Supporting Information for

N6-Methyladenosine Modification of Hepatitis B and C Viral RNAs Attenuates Host Innate Immunity via RIG-I Signaling

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Running title: m^6A modification of viral RNA regulates RIG-I signal pathway

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Supplementary Figure legends

Supplementary Fig 1. Related to Figure 1. (A and B) HepG2 cells treated with siRNAs of METTL3 and 14 were transfected with pHBV 1.3-mer 5′-MT or 3′-MT. After 72 h, cells were harvested to assess the expression level of p-IRF-3 levels. Each right panel shows that p-IRF-3 protein levels relative to the IRF-3 from three independent experiments were quantified using ImageJ. (C - D) The HBV pgRNA levels, in Fig 1B and D, were quantified by qRT-PCR. (E - J) The METTL3 or METTL14 expression levels, in Fig 1E, S1 Fig A and B, were quantified using ImageJ. In (A - H), the error bars are the standard deviations of three independent experiments. *P* values were calculated using an unpaired *t*-test. **P* < 0.05; ***P* < 0.01; ****P* < 0.001; n.s., not significant by unpaired Student's *t*-test.

Supplementary Fig 2. Related to Figure 2. (A - C) Huh7 cells treated with siMETTL3 and 14 were transfected with HCV GND A8766C RNA for 16 h. IFN-β mRNA levels relative to input HCV RNA (B) were quantified by qRT-PCR (A). The indicated proteins were analyzed by immunoblotting (C). n.s, not significant by unpaired Student's *t*-test. (D - F) The depleted m^6A methyltransferases Huh7 cells were transfected with HCV GND A331C RNA. After 16 h, cells were harvested to analyze IFN-β mRNA (D) and p-IRF-3 (F) levels. (G - L) The METTL3 or METTL14 expression levels, in Fig 2C, S2 Fig C, and F, were quantified using ImageJ. The data for this figure are from three independent experiments and the bars represent the mean ± SD. *P* values were calculated using an unpaired *t*-test. **P* < 0.05; ***P* < 0.01; ****P* < 0.001; n.s., not significant by unpaired Student's *t*-test.

Supplementary Fig 3. Related to Figures 1 and 2. (A) HepG2 cells depleted METTL3/14 were treated with poly(I:C) for 16 h. Cells were harvested to assess IFN-β mRNA (in the left panel) or p-IRF-3 (in the right panel) levels. n.s., not significant by unpaired Student's *t-test*.

Supplementary Fig 4. Related to Figure 3. (A) The input HCV genome levels were calculated for

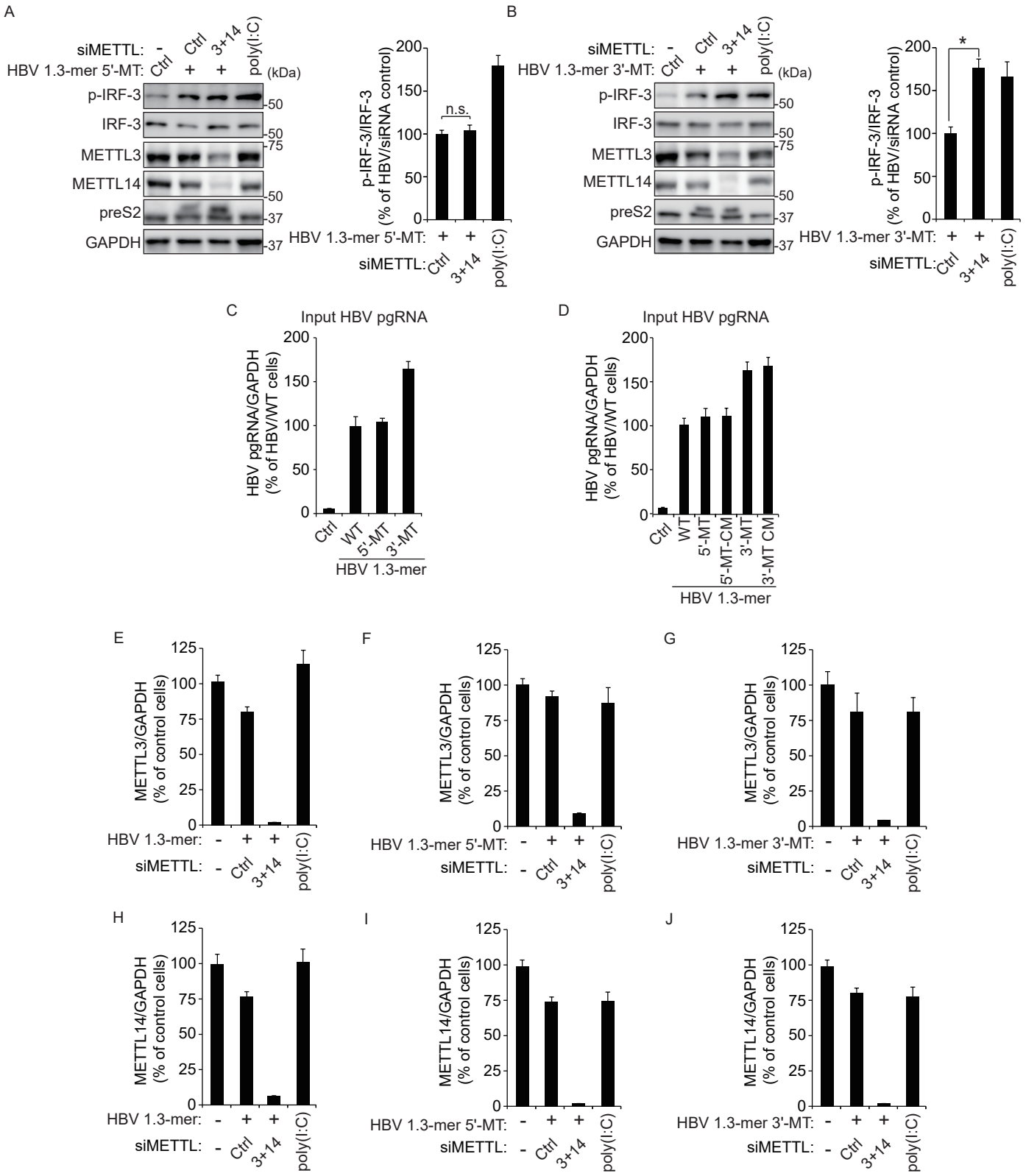
normalizing IFN-β mRNA in Fig 3B. (B) The input HBV pgRNA levels were quantified for calculating enriched HBV pgRNA levels in Fig 3D. (C) The transfected HCV genome levels were quantified for normalizing enriched HCV genome in Fig 3E. In this figure, error bars represent the standard deviations of three independent experiments.

Supplementary Fig 5. Related to Figure 4. (A - H) The p-IRF-3 expression levels, in Fig 4A to H, relative to IRF-3 were quantified using ImageJ. (I - L) The HCV genome copy numbers were calculated for normalizing IFN-β mRNA in Fig 4C to D. In this figure, error bars represent the standard deviations of three independent experiments. *P* values were calculated using an unpaired *t*-test. **P* < 0.05; ***P* < 0.01; ****P* < 0.001; n.s., not significant by unpaired Student's *t*-test.

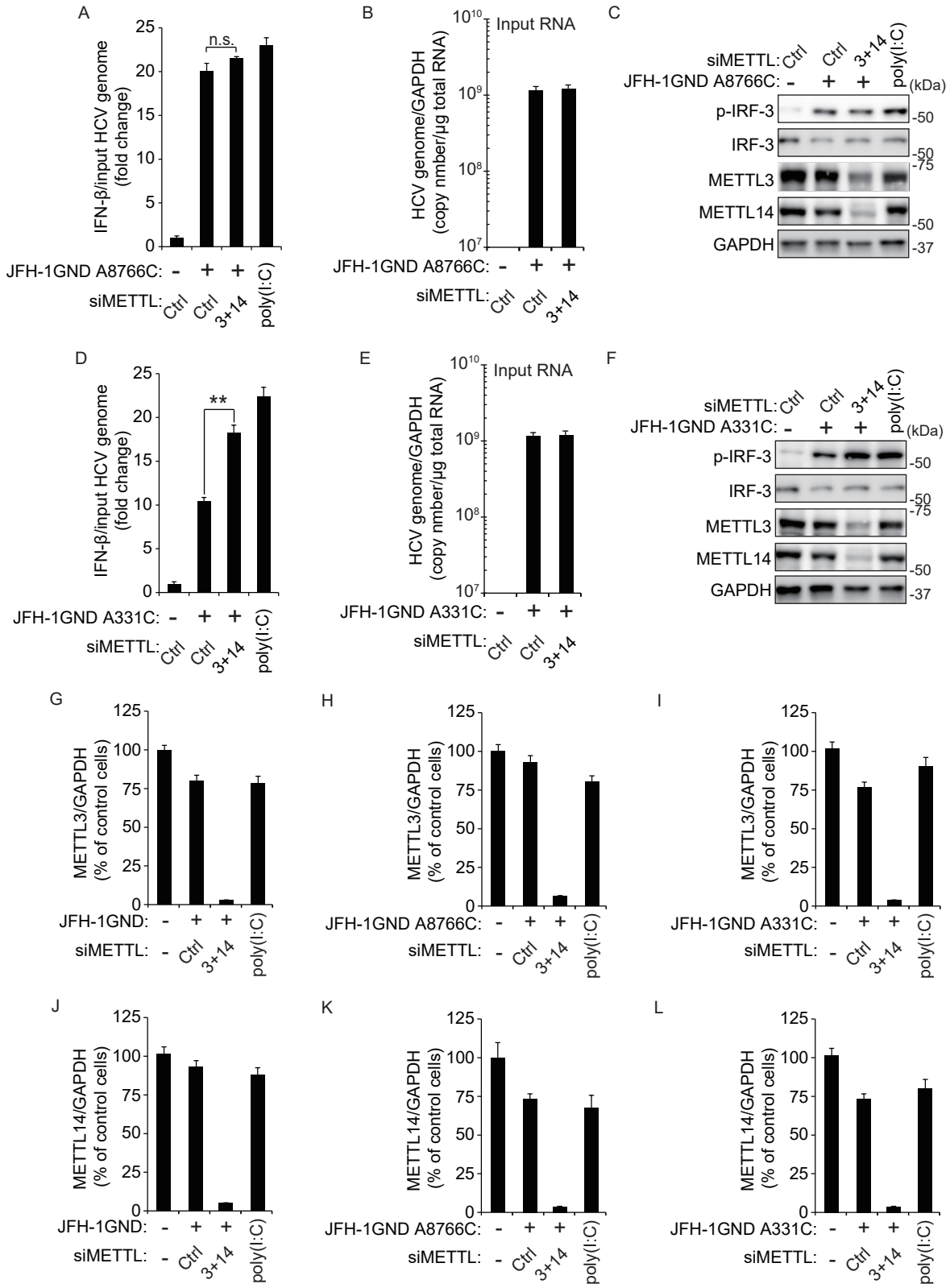
Supplementary Fig 6. Related to Figures 4 and 5. (A and B) RNA immunoprecipitation (IP) from FLAG-YTHDF1, -YTHDF2 and -YTHDF3 transfected with HBV 1.3-mer or HBV 1.3-mer 5′-MT expression cells using an anti-FLAG antibody. Enriched HBV pgRNA levels were quantified by qRT-PCR as fold enrichment relative to control. The enriched HBV pgRNA levels were normalized by input HBV pgRNA levels (B). CREBBP and HPRT1 serve as positive and negative controls, respectively. (B and C) Enrichment of HCV genome following immunoprecipitation of FLAG-YTHDFs from extracts of Huh7 cells after 48 h transfection. Enriched HCV genome was quantified by qRT-PCR as fold enrichment relative to control. FLAG-tagged RIG-I levels in input and IP were assessed by immunoblotting (in the right panel). The transfected HCV GND RNA levels were quantified by qRT-PCR (C). Data here are presented from three independent experiments and the bars represent mean ± SD. *P* values were calculated using an unpaired *t*-test. **P* < 0.05; ***P* < 0.01; ****P* < 0.001

Supplementary Fig 7. Related to Fig 5. (A) The input HBV pgRNA levels were quantified for normalizing enrichment HBV pgRNA in Fig 5A. (B) The transfected HCV genome copy numbers were analyzed for calculating enriched HCV genomes in Fig 5B. The error bars represent the standard deviations of three

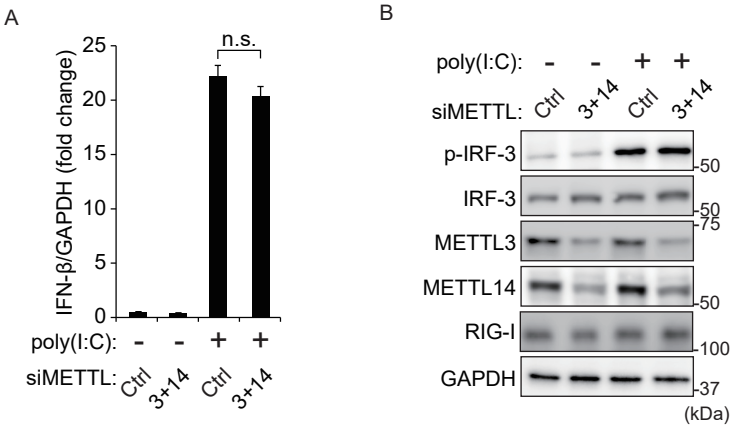
independent experiments. (C and D) The YTHDF2 expression levels, in Fig 5A and B, were quantified using ImageJ.



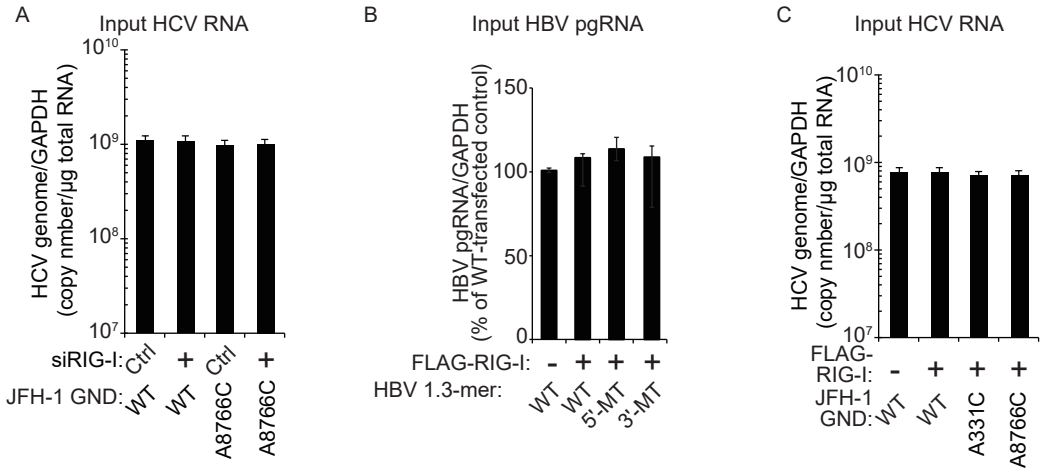
Supplementary Fig 1. Related to Figure 1.



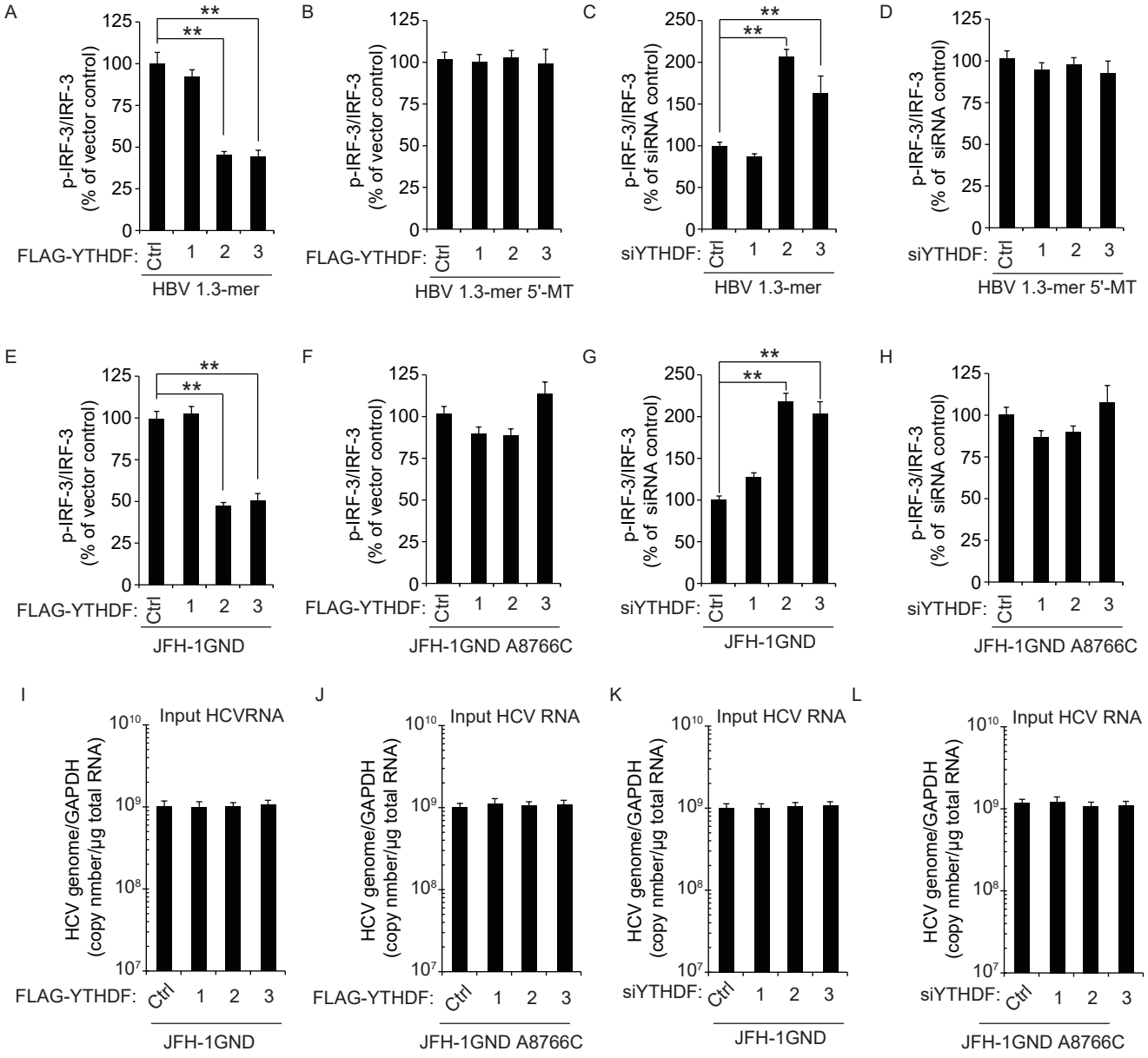
Supplementary Fig 2. Related to Figure 2.



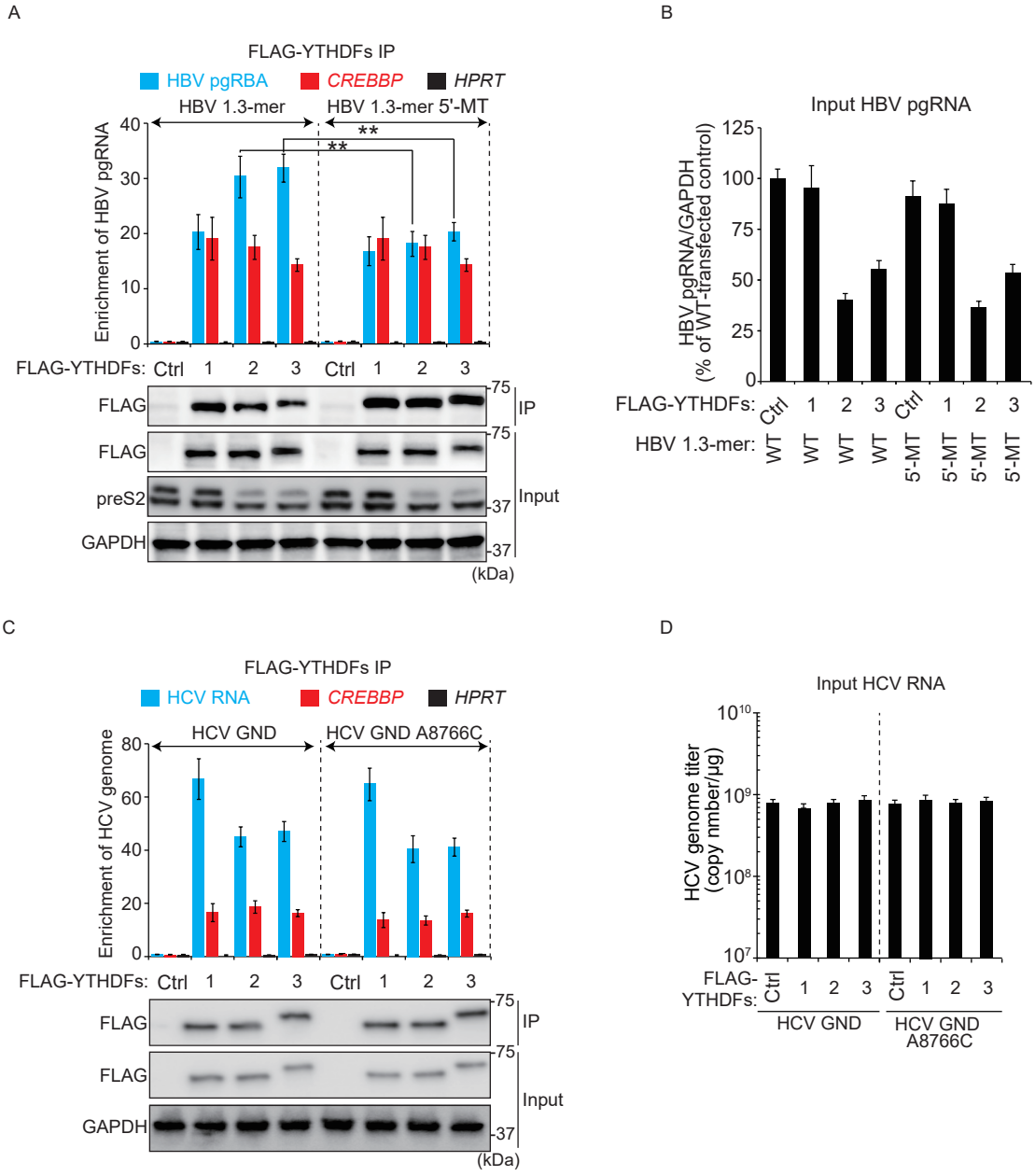
Supplementary Fig 3. Related to Figures 1 and 2.



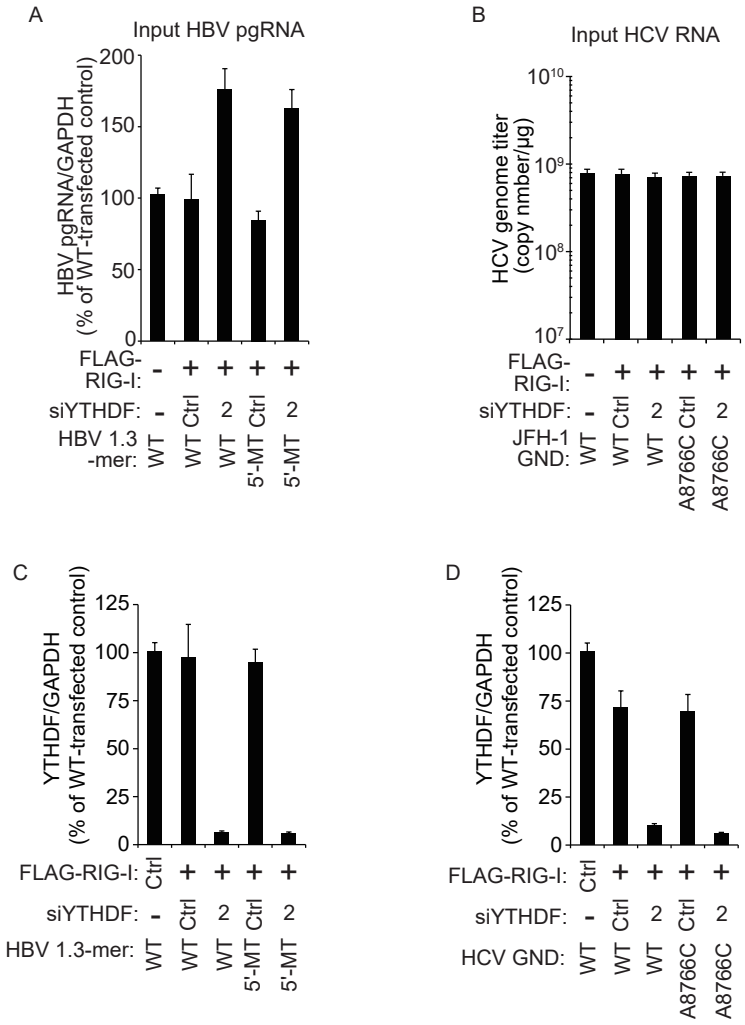
Supplementary Fig 4. Related to Figure 3.



Supplementary Fig 5. Related to Figure 4.



Supplementary Fig 6. Related to Figures 4 and 5.



Supplementary Fig 7. Related to Fig 5.