Expanded View Figures

Figure EV1. IFNα induces APOBEC3 proteins, of which A3A is knocked down by siRNA.

A dHepaRG cells were infected with HBV at a multiplicity of infection (MOI) of 100 virions/cell, transfected with siRNA against A3A (siA3A) or control siRNA (–) on day 6 postinfection (p.i.), and treated with 300 U/ml IFNα 1 day later for 6 h. Expression of indicated genes was analyzed by qRT–PCR relative to TATA-box binding protein (TBP) mRNA (n = 3 biological replicates). mRNA from PBMCs was used as positive control in qRT–PCR for AICDA.

B–D dHepaRG cells were infected with HBV at a multiplicity of infection (MOI) of 100 virions/cell and transfected with siRNA against A3A (siA3A) or control siRNA (–) on day 8 postinfection (p.i.). Cells were treated with 300 U/ml IFNα from days 9 to 15 p.i., and cell viability was determined thereafter by CellTiter-Blue (CTB) assay (B), total intracellular HBV DNA by qPCR relative to the prion protein (Prnp) gene (C), and HBeAg by ELISA (D) (n = 4 biological replicates).

Data information: Data are represented as mean ± standard deviation (SD), nd: not detected. *P < 0.05, **P < 0.01, ***P < 0.001, and ns: not significant by Student's unpaired t-test with Welch’s correction.
Figure EV1.
Figure EV2. ISG20 is expressed in HCV infection.

A Liver tissue samples obtained from HBV-negative patients undergoing metastasis resection (control), with chronic hepatitis B (HepB) and HCV coinfection or acute hepatitis C (HepC) were stained for ISG20 by immunohistochemistry. For each clinical entity, tissue sections from three different patients are shown; scale bar: 50 µm.

B ISG20-positive area of each sample was determined by Tissue IA image analysis software (n = 3 biological replicates) and is given in % of total tissue area scanned.

Data information: Data are represented as mean ± SD. ns: not significant by one-way ANOVA.
Figure EV3. ISG20 co-localizes with nucleolar proteins and is expressed in HBV-replicating cells.

A-D dHepG2H1.3 cells were treated with 1,500 U/ml IFNα or 1,000 U/ml IFNγ from days 3 to 4 after differentiation start and stained by immunofluorescence for ISG20 and PML (A), PML and Daxx (B), ISG20 and nucleophosmin (C), or ISG20 and a nucleolar marker protein (D). Scale bar: 10 µm.

E Primary human hepatocytes were infected with an MOI of 200 virions/cell, treated with 500 U/ml IFNα or 200 U/ml IFNγ from days 5 to 6 p.i., and stained for ISG20 and HBV core protein. Scale bar: 50 µm.
Figure EV3.
**Figure EV4. ISG20 is knocked down by siRNA and binds and degrades ssDNA.**

A–D  dHepaRG cells were infected with HBV at an MOI of 100 virions/cell and transfected with siRNA targeting ISG20 (siISG20) or control siRNA (C0) at day 7 p.i. and treated with 300 U/ml IFNa from days 8 to 11 p.i. ISG20 mRNA (A) was measured by qRT–PCR relative to TBP mRNA (n = 3 biological replicates). cccDNA (B) and total intracellular HBV DNA (C) were determined by qPCR relative to Prnp, and HBeAg (D) was determined by ELISA (n = 5–6 biological replicates from two independent experiments).

E  ssDNA oligomers with and without dU modifications (left panel) or ssRNA oligomers (right panel) were digested in vitro for 4 h using 7 µM oligomer and 2.5 µM recombinant ISG20 each and separated by polyacrylamide gel electrophoresis.

F  NMR spectra of 15N-labeled ISG20 bound to the (C0) ssDNA and dU-containing (C0) ssDNA oligomers are shown. At a 1:1 ratio of oligomer to ISG20, both oligomers induce similar chemical shift perturbations in ISG20 amide resonances, as observed in the 1H,15N correlation NMR spectra.

Data information: Data are represented as mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001, and ns: not significant by Student’s unpaired t-test with Welch’s correction.
Figure EV4.
Figure EV5. ISG20 overexpression together with A3A reduces cccDNA similar to interferon treatments.

A, B dHepG2H1.3-A3A cells constantly expressing A3A (A3A Ctrl) were transduced 10 days after differentiation with an empty adenoviral vector (AdV) or AdV-ISG20. ISG20 levels (A) were determined by qRT–PCR relative to TBP mRNA 1 day after transduction (n = 4 biological replicates). cccDNA (B) was measured after T5 digest 7 days after transduction by qPCR relative to Prnp (n = 4 biological replicates).

C dHepaRG-TR-A3A cells were infected with HBV at an MOI of 300 virions/cell and transduced on day 7 p.i. with AdV-ISG20 and treated with tetracycline to induce A3A expression, 300 U/ml IFNα or 200 U/ml IFNγ. Seven days after transduction, cccDNA was measured after T5 digest by qPCR relative to Prnp (n = 5 biological replicates from two independent experiments).

D, E dHepaRG-TR-A3A cells were infected with wild-type HBV (HBVwt) or HBVαx at an MOI of 300 virions/cell and transduced on day 7 p.i. with AdV-ISG20 and treated with tetracycline to induce A3A expression as indicated (+ Tet (A3A)) for 7 days. Cell viability (D) was measured by CTB assay and total intracellular HBV DNA (E) by qPCR relative to Prnp (n = 6 biological replicates from two independent experiments).

Data information: Data are represented as mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001, and ns: not significant by Student’s unpaired t-test with Welch’s correction.