Figure S1. HBx increases CPAP promoter activity in HCC cell lines. Huh7, SK-Hep1, HepG2 and Hep3B cells were transiently transfected with GFP or GFP-HBx with CPAP promoter for 24 h prior to a reporter assay. GFP-HBx was detected by Western blot analysis. The error bars represent the mean ± SD of three independent experiments, each performed in triplicate (**, p < 0.01; ***, p < 0.001).
Figure S2. Expression of CPAP is increased in HBV genome-expressing HCC cells. (A) HBV genome is induced to express in cells without (-) tetracyclin. The expression of HBV core antigen (HBC) was detected by RT-PCR and Western blot analysis. HBC is an indicator for HBV genome expression. (B) Total cell lysates from HepAD38 cells with (+) or without (-) tetracyclin were collected to analyze the expression of CPAP. Triplicated cell lysates were analyzed.
Figure S3. The CREB binding site is crucial for CPAP promoter activity. pGL2-CPAP/WT or pGL2-CPAP/M1, M2 or M1+2 (for detail, please see Figure 1C) were transiently transfected into Huh7 and Hep3B cells prior to a reporter assay. Data presents as the mean ± SEM in three independent experiments, each performed in triplicate (*, p < 0.05; **, p < 0.01; N.S., no significance).
Figure S4. CREB is essential for HBx-mediated CPAP promoter activity. (A) Hep3B cells with GFP-CREB or/and HA-HBx expression were used in a reporter assay to determine the CPAP promoter activity. Expression of GFP-CREB and HA-HBx was detected by Western blot analysis. (B-C) CPAP promoter activity in Huh7 cells (B) or Hep3B (C) with HA-HBx or/and wild type GFP-CREB (WT) or GFP-CREB/S133A dominant-negative mutant (DN) was determined by reporter assay. (D) pGL2-CPAP/WT or pGL2-CPAP/M1, M2 or M1+2 were transiently transfected into Hep3B cells with GFP-CREB or HA-HBx, and luciferase activity and Western blot analysis were performed. Data presents as the mean ± SEM in three independent experiments, each performed in triplicate (*, p < 0.05; **, p < 0.01; ***, p < 0.001; N.S., no significance).
**Figure S5.** Evaluation of *CPAP* promoter activity in sh*CREB* transfected Hep3B cells. (A-B) Three different sh*CREB* (#1, #2 and #3) were used to knock down the expression of endogenous CREB (A), and *CPAP* promoter activity was determined in sh*CREB* #2 and #3 transfected Hep3B cells. (C) pGL2-CPAP/WT and HA-HBx were co-transfected into shGFP or sh*CREB* (#2 or #3) knocked-down Hep3B cells and then the reporter assay was performed as described above. The numbers indicate the fold change versus shGFP control. Three independent experiments were performed (**, *p* < 0.01).
Figure S6. CPAP is involved in HBx-induced transcriptional activation of NF-κB. NF-κB-responsive transcriptional activity (A) and IL-8 promoter activity (B, C) were examined in Hep3B (B) or Huh7 cells (C) using a reporter assay. Cells were transiently transfected with GFP-HBx and HA-CPAP or CPAP siRNA (siCPAP) (A), or HA-HBx and GFP-CPAP or pSuper-CPAP (B, C) prior to a reporter assay. The expression levels of individual proteins were detected by Western blot analysis. pSuper/NS or control siRNA (siCtrl) was the siRNA control. The expression of CPAP and HBx was detected by Western blot analysis. RLA, relative luciferase activity. The data presents as the mean ± SEM in three independent experiments, each performed in triplicate (*, p < 0.05; **, p < 0.01; ***, p < 0.001).
Figure S7. The interaction between CPAP and HBx is increased upon TNF-α treatment. (A) The interaction between CPAP and HBx was determined by in situ proximal ligation assay (PLA). Anti-CPAP and anti-GFP antibodies (CPAP+, GFP+) were used to detect the HA-CPAP/wild type (WT) or HA-CPAP/double mutant (DM, K921.975R) and GFP-HBx in SK-Hep1 cells. The red spots represent interacting complexes of CPAP and HBx. Cells stained with anti-GFP antibody only (CPAP-, GFP+) were used as a negative control. The nuclei were stained with DAPI (blue). Expression of HA-CPAP/WT, HA-CPAP/DM and GFP-HBx was detected by Western blot analysis. (B) Huh7 cells transfected with HA-HBx and GFP-CPAP or GFP were treated with TNF-α and then harvested for IP assay using anti-HA antibody. The expression level of HA-HBx and the interaction ability between GFP-CPAP and HA-HBx are indicated as ratio.
Figure S8

(A) Cell proliferation assay (left), BrdU incorporation (middle) and colony-formation assay (right) were examined in HepG2 cells stably expressed with GFP, GFP-CPAP/WT or GFP-CPAP/DM. The data are presented as the mean ± SEM, and three independent experiments were performed (*, p < 0.05; **, p < 0.01; ***, p < 0.001).

(B) GFP, GFP-CPAP/WT or GFP-CPAP/DM stably expressed Hep3B cells were injected subcutaneously into the right flank of NOD-SCID mice (n=8). Tumor size and weight were examined. The Ki-67 proliferation index of tumor cells is shown. The protein expression of GFP, GFP-CPAP/WT and GFP-CPAP/DM in the xenograft tumors was determined by Western blot analysis.

Figure S8. CPAP promotes proliferation, colony formation, and tumorigenicity of HCC. (A) Cell proliferation assay (left), BrdU incorporation (middle) and colony-formation assay (right) were examined in HepG2 cells stably expressed with GFP, GFP-CPAP/WT or GFP-CPAP/DM. The data are presented as the mean ± SEM, and three independent experiments were performed (*, p < 0.05; **, p < 0.01; ***, p < 0.001). (B) GFP, GFP-CPAP/WT or GFP-CPAP/DM stably expressed Hep3B cells were injected subcutaneously into the right flank of NOD-SCID mice (n=8). Tumor size and weight were examined. The Ki-67 proliferation index of tumor cells is shown. The protein expression of GFP, GFP-CPAP/WT and GFP-CPAP/DM in the xenograft tumors was determined by Western blot analysis.
Figure S9. NF-κB/p65 is essential for CPAP-mediated colony formation of HCC cells. Hep3B cells with stably expressed GFP or GFP-CPAP were transfected with *NF-κB/p65* (si*p65*) or control (siCtrl) siRNA, and then performed the colony-formation assay. The mean ± SEM were obtained from three independent experiments (**, $p < 0.01$; ***, $p < 0.001$).
Figure S10. Overexpression of CPAP/WT significantly increased tumor growth in a xenograft animal model. (A) HepG2 (n=6) or GFP stably expressed HepG2 (n=7) cells were injected subcutaneously into the right flank of NOD-SCID mice. Tumor weight was examined at the 35th day after injection. (B-C) GFP-CPAP/WT or GFP-CPAP/DM stably expressed HepG2 cells were injected subcutaneously into the right flank of NOD-SCID mice (n=6). Tumor volume (B) and weight (C) were examined at the 28th day after injection. Tumor volume was measured using the formula: length × width² × 0.5. *, p < 0.05; **, p < 0.01.
Figure S11. CPAP increases TNF-α-mediated HBx protein stabilization. (A) TNF-α increases GFP-HBx expression. (Upper) Huh7 cells were transiently transfected with GFP-HBx and treated with TNF-α and then harvested for Western blot analysis at indicated time points. (Lower) Quantification showed GFP-HBx expression in TNF-α-treated cells. (B) Huh7 cells co-transfected with GFP-HBx and HA-CPAP or HA control were treated with TNF-α for 1 h and then incubated with proteasome inhibitor cycloheximide (200 μg/ml) for 0.5, 1, or 2 h. The cells were harvested for Western blot analysis to detect the expression of HA-CPAP and GFP-HBx. The level of GFP-HBx is indicated as ratio.
Figure S12. The clinical outcome of overexpressed CPAP, HBx and activated NF-κB (p65) in HCC. A total of 23 cases of HCC were collected to analyze. Kaplan-Meier (p=0.0086) and Cox regression (p=0.018) models were used to calculate the disease-free survival rate (DFS) of HCC patients with overexpressed CPAP, HBx and activated NF-κB (p65).
Figure S13

(A) Co-overexpression of CPAP and CREB is positively correlated with a poor disease-free survival rate in HBx-positive HCC. (A) Patients with overexpressed CPAP/CREB (T/NT >1, n=12) HCC tissues have a poor disease-free survival rate compared with HCC patients with lower expression level of CPAP/CREB (T/NT <1, n=4). All of these HCC are HBx-positive. (B) (Left) The HCC data set from TCGA (n=361) was split into two maximized risk groups, low-risk (green) and high-risk (red), according their prognostic index. P-values correspond to log-rank test and t-test for the box plot. (Right) Overexpression of CPAP and CREB mRNAs exists in the high-risk group with a lower overall survival rate (red).