PRMT5 Restricts Hepatitis B Virus Replication via Epigenetic Repression of cccDNA Transcription and Interference with pgRNA Encapsidation

Short title: PRMT5 Inhibits HBV cccDNA Transcription and pgRNA Encapsidation

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List of Abbreviations:

HBV, hepatitis B virus; cccDNA, covalently closed circular DNA; H3, histone 3; H4, histone 4; PRMT5, protein arginine methyltransferase 5; H4R3me2s, symmetric dimethylation onhistone 4 arginine 3; pgRNA, pre-genomic RNA; rcDNA, relaxed circular DNA; HBV Pol, HBV polymerase; IT, immunetolerant; IC, inactive carrier; HBc, HBV core protein; dHepaRG, differentiated HepaRG; shPRMT5, PRMT5-shRNA; ChIP, chromatin immunoprecipitation; H3Ac, H3 acetylation; hSWI/SNF, human SWI/SNF; RIP, RNA immunoprecipitation; H4R3me2a, asymmetric dimethylation on H4R3; H3K9me3, H3K9 trimethylation

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Abstract

Chronic hepatitis B virus (HBV) infection remains a major health problem worldwide. The covalently closed circular DNA (cccDNA) minichromosome, which serves as the template for the transcription of viral RNAs, plays a key role in viral persistence. While accumulating evidence suggests that cccDNA transcription is regulated by epigenetic machinery, particularly the acetylation of cccDNA-bound histone 3 (H3) and H4, the potential contributions of histone methylation and related host factors remain obscured. Here, by screening a series of methyltransferases and demethylases, we identified protein arginine methyltransferase 5 (PRMT5) as an effective restrictor of HBV transcription and replication. In the cell culture-based models for HBV infection and the liver tissues of patients with chronic HBV infection, we found that symmetric dimethylation of arginine 3 on H4 (H4R3me2s) on cccDNAwas a repressive marker of cccDNA transcription and was regulated by PRMT5 depending on its methyltransferase domain. Moreover, PRMT5-triggered H4R3me2s on the cccDNA minichromosome involved an interaction with the HBV core protein and the Brg1-based hSWI/SNF chromatin remodeler, which resulted in the downregulation of the binding of RNA Pol II to cccDNA. In addition to the inhibitory effect on cccDNA transcription, PRMT5 inhibited HBV core particle DNA production independent of its methyltransferase activity. Further study revealed that PRMT5 interfered with pre-genomic RNA (pgRNA) encapsidation by preventing its interaction with viral polymerase protein through binding to the RT-RH region of polymerase which is crucial for the polymerase-pgRNA interaction. *Conclusion*: PRMT5 restricts HBV replication via two-part mechanisms including epigenetic suppression of cccDNA transcription and interference with pgRNA encapsidation. These findings improve the understanding of epigenetic regulation of HBV transcription and

host-HBV interaction, thus provide new insights into targeted therapeutic intervention.

Hepatitis B virus (HBV) is a hepatotropic partially double-stranded DNA virus. HBV infection remains a serious global health problem with more than 240 million people chronically infected. Upon infection, relaxed circular DNA (rcDNA) in the HBV virion is delivered into the nucleus and converted into covalently closed circular DNA (cccDNA), which then serves as a template to transcribe pre-genomic RNA (pgRNA) and other subgenomic viral RNAs. The 5'-ε signal within pgRNA is recognized and bound by HBV polymerase (Pol), which is required for subsequent pgRNA encapsidation into a newly forming capsid with HBV core proteins in the cytoplasm. Mature nucleocapsids with HBV rcDNA which is reversely transcribed from pgRNA are either released from the cell after being enveloped with HBs proteins or recycled to the cccDNA reservoir in the nucleus. The cccDNA is crucial for persistent HBV infection and recurrence upon the discontinuation of treatment. Current therapies successfully suppress viral replication, but their inhibitory effects on stably existing cccDNA are limited. Identification of the mechanisms and factors involved in silencing or elimination of cccDNA could allow the development of more precisely targeted therapeutic strategies(1).

The cccDNA organizes intoa minichromosome with histone 3 (H3) and H4 proteins and non-histone proteins, such as HBV core protein (HBc), HBx and host transcription factors(2-4). Accumulating evidences have suggested that the host epigenetic mechanisms, including DNA methylation and DNA-bound histones modifications, play vital roles in the regulation of cccDNA transcriptional activity(5-6).Previous studies have suggested that the acetylation (Ac) status of H3 and H4 on the HBV minichromosome is associated with cccDNA transcriptional activity, and

histone acetyltransferases CBP/p300 and the deacetylase HDAC1 are involved in the regulation of HBV transcription(4, 7). Moreover, it has been shown that IFN-α can result in hypoacetylation of cccDNA-bound histones and recruitment of transcriptional co-repressors to cccDNA(7-9). In addition to histone acetylation, histone methylation can also regulate DNA transcription and has been shown to be related to the replication of viruses such as HSV-1, EBV and HCMV, whose nucleic acids also organize into nucleosomal structures(10-12). However, the effect of histone methylation on regulation of HBV cccDNA function and the host factor(s) involved remain obscured.

Here, by screening a series of methyltransferases and demethylases, we identified protein arginine methyltransferase 5 (PRMT5) as a restrictor of HBV replication that can preferentially catalyze the symmetric dimethylation of arginine 3 on H4 (H4R3me2s) on cccDNA through its methyltransferase activity to epigenetically silence cccDNA transcription, and, in a methyltransferase-independent manner, interferes with HBV pre-genomic RNA encapsidation and thus inhibits HBV DNA production.

Materials and Methods

Cell culture, Viruses and Infection The HBV-producing stable cell line HepG2.117 and the HepG2-NTCPwere kindly provided by Prof. Mengji Lu (University Hospital Essen) and Prof.Stephan Urban (University Hospital Heidelberg), respectively. The HepaRG cells and Primary

Human Hepatocytes (PHHs) were purchased from Biopredic International (France) and RIDL (China), respectively. The Huh7 and HEK293 cells were obtained from the Cell Bank of the Chinese Academy of Sciences. The culture of the cells are described in the supplementary materials. The HBc protein deficient virus (HBV-ΔHBc), Adeno-PRMT5, Adeno-PRMT5Δ, Adeno-shPRMT5, Adeno-NES-PRMT5 and Adeno-shBrg1 were produced as described in the supplementary materials. HBV infection of dHepaRG cells mainly followed the published protocols(13).The dHepaRG cells were transduced by incubating with the indicated adenovirus vectors for 16 hrs.

Plasmids and Reagents Plasmidsused for transfection are listed in Supporting Table 3. The constructions of the plasmids are described in the supplementary materials**.** The antibodies used in this study are listed in Supporting Table 4. The small interfering RNAs (siRNAs) targeting the indicated genes (Supporting Table 5) and the control siRNA were purchased from Ribobio.

Patients The clinical and virological characteristics of the patients enrolled in this study are summarized in Supporting Table 2. The liver tissue samples were obtained by percutaneous needle biopsy, immediately frozen in liquid nitrogen and stored at -80°C. Written informed consent was obtained from all subjects prior to participation, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved and supervised by the ethics committee of the Shanghai Public Health Clinical Center of Fudan University.

HBV cccDNA and HBV core particle DNA extraction and quantitation by qPCR HBV cccDNA was extracted according to the method described previously with minor modifications(8). An aliquot of the extracted nuclear DNA was treated with Plasmid-safe DNase (Epicentre, USA) for 45 mins at 37°C, 30 mins at 65°C and then analyzed for cccDNA by Taqman PCR amplification (Toyobo, Japan). The cytoplasmic cell lysate was digested by DNase I and then precipitated with PEG8000 for overnight, the core particles were incubated in PK buffer at 56°C for 2 hrs. The precipitated core particle DNA was analyzed by qPCR or Southern blot. The levels of HBV cccDNA and core particle DNA are normalized to genomic GAPDH.

HBV core particle RNA extraction Encapsidated viral pgRNA was purified as described in the supplementary materials and the core particle pgRNA was extracted using TRIzol reagent and was detected by Northern blot.

Analysis of core capsids The cytoplasmic cell lysates containing HBV capsids were directly resolved on a 1%agarose gel in 0.5×TBE buffer and transferred to nitrocellulose membranes. The proteins were then detected by Western blot using anti-HBcAg antibody (DAKO) (13).

Southern blot/Northern blot The extracted DNA and extracted RNA was loaded on a 1.2%

agarose gel in 1×TAE buffer or on formaldehyde denaturing agarose gel, respectively, and then transferred onto a positively charged nylon membrane (Roche, Switzerland) using a vacuum blotter (Bio-Rad, USA). HBV DNA and RNA were hybridized and detected using a DIG Northern Starter Kit (Roche, Switzerland) according to the manufacturer's instructions(14).

Chromatin immunoprecipitation (ChIP) assay ChIP assays were performed using the method described previously with minor modifications(8).The protein-DNA complexes were immunoprecipitated with the indicated antibodies and normal IgG was conducted in each experiment to exclude the non-specific binding. The precipitated DNA was analyzed by qPCR and the results were presented as percentages of input DNA. The strategies for quantification and calculation are described in Fig S4.

Real-time reverse transcription-PCR (RT-qPCR) Total RNA was extracted using TRIzol reagent and reverse transcribed using ReverTra Ace qPCR RT Master Mix with gDNA Remover kit followed by quantitative PCR using Thunderbird SYBR qPCR Mix (both from Toyobo, Japan). The primers used are listed in Supporting Table 6. The pgRNA primers can also amplify HBV preC RNA. The quantification of HBV pgRNA is normalized to 18 s rRNA.

Co-immunoprecipitation (CoIP) and Western blot The cells were lysed on ice in CoIP lysis buffer.

After centrifugation, the supernatant was pre-cleared with protein A/G plus agarose beads. The protein complexes were then immunoprecipitated with the indicated antibodies and analyzed by Western blot using the specific antibodies (Supporting Table 4).

Immunofluorescence assay Immunofluorescence assay was performed as described previously (15). The fluorescence was observed under a confocal fluorescence microscope (Leica TCS SP2).

RNA immunoprecipitation (RIP) assay RIP assay was performed using the method described previously with minor modifications(16). Immunoprecipitated pgRNA was quantified by qPCR and represented as percentages of input pgRNA.

Statistics The data are presented as the mean±SEM. Statistical analyses were performed using Student's t test or Mann-Whitney U test. A value of $P<0.05$ was considered statistically significant (*$P<0.05$; **$P<0.01$).

Results

Identification of PRMT5 as an anti-HBV host factor that restricts cccDNA transcription and core particle DNA production

To examine whether HBV transcription and replication is associated with histone methylation and to identify the host factors involved, we applied the RNA interference-based approach to screen a panel of the known histone methyltransferases or demethylases, including SUV39H, G9a, EZH2, PRMT5, LSD1, KDM5A and KDM2A (Fig S1A and Supporting Table 1, asterisk), which have been reported to catalyze histone methylation or demethylation to result in transcriptional repression(17-19). The monomeric linear HBV DNA-transfected model was used (4) in which the HBV minichromosome is proved to be formed and the expression levels of HBe and HBs antigens could serve as surrogate markers for the transcription of cccDNA. Since the result suggested that PRMT5 knockdown led to significant up-regulation of both HBeAg and HBsAg (Fig 1A) and PRMT5 inhibits the production of pgRNA and core particle DNA in a dose-dependent manner (Fig S1B-D), we further focused on the PRMT5-mediated suppression of HBV. By using the HepaRG model for HBV infection, we showed that overexpression or knockdown of PRMT5 significantly decreased or increased the level of HBeAg and HBsAg in the supernatant and the intracellular pgRNA and the HBV core particle DNA in the cytoplasm (Fig 1B), respectively, and regulated the cccDNA transcriptional activity (Fig 1C), which was calculated as the ratio of pgRNA to cccDNA. Considering that the efficacy of HBV infection in dHepaRG cells was relative low which might lead to an underestimated anti-HBV effect of PRMT5 when we analyzed at the whole cell lysate level, we further evaluated the effect at the single cell level by immunofluorescence assay (Fig 1D and S1F) and in HBV-infected HepG2-NTCP cells (Fig 1E) and PHHs (Fig S1E). The results confirmed the role of PRMT5 in suppression of the cccDNA transcription and core particle DNA production, and suggested that the efficacy of PRMT5 for HBV suppression is greater than 80% at the single cell level.

Considering that PRMT5, a member of type II protein arginine methyltransferases, epigenetically regulates gene expression mainly through methylating histone and non-histone targets, we next examined whether the enzymatic activity of PRMT5 is essential for its inhibitory effect on cccDNA transcription and HBV DNA production. The mutation of the methyltransferase domain of PRMT5 (PRMT5△, Fig 2A and S2A) markedly diminished the inhibitory effect of PRMT5 on viral antigen expression and cccDNA transcription (Fig 2B-D and S1G). Surprisingly, PRMT5△ retained the majority of its capability to decrease the level of cytoplasmic core particle DNA as the wildtype PRMT5 (Fig 2E and S2B), suggesting that PRMT5 inhibits cccDNA transcription dependent on its methyltransferase activity while inhibits HBV core particle DNA production in a methyltransferase-independent manner.

PRMT5 increases the symmetric dimethylation of cccDNA-bound H4R3 to inhibit cccDNA transcription in a methyltransferase activity-dependent manner.

Since H3R8me2s and H4R3me2s are reported to be catalyzed by PRMT5(17, 20), we speculated whether the status of H3R8me2s and H4R3me2s on cccDNA correlate with the cccDNA transcriptional activity. In HBV-infected dHepaRG model, the cccDNA transcriptional activity (pgRNA/cccDNA) was shown to fluctuate during the natural HBV infection (Fig 3A, S3A and S3B). By chromatin immunoprecipitation (ChIP) analysis, we found that the status of H4R3me2s on the HBV minichromosome was negatively correlated with cccDNA transcriptional activity

during HBV replication (Fig 3B, red line and black line) whereas H3R8me2s was almost undetectable on the HBV minichromosome throughout the whole period of viral infection (Fig S3C). As the control, the status of H4R3me2s on the transcription start site (TSS) of host genes such as GAPDH was relatively stable (Fig 3B, blue line). We repeated this experiment three times with sera from different chronic HBV patients. Although the kinetics of cccDNA transcriptional activity varied a bit in the three experiments, they were always negatively associated with the status of H4R3me2s on the HBV minichromosome.

To further validate the effect of cccDNA-bound H4R3me2s on HBV transcription *in vivo*, we collected liver biopsies from ten chronic HBV patients including five HBeAg-positive patients in immune-tolerant phase (IT) and five HBeAg-negative inactive carriers (IC) to analyze the association of the status of cccDNA-bound H4R3me2s with the levels of cccDNA transcriptional activity. The level of pgRNA/cccDNA in IC patients was, as expected, significantly lower than that in IT patients (Fig 3C), whereas the average level of cccDNA-bound H4R3me2s was about 3-fold higher in IC patients than in IT patients. Moreover, the level of H4R3me2s on cccDNA varied more greatly than that on the host GAPDH DNA (Fig 3D). The level of H3Ac on cccDNA, which was reported to be involved in the regulation of HBV replication in the liver tissue (7), was no significant correlation with the cccDNA transcriptional activity in the ten patients (Fig S5A), which might be attributed to different inclusion criteria for HBeAg status and the ALT level of the patients.

We further examined whether alteration of PRMT5 expression affects the status of cccDNA-bound H4R3me2s. Overexpression or knockdown of PRMT5 led to increased or reduced binding of PRMT5 to cccDNA and accordingly increased or reduced the level of H4R3me2s on the minichromosome and cccDNA transcription activity, respectively (Fig 4A and 4B). In addition, although the level of cccDNA-bound PRMT5△ was comparable with that of cccDNA-bound wildtype PRMT5, neither the status of H4R3me2s nor the cccDNA transcriptional activity was affected by the increased expression of PRMT5△ (Fig 4C). Together, these results implicate that cccDNA-bound H4R3me2s is a repressive marker of cccDNA transcription and PRMT5 can be recruited to cccDNA and, in a methyltransferase-dependent manner, catalyzes H4R3me2s modification on cccDNA to repress cccDNA transcription.

PRMT5 preferentially regulates H4R3me2s on cccDNA and interacts with the HBV core protein

Since H4R3me2s was present not only on cccDNA minichromosome but also on host genome, we next compared the effect of PRMT5 on cccDNA- or host genes-bound H4R3me2s. The histone-DNA complexes were immunoprecipitated using antibodies specifically against H4R3me2s followed by qPCR analysis with primers designed to detect cccDNA and three host genes including RPL30, MYOD1 and GAPDH. The results showed that overexpression or knockdown of PRMT5 significantly elevated or reduced the level of H4R3me2s as well as PRMT5 on HBV cccDNA minichromosome, respectively, but had much less impact on those host

genes studied here (Fig 5Aand5B), implying that PRMT5 can, to some extent, preferentially bind to cccDNA and catalyze cccDNA-bound H4R3me2s.

The HBV core and HBx proteins have been shown to be associated with the HBV minichromosome (3-4, 21) (Fig S8A) and thus were candidates to mediate the preferential binding of PRMT5 to cccDNA. Therefore, we further clarified whether there is any interaction between PRMT5 and HBc or HBx proteins. While no interaction between PRMT5 and HBx (Fig S8B) was observed, the CoIP results suggested that both ectopically-expressed and endogenous PRMT5 interacted with the HBV core protein (Fig 5C and S8C-F). In addition, the ectopically-expressed HBc was mainly distributed in the nucleus and was partially co-localized with the nucleus-distributed PRMT5 (Fig 5D). To further examine the role of HBc in the binding of PRMT5 to cccDNA, we constructed the HBc-deficient virus (HBV-△HBc) and found that less PRMT5 was bound to the cccDNA of HBV-△HBc compared to that of HBV-WT. Moreover, overexpression of PRMT5 resulted in significant upregulation of the level of cccDNA-bound PRMT5 in the HBV-WT group but not in the HBV-△HBc group (Fig 5E). These results provide a possibility that PRMT5 is targeted to HBV cccDNA through its interaction with the viral core protein.

PRMT5-mediated suppression of cccDNA transcription involves the Brg1-based hSWI/SNF chromatin remodeler and RNA Pol II

It has been reported that PRMT5 acts as a part of a variety of complexes to regulate gene transcription and cell signal transduction(22). Among these complexes, the human SWI/SNF (hSWI/SNF) chromatin remodeler is mainly involved in PRMT5-catalyzed histone methylation, which results in epigenetic gene silencing(23). The hSWI/SNF chromatin remodeler is a multi-subunit complex that can be divided into two distinct complexes called PBAF and BAF. The central core of the PBAF complex is the Brg1 ATPase subunit, and the BAF complex contains either hBrm or Brg1 ATPase subunits(24). Since HBV cccDNA transcriptional activity is suppressed by PRMT5-catalyzed H4R3me2s, we speculated whether the hSWI/SNF chromatin remodeler is involved in the inhibition of cccDNA transcription by PRMT5. We thus evaluated the changes in the association of Brg1 and hBrm with cccDNA when we altered the expression levels of PRMT5 in HBV-infected dHepaRG cells. The results showed that the binding of Brg1 to cccDNA significantly increased or reduced when PRMT5 was overexpressed or knockdown, respectively(Fig 6A). Unlike Brg1, the binding of hBrm to cccDNA did not significantly change with the alteration of PRMT5 expression (Fig S9). Furthermore, knockdown of the Brg1 significantly restored the PRMT5-mediated suppression of cccDNA transcription (Fig 6B), which confirms the role of Brg1 in PRMT5-mediated repression of HBV cccDNA transcription.

Since cccDNA is transcribed by the host RNA polymerase II (Pol II) (25), whose enrichment on cccDNA is reported to be associated with the active posttranscriptional modifications on cccDNA-bound histones (26), we further examined whether PRMT5 could regulate its binding to cccDNA. As expected, the binding of RNA Pol II to cccDNA was negatively regulated by PRMT5

in HBV-infected dHepaRG cells, while the enrichment of RNA Pol II on the three host genes was not much affected (Fig 6C). Together, the above data suggest that PRMT5 regulates the binding of the host Brg1-based hSWI/SNF chromatin remodeler and RNA Pol II to cccDNA, which may contribute to the PRMT5-mediated epigenetic suppression of cccDNA transcription.

PRMT5 inhibits HBV core particle DNA production in a methyltransferase activity-independent manner

As indicated above, PRMT5△ maintained the activity of inhibiting the production of core particle DNA but did not affect cccDNA transcription (Fig 2)**.** To confirm this, we further tested the levels of HBV core capsids, RNAs and core particle DNA in a HBV-replicating cell model, in which the viral pgRNA is transcribed under the CMV promoter. Southern blot results showed that PRMT5△ expression or knockdown of endogenous PRMT5 decreased or increased the level of core particle DNA in a dose-dependent manner, respectively, while it did not markedly affect the levels of HBV total RNA and core capsids (Fig 7A, S10A). Similar results were obtained in stable HBV replicating cell line HepG2.117 (Fig 7B). To further clarify the step at which PRMT5 inhibits HBV core particle DNA production, we constructed cytoplasm-or nucleus-localized PRMT5 △ . The expression of both NES-PRMT5 △ and NLS-PRMT5 △ in CMV-HBV-replicating Hun7 cells did not reduce the levels of HBV RNAs and core capsids, however, the cytoplasm-localized, but not the nucleus-localized PRMT5△, significantly decreased the core particle DNA levels (Fig 7C). In HBV-infected dHepaRG cells, the cytoplasm-localized PRMT5 decreased the level of viral core particle-DNA but did not affect the

production of viral antigens and pgRNA and the level of cccDNA-bound H4R3me2s and PRMT5 proteins (Fig 7D). These results suggest that PRMT5 does not affect the HBV RNA transcription or the formation of the core capsid itself, but inhibits HBV core particle DNA production at the cytoplasmic level in a methyltransferase activity-independent manner.

PRMT5 interferes with pgRNA encapsidation by counteracting the interaction between pgRNA and HBV Pol through binding to the RT-RH region of Pol

Because pgRNA encapsidation is an essential step before core particle DNA synthesis, we further evaluated whether PRMT5 prevents pgRNA encapsidation to block viral DNA synthesis. To address this, we transfected Huh7 cells with the pCMV-HBV YMHD vector, which lacks the polymerase catalytic active site and thus leads to the accumulation of RNA-containing capsids in the cells. The Northern blot results showed that while the levels of HBV total RNA and capsids were not affected by PRMT5△, it dramatically reduced the level of encapsidated RNA in a dose-dependent manner (Fig 8A), indicating that PRMT5△ inhibits pgRNA encapsidation rather than directly targeting the cytoplasmic viral pgRNA for degradation.

Since the HBc, Pol and pgRNA are the three key factors involved in the process of pgRNA encapsidation and we found that PRMT5 interacts with the HBc, we speculated whether PRMT5 affects the associations of HBc with Pol and with pgRNA. The CoIP assay suggested that the interaction between HBc and Pol was not affected by PRMT5△ (Fig S10B). In addition, the

RNA immunoprecipitation (RIP) assay showed that in the absence of Pol, the interaction between HBc and pgRNA was weak, even without PRMT5△ expression (Fig S10C), which is consistent with previous studies showing that the binding between HBV Pol and the 5'-ε signal on pgRNA is prerequisite for pgRNA encapsidation and subsequent viral DNA production(27-28). These results suggest that the interaction between PRMT5 and HBc has little contribution to PRMT5-mediated inhibition of pgRNA encapsidation. We then investigated the impact of PRMT5 on the binding of Pol to pgRNA. The RIP assay clearly suggested that the ectopically-expressed PRMT5 and PRMT5△ disrupted the interaction between Pol and pgRNA, whereas knockdown of endogenous PRMT5 significantly enhanced the Pol-pgRNA interaction (Fig 8B). Thus, we further investigated how PRMT5 counteracted the Pol-pgRNA interaction. While there was no direct interaction between PRMT5 and pgRNA(Fig S10D), a co-localization of PRMT5 with HBV Pol in the cytoplasm (Fig 8C) and a physical interaction between HBV Pol and endogenous or ectopic PRMT5 or PRMT5△ (Fig 8D) were observed. Further studies indicated that PRMT5 interacted with the RT-RH region of Pol (Fig 8E) which has been recognized as the domain that is responsible for the Pol-pgRNA interaction(28). Together, these results suggest that PRMT5 interferes with pgRNA encapsidation through disrupting the Pol-pgRNA interaction possibly by competitively binding to the RT-RH region of Pol, which provides a mechanism of how PRMT5 inhibits core particle DNA production.

Discussion

Here, we identified PRMT5 as a potent host restrictor of HBV replication. We demonstrate that

PRMT5, in a methyltransferase-dependent manner, epigenetically silence cccDNA transcription by triggering H4R3me2s on the cccDNA minichromosome, which involved the regulation of the binding of the Brg1-based hSWI/SNF chromatin remodeler and RNA Pol II to cccDNA. Importantly, the negative correlation between the cccDNA-bound H4R3me2s and cccDNA transcription was validated *in vitro* and *in vivo*. In addition, PRMT5, in a methyltransferase-independent manner, disrupt pgRNA encapsidation by competitively binding to the RT-RH region of HBV Pol, which further inhibit HBV DNA production and may affect the cccDNA replenishment (Fig S11).

In recent years, a number of epigenetic markers on the HBV cccDNA minichromosome associated with viral transcription have been identified. While high levels of post-transcriptional modifications (PTMs) of the cccDNA-bound histones associated with active transcription are observed across the HBV genome and the role of acetylation of cccDNA-bound histones in the regulation of HBV transcription has been recognized (7, 9, 26), the PTMs associated with transcriptional repression, including H3K9me3 and H3K27me3, were hardly detected on HBV DNA(26), thus the role of histone methylation remains elusive and controversial. Here, we reported that both *in vitro* and *in vivo*, cccDNA-bound H4R3me2s could be detected and was significantly associated with the repressed cccDNA transcription, and could be manipulated by the alteration of PRMT5 expression. Since the previous study with a ChIP-sequencing approach did not include H4R3me2s, it will be interesting to map its distribution on cccDNA in the future. In addition to the present study, the role of methyltransferases including PRMT1 and SETDB1 in

regulating HBV transcription has been reported(29-30). PRMT1, a member of the type I protein arginine methyltransferase family, was found to be a binding partner of HBx and could inhibit HBV transcription. Considering that the asymmetric dimethylation on H4R3 (H4R3me2a), known to be catalyzed by PRMT1, has been recognized as a gene activation marker(19), and our results from an HBV infection system revealed that there was no positive correlation between the status of H4R3me2a and cccDNA transcriptional activity (Fig S3C), the inhibitory effect of PRMT1 on HBV transcription may not act through its catalysis of cccDNA-bound H4R3me2a but through other target proteins. Riviere et al suggested that histone methyltransferase SETDB1-mediated H3K9 trimethylation (H3K9me3) was involved in the transcriptional repression of HBV cccDNA. Notably, this repressive effect could only be observed in the absence of HBx (HBVX-) (26, 30), which might explain why we did not observe H3K9me3-associated transcriptional repression of cccDNA in dHepaRG cells infected with wild-type HBV (Fig S3C), thus argues the role of H3K9me3 in regulating cccDNA transcription in the presence of the HBx protein during natural HBV infection. Together, these data suggest that, compared with H4R3me2a and H3K9me3, PRMT5-catalyzed H4R3me2s may play a vital role in the restriction of HBV transcription during HBV infection. However, whether other factor(s) that can regulate the status H4R3me2s and factor(s) that modulate the activity of PRMT5 remains to be investigated. Besides, from the perspective of the virus, such epigenetic mechanism with repressive effect might be a strategy for maintaining chronic infection.

Previous studies have suggested that PRMT5 methylates histones H4R3 and H3R8 as a part of the

Brg1- or hBrm-based SWI/SNF chromatin remodeling complexes(23), and this, in turn, facilitates the binding of Brg1 to the target gene for subsequent chromatin remodeling. Here, we showed that altering PRMT5 expression accordingly regulated the binding of Brg1, but not hBrm to cccDNA. We thus speculated that the Brg1-based hSWI/SNF chromatin remodeler complex may be associated with PRMT5 to promote H4R3me2s on cccDNA as well as to remodel the nucleosome structure of the HBV minichromosome to coordinately impede the binding of RNA Pol II to cccDNA(31). Since PRMT5-mediated methylation of H4R3 can recruit the DNA methyltransferase DNMT3A(20) and HBV DNA methylation has been shown to suppress cccDNA transcription, it will be interesting to further determine whether the methylation of cccDNA is increased along with the PRMT5-mediated up-regulation of cccDNA-bound H4R3me2s and coordinately contributes to the repression of HBV transcription by PRMT5.

Although both the host chromosomes and the HBV cccDNA minichromosome are organized with the host histone proteins, we showed that the status of H4R3me2s varied more greatly on cccDNA than that on the host GAPDH gene during HBV infection and the alteration of the expression of PRMT5 significantly changed the level of cccDNA- but not the host genes-bound H4R3me2s and PRMT5, implying that PRMT5 can, to some extent, specifically bind to cccDNA and catalyze cccDNA-bound H4R3me2s to repress cccDNA transcription. Based on the evidences that PRMT5 interacted and co-localized with HBc in the nucleus and knockdown of HBc decreased the binding of PRMT5 to cccDNA, we proposed that HBc plays a role in targeting PRMT5 to cccDNA. The factors that have been reported to mediate the binding of PRMT5 to host chromosomes, such as

the histone-binding protein COPR5, may also be involved in the binding of PRMT5 to cccDNA, which remains to be further studied. The preference of PRMT5 could also be attributed to the differences in chromatin protein composition and the chromosome structure between cccDNA and host genes (3, 32), which may lead to different sensitivities to the alteration of PRMT5 expression levels.

Until now, a number of host restrictors that target different steps of HBV replication have been identified, including the APOBEC cytidine deaminases (33), the IFN-induced MyD88 and Zinc finger protein (14, 34), the TNF-α-induced cellular inhibitor of apoptosis protein 2 (cIAP2) (35), and RIG-I (16). Here, we reported that PRMT5, in addition to suppressing HBV transcription, has a novel inhibitory effect on HBV pgRNA encapsidation through binding to the RT-RH region of Pol which is crucial for the pgRNA-Pol interaction as well as for the subsequent RNA reverse transcription (28, 36). Because the pgRNA is reverse transcribed within the capsids, which are subsequently used for the formation of mature virions or recycling to the nucleus for cccDNA maintenance, there is a possibility that by binding to the viral Pol, PRMT5 can also be incorporated into the viral nucleocapsids to interfere with HBV reverse transcription and prevent the replenishment of cccDNA, which requires further investigation. Another possibility we cannot exclude is that the binding of PRMT5 to core protein affects the packaging of Pol-pgRNA ribonucleoprotein (RNP) complex into capsid.

Besides histones, PRMT5 can target a series of other host proteins and has been implicated in various cellular processes, including pluripotency, tumorigenesis (37) and regulation of viral replication and pathogenesis by targeting a variety of cellular substrates and viral proteins(38). We showed that the anti-HBV effect of PRMT5 is not dependent on the NF-κB activation (Fig S7) (39) or alteration of the expression levels of HBV transcription-related host transcription factors like HNF4 and C/EBPα (Fig S5B and S6), but other possibilities remain to be further studied, such as whether PRMT5 could directly methylate HBV Pol or HBc to influence their function or PRMT5 inhibits HBV replication through indirect mechanism(s). Moreover, although the levels of PRMT5 mRNA is not affected by HBV infection (Fig S5C-D), they are much higher in the hepatoma HepG2-NTCP cells than PHHs (data not shown), which is consistent with the reports that PRMT5 expression is upregulated in tumor tissues. Considering that HBV replication is often robust in PHHs while low in tumor cells, we determined whether there is any association between the anti-HBV effect of PRMT5 and the low HBV replication efficacy in tumor-derived cells. Our recent data showed that the HBV transcription activity was lower in liver tumor cells than that in the paired para-tumor tissues, while, interestingly, the levels of PRMT5 mRNAs and the cccDNA-bound PRMT5 and H4R3me2s were higher in tumor cells (unpublished). Although more validations are required, these data have provided more insights into the physiological significance of PRMT5-mediated restriction of HBV replication *in vitro* and *in vivo*.

In conclusion, the present study revealed a novel two-part mechanism of the regulation of HBV gene transcription and viral replication by PRMT5, which promotes the understanding of the

epigenetic modulation of cccDNA transcription and the control of HBV replication and may provide new insight into non-cytolytic mechanism of cccDNA elimination.

References

1. Ganem D, Prince AM. Hepatitis B virus infection--natural history and clinical consequences. N Engl J Med 2004;350:1118-1129.

2. Bock CT, Schranz P, Schroder CH, Zentgraf H. Hepatitis B virus genome is organized into nucleosomes in the nucleus of the infected cell. Virus Genes 1994;8:215-229.

3. Bock CT, Schwinn S, Locarnini S, Fyfe J, Manns MP, Trautwein C, et al. Structural organization of the hepatitis B virus minichromosome. J Mol Biol 2001;307:183-196.

4. Belloni L, Pollicino T, De Nicola F, Guerrieri F, Raffa G, Fanciulli M, et al. Nuclear HBx binds the HBV minichromosome and modifies the epigenetic regulation of cccDNA function. Proc Natl Acad Sci U S A 2009;106:19975-19979.

5. **Guo YH**, **Li YN**, Zhao JR, Zhang J, Yan Z. HBc binds to the CpG islands of HBV cccDNA and promotes an epigenetic permissive state. Epigenetics 2011;6:720-726.

6. Zhang Y, Mao R, Yan R, Cai D, Zhu H, Kang Y, et al. Transcription of hepatitis B virus covalently closed circular DNA is regulated by CpG methylation during chronic infection. PLoS One 2014;9:e110442.

7. Pollicino T, Belloni L, Raffa G, Pediconi N, Squadrito G, Raimondo G, et al. Hepatitis B virus replication is regulated by the acetylation status of hepatitis B virus cccDNA-bound H3 and H4 histones. Gastroenterology 2006;130:823-837.

8. Belloni L, Allweiss L, Guerrieri F, Pediconi N, Volz T, Pollicino T, et al. IFN-alpha inhibits HBV transcription and replication in cell culture and in humanized mice by targeting the epigenetic regulation of the nuclear cccDNA minichromosome. J Clin Invest 2012;122:529-537.

9. Liu F, Campagna M, Qi Y, Zhao X, Guo F, Xu C, et al. Alpha-interferon suppresses hepadnavirus

transcription by altering epigenetic modification of cccDNA minichromosomes. PLoS Pathog 2013;9:e1003613.

10. **Huang J**, **Kent JR**, Placek B, Whelan KA, Hollow CM, Zeng PY, et al. Trimethylation of histone H3 lysine 4 by Set1 in the lytic infection of human herpes simplex virus 1. J Virol 2006;80:5740-5746.

11. Murata T, Kondo Y, Sugimoto A, Kawashima D, Saito S, Isomura H, et al. Epigenetic histone modification of Epstein-Barr virus BZLF1 promoter during latency and reactivation in Raji cells. J Virol 2012;86:4752-4761.

12. Nitzsche A, Steinhausser C, Mucke K, Paulus C, Nevels M. Histone H3 lysine 4 methylation marks postreplicative human cytomegalovirus chromatin. J Virol 2012;86:9817-9827.

13. Liu Y, Li J, Chen J, Li Y, Wang W, Du X, et al. Hepatitis B virus polymerase disrupts K63-linked ubiquitination of STING to block innate cytosolic DNA-sensing pathways. J Virol 2015;89:2287-2300.

14. Li JH, Lin SS, Chen QY, Peng L, Zhai JW, Liu YH, et al. Inhibition of Hepatitis B Virus Replication by MyD88 Involves Accelerated Degradation of Pregenomic RNA and Nuclear Retention of Pre-S/S RNAs. Journal of Virology 2010;84:6387-6399.

15. **Yu S**, **Chen J**, Wu M, Chen H, Kato N, Yuan Z. Hepatitis B virus polymerase inhibits RIG-I- and Toll-like receptor 3-mediated beta interferon induction in human hepatocytes through interference with interferon regulatory factor 3 activation and dampening of the interaction between TBK1/IKKepsilon and DDX3. J Gen Virol 2010;91:2080-2090.

16. Sato S, Li K, Kameyama T, Hayashi T, Ishida Y, Murakami S, et al. The RNA sensor RIG-I dually functions as an innate sensor and direct antiviral factor for hepatitis B virus. Immunity 2015;42:123-132.

17. Greer EL, Shi Y. Histone methylation: a dynamic mark in health, disease and inheritance. Nat Rev

Genet 2012;13:343-357.

18. Klose RJ, Zhang Y. Regulation of histone methylation by demethylimination and demethylation. Nat Rev Mol Cell Biol 2007;8:307-318.

19. Peterson CL, Laniel MA. Histones and histone modifications. Curr Biol 2004;14:R546-551.

20. Zhao Q, Rank G, Tan YT, Li H, Moritz RL, Simpson RJ, et al. PRMT5-mediated methylation of histone H4R3 recruits DNMT3A, coupling histone and DNA methylation in gene silencing. Nat Struct Mol Biol 2009;16:304-311.

21. Nassal M. HBV cccDNA: viral persistence reservoir and key obstacle for a cure of chronic hepatitis B. Gut 2015.

22. Karkhanis V, Hu YJ, Baiocchi RA, Imbalzano AN, Sif S. Versatility of PRMT5-induced methylation in growth control and development. Trends Biochem Sci 2011;36:633-641.

23. Pal S, Vishwanath SN, Erdjument-Bromage H, Tempst P, Sif S. Human SWI/SNF-associated PRMT5 methylates histone H3 arginine 8 and negatively regulates expression of ST7 and NM23 tumor suppressor genes. Mol Cell Biol 2004;24:9630-9645.

24. Euskirchen G, Auerbach RK, Snyder M. SWI/SNF chromatin-remodeling factors: multiscale analyses and diverse functions. J Biol Chem 2012;287:30897-30905.

25. Rall LB, Standring DN, Laub O, Rutter WJ. Transcription of hepatitis B virus by RNA polymerase II. Mol Cell Biol 1983;3:1766-1773.

26. Tropberger P, Mercier A, Robinson M, Zhong W, Ganem DE, Holdorf M. Mapping of histone modifications in episomal HBV cccDNA uncovers an unusual chromatin organization amenable to epigenetic manipulation. Proc Natl Acad Sci U S A 2015;112:E5715-5724.

27. Bartenschlager R, Schaller H. Hepadnaviral assembly is initiated by polymerase binding to the

encapsidation signal in the viral RNA genome. EMBO J 1992;11:3413-3420.

28. Jones SA, Hu J. Hepatitis B virus reverse transcriptase: diverse functions as classical and emerging targets for antiviral intervention. Emerg Microbes Infect 2013;2:e56.

29. Benhenda S, Ducroux A, Riviere L, Sobhian B, Ward MD, Dion S, et al. Methyltransferase PRMT1 is a binding partner of HBx and a negative regulator of hepatitis B virus transcription. J Virol 2013;87:4360-4371.

30. Riviere L, Gerossier L, Ducroux A, Dion S, Deng Q, Michel ML, et al. HBx relieves chromatin-mediated transcriptional repression of hepatitis B viral cccDNA involving SETDB1 histone methyltransferase. J Hepatol 2015.

31. Wilson BG, Roberts CW. SWI/SNF nucleosome remodellers and cancer. Nat Rev Cancer 2011;11:481-492.

32. Liping Shi SL, Fang Shen, Haodong Li, Shuiming Qian, Daniel H.S. Lee, Jim. Z Wu, Wengang Yang. characterization of the nucleosome positioning in hepadnaviral cccDNA minichromosome. Journal of Virology 2012.

33. **Lucifora J**, **Xia Y**, Reisinger F, Zhang K, Stadler D, Cheng X, et al. Specific and nonhepatotoxic degradation of nuclear hepatitis B virus cccDNA. Science 2014;343:1221-1228.

34. **Mao R**, **Nie H**, Cai D, Zhang J, Liu H, Yan R, et al. Inhibition of hepatitis B virus replication by the host zinc finger antiviral protein. PLoS Pathog 2013;9:e1003494.

35. Wang Z, Ni J, Li J, Shi B, Xu Y, Yuan Z. Inhibition of Hepatitis B Virus Replication by cIAP2 Involves Accelerating the Ubiquitin-Proteasome-Mediated Destruction of Polymerase. J Virol 2011;85:11457-11467.

36. Hu J, Boyer M. Hepatitis B virus reverse transcriptase and epsilon RNA sequences required for

specific interaction in vitro. J Virol 2006;80:2141-2150.

37. Stopa N, Krebs JE, Shechter D. The PRMT5 arginine methyltransferase: many roles in development, cancer and beyond. Cell Mol Life Sci 2015.

38. Shire K, Kapoor P, Jiang K, Hing MN, Sivachandran N, Nguyen T, et al. Regulation of the EBNA1 Epstein-Barr virus protein by serine phosphorylation and arginine methylation. J Virol 2006;80:5261-5272.

39. Wei H, Wang B, Miyagi M, She Y, Gopalan B, Huang DB, et al. PRMT5 dimethylates R30 of the p65 subunit to activate NF-kappaB. Proc Natl Acad Sci U S A 2013;110:13516-13521.

Author names in bold designate shared co-first authorship

Figure Legends

Fig 1. PRMT5 inhibits HBV transcription and core particle DNA production. (A) The indicated siRNAs were co-transfected with monomeric linear HBV DNA into Huh7 cells. The culture supernatants were collected to detect HBeAg and HBsAg by ELISA. (B-D) PRMT5 was transduced into HBV-infected dHepaRG cells for 6 days and shPRMT5 was transduced for 5 days. The expressions of PRMT5 were detected by Western blot. The levels of HBV antigens in the supernatant were detected by ELISA. HBV pgRNA, core particle DNA and cccDNA were analyzed by qPCR. The levels of pgRNA and cccDNA were used to calculate the ratio of pgRNA/cccDNA (B, C). HBV core protein and endogenous PRMT5 were observed by immunofluorescence assay using the specific antibodies (D). (E) PRMT5 expression plasmids or 100 nM of siPRMT5 were transfected into HBV-infected HepG2-NTCP cells. Cells were collected to examine the level of HBV antigens, core particle DNA and pgRNA/cccDNA at the 4th day post transfection.

Fig 2. The effects of PRMT5△ on HBV transcription and core particle DNA production. (A) A schematic representation of the catalytically inactive form of human PRMT5 (PRMT5△) and the deleted amino acids in PRMT5△ are shown. (B-E) PRMT5△ was transduced into HBV-infected dHepaRG cells for 6 days. (B) The expressions of PRMT5△ were detected by Western blot. (C) The culture supernatants were collected to detect HBeAg and HBsAg by ELISA. (D) HBV pgRNA and cccDNA were extracted and quantified by qPCR for calculating the ratio of pgRNA/cccDNA. (E) The level of core particle DNA was analyzed by qPCR.

Fig 3. The cccDNA transcriptional activity was negatively correlated with the symmetric dimethylation of cccDNA-bound H4R3. (A, B) HBV-infected dHepaRG cells in 100 mm dishes were harvested by trypsinization on the indicated days. One-eighth of the cells were used to extract HBV cccDNA and 1/16 of the cells were used to extract HBV pgRNA, followed by qPCR analysis. (A) The ratio of pgRNA to cccDNA at 4 dpi was set as 1. (B) Three-quarters of HBV-infected dHepaRG cells harvested at 7, 13 and 19dpi were used for ChIP assays with the indicated antibody. The levels of the specific modified histones on HBV cccDNA or the host gene GAPDH were analyzed by qPCR. The ratio of pgRNA to cccDNA at 7 dpi was set as 1. (C, D) Each liver biopsy was used to conduct RNA extraction and ChIP assay with the indicated antibody. Relative HBV pgRNA/cccDNA was calculated as the ratio of normalized HBV pgRNA to cccDNA. The levels of H4R3me2s on HBV cccDNA or the host GAPDH DNA were examined by qPCR.

Fig 4. PRMT5 increases the symmetric dimethylation of cccDNA-bound H4R3 to inhibit cccDNA transcription. (A, C) PRMT5 or PRMT5Δ was transduced into HBV-infected dHepaRG cells in 100 mm dishes for 6 days. (B) shPRMT5 was transduced into HBV-infected dHepaRG cells in 100 mm dishes for 5 days. One-eighth of the cells were used to extract HBV cccDNA and 1/16 of cells were used to extract total RNA, followed by qPCR analysis. Three-quarters of the cells were harvested and used for Chip assays with the indicated antibodies. The levels of the specific proteins on HBV cccDNA was analyzed by qPCR.

Fig 5. PRMT5 preferentially regulates H4R3me2s on cccDNA and interacts with the HBV core protein. (A, B) PRMT5 was transduced into HBV-infected dHepaRG cells in 100 mm dishes for 6 days. shPRMT5 was transduced into HBV-infected dHepaRG cells in 100 mm dishes for 5 days. Three-quarters of the cells were used for ChIP assays with the indicated antibodies. The levels of H4R3me2s or PRMT5 on HBV cccDNA or the host RPL30, MYOD1 and GAPDH genes were analyzed by qPCR. (C) Huh7 cells were co-transfected as indicated and the cells were subjected to CoIP assay with indicated antibody. The expression of indicated proteins was analyzed by Western blot (β-actin as a loading control). (D) Huh7 cells were co-transfected with plasmids encoding PRMT5 and 1.3-fold HBV genome. The cells were harvested for the immunofluorescence assay. PRMT5 and HBc were detected by the specific antibodies. (E) dHepaRG cells in 100 mm dishes were infected by wildtype HBV (HBV WT) and HBc protein deficient virus (HBV-△HBc).Three-quarters of the cells were used for ChIP assays with the anti-PRMT5antibody. The rest of cells were subjected to analyze the expression levels of HBc and PRMT5 by Western blot.

Fig 6. The effects of PRMT5 on the binding of Brg1 and RNA Pol II to cccDNA. (A, C) PRMT5 was transduced into HBV-infected dHepaRG cells in 100 mm dishes for 6 days.shPRMT5 was transduced into HBV-infected dHepaRG cells in 100 mm dishes for 5 days. Three-quarters of the cells were used for ChIP assay with the indicated antibodies. The levels of Brg1 or Pol II on

the indicated genes were analyzed by qPCR. (B) PRMT5 was transduced into HBV-infected dHepaRG cells at Day 7 post HBV infection. Adeno-shBrg1 was added one day later and the cells were cultured for another 5 days before subjected to qPCR analysis. The expressions of PRMT5 and Brg1 were detected by Western blot. HBV pgRNA and cccDNA were quantified by qPCR for calculating the ratio of pgRNA/cccDNA.

Fig 7. Cytoplasmic PRMT5Δ reduces HBV core particle DNA production. (A) PRMT5Δexpression plasmids were co-transfected with pCMV-HBV into Huh7 cells. The pcDNA3.1a vector was used as a negative control. The cells were harvested at 72 hrs. post-transfection. (B) PRMT5△ was transduced into HepG2.117 cells and the cells were harvested at 5 days after transduction. Core particle DNAs were analyzed by Southern blot. The positions of HBV relaxed circular DNA (RC) and single stranded DNA (SS) are indicated. Numbers below the lanes indicate the intensity of viral DNA bands relative to the vector group. HBV RNA was analyzed by Northern blot. 28s and 18s rRNAs serve as loading controls. The position of the 3.5 kb and 2.4/2.1 kb HBV RNAs are indicated. An aliquot of cell lysates was loaded onto an agarose gel to detect cytoplasmic core capsids. The overexpression of PRMT5△, NES-PRMT5△ and NLS-PRMT5△ was detected by Western blot and β-actin was used as a loading control. (C) Huh7 cells transfected with NES-PRMT5△ and NLS-PRMT5△, followed by an immunofluorescence assay with anti-PRMT5 antibody. (D-E) NES-PRMT5 was transduced into HBV-infected dHepaRG cells in 100 mm dishes for 6 days. The expressions of NES-PRMT5 were detected by Western blot. The levels of HBV antigens in the supernatant were detected by

ELISA. HBV pgRNA, core particle DNA and cccDNA were analyzed by qPCR. Three-quarters of the cells were harvested and used for ChIP assays with the indicated antibodies. The levels of the specific proteins on HBV cccDNA were analyzed by qPCR.

Fig 8. PRMT5 interferes with pgRNA encapsidation by counteracting the interaction between pgRNA and HBV Pol through binding to the RT-RH region of Pol. (A) PRMT5△ was co-transfected with pCMV-HBV YMHD into Huh7 cells. The cells were harvested at 72 hrs post-transfection. Core particle RNAs were analyzed by Northern blot. Numbers below the lanes indicate the intensity of the core particle RNA bands relative to the vector group. Total HBV RNA was analyzed by Northern blot. An aliquot of cell lysates was loaded onto an agarose gel to detect the cytoplasmic core capsids. The overexpression of PRMT5△ was detected by Western blot. (B) Huh7 cells were co-transfected as indicated, and pCMV-HBV-△Pol was transfected in all groups to transcribe pgRNA. At 36 hrs post-transfection, the cells were treated with 10 μM of MG132 for 9 hrs. and harvested for a RIP assay. (C) PRMT5was co-transfected with HA-Pol into Huh7 cells. After 48 hrs, the cells were harvested for an immunofluorescence assay. Anti-PRMT5 and anti-HA antibodies were used. (D) Huh7 cells were co-transfected as indicated. At 36 hrs post-transfection, the cells were treated with 10 μM of MG132 for 9 hrs. and then harvested for CoIP assay with anti-Flag antibody. (E) Schematic diagram of the full-length and truncated constructs of HBV Pol (upper panel). Huh7 cells were co-transfected with Flag-PRMT5 and the indicated Myc-tagged HBV Pol constructs or the negative control, Myc-Rluc. At 36 hrs post-transfection, the cells were treated with 10 μM of MG132 for 9 hrs. and then harvested for CoIP assay with anti-Flag antibody

(lower panel).

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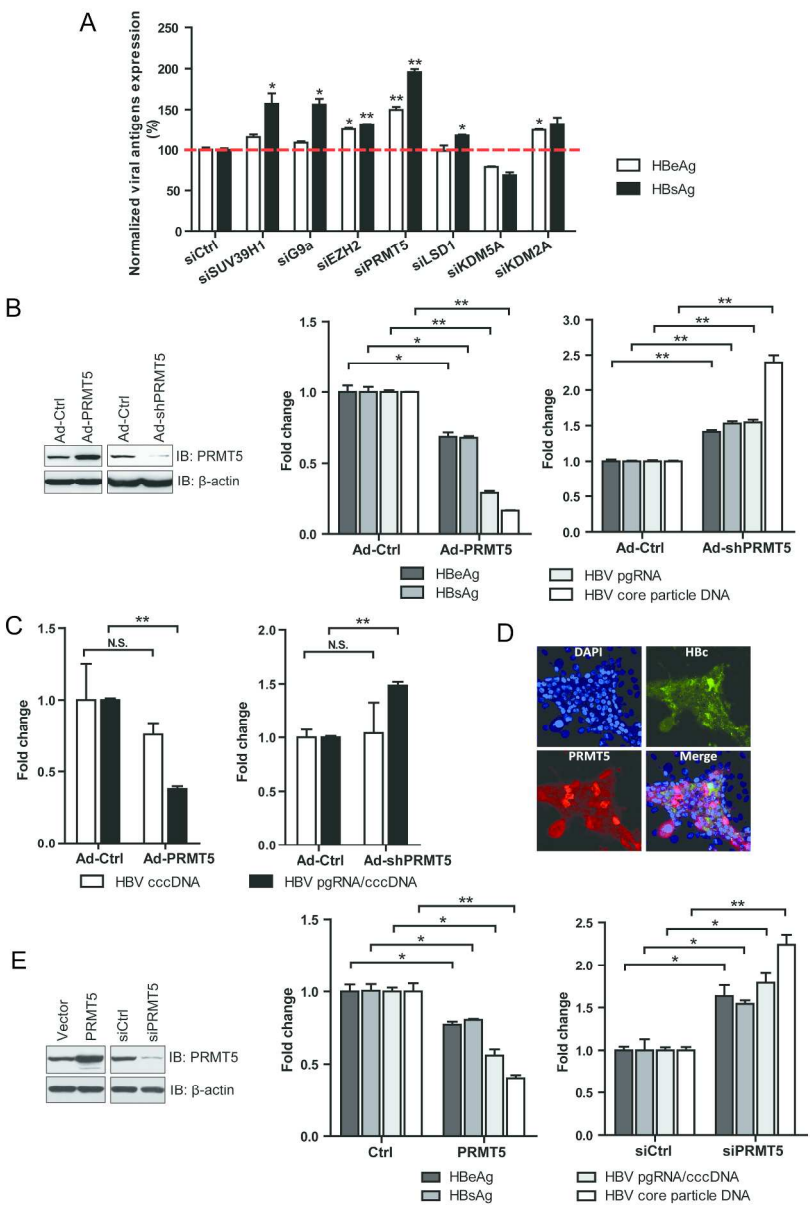

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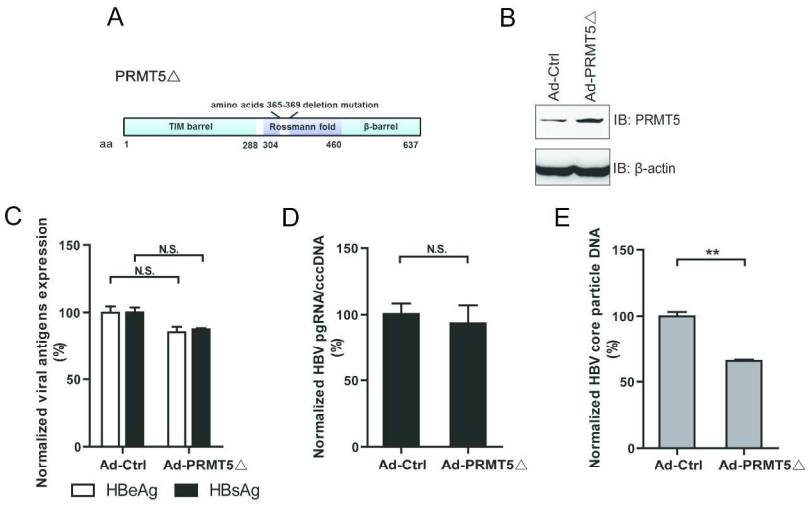

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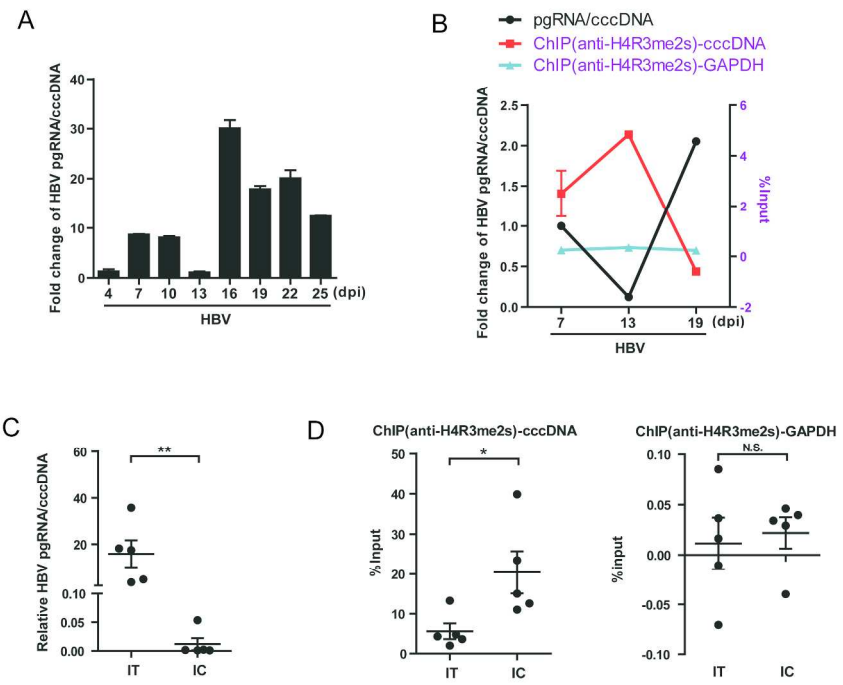

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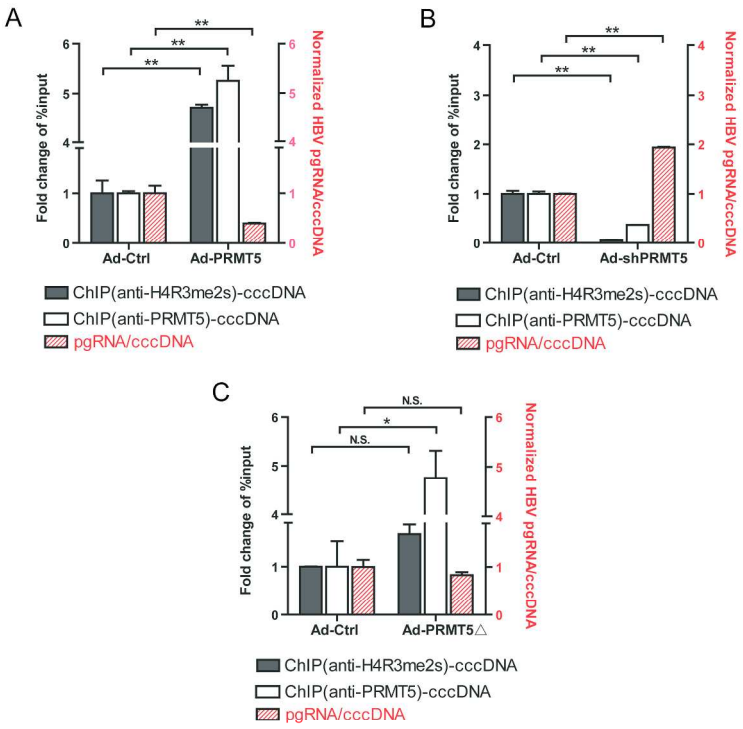

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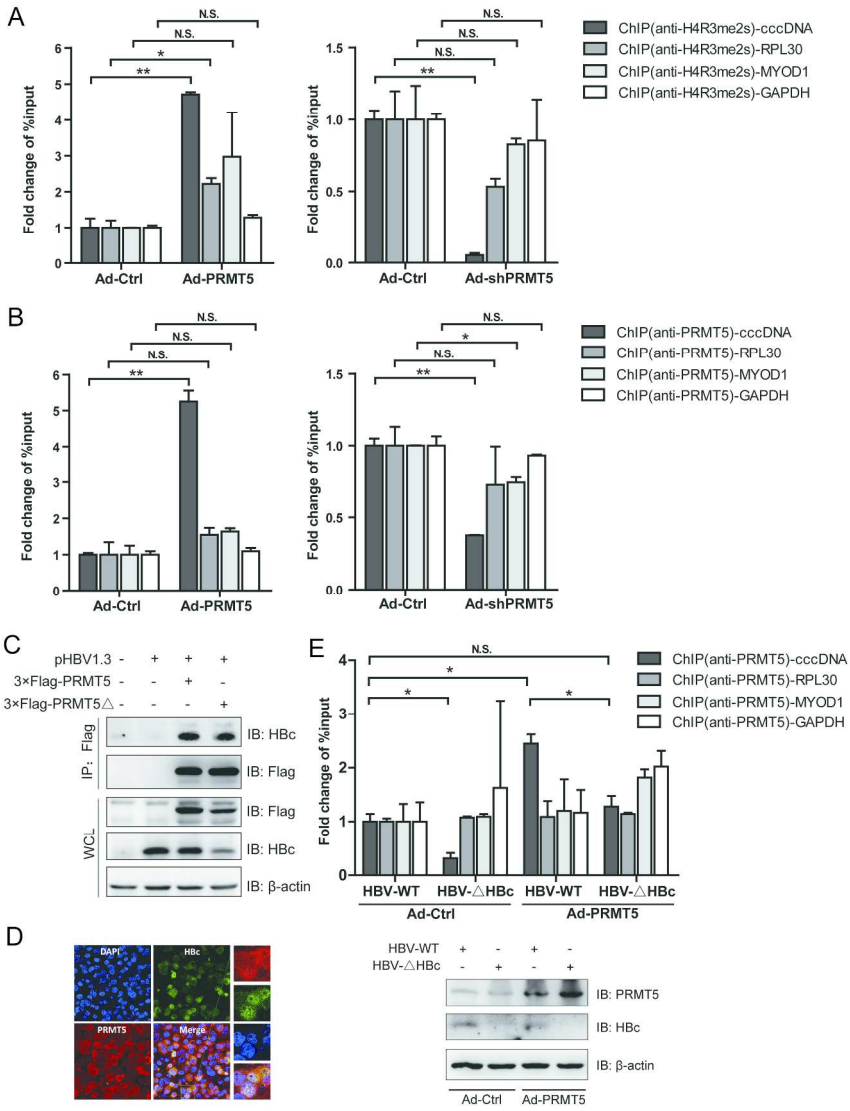

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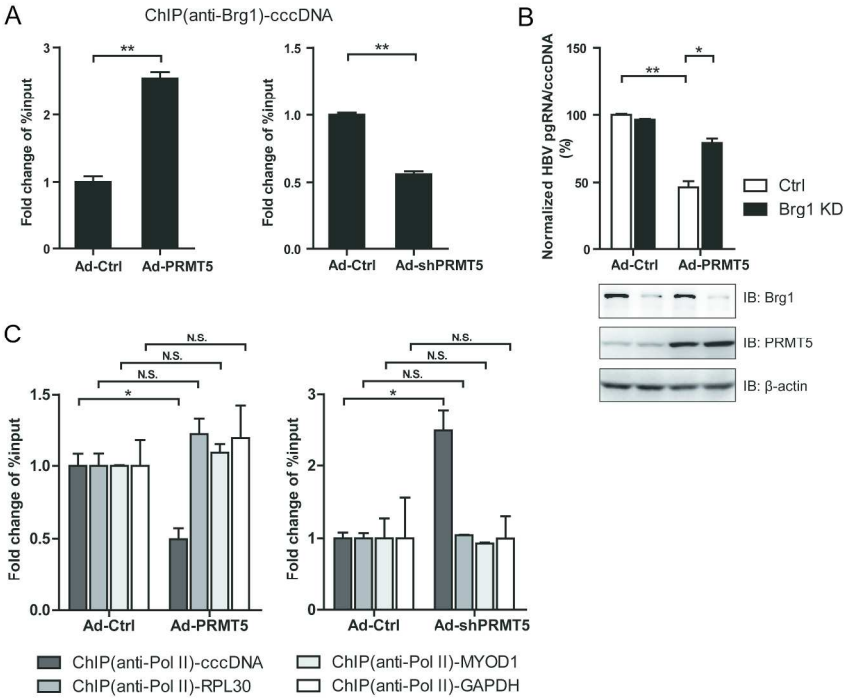

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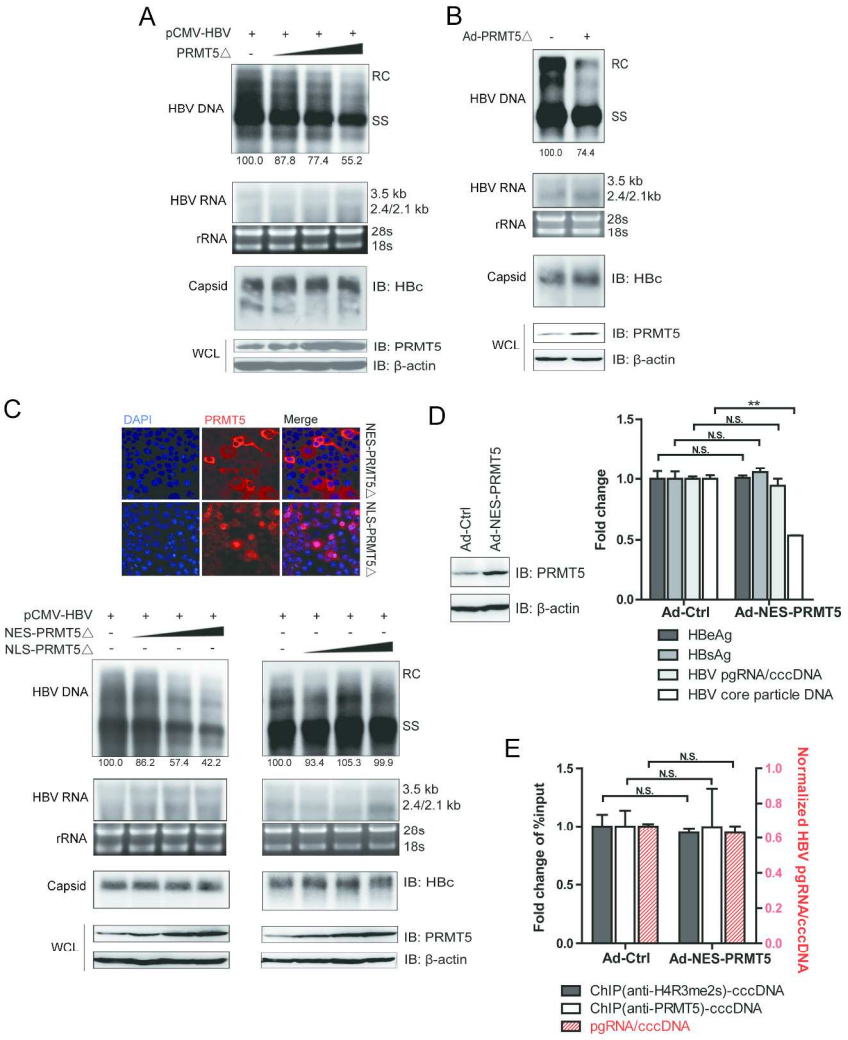

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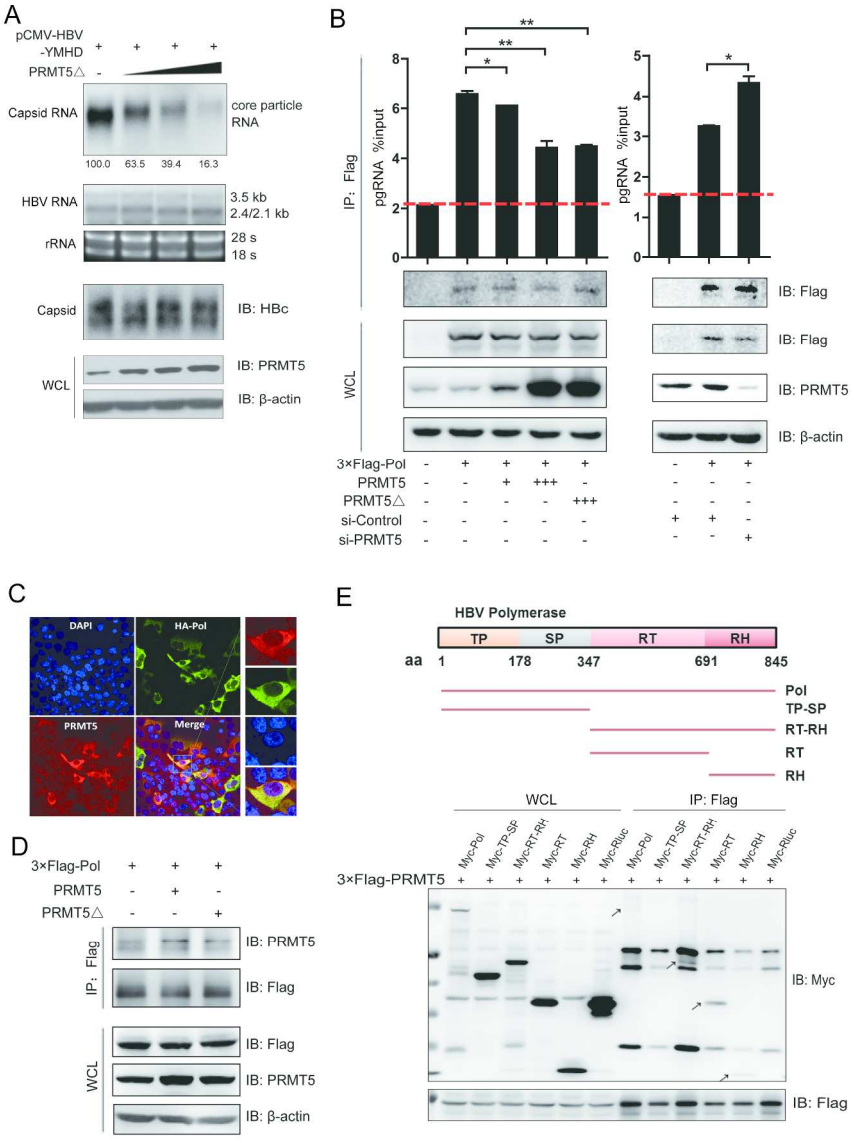

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Supporting Information

PRMT5 Restricts Hepatitis B Virus Replication via Epigenetic Repression of cccDNA Transcription and Interference with pgRNA Encapsidation

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Materials and Methods

Plasmids The pCR.HBV.A.EcoRI plasmid contains the ~3.2 kb complete HBV genome (genotype A, adw)**.**The preparation of monomeric linear full-length HBV DNA for cell transfection using pCR.HBV.A.EcoRI has been previously described (1). pCMV-HBV-ΔPol-ΔCore, lacking the expression of the HBV core protein, was derived from pCMV-HBV-ΔPol by introducing a frameshift mutation (2).pcDNA3.1a-PRMT5Δexpressed mutant PRMT5 with the deletion of amino acids 365-369.The oligonucleotide used to introduce the deletion was 5'-CTGATGGTGCTGCCCCTGGTGAACGCTTCCC-3'(3).pcDNA3.1a-NES-PRMT5Δ and pcDNA3.1a-NLS-PRMT5Δ were constructed by inserting the NES signal sequence 5'-ATGAACGAGCTAGCACTAAAGCTAGCAGGTCTAGACATCAACAAG-3' and the NLS signal sequence 5'-ATGCCAAAGAAGAAGCGAAAGGTG into pcDNA3.1a-PRMT5Δ, respectively.

Cell culture To obtain the differentiated HepaRG cells (4), the cells were cultured for 2 weeks in standard medium and then for two more weeks in medium supplemented with 1.8% dimethyl sulfoxide (differentiation medium) according to previous studies (4, 5). The Huh7 and HepG2.117(6) cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS) (BI), penicillin (100 IU/ml) (Gibco), and streptomycin (100 μg/ml) (Gibco) in a 5% CO_2 atmosphere at 37°C. HepG2-NTCP cells were cultured as described (7).

Viruses and infection Adeno-PRMT5 and Adeno-PRMT5Δ were produced by transfecting PacI-digested pAdPLDest-PRMT5 (constructed in Prof. Ulrike Protzer's laboratory in Munich, Germany) and pAdPLDest-PRMT5Δ, respectively, into the HEK293 cells. pLKO.1-shPRMT5-001 and -005 were constructed according to the manufacturer's instructions, and then the U6 promoter and shPRMT5 sequences were cloned into pAdPLDest-shPRMT5-001/005. The adeno-PRMT5/PRMT5Δ/shPRMT5 were titrated using TCID50. The construction of pAdPLDest-shBrg1 and the production of adeno-shBrg1 were similar to those of pAdPLDest-shPRMT5 and adeno-shPRMT5, respectively. HBV WT virus were produced by transient transfection of 1.3 copies of HBVgenome (genotype D) HBV1.3-pBR322 (provided by Dr. Qiang Deng) to HepG2 cells. HBc protein deficient virus (HBV-ΔHBc) was generated by co-transfection of HepG2 cells with pBR322 plasmid harboring 1.3 copies of HBV genome with mutant site at the starting code of core gene open reading frame (M1V mutant), and pcDNA3.1 vector expressing of HBc protein. The supernatant were collected after transfection, and HBV virion particle were concentrated by ultra-centrifuge at 32000 RPM for 18 hours in 20% sucrose medium. HBV DNA was extracted by Viral DNA Kit, and genome levels was quantified by qPCR

Virus stocks were alquoted and stored at -80℃. For HBV infection, the dHepaRG cells were incubated with differentiation medium containing 10% HBV-positive sera (approximately 100 genome equivalent copies of HBV per cell) or concentrated HBV virions (MOI 200) and 5% polyethylene glycol (PEG) 8000 for 16 hrs and then rinsed three times with PBS.To make sure that the HBV infection is successful and the cell viability is not affected by different treatments, the viral HBs and HBe antigens and HBV DNA in the supernatant and the cell viability (using the CCK8 kit, Dojindo (Japan)) were examined before cell extraction in every experiment.

HBV cccDNA isolation The cells were lysed in lysis buffer A (50 mM Tris-HCl pH 7.4, 1 mM EDTA, 1% NP-40) containing complete protease inhibitor cocktail (Roche) for 30 mins on ice. After centrifugation, the pelleted nuclei were resuspended in lysis buffer B (10 mM Tris-HCl, 10 mM EDTA, 150 mM NaCl, 0.5% SDS, Proteinase K 0.5 mg/ml) and incubated overnight at 37°C. Nucleic acids were purified by phenol-chloroform (1:1) extraction and ethanol precipitation.

HBV core particle RNA extraction The cells were lysed in 600 µl of lysis buffer A, and the nuclei were removed by centrifugation. The cytoplasmic cell lysate was incubated with DNase I, RNase A and 10 mM $MgCl_2$ and incubated for 60 min at 37°C to digest free nucleic acids. The reaction was stopped with 20 mM EDTA, and the core capsids were precipitated with 7% PEG8000 in 1.75 M NaCl. The pellets were resuspended in 50 µl of TNE buffer (10 mM Tris-HCl pH 8, 100 mM NaCl, 1 mM EDTA). The core particle RNA was then extracted using Trizol reagent(8).

Chromatin immunoprecipitation (ChIP) assay The dHepaRG cells were resuspended in ChIP lysis bufferand incubated for 30 min on ice and then the pelleted nucleiwere fixed in 1% formaldehyde for 30 min on ice. The isolated cross-linked nuclei were lysed in SDS lysis buffer and sheared by sonication to generate 500-1000 bp DNA fragments. HBV cccDNA is resistant to sonication (9). After centrifugation, the supernatant was diluted 1:10 in a dilution buffer and precleared with protein A/G plus agarose beads. 50 µl of precleared sample was put aside as input, and the rest of thechromatin was then subjected to immunoprecipitation for 14–16 hours at 4°C using the indicated antibodies. Immunoprecipitation with normal IgGwas conducted in each experiment to exclude the non-specific binding. The protein-DNA complexes were precipitated with protein A/G plus agarose beads and washed. After elution and reverse cross-linking, the immunoprecipitated chromatin was purified by phenol/chloroform (1:1) extraction and ethanol precipitationand analyzed by qPCR for HBV cccDNA and host genes. The results were presented as percentages of input DNA. For the ChIP assay using liver samples, the liver tissues were homogenized in 500 µl of tissue lysis buffer (5 mM PIPES, 85 mM KCl, 0.5%

NP-40) andthe pelleted nuclei were fixed in 1% formaldehyde for 15 min at 4°C. The subsequent steps were the same as described above.

RNA immunoprecipitation (RIP) assay The transfected Huh7 cells were lysed on ice in RIP lysis buffer for 30 min and scraped off. Flag-tagged proteins were immunoprecipitated from the supernatant with anti-Flag M2 antibody overnight at 4°C, followed by incubating with protein A/G plus agarose beads for another 2 hrs. The beads were washed 5 times at 4°C with RIP lysis buffer. During the final wash, the beads were equally divided for western blotting and RNA extraction. Immunoprecipitated pgRNA was quantified by qPCR and represented as percentages of input pgRNA. An aliquot of the cell lysate and the immunoprecipitated proteins were subjected to western blotting(10).

Enzyme-linked immunosorbent assay (ELISA) The supernatants were collected and centrifuged at 3,000 rpm for 5 min. The HBeAg and HBsAg in the supernatants were examined by ELISA (Kehua, Shanghai, China) according to the manufacturer's instructions.

Supporting Results

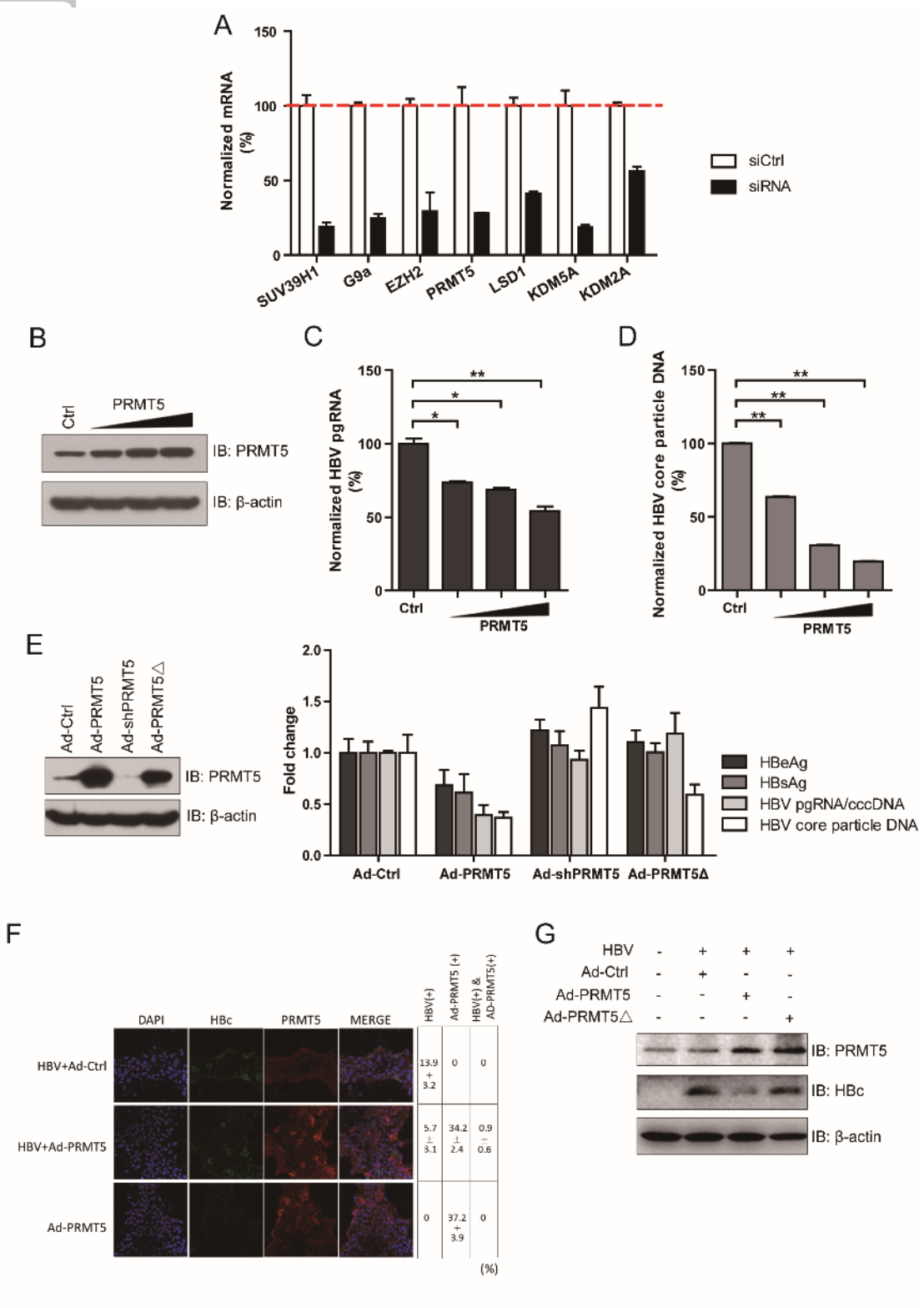

Fig S1. The effects of PRMT5 on HBV replication.

(A) The mRNA levels of the indicated genes in Fig 1A were quantified by RT-qPCR. (B-D) Monomeric linear HBV DNA was co-transfected with a PRMT5-expressing plasmid into Huh7

cells. The dose ratios of PRMT5 to HBV DNA were 1:1, 2:1 and 3:1. The pcDNA3.1a vector was used as a negative control. A GFP-expressing plasmid was included as a control for transfection efficiency. At 48 hrs post-transfection, the cells were harvested for Western blot to detect PRMT5 expression (B) or for RT-qPCR analysis to quantify the levels of pgRNA (C) and core particle DNA (D). The results are representative of three independent experiments, each performed in duplicate. (E) PRMT5 and shPRMT5 were transduced into HBV-infected PHHs. The expressions of PRMT5 were detected by Western blot. The levels of HBV antigens in the supernatant were detected by ELISA. HBV pgRNA, core particle DNA and cccDNA were analyzed by qPCR. The levels of pgRNA and cccDNA were used to calculate the ratio of pgRNA/cccDNA. (F) dHepaRG cells were infected by HBV and adeno-PRMT5 as indicated. The HBc protein and PRMT5 were detected by the immunofluorescence assay using mouse anti-HBc Abs (provided by Prof. Ningshao Xia) and rabbit anti-PRMT5 Abs. The percentage of cells with HBc or ectopically-expressed PRMT5 from three different fields (covering approximately 40-80 cells for each field) was calculated (mean ± SD).(G) PRMT5 and PRMT5△ were transduced into HBV-infected dHepaRG cells, respectively. The expressions of HBc and PRMT5 were determined by Western blot.

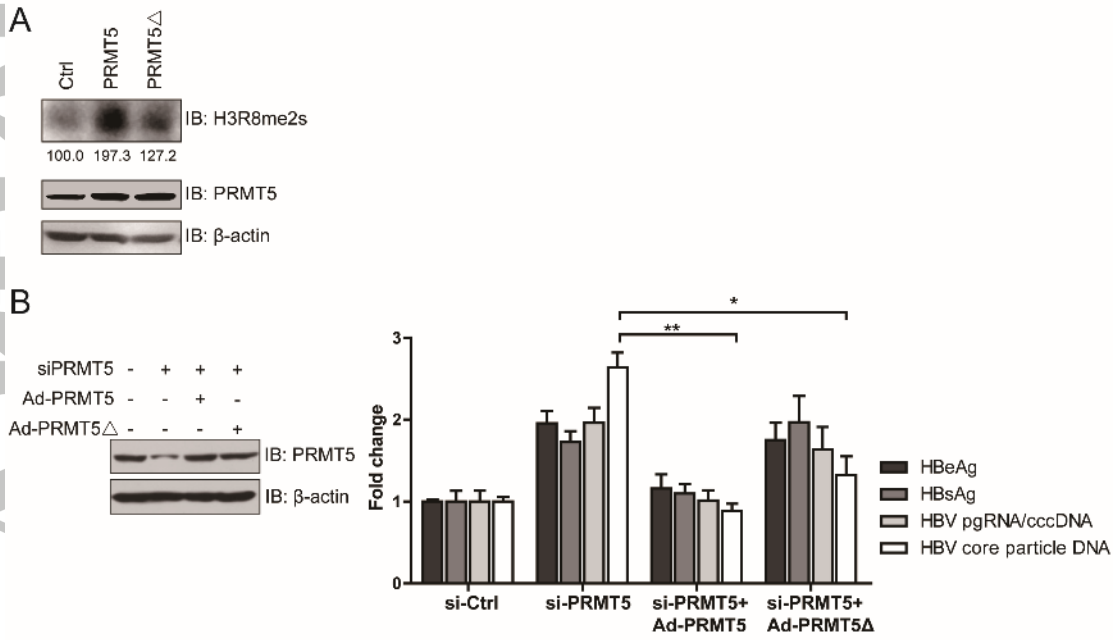

Fig S2. The effect of PRMT5△on H3R8me2s levels and HBV replication.

(A) pcDNA3.1a-vector, PRMT5 and PRMT5△ were transfected into HepG2 cells. H3R8me2s were detected by Western blot using H3R8me2s antibody. The expression levels of PRMT5 and PRMT5△ were detected by PRMT5 antibody. β-actin was used as a loading control. The level of the symmetric dimethylation of H3R8 (H3R8me2s), which is known to be catalyzed by PRMT5, was significantly lower in cells with PRMT5△ expression compared with that in cells with wildtype PRMT5 expression, suggesting the methyltransferase activity of PRMT5△ is extensively impaired compared to that of wildtype PRMT5. Although the level of H3R8me2s was slightly higher compared with that in control cells, this could be caused by the presence of a small amount of dimers consisting of endogenous PRMT5 and PRMT5△ when PRMT5△was overexpressed (11).(B) siPRMT5 was transfected into HBV-infected HepG2-NTCP cells at D1 post HBV infection and the cells were re-plated and transduced with PRMT5 and PRMT5△ at D3.The cells were extracted at D7. The expressions of PRMT5 were detected by Western blot. The levels of HBV antigens in the supernatant were detected by ELISA. HBV pgRNA, core particle DNA and cccDNA were analyzed by qPCR. The levels of pgRNA and cccDNA were used to calculate the ratio of pgRNA/cccDNA.

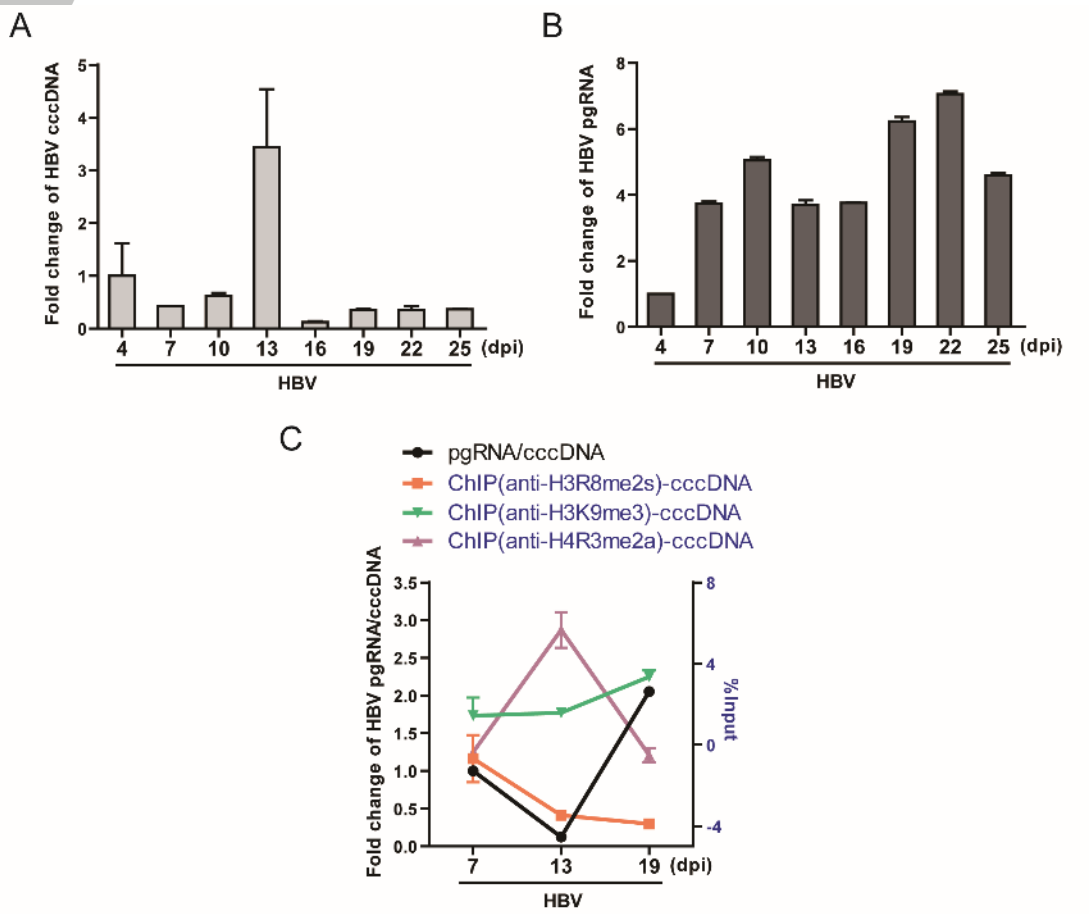

Fig S3. The dynamic changes in the cccDNA and pgRNA levels in dHepaRG cells and the modification status of histones on cccDNA.

(A) cccDNA quantification in the experiment shown in Fig. 3A. (B) pgRNA quantification in the experiment shown in Fig. 3A. The dHepaRG cells which were infected with the sera of CHB patients were harvested every three days and examined the expression levels of intracellular cccDNA and pgRNA by qPCR since the Day 4 post HBV infection.(C) The cells in Fig. 3B were also used for ChIP assays with antibodies specific for H3R8me2s, H3K9me3 or H4R3me2a. ChIP results were presented as percentages of input cccDNA.

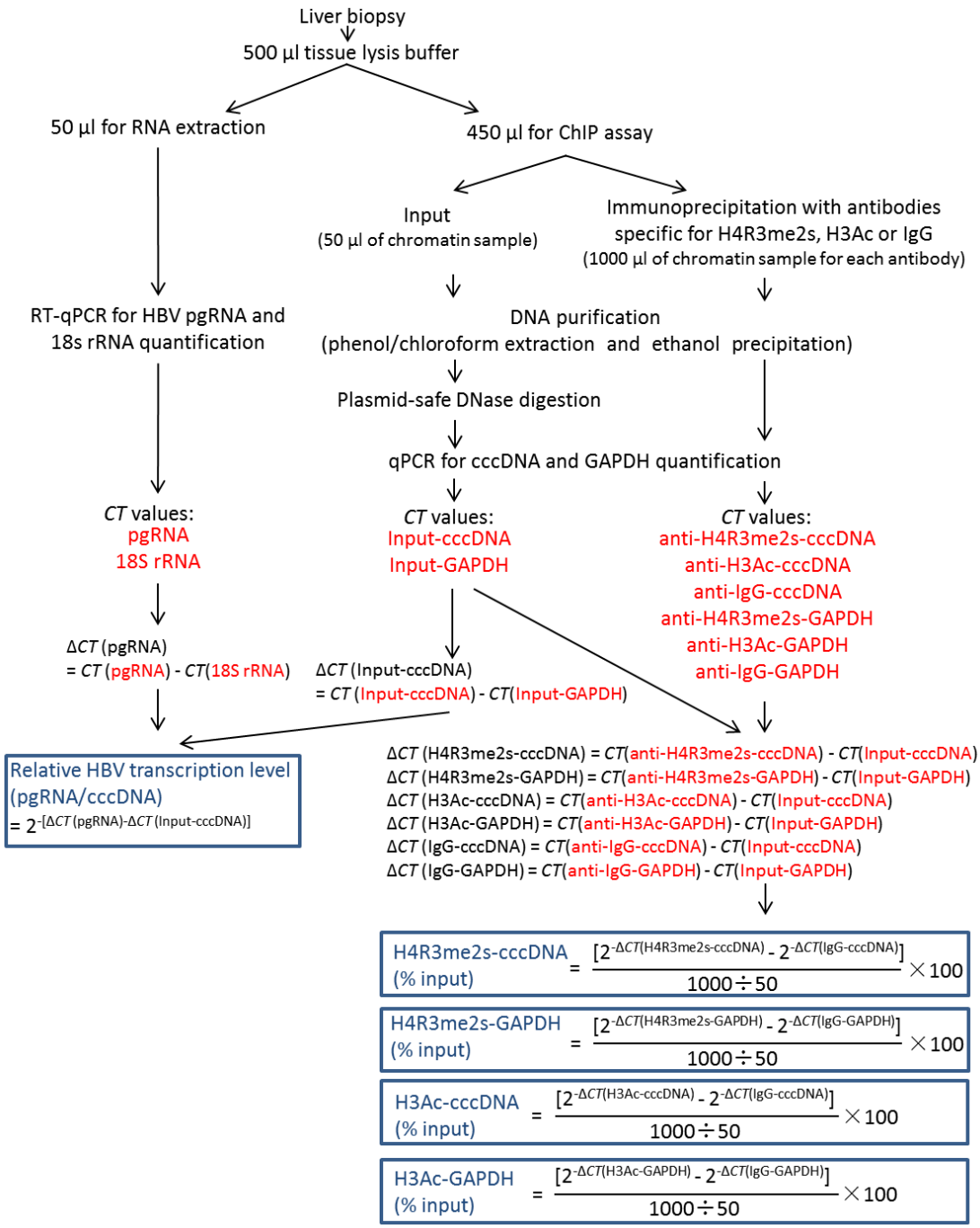

Fig S4. Strategy for quantification of pgRNA, cccDNA and cccDNA- or GAPDH-bound H4R3me2s and H3Ac in liver biopsies from CHB patients.

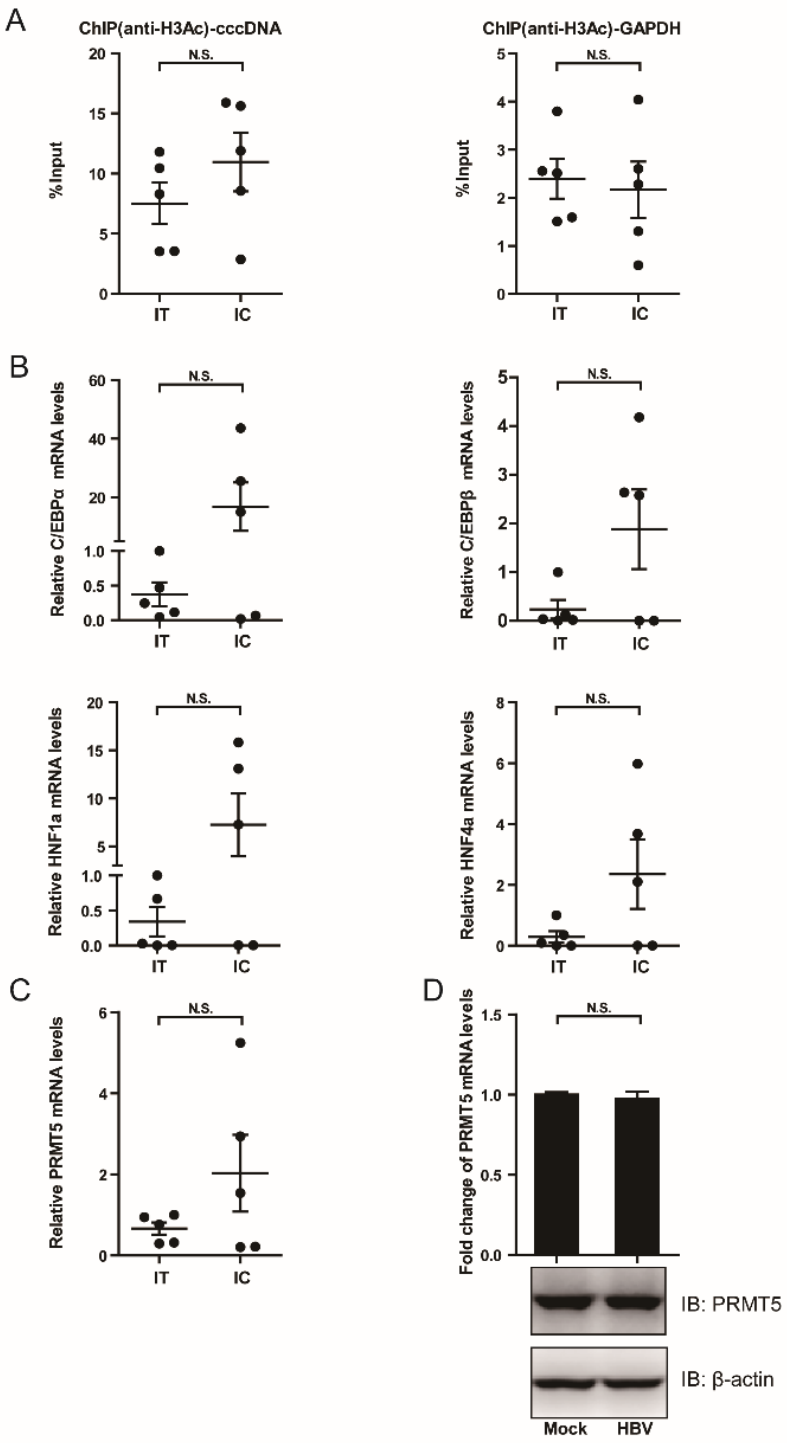

Fig S5. The effect of HBV infectionon histones modifications and host factors expressions in liver biopsies and dHepaRG cells.

(A-C) The samie liver biopsies analyzed in Fig. 3C and 3D were also used here. (A)The biopsies were subjected for ChIP assays with an antibody specific for H3Ac. The data analysis method was the same as that in Fig. 3C and 3D.(B, C) C/EBPα, C/EBPβ, HNF1α, HNF4α and PRMT5 expressions were quantified. (D) The expression levels of PRMT5 in dHepaRG cells with or without HBV infection were determined by qPCR and Western blot.

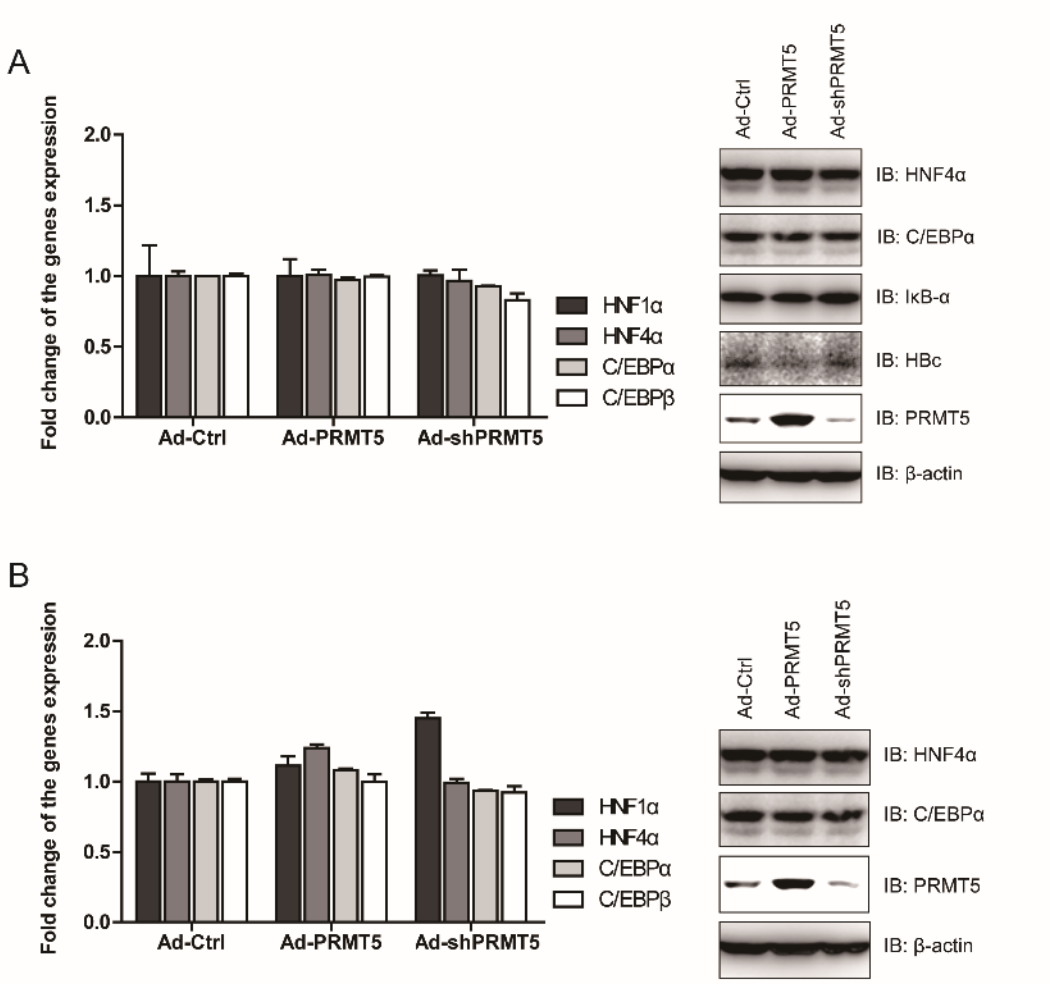

Fig S6. The effect of PRMT5 on the expressions of host factors including HNF1α, HNF4α, C/EBPα, C/EBPβ and IkB-α in HBV-infected or control dHepaRG cells.

(A) PRMT5 or shPRMT5 was transduced into HBV-infected dHepaRG cells.The expressions of HNF1α, HNF4α, C/EBPα, C/EBPβ, IkB-α, HBc and PRMT5 were determined by qPCR and/or Western blot.(B) PRMT5 or shPRMT5 was transduced into dHepaRG cells without HBV infection. The expressions of HNF1α, HNF4α, C/EBPα, C/EBPβ, IkB-α and PRMT5 were determined by qPCR and/or Western blot.

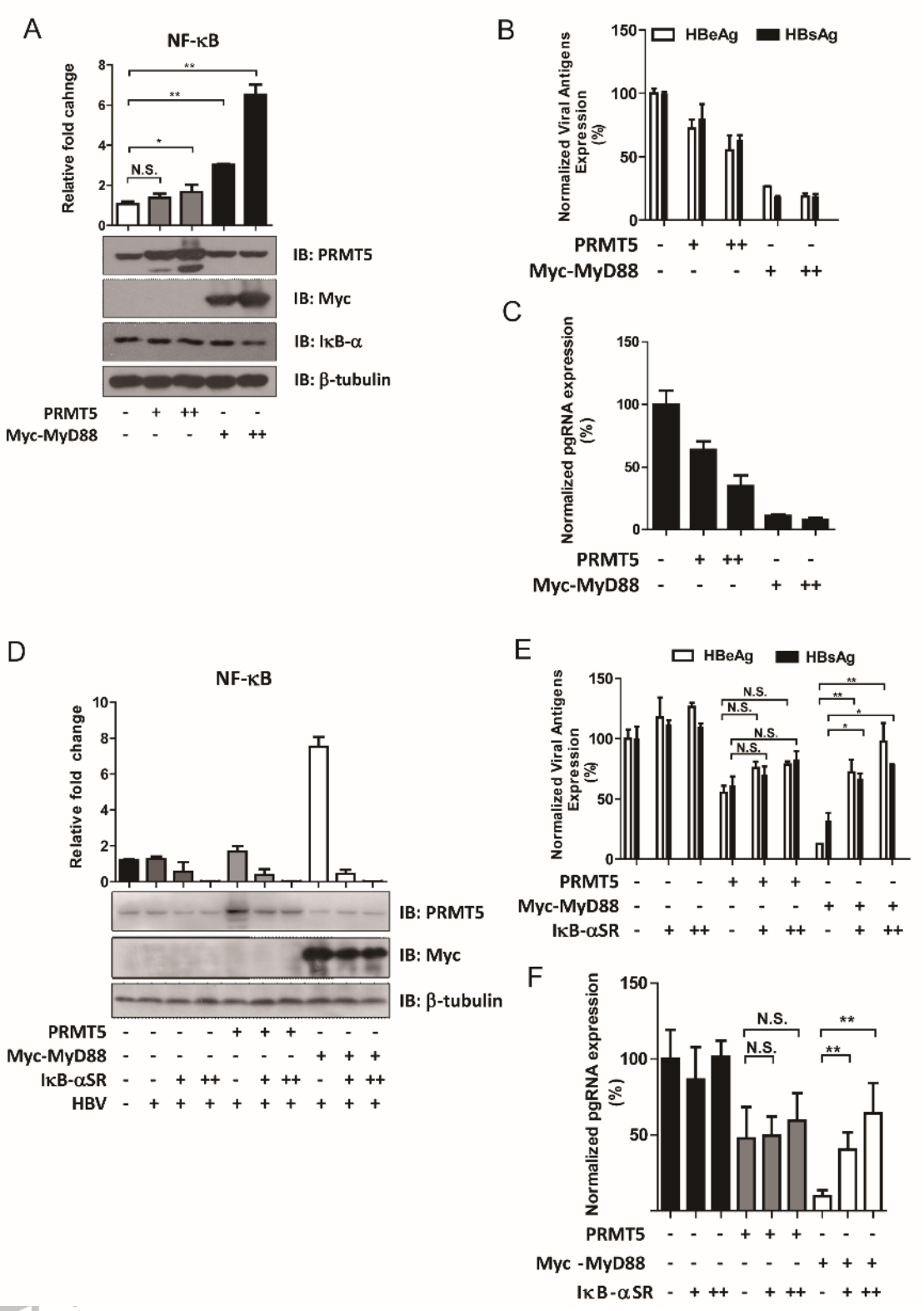

Fig S7. The role of NF-kB pathway in PRMT5's anti-HBV effect.

Huh7 cells in 24-well plates were co-transfected as indicated. The cells were extracted for luciferase reporter assay and HBV pgRNA quantification 48 hrs later and the supernatants were collected to detect HBeAg and HBsAg by ELISA. IκB-αSR is a dominant negative mutant of IκB-αSR.

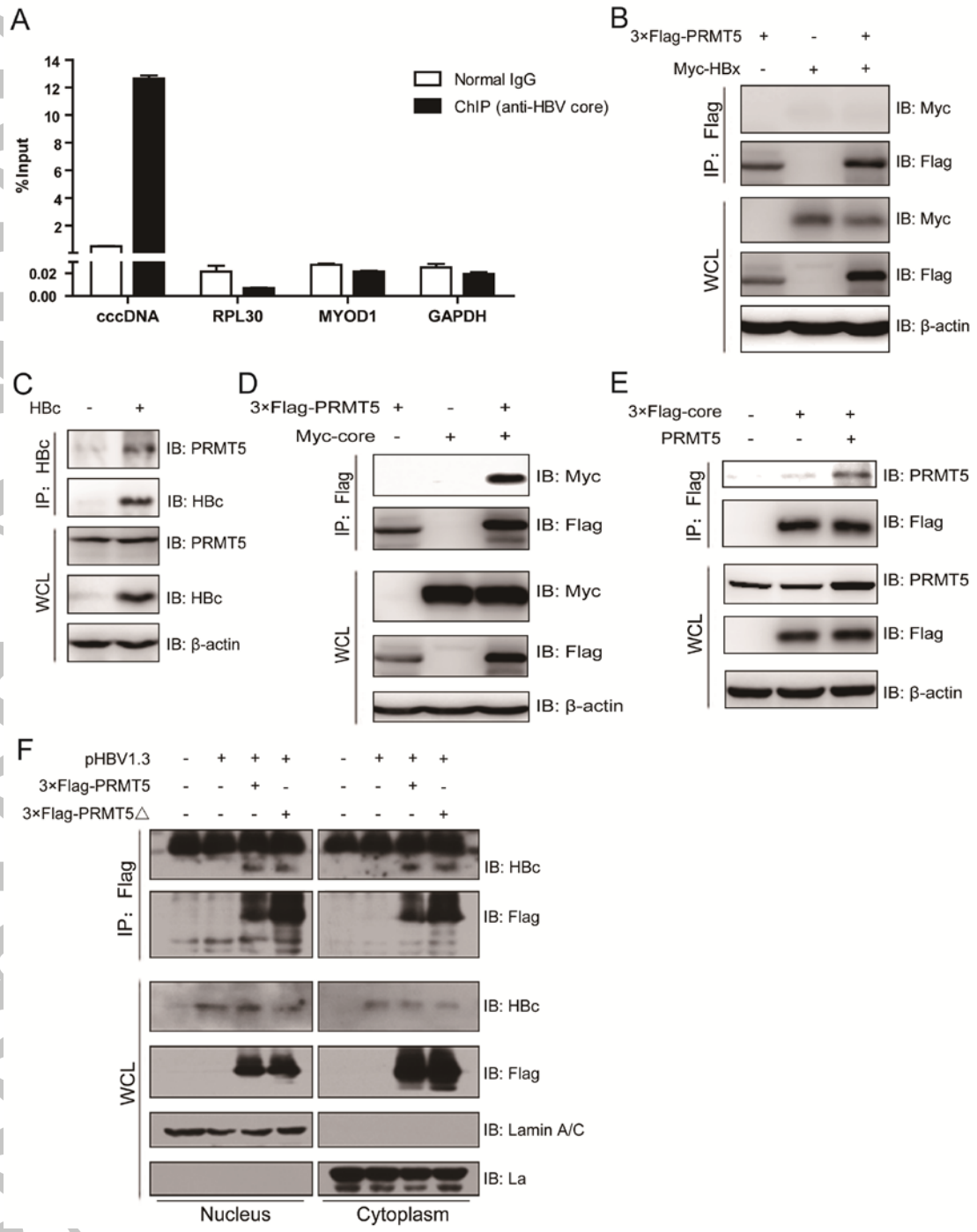

Fig S8. Analysis of the bingding of HBc to cccDNA and the interaction between PRMT5 and HBc or HBx.

(A) The HBV-infected HepaRG cells were conducted ChIP assay with anti-HBc antibody. qPCR was performed to analyze the levels of HBc protein on HBV cccDNA or the host genes RPL30, MYOD1 and GAPDH.(B-E) Huh7 cells were co-transfected as indicated and the cells were harvested for CoIP assay with anti-Flag Abs at 48 hrs post-transfection. (F) Huh7 cells were co-transfected as indicated and the cells were harvested to conduct CoIP assay with anti-Flag antibody for the nuclear and the cytoplasmic fractions respectively. The proteins were detected by

Western blot.

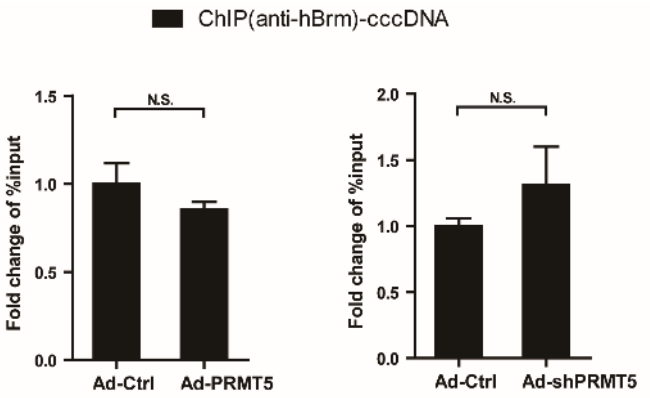

Fig S9. The effect of PRMT5 on the binding of hBrm to cccDNA.

PRMT5 was transducedinto HBV-infected dHepaRG cells in 100 mm dishesfor 6 days.shPRMT5 was transducedinto HBV-infected dHepaRG cells in 100 mm dishesfor 5 days. Three-quarters of the cells were used for a ChIP assay with anti-hBrm antibody. qPCR was performed to analyze the levels of hBrm on HBV cccDNA.

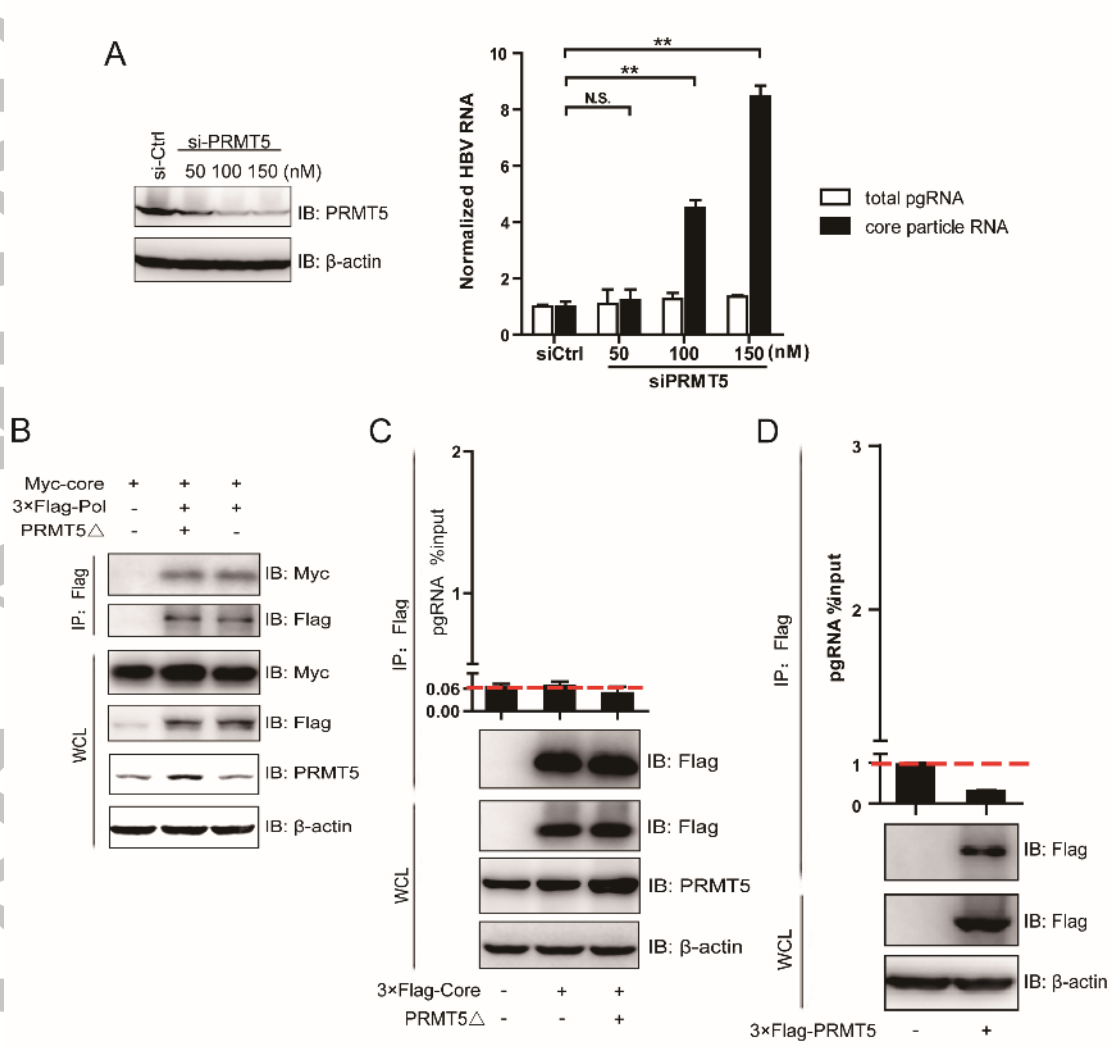

Fig S10. The effect of PRMT5△on the interactions between HBV core and Pol or pgRNA. (A) Huh7 cells were transfected with siPRMT5and harvested for RNA extraction and core particle RNA isolation, followed by HBV pgRNA quantification by qPCR assay. The expression levels of PRMT5 were detected by Western blot. (B) Huh7 cells were co-transfected as indicated and the cells were conducted CoIP assay with anti-Flag antbody. (C) Huh7 cells were co-transfected as indicated, and pCMV-HBV-ΔPol-ΔCore was tranfected in all groupsto transcribepgRNA. At 48 hrs post-transfection, the cells were harvested forRIP assay using anti-Flag antibody. (D) Huh7 cells were co-transfected with pCMV-HBV-ΔPol-ΔCore and pcDNA3.1a-3×Flag-PRMT5 or vector. At 48 hrs post-transfection, the cells were conducted RIP assay with anti-Flag antibody.

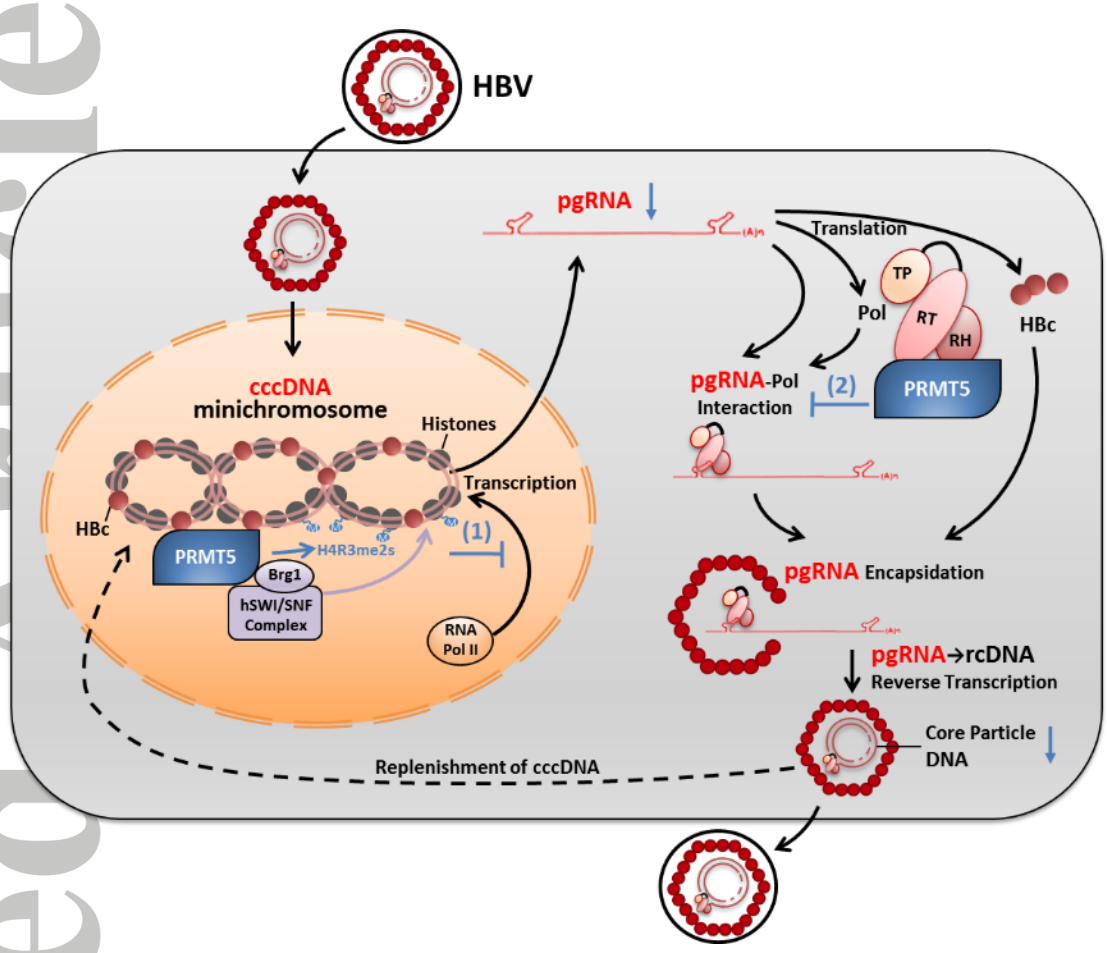

Fig S11. Working model of the anti-HBV effects of PRMT5.

In the nucleus, PRMT5 suppresses cccDNA transcription through symmetrically dimethylating H4R3 on the minichromosome, which involves the HBV core, the Brg1-based hSWI/SNF chromatin remodeler and RNA Pol II. In the cytoplasm, PRMT5 interacts with HBV Pol at RT-RH region to counteract the binding between Pol and pgRNA to interfere with pgRNA encapsidation and thus reduce viral DNA production.

Supporting Table 1. Histone methylation sites and the main related methyltransferases and demethylases

Residues on Histones	Effect of methylation on gene transcription	Methyltransferases	Demethylases
H3K9	Repression	SUV39H1*, G9a*	KDM3A, KDM4A
H3K27	Repression	EZH2*	KDM6A, JMJD3
H4R3#	Repression	PRMT5*	PADI4, JMJD6
H3R8	Repression	PRMT5*	PADI4
H3K4	Activation	SETD1A	LSD1*, KDM5A*
H3K36	Activation	SETD2, NSD3	KDM2A*

#symmetric dimethylation on histone 4 arginine 3 (H4R3)is a repressive mark and canbe catalyzed by PRMT5.

*the factors studied here

SupportingTable 2. Characteristics of the studied patients and the levels of HBV pgRNA/cccDNA and H4R3me2s on the HBV minichromosome

Patient	Age (y)	Gender (F/M)	HBV genotype (A-F)	ALT (IU/ml)	HBeAg	Pathological diagnosis (G0-G4, S0-S4)	Serum HBV DNA (copies/ml)	Relative HBV transcription level (pgRNA/cccDNA)	H4R3me2s -cccDNA (%input)
1	45	F	B	45	+	G1/S1	2.55×10^{7}	35.691	1.9992
2	52	M	C	19	+	G1/S1	1.16×10^{6}	18.225	4.7598
3	19	F	B	18	+	G1/S1-2	5.00×10^{7}	17.515	3.6231
4	29	M	C	42	+	G1/S1	2.06×10^{7}	4.6527	13.244
5	26	M	B	9	+	G1/S2	1.09×10^{7}	3.4252	4.3199
6	30	F	B	14	-	G1/S1	3.14×10^{3}	0.0535	12.585
7	34	F	B	14	-	G1/S0-1	1.11×10^{3}	0.0022	39.899
8	30	F	C	13	-	G1/S2	9.91×10^{3}	0.0016	11.016
9	51	F	C	13	-	G1/S1	2.80×10^{3}	0.0010	23.482
10	43	F	B	14	-	G1/S1	1.18×10^{3}	0.0009	15.019

Abbreviations: F, female; M, male; ALT, alanine aminotransferase, ALT normal values ≤ 40IU/ml; pgRNA, pre-genomic RNA; cccDNA, covalently closed circular DNA; H4R3me2s, symmetric dimethylation of histone 4 arginine 3

Pathological diagnosis of liver biopsies including inflammation grade (G) and fibrosis stage (S) was determined according to the Scheuer scoring system.

Themaximum detection limit of serum HBV DNA level is 5.00×10^{7} copies/ml.

The exclusion criteria included co-infection with HCV or HIV and previous treatment with antiviral or immunosuppressive drugs.

Supporting Table 3. Plasmids used for transfection

Plasmids	Origin
pCR.HBV.A.EcoRI	Described in J Clin Invest 122: 529-537
pCMV-HBV	kindly provided by Prof. Jianming Hu (Pennsylvania State University, PA, USA)
pCMV-HBV-ΔPol	
pCMV-HBV YMHD	
pCMV-HBV-ΔPol-ΔCore	Subcloned from the pCMV-HBV-ΔPol
pHBV1.3	Described in J Virol 2011;85:6319-6333.
pcDNA3.1a-PRMT5	Amplified from the PRMT5 cDNA and inserted into pcDNA3.1a-vector
pcDNA3.1a-3×Flag-PRMT5	Amplified from the PRMT5 cDNA and inserted into pcDNA3.1a-3×Flag-vector
pcDNA3.1a-PRMT5Δ	Subcloned from the pcDNA3.1a-PRMT5
pcDNA3.1a-NES-PRMT5Δ	Subcloned from the pcDNA3.1a-PRMT5Δ
pcDNA3.1a-NLS-PRMT5Δ	Subcloned from the pcDNA3.1a-PRMT5Δ
pcDNA3.1a-3×Flag-vector	Described in J Gen Virol 2010;91:2080-90.
pcDNA3.1a-3×Flag-Pol	
pCMV-Myc-Pol	Amplified HBV Pol from pcDNA3.1a-3×Flag-Pol and inserted into pCMV-Myc-vector
pCMV-Myc-TP-SP	Subcloned from the pCMV-Myc-Pol
pCMV-Myc-RT-RH	
pCMV-Myc-RT	
pCMV-Myc-RH	

Supporting Table 4. Antibodies used in this study

Antibodies	Origin
Anti-Histone H4R3me2s(ab5823)	Abcam
Anti-PRMT5[EPR5772](ab109451)	
Anti-RNA polymerase II(ab817)	
Anti-HBcAg (ab-8638)	
Anti-Histone H3R8me2s(600-401-I68)	Rockland
Anti-H4R3me2a(A2376)	ABclonal
Anti-Brg1 (H-88)(sc-10768/x)	Santa Cruz
Anti-HBcAg (sc-23945)	
Goat anti-mouse secondary Abs-HRP	
Goat anti-rabbit secondary Abs-HRP	
Normal mouse IgG	
Normal rabbit IgG	
Normal goat IgG	
Anti-Histone H3K9me3(39161)	Active Motif
Anti-H3Ac (39139)	
Anti-HBcAg(B0586)	DAKO
Anti-HBcAg (mouse, for IFA)	Prof. Ningshao Xia
Anti-HBcAg (mouse, for WB)	Prof. Ningshao Xia
Anti-Flag M2	Sigma
Anti-Flag M2 affinity beads	
Anti-Myc	
Anti-β-actin	
Anti-HA	
DAPI	Invitrogen
AlexaFlour-488-coupled secondary Abs	Jackson Immunologicals
AlexaFlour-594-coupled secondary Abs	

Supporting Table 5. Target sequences of siRNAs

siRNAs	Target sequence (5'-3')
siLSD1	GTGATACTGTGCTTGTCCA
siKDM5A	CCATTTGCCTGTGAAGTAA
siSUV39H1	ACCTCTTTGACCTGGACTA
siG9a	CCATGCTGTCAACTACCAT
siEZH2	GACTCTGAATGCAGTTGCT
siKDM2A	GAAGAAGAAAGGATTCGTT
siPRMT5	001: GGACCTGAGAGATGATATA 005:GAGGATTGCAGTGGCTCTT
siBrg1	001: CCAAGGATTTCAAGGAATA 002: GGACCTGAATGAGGAGGAA

Supporting Table 6. Primer sequences used for RT-qPCR

Gene	Primer Sequences(5'→3')
LSD1	s:GAAACTATGTAGCTGATCTTGGAGC as:TGATCTTGGCCAGTTCCATATTTAC
KDM5A	s: CCGTCTTTGAGCCGAGTTGG as:GCTGGACTCTTGGAGTGAAACG
SUV39H1	s:CTGCCCATCTACGAGTGCAA as:GCTCTGCCTCCTCTGAGGTAATG
G9a	s:GAGGTGTACTGCATAGATGCCC as:CAGACGGCTCTGCTCCAGG
EZH2	s:GCCAGACTGGGAAGAAATCTG as:TGTGTTGGAAAATCCAAGTCAC
KDM2A	s:GACTTGGAAGAGAAACTGCACACC as:CTCCCCACACACATTTTGACATC
PRMT5	s:CTCTCAGTACCAGCAGGCCATC as:GCGTCACCACGGCATTTG
cccDNA	s:CTCCCCGTCTGTGCCTTCT as:GCCCCAAAGCCACCCAAG Probe:FAM-AGCGAAGTGCACACGGACCGGCAGA-TAMRA
pgRNA	s:GCCTTAGAGTCTCCTGAGCA(genotype A/D) s:GCCTTAGAGTCTCCGGAACA (genotype B/C) as:GAGGGAGTTCTTCTTCTAGG
core particle DNA	s:CCGTCTGTGCCTTCTCATCTG as:AGTCCAAGAGTYCTCTTATGYAAGACCTT Probe: FAM-CCGTGTGCACTTCGCTTCACCTCTGC-TAMRA
G188	s:GGTATCGTGGAAGGACTCATGA as:ATGCCAGTGAGCTTCCCGTTCAGC
18s rRNA	s:CAGCCACCCGAGATTGAGCA as:TAGTAGCGACGGGCGGTGTG
GAPDH-ChIP	s:TCGACAGTCAGCCGCATCT as:CTAGCCTCCCGGGTTTCTCT
RPL30-ChIP	s:CAAGGCAAAGCGAAATTGGT as:GCCCGTTCAGTCTCTTCGATT
MYOD1-ChIP	s:CCGCCTGAGCAAAGTAAATGA as:GGCAACCGCTGGTTTGG

References

1. Chen J, Zhang W, Lin J, Wang F, Wu M, Chen C, Zheng Y, et al. An efficient antiviral strategy for targeting hepatitis B virus genome using transcription activator-like effector nucleases. Mol Ther 2014;22:303-311.

2. Ning X, Nguyen D, Mentzer L, Adams C, Lee H, Ashley R, Hafenstein S, et al. Secretion of genome-free hepatitis B virus--single strand blocking model for virion morphogenesis of para-retrovirus. PLoS Pathog 2011;7:e1002255.

3. Branscombe TL, Frankel A, Lee JH, Cook JR, Yang Z, Pestka S, Clarke S. PRMT5 (Janus kinase-binding protein 1) catalyzes the formation of symmetric dimethylarginine residues in proteins. J Biol Chem 2001;276:32971-32976.

4. Gripon P, Rumin S, Urban S, Le Seyec J, Glaise D, Cannie I, Guyomard C, et al. Infection of a human hepatoma cell line by hepatitis B virus. Proc Natl Acad Sci U S A 2002;99:15655-15660.

5. Hantz O, Parent R, Durantel D, Gripon P, Guguen-Guillouzo C, Zoulim F. Persistence of the hepatitis B virus covalently closed circular DNA in HepaRG human hepatocyte-like cells. J Gen Virol 2009;90:127-135.

6. Sun D, Nassal M. Stable HepG2- and Huh7-based human hepatoma cell lines for efficient regulated expression of infectious hepatitis B virus. J Hepatol 2006;45:636-645.

7. Ni Y, Lempp FA, Mehrle S, Nkongolo S, Kaufman C, Falth M, Stindt J, et al. Hepatitis B and D Viruses Exploit Sodium Taurocholate Co-transporting Polypeptide for Species-Specific Entry into Hepatocytes. Gastroenterology 2014;146:1070-1083 e1076.

8. Guo JT, Pryce M, Wang X, Barrasa MI, Hu J, Seeger C. Conditional replication of duck hepatitis B virus in hepatoma cells. J Virol 2003;77:1885-1893.

9. Pollicino T, Belloni L, Raffa G, Pediconi N, Squadrito G, Raimondo G, Levrero M. Hepatitis B virus replication is regulated by the acetylation status of hepatitis B virus cccDNA-bound H3 and H4 histones. Gastroenterology 2006;130:823-837.

10. Sato S, Li K, Kameyama T, Hayashi T, Ishida Y, Murakami S, Watanabe T, et al. The RNA Sensor RIG-I Dually Functions as an Innate Sensor and Direct Antiviral Factor for Hepatitis B Virus. Immunity 2015;42:123-132.

11. Zhao Q, Rank G, Tan YT, Li H, Moritz RL, Simpson RJ, Cerruti L, et al. PRMT5-mediated methylation of histone H4R3 recruits DNMT3A, coupling histone and DNA methylation in gene silencing. Nat Struct Mol Biol 2009;16:304-311.