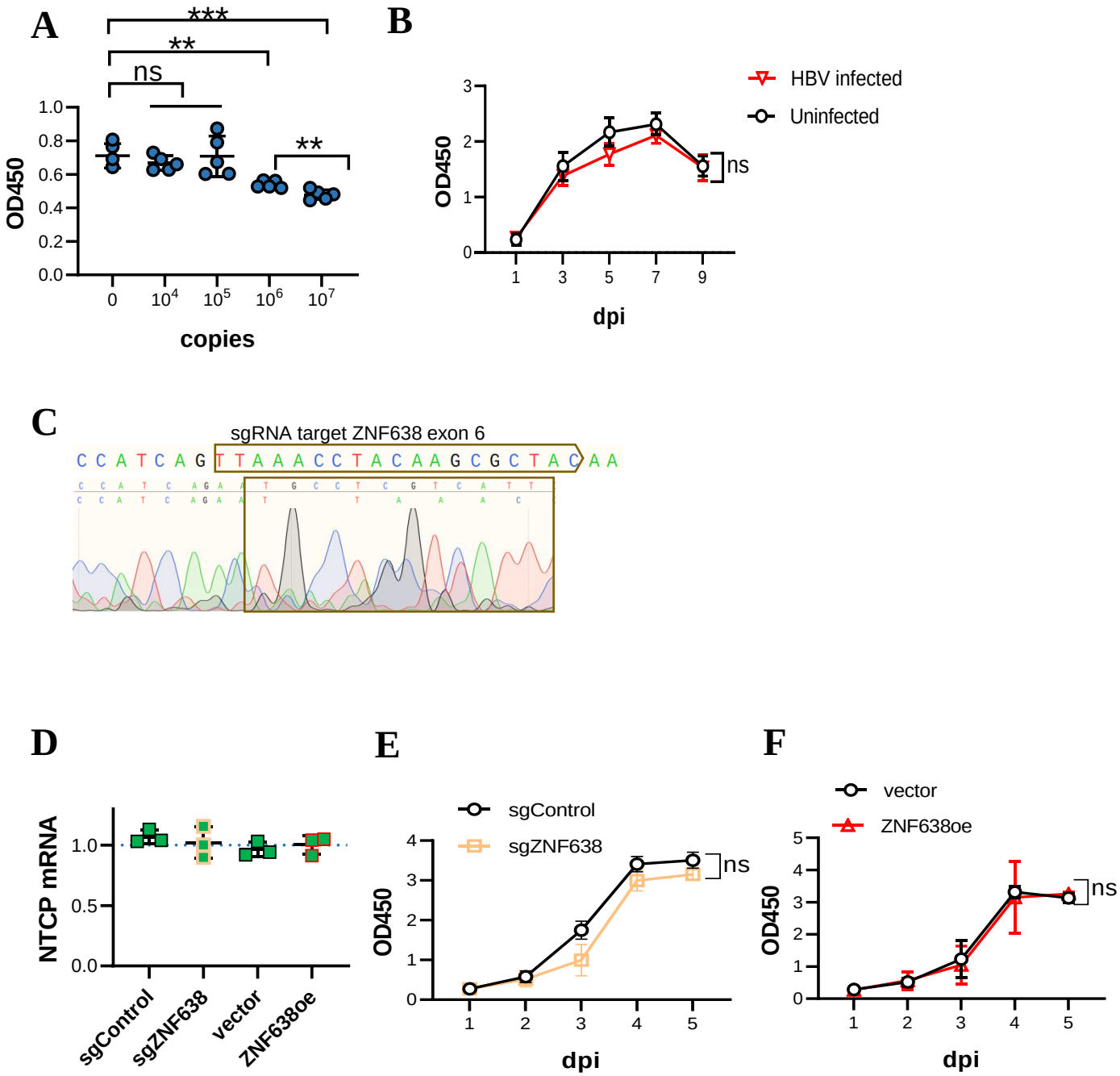
Fig. S1

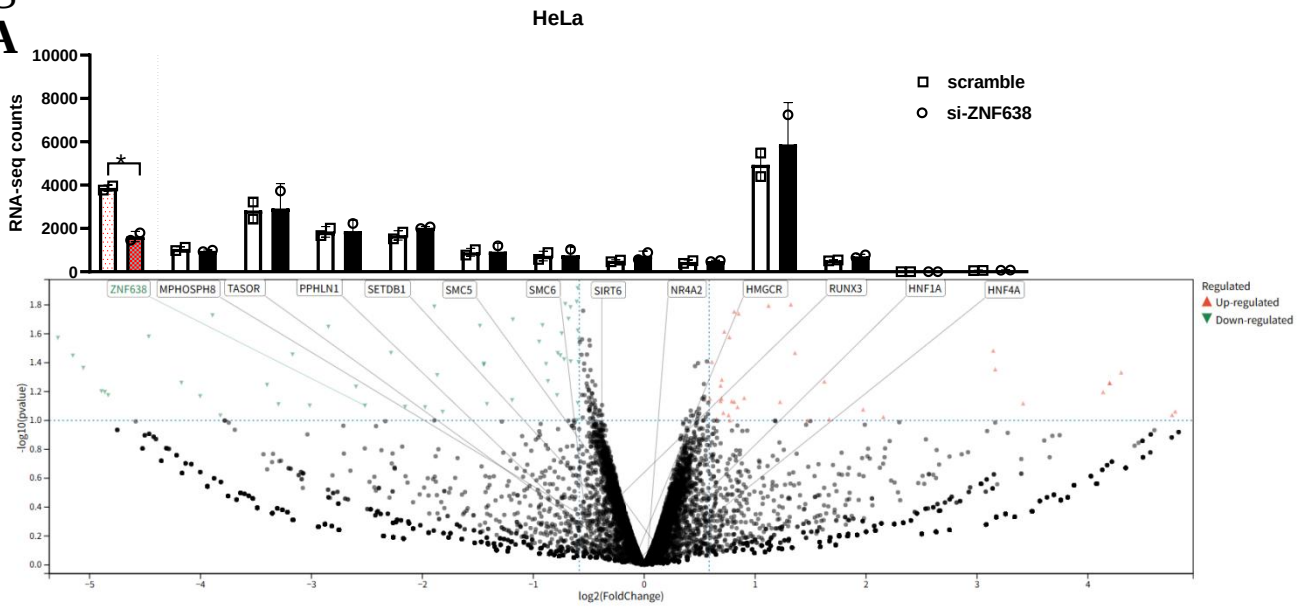


Low dose HBV infection and knockout ZNF638 expression in HepG2-NTCP cells did affected neither NTCP levels nor cell growths.

A CCK8 assay for cell growth in HepG2-NTCP cells infected with HBV prepared from patient serum at increasing doses (6× 10^4, 10^5, 10^6 and 10^7 copies/ml). **B** CCK8 assay for cell growth in HepG2-NTCP cells infected with HBV at a concentration of 6×10^5 copies/ml, compared to vehicle control cells. **C** Sanger sequencing on target sites in knockout produced ZNF638 knockdown cells. **D** NTCP mRNA levels in sgZNF638 or ZNF638oe HepG2-NTCP cells as determined by of RT-qPCR. **E-F** CCK8 assay for cell growth in sgZNF638 or ZNF638oe HepG2-NTCP cells.

Fig. S2

A

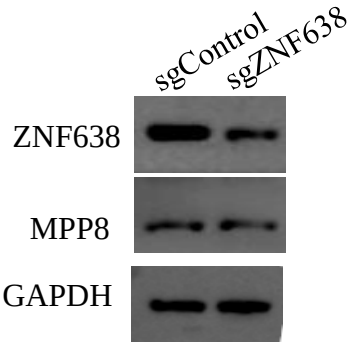


B

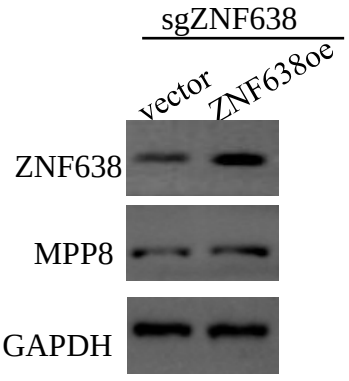
Relative mRNA level (Fold GAPDH)

	HepG2-NTCP				HepAD38			
			sgZNF638				sgZNF638	
	sgControl	sgZNF638	vector	ZNF638oe	sgControl	sgZNF638	vector	ZNF638oe
ZNF638	1.00±0.09	0.50±0.02**	1.00±0.01	1.84±0.19**	1.00±0.07	0.55±0.01**	1.00±0.02	1.93±0.11**
MPP8	1.00±0.09	0.97±0.13	1.00±0.03	1.10±0.11	1.00±0.08	0.96±0.07	1.00±0.04	1.05±0.12
TASOR	1.00±0.10	0.91±0.17	1.00±0.09	0.99±0.23	1.00±0.05	0.96±0.08	1.00±0.03	0.92±0.02
PPHLN1	1.00±0.02	0.89±0.14	1.00±0.11	0.89±0.21	1.00±0.02	1.02±0.14	1.00±0.11	1.02±0.03
SETDB1	1.00±0.03	0.99±0.04	1.00±0.04	0.95±0.06	1.00±0.01	1.04±0.08	1.00±0.03	0.99±0.11
HMGCR	1.00±0.02	0.95±0.12	1.00±0.03	1.02±0.04	1.00±0.02	1.00±0.09	1.00±0.08	0.91±0.21

C



D

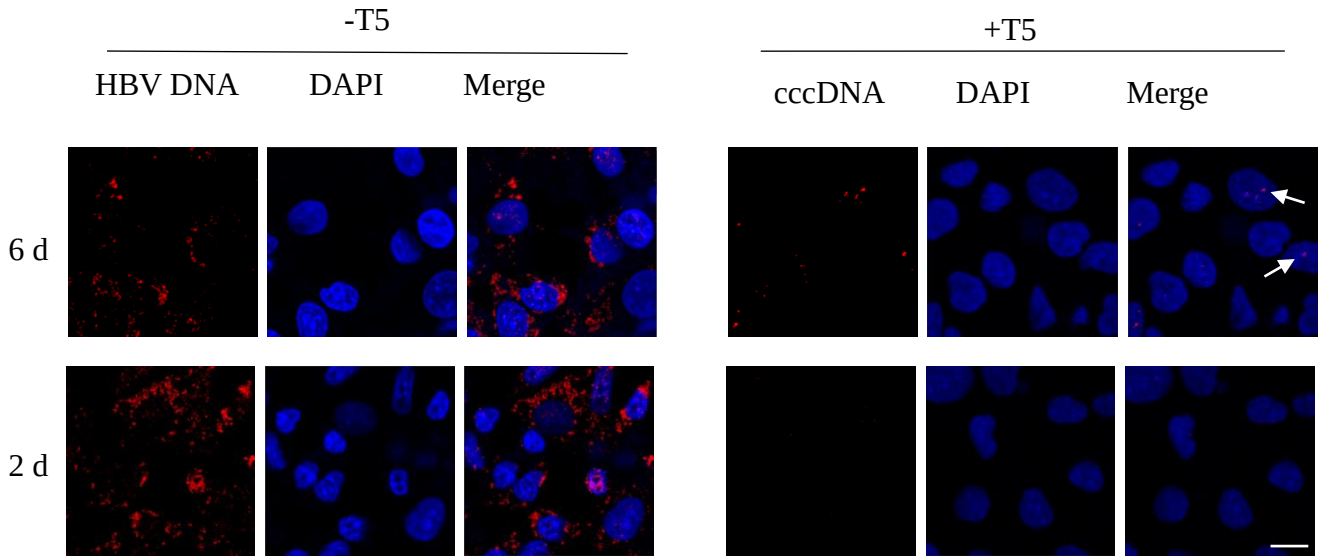


The effect of ZNF638 on the expression levels of a selection of genes that have been correlated with HBV transcription within our *in vitro* cell models.

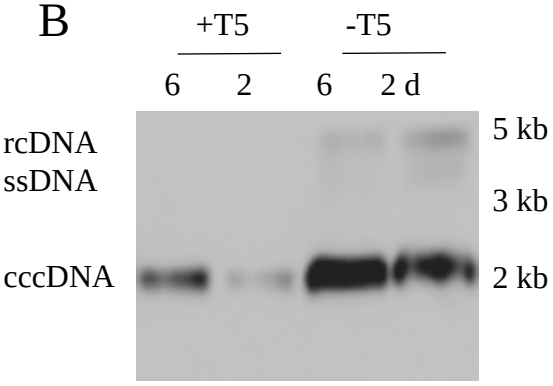
A Top: RNA-seq read counts for a curated panel of genes implicated in HBV transcription in HeLa cells subjected to ZNF638 knockdown. Bottom: Volcano plot derived from RNA-seq data following the transfection of HeLa cells with ZNF638 siRNA for 48 hours. **B** qPCR analysis of genes implicated in HBV transcription in sgZNF638 HepG2-NTCP and HepAD38 cells after 48 hours of ZNF638 overexpression. Western blot for MPP8 protein expression in sgControl and sgZNF638 HepG2-NTCP cells **C** or after 48 hours of ZNF638 overexpression **D.**

Fig. S3

A



B

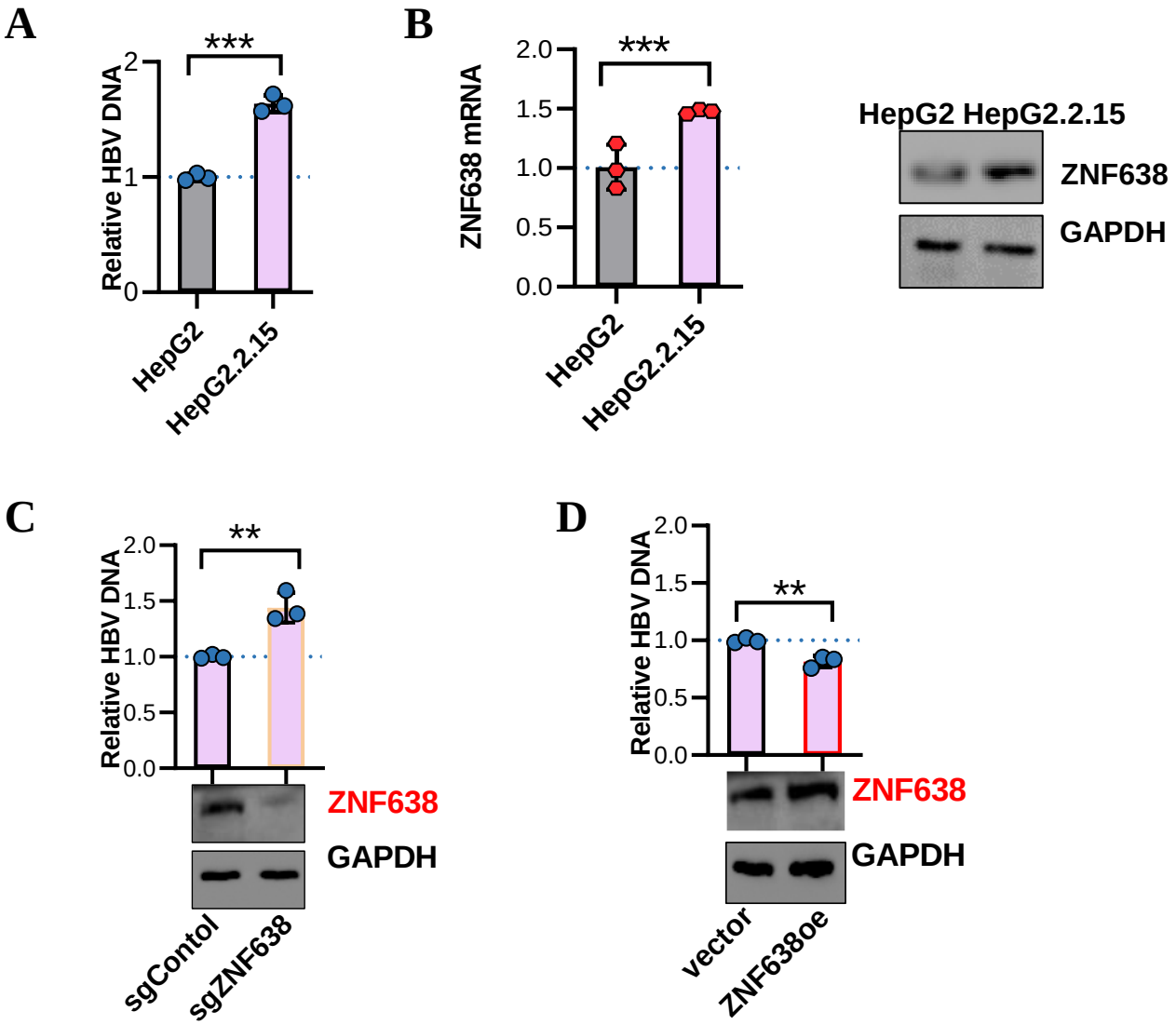


C

DNA copies from qPCR		
HepG2-NCTP	**6 d**	**2 d**
HBV DNA	1.10×10^3	3.65×10^3
cccDNA	4.97×10^3	below limit

Analyses of HBV DNA and cccDNA in HBV-infected HepG2-NTCP cells.
A FISH microscopy of HBV of HepG2-NTCP cells for DNA or cccDNA at 2 and 6 days post-infection (dose?). **B** Southern blot of HBV DNA from HepG2-NTCP with or without the treatment of T5 exonuclease. **C** Quantification of obtained HBV DNAs

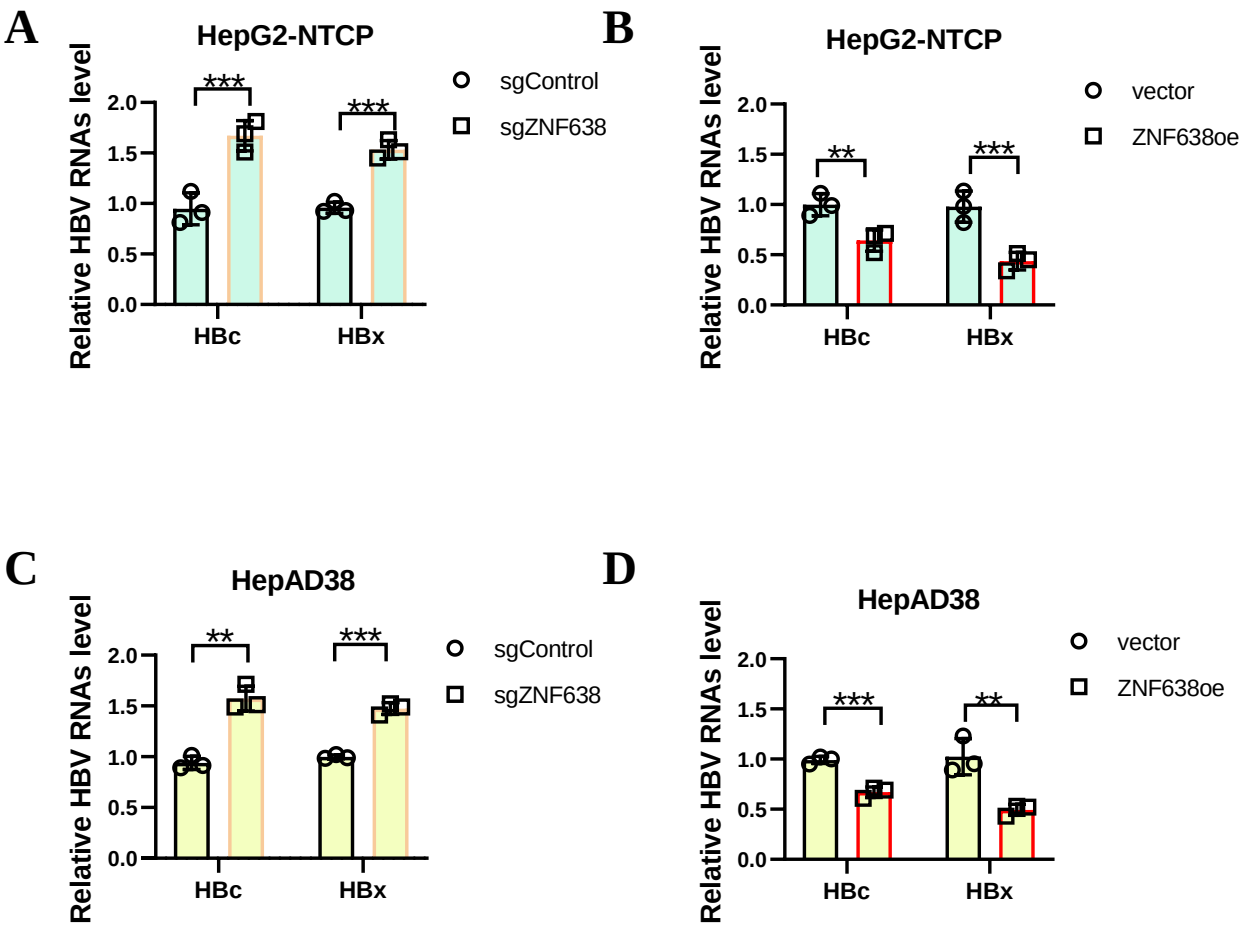
Fig. S4



Role of ZNF638 on HBV gene expression in HepG2.2.15 cells.

A HBV DNA levels detected in the supernatant of HepG2 and HepG2.2.15 cells. **B** ZNF638 expression in HepG2 and HepG2.2.15 cells from RT-qPCR (left) and western blot (right). **C-D** HBV DNA levels in sgZNF638 or ZNF638oe HepG2.2.15 cells.

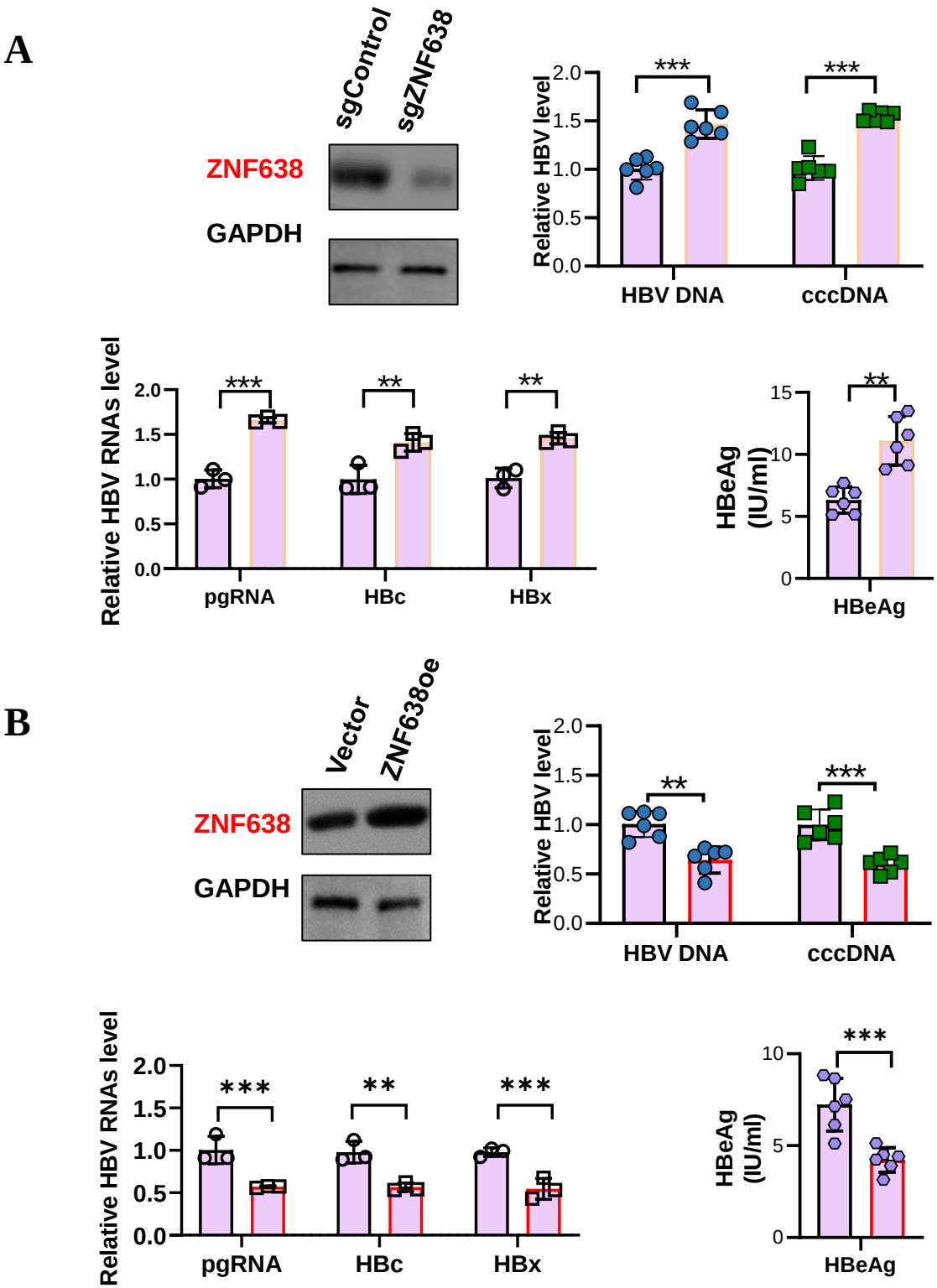
Fig. S5



Levels of HBc and HBx RNAs were detected in sgZNF638 and ZNF638oe HepG2-NTCP and HepAD38 cells.

A-B In sgZNF638 or ZNF638oe HepG2-NTCP cells were infected with HBV at 6 dpi. **C-D** The levels of HBV RNA were detected. The levels of HBV in sgZNF638 or ZNF638oe HepAD38 cells.

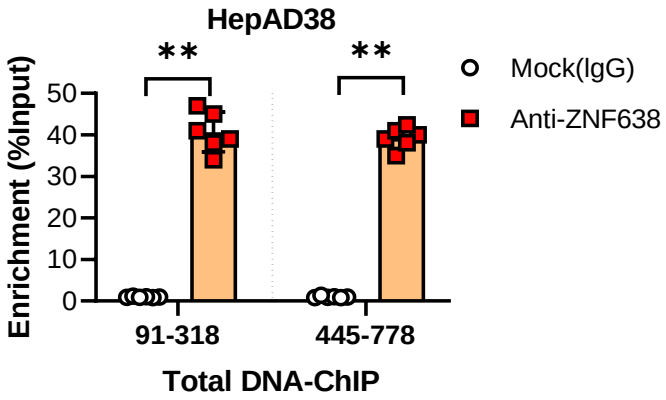
Fig. S6



Repression of HBV cccDNA transcription in HepG2.2.15 cells is dependent to ZNF638 expression levels.

A Levels of HBV DNA, cccDNA, HBV RNA (pgRNA, HBc, HBx) and E antigen were detected in sgZNF638 HepG2.2.15 cells. **B** Levels of HBV DNA, cccDNA, HBV RNA (pgRNA, HBc, HBx) and E antigen were detected in ZNF638oe HepG2.2.15 cells.

Fig. S7

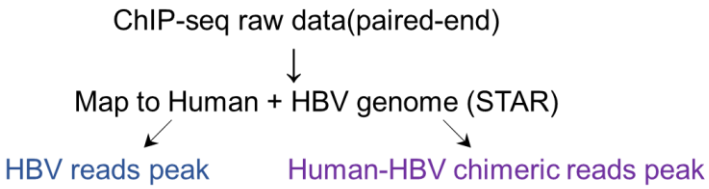


ChIP-qPCR assays with total DNA isolated from HepAD38 cultures for enrichment of ZNF638 at *PreS2* and *S* gene regions.

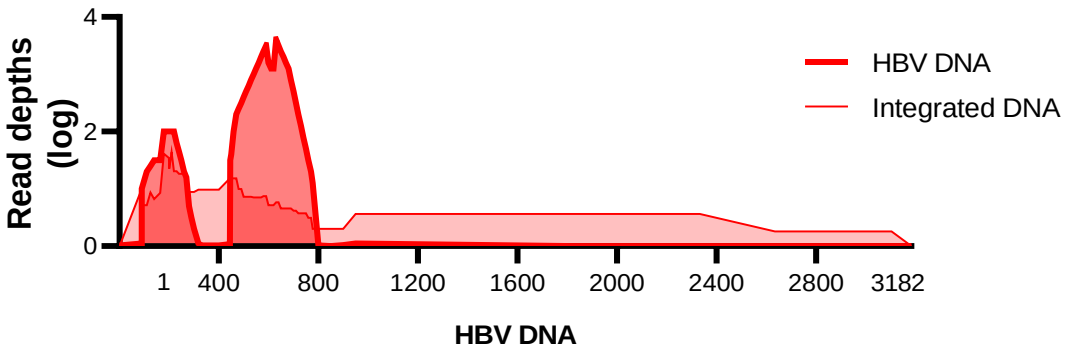
Validation of identified ZNF638 binding peaks by ChIP-qPCR for ChIP-seq enriched HBV sequence.

Fig. S8

A



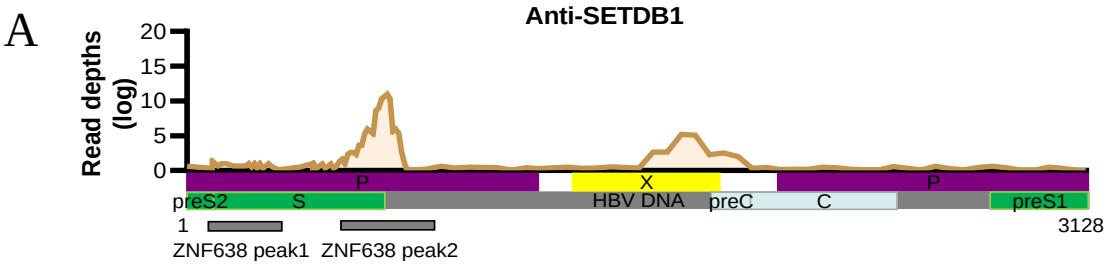
B

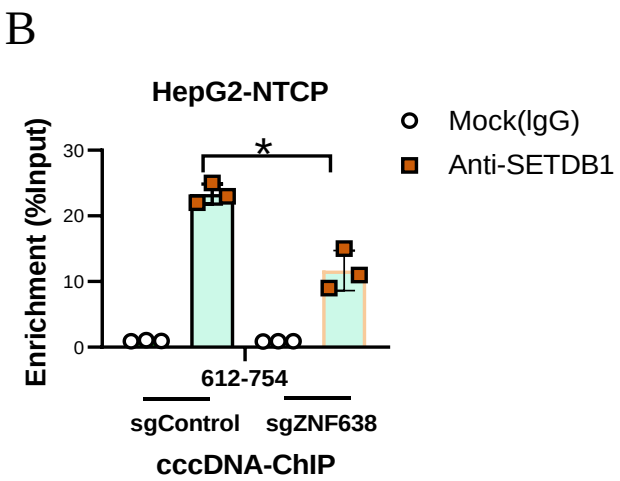


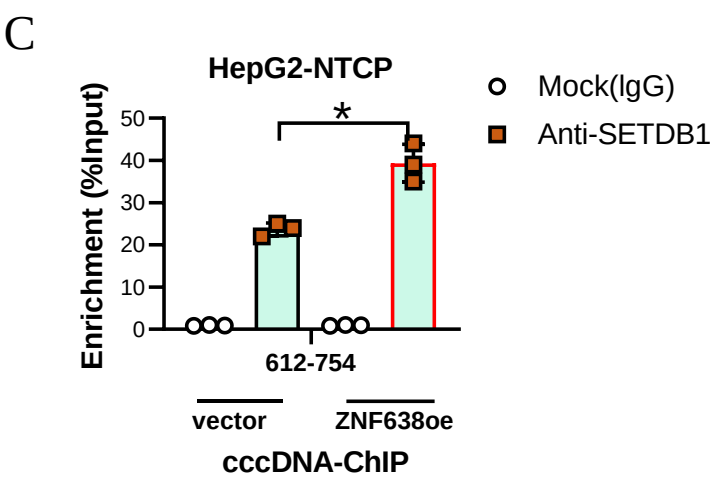
Evaluation of ZNF638 binding on HBV cccDNAs and genome integrated HBV sequences in HepAD38 cells.

A Data analysis workflow. **B** ChIP-seq for ZNF638 binding peaks in non-induced HepAD38 cells (harvest at day 6 with 0.3 μg/ml tetracycline).

Fig. S9

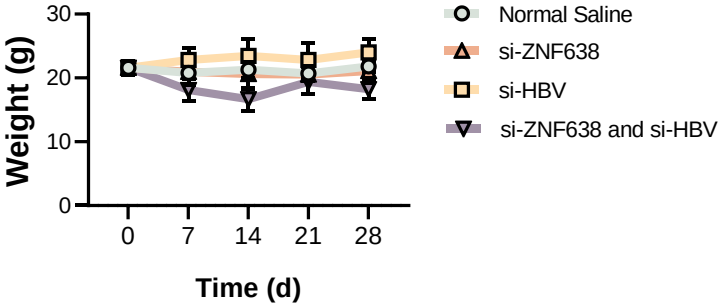






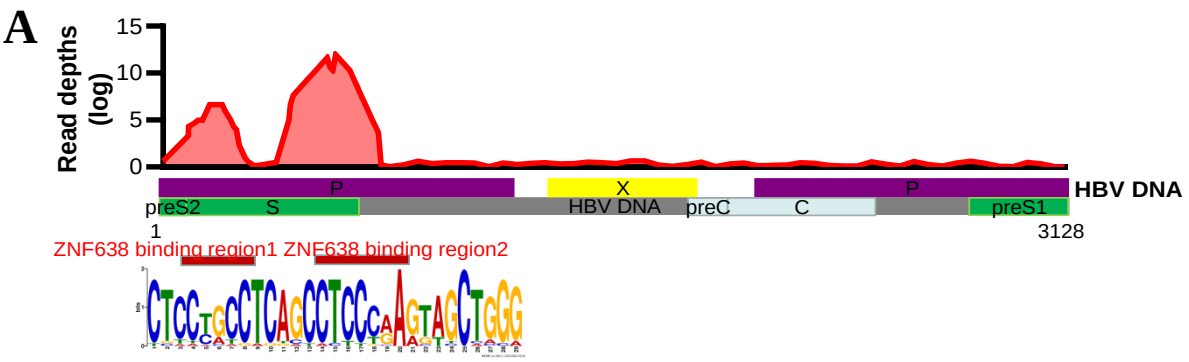
Evaluation of SETDB1 binding on cccDNAs in HBV infected HepG2-NTCP cells.
A SETDB1 peaks as determined by ChIP-seq on cccDNA from HepG2-NTCP cells at 6 d post HBV infection using an anti- SETDB1 antibody. **B-C** ChIP-qPCR at SETDB1 peak (nt 612-754) of cccDNAs isolated from sgZNF638 or ZNF638oe HepG2-NTCP cells at 6 d post HBV infections.

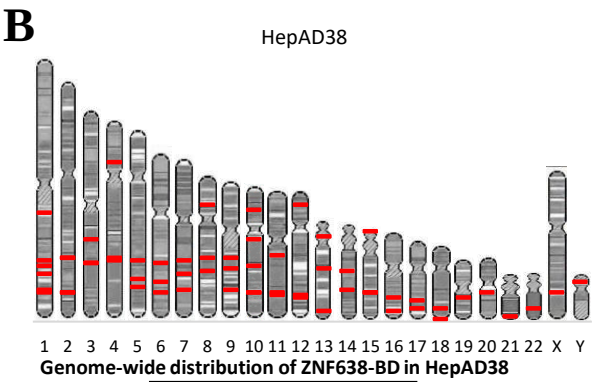
Fig. S10



Body weights of HBV transgenic mice during a 29-day period of siRNA treatments.

Fig. S11





Genome-wide distribution of ZNF638-BD in HepAD38

Location	Counts
3UTR	4
ncRNA	1
TTS	6
LINE	57
SINE	103
pseudo	2
DNA	9
Exon	10
Intron	117
Intergenic	77
Promoter	17
CpG-Island	2
Low_complexity	3
LTR	20
Simple_repeat	4
Satellite	147

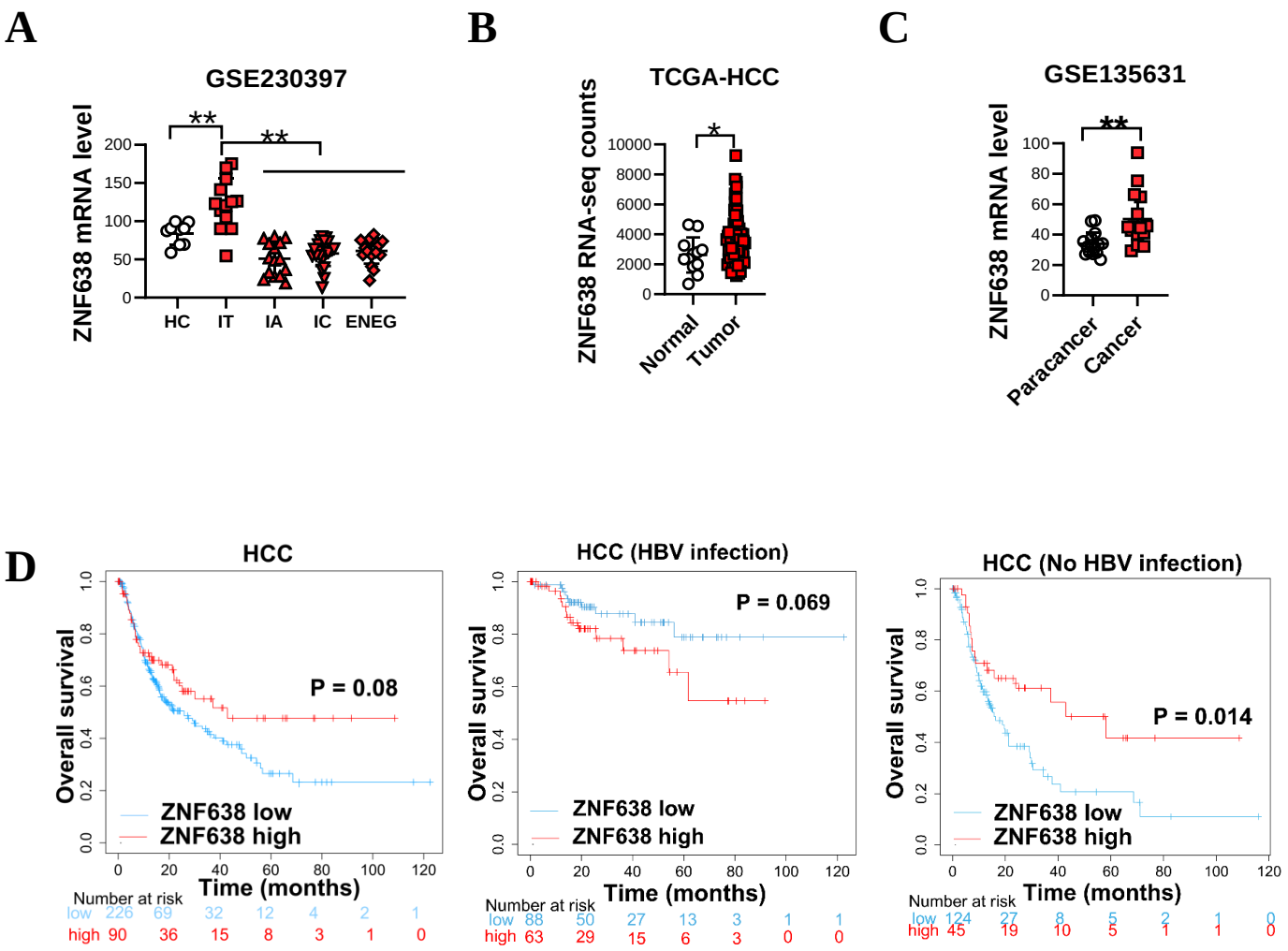
C

Alterations in potential ZNF638-regulated downstream gene expression in ZNF638 knockdown Hela cells

Gene	logFC	pvalue
WFIKKN1	-2.712538004	0.108751643
APOA1	3.082075808	0.526279271
PGLS	-0.295289544	0.362365605
PRKD3	0.107520305	0.700960277
NRG2	3.082075808	0.526279271
CFAP300	0.384902979	0.504375159
OAS3	-0.131650603	0.575389124
ACTR3B	0.08699104	0.760659796
B4GALNT3	-3.352732038	0.438517959

ZNF638 binding motif on HBV cccDNA and its chromatin occupancy and transcriptional impact. **A** Top: ZNF638 ChIP-seq read density across HBV cccDNA (preS/S) defining the 560 bp binding fragment (ZNF638-BD); bottom: WebLogo showing the ZNF638 motif within the peak. **B** Genome-wide enrichment and distribution of ZNF638-binding sequences across chromosomal loci in HepAD38 cells. **C** RNA-seq overview of transcriptional changes in putative ZNF638-regulated downstream genes upon ZNF638 knockdown in HeLa cells.

Fig. S12



ZNF638 expression levels associate with HBV infection related phenotypes and have prognosis values to predict disease progression.

A ZNF638 expression in liver biopsies of patients with Chronic HBV (GSE230397). Clinical phases of immunotolerant (IT), immune-active (IA), inactive carrier (IC) and HBeAg-negative hepatitis (ENEG) are categorized summarizing serum HBV DNA, HBeAg and alanine aminotransferase (ALT) levels. **B** TCGA statistics data on ZNF638 expression in HCC (https://portal.gdc.cancer.gov/). **C** ZNF638 expression in HBV-related HCC from GEO database (GSE135631). **D** Kaplan-Meier analyses for overall survival prognosis based on ZNF638 expression in HBV-related HCC (https://kmplot.com/analysis/).