De novo synthesis of hepatitis B virus nucleocapsids is dispensable for the maintenance and transcriptional regulation of cccDNA

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Table of contents

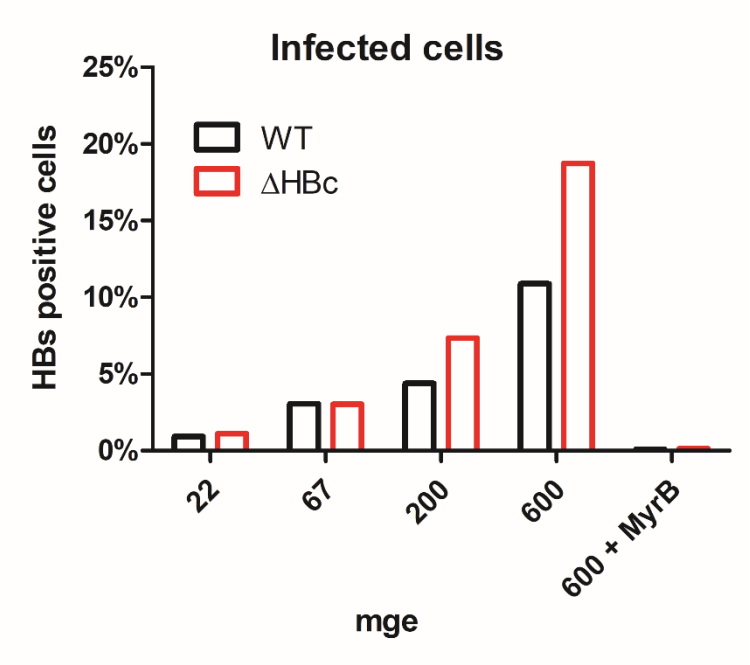

Fig. S1. Quantification of HBs-positive cells

HepG2-NTCP cells were infected with WT (black) or ΔHBc (red) HBV with increasing mge. Infection rates were determined by immunofluorescence for HBs, followed by quantification of HBs-positive cells using ilastik software [1].

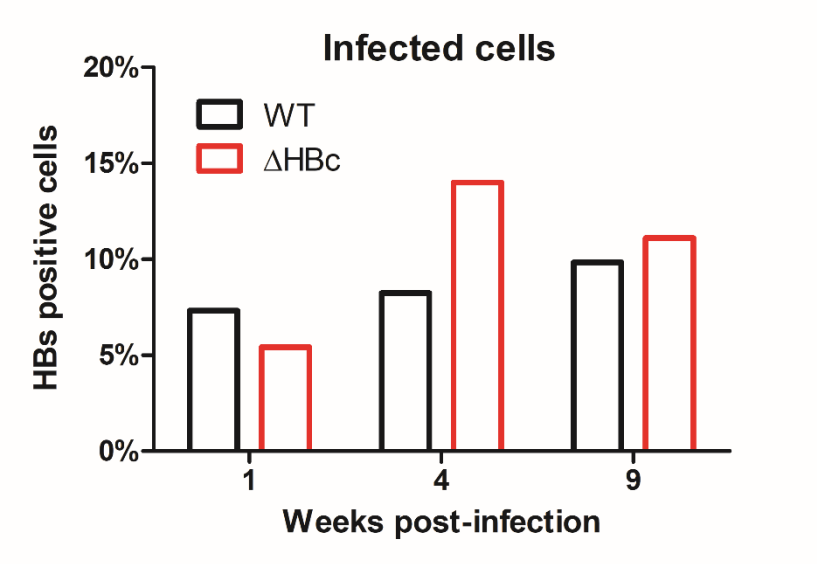

Fig. S2. Quantification of HBs-positive HepG2-NTCP cells in long-term infection

HepG2-NTCP cells were infected with WT (black) or ΔHBc (red) HBV at mge 40. Cells were fixed 1, 4, and 9 weeks post infection and infection rates were determined by immunofluorescence for HBs, followed by quantification of HBs-positive cells using ilastik software [1].

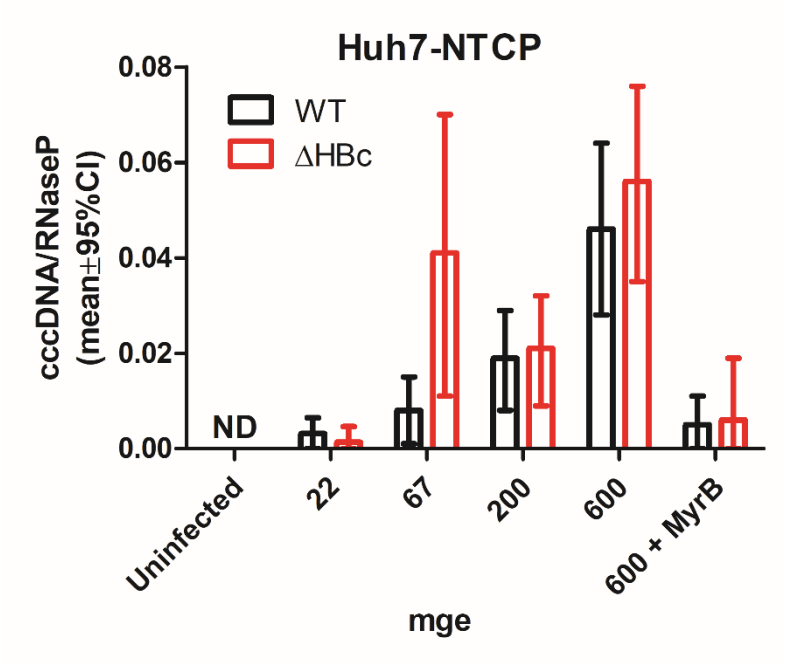

Fig. S3. HBc expression is not required for the establishment of HBV cccDNA in Huh7-NTCP cells

Huh7-NTCP cells were infected with WT (black) or ΔHBc (red) HBV with increasing inoculating doses. Total cellular DNA extracted at 7dpi was analysed by cinqPCR to detect cccDNA levels relative to the single-copy cellular gene RNaseP. The error bars (Poisson 95% confidence interval) represent the technical error of the ddPCR assay. No significant differences in cccDNA levels were detected between WT and ΔHBc HBV-infected cells at any inoculating dose. Results are representative of 2 independent experiments.

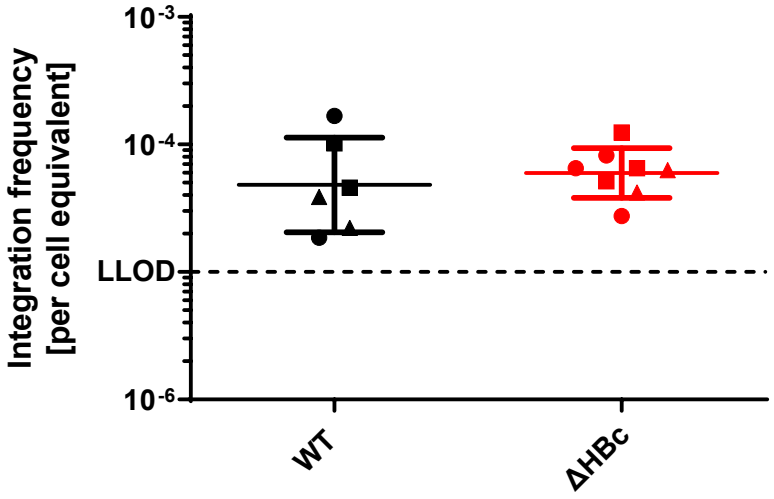

Fig. S4. HBc expression is not required for the integration of HBV DNA

Huh7-NTCP cells were infected with WT (black) or ΔHBc (red) HBV at mge 200. The frequency of integrated HBV DNA at 5dpi was determined by inverse nested PCR and end-point titration. The results of 3 independent infections are shown (squares, circles, and triangles, line = mean ± 95% confidence interval). Each point represents the calculated integration frequency for a separate dilution factor of cellular DNA multiplied by number of integrations detected at that dilution by inverse nested PCR. LLOD = Lower limit of detection.

Supplementary references

[1] Berg S, Kutra D, Kroeger T, Straehle CN, Kausler BX, Haubold C, et al. ilastik: interactive machine learning for (bio)image analysis. Nat Methods 2019;16:1226-1232.