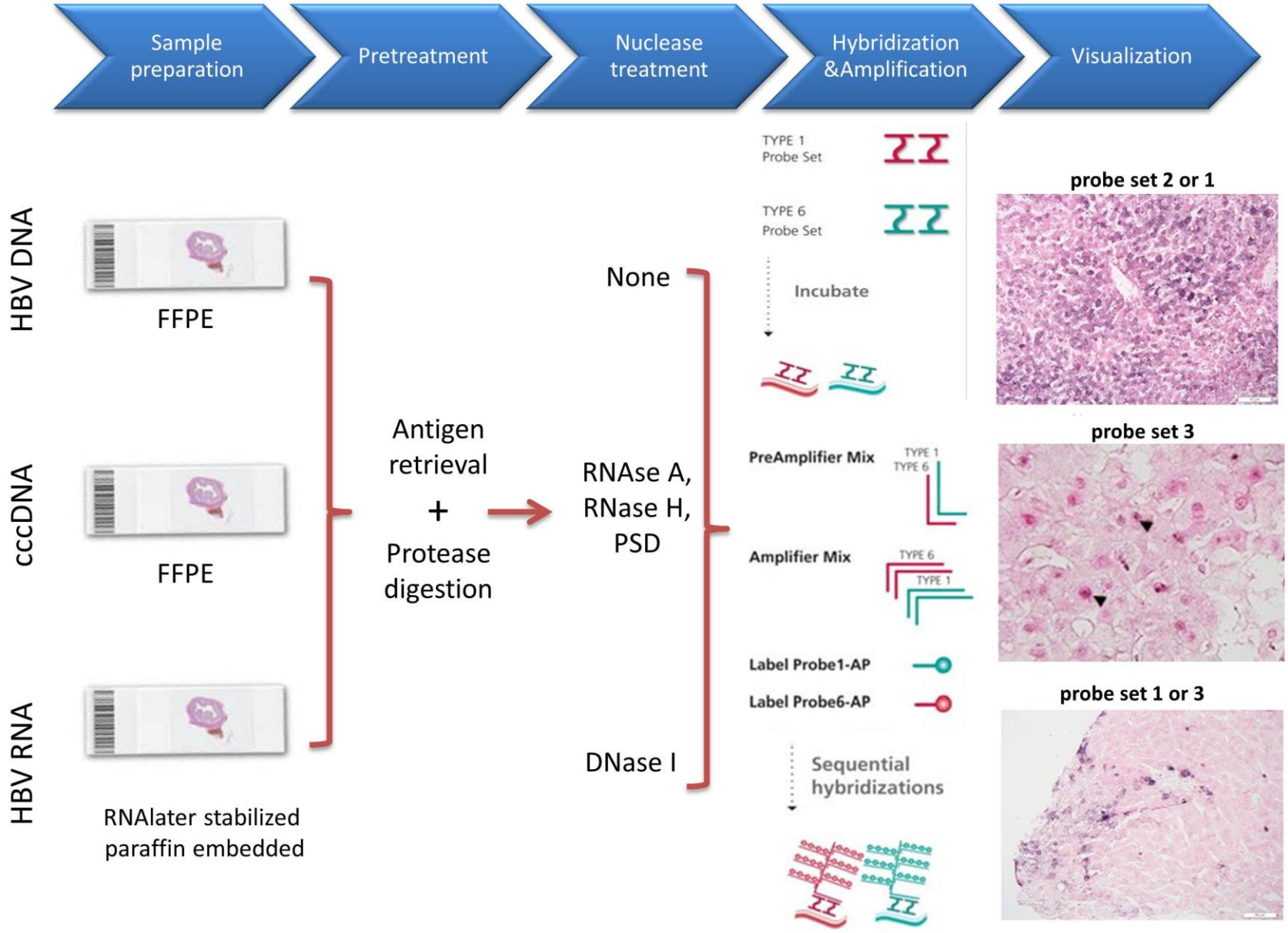
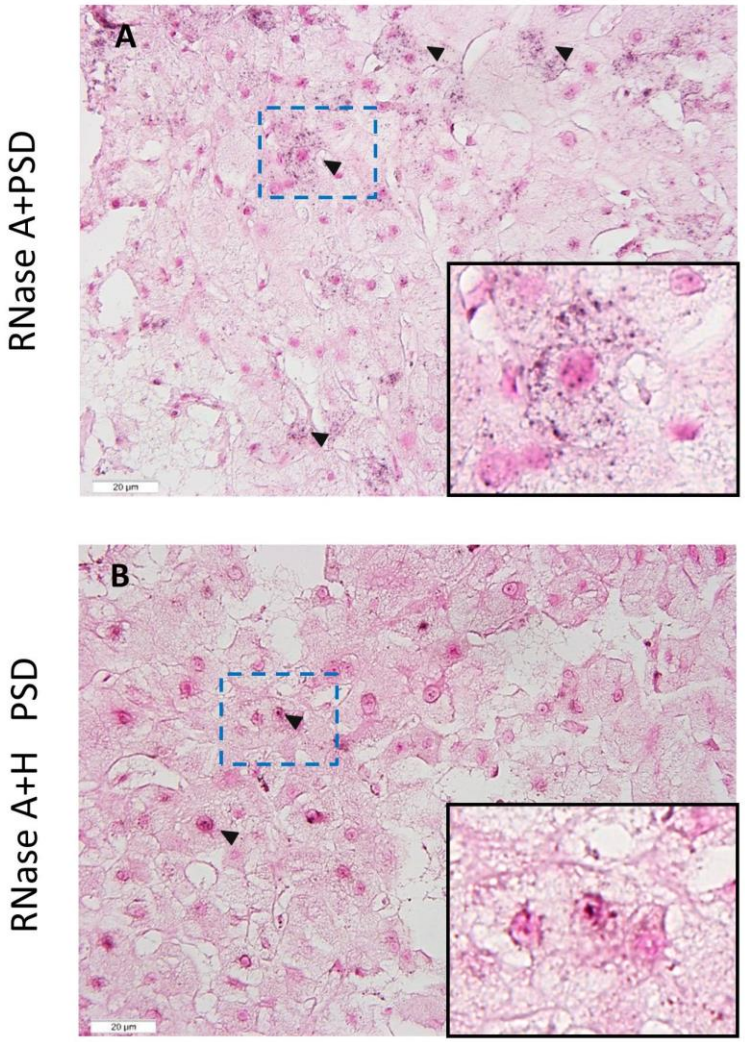
Supplementary figure 1



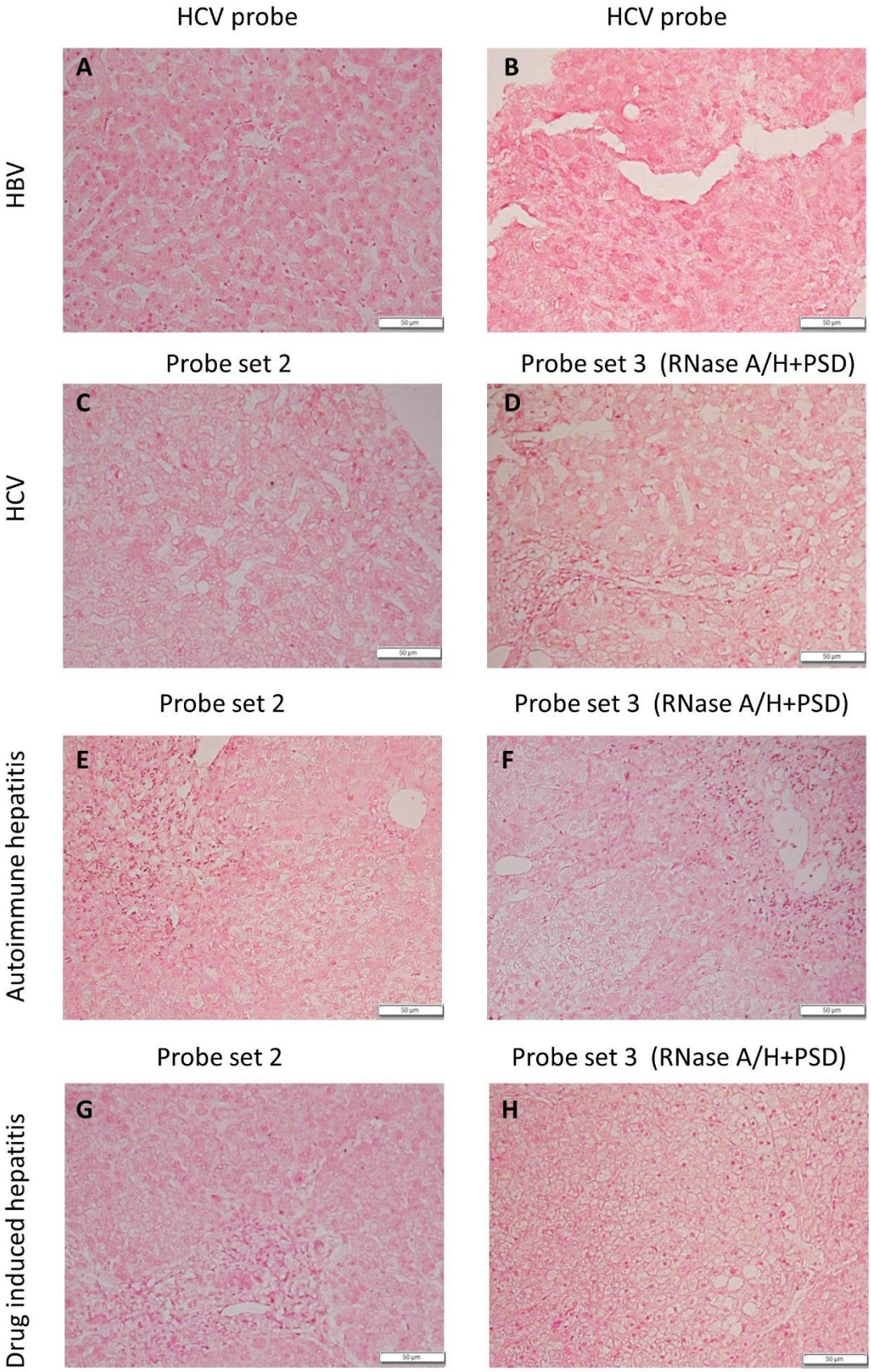
Supplementary Figure 1. Flowchart of experimental procedures of in situ hybridization using ViewRNA technology.

Supplementary figure 2



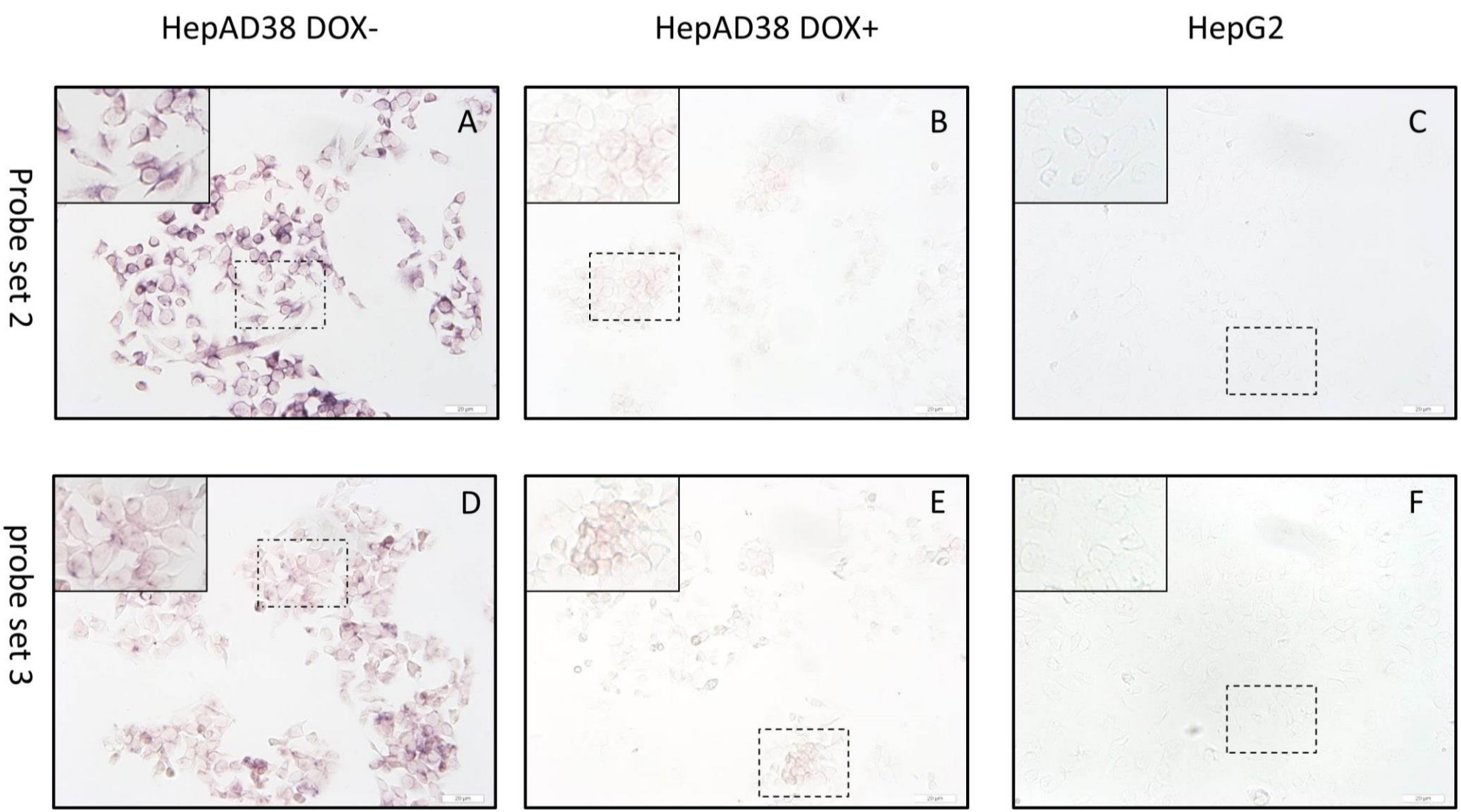
Supplementary Figure 2. Evidence for existence of HBV DNA-RNA hybrid in clinical HBV infection. Liver sections were pre-treated with RNase A+PSD (A) or RNase A, RNase H and PSD (B) and hybridized with probe set 3 and visualized with NBT/BCIP followed by counter staining with nuclear fast red. Magnification, 400×.

Supplementary figure 3



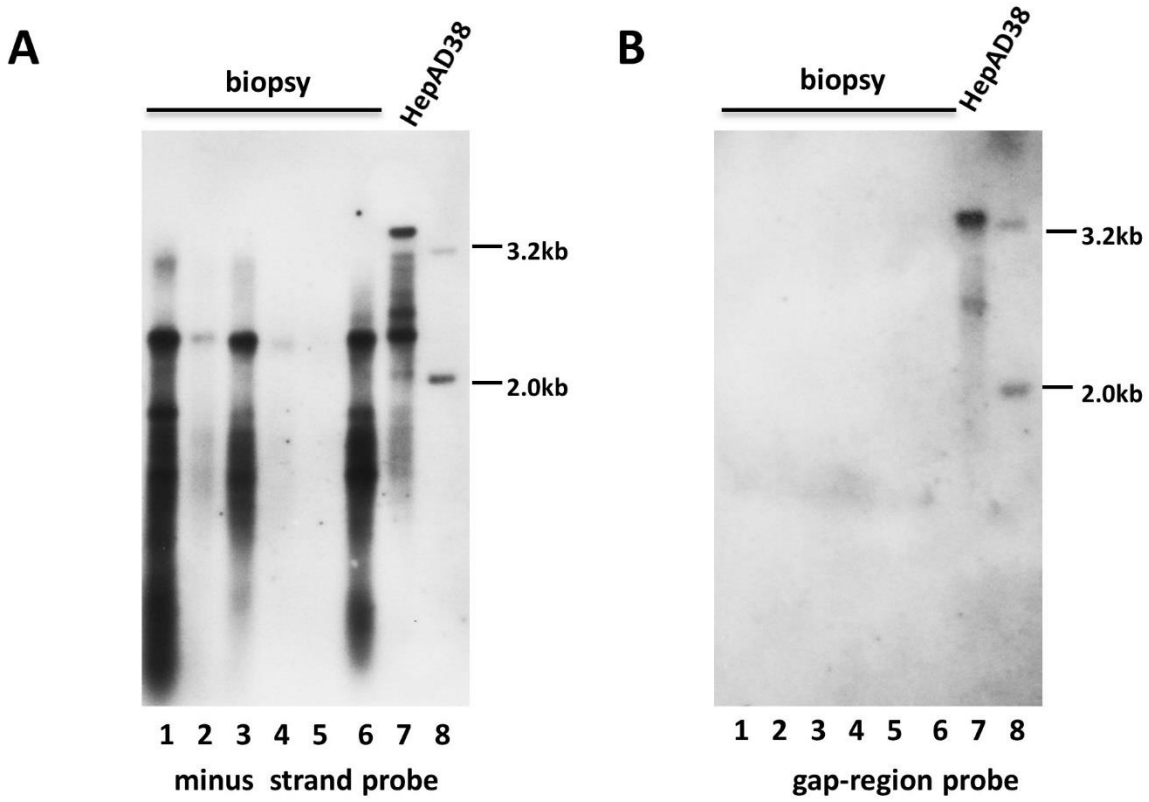
Supplementary Figure 3. Control experiments for in situ hybridization. (A-B) Liver sections from two CHB patients were hybridized to HCV probe; sections from a HCV patient (C, D), a patient with autoimmune hepatitis (E, F) and a patient with drug-induced hepatitis (G, H) were hybridized to probe set 2 (C, E, G) or probe set 3 (predigested with RNase A/H and PSD, D, F, H), visualized by NBT-BCIP and counterstained by nuclear fast red. Magnification, 200×.

Supplementary figure 4



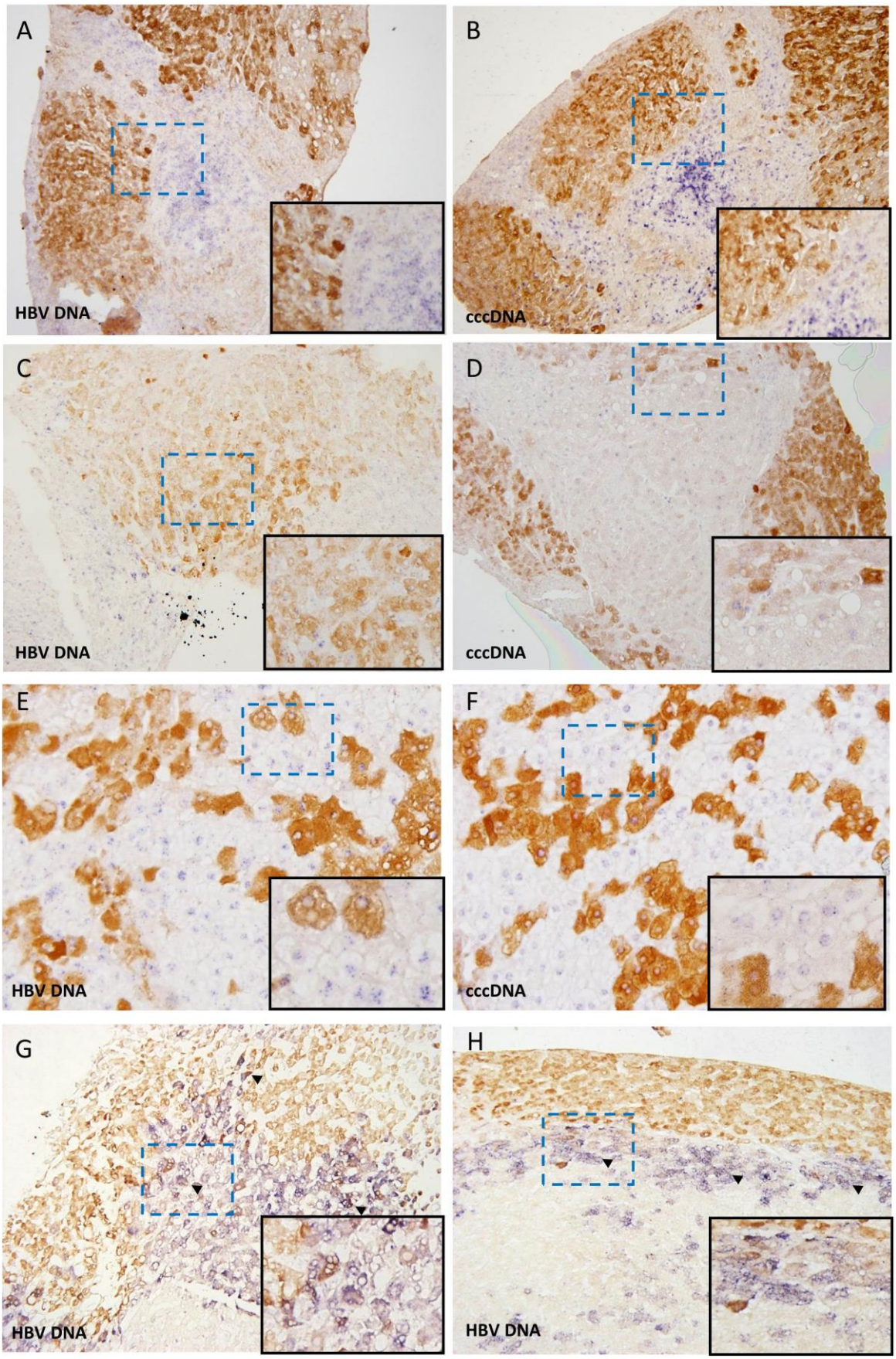
Supplementary Figure 4. In situ hybridization of probe set 2 and 3 in HepAD38 cells. Probe set 2 and 3 were used to visualize HBV DNA and cccDNA in HepAD38 cells in the absence (A,D) or presence (B, E) of doxycycline and in HepG2 cells (C, F). Magnification 400×

Supplementary figure 5



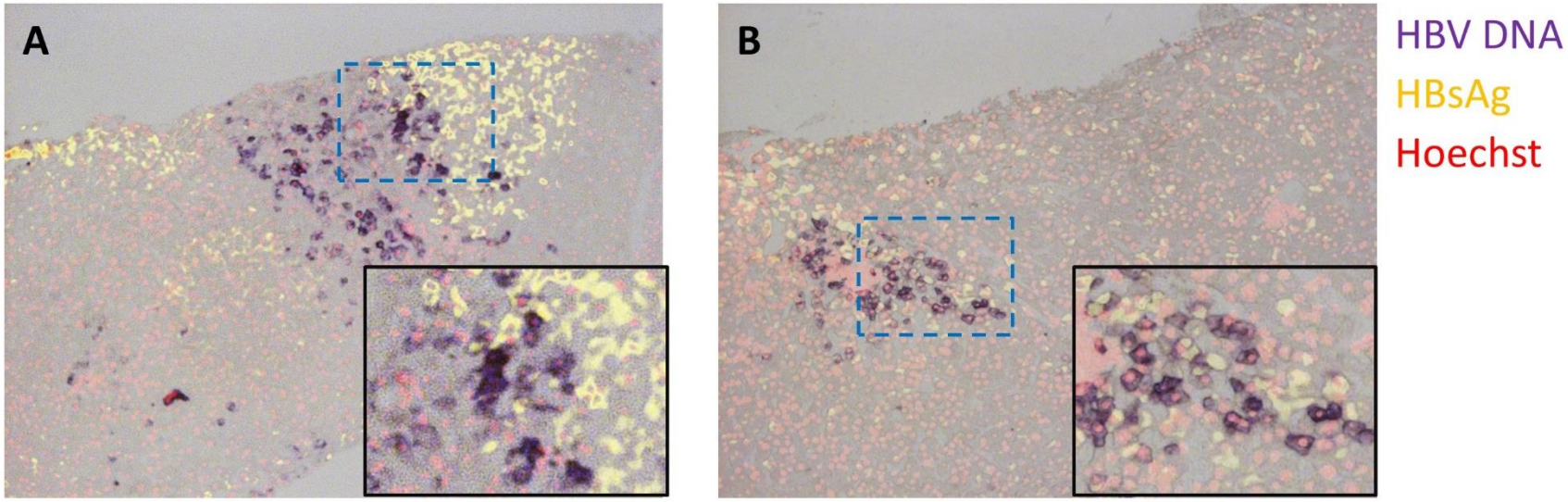
Supplementary Figure 5. Southern blot analysis of core particle DNA from liver biopsies and HepAD38 cells. Core particle DNA from six liver biopsies and HepAD38 cells were subjected to Southern blot analysis using minus strand specific riboprobe 1 (A) or gap-region specific riboprobe 2 (B).

Supplementary figure 6



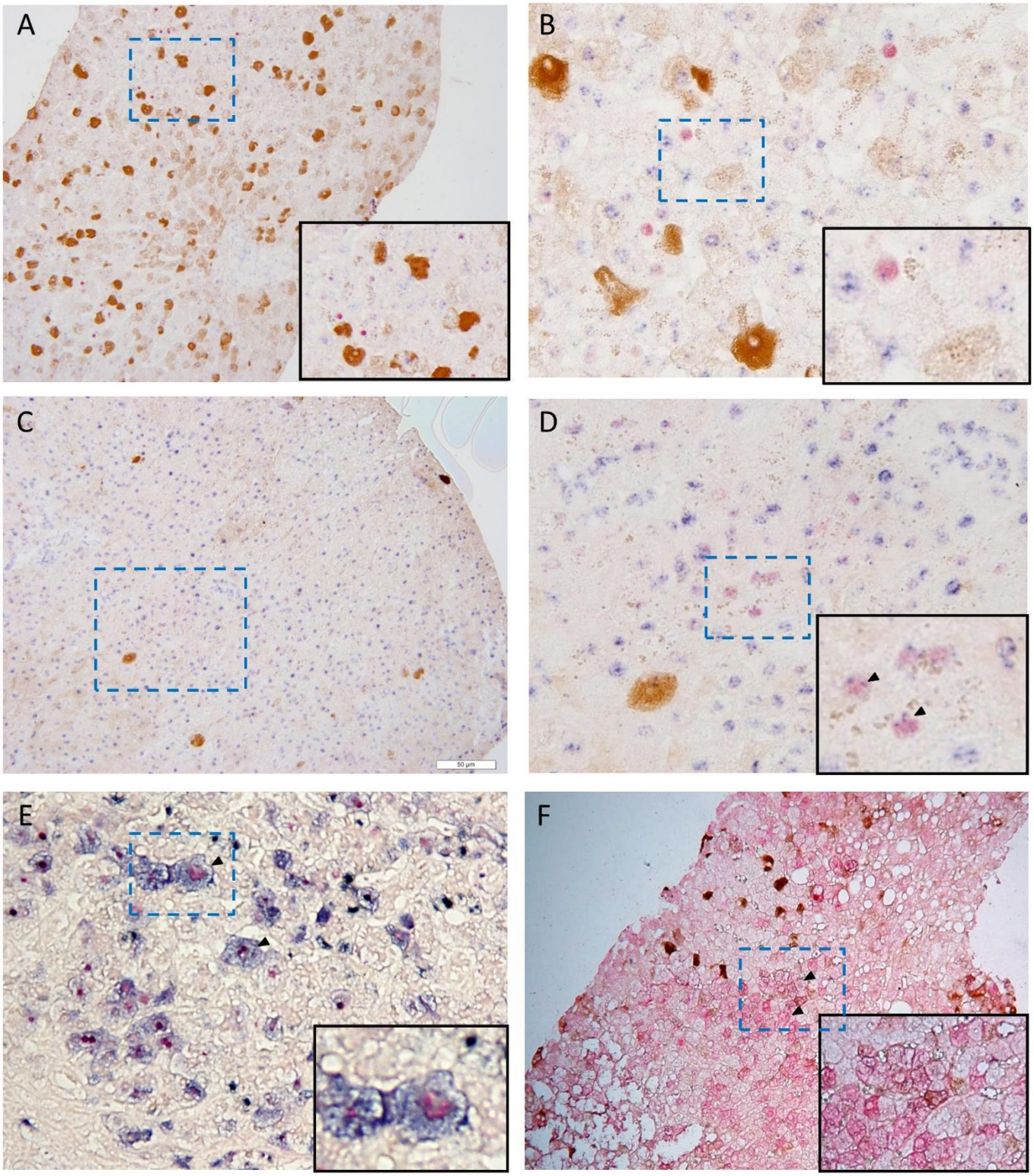
Supplementary Figure 6. The spatial relationship between intrahepatic viral nucleic acids (HBV DNA and cccDNA) and surface antigen. Blue purple, DNA or cccDNA; brown, HBsAg. Magnification, 100× for A, B, 200× for C, D, G, H, 400×for E,F. For A-F, images from the same row were from the same specimens. The selected regions were magnified in the insets.

Supplementary figure 7



Supplementary Figure 7. Combined in situ hybridization and immunofluorescence staining. Liver sections from CHB patients were hybridized to probe set 2 and visualized by NBT-BCIP colorization. Immunofluorscence stainig against HBsAg was performed after in situ hybridization using Alexa fluore 594 conjugated secondary antibody and counterstained with Hoechst. Sections were finally mounted in fluorsave (Calbiochem). Images taken from bright field and fluorescence channels were merged. The immunofluorescence and hoechst 33342 signal were pseudo-colored yellow and red respectively. Magnification, 200×.

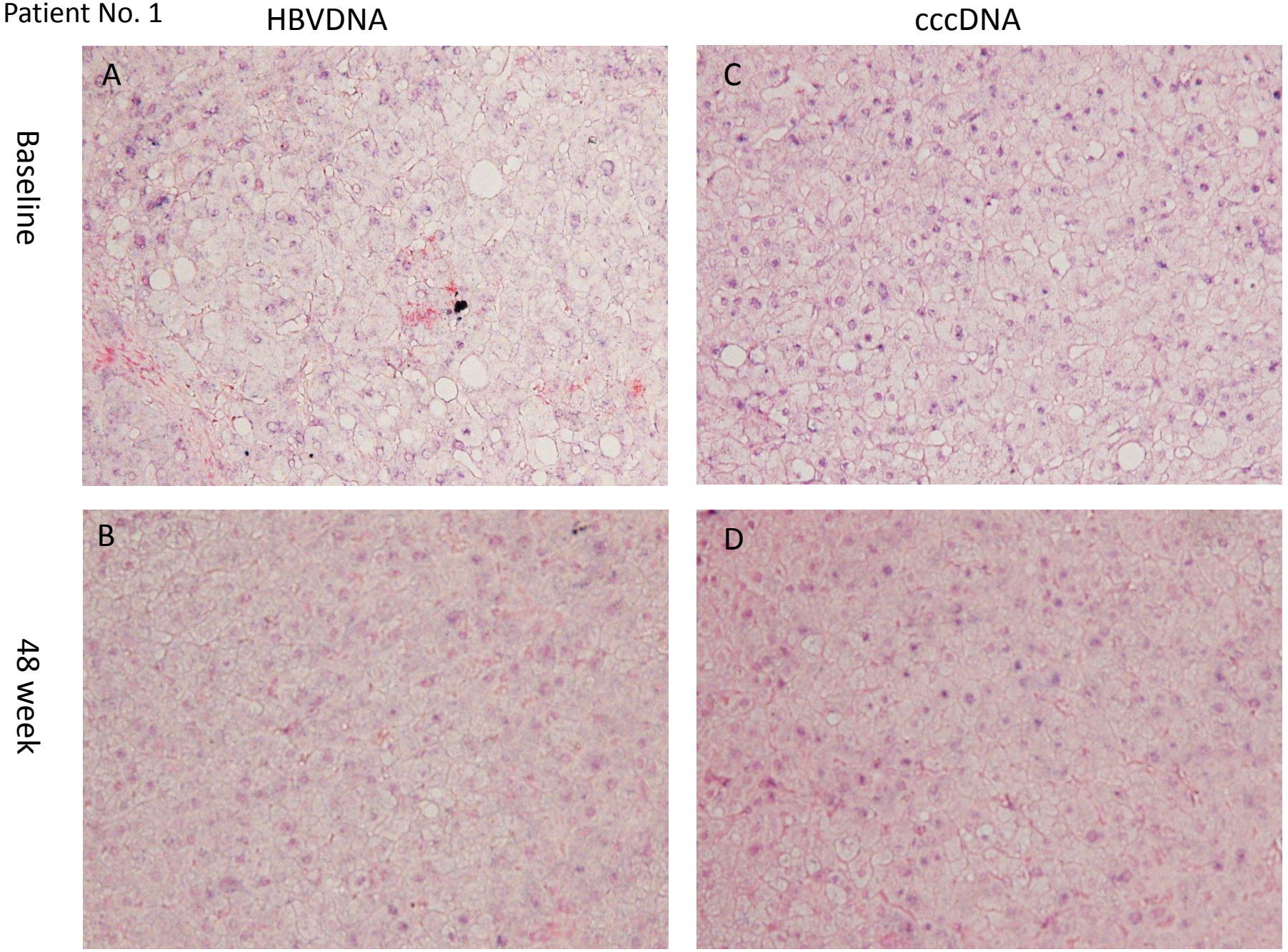
Supplementary figure 8



Supplementary Figure 8. Highly complex distribution of HBsAg, HBcAg and viral DNA. In situ hybridization of HBV DNA with probe set 2 in conjunction with HBsAg/HBcAg double immunohistochemistry was performed and presented. Magnification, 200× for A, C and F, 400× for B, D and E. A and B, C and D were from the same specimens respectively. Image D was the magnification of the rectangle area in image C. The selected regions were magnified in the insets.

Supplementary figure 9

Patient No. 1



Supplementary figure 9

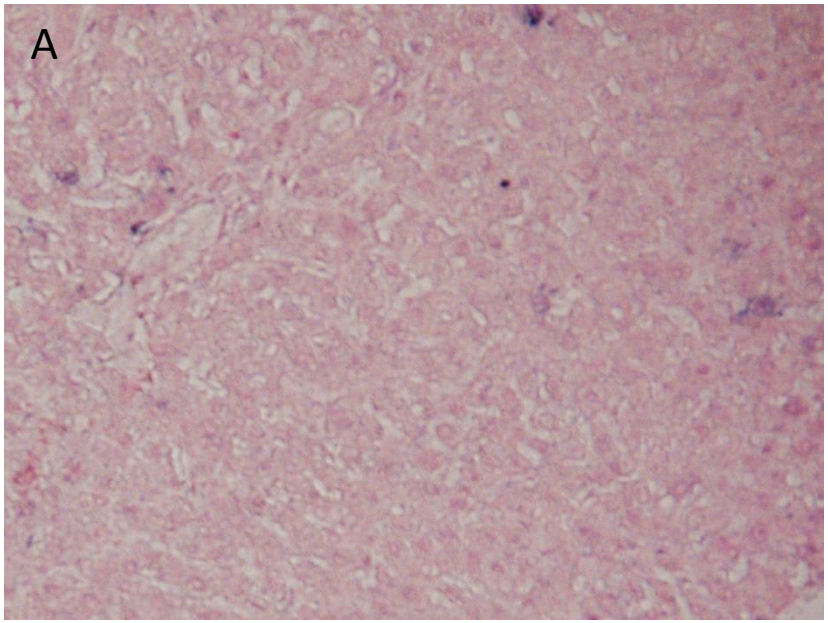
Patient No. 2

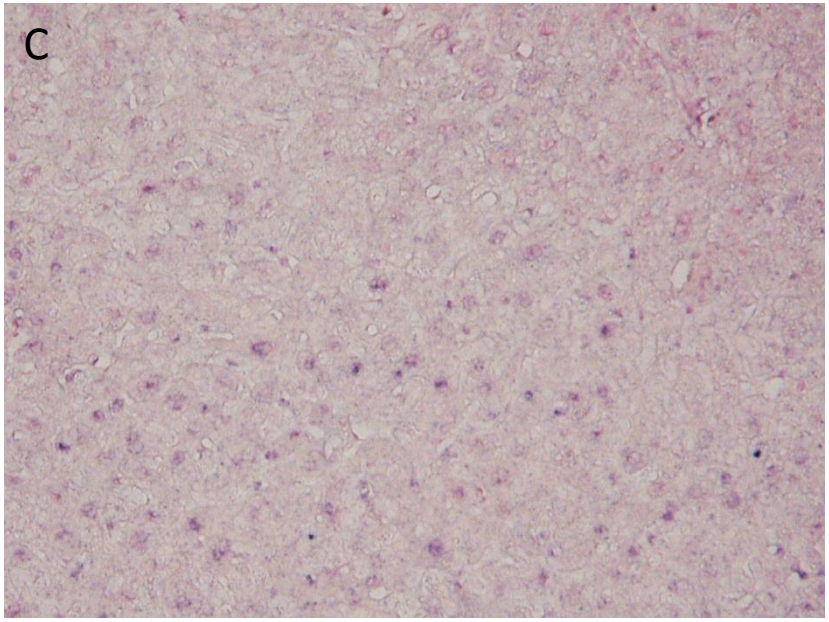
HBVDNA

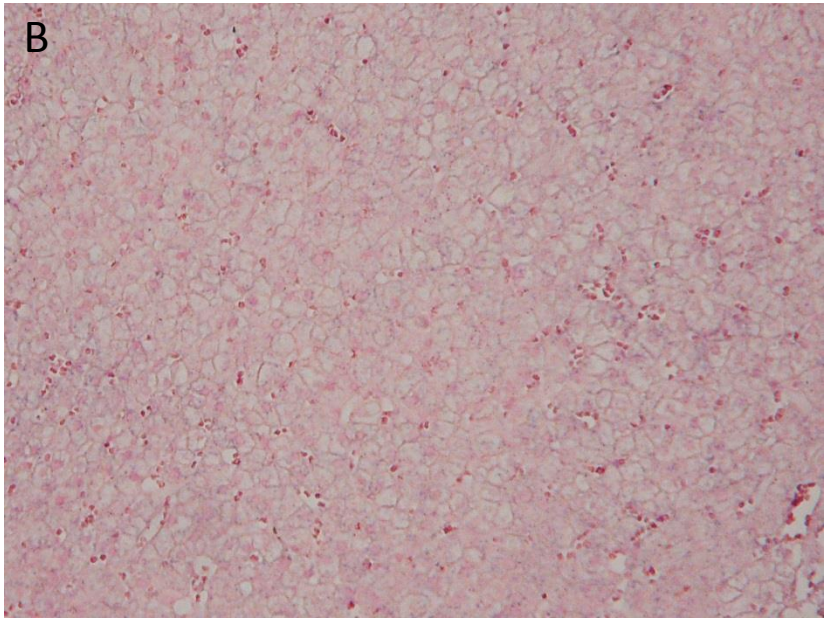
cccDNA

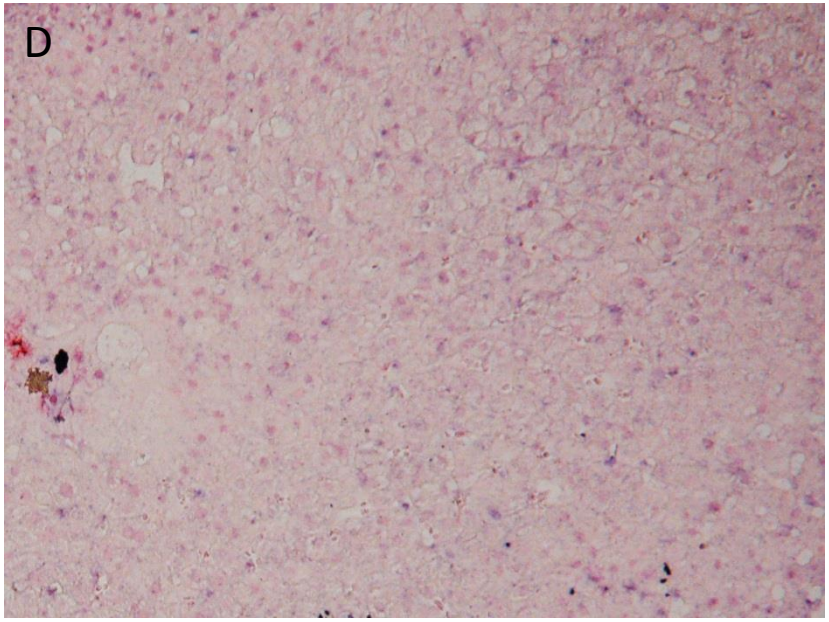
Baseline

48 week









Supplementary figure 9

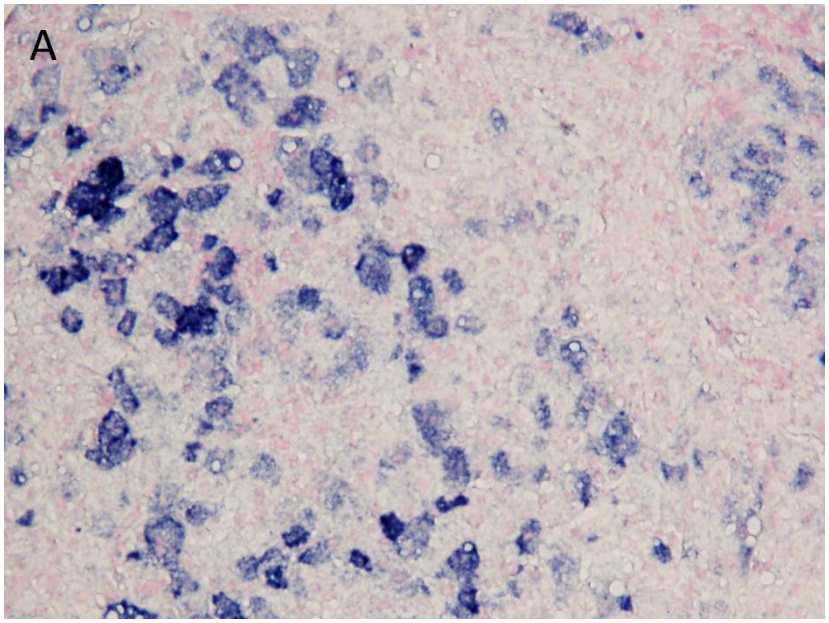
Patient No. 3

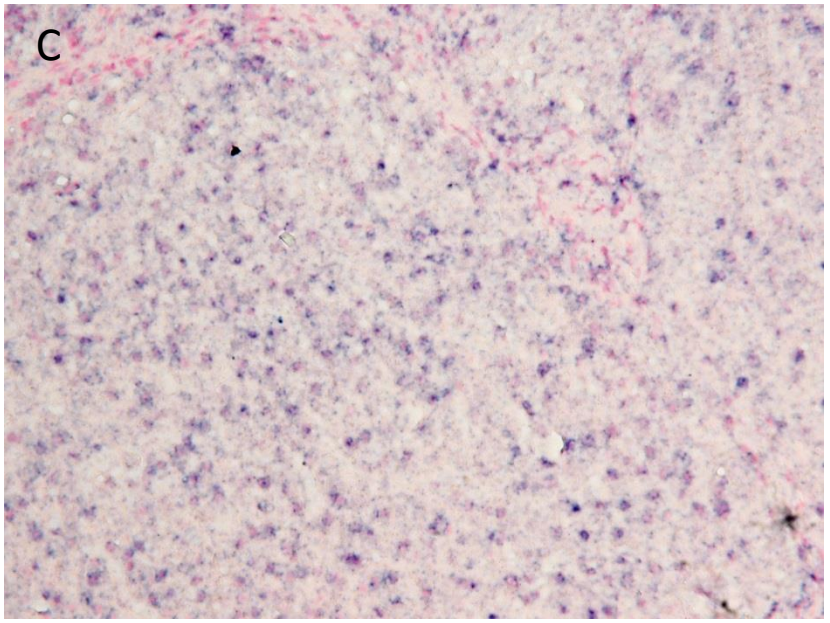
HBVDNA

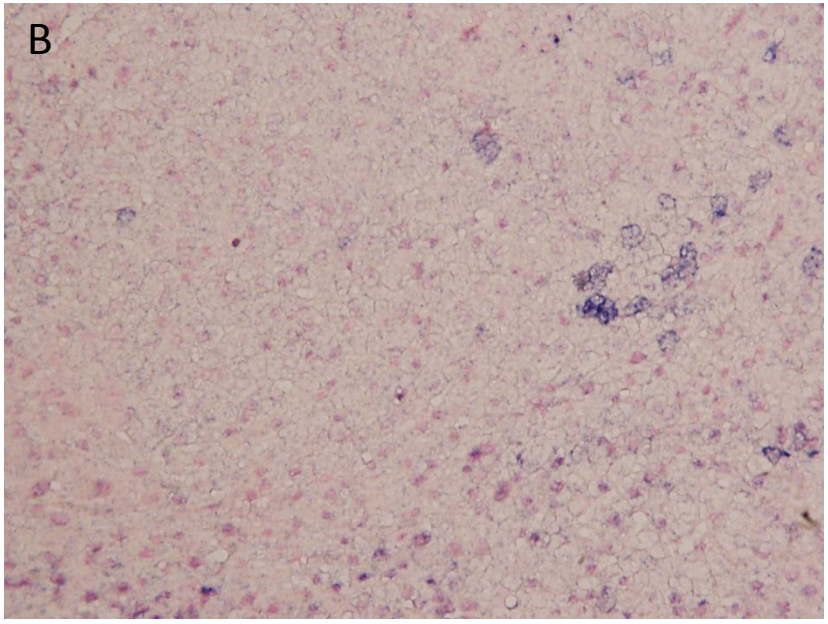
cccDNA

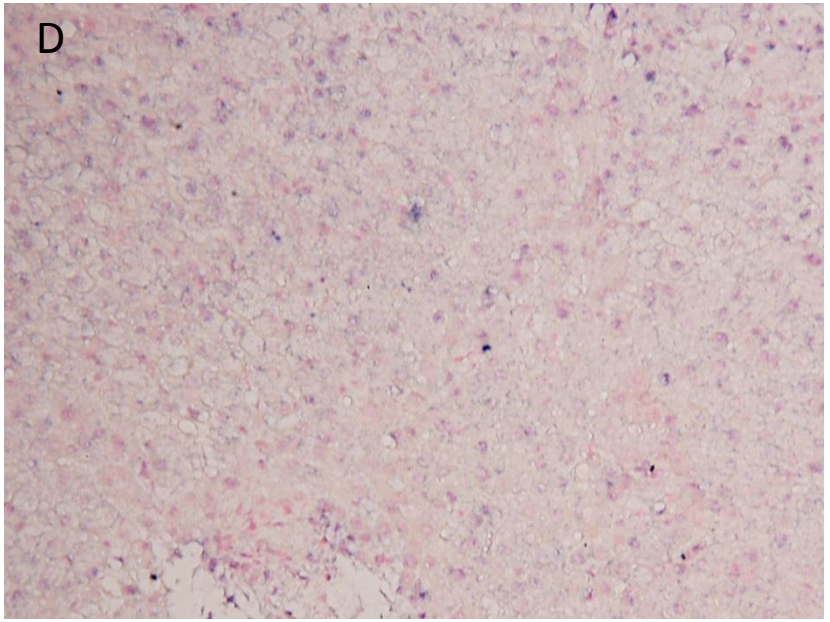
Baseline

48 week









Supplementary figure 9

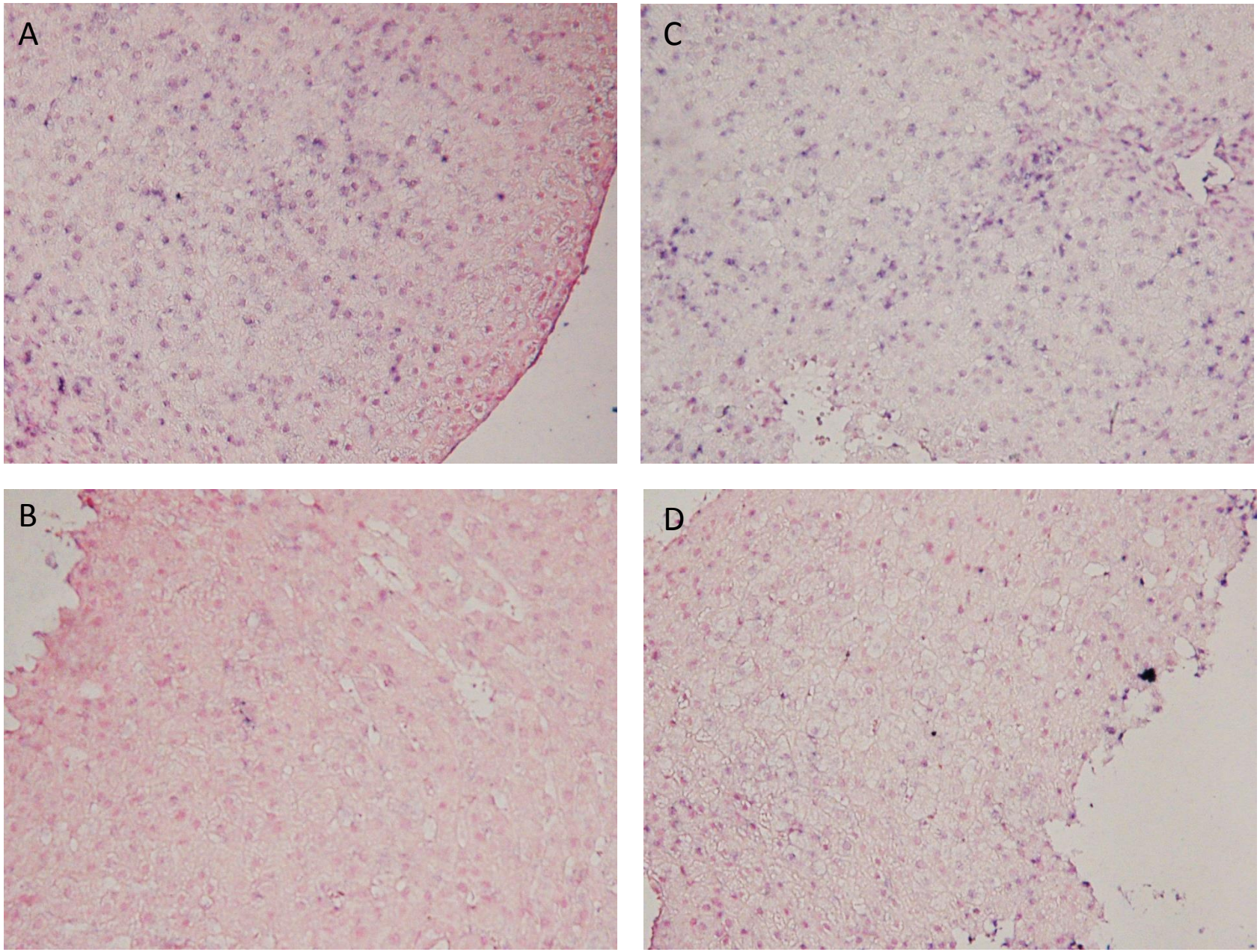
Patient No. 4

HBVDNA

cccDNA

Baseline

48 week



Supplementary figure 9

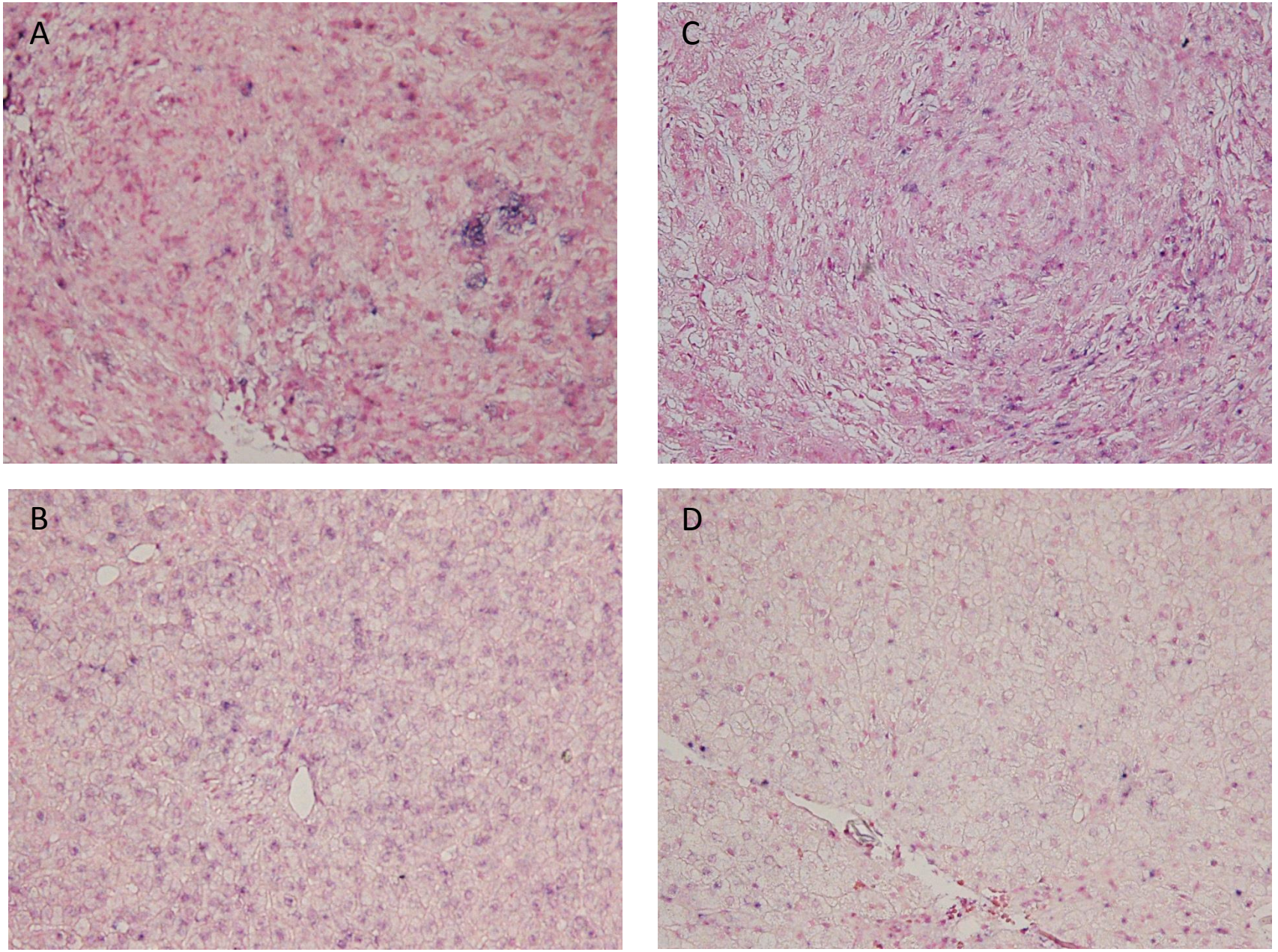
Patient No. 5

HBVDNA

cccDNA

Baseline

48 week



Supplementary figure 9

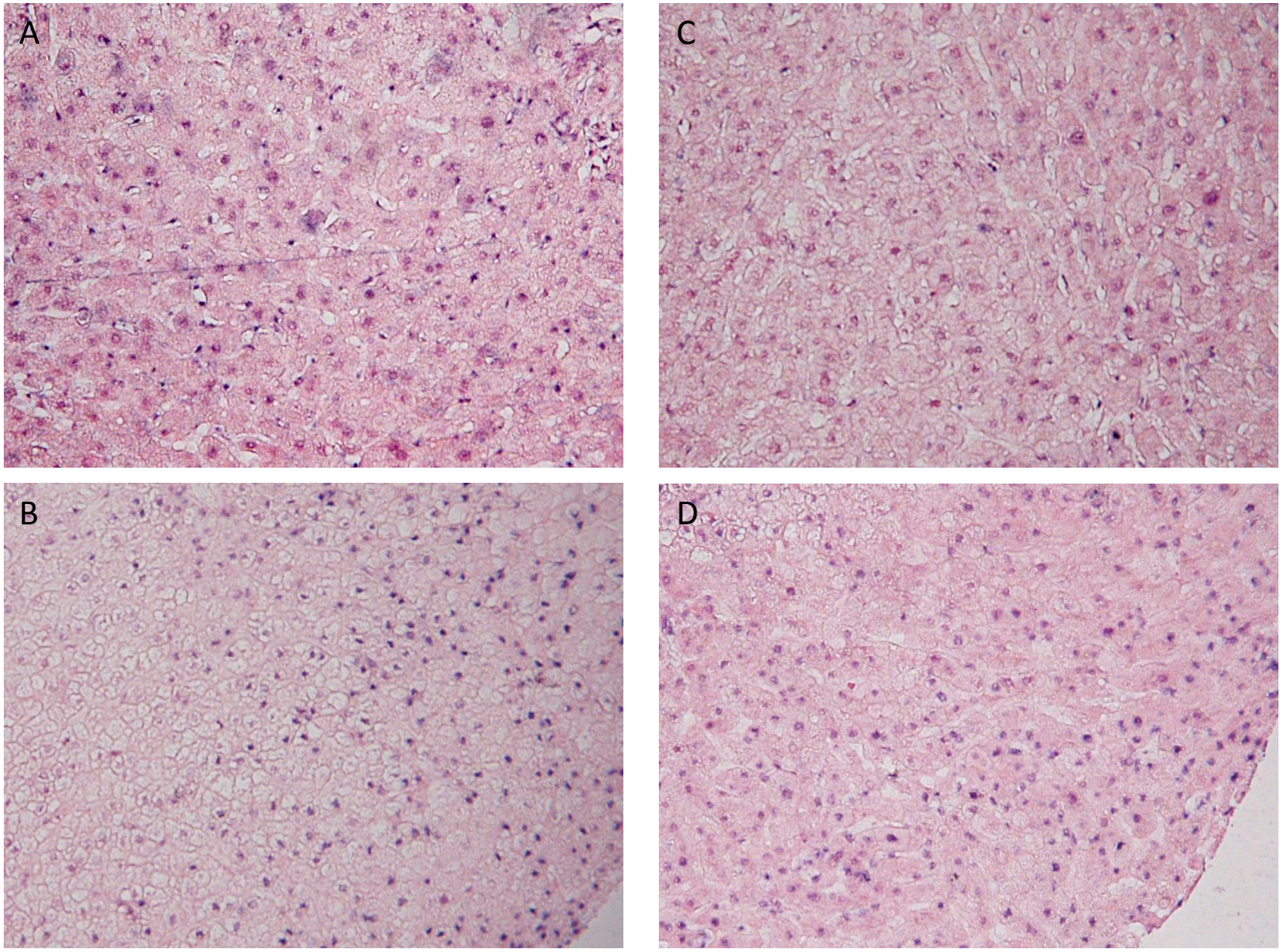
Patient No. 6

HBVDNA

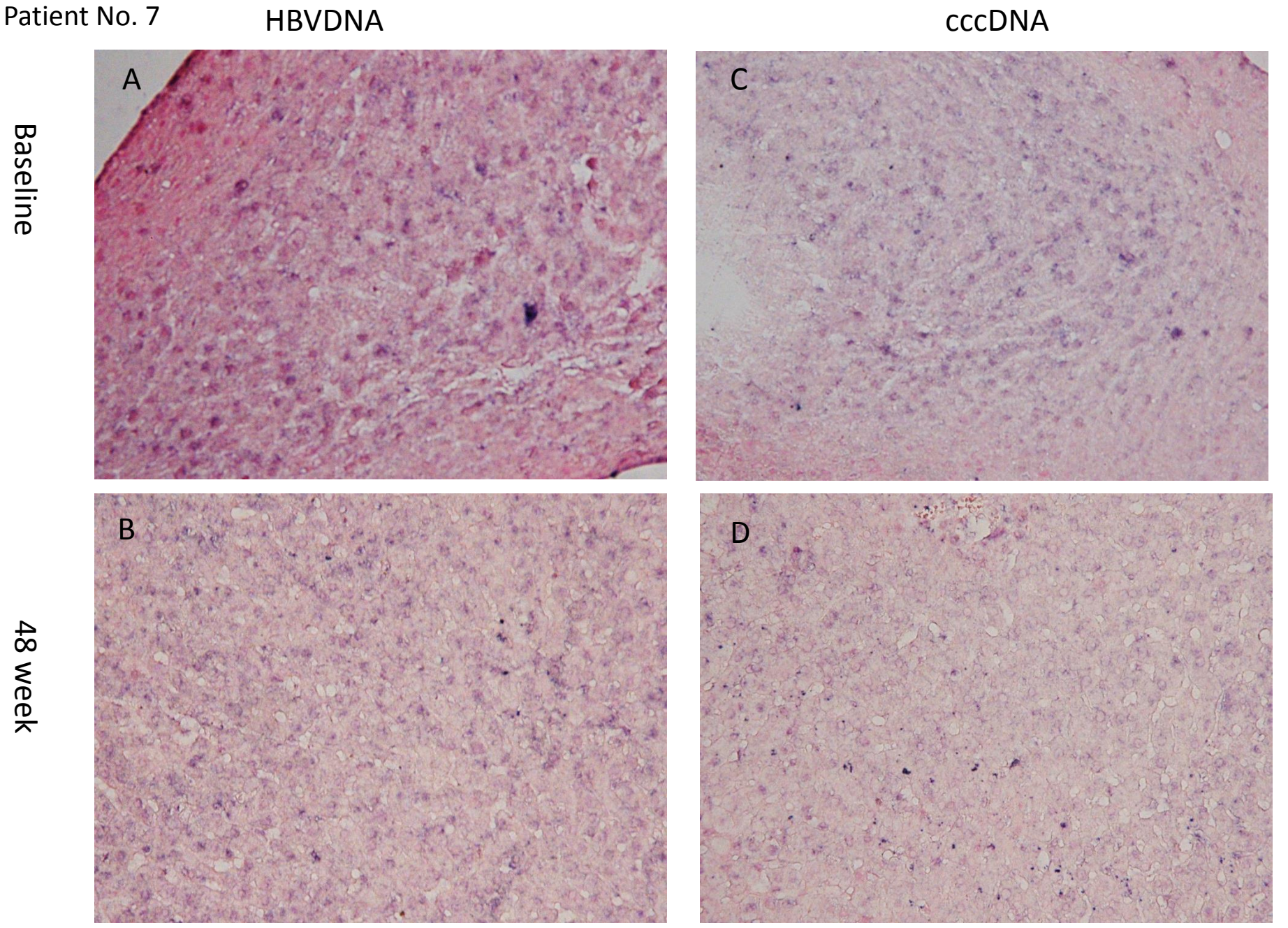
cccDNA

Baseline

48 week

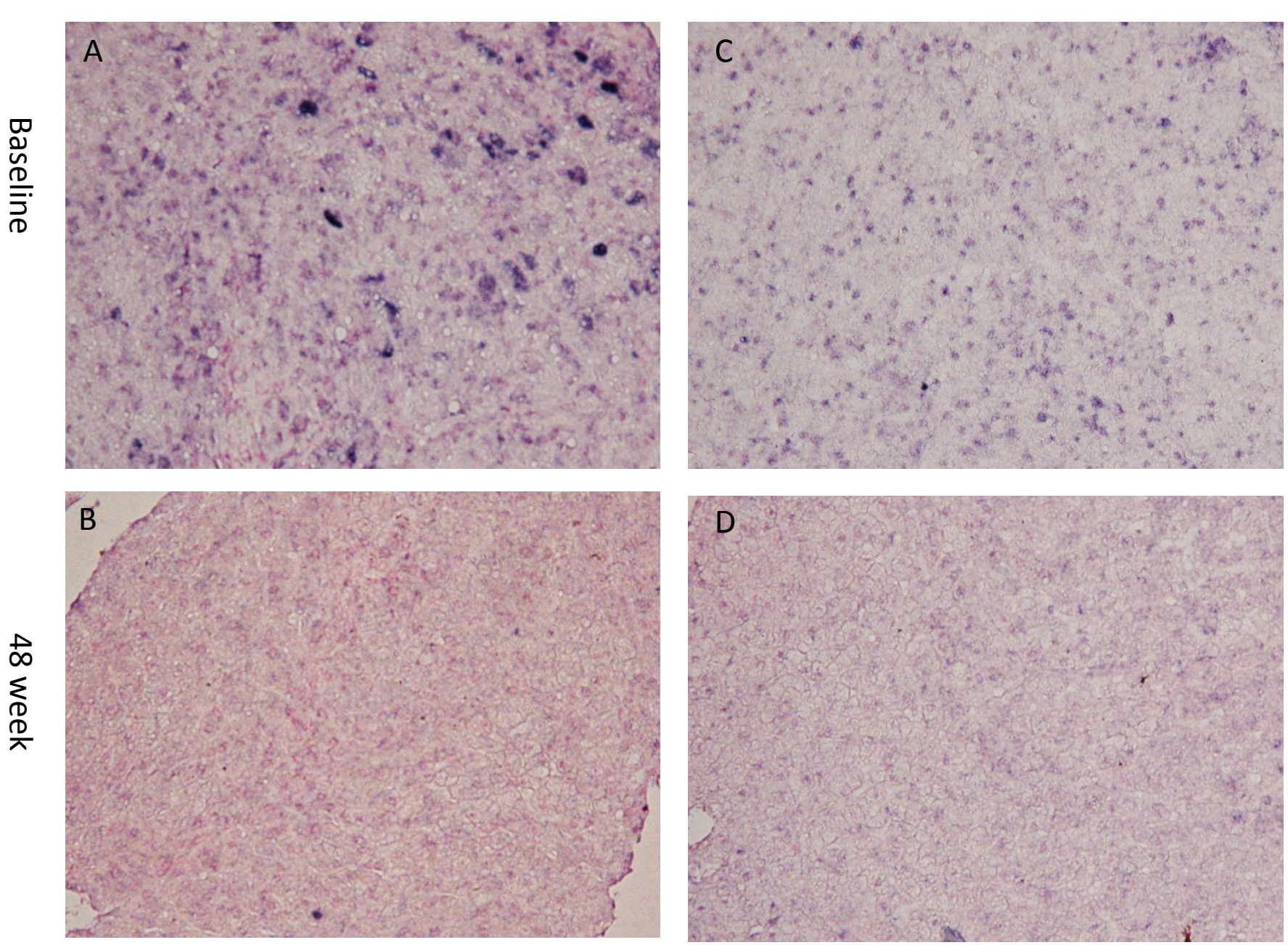


Supplementary figure 9



Supplementary figure 9

Patient No. 8



Supplementary figure 9

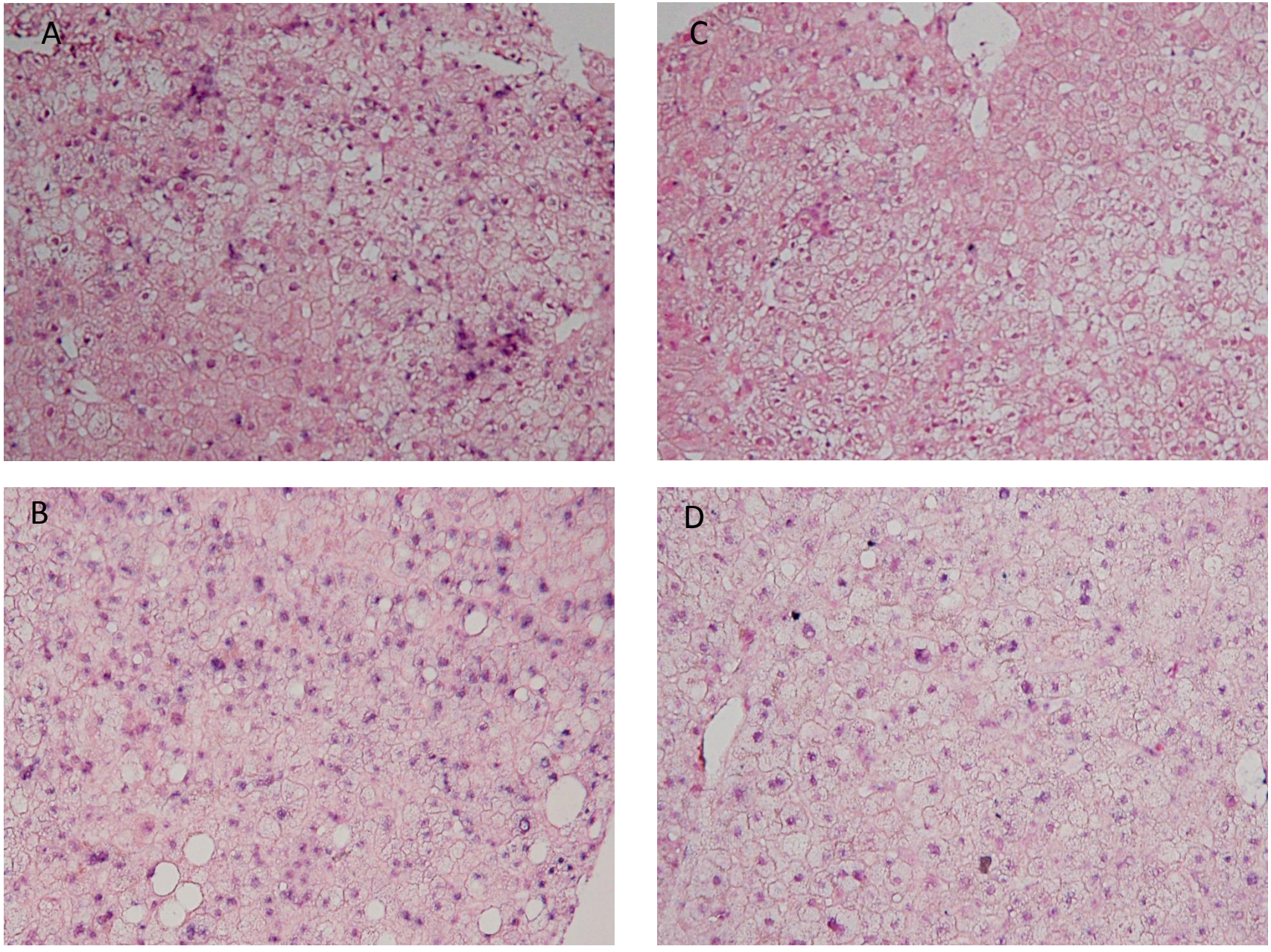
Patient No. 9

HBVDNA

cccDNA

Baseline

48 week



Supplementary Figure 9. The distribution of HBV DNA and cccDNA before and after adeforvir therapy. Serial liver sections from nine CHB patients before and 48 week after adefovir therapy were hybridized with probe set 2 or 3 to visualize HBV DNA (A, B) or cccDNA (C, D). Results from eight patient (No. 1-2, 4-9) were shown. 200×magnification.