

Expanded View Figures

Figure EV1. IFN α 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 IFN α 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 IFN α 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 $\pm$ 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.

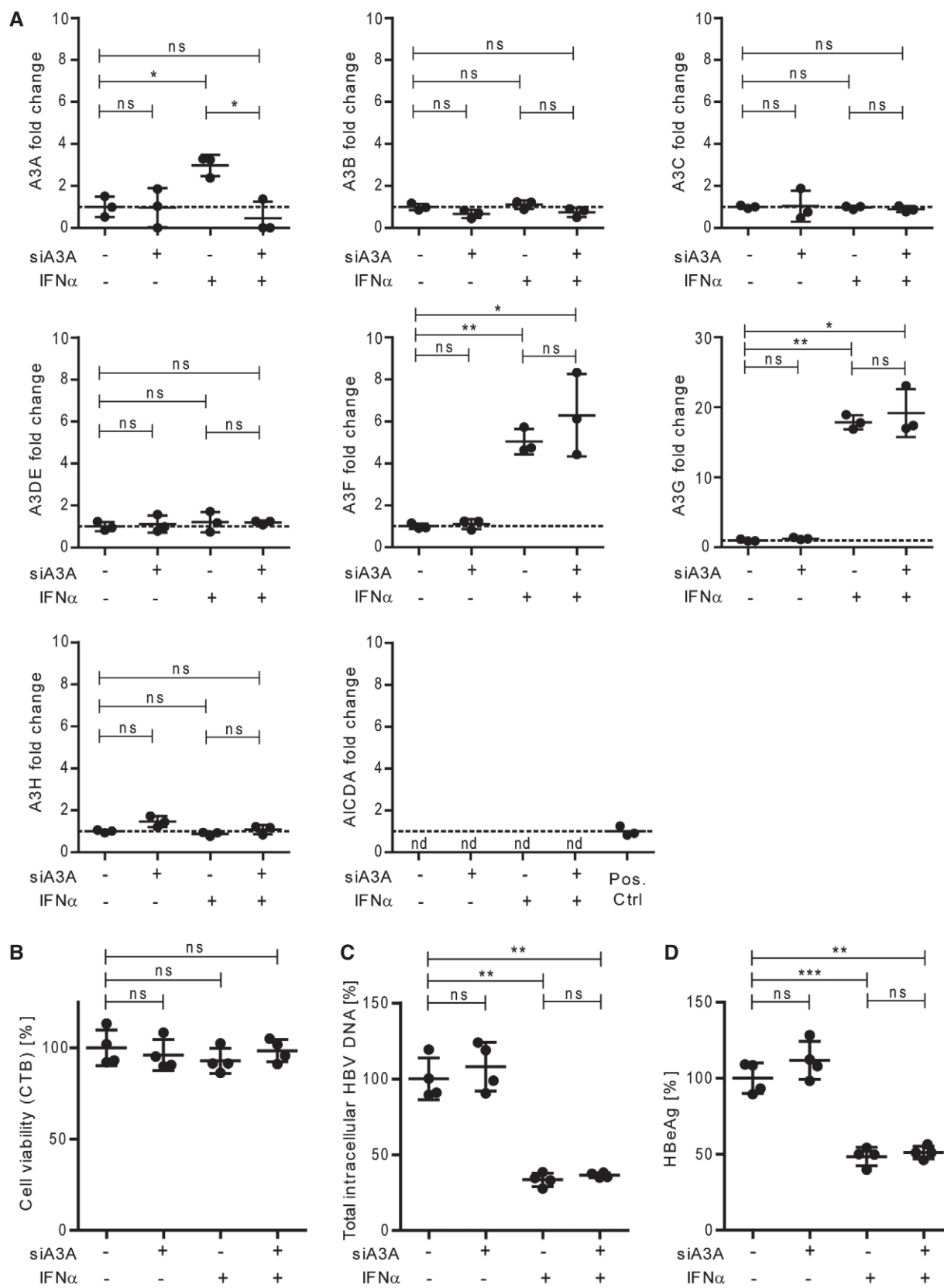


Figure EV1.

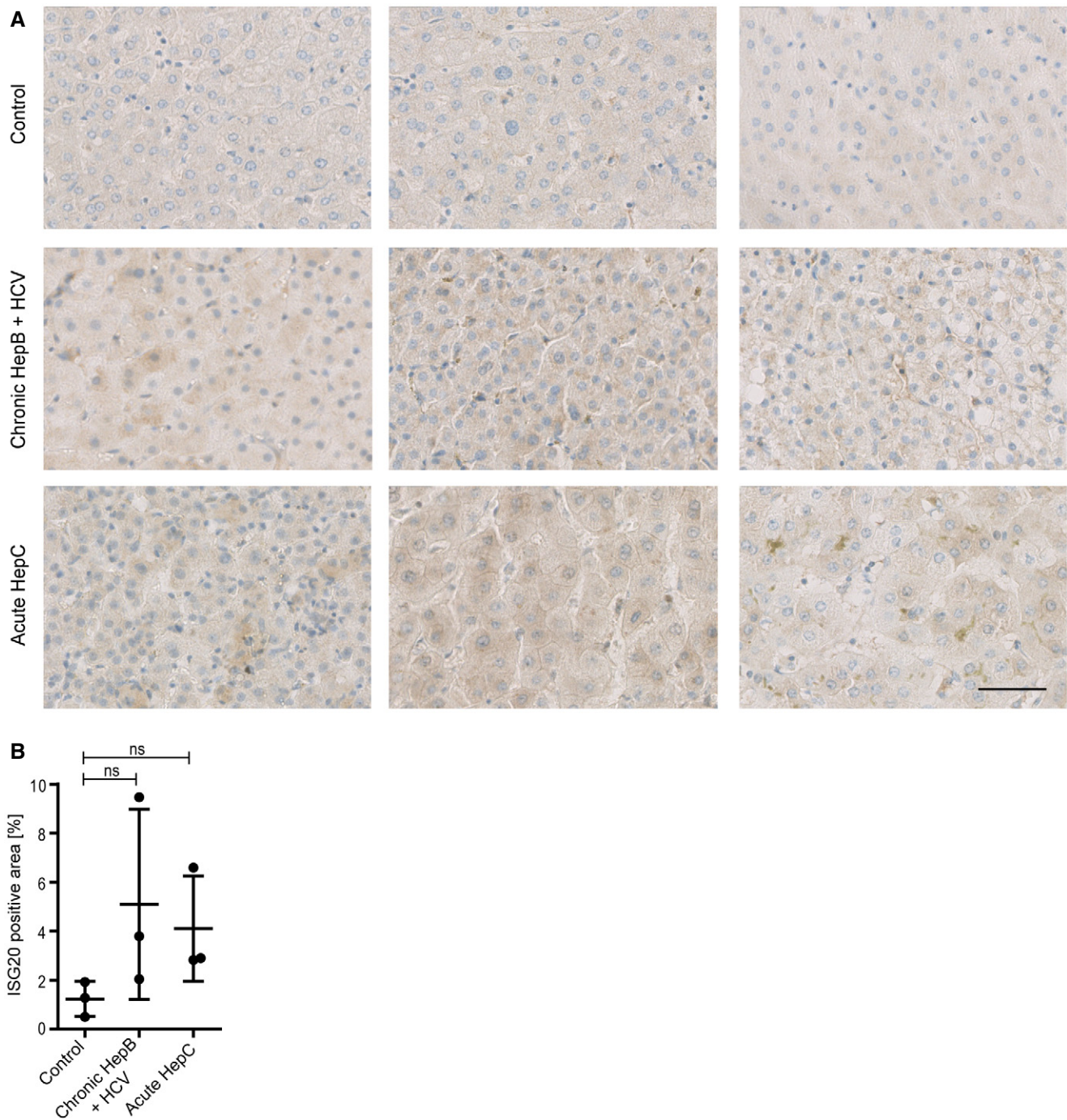


Figure EV2. ISG20 is expressed in HCV infection.

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 μ m.

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 $\pm$ 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 IFN α or 1,000 U/ml IFN γ 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 μ m.
- E Primary human hepatocytes were infected with an MOI of 200 virions/cell, treated with 500 U/ml IFN α or 200 U/ml IFN γ from days 5 to 6 p.i., and stained for ISG20 and HBV core protein. Scale bar: 50 μ m.

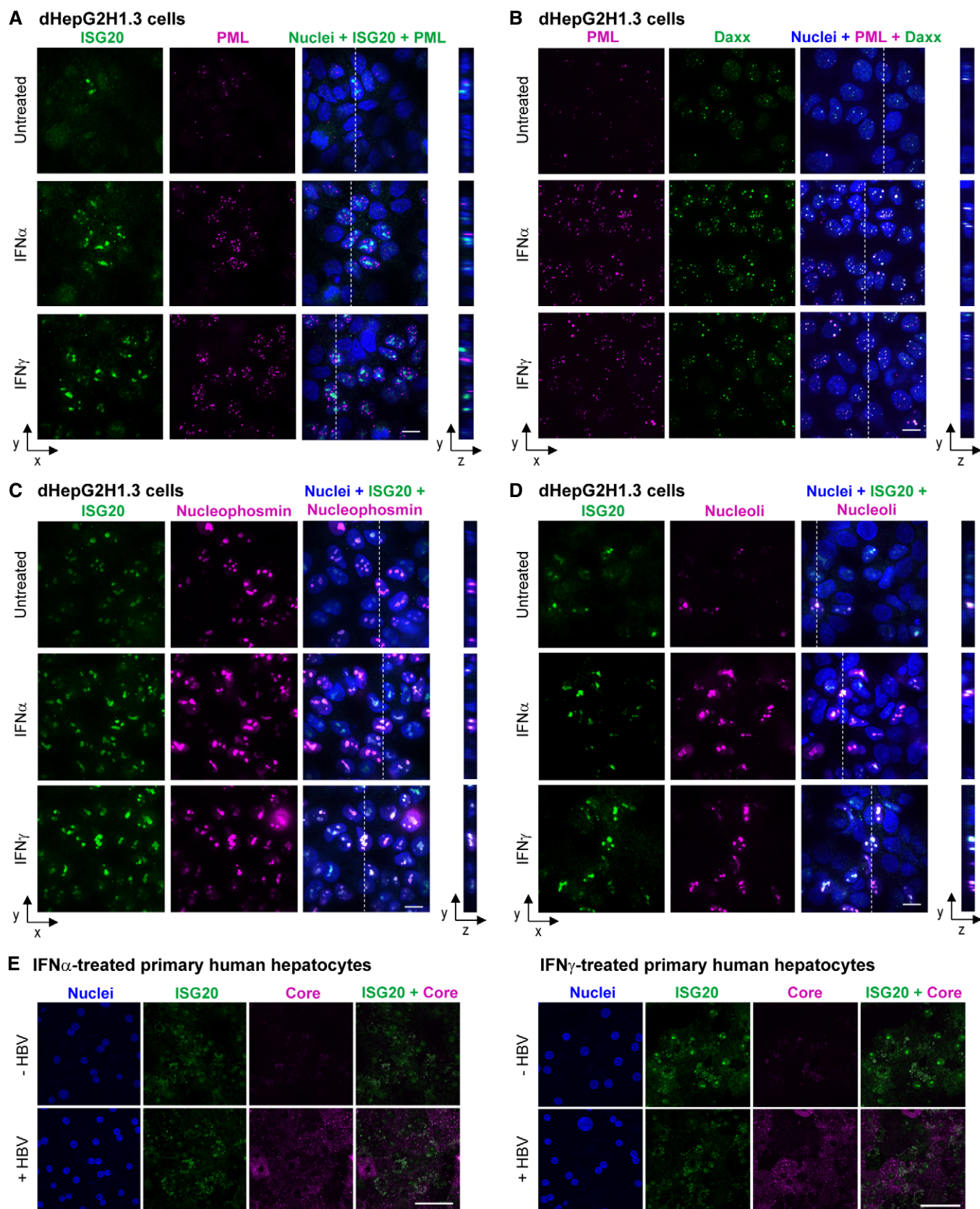


Figure EV3.

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 (–) at day 7 p.i. and treated with 300 U/ml IFN α 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 μ M oligomer and 2.5 μ M recombinant ISG20 each and separated by polyacrylamide gel electrophoresis.
- F NMR spectra of ^{15} N-labeled ISG20 bound to the (–) ssDNA and dU-containing (–) 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 , ^{15}N correlation NMR spectra.

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

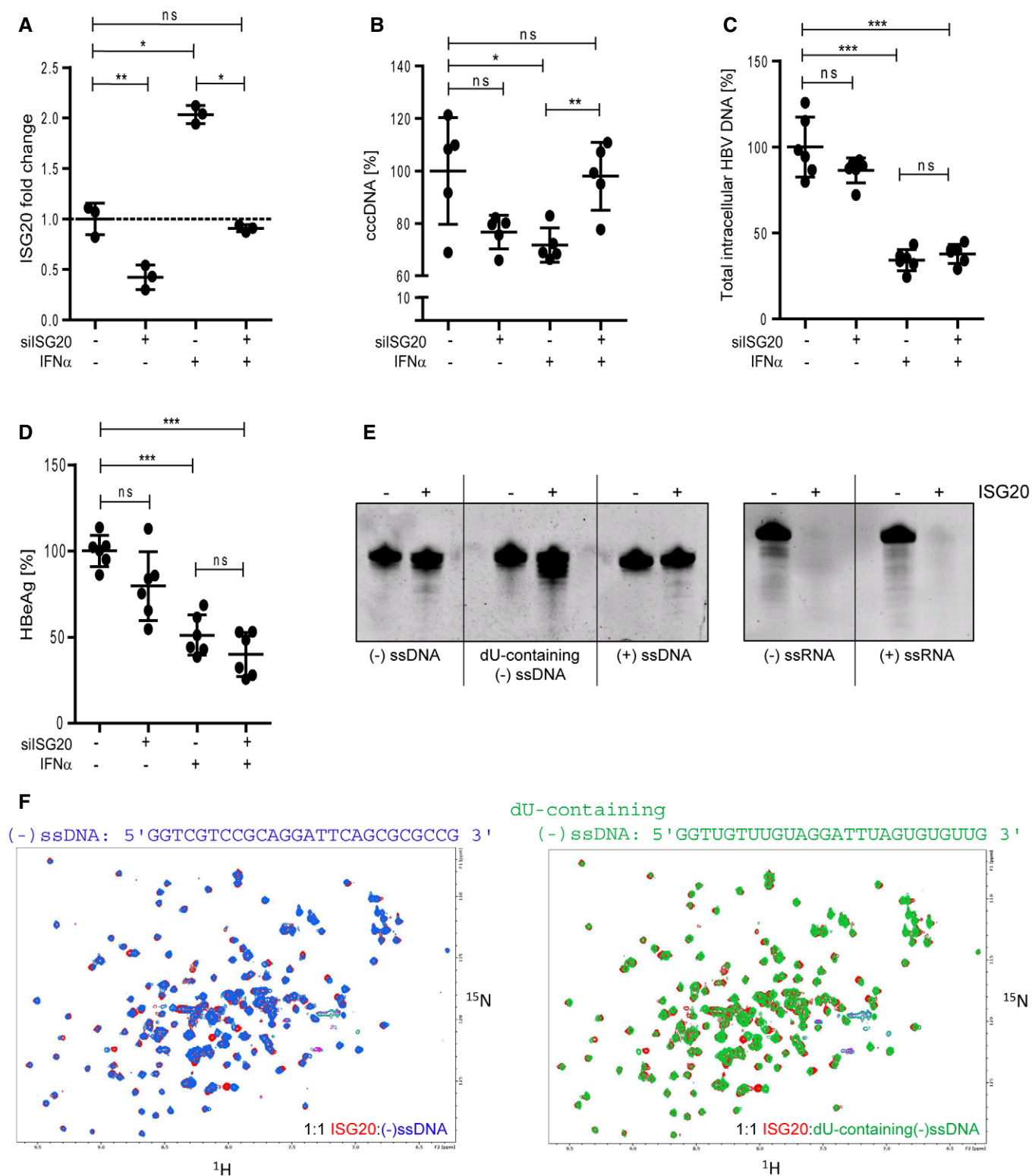


Figure EV4.

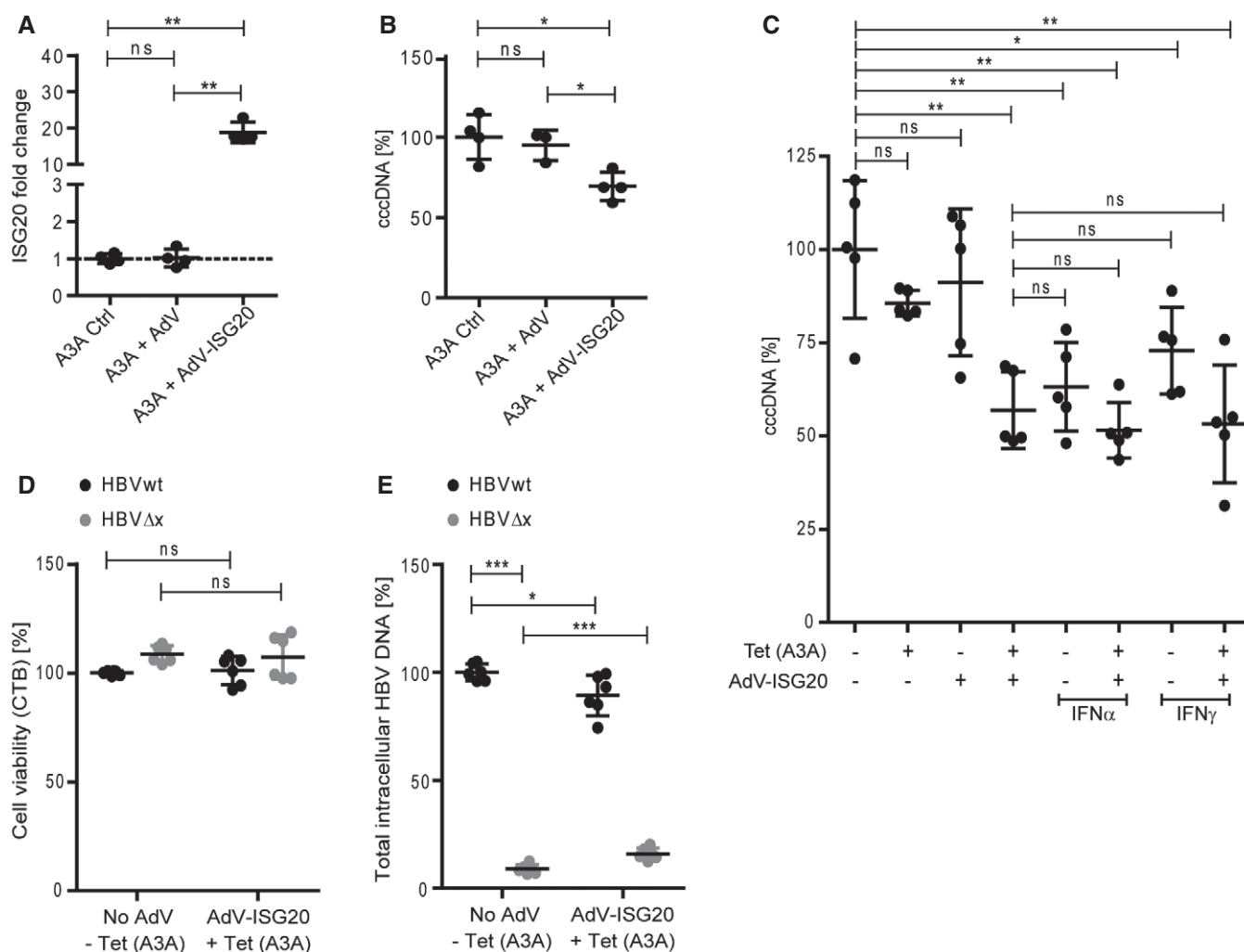


Figure EV5. ISG20 overexpression together with A3A reduces cccDNA similar to interferon treatments.

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 IFN α or 200 U/ml IFN γ . 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 HBV Δ x 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 ($\pm$ 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 $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and ns: not significant by Student's unpaired *t*-test with Welch's correction.