



RESEARCH ARTICLE

# Mechanisms and Effects on HBV Replication of the Interaction between HBV Core Protein and Cellular Filamin B

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## Abstract

Hepatitis B virus (HBV) infection is one of the major problems that threatens global health. There have been many studies on HBV, but the relationship between HBV and host factors is largely unexplored and more studies are needed to clarify these interactions. Filamin B is an actin-binding protein that acts as a cytoskeleton protein, and it is involved in cell development and several signaling pathways. In this study, we showed that filamin B interacted with HBV core protein, and the interaction promoted HBV replication. The interaction between filamin B and core protein was observed in HEK 293T, Huh7 and HepG2 cell lines by co-immunoprecipitation and co-localization immunofluorescence. Overexpression of filamin B increased the levels of HBV total RNAs and pre-genome RNA (pgRNA), and improved the secretion level of hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg). In contrast, filamin B knockdown inhibited HBV replication, decreased the level of HBV total RNAs and pgRNA, and reduced the secretion level of HBsAg and HBeAg. In addition, we found that filamin B and core protein may interact with each other via four blocks of arginine residues at the C-terminus of core protein. In conclusion, we identify filamin B as a novel host factor that can interact with core protein to promote HBV replication in hepatocytes. Our study provides new insights into the relationship between HBV and host factors and may provide new strategies for the treatment of HBV infection.

**Keywords** Filamin B · Core · HBV replication

## Introduction

Filamin belongs to the actin-binding protein family and other family members include talin, vinculin, and  $\alpha$ -actinin (Hartwig and Stossel 1975; Wang *et al.* 1975). Filamin is

usually in the form of dimers that can link actin filaments into orthogonal networks or parallel bundles according to the actin:filamin ratio and the flexibility of the filamin isoform (van der Flier and Sonnenberg 2001). The N-terminus of filamin B encodes an actin-binding domain (ABD), which contains two calponin homology domains, known as CHD1 and CHD2. The ABD is followed by a series of 24 repeats that are folded into antiparallel  $\beta$ -sheets, with each repeat containing 96 amino acids (aa) (Gorlin *et al.* 1990). These repeats are interrupted by two hinge regions. Hinge I is located between the 15th and 16th repeats (Xie *et al.* 1998; Xu *et al.* 1998), and hinge II is located between the 23th and 24th repeats (Gorlin *et al.* 1990). It has been reported that filamin can interact with over 30 kinds of proteins, such as integrin (van der Flier *et al.* 2002), caveolin, smad, glycoprotein Ib-IX complex (Xu *et al.* 1998), insulin receptor and tissue factor (Stossel *et al.* 2001).

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There are three types of filamin: filamin A, filamin B and filamin C. They are highly conserved in term of their nucleic acid sequences, but quite different in terms of their hinge area (Patrosso *et al.* 1994; Chakarova *et al.* 2000). Filamin A is found in all tissues, while filamin B (FLNB) mainly exists in the uterus, liver, thyroid and vascular endothelial cells. The gene encoding filamin B, which is also known as ABP278, is located in chromosome 3p14.3 (Brocker *et al.* 1999), and the protein has a variety of functions. For instance, it serves as a molecular scaffold for the type I interferon-induced c-jun N-terminal kinase signaling pathway (Jeon *et al.* 2008), regulates chondrocyte proliferation and differentiation (Hu *et al.* 2014), and plays a key role in vascular endothelial growth factor-induced endothelial cell motility (del Valle-Perez *et al.* 2010). Filamin B mutations cause skeleton-related diseases including Larsen syndrome (LS) (Bicknell *et al.* 2007), atelosteogenesis I phenotypes (AOI), atelosteogenesis III phenotypes (AOIII) (Krakow *et al.* 2004), and Boomerang dysplasia (BD) (Bicknell *et al.* 2005).

Hepatitis B virus (HBV) infection can cause chronic hepatitis, which can develop into liver cirrhosis, liver fibrosis, and hepatocellular carcinoma (Cougot *et al.* 2005; Lupberger and Hildt 2007). HBV belongs to the *Hepadnaviridae* family (Beck and Nassal 2007), a family of small DNA viruses, and has four open reading frames (ORFs): S, P, X, and C. They encode a variety of viral protein including viral envelope (pre-S1/pre-S2/S), core proteins (pre-C/C), viral polymerase, and HBx protein (Schadler and Hildt 2009).

Core protein is encoded by the C ORF and contains 183–185 aa (Scaglioni *et al.* 1997). Its N-terminal domain (NTD) consists of abundant  $\alpha$ -helices (Dryden *et al.* 2006), and this domain regulates the assembly of core protein. The C-terminal domain (CTD) is rich in arginine and contains three SPRRR sequences (Salfeld *et al.* 1989). The CTD plays an important role in pre-genome/polymerase complex packing (Jung *et al.* 2014).

The HBV life cycle is a complex process. Core protein is involved in almost every step of the HBV life cycle. Firstly, HBV binds to Na<sup>+</sup>-taurocholate cotransporting polypeptide (NTCP) to enter the cytoplasm and releases the nucleocapsid (containing the viral genome) (Rabe *et al.* 2003; Yan *et al.* 2012). The function of core protein is to form the nucleocapsid, which moves to the nucleus and releases the viral genome (Kann *et al.* 2007). Later in the process, core protein packages the new made pre-genome/polymerase complex (Bartenschlager *et al.* 1990) and this complex gradually forms the mature nucleocapsid (Lambert *et al.* 2007). Core protein promotes the transcriptional activity and stability of covalently closed circular DNA (ccc DNA), and interacts with the viral DNA to influence epigenetics (Guo *et al.* 2011). In the development

of liver disease, core protein can induce tumor necrosis factor- $\alpha$  to mediate an immune response (Tzeng *et al.* 2014), sensitize hepatocytes to tumor necrosis factor-induced apoptosis (Jia *et al.* 2015), and regulates the replication of HBV (Liu *et al.* 2015).

Core protein plays an important role in the HBV life cycle, but there is little knowledge on how core protein interacts with host factors (Nguyen *et al.* 2008). Further study of the interaction between the virus and host is important to understand the HBV life cycle and discover new antiviral targets. A yeast two-hybrid system has shown that the CTD of core protein interacts with the CTD of human filamin B (Huang *et al.* 2000). This interaction has been further confirmed both *in vitro* and *vivo* (Huang *et al.* 2000). It is unknown where the interaction participates in HBV genome packaging, DNA replication, and virion morphogenesis. Filamin A can serve as an adaptor protein to connect the human immunodeficiency virus type I (HIV-1) receptor and the cytoskeleton, promoting HIV infection (Jimenez-Baranda *et al.* 2007; Dotson *et al.* 2016). These studies suggest that filamin B may be involved in HBV infection, so the potential function between the interaction of core protein and filamin B needs to be studied.

In this study, we tested the interaction between filamin B and core protein in different cell lines using co-immunoprecipitation and co-localization immunofluorescence assay. And the influence of this interaction on HBV replication was examined at both RNA and protein levels using overexpression and knockdown strategies. After evaluation of the levels of HBV total RNAs, pre-genome RNA (pgRNA), and the expression levels of hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg), we found that the interaction of filamin B and core protein promotes HBV replication, impairing the interaction down-regulates HBV transcription, replication and protein expression. Lastly, we identified the potential interaction sites in core protein. Taken together, these results strongly indicated that the interaction between filamin B and core protein plays an important role in regulating HBV replication. These data provide new insight into the relationship between HBV and host factors, which may provide new strategies for the treatment of HBV infection.

## Materials and Methods

### Plasmid Constructions and Filamin B siRNAs

The pHBV1.3 (serotype *adw*, genotype B) plasmid was generated from the HBV genome (GenBank accession number JN406371) inserted into the pUC18 plasmid. The Core-HA plasmid contained the HBV core protein gene,

was digested with *Kpn I/Hind III* and inserted into pXJ40-HA. Filamin B-GFP (designated FLNB-GFP) was provided as a generous gift from Professor Arnoud Sonnenberg (Netherlands Cancer Institute). We used the wild-type Core-HA plasmid (designated WT-HA) as a template to generate mutated versions of the Core-HA plasmid (designated M1-HA, M2-HA, M3-HA, M4-HA; each involving one of the four blocks of arginine residues), and the primers for the mutants are listed in Supplementary Table S1. Small interfering RNAs (siRNAs; siFLNB-1, siFLNB-2, siFLNB-3) and the negative control (siNC) were purchased from RiboBio (Guangzhou, China).

### Cell Culture and Transfection

HEK 293T cells, Huh7 cells and HepG2 cells (from the American Type Culture Collection [ATCC]) were maintained in Dulbecco's modified Eagle's medium (Gibco, Shanghai, China) supplemented with 10% fetal bovine serum (Gibco) at 37 °C and 5% CO<sub>2</sub>. Cells were seeded in 6-well plates (4 × 10<sup>5</sup> cells/well) or 12-well plates (2 × 10<sup>5</sup> cells/well) and grown to ~ 70% confluence at the time of transfection. The siRNA and plasmids were transfected into cells using Lipofectamine 2000 reagent (Invitrogen, Shanghai, China).

### Co-Immunoprecipitation Assay

Cells grown in 10-cm<sup>2</sup> culture dishes, and FLNB-GFP or GFP (10 µg) were co-transfected with the Core-HA plasmid (10 µg) using Lipofectamine 2000 reagent according to the manufacturer's protocol. Transfected cells were harvested at 48 h post transfection, then washed with PBS and lysed with 1 mL Nonide P-40 supplemented the 10 µL 100× Cocktail (Biyuntian, Shanghai, China) and 10 µL 100× PMSF (100 mmol/L) (Biyuntian). Cell lysates were centrifuged (13,000 ×g) at 4 °C for 10 min to remove the nuclei, and then incubated with 40 µL proteinA/G (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and 8 µg anti-GFP antibody at 4 °C overnight. After centrifugation, the sediment was washed three times with washing buffer (50 mmol/L Tris [pH 7.4] supplemented with 150 mmol/L NaCl).

### Western Blot Analysis

Cells were lysed in radioimmunology precipitation assay (RIPA) buffer with SDS. The protein samples were then separated by SDS polyacrylamide gel electrophoresis (PAGE) and transferred onto a nitrocellulose membrane (GE Healthcare, Waukesha, WI, USA). The membrane was then blocked with skim milk and probed with antibodies. The antibodies were anti-HA (Santa Cruz Biotechnology),

anti-GFP (Santa Cruz Biotechnology), anti-filamin B (GeneTex, CA, USA), anti-GAPDH (Sigma, St. Louis, MO, USA) and anti-tubulin (Sigma). The immunoblot signals were detected using an enhanced chemiluminescence (ECL) substrate (Bio-Rad, Shanghai, China).

### Immunofluorescence

Cells were cultured on glass coverslips and transfected with FLNB-GFP and Core-HA plasmids respectively, or co-transfected pair-wise, using Lipofectamine 2000 for 24 h. Cells were rinsed three times with PBS, fixed with 4% paraformaldehyde for 30 min, permeabilized with 0.2% Triton X-100 for 15 min, and blocked with 5% bovine serum albumin in PBS for 30 min at room temperature. Then cells were then incubated with polyclonal rabbit anti-HA antibody (Santa Cruz Biotechnology), followed by 5 µg/mL Alexa 568 anti-rabbit antibody (Invitrogen) for 1 h at room temperature. At last, cells were permeabilized and stained with DAPI. Coverslips were mounted using Gel Mount (Biomedica), and the fluorescence was viewed with a Leica confocal microscope (LEICA Spectral Confocal TCS-SL, Serveis Científics Te'cnics de la Universitat de Barcelona). Colocalization between filamin B and core protein was quantified by using Leica confocal microscope software. R values are shown as Pearson correlation coefficient ± SD averaged from 5 to 8 cells.

### Quantitative Real-Time PCR Analysis

Quantitative real-time PCR analysis was performed to determine the level of HBV total RNAs and HBV pgRNA. Cells were grown in 6-well plates and transfected with the following plasmids: pHBV1.3, GFP, and pXJ40-HA; pHBV1.3, Core-HA, and GFP; pHBV1.3, FLNB-GFP, and pXJ40-HA; pHBV1.3, FLNB-GFP, and Core-HA. Total RNAs were isolated from cells using TRIzol Reagent (Invitrogen). The RNA (1 µg) was reverse transcribed with RT primer mix (oligo dT primer and random 6 mers) (TaKaRa). Quantitative PCRs were performed with SYBR Select Master Mix (Life Technologies) using the StepOne Real-Time PCR System (Applied Biosystems). The mRNAs levels were normalized to the *GAPDH* expression level.

PCR was performed at 95 °C for 3 min followed by 40 cycles at 95 °C for 15 s, 58 °C for 30 s, and 72 °C for 30 s. Each set of PCRs was performed in triplicate, and the C<sub>T</sub> values were obtained for each PCR. The ΔΔC<sub>T</sub> method was used to calculate the ratios of gene expression relative to the control set (pHBV1.3, GFP, and pXJ40-HA group) in the experiments. The primers used are list in Supplementary Table S2.

## Enzyme-Linked Immunosorbent Assay (ELISA)

Cells were grown in 6-well plates and transfected with the following plasmids: pHBV1.3, GFP, and pXJ40-HA; pHBV1.3, Core-HA, and GFP; pHBV1.3, FLNB-GFP, and pXJ40-HA; pHBV1.3, FLNB-GFP, and Core-HA. The culture medium was collected at 48 h post transfection. The level of HBsAg and HBeAg in the culture medium was determined using ELISA kits (Beijing Wantai Biotech, Beijing, China) according to the manufacturer's instructions.

## Southern Blot Analysis

Cells were grown in 12-well plates and transfected with the following plasmids: pHBV1.3, GFP, and pXJ40-HA; pHBV1.3, Core-HA, and GFP; pHBV1.3, FLNB-GFP, and pXJ40-HA; pHBV1.3, FLNB-GFP, and Core-HA. For the Southern blot analysis, HBV DNA from intracellular core particles was extracted at 96 h post-transfection. Cells were lysed in 400  $\mu$ L lysis buffer (50 mmol/L Tris–Cl [pH 7.4], 1% Nonidet P-40, 1 mmol/L EDTA) at 4 °C for 30 min and subsequently centrifuged (13,000  $\times$ g) at room temperature. The supernatant was then transferred to fresh tubes and incubated with 10  $\mu$ L DNase I (1 U/ $\mu$ L; Sigma) and 5  $\mu$ L RNase A (10 mg/mL; Sigma) at 37 °C for 2 h. Subsequently, 20  $\mu$ L EDTA (0.5 mol/L) was added to inactivate DNase I, and the mixture was then incubated with 10  $\mu$ L protease K (20 mg/mL Sigma) and 20  $\mu$ L 20% SDS at 55 °C overnight. The digestion mixture was extracted with phenol/chloroform, and DNA was precipitated with ethanol and dissolved in 15  $\mu$ L TE buffer (10 mmol/L Tris–HCl [pH 8.0], 1 mmol/L EDTA). All the extracted DNA was separated on 0.8% agarose gels. After standard denaturation and neutralization procedures, the DNA was transferred onto a positively charged nylon membrane (GE Healthcare, Waukesha, WI, USA) and probed with a digoxigenin (DIG)-labeled HBV RNA probe. The probe preparation and subsequent DIG detection were conducted using the DIG Northern Starter Kit (Roche Diagnostics, Indianapolis, IN, USA) according to the manufacturer's instructions. pHBV1.3 plasmid was used as HBV DNA marker. HBV DNA marker bands are shown for relaxed circular DNA (rcDNA), double stranded DNA (dsDNA), and single stranded DNA (ssDNA). Ratios were quantified by gray analysis using GeneTools from Syngene software (GeneGnome5).

## Results

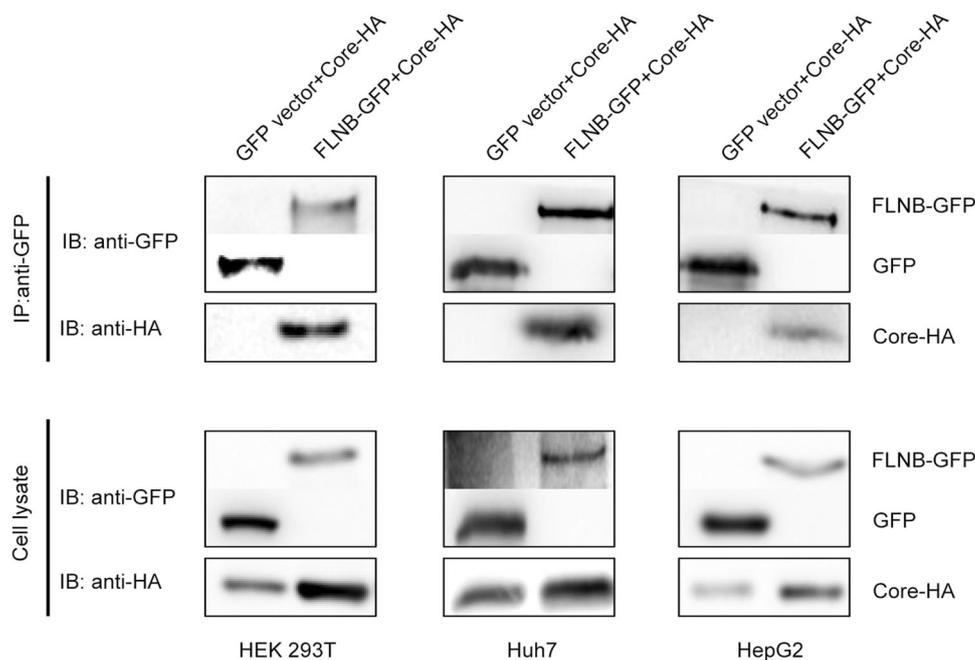
### Filamin B Interacts with Core Protein in Different Cell Lines

It has previously been shown that filamin B interacts with HBV core protein. This interaction between the C-terminal region of filamin B and core protein has been demonstrated both by a pull-down assay *in vitro* and by a co-immunoprecipitation *in vivo* (Huang *et al.* 2000). To verify the interaction between filamin B and core protein, we used co-immunoprecipitation analysis in several cell lines. In this co-immunoprecipitation experiment, filamin B was tagged with GFP (designated FLNB-GFP), and core protein was tagged with HA (designated Core-HA). FLNB-GFP or GFP vectors were co transfected with the Core-HA plasmid into HEK 293T cells (Fig. 1). We used anti-GFP antibody to pull down FLNB-GFP and GFP. The aimed bands were detected by anti-GFP and anti-HA antibodies. The result demonstrated that FLNB-GFP could pull down Core-HA. In contrast, there was no interaction between GFP and Core-HA (Fig. 1). Similarly, this interaction was also detected in Huh7 cells and HepG2 cells (Fig. 1), which are both hepatoma cell lines. These data suggest that full-length filamin B interacted with core protein in all three cell lines.

### Filamin B Colocalizes with Core Protein

To determine whether filamin B colocalizes with core protein in human cells, we performed confocal immunofluorescence microscopy. Filamin B is a cytoskeletal protein and widely distributed across the whole cell, but it is concentrated more in the cytoplasm than the nucleus (Feng and Walsh 2004). Indeed, in cells without core protein expression, filamin B was mainly detected in the cytoplasm (Fig. 2A). The localization of core protein is related to the cell cycle (Yeh *et al.* 1993; Weigand *et al.* 2010) and the level of HBV replication (Chu *et al.* 1997; Serinoz *et al.* 2003), and it existed both in the cytoplasm and nucleus (Fig. 2B). We examined the colocalization of filamin B and core protein. In our study, filamin B and core protein expression plasmids were co-transfected pair-wise into HEK 293T cells, and a significant portion of core protein colocalized with filamin B in the cytoplasm (Fig. 2C). We also found colocalization in Huh7 and HepG2 cells (Fig. 2C). The colocalization rates were examined and Pearson correlation coefficient R values were 0.71, 0.83, and 0.79 for 293T, Huh7 and HepG2 cells, respectively. This revealed that filamin B colocalized with core protein in human cells, and further confirmed that filamin B interacted with core protein.

**Fig. 1** Filamin B interacts with core protein in different cell lines. Co-immunoprecipitation of filamin B and core protein is shown. Either FLNB-GFP plasmid or GFP vector (10  $\mu$ g) was co-transfected with 10  $\mu$ g Core-HA plasmid into HEK 293T, Huh7, and HepG2 cells. Filamin B was immunoprecipitated using GFP. Co-immunoprecipitated core protein was detected by immunoblotting with anti-HA antibodies (top). Western blot analysis of the precipitated filamin B and GFP using anti-GFP antibodies is shown (top). Cell lysates before immunoprecipitation were probed with anti-GFP and anti-HA antibodies (bottom).



### Interaction between Filamin B and Core Protein Promotes HBV Replication

Real-time PCR revealed that total RNAs and pgRNA of HBV were remarkably up-regulated 6–8-fold (pHBV1.3, FLNB-GFP, and Core-HA group) in comparison to the control (pHBV1.3, GFP, and pXJ40-HA group) in Huh7 and HepG2 cells (Fig. 3A, B). ELISA assay showed that the secretion level of HBeAg and HBsAg in the culture medium of Huh7 and HepG2 cells increased (Fig. 3C, D). Furthermore, Southern blot analysis suggested that the interaction significantly promoted HBV DNA replication (Fig. 3E). The control group (pHBV1.3, GFP, and pXJ40-HA) was set at a value of 1, and the other two groups (pHBV1.3, FLNB-GFP, pXJ40-HA group and pHBV1.3, FLNB-GFP, Core-HA group) increased 1.7–2.8-fold in comparison to the control group. The Western blot analysis indicated that filamin B was overexpressed. The real-time PCR and ELISA data showed that co-transfection with pHBV1.3 and Core-HA had no significant effect on HBV replication, so the Southern blot analysis did not involve this control experiment. These results indicated that the interaction between filamin B and core protein contributed to viral replication.

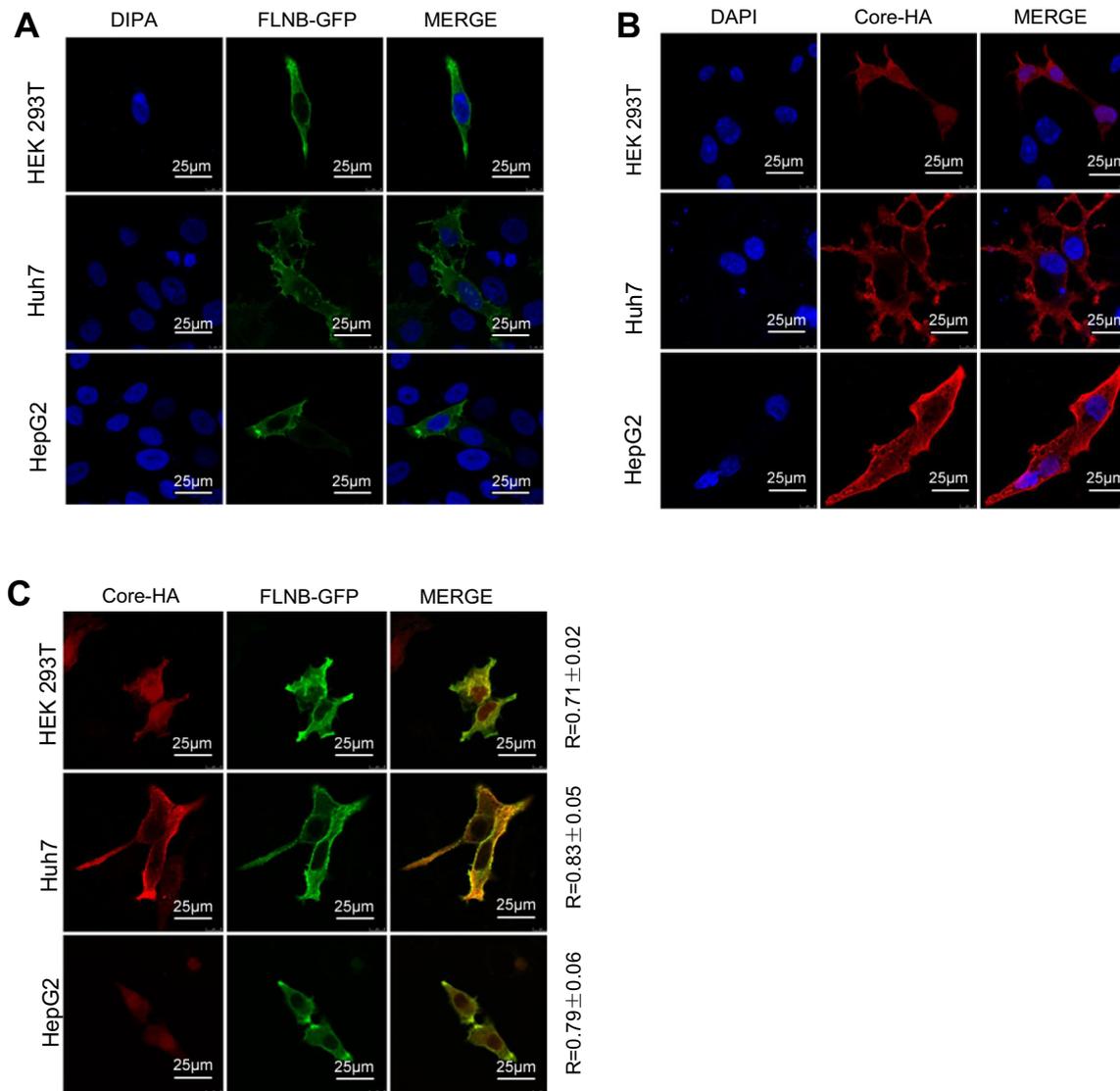
### Filamin B Knockdown Inhibits the Replication of HBV

To further elucidate the role of the interaction in HBV replication, the Huh7 and HepG2 cells were transfected with filamin B-targeting siRNAs (siFLNB-1, siFLNB-2,

and siFLNB-3) or siRNA negative control (siNC). The knockdown efficiency was detected using Western blotting, which indicated that all the siRNAs showed strong knockdown effects but siFLNB-2 was more effective than siFLNB-1 and siFLNB-3. This was consistent between Huh7 and HepG2 cells (Fig. 4A). We chose to use siFLNB-2 in our subsequent experiments. Real-time PCR results revealed that knockdown of filamin B remarkably reduced the level of HBV total RNAs and pgRNA in Huh7 and HepG2 cells (Fig. 4B, C). Consistently, the secretion level of HBeAg and HBsAg in culture supernatant (pHBV1.3 and siFLNB-2 group) decreased compared with the control cells (pHBV1.3 and siNC group) (Fig. 4D, E). In addition, the Southern blot results suggested that HBV replication decreased in filamin B knockdown cells (Fig. 4F). The gray analysis was executed by Syngene software (GeneGnome5), and the pHBV1.3 and siNC group was used as the control group and set at a value of 1. The other group (pHBV1.3 and siFLNB-2 group) decreased by 0.38–0.55-fold in comparison to the control group. The Western blot results indicated that filamin B was knocked down. Taken together, these data suggested that filamin B knockdown inhibits HBV replication.

### Potential Interaction Sites in Core Protein Are Identified

The C-terminus of core protein contains four blocks of arginine residues, which play an important role in viral DNA synthesis and genome packing (Yu and Summers 1991; Hatton *et al.* 1992). Therefore, we constructed four

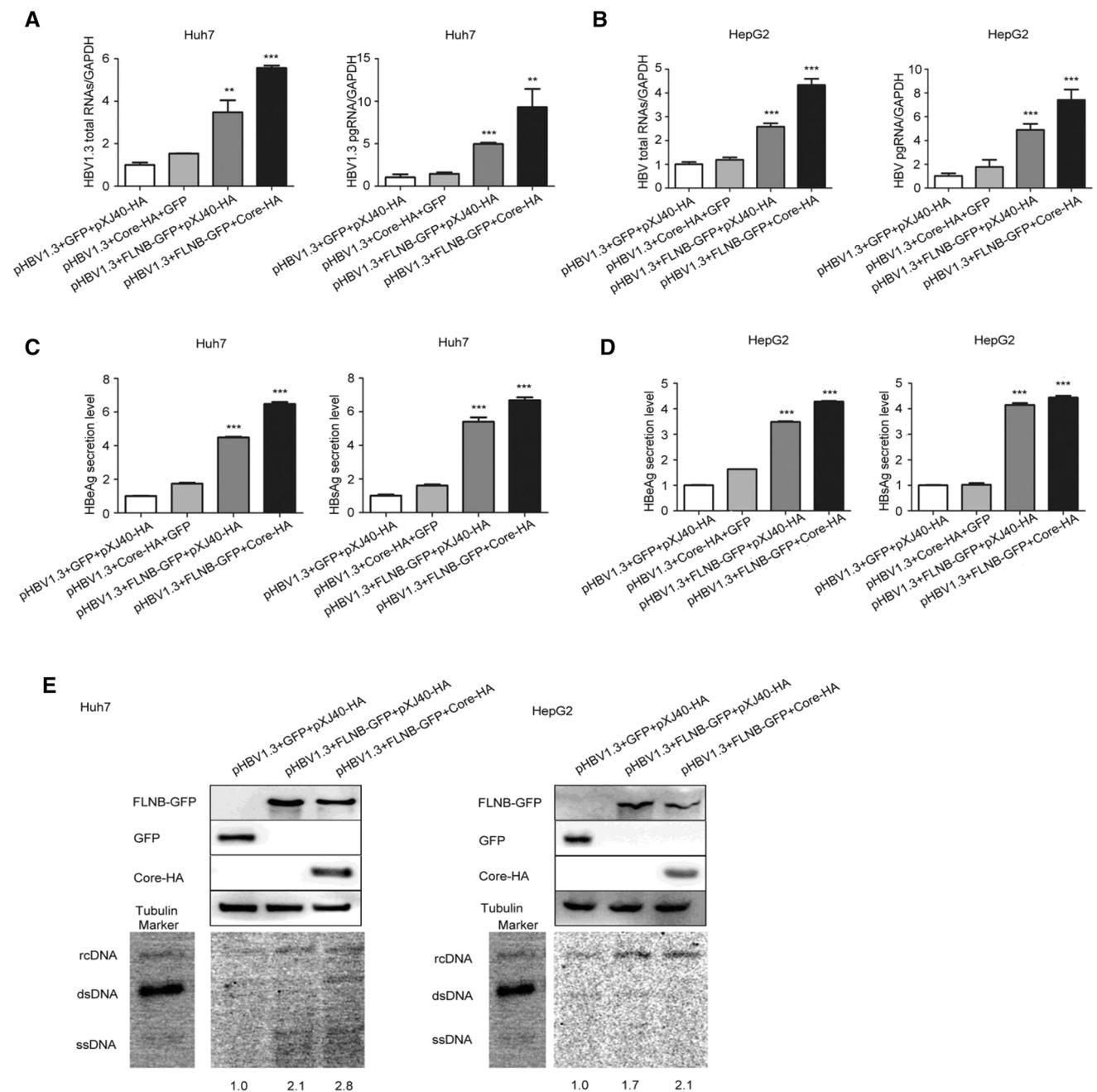


**Fig. 2** Filamin B colocalizes with core protein. (A) Confocal analysis of filamin B subcellular localization is shown in green. Cells were transfected with 2  $\mu$ g FLNB-GFP plasmid. At 24 h post transfection, fixed cells were permeabilized and stained with DAPI. (B) Confocal analysis of core protein subcellular localization is shown in red. Cells were transfected with 2  $\mu$ g of Core-HA plasmid. At 24 h post transfection, fixed cells were permeabilized and immunostained for core protein using anti-HA antibodies and fluorescent secondary antibodies. (C) Immunofluorescence analysis of the colocalization of

filamin B and core protein is shown. Cells were transfected with 1  $\mu$ g FLNB-GFP plasmid and 1  $\mu$ g Core-HA plasmid. At 24 h posttransfection, fixed cells were permeabilized and immunostained for core protein using anti-HA antibodies and fluorescent secondary antibodies. Filamin B is shown in green (left), core protein in red (center), and colocalization in yellow (right). Scale bars represent 10  $\mu$ m. Colocalization between filamin B and core protein was quantified using Leica confocal microscope software. R values are the Pearson correlation coefficient  $\pm$  SD averaged from 5 to 8 cells.

mutants, each involving one of the four blocks of arginine residues (Fig. 5A). Western blot results confirmed that each mutant was expressed normally (Fig. 5B). In the immunoprecipitation experiment, core protein mutants were tagged using HA, and they were designated M1-HA, M2-HA, M3-HA and M4-HA. FLNB-GFP or GFP vector were co transfected with the Core-HA plasmid or one of the mutant plasmid into HEK 293T cells. This immunoprecipitation result demonstrated that FLNB-GFP could pull down Core-HA, but it not the mutants (Fig. 5C). The

result indicates that a mutation in any of the arginine residue blocks can prevent the interaction between filamin B and core protein. Based on this result, we can preliminarily conclude that the four blocks of arginine residues in core protein are potential interaction sites.



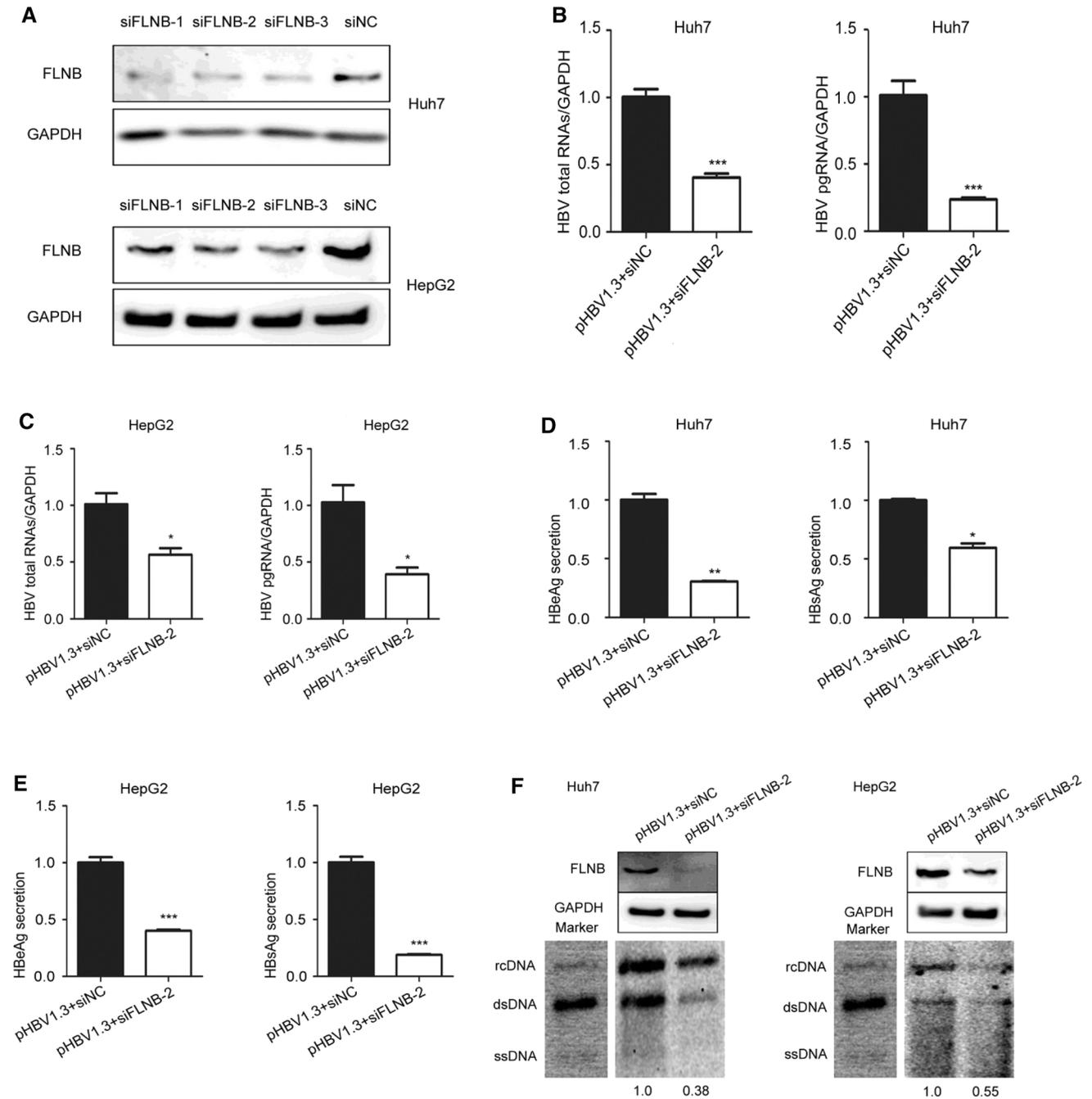
**Fig. 3** Interaction of filamin B and core protein promotes HBV replication. (A) Levels of HBV total RNAs and pgRNA in Huh7 cells were determined by real-time PCR. (B) HBV total RNAs and pgRNA levels in HepG2 cells were determined by real-time PCR. (C) Levels of HBeAg and HSbAg in culture medium supernatant of Huh7 cells were detected with ELISA. (D) Levels of HBeAg and HSbAg in culture medium supernatant of HepG2 cells were detected with ELISA. (E) HBV DNA was extracted at 96 h post-transfection and

subjected to Southern blotting. Western blot showed that all proteins were overexpressed. Co-transfection with pHBV1.3, GFP and pXJ40-HA was used as a loading control. Ratios were quantified by gray analysis with GeneTools from Syngene software (GeneGnome5). HBV DNA marker bands are shown for relaxed circular DNA (rcDNA), double stranded DNA (dsDNA), and single stranded DNA (ssDNA). All results are shown as mean  $\pm$  SD. (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ).

## Discussion

Filamin B, a cytoskeleton protein, serves as an important molecular scaffold for the connection and coordination of various cellular processes (Stossel *et al.* 2001). It has been

reported that the C-terminal region of filamin B interacts with HBV core protein. In addition, core protein plays an important role in the HBV life cycle (Hatton *et al.* 1992), which necessitates that the function of the interaction between filamin B and HBV core protein be clarified. Our

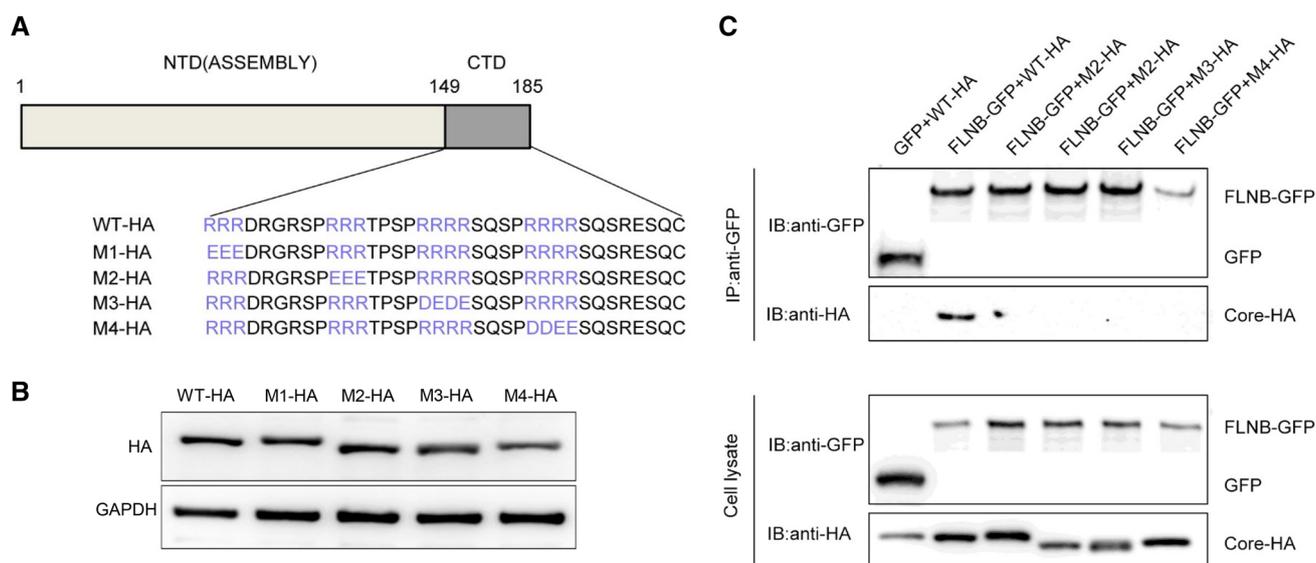


**Fig. 4** Filamin B knockdown inhibits the replication of HBV. (A) Huh7 and HepG2 cells were transfected with siNC, filamin B-targeting siFLNB-1, siFLNB-2 or siFLNB-3 separately. Western blot analysis of filamin B was used to confirm the silencing efficiency. (B) Levels of HBV total RNAs and pgRNA in Huh7 cells were determined by real-time PCR. (C) Levels of HBV total RNAs and pgRNA in HepG2 cells were determined by real-time PCR. (D) Levels of HBsAg and HBsAg in culture medium supernatant of Huh7 cells were detected with ELISA. (E) Levels of HBsAg and

HBsAg in culture medium supernatant of HepG2 cells were detected with ELISA. (F) HBV DNA was extracted at 96 h post transfection, and then subjected to Southern blotting. Western blot showed that filamin B was successfully knocked down. Co-transfection with pHBV1.3 and siNC was used as a loading control. Ratios were quantified by gray analysis with GeneTools from Syngene software (GeneGnome5). rcDNA, relaxed circular DNA; dsDNA, double stranded DNA; ssDNA, single stranded DNA. All results are shown as mean  $\pm$  SD. (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ).

findings confirm filamin B is an important host factor in the HBV life cycle, and strongly indicate that the interaction between filamin B and core protein plays an important role

in regulating HBV replication, which provide new insight into the relationship between HBV and host factors.



**Fig. 5** Potential interaction sites in core protein are shown. (A) Schematic presentation of Core-HA mutants. (B) Western blot analysis was performed to detect the expression of the mutants. (C) Co-immunoprecipitation assay was performed to confirm the interaction between filamin B and core protein mutants. A filamin B plasmid was co-transfected with a wild-type or mutated core protein

To confirm the interaction between filamin B and HBV core protein, we also used Core-HA to pull down FLNB-GFP to examine the specific interaction between them, but the immunoprecipitation experiment failed. We propose that the molecular weight of FLNB-GFP (305 kD) and core protein (25 kD) might be too different, making it difficult for the small core protein to pull down the large FLNB-GFP. Zhao *et al.* investigated the specific interaction between the host factor ceruloplasmin (CP, 130 kD) and HBV middle surface protein (MHB, 35 kD), and they had similar problems with the reverse immunoprecipitation experiment, which indicated that MHB could not pull down CP (Zhao *et al.* 2017). Based on our results and the results from the other lab, it is hypothesized that molecular weight may affect the pull-down result during immunoprecipitation experiments, which should be investigated in a further study.

In this study, we found that the co-transfection groups (FLNB-GFP, Core-HA group) expressed a higher level of core protein than the control group (GFP, Core-HA group). We propose that filamin B may be involved in modulating capsid assembly and stability and the expression of core protein. As reported by Liu *et al.*, the interaction between Dicer-2 (DCR-2) and *Caenorhabditis elegans* RNAi protein RDE-4 homolog (R2D2) could stabilize both proteins in *Drosophila* S2 cells (Liu *et al.* 2003). Therefore, it is reasonable to hypothesize that filamin B promotes the stability of core protein. Through further efforts are needed to clarify this conclusion. This study showed that the

plasmid into HEK 293T cells. Filamin B was immunoprecipitated using GFP. Co-precipitated core protein was detected by immunoblotting with anti-HA antibodies (top). Western blot analysis of the co-precipitated filamin B and GFP is shown using anti-GFP antibodies (top). Cell lysates before immunoprecipitation were probed with anti-GFP antibodies (bottom).

interaction between filamin B and core protein promotes HBV replication. However, whether filamin B, core protein, or both influence each other during this process is still unknown. However, we know that the ubiquitination of filamin B is different between a normal hepatoma cell line and an HBV-expressing hepatoma cell line. Thus, core protein may regulate HBV replication by influencing filamin B.

Filamin A is similar to filamin B, and it can interact with HIV-1 Gag protein. Subsequently, the interaction could contribute to viral particle assembly (Cooper *et al.* 2011). Gag protein is an essential HIV component in terms of HIV assembly and release (Wills and Craven 1991; Freed 1998), which is similar to the function of core protein in HBV life cycle. Therefore, filamin B may contribute to the assembly of core protein into a nucleocapsid.

As a pleiotropic keystone in the HBV life cycle, core protein can self-assemble to form the viral capsid, which can be targeted by antivirals (Zlotnick *et al.* 2015). NVR3-778, a new drug targeting the HBV capsid, has high antiviral activity in mice with humanized livers suffering from stable HBV infection (Klumpp *et al.* 2017). Similarly, filamin B is a potential drug target, as targeting it would disrupt the formation of the HBV capsid.

In conclusion, this study further validated the interaction between filamin B and HBV core protein, suggesting that filamin B may be an important host factor that targets core protein, which provides a new insight to understand the interaction between HBV and host factors. In addition, due

to the properties of the interaction between filamin B and HBV core protein, filamin B is a potential drug target.

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## Compliance with Ethical Standards

**Conflicts of interest** The authors declare that they have no conflict of interest.

**Animal and Human Rights Statement** This article does not contain any studies with human or animal subjects performed by any of the authors.

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