Materials and methods

Cell culture, HBV production, infection and transfection

Cell lines such as HEK 293T, HepG2, HepG2-NTCP and HepG2.2.15 were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) [1]. HepG2-NTCP and HepG2.2.15 cells were cultured in 400 μg/mL G418. HepG2 cells were transfected with the hNTCP expression plasmid using TransIT-LT1 (Mirus, USA), and HepG2-NTCP cells were generated according to the manufacturer's instructions. HepAD38 (tet-off) cells were cultured in DMEM/F12 medium supplemented with 10% FBS and 400 μg/mL G418 (for induction of HBV replication). Primary human hepatocytes (PHHs) were purchased from Shanghai RILD Inc. (Shanghai, China). These cells were cultured in a similar manner using the same plating strategy and incubation medium as previously described [2, 3]. All cell lines were treated with 100 U/mL penicillin and 100 mg/mL streptomycin and incubated in a humidified incubator equilibrated with 5% CO_2 at 37 °C. Cells were cultured in a 100 mm dish, 6-well plate or 24-well plate for 12 h and then transfected with plasmids, siRNAs, or their corresponding negative controls (unless otherwise indicated, a concentration of 100 nM was used for siRNA transfection, and cells were transfected with 10, 2, or 0.5 μg of plasmids in a 100 mm dish, 6-well plate or 24-well plate). Transfection was performed using Lipofectamine 3000 reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol.

HBV particle collection and infection

The HBV particles used in this study were mainly derived from HepAD38 cells, and prepared and quantified by a previously described method [4, 5]. HBV infection was performed as previously described [2, 3, 5].

RNA extraction and quantitative real-time PCR (RT–qPCR)

Total RNA was extracted from cells (or liver tissues from human liver chimeric mice) using TRIzol reagent (Solarbio, Beijing, China). First-strand cDNA was synthesized using Hifair III 1st Strand cDNA Synthesis SuperMix (Yeasen Biotech, Shanghai, China). Quantitative real-time PCR was performed in a StepOnePlus real-time PCR system (Bio–Rad) using SYBR Green qPCR Master Mix (Yeasen Biotech, Shanghai, China). Relative fold differences in transcription were calculated by the $2^{-\Delta\Delta Ct}$ method. GAPDH was used as the internal control for normalization. The primers used are listed in Supporting Information Table 4.

Dual-luciferase reporter assay

Cells were transferred into 24-well plates at 3×10^4 cells/well. After 12 h, the cells in each well were transiently co-transfected with 0.2 μg of the pRL-TK plasmid (Promega, Madison, WI, USA) containing the Renilla luciferase gene used for internal normalization and the pGL3-DTX4 promoter, pGL3-MSL2 promoter, pGL3-DTX4 promoter mut or pGL3-MSL2 promoter mut plasmid (lost the ability to interact with STAT3, termed mutant luciferase reporter) (Supporting Information Table 5). Luciferase activities were measured as previously described [6]. All experiments were performed at least three times.

HBV cccDNA extraction and quantification

The procedure for the extraction of HBV cccDNA from HepAD38 (tet-off) has been reported previously [4, 5]. Briefly, aliquots of each DNA sample extracted from cell pellets were treated for 1 hour at 37 °C with 10 U of Plasmid-Safe ATP-Dependent DNase (Epicentre, Madison, WI). qPCR analysis was carried out in a Mastercycler ep realplex instrument (Eppendorf, Germany) to quantify the cccDNA. The primers used in this experiment are listed in Supporting Information Table 4.

Quantification of HBV antigens and serum HBV DNA

Hepatitis B virus surface antigen (HBsAg) and hepatitis B virus e antigen (HBeAg) in culture supernatants were assayed with commercial enzyme-linked immunosorbent assay (ELISA) kits (Kehua Bioengineering, Shanghai, China). HBV DNA in the supernatants of HepAD38 cells and HBV-infected HepG2-NTCP cells was quantified by using a diagnostic kit with a lower limit of detection of 400 copies/mL (Sansure Biotech, Changsha, China).

Immunofluorescence analysis

Cells in 6-well plates were washed three times with precooled PBS, fixed with 4% paraformaldehyde for 10 min, and permeabilized with 0.5% Triton X-100 for 10 min at room temperature. After incubation for 1 h with 5% BSA to block nonspecific binding, primary

antibodies were added for incubation overnight at 4 °C. The bound antibodies were detected by incubation with secondary antibodies. Images were acquired using fluorescence microscopy. The antibodies used for immunofluorescence analysis are listed in Supporting Information Table 6.

Co-immunoprecipitation (Co-IP)

Whole-cell extracts were prepared using IP lysis buffer (Beyotime) supplemented with a complete protease inhibitor cocktail (Solarbio) according to the manufacturer's instructions. Cell lysates were then incubated with the IP antibody overnight at 4 ℃ on a rotating wheel before they were incubated with Protein A/G magnetic beads (Millipore) for 4 h at 4 ℃ on a rotating wheel. The PPIs were analysed by immunoblotting.

Immunohistochemical (IHC) staining

Mice were sacrificed, and their liver tissues were fixed and embedded in paraffin. First, the sections were dewaxed. Then, antigen retrieval was performed at 95 °C with citrate buffer (pH 6.0) for 15 min. The slides were treated with 3% H_2O_2 for 10 min and blocked with goat serum for 1 h. Then, the slides were incubated with a monoclonal antibody at 4 ℃ overnight. After washing three times with 0.01 M PBS, the sections were incubated with horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse IgG (OriGene, Beijing, China) for 30 min at 37 °C. Immunohistochemistry staining was performed using the chromogen 3,3′-diaminobenzidine (DAB), and counterstaining was performed with Mayer's

haematoxylin (ZSGB-BIO, China). The sections were then dehydrated and covered with a coverslip. The antibodies used for IHC staining are listed in Supporting Information Table 6.

Chromatin immunoprecipitation (ChIP)

ChIP was performed by using a SimpleChIP® Plus Sonication CHIP Kit 4C and RT reagents according to the manufacturer's instructions (Cell Signaling Technology, MA, USA) [7]. DNA synthesis was analyzed by quantitative PCR. Precipitated DNA was analyzed by quantitative PCR, and the primers used are listed in Supporting Information Table 4.

TCGA database

Public TCGA (https://portal.gdc.cancer.gov/) data repositories for liver hepatocellular carcinoma (LIHC) (Cancer Genome Atlas Network, 2014) were used for analysis of STAT3 and MSL2 expression.

Western blot analysis

Total protein was extracted by lysis of hepatoma cells or tissues with RIPA buffer. Protein concentrations were measured using the Bradford assay, and 20-50 μg of protein extracts were subjected to SDS–PAGE. Then, proteins were transferred to a nitrocellulose membrane, blocked with 5% nonfat milk and incubated with primary antibodies overnight at 4 °C. After incubation with an anti-mouse (1:10,000) or anti-rabbit (1:10,000) secondary antibody for 1 h

at 37℃, immunocomplexes were visualized with Super ECL Detection Reagent (Yeasen Biotech, Shanghai, China). The primary antibodies used for Western blot analysis are listed in Supporting Information Table 6.

RNA-Seq analysis

The cells were collected in TRIzol reagent, and RNA-Seq analysis was performed at Shanghai Majorbio Bio-pharm Technology Co. Ltd. The data were analyzed on the free online platform Majorbio Cloud Platform (https://www.majorbio.com/). Genes with an FDR-adjusted $P < 0.05$ were considered significantly differentially expressed.

Bioinformatics analysis

The public web servers JASPAR (https://jaspar.elixir.no/) was used to conduct bioinformatics analysis, JASPAR was applied to the binding sites of transcription factor on the STAT3, DTX4 or MSL2 promoter.

References

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APOBEC3B to enhance hepatocarcinogenesis. Hepatology. 2017; 66: 1413-29.

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6. Zhang T, Zhang J, You X, Liu Q, Du Y, Gao Y, et al. Hepatitis B virus X protein modulates oncogene Yes-associated protein by CREB to promote growth of hepatoma cells. Hepatology. 2012; 56: 2051-9.
7. Yang Y, Zhao X, Wang Z, Shu W, Li L, Li Y, et al. Nuclear Sensor Interferon-Inducible Protein 16 Inhibits the Function of Hepatitis B Virus Covalently Closed Circular DNA by Integrating Innate Immune Activation and Epigenetic Suppression. Hepatology. 2020; 71: 1154-69.

Supplementary figure legends

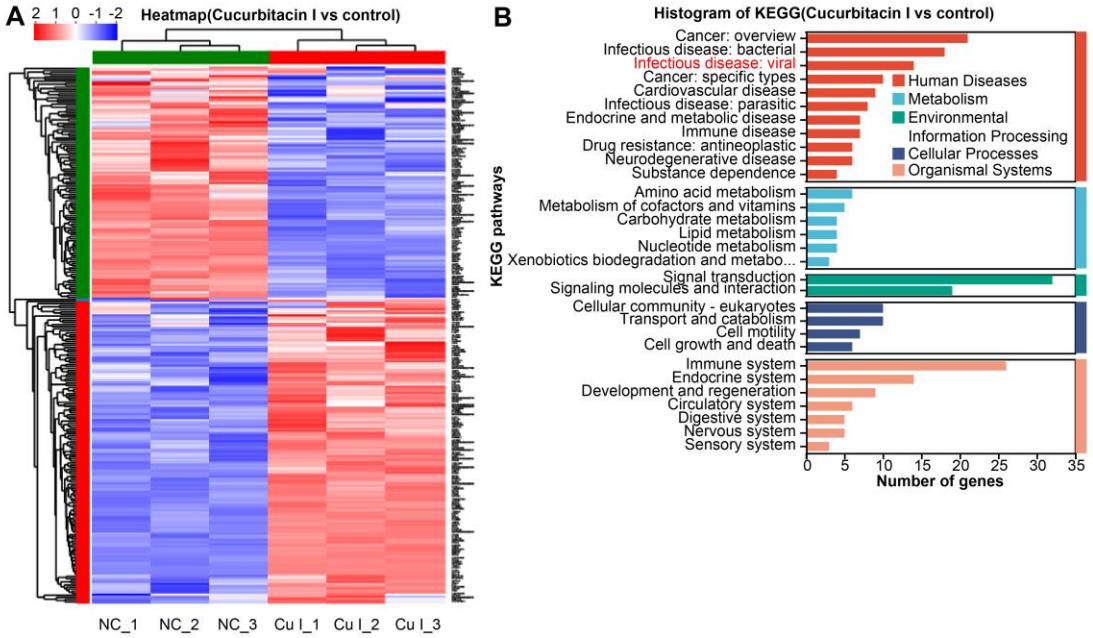

FIGURE S1 RNA-Seq profiling of the effect of Cucurbitacin I on HBV-infected HepG2-NTCP cells. (A) Heatmap of RNA-Seq data from HBV-infected HepG2-NTCP cells treated with cucurbitacin I. (B) KEGG pathway annotation of the RNA-Seq data. The mean ± SD of at least three experiments is shown. Abbreviation: Cu I, Cucurbitacin I.

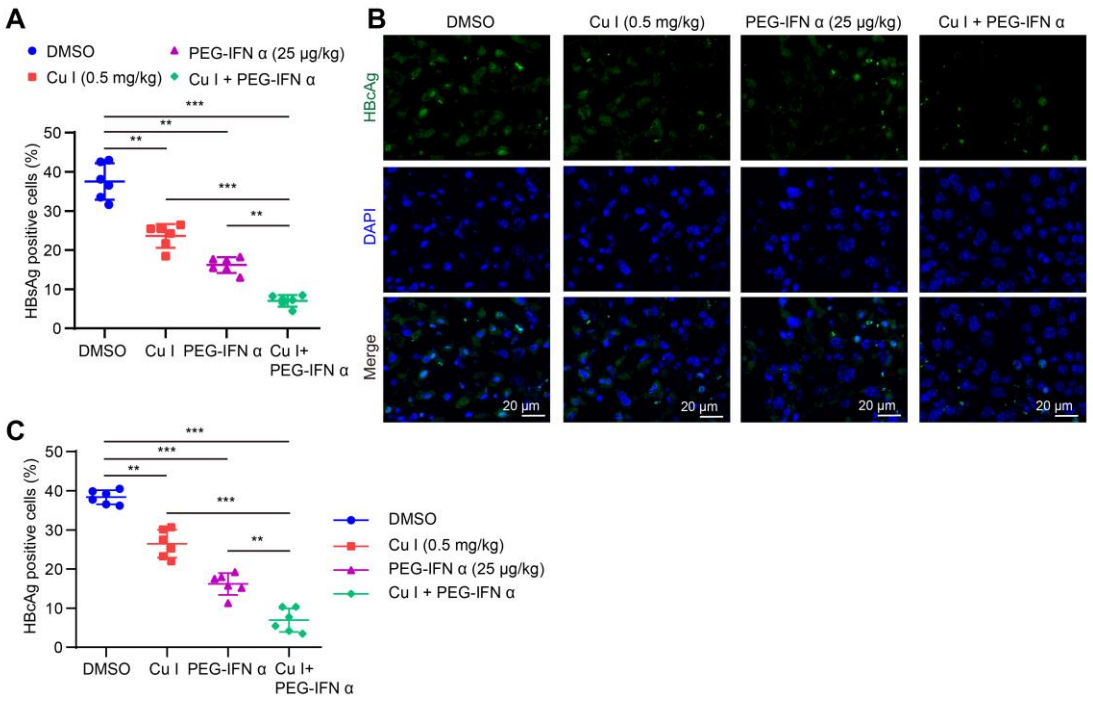

FIGURE S2 Cucurbitacin I attenuates the HBV replication and enhances the sensitivity of PEG-IFN α against HBV *in vivo*. (A) The positive staining rate of HBsAg was assessed by immunohistochemical staining. The HBsAg expression levels in liver tissue samples were quantified by using the software of ImageJ. (B) The HBcAg expression levels were examined by fluorescent staining in liver tissues. Scale bars: 20 μm. (C) ImageJ was used to quantitate the protein expression levels in the fluorescent staining analysis. The mean ± SD of at least three experiments is shown. Statistically significant differences are indicated as follows: $^{**}P < 0.01$, $^{***}P < 0.001$. Abbreviation: Cu I, Cucurbitacin I.

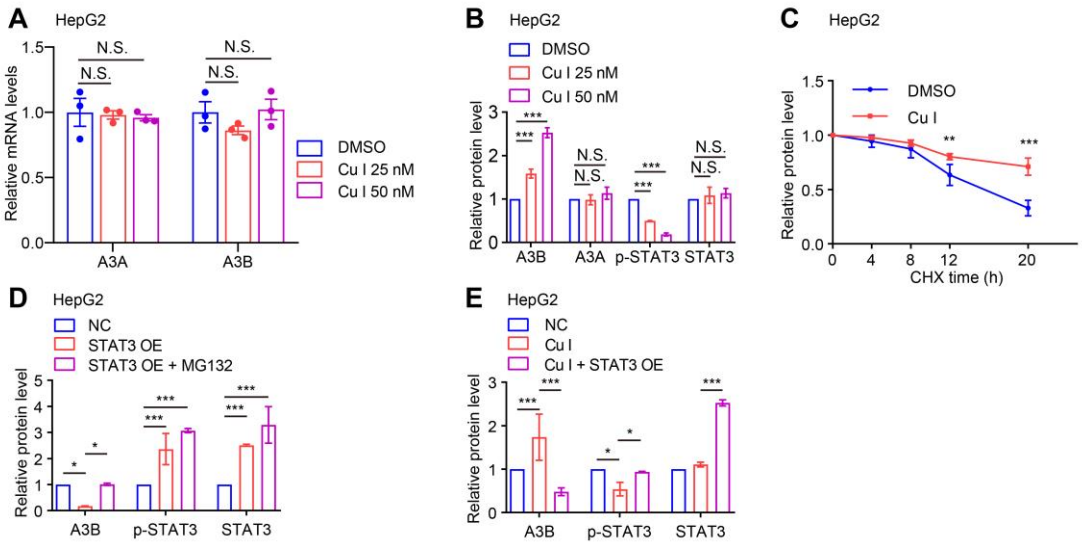

FIGURE S3 The effect of Cucurbitacin I on APOBEC3A and APOBEC3B. (A) The mRNA expression levels of APOBEC3A and APOBEC3B were detected by RT-qPCR in HepG2 cells treated with cucurbitacin I. Cells treated with IL-6 to induce STAT3 activation for 16 hours. (B-E) ImageJ was used to quantitate the protein expression levels from Western blot analysis of Figure 3C, 3E-G. The mean ± SD of at least three experiments is shown. Statistically significant differences are indicated as follows: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Abbreviation: N.S., not significant, Cu I, Cucurbitacin I, A3A, APOBEC3A, A3B, APOBEC3B.

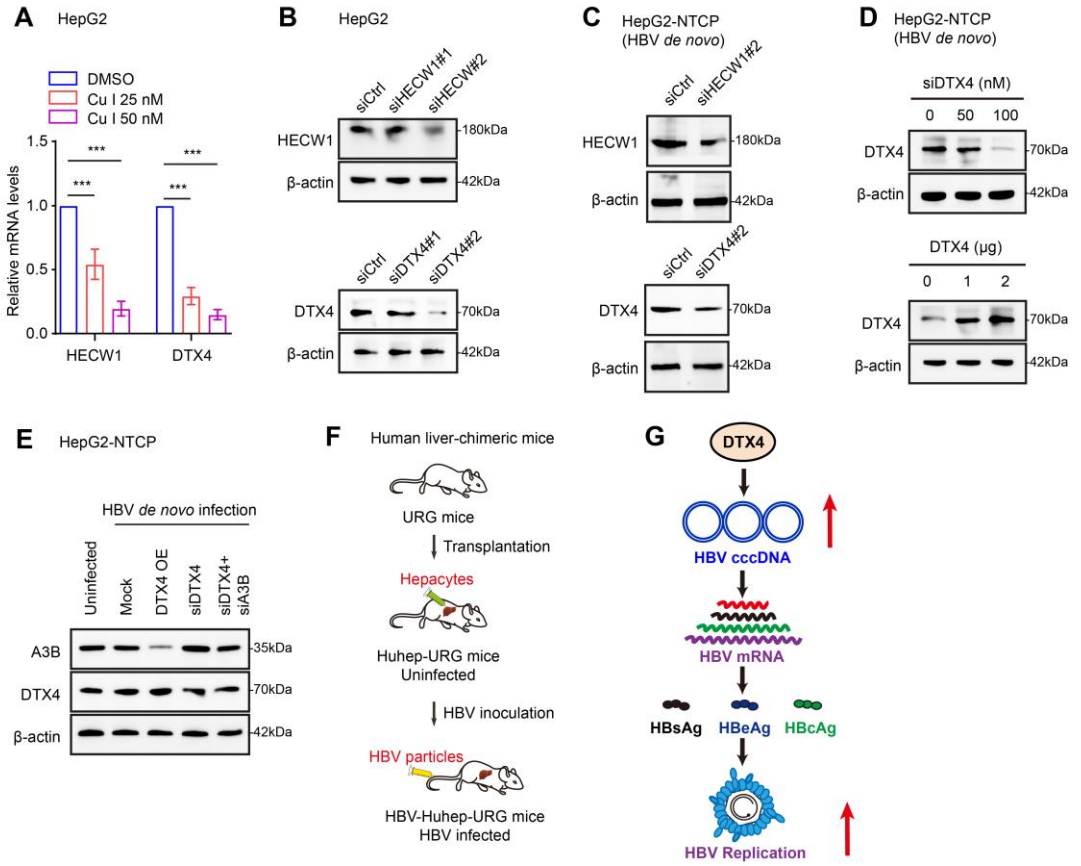

FIGURE S4 E3 ubiquitin ligase DTX4 contributes to HBV cccDNA and HBV replication. (A) ImageJ was used to quantitate the protein expression levels from Western blot analysis of Figure 4B. (B, C) The interference efficiency of siDTX4 and siHECW1 were validated by Western blot analysis in HepG2 cells treated with IL-6 to induce STAT3 activation for 16 hours and HBV-infected HepG2-NTCP cells. (D) The interference and overexpression efficiency of DTX4 were verified by Western blot analysis in HBV-infected HepG2-NTCP cells. (E) The interference and overexpression efficiency of DTX4 and the interference efficiency of APOBEC3B were examined by Western blot analysis in HepG2-NTCP cells. (F) Schematic diagram of human liver-chimeric mouse model (Huhep-URG mouse model)

establishment. (G) A model of E3 ubiquitin ligase DTX4 inhibiting HBV cccDNA and HBV replication. The mean ± SD of at least three experiments is shown. Statistically significant differences are indicated as follows: $^{***}P < 0.001$. Abbreviation: A3B, APOBEC3B.

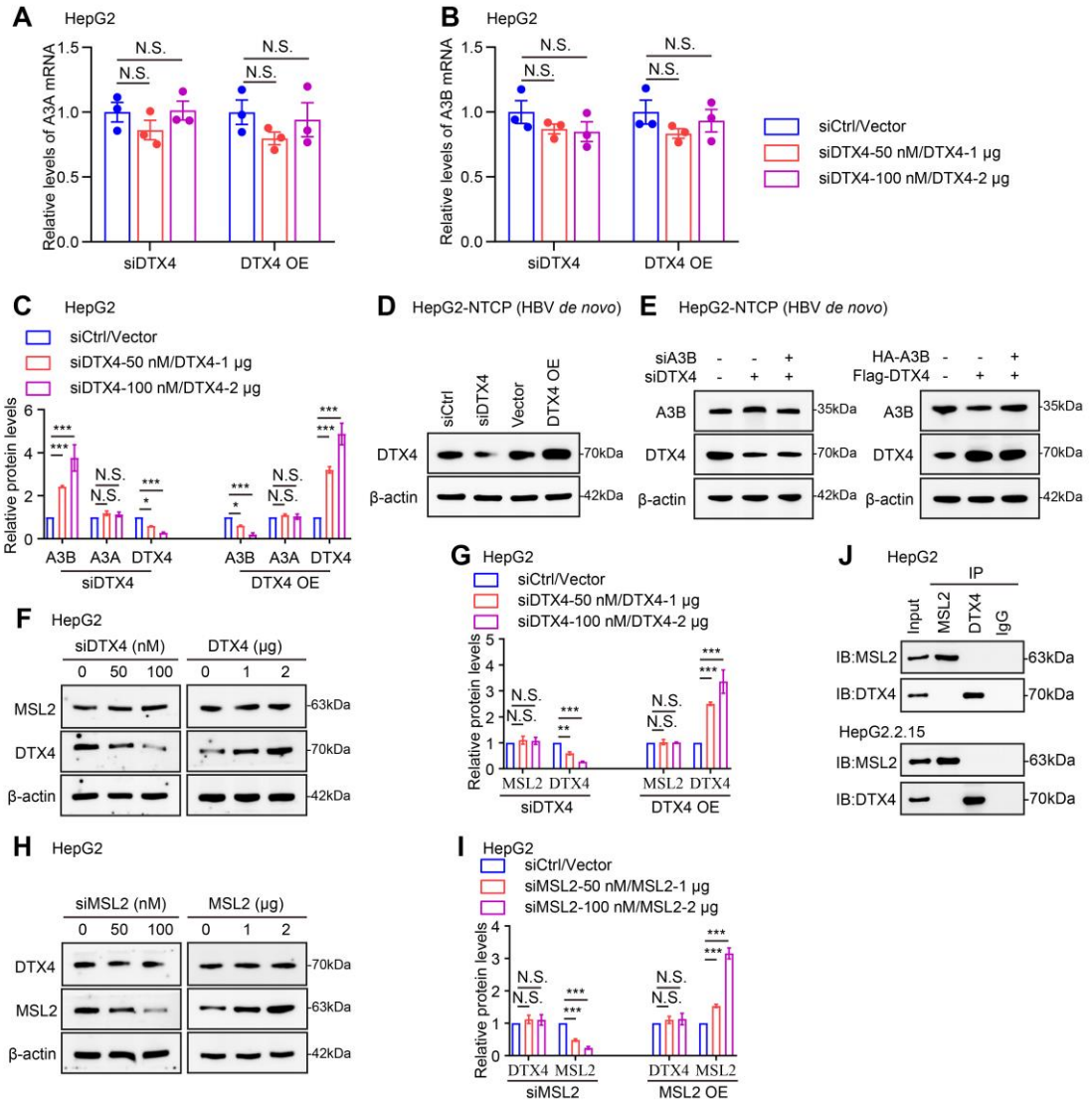

FIGURE S5 The effect of DTX4 on APOBEC3A and APOBEC3B and the relationship between DTX4 and MSL2. (A, B) The mRNA expression levels of APOBEC3A or APOBEC3B were tested by RT-qPCR in HepG2 cells treated with knockdown or overexpression of DTX4. (C) ImageJ was used to quantitate the protein expression levels from Western blot analysis of Figure 5A. (D) The interference and overexpression efficiency

of DTX4 were evaluated by Western blot analysis in HBV-infected HepG2-NTCP cells. (E) The interference and overexpression efficiency of DTX4 and APOBEC3B were measured by Western blot analysis in HBV-infected HepG2-NTCP cells. (F) The effects of DTX4 on the expression levels of MSL2 were detected by Western blot in HepG2 cells. (G) ImageJ was used to quantitate the protein expression levels from Western blot analysis of Figure S5F. (H) The effects of MSL2 on the expression levels of DTX4 were detected by Western blot in HepG2 cells. (I) ImageJ was used to quantitate the protein expression levels from Western blot analysis of Figure S5H. (J) The binding affinity between endogenous DTX4 and MSL2 was evaluated by a Co-IP assay in HepG2 and HepG2.2.15 cells. The mean ± SD of at least three experiments is shown. Statistically significant differences are indicated as follows: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Abbreviation: N.S., not significant, A3B, APOBEC3B.

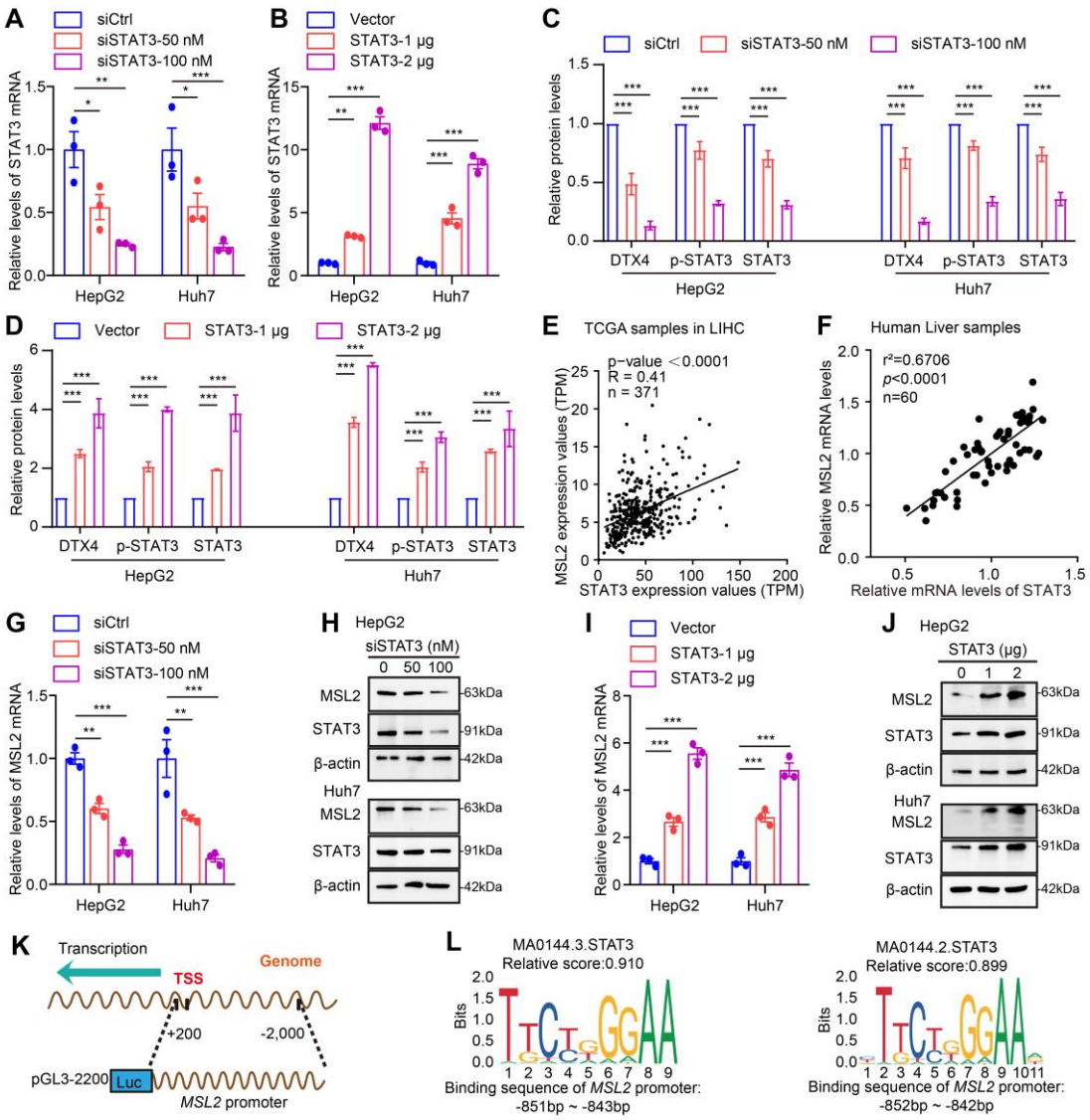

FIGURE S6-1 STAT3 transcriptionally activates E3 ubiquitin ligase DTX4. (A, B) The interference and overexpression efficiency of STAT3 were detected by RT-qPCR in HepG2 and Huh7 cells treated with IL-6 to induce STAT3 activation for 16 hours. (C, D) ImageJ was used to quantitate the protein expression levels from Western blot analysis of Figure 6C and E. (E, F) Correlation analysis of the mRNA expression levels of STAT3 and MSL2 in the TCGA-LIHC (liver hepatocellular carcinoma) dataset (*n*=371) and paracancerous tissue of human clinical hepatocellular carcinoma samples (*n*=60). (G, H) The effects of STAT3 interference on MSL2 mRNA and protein expression levels were validated by RT-qPCR and

Western blot in HepG2 and Huh7 cells treated with IL-6 to induce STAT3 activation for 16 hours. (I, J) The effects of overexpression of STAT3 on MSL2 mRNA and protein expression were demonstrated by RT-qPCR and Western blot analysis in HepG2 and Huh7 cells treated with IL-6 to induce STAT3 activation for 16 hours. (K) Schematic illustration shows the pGL3-MSL2 promoter plasmid containing the -2,000 to +200 bp sequence of the 5' flanking region of MSL2. (L) STAT3 sequence motif logos and the binding elements in the promoter regions of MSL2 gene were identified by JASPAR database with a relative score higher than 80%. The mean ± SD of at least three experiments is shown. Statistically significant differences are indicated as follows: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

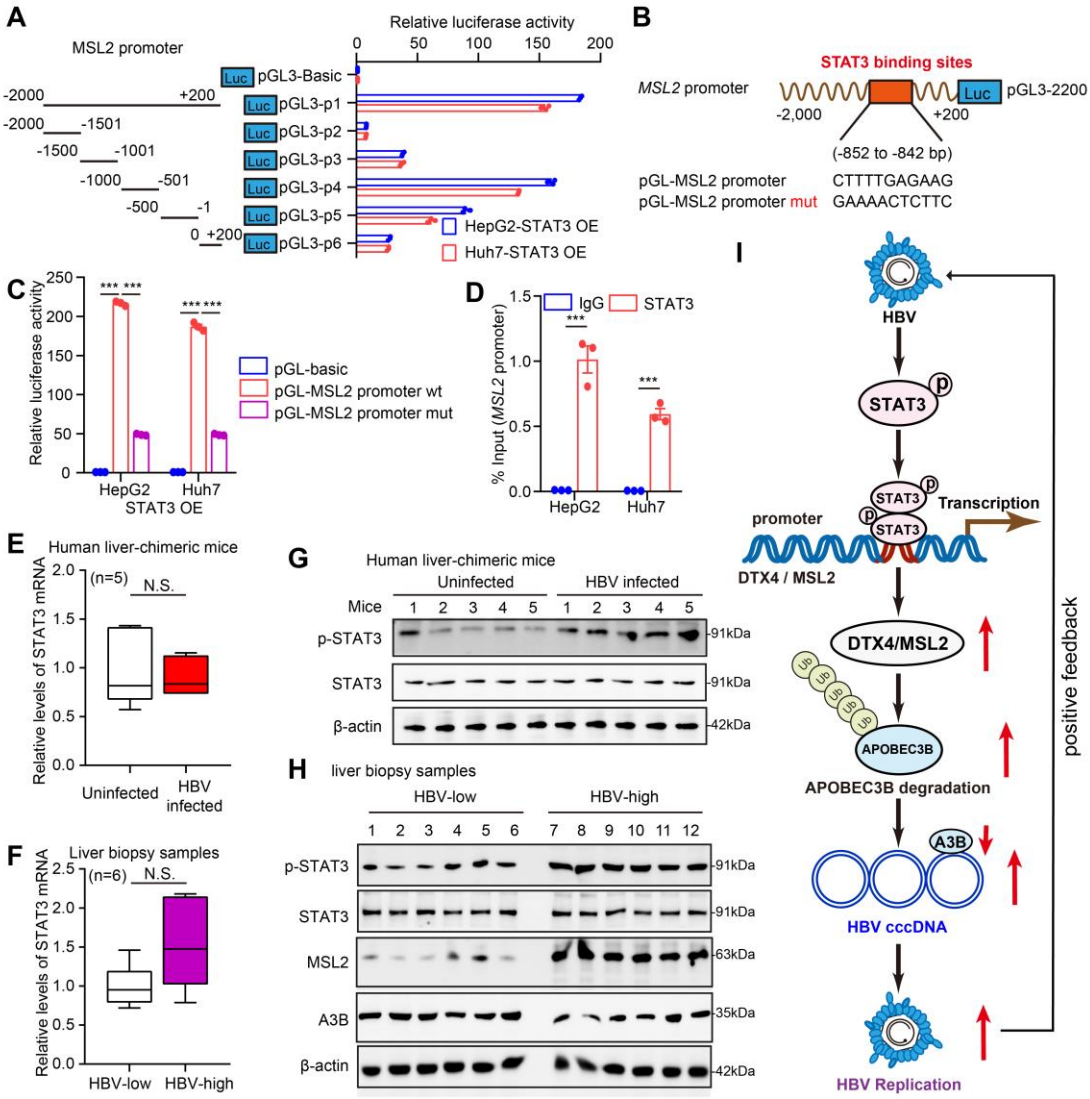

FIGURE S6-2 STAT3 transcriptionally activates E3 ubiquitin ligase DTX4 and MSL2. (A) Luciferase activities of MSL2 promoter reporter gene vectors in HepG2 and Huh7 cells transfected with STAT3 were examined by dual-luciferase reporter assays. Cells treated with IL-6 to induce STAT3 activation for 16 hours. (B) A model demonstrates the predicted STAT3 binding site at -852 to -842 bp of the MSL2 promoter region. The generated mutant sites at the STAT3 binding site region were indicated. The wild-type MSL2 promoter (or mutant) was inserted into the upstream of the luciferase reporter gene in the pGL3-control vector. (C) Luciferase activities of MSL2 promoter wt or MSL2 mutant promoter reporter gene vectors in HepG2 and Huh7 cells transfected with STAT3 were examined by

dual-luciferase reporter assays. Cells treated with IL-6 to induce STAT3 activation for 16 hours. (D) The enrichment of STAT3 on the MSL2 region of -950 bp to -750 bp was examined by ChIP- qPCR assays in HepG2 and Huh7 cells treated with IL-6 to induce STAT3 activation for 16 hours. (E, F) The mRNA levels of STAT3 were analyzed by RT-qPCR analysis in liver tissues of HBV-infected (*n*=5) and uninfected (*n*=5) human liver chimeric mice and in liver biopsy specimens from clinical patients with a high HBV load (*n*=6) and with a low HBV load (*n*=6). (G) The protein levels of STAT3 and its phosphorylated levels were analyzed by Western blot analysis in liver tissues of HBV-infected (*n*=5) and uninfected (*n*=5) human liver chimeric mice. (H) The protein levels of STAT3, MSL2 and APOBEC3B, and the levels of p-STAT3 were evaluated by Western blot analysis in liver biopsy specimens from clinical patients with a high HBV load (*n*=6) and with a low HBV load (*n*=6). (I) A model of the mechanism by which HBV-elevated the transcription factor STAT3 promotes the transcription and expression of E3 ubiquitin ligases DTX4 and MSL2, thereby increasing the stability of HBV cccDNA and promoting HBV replication in a positive feedback manner. The mean ± SD of at least three experiments is shown. Statistically significant differences are indicated as follows: $^{***}P < 0.001$. Abbreviation: N.S., not significant, A3B, APOBEC3B.

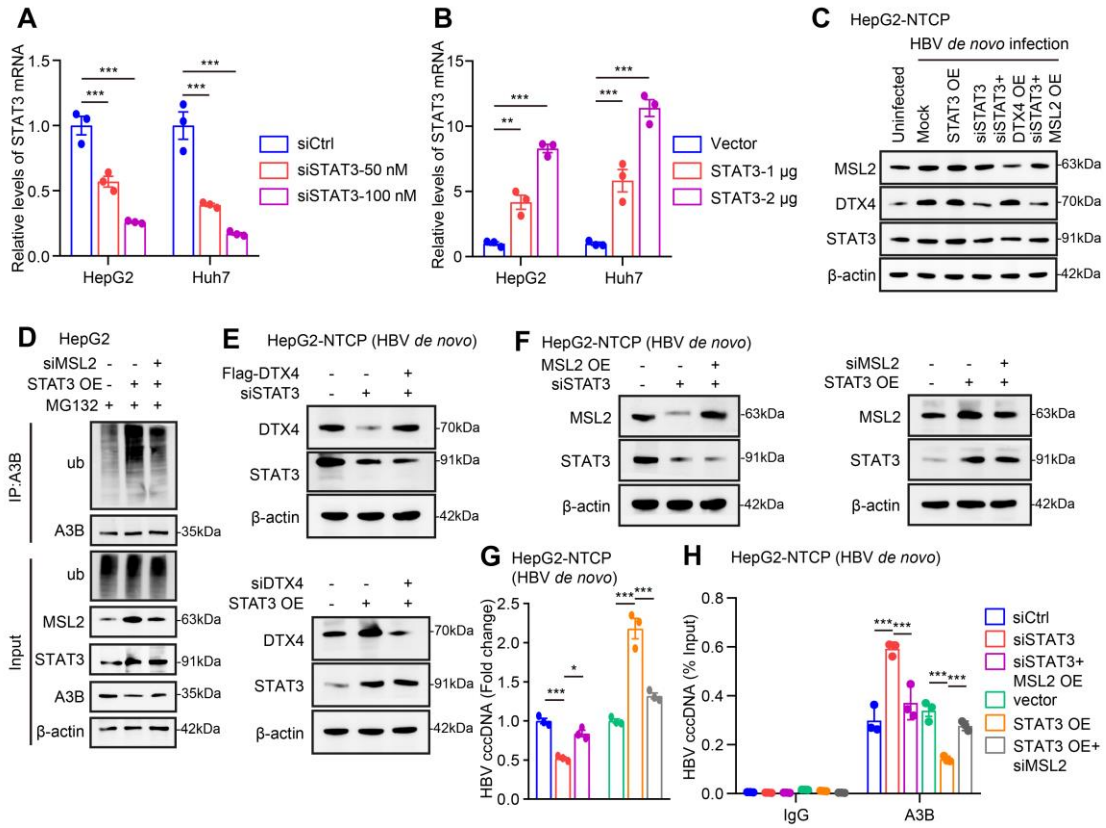

FIGURE S7 STAT3 enhances the stability of HBV cccDNA and HBV replication through DTX4/MSL2-mediated ubiquitination of APOBEC3B. (A, B) The interference and overexpression efficiency of STAT3 were measured by RT-qPCR in HepG2 and Huh7 cells treated with IL-6 to induce STAT3 activation for 16 hours. (C) The overexpression efficiency of STAT3, DTX4 and MSL2 and the interference efficiency of STAT3 were detected by Western blot analysis in HepG2-NTCP cells. (D) Ubiquitination analysis of APOBEC3B in HepG2 cells when STAT3 were co-transfected with siMSL2 or not; cells were treated with MG132 (2 mM) for 24 h. Co-IP and Western blot analysis was performed to determine whether the effect of STAT3 on APOBEC3B protein expression through MSL2 in cells treated with IL-6 to induce STAT3 activation for 16 hours. (E, F) Interference and overexpression efficiency of DTX4, MSL2 and STAT3 were analyzed by Western blot analysis in HBV-infected HepG2-NTCP cells. (G, H) HepG2-NTCP cells were subjected to

HBV infection (MOI = 500) and treated as indicated at 1 and 4 dpi (days post-infection). Analysis was performed 7 days post-infection. qPCR analysis was performed to determine whether the effect of STAT3 on the level of HBV cccDNA through MSL2 in the cells (G). ChIP-qPCR analysis was performed to determine whether the effect of STAT3 on binding of APOBEC3B to HBV cccDNA micro-chromosome through MSL2 in the cells (H). The mean ± SD of at least three experiments is shown. Statistically significant differences are indicated as follows: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Abbreviation: A3B, APOBEC3B.

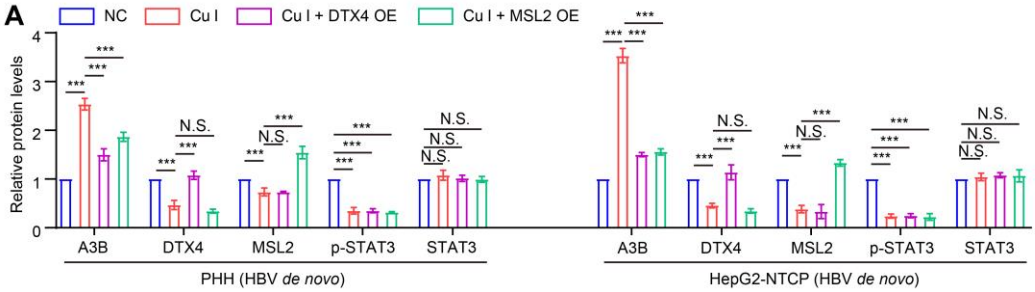

FIGURE S8 Cucurbitacin I limits the expression of APOBEC3B by inhibiting p-STAT3/DTX4/MSL2 signalling. (A) ImageJ was used to quantitate the protein expression levels from Western blot analysis of Figure 8A. The mean ± SD of at least three experiments is shown. Statistically significant differences are indicated as follows: $^{***}P < 0.001$. Abbreviation: A3B, APOBEC3B, N.S., not significant.

Supporting Information Tables

Table 1. The characteristics of patients with biopsy.

Case No.	Age (year)	Gender	Diagnosis	Organ	HBV (copy/mL)
1	55	Male	NA	Liver	$< 5.6\times10^{2}$
2	70	Male	NA	Liver	0
3	67	Male	NA	Liver	$< 5.6\times10^{2}$
4	74	Male	NA	Liver	$< 5.6\times10^{2}$
5	68	Male	NA	Liver	0
6	65	Male	NA	Liver	$< 5.6\times10^{2}$
7	58	Male	Viral hepatitis type B	Liver	8.29×10^{7}
8	58	Male	Viral hepatitis type B	Liver	1.57×10^{8}
9	68	Male	Viral hepatitis type B	Liver	1.93×10^{7}
10	36	Male	Viral hepatitis type B	Liver	1.08×10^{7}
11	59	Male	Viral hepatitis type B	Liver	6.89×10^{5}
12	57	Male	Viral hepatitis type B	Liver	2.60×10^{5}

Abbreviations: "NA" refers to not available.

Table 2. Clinical characteristics of 60 pairs of clinical liver cancer tissues.

Clinicopathological features		
	No. of patients	**Proportion (%)**
Age		
⩽60	35	58.3
>60	25	41.7
Gender		
Male	50	83.3
Female	10	16.7
Cirrhosis status		
Yes	32	53.3
No	28	46.7
Satellite nodule		
Positive	14	23.3
Negative	46	76.7
Microvascular invasion		
M0	37	61.7
M1	15	25
M2	8	13.3
Differentiation degree		
Well-differentiated	3	5
Moderately-differentiated	41	68.3
Poorly-differentiated	16	26.7

Table 3. The information of mice with humanized liver.

Mice	HSA (μg/mL)	HBV DNA (×10^6 copy/mL)	HBsAg (IU/mL)	HBeAg (PEIU/mL)
Mice-Uninfected-1	1844	0	0	0
Mice-Uninfected-2	1916	0	0	0
Mice-Uninfected-3	2065	0	0	0
Mice-Uninfected-4	3243	0	0	0
Mice-Uninfected-5	4274	0	0	0
Mice-HBV infected-1	1128	11.50	54.78	22.85
Mice-HBV infected-2	3289	1.34	25.24	5.86
Mice-HBV infected-3	3662	1.28	25.51	6.85
Mice-HBV infected-4	1094	4.38	27.28	8.13
Mice-HBV infected-5	1571	23.7	62.59	24.18

Abbreviations: “HSA” refers to “Human Serum Albumin”.

Table 4. List of Oligonucleotide Sequences used in this article.

Gene	Primer	Sequence (5'-3')
Primers for RT-qPCR		
GAPDH	Forward	GAGTCAACGGATTTGGTCGT
	Reverse	TTGATTTTGGAGGGATCTCG
DTX4	Forward	TTAAGGCAGCCGTGGTCAATG
	Reverse	CTTCAGTGGGCCTCGAATGG
HECW1	Forward	GTTTTGTGTCCTTGCCCACT
	Reverse	GAATTGCAGCTGTCCACTCA
MSL2	Forward	ACAGTGAGAAAGTTCAGCCA
	Reverse	AGCACGCCCACATTTACA
APOBEC3A	Forward	GAGAAGGGACAAGCACATGG
	Reverse	TGGATCCATCAAGTGTCTGG
APOBEC3B	Forward	TGACCCTTTGGTCCTTCGAC
	Reverse	CATGGCGGCCGTAAAAGC
STAT3	Forward	CAGCAGCTTGACACACGGTA
	Reverse	AAACACCAAAGTGGCATGTGA
mouse-gapdh	Forward	CCTGCCAAGTATGATGACAT
	Reverse	GTTGCTGTAGCCGTATTCA
mouse-dtx4	Forward	TGTGCCTGTGAAAAACTTGAATG
	Reverse	TGGGATGGACTTTATCTCACTCT
mouse-stat3	Forward	AGCTGGACACACGCTACCT
	Reverse	AGGAATCGGCTATATTGCTGGT
cccDNA	Forward	CGTCTGTGCCTTCTCATCTGC
	Reverse	GCACAGCTTGGAGGCTTGAA
HBV pgRNA	Forward	CTCCTCCAGCTTATAGACC
	Reverse	GTGAGTGGGCCTACAAA
GAPDH (ChIP)	Forward	TCGACAGTCAGCCGCATCT
	Reverse	CTAGCCTCCCGGGTTTCTCT

cccDNA (ChIP)	Forward	CTCCCCGTCTGTGCCTTCT
	Reverse	GCCCCAAAGCCACCCAAG
DTX4-promoter (ChIP)	Forward	TAGCCCAATAAATTTTTAA
	Reverse	TTCAAAATTTAGTTGGGCT
MSL2-promoter (ChIP)	Forward	GGTTGGTCTCGCCCTCTTG
	Reverse	GTCCAACATTCAGAATCTA
siRNA sequence		
DTX4#1		UCAAUUACGUAGCUAAAGCCA
DTX4#2		GGAUCGACCUCACUUCCAUUU
HECW1#1		UCAUCAAUGAGGUACAUGCCA
HECW1#2		GACCUCACUUUCACUGUUAAU
APOBEC3B		CCUGAUGGAUCCAGACACA
STAT3		UCCAGUUUCUUAAUUUGUUGACGGGUU
MSL2		GUGCAUUAUAUCCCAGCAGAA
Control		UUCUCCGAACGUGUCACGU

Table 5. List of plasmids used in this article.

Plasmids	Note
pcDNA3.1-vector	Vector
pcDNA3.1-HA-Vector	Vector
pCMV-Flag-Vector	Vector
pcDNA3.1-STAT3	STAT3 (no tag)
pcDNA3.1-APOBEC3B	APOBEC3B (no tag)
pcDNA3.1-APOBEC3B-HA	HA-tagged APOBEC3B
pcDNA3.1-MSL2	MSL2 (no tag)
pCMV-DTX4-Flag	Flag-tagged DTX4
pCMV-ub WT-Flag	Flag-tagged ub WT
pCMV-ub K48R-Flag	Flag-tagged ub K48R
pCMV-ub K63R-Flag	Flag-tagged ub K63R
pGL3-Basic	Vector
pRL-TK	Vector
pGL3-P1	DTX4 promoter -2000 ~ +200
pGL3-P2	DTX4 promoter -2000 ~ -1501
pGL3-P3	DTX4 promoter -1501 ~ -1001
pGL3-P4	DTX4 promoter -1001 ~ -501
pGL3-P5	DTX4 promoter -501 ~ -1
pGL3-P6	DTX4 promoter 0 ~ +200
pGL3-DTX4 promoter mut	DTX4 promoter mutation
pGL3-P1	MSL2 promoter -2000 ~ +200
pGL3-P2	MSL2 promoter -2000 ~ -1501
pGL3-P3	MSL2 promoter -1501 ~ -1001
pGL3-P4	MSL2 promoter -1001 ~ -501
pGL3-P5	MSL2 promoter -501 ~ -1
pGL3-P6	MSL2 promoter 0 ~ +200
pGL3-MSL2 promoter mut	MSL2 promoter mutation

Table 6. List of antibodies used in this article.

Antibody	Manufacture	Catalog Number
HBc	Abcam	Ab115992
APOBEC3A	Abclonal	A12399
APOBEC3B	Abcam	ab184990
p-STAT3	Santa Cruz	sc-8059
STAT3	Santa Cruz	sc-8019
HECW1	proteintech	24695-1-AP
DTX4	proteintech	25222-1-AP
Ubiquitin	Abcam	ab134953
MSL2	Novus	H00055167- D01P
HBsAg	Abcam	ab68518
HBeAg	Abcam	ab8639
Flag tag	MBL	M185-3L
Flag tag	CST	14793S
β-actin	Sigma-Aldrich	A2228