The effect of plasma protein binding on the anti-HBV activity and pharmacokinetic properties of NVR 3-778.

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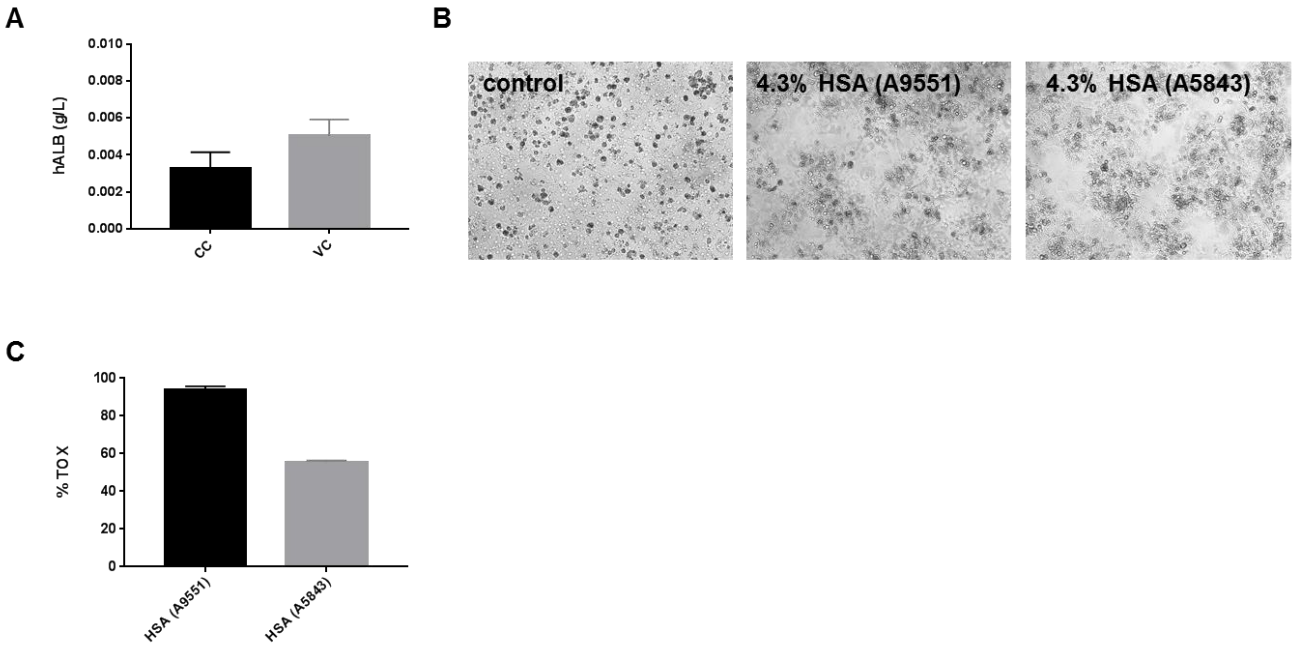
Supplementary Table

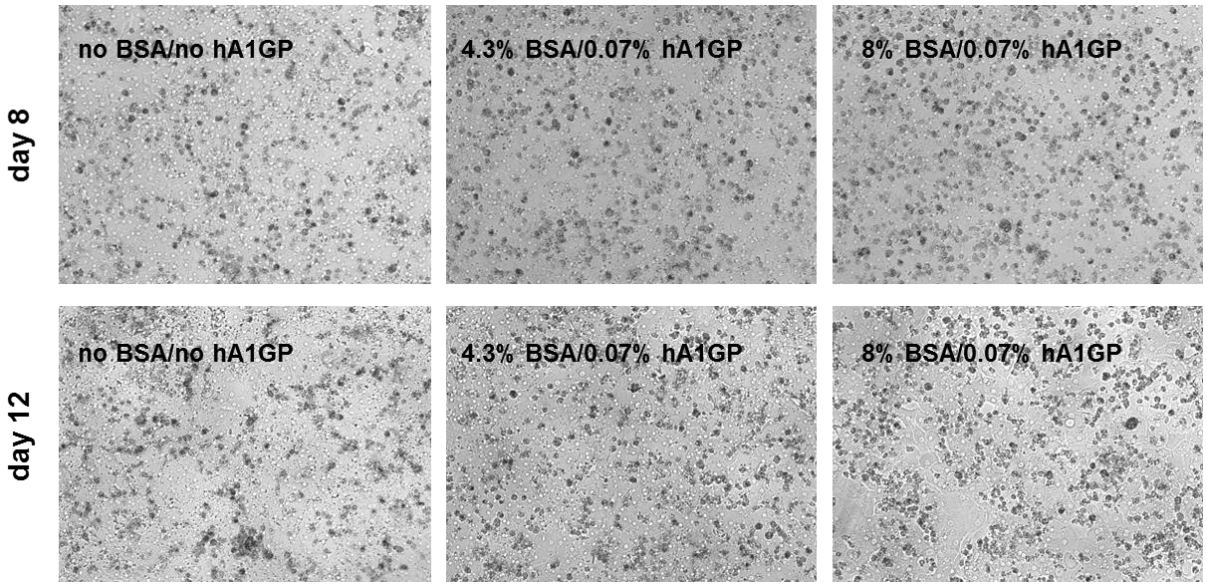
Antibody	Catalogue number	Company	Dilution
HBV core antigen	B0586	Dako	1:1600
HBsAg	In-house	In-house	/
Hoechst	H3570	Life Technologies	1:2000

Table 1: Antibody list.

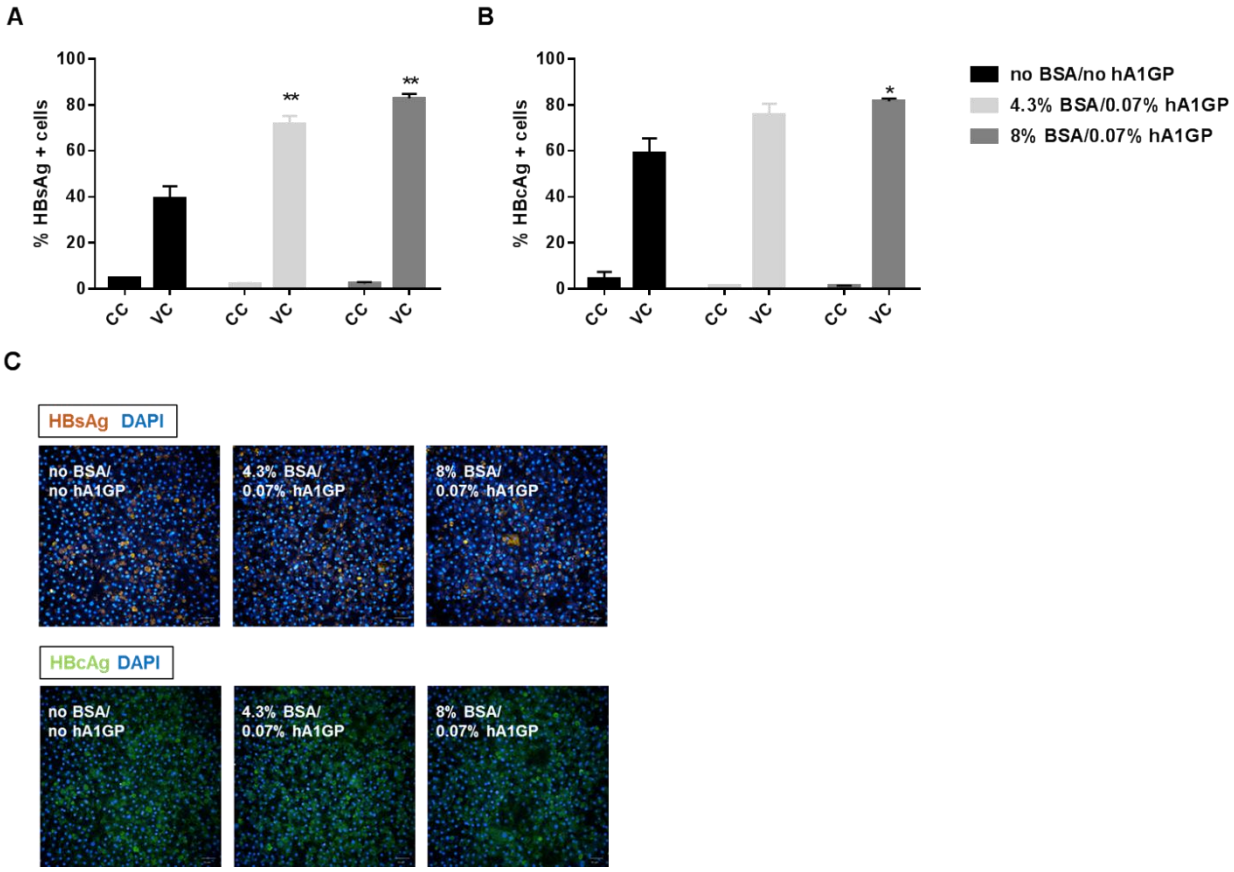
Supplementary Figures



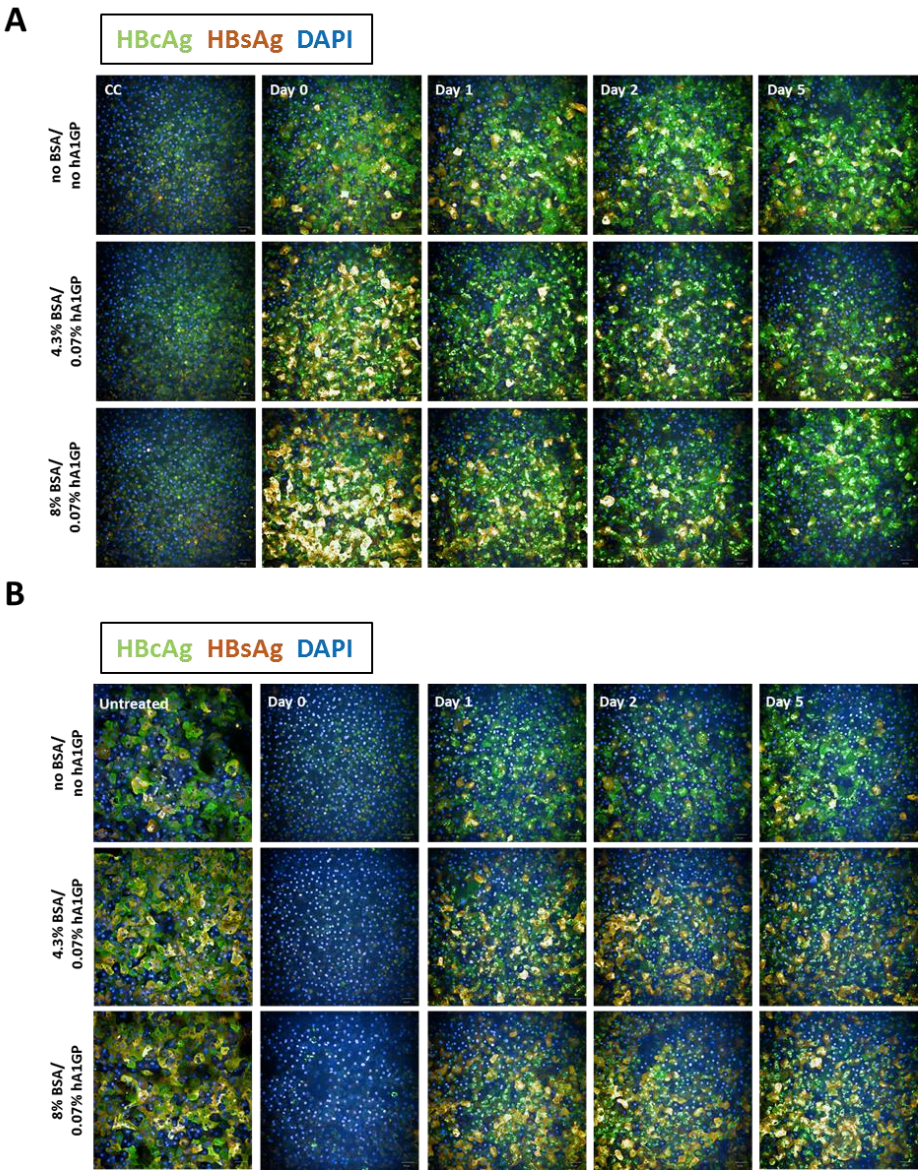
Supplementary Figure 1: Production of human albumin by PHHs and effect of HSA on PHHs. (A) Secretion of human albumin (hALB) by *in vitro* cultured PHHs. CC, cell control; VC, virus control. Results represent mean of five independent experiments ± SEM. (B) Pictures taken 8 days post plating of PHHs cultured in control HCM or in HCM spiked with two different batches of HSA (Sigma). (C) Cell toxicity assessed by ATPLite (Promega). Percent toxicity (% TOX) was relative to the ATP content of PHHs cultured in HCM without plasma proteins.



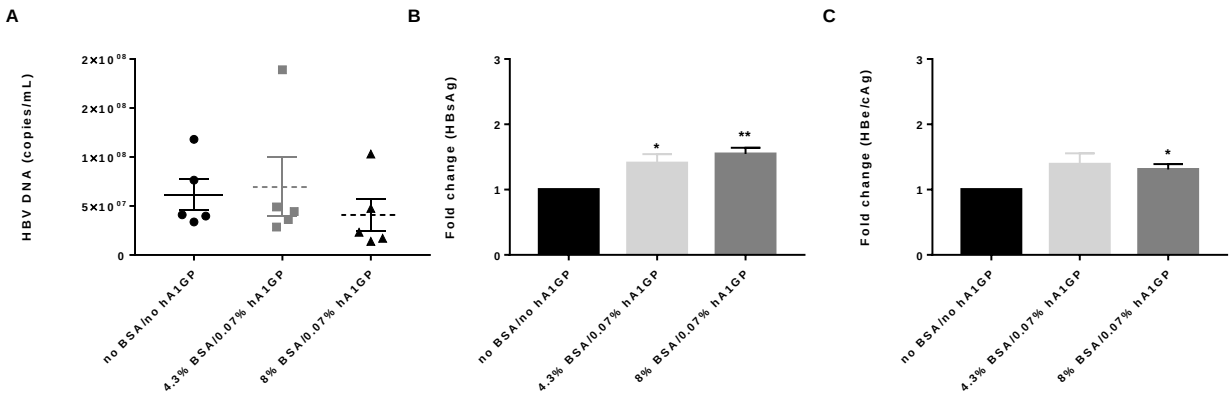
Supplementary Figure 2: Effect of BSA and hA1GP on PHHs. Representative images of *in vitro* cultured PHHs in HCM without plasma proteins or spiked with 4.3% BSA/0.07% hA1GP or 8% BSA/0.07% hA1GP. Pictures were taken both, 8 and 12 days post seeding. Representative image of more than three independent experiments.



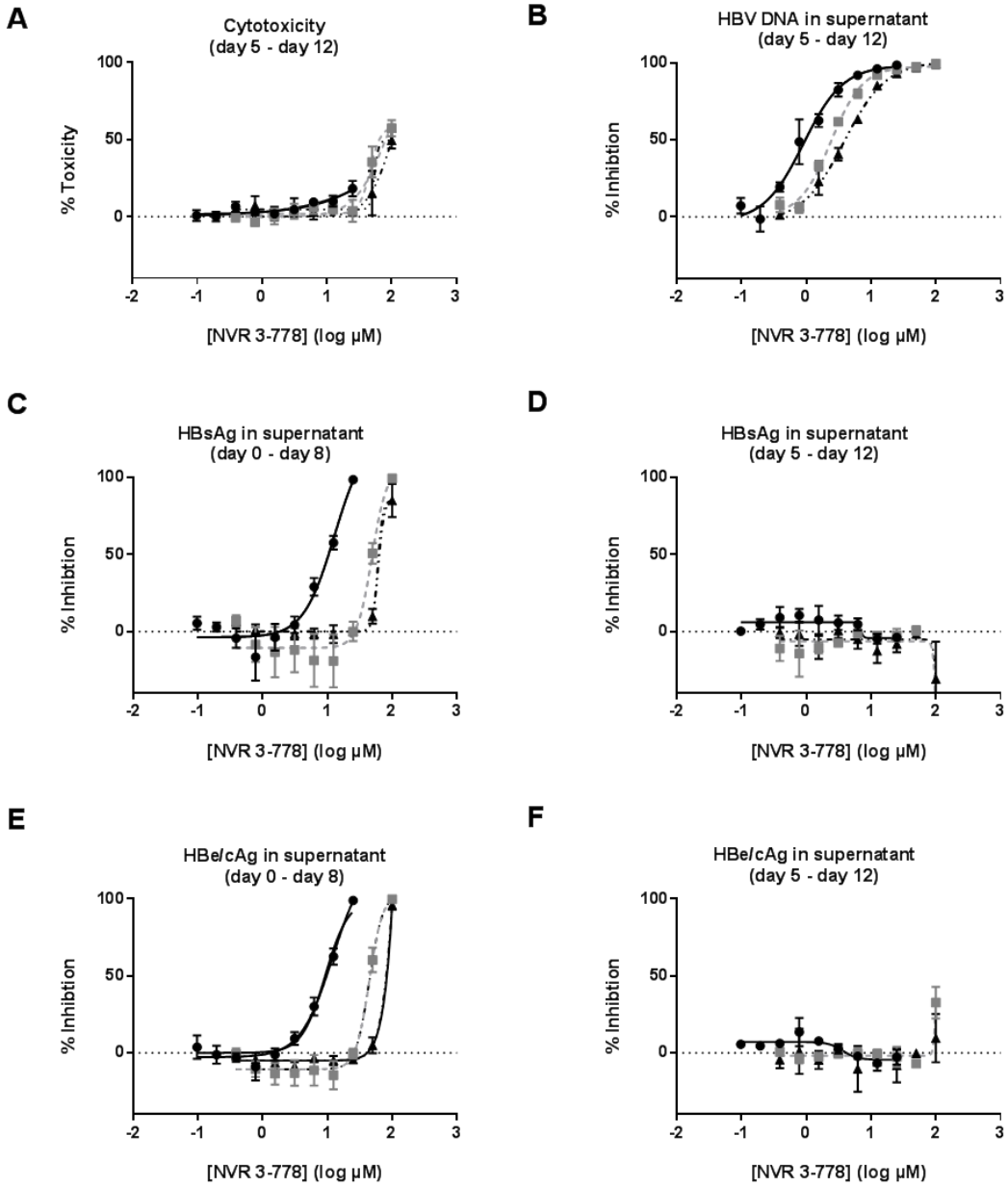
Supplementary Figure 3: Effect of BSA and hA1GP on the susceptibility of PHHs to HBV. (A) Quantification of the percentage of HBsAg positive cells. PHHs were infected in the absence or presence of additional plasma proteins and the percentage of infected cells was quantified 8 days post infection. (B) Quantification of the percentage of HBcAg positive cells. PHHs were infected in the absence or presence of additional plasma proteins and the percentage of infected cells was quantified 8 days post infection. CC: uninfected cell control; VC: infected virus control. All data represent the mean of three or more independent experiments ± SEM. (C) PHHs were mock-infected and cultured in either HCM without plasma proteins or in HCM spiked with plasma proteins. After 8 days, PHHs were stained for HBsAg (upper panel) or HBcAg (lower panel). Representative image of three independent experiments. Scale bar = 50 µM.



Supplementary Figure 4: Time-of-addition of plasma proteins and the preS1 – peptide. (A) PHHs were either HBV-infected or mock-infected (CC; cell control). For HBV-infected PHHs, plasma proteins were added from day 0, day 1, day 2 or day 5 onwards until day 8. After 8 days post infection, PHHs were stained for HBsAg and HBcAg. Representative images from three independent experiments. Scale bars = 50 µM. (B) HBV-infected PHHs were treated with 0.5 µM of the preS1 – peptide from day 0 until day 1 or from day 1, day 2 or day 5 onwards until day 8. After 8 days post infection, PHHs were stained for HBsAg and HBcAg. Representative images from three independent experiments. Scale bars = 50 µM.

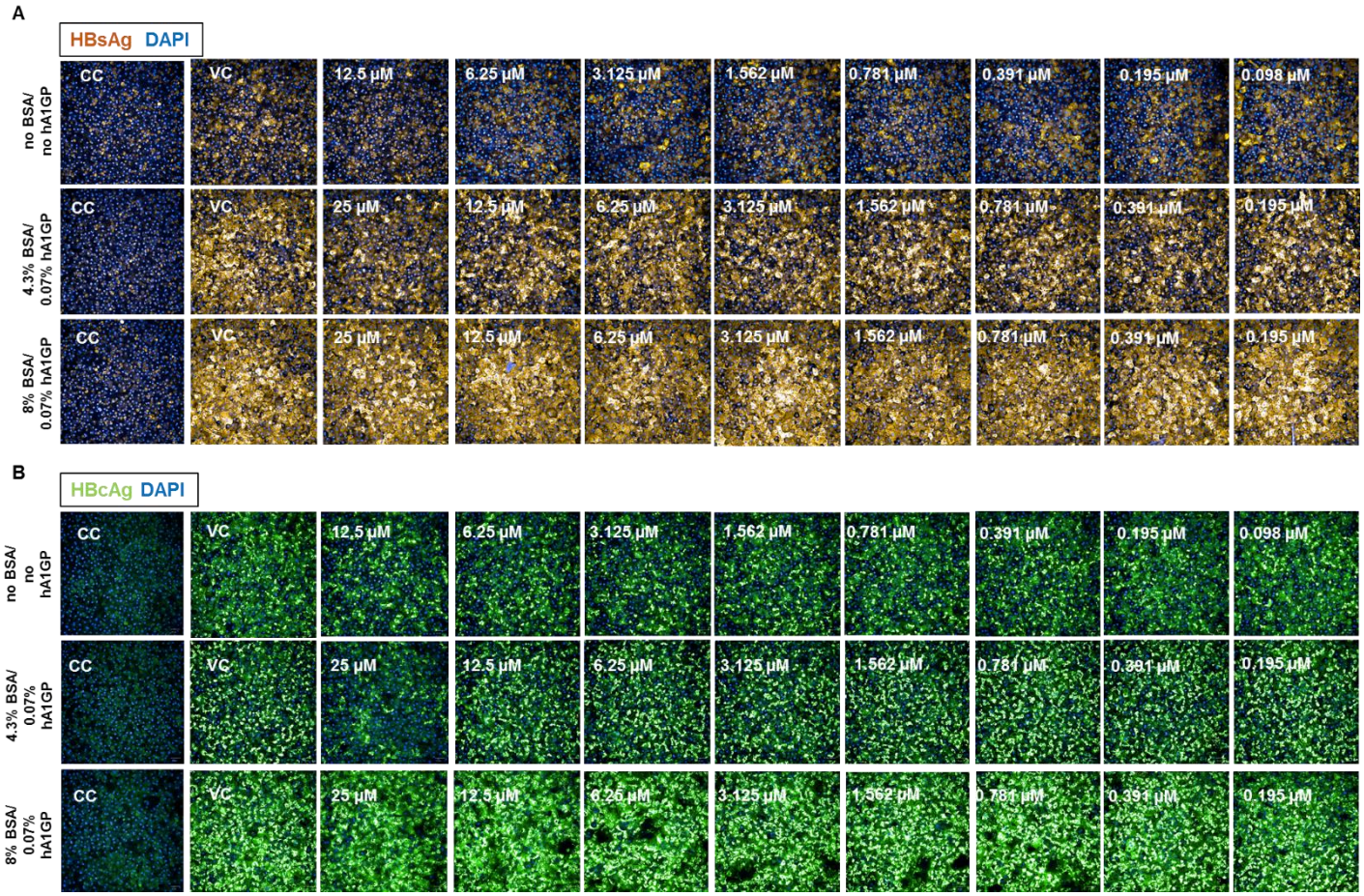


Supplementary Figure 5: Effect of plasma proteins on HBV DNA, HBsAg and HBe/cAg in the supernatant of HBV-infected PHHs after 12 days post infection. (A) HBV DNA secretion was analyzed by qPCR on DNA extracted from the cell culture supernatant of HBV-infected PHHs cultured in HCM with or without the addition of plasma proteins. (B) AlphaLisa was used to determine the amount of HBsAg that was secreted from HBV-infected PHHs cultured in the absence and presence of plasma proteins. Results are expressed as fold change compared to HBV-infected PHHs cultured in medium without BSA or hA1GP. (C) AlphaLisa was used to determine the amount of HBe/cAg that was secreted from HBV-infected PHHs cultured in the absence and presence of plasma proteins. Results are expressed as fold change compared to HBV-infected PHHs cultured in medium without BSA or hA1GP. All data represent the mean of three or more independent experiments ± SEM.



Supplementary Figure 6: Cell cytotoxicity and anti-HBV effect of NVR 3-778 on HBV DNA, HBsAg and HBe/cAg in the supernatant. NVR 3-778 was either added together with the viral inoculum until day 8 or from day 5 onwards until day 12. HBV-infected PHHs were incubated with a 2-fold dilution series of NVR 3-778 in HCM without plasma proteins (●) or in HCM spiked with either 4.3% BSA/0.07% hA1GP (■) or 8% BSA/0.07% hA1GP (▲). (A) Cell cytotoxicity was assessed by ATP content after incubation of HBV-infected PHHs with NVR 3-778. Percent toxicity was related to the ATP content of untreated PHHs cultured in the

corresponding HCM. (B) HBV DNA production in the cell culture supernatant was quantified by qPCR. Percent inhibition was compared to HBV DNA produced by untreated cells cultured in the corresponding HCM. (C & D) Secretion of HBsAg in the supernatant was measured by an in-house AlphaLisa after 8 and 12 days post infection. Percent inhibition was compared to the secretion of HBsAg by untreated cells cultured in the corresponding HCM. (E & F) Secretion of HBe/cAg in the supernatant was measured by an in-house AlphaLisa after 8 and 12 days post infection. Percent inhibition was compared to the secretion of HBe/cAg by untreated cells cultured in the corresponding HCM. All data represent the mean of three or more independent experiments ± SEM.



Supplementary Figure 7: Anti-HBV effect of NVR 3-778 on intracellular HBsAg and HBcAg. PHHs were treated with NVR 3-778 from day 0 until day 8. At day 8, cells were stained for HBsAg (A), HBcAg (B) and Hoechst. PHHs cultured in HCM without plasma proteins were incubated with NVR 3-778 ranging from 0 – 12.5 µM while PHHs cultured in HCM with plasma proteins were treated with NVR 3-778 ranging from 0 – 25 µM. Representative images from three independent experiments. CC: mock-infected cell control; VC: virus control. Scale bars = 50 µM.