Novel potent capsid assembly modulators regulate multiple steps of the Hepatitis B virus life-cycle

Thomas Lahlali [1], Jan Martin Berke[2], Karen Vergauwen[2], Adrien Foca[1], Koen Vandyck[2], Frederik Pauwels[2], Fabien Zoulim [1,3], and David Durantel [1,*]

[1]*INSERM, U1052, Cancer Research Center of Lyon (CRCL), Université de Lyon (UCBL1), CNRS UMR_5286, Centre Léon Bérard, Lyon, France;*

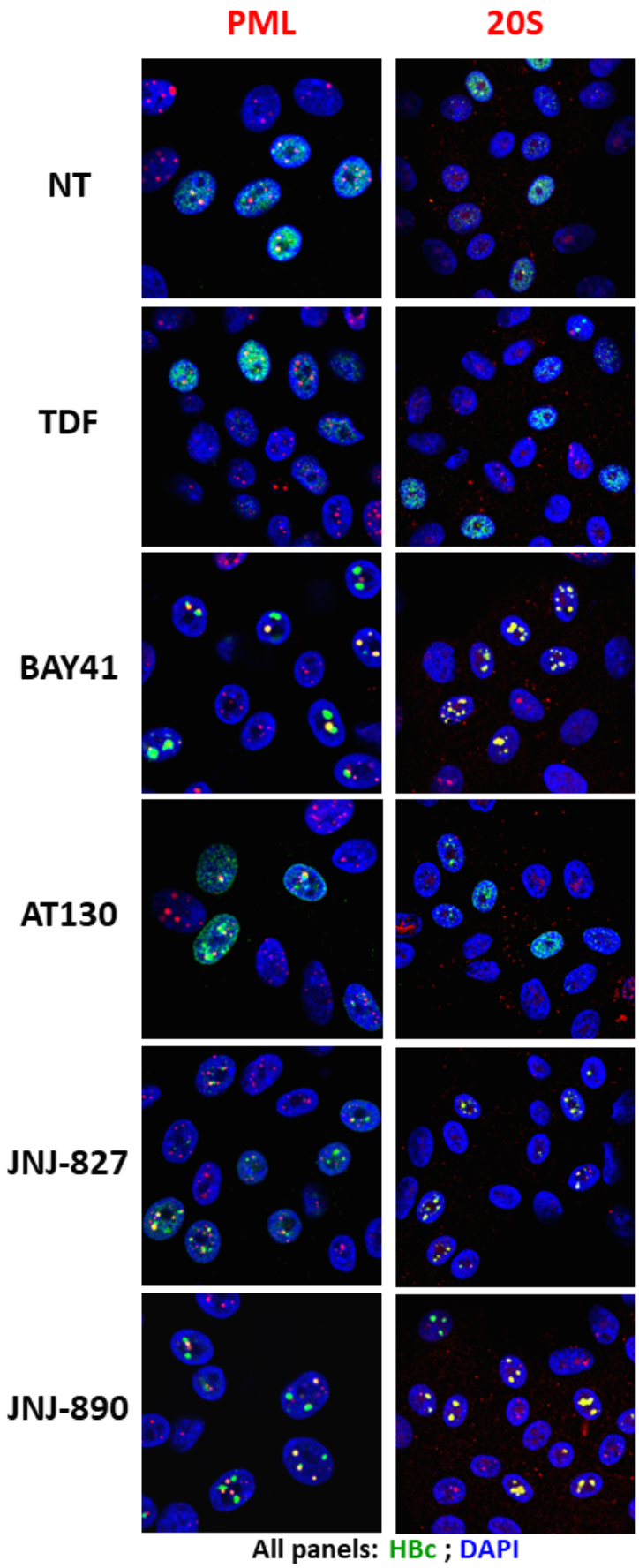
[2]*Janssen Research & Development, Beerse, Belgium;*

[3]*Liver Unit, Hospices Civils de Lyon (HCL), Lyon, France.*

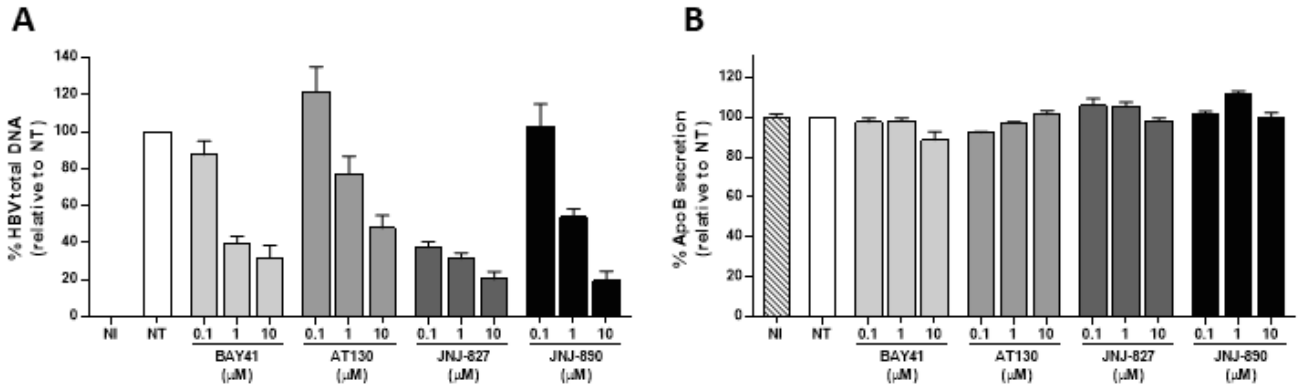
***Correspondence:** David Durantel, PhD, HDR

Mailing address: INSERM U1052, 151 cours Albert Thomas, 69424 Lyon Cedex 03, France. **Phone:** + 33 4 72 68 19 70**; Fax:** +33 4 72 68 19 71; **E-mail:** david.durantel@inserm.fr

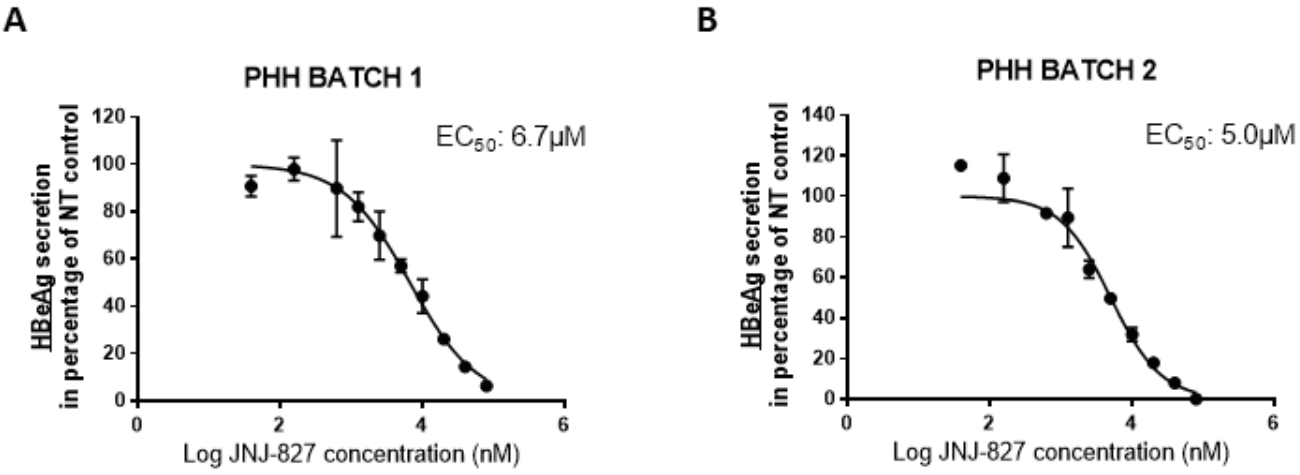
Supplementary figures and figure legends



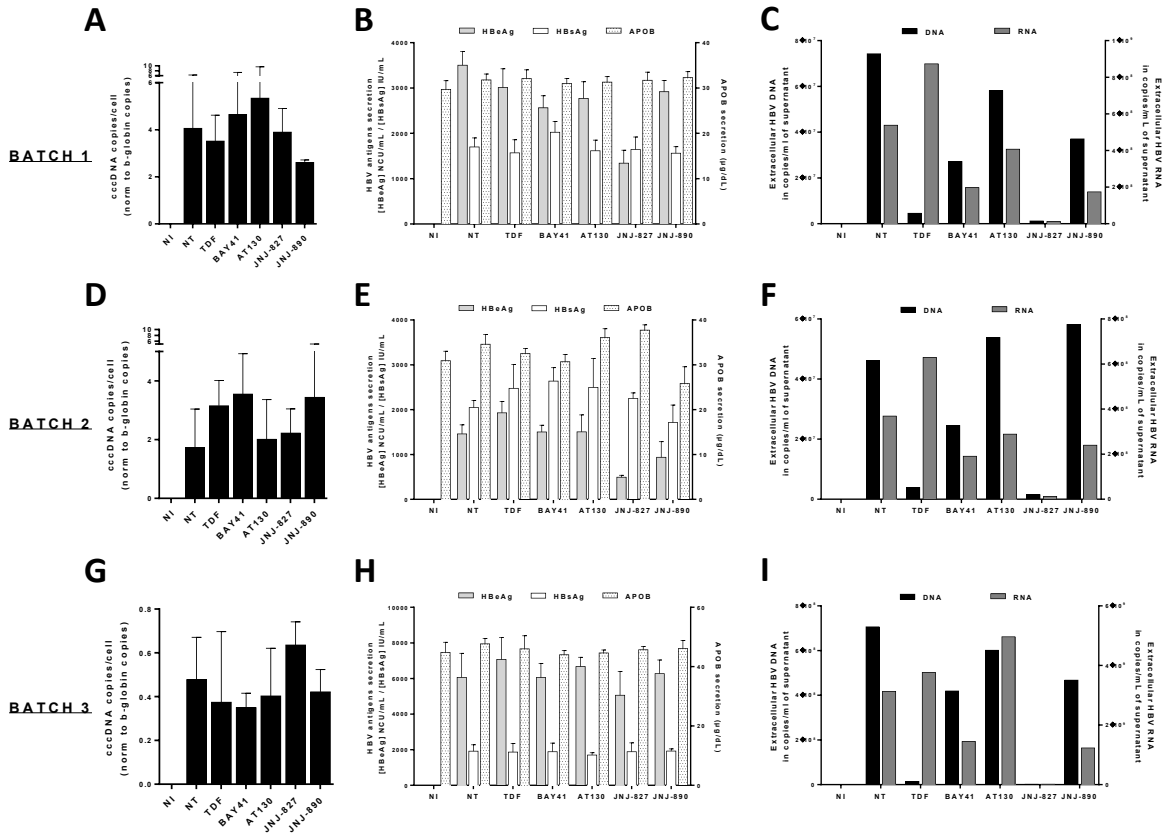
Supplementary figure 1. Effect of JNJ-827 and JNJ-890 on subnuclear localisation of capsids. Differentiated HepaRG were infected with HBV (500 vge/mL), then treated starting at day 7 post-infection (p.i.) for 7 days every two or three other days (3 treatments) with 10 µM of indicated compounds. Protein subcellular distribution was evaluated by immuno-fluorescence. At the end of the treatment procedure, cells were fixed by 2% PFA and stained with indicated primary antibodies and with fluorescently labeled secondary antibodies. An antibody recognizing only assembled capsid was used (ab8637). Green: HBc; red: PML (Promyelocytic Leukemia) protein or 20S (proteasomal 20 subunit) ; blue: DAPI. The pictures are representatives of 3 independent experiments.



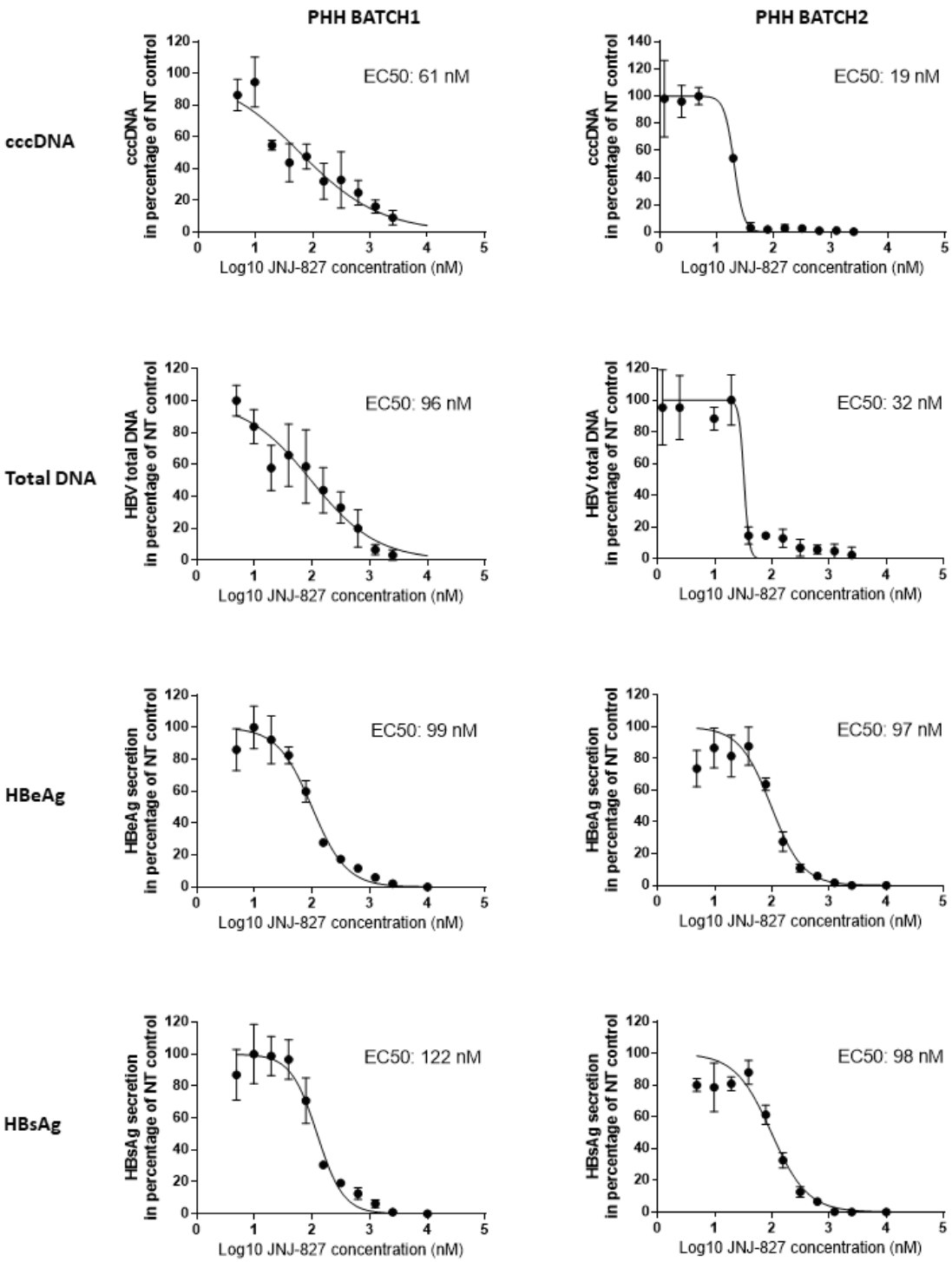
Supplementary figure 2. Dose-response effect of JNJ-827 and JNJ-890 in infected dHepaRG cells. dHepaRG cells were infected with HBV (200 vge/cell), cultured for one week then treated 7 days (treatment each 2/3 days) with 0.1, 1 or 10 µM of indicated compound. (A) Intracellular HBV total DNA level was quantified by qPCR. (B) ApoB secretion levels were assessed by ELISA. Results are the mean + SEM of n=3.



Supplementary figure 3. Determination of JNJ-827 EC_{50} on HBeAg secretion. PHH were infected with HBV (200 vge/mL), cultivated for 5 days post infection, treated three times for 7 days (treatment each 2/3 days) with increasing amount of JNJ-827 ranging from 40 nM to 80 µM. HBeAg levels in cell culture supernatant were monitored by ELISA. Results obtained with 2 different PHH donors/batches are represented independently.

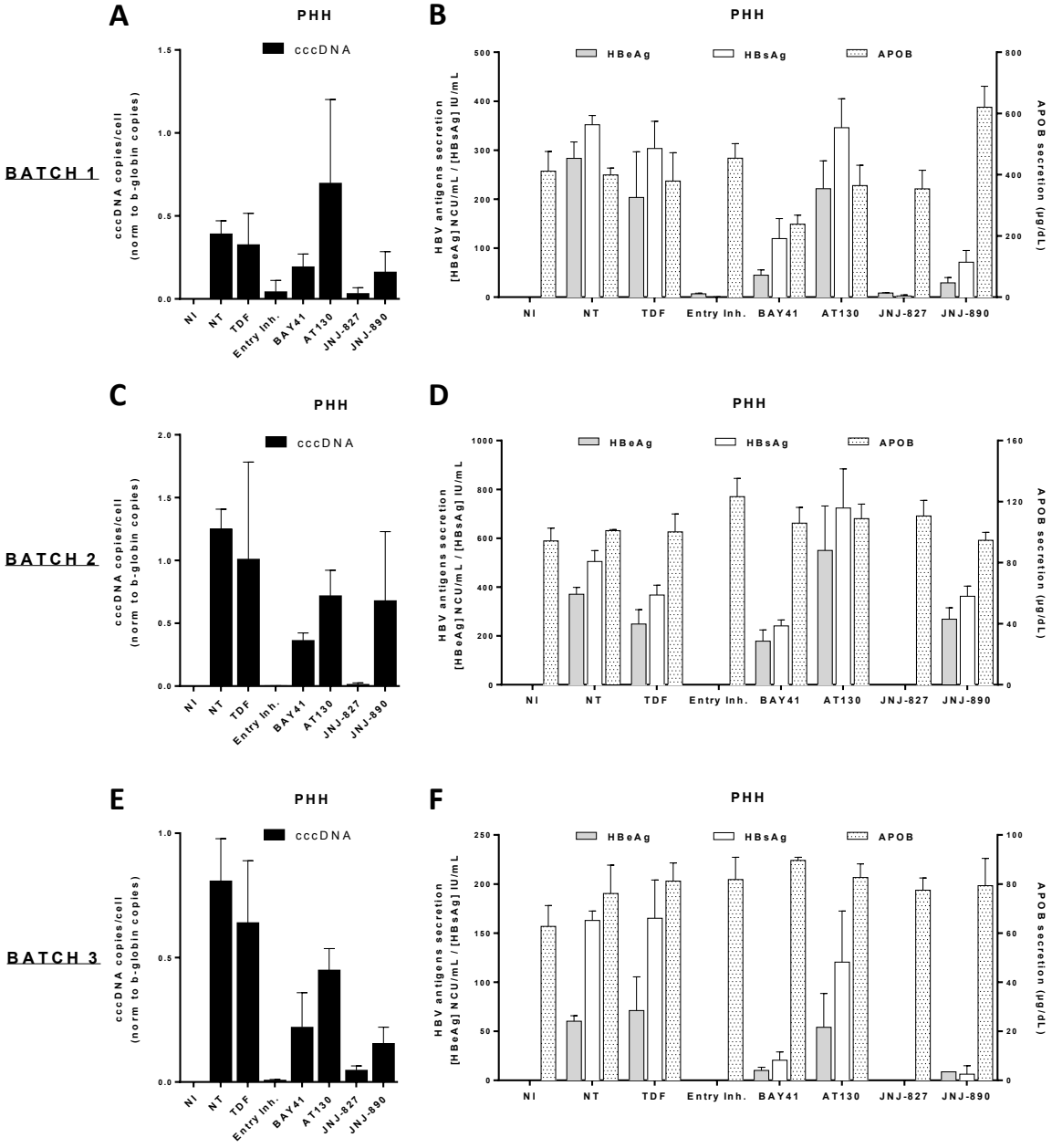


Supplementary figure 4. Antiviral properties of JNJ-827 and JNJ-890 in primary human hepatocytes. PHH were mock (NI condition) or infected with HBV (200 vge/mL), cultivated during 5 days post infection, then non-treated (NT condition) or treated three times for 7 days (treatment every 2/3 days) with 10 µM of indicated compound. (A, D, G) CccDNA, was quantified by qPCR. (B, E, H) HBeAg, HBsAg and ApoB levels in cell culture supernatant were monitored by ELISA. (C, F, I) Extracellular HBV DNA and RNA levels were quantified by qPCR or RT-qPCR after nucleic acid extraction from supernatant. Results obtained with 3 different PHH donors/batches are represented independently. Results are the mean + SD of technical replicates (n=2 or 3).



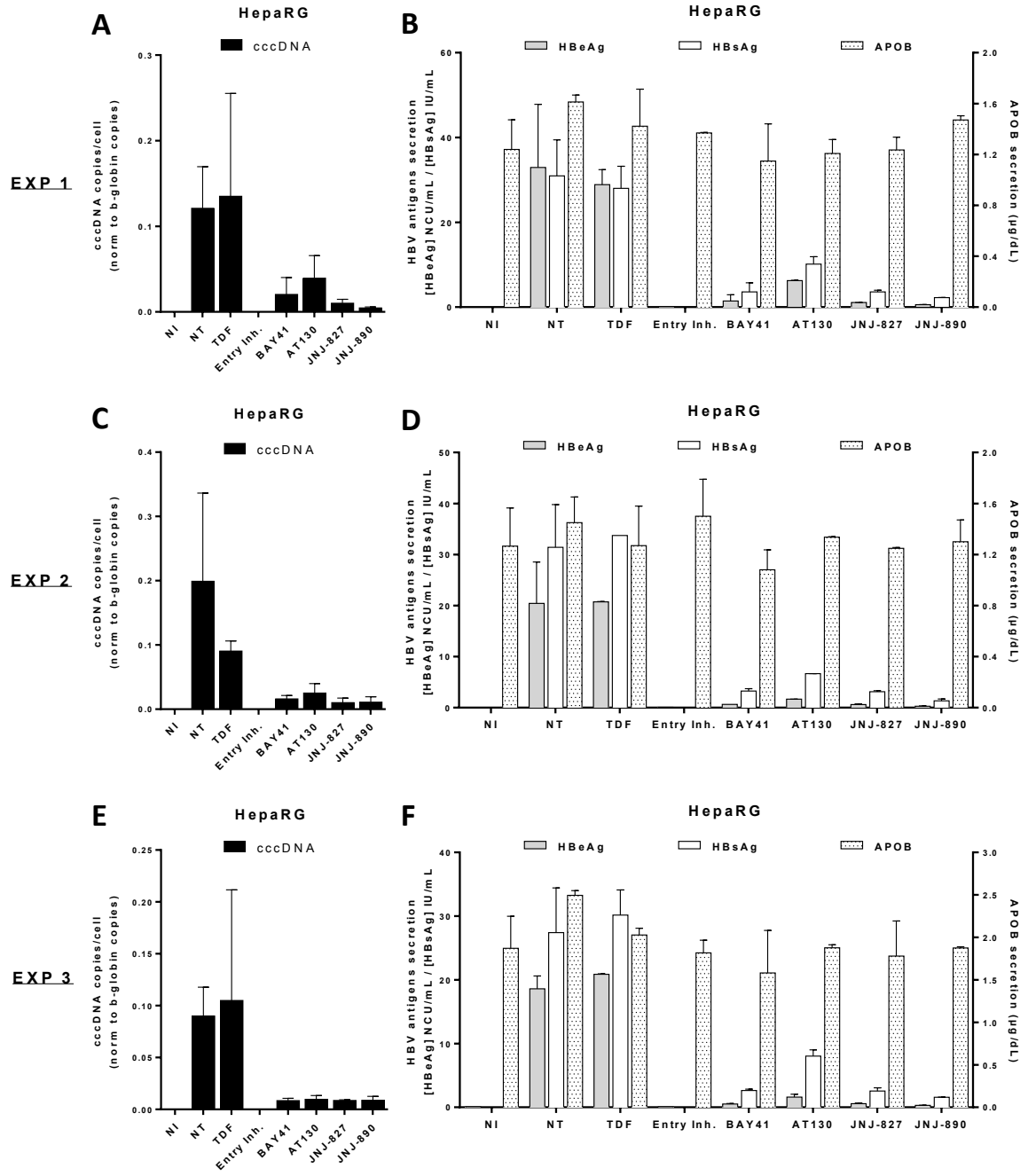
Supplementary figure 5. Determination of JNJ-827 EC_{50} on cccDNA establishment. PHH were pre-treated for 24h with increasing amount of JNJ-827 ranging from 5 nM to 10 µM then inoculated with HBV (200 vge/mL) in presence of drug for 24h. One day after infection, medium was changed and cells were cultured for one week. Intracellular cccDNA and HBV total DNA levels were quantified by qPCR FRET and qPCR respectively. HBeAg and HBsAg

levels in cell culture supernatant were monitored by ELISA. Results obtained with 2 different PHH donors/batches are represented independently.



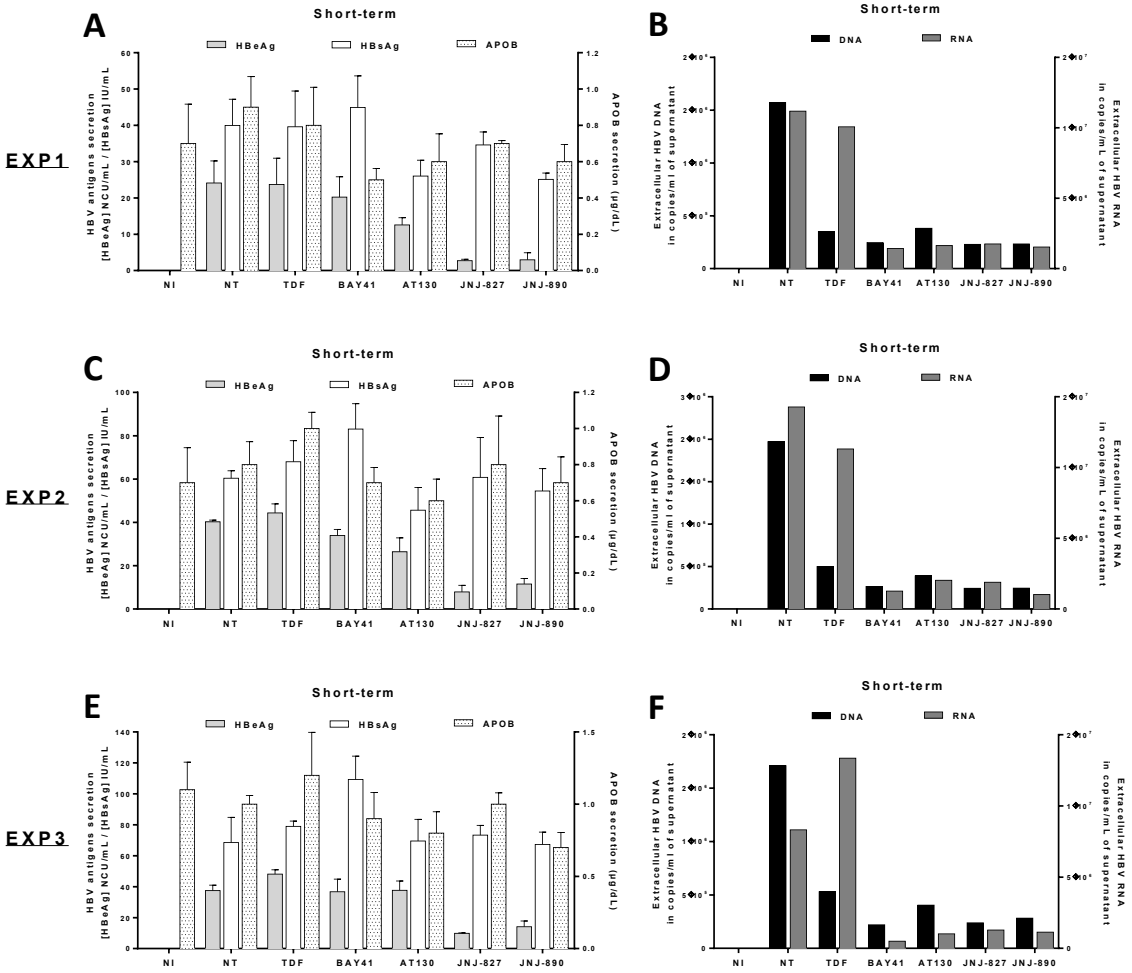
Supplementary figure 6. Effect of JNJ-827 and JNJ-890 on HBV infection establishment in PHH. PHH cells were pre-treated for 24h with 10 µM of indicated CAMs or 100 nM of preS1 peptide entry inhibitor, then inoculated with HBV (200 vge/mL) in presence of drug for 24h. One day after infection, medium was changed and cells were cultured for one week. For the TDF control, cells were treated twice after infection. A non-infected (NI) control was included. (A, C, E) CccDNA, was quantified by qPCR. (B, D, F) HBeAg, HBsAg and ApoB levels in cell culture supernatant were monitored by ELISA. Results obtained with 3 different PHH

donors/batches are represented independently. Results are the mean + SD of technical replicates (n=2 or 3).

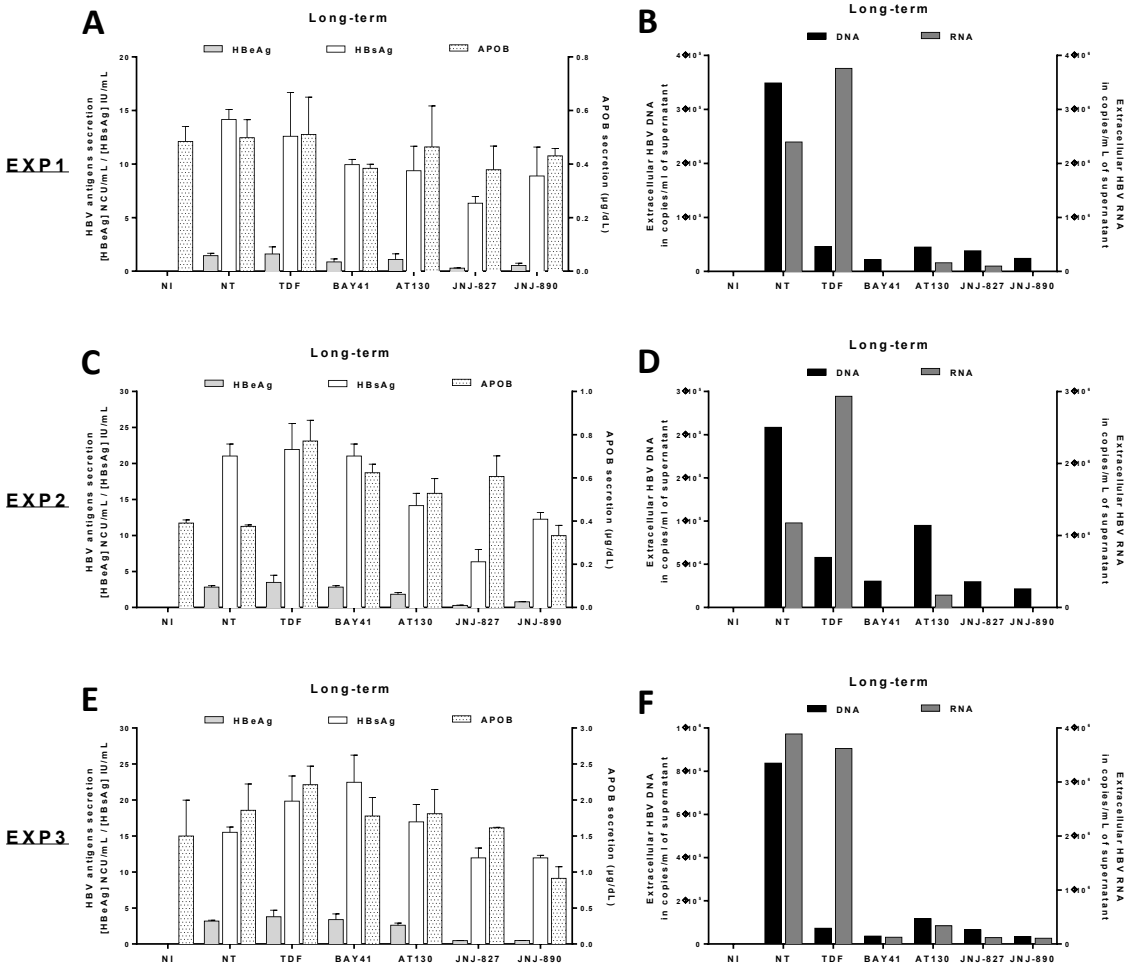


Supplementary figure 7. Effect of JNJ-827 and JNJ-890 on HBV infection establishment in dHepaRG. Differentiated HepaRG cells were pre-treated for 24h with 10 µM of indicated CAMs or 100 nM of preS1 peptide entry inhibitor, then inoculated with HBV (200 vge/mL) in presence of drug for 24h. One day after infection, medium was changed and cells were

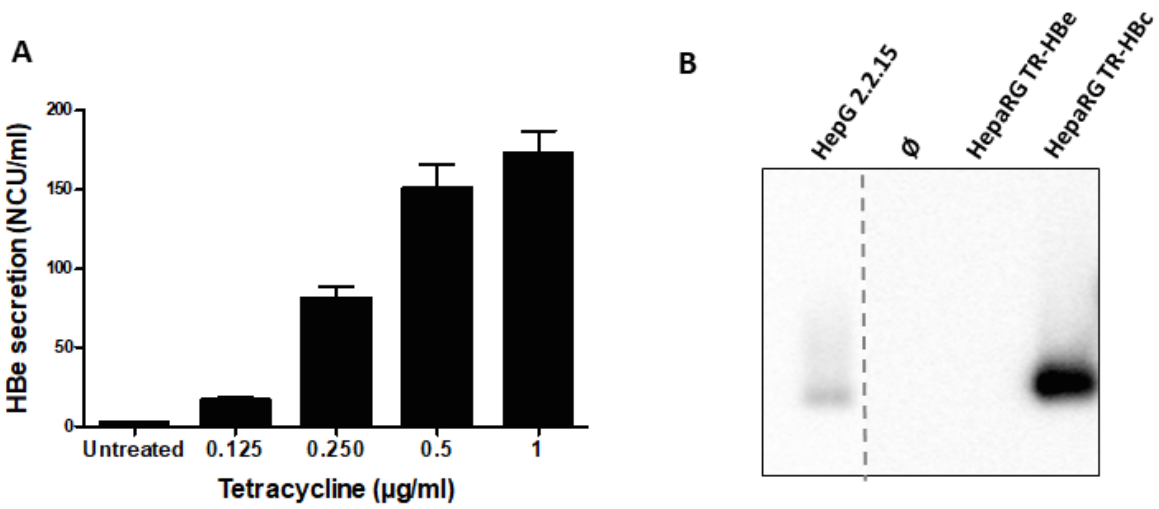
cultured for one week. For the TDF control, cells were treated twice after infection. A non-infected (NI) control was included. (A, C, E) CccDNA, was quantified by qPCR. (B, D, F) HBeAg, HBsAg and ApoB levels in cell culture supernatant were monitored by ELISA. Results obtained with 3 different HepaRG differentiations are represented independently. Results are the mean + SD of technical replicates (n=2 or 3).



Supplementary figure 8. Antiviral properties of JNJ-827 and JNJ-890 in dHepaRG: short versus long-term treatment; Part 1: short-term results. dHepaRG were infected with HBV (200 vge/mL), cultured during one week then treated for 7 days (treatment each 2/3 days; 3 treatments in total) with 10 µM of indicated compound. The antiviral effects of the compounds were monitored at end point. (A, C, E) HBeAg, HBsAg and ApoB levels in cell culture supernatant were monitored by ELISA. (B, D, F) Extracellular HBV DNA and RNA levels were quantified by qPCR or RT-qPCR after nucleic acid extraction from supernatant. Results obtained with 3 different HepaRG differentiations are represented independently. Results are the mean + SD of technical replicates (n=2 or 3).



Supplementary figure 9. Antiviral properties of JNJ-827 and JNJ-890 in dHepaRG: short versus long-term treatment; Part 2: long-term results. dHepaRG were infected with HBV (200 vge/mL), cultured during one week then treated for one month (treatment each 2/3 days; 15 treatments in total) with 10 µM of indicated compound. The antiviral effects of the compounds were monitored at end point. (A, C, E) HBeAg, HBsAg and ApoB levels in cell culture supernatant were monitored by ELISA. (B, D, F) Extracellular HBV DNA and RNA levels were quantified by qPCR or RT-qPCR after nucleic acid extraction from supernatant. Results obtained with 3 different HepaRG differentiations are represented independently. Results are the mean + SD of technical replicates (n=2 or 3).



Supplementary figure 10. Characterization of the HepaRG-TR-HBe cell line. (A) The secretion of HBeAg by cells was done by ELISA on supernatant recovered 48 hours after induction of gene expression by the indicated concentration of tetracycline. (B) Capsid migration assay. HepaRG TR-HBe and a positive control expressing HBc, HepaRG-TR-HBc, were induced using 1 µg/mL of tetracyclin for 48hrs. Cell lysates were loaded in a native agarose gel for visualization of intracellular capsid formation. Capsid formation was monitored by immunoblotting using an anti-HBc antibody.