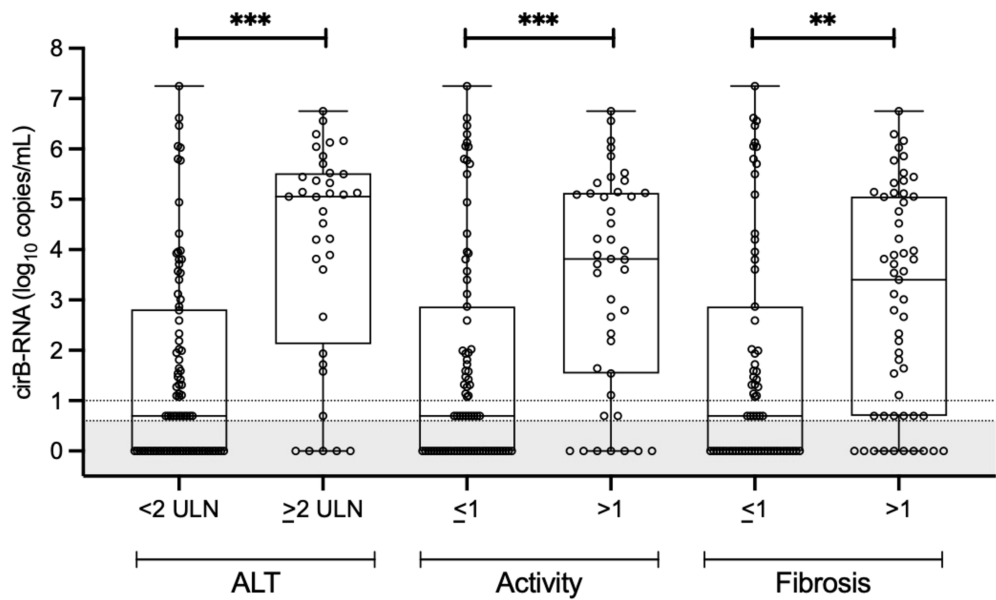
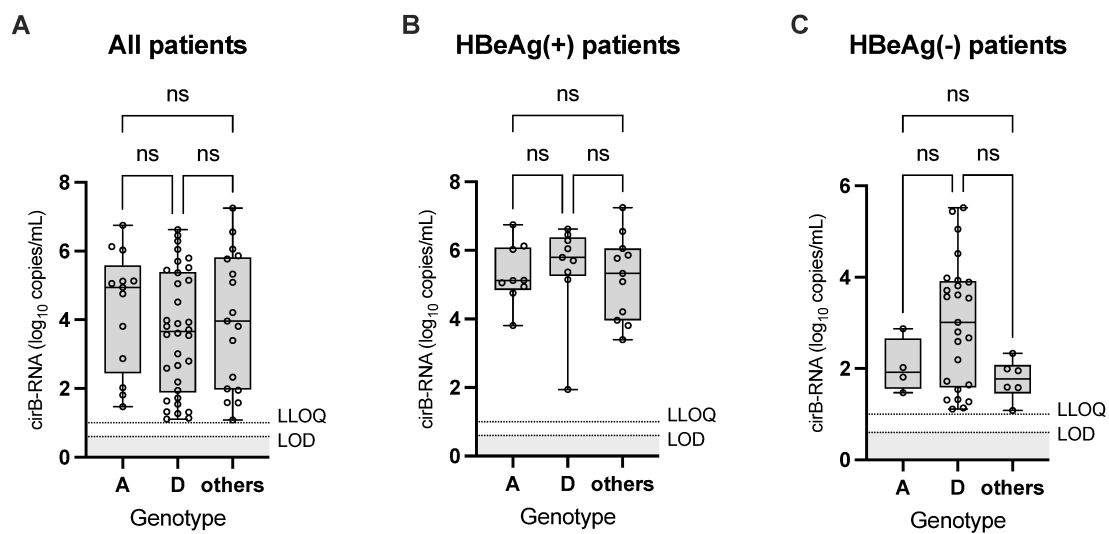


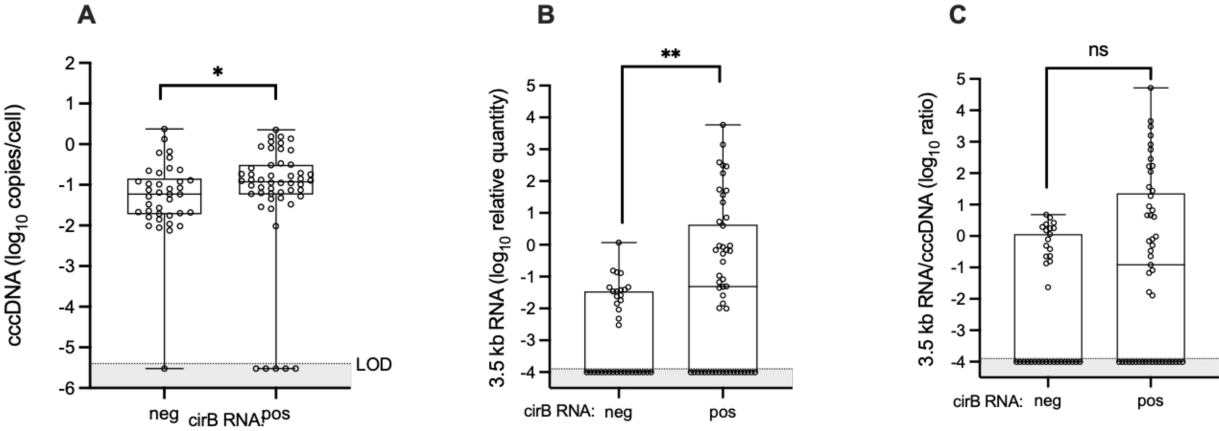
**Figure S1. Serum viral markers distribution across CHB phases in untreated patients. (A) serum HBV DNA (B) HBcrAg (C) HBsAg.** Boxes span the 25<sup>th</sup>-75<sup>th</sup> percentile, whiskers span the range, and horizontal bar in the box represents the median. CH: chronic hepatitis. CI: chronic infection. \* p < 0.05; \*\*\*\* p < 0.0001; ns: not significant



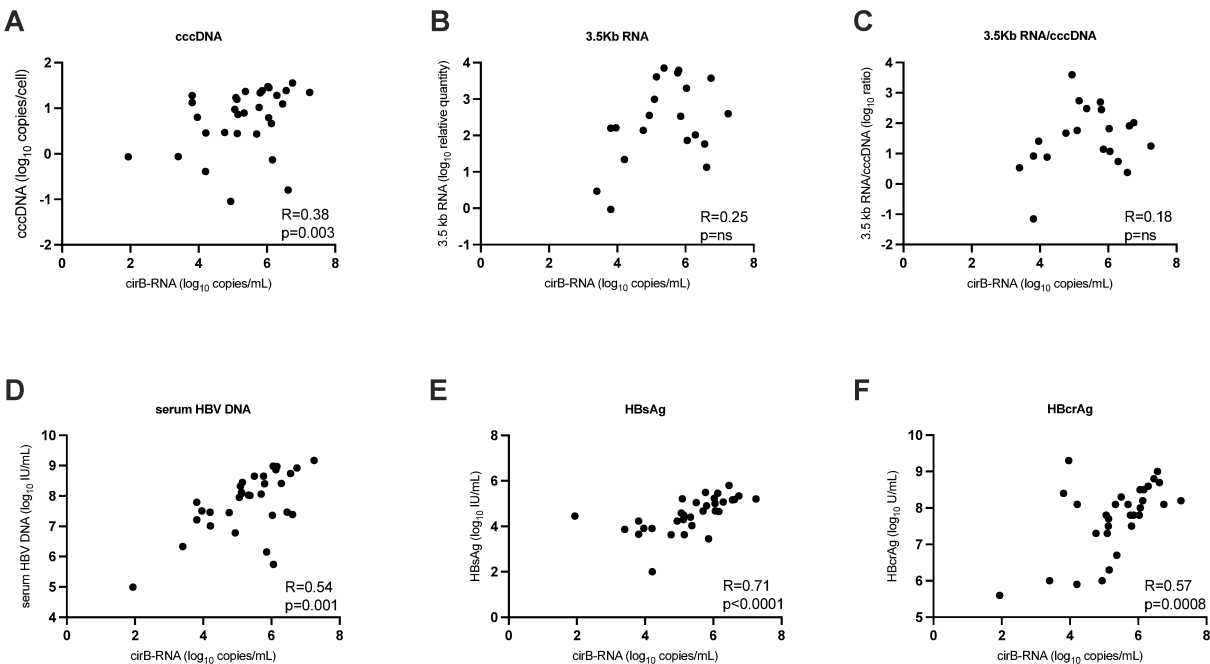
**Figure S2. cirB-RNA values according to markers of liver damage in untreated patients.** Boxes span the 25<sup>th</sup>-75<sup>th</sup> percentile, whiskers span the range, and horizontal bar in the box represents the median. \*\* p < 0.01; \*\*\* p < 0.001; ns: not significant; ULN: upper limit of normal



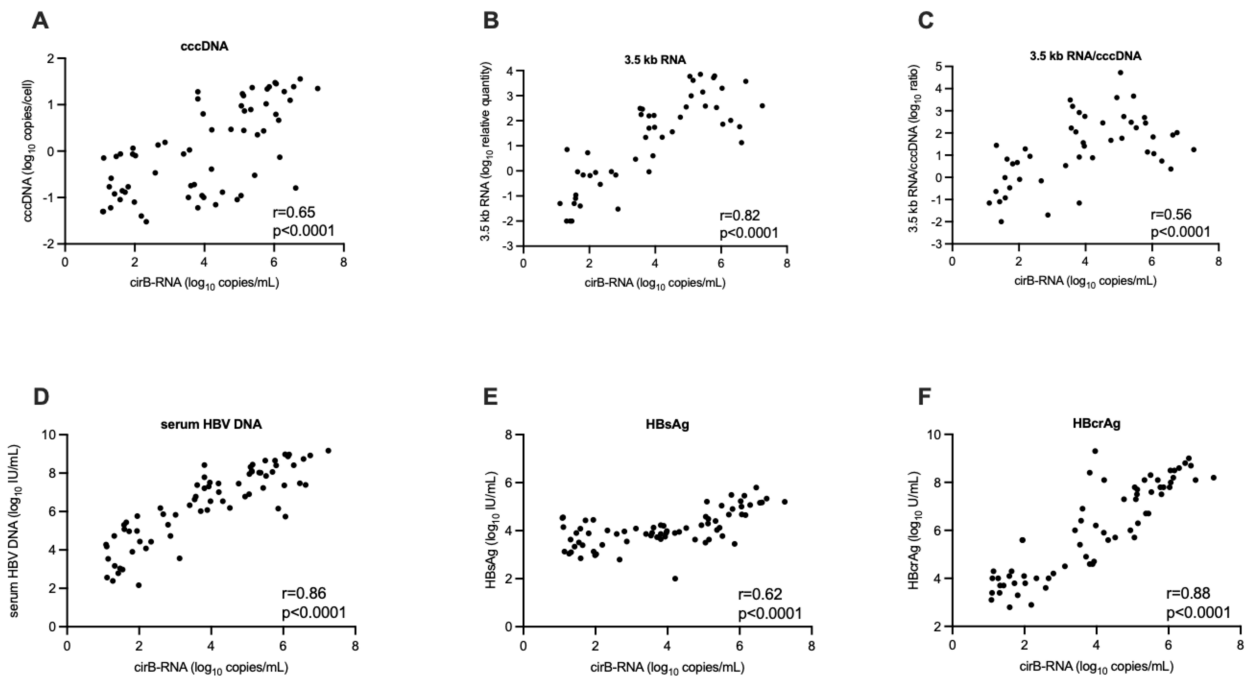
**Figure S3. cirB-RNA values according to HBV genotypes in untreated patients.** Boxes span the 25<sup>th</sup>-75<sup>th</sup> percentile, whiskers span the range, and horizontal bar in the box represents the median. ns: not significant.



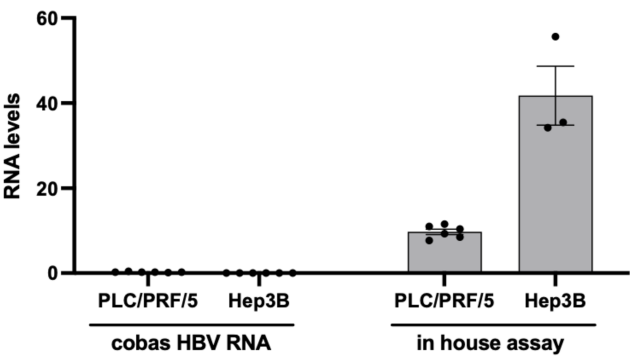
**Figure S4. Intrahepatic viral markers vs. cirB-RNA (untreated HBeAg- patients only).** (A) cccDNA (B) 3.5 kb RNA (C) 3.5 kb RNA/cccdNA ratio. Intrahepatic HBV markers quantification was available for 88/90 HBeAg(-) patients. cccDNA was negative in 6 patients [5 cirB-RNA(+) and 1 cirB-RNA(-)], while 3.5kb RNA was negative in 41 patients [22 cirB-RNA(+) and 19 cirB-RNA(-)]. 3.5Kb RNA/cccdNA ratio could be calculated for 82 patients and different from zero in 46 patients [29 cirB-RNA(+) and 17 cirB-RNA (-) patients]. \* p < 0.05; \*\* p < 0.01.



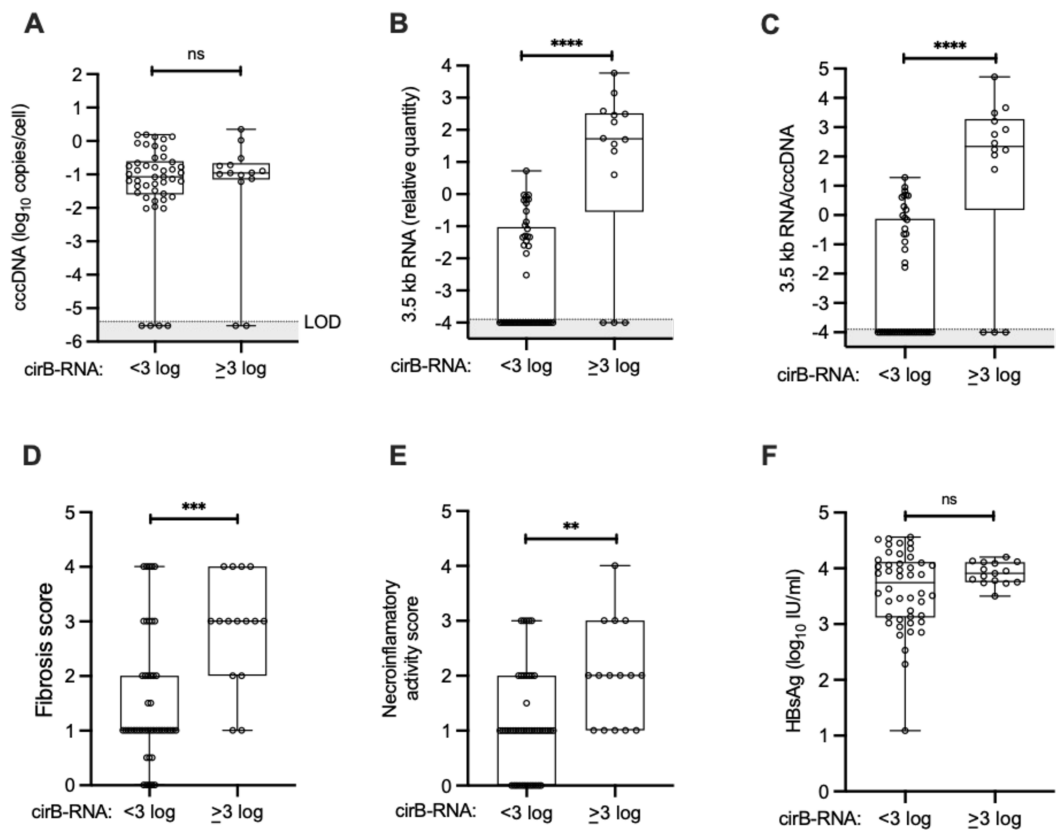
**Figure S5. Correlations between cirB-RNA and intrahepatic viral markers in HBeAg (+) untreated patients.** (A) cccDNA (B) 3.5 kb RNA (C) 3.5 kb RNA/cccdNA ratio (D) serum HBV DNA (E) HBsAg (F) HBcrAg. Intrahepatic HBV markers and serum HBcrAg quantifications were available for 32 patients. 3.5Kb RNA/cccdNA ratio could be calculated for 20 patients. Only samples having quantifiable values for both intrahepatic HBV markers, serum HBV DNA, HBsAg or HBcrAg and cirB-RNA (n=31 for cccDNA, n=20 for 3.5kb RNA, n=20 for 3.5kb RNA/cccdNA, n=32 for serum HBV DNA, n=32 for HBsAg, n=31 for HBcrAg) were included in the analysis.



**Figure S6. Correlations between cirB-RNA and intrahepatic viral markers (untreated patients).** (A) cccDNA (B) 3.5 kb RNA (C) 3.5 kb RNA/cccdNA ratio (D) serum HBV DNA (E) HBsAg (F) HBcrAg. Intrahepatic HBV markers and serum HBcrAg quantifications were available for 120/122 patients. 3.5Kb RNA/cccdNA ratio could be calculated for 113 patients. Only samples having quantifiable values for both intrahepatic HBV markers, serum HBV DNA, HBsAg or HBcrAg and cirB-RNA (n=64 for cccDNA, n=50 for 3.5kb RNA, n=48 for 3.5kb RNA/cccdNA, n=71 for serum HBV DNA, n=70 for HBsAg, n=66 for HBcrAg) were included in the analysis.



**Figure S7. cobas® HBV RNA assay preferentially recognizes RNA derived from cccDNA in cell culture.** Total RNA was extracted from cell lysates using the Manual Workflow for cobas HBV RNA or Trizol reagent for the in-house protocol and then quantified by ddPCR (Scholtes et al. J Clin Virol 2022;150-151:105150). The in-house assay uses primers and a TaqMan probe located in the HBx open reading frame (Pa03453406\_s1, ThermoFisher Scientific), and is thus capable of recognizing viral RNA produced both by cccDNA and integrated sequences.  $\beta$ -glucuronidase (GUSB) RNA quantification served as internal references for RNA relative quantity expression.



**Figure S8. Intrahepatic viral markers in HBeAg(-) CH patients with high or low cirB RNA levels.** Boxes span the 25<sup>th</sup>-75<sup>th</sup> percentile, whiskers span the range, and the horizontal bar in the box represents the median. Assay detection limits are marked with dotted lines; the shaded area represents undetectability. Intrahepatic cccDNA (A) and 3.5 kb RNA (B) results were available for 60 of 61 HBeAg(-) CH patients. 3.5 kb RNA/cccdNA ratio (C) could be calculated for 56 patients and was different from zero in 25 patients (14 cirB-RNA <3 log<sub>10</sub> and 11 cirB-RNA >3 log<sub>10</sub> patients). cccDNA was negative in 4 patients (2 cirB-RNA <3 log<sub>10</sub> and 2 cirB-RNA >3 log<sub>10</sub>), while 3.5 kb RNA was negative in 18 patients (14 cirB-RNA <3 log<sub>10</sub> and 4 cirB-RNA >3 log<sub>10</sub>). (D) Fibrosis score; (E) necroinflammatory activity score; (F) HBsAg levels. \*\*\*\* p<0.0001; \*\*\* p<0.001; \*\* p<0.01.