Supplementary Information for

N6-methyladenosine Modification of the 5′ epsilon Structure of HBV pre-genome RNA is Required for its Encapsidation by the Viral Core Protein

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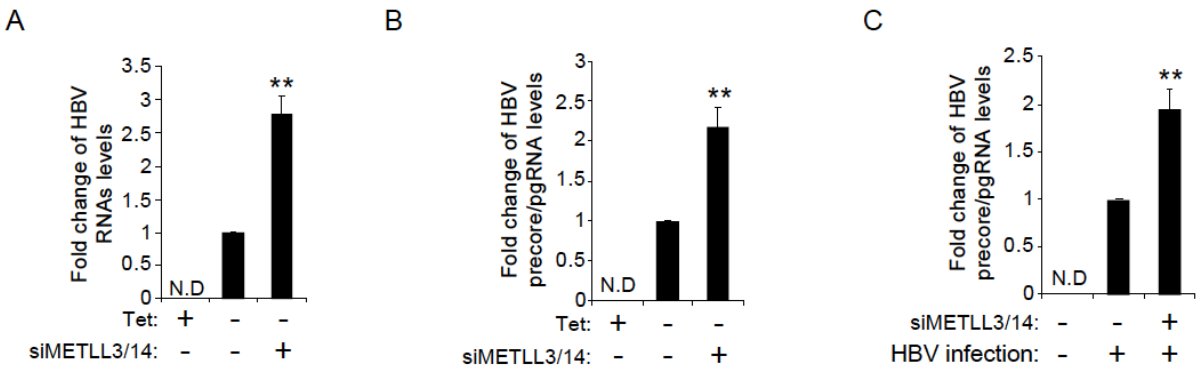
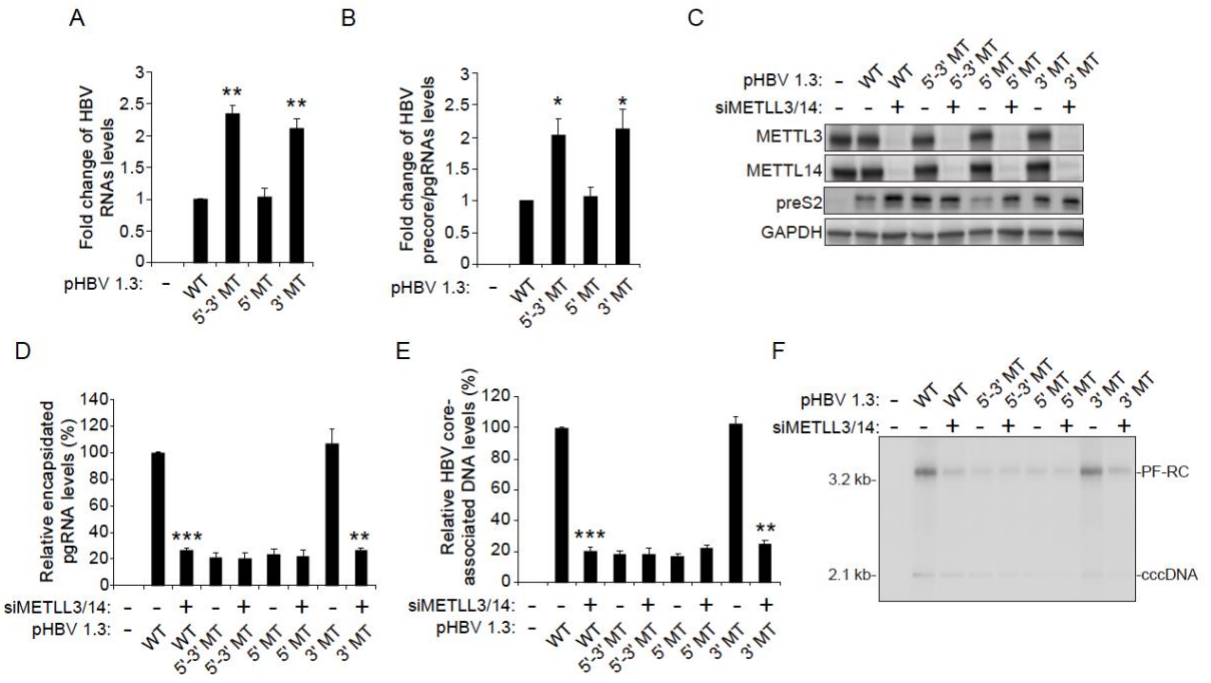
This PDF file includes:

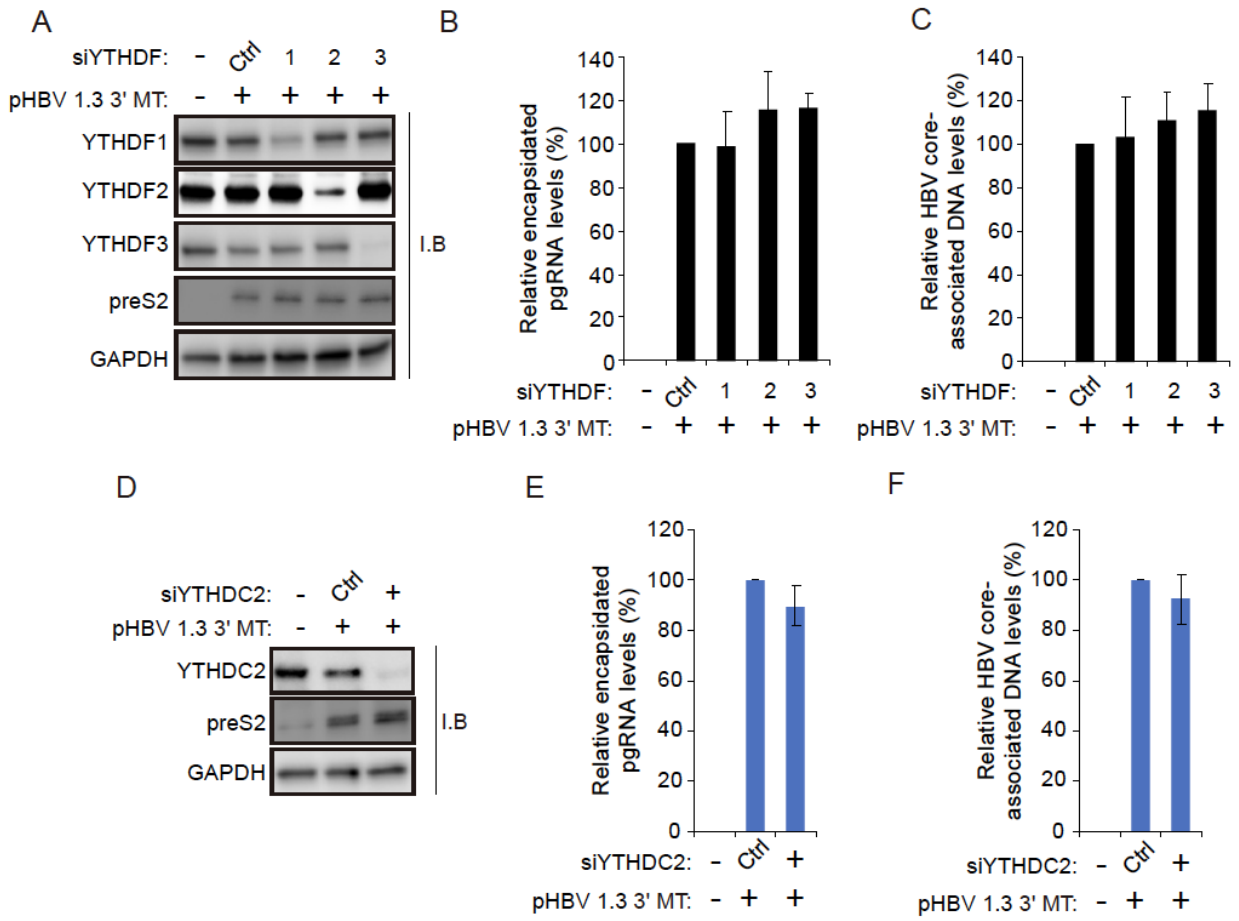
Supplementary Figure 1 to 4.



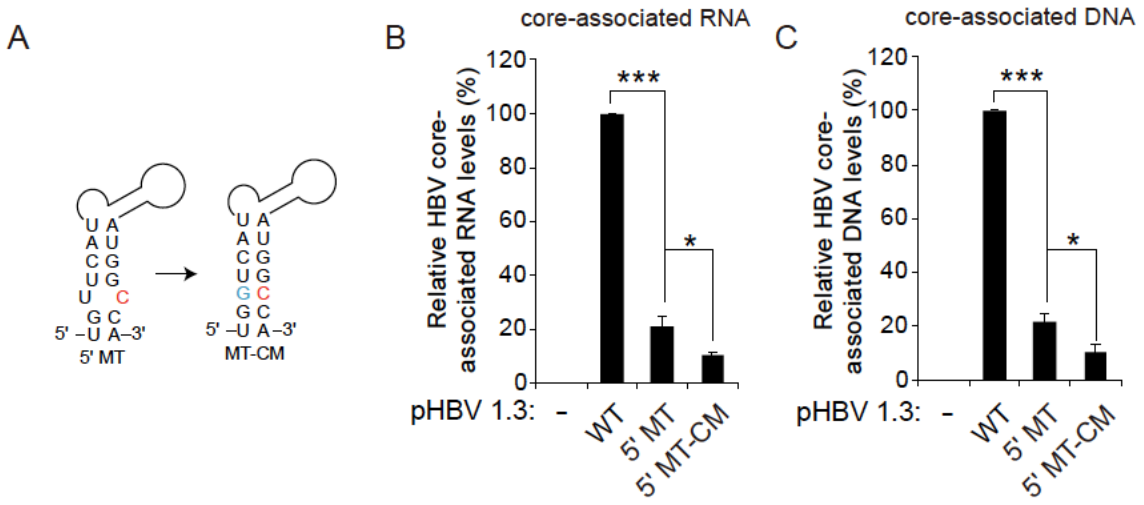
Supplementary Fig. 1 Related to Figure 1. (A and B) The siRNAs of METTL3/14 were transfected into HepAD38 cells grown in the absence or presence of tetracycline for 48 h. Total RNA was extracted. HBV RNA and HBV precore/pgRNA levels were analyzed by RT-qPCR. (C) HepG2-NTCP cells were infected with 2.5 x 10^3 genome equivalents per cell of HBV particles. After 10 days, total RNA was extracted and HBV precore/pgRNA was analyzed by RT-qPCR. In all panels, the error bars represent the SDs of three independent experiments. The *P* values are calculated via an unpaired Student's *t*-test. $^{**}P < 0.01$.



Supplementary Fig. 2 Related to Figure 2. (A and B) The indicated pHBV 1.3 plasmids were transfected into Huh7 cells. After 72 h, total RNA was isolated. HBV RNA and HBV precore/pgRNA were analyzed by RT-qPCR. (C-F) The siRNAs of METTL3/14 were transfected into Huh7 cells expressing indicated plasmids. After 48 h, cell lysates, encapsidated HBV RNA, HBV core-associated DNA, and HBV DNA were isolated from these cells. The indicated proteins were analyzed by Western blotting (C). The encapsidated HBV RNA levels were analyzed by RT-qPCR (D). The HBV core-associated DNA levels were assayed by qPCR (E). HBV rcDNA and cccDNA were detected by Southern blotting (F). In (A and B) and (D and E), the error bars represent the SDs of three independent experiments. The *P* values are calculated via an unpaired Student's *t*-test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.



Supplementary Fig. 3 The effect of m^{6}A reader proteins on HBV pgRNA packaging. (A-F) The indicated siRNAs were transfected into pHBV 1.3-mer 3′ MT plasmid expressed Huh7 cells for 48 h. Cell lysates, encapsidated HBV RNA, and HBV core-associated DNA were extracted from these cells. The indicated proteins were analyzed by Western blotting (A and D). The encapsidated HBV pgRNA was analyzed by RT-qPCR (B and E). HBV core-associated DNA was analyzed by qPCR (C and F). In (B and C) and (E and F), the error bars represent the SDs of three independent experiments.



Supplementary Fig. 4 Related to Figure 3. (A) The A1907C mutation in the epsilon structure is predicted to create a bubble. The compensatory U1851G mutation (blue) was generated in the 5′ epsilon elements (pHBV-5′-MT-CM). (B and C) The indicated pHBV 1.3-mer plasmids were transfected into Huh7 cells. After 72 h, cells were harvested to analyzed encapsidated HBV pgRNA and HBV core-associated DNA levels. In (B) and (C), the error bars represent the SDs of three independent experiments. The *P* values are calculated via an unpaired Student's *t*-test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.