

**Cellular Id1 inhibits hepatitis B virus transcription by
interacting with the novel covalently closed circular
DNA-binding protein E2F4**

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Supplementary Table

Table S1. Patient characteristics (N = 25)

Age (years)*	53.4 (22-77)
Sex (female/male)	4/21
HBsAg (-/+)*	7/18
HBV DNA (</>1.0*10 ³ copies/ml)*	11/14
AST (IU/L)*	156.88(24-1608)
ALT (IU/L)*	135.46(16-1491)
AFP (ng/ml) *	18494.5(0-242611)
Cirrhosis (presence/absence)**	15/10

Table S2. Primer sequences used for clone and site-specific mutagenesis

Gene	Primer Sequence (5'→3')
Id1Ad	Sense:CGGGATCCATGAAAGTCGCCAGTGGCAGC Anti-sense:CCCAAGCTTTCAGCGACACAAGATGCGATCG
pcDNA3.1-Id1	Sense:TTAGGATCCATGAAAGTCGCCAGTGGCAGC Anti-sense:ATTCTCGAGTCAGCGACACAAGATGCGATCG
E2F4Ad/ pcDNA3.1-E2F4	Sense: ATTGGATCCATGGCGGAGGCCGGGCCACAG Anti-sense:TCCCTCGAGTCAGAGGTTGAGAACAGGCAC
pcDNA3.1-CLO CK	Sense:ATTGCTAGCATGTTGTTTACCGTAAGCTG Anti-sense:TCCCTCGAGCTACTGTGGTTGAACCTTGG
pcDNA3.1-TCF3	Sense:ATTGGATCCATGAACCAGCCGCAGAGGAT Anti-sense:TCCTCTAGATCACATGTGCCCGGCGGGGT
pcDNA3.1-E40	Sense:TTAGAATTCATGGAGCGGATCCCCAGCGC Anti-sense:TGCTCTAGATTAGTCTTTGGTTTCTAAG
pcDNA3.1-USF1	Sense:ATCGGATCCATGTACAGGGTGATCCAGGTG Anti-sense:TGCTCTAGATTAGTTGCTGTCATTCTTG
pGEX-E2F4(GS T-E2F4)	Sense:ATTGGATCCATGGCGGAGGCCGGGCCACAG Anti-sense:TCCCTCGAGTCAGAGGTTGAGAACAGGCAC
pET-Id1(Id1-His)	Sense:CGGGATCCATGAAAGTCGCCAGTGGCAGC Anti-sense:CCCAAGCTTTCAGCGACACAAGATGCGATCG
pEGFP-E2F4	Sense:TCAGATCTCGAGCTCAAGCTTGCCACCATGGCGGA GGCCGGGCCACAG Anti-sense:CATGGTGGCGACCGGTGGATCCCGGAGGTTGA GAACAGGCAC

pMcherry-Id1	Sense:TCAGATCTCGAGCTCAAGCTTGCCACCATGAAAGT CGCCAGTGGCAGC Anti-sense:CATGGTGGCGACCGGTGGATCCCGGCGACACA AGATGCGATCG
pET-E2F4 ₁₋₁₈₀	Sense:ATTGGATCCATGGCGGAGGCCGGGCCACAG Anti-sense:TCCAAGCTTCTGGTACTTCTTCTGCCC
pcDNA3.1-E2F4 ₁₋₁₈₀	Sense:ATTGGATCCATGGCGGAGGCCGGGCCACAG Anti-sense:TCCCTCGAGCTGGTACTTCTTCTGCCC
pGL3-Cp Δ site1	Sense:GAACGCCACCGAATAAGGTCTTACATAAG Anti-sense:CTTATGTAAGACCTTATTCGGTGGGCGTTC
pGL3-Cp Δ site2	Sense:GGAGGAGATTAGATTTGTAAGGAGGCTG Anti-sense:CAGCCTCCTAGTACAAATCTAATCTCCTCC
pGL3-Cp Δ site3	Sense:TGTAGGCATAAATTGACCAGCACCATGCAAC Anti-sense:GTTGCATGGTGTGCTGGTCAATTTATGCCTACA
HBV1.3 mut	Sense:GGAGATTAGATTAAATTTCTTTGTAAGGAG Anti-sense:CTCCTAGTACAAAGAAATTTAATCTAATCTCC
Cp(for EMSA)	Sense:TCACCTCTGCACGTTCGCATG Anti-sense:AGTTGCATGGTGTGCTGGTGCG
Biotin-Cp(for EMSA)	Sense:5'-Biotin-TCACCTCTGCACGTTCGCATG Anti-sense:AGTTGCATGGTGTGCTGGTGCG
Control-DNA-biotin(for SPR)	Sense:5'-Biotin-ATGATGTCACCACAAGAAGT Anti-sense:ACTTCTTGTGGTGACATCAT
Biotin-Cp2(for SPR)	Sense:5'-Biotin-TTAGATTAAAGGTCTTTGT Anti-sense:ACAAAGACCTTTAATCTAA
Cp2(for ITC)	Sense:TTAGATTAAAGGTCTTTGT Anti-sense:ACAAAGACCTTTAATCTAA
Cp2-mut(for ITC)	Sense:TTAGATTAAATTTCTTTGT Anti-sense:ACAAAGAAATTTAATCTAA

Table S3. Target sequences of shRNAs.

Name of shRNAs	Target sequence (5'-3')
shId1-1	Sense:GGCGAGATCAGCGCCCTGA Anti-sense:TCAGGGCGCTGATCTCGCC
shId1-2	Sense:CGGCATGCGTTCCTGCGGA Anti-sense:TCCGCAGGAACGCATGCCG
shE2F4-1	Sense:CGGCGGATTTACGACATTA Anti-sense:TAATGTTCGTAAATCCGCCG
shE2F4-2	Sense:CTCACGTCCAAATAGTCCT Anti-sense:AGGACTATTTGGACGTGAG
siE2F4Ad	Sense:CGGCGGATTTACGACATTA Anti-sense:TAATGTTCGTAAATCCGCCG

Table S4. Primer sequences used for qPCR.

Gene	Primer Sequence (5'→3')
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Id1	Sense: TGTTACTCACGCCTCAAGG Anti-sense: AACTGAAGGTCCCTGATGTAG
p16(CDKN2A)	Sense: ACCAGAGGCAGTAACCATGC Anti-sense: AAGTTTCCCGAGGTTTCTCAG
p21(CDKN1A)	Sense: TGTCCGTCAGAACCCATGC Anti-sense: AAAGTCGAAGTTCCATCGCTC
p53(TP53)	Sense: GGAAATTTGCGTGTGGAGTATTT Anti-sense: GTTGTAGTGGATGGTGGTACAG
NFκB-P50	Sense: CAAGGACATGGTGGTCGGCTTC Anti-sense: CGCCTCTGTCATTCGTGCTTCC
NFκB-P65	Sense: TGATAGCGACTCGGACTCTGAAGG Anti-sense: CAAGTGAGGTCAAGAGGCGTGTG
E2F1	Sense: GCTGGACCACCTGATGAATATC Anti-sense: GTCTGCAATGCTACGAAGGT
E2F3	Sense: TGTCAGAAAGAGACTGAAACAC Anti-sense: GCTATGTCCTGAGTTGGTTGAAG
E2F5	Sense: GGCTGCTCACTACCAAGTTC Anti-sense: TGCCTCACAGCCAAAGTATC
E2F8	Sense: GTGTCAACGCCTCAGATAGTAAG Anti-sense: GATCCAGGCTACTCAGAACATTAG
ZEB2	Sense: TTTCAGGGAGAATTGCTTGA Anti-sense: CACATGCATACATGCCACTC
SNAIL1	Sense: CTCTTTCCTCGTCAGGAAGC Anti-sense: GCTGGAAGGTAAACTCTGGATTA
HNF6a	Sense: CACAGATGTCCAGCGTCGAACTC Anti-sense: TCCTGCCACTTGTCCAGACTCC
PPARα	Sense: TCCTCGGTGACTTATCCTGT Anti-sense: GCGTGGACTCCGTAATGATAG
RXRα	Sense: CATCGTCCTCTTTAACCCTGAC Anti-sense: GGGTACTTGTGCTTGCAGTA
TR2(NR2C1)	Sense: CCTCCAGACTGCTGTTCTTATC Anti-sense: CTGGGCAAGACCAAGAGTAAA
CREB2(ATF4)	Sense: GGAGATAGGAAGCCAGACTACA Anti-sense: GGCTCATACAGATGCCACTATC
SOX7	Sense: TCAGCAAGATGCTGGGAAAG Anti-sense: CGGCCGGTACTTGTAGTTG
HNF4a	Sense: CCTACCTCAAAGCCATCATCTT Anti-sense: GTCGTTGATGTAGTCCTCCAAG
VPS4b	Sense: GCACTGAAAGAGGCTGTGATA Anti-sense: CCAGGCGGCCCAAATAATA
ARNT	Sense: CTAAGAGACAGCTTCCAACAGG Anti-sense: GTTCTCATCCAGAGCCATTCTT
ARNT2	Sense: TTTGGACCACCTTCTCTAAAC Anti-sense: GTACAGTGGACCACAGCATATT

HIF1a	Sense:GTCTGCAACATGGAAGGTATTG Anti-sense:GCAGGTCATAGGTGGTTTCT
E40(BHLHE40)	Sense:GACAGCAAGGAGACCTACAAAT Anti-sense:GTTTGAGATGTTCCGGGTAGGAG
Hes1	Sense:CGACACCGGATAAACCAAAG Anti-sense:CGCGAGCTATCTTTCTTCAGAG
Hes6	Sense:GACCTGTGCTCCGACCTG Anti-sense:CAGACACTCCGGGCAATTTG
Hey1	Sense:CGACGAGACCGGATCAATAAC Anti-sense:GCAGATCCCTGCTTCTCAA
MXD1	Sense:GACTCCGACAGGGAAGAAATC Anti-sense:GCTGGAATAGCCCTCATCAC
MXL	Sense:GATTCAGAGCGAGAGGAGATTG Anti-sense:CACTGATGCTGGTGGTACTTAT
NOCA3	Sense:CGGCAGAATGGAACCTATGAA Anti-sense:GAAGAGGCAATGTGGGAATAGAG
NPAS2	Sense:CACAGAGCACCTCCAATCATAG Anti-sense:CTCCAGGTCATCAATGTGGTAG
Clock	Sense:CTCTACTCATCTGCTGGAAAGTG Anti-sense:ATGGCTCCTTTGGGTCTATTG
TCF3	Sense:CCCGGATCACTCAAGCAATAAC Anti-sense:GTCGTAGCTGGGCGATAAGGC
USF1	Sense:CAGGGCTCAGCATAATGAAGT Anti-sense:TCCATAGAGCAGTCTGGGATT
USF2	Sense:CGTCCAGTGTGGGAGATACT Anti-sense:TGTTCCCTGTCTGAAGCACATC
Rb1	Sense:CTGTCTGAGCACCCAGAATTAG Anti-sense:GTCCAAATGCCTGTCTCTCAT
SR-1(SREBF1)	Sense:CACTGAGGCAAAGCTGAATAAAT Anti-sense:TAGGTTCTCCTGCTTGAGTTTC
pgRNA	Sense:GCCTTAGAGTCTCCTGAGCA Anti-sense:GAGGGAGTTCTTCTTCTAGG
HBV DNA	Sense:TGCGGCGTTTTATCATATTCC Anti-sense:ATACCTTGGTAGTCCAGAAGAACCA
HBV cccDNA	Sense:GTGCACTTCGCTTCACCTCT Anti-sense:AGCTTGGAGGCTTGAACAGT
GAPDH	Sense:GTATGACAACGAATTTGGCTACAG Anti-sense:TGAGGGTCTCTCTCTTCTCTTGT
β -actin	Sense:CCTGGCACCCAGCACAAT Anti-sense:GCCGATCCACACGGAGTA
CA2(Cyclin A2)	Sense:TTATTGCTGGAGCTGCCTTT Anti-sense:CTCTGGTGGGTTGAGGAGAG
Cp(for CHIP)	Sense: TCACCTCTGCACGTCGCATG Anti-sense:AGTTGCATGGTGCTGGTGCG

Table S5. Sequence of E2F4₁₋₈₈

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ATGGCGGAAGCGGGCCCGCAGGCGCCGCCGCCGGGCACCCCGAGCC
GTCACGAAAAAAGCCTGGGCCTGCTGACCACCAAATTCGTTAGCCTGCTG
CAGGAAGCGAAAGATGGCGTTCTGGATCTGAAACTGGCGGCGGATAACCT
GGCGGTTCGTCAGAAACGTCGTATCTACGATATCACCAACGTTCTGGAAGG
CATCGGTCTGATCGAAAAGAAAAGCAAAAACAGCATCCAGTGGAAAGGCG
TTGGTCCGGGCTGC
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Supplementary figures

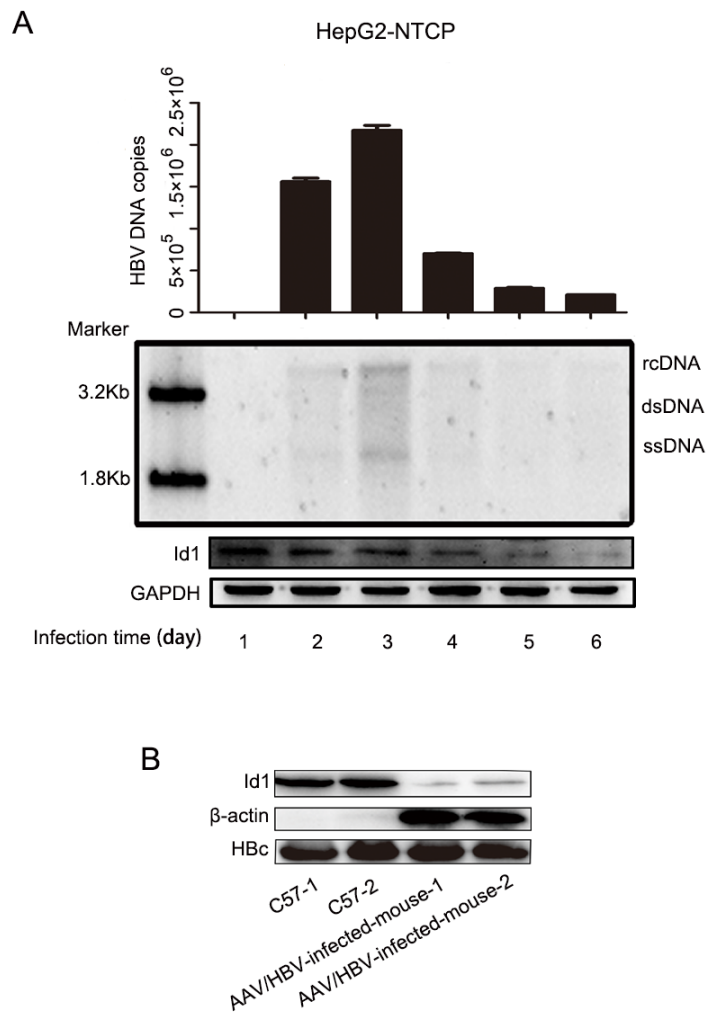


Figure S1: (A) 3.5×10^5 Cells infected with 800 HBV particles/cell were subjected to qPCR, southern blotting and western blotting at 1-6 day s post infection. In all conditions, 4% PEG8000 was added in the inoculum for promoting HBV infection, then washed out 24 hours post-infection. **(B)** Expression of Id1 in liver of **AAV/HBV-infected mice**. Livers were extracted from C57 black/6J (no HBV replication) or **AAV/HBV-infected mice** (HBV replication) and subjected to Western blotting with β -actin used as the control gene.

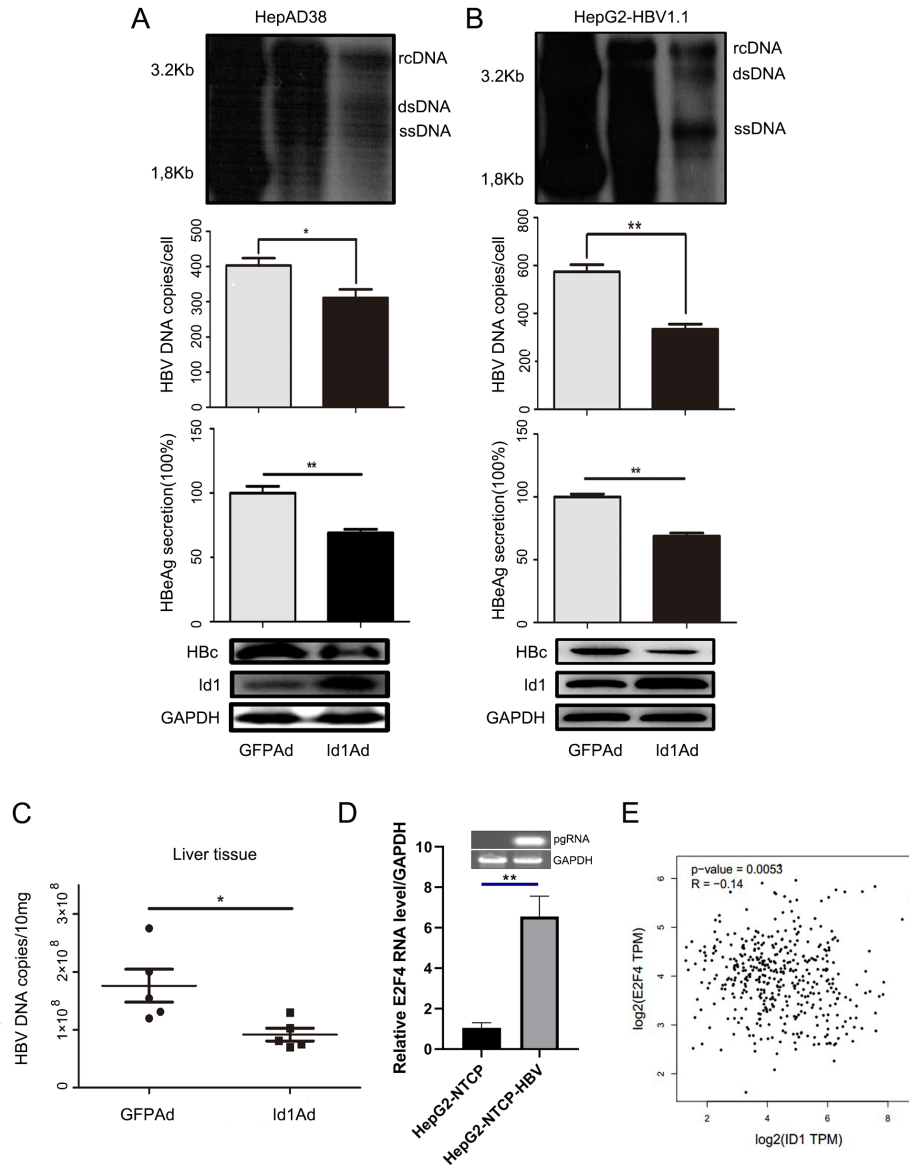


Figure S2: (A-B) Detection of inhibition of Id1 on HBV transcription and replication in HepAD38 and HepG2-HBV1.1 cells. Intracellular HBV DNA was extracted and subjected to qPCR and Southern blotting 72 h after addition of Id1Ad to the cells. HBeAg and HBc protein were detected by ELISA and western blotting, respectively. GAPDH was used as a loading control. GFPAd was used as control adenovirus. **(C)** Intracellular HBV DNA extracted from 10 mg liver tissue of **AAV/HBV-infected mice** was analyzed by absolute quantification PCR. **(D)** Enhancement of E2F4 mRNA when HepG2-NTCP being infected with HBV was detected. **(E)** The correlation coefficient between E2F4 and Id1 in LIHC tumour and normal from GEPIA database was visualized by Spearman Analysis.

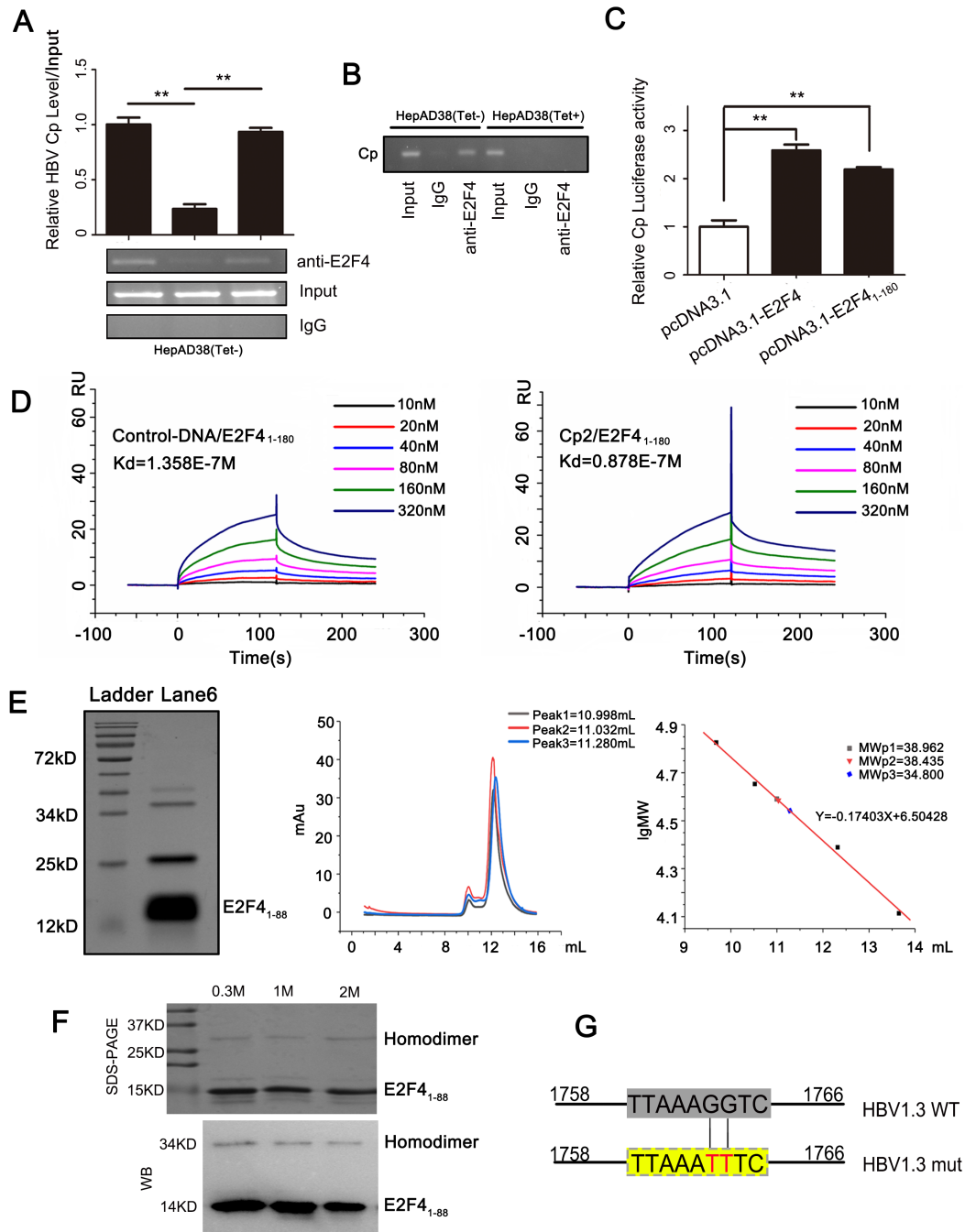


Figure S3: (A) ChIP assays were performed to confirm the interaction between E2F4 and HBV Cp in HepAD38 cells. (B) ChIP assays were performed to confirm the interaction between E2F4 and HBV Cp in HepAD38 cells with or without tetracycline. (C) Increase in the luciferase activity of Cp induced by E2F4₁₋₁₈₀ was slightly lower than E2F4 full-length protein, which revealed that E2F4₁₋₁₈₀ retained the ability to recognize and bind Cp DNA fraction. (D) Interaction of HBV core promoter with truncated E2F4₁₋₁₈₀ protein by SPR-binding analysis. E2F4₁₋₁₈₀ protein was injected

over a sensor chip immobilised with biotinylated-Cp DNA (bottom) or biotinylated-control DNA (top) as random control, and real-time binding profiles for increasing concentrations of E2F4₁₋₁₈₀ protein (black: 10 mM, red: 20 mM, light blue: 40 mM, pink: 80 mM, green: 160 mM, dark blue: 320 mM) were assessed. **(E-F)** Truncated E2F4₁₋₈₈ protein was purified. The purity of E2F4₁₋₈₈ in the total elution protein was higher than 80%. Elution Profile of E2F4₁₋₈₈ protein. The protein was separated and eluted with 50 mM Tris-HCl pH8.0 and 0.3M/1M/2M NaCl on a Superdex 75 gel filtration column. **(G)** The graphical representation of nt1758-1766 of HBV1.3 wild-type and mutant plasmid.

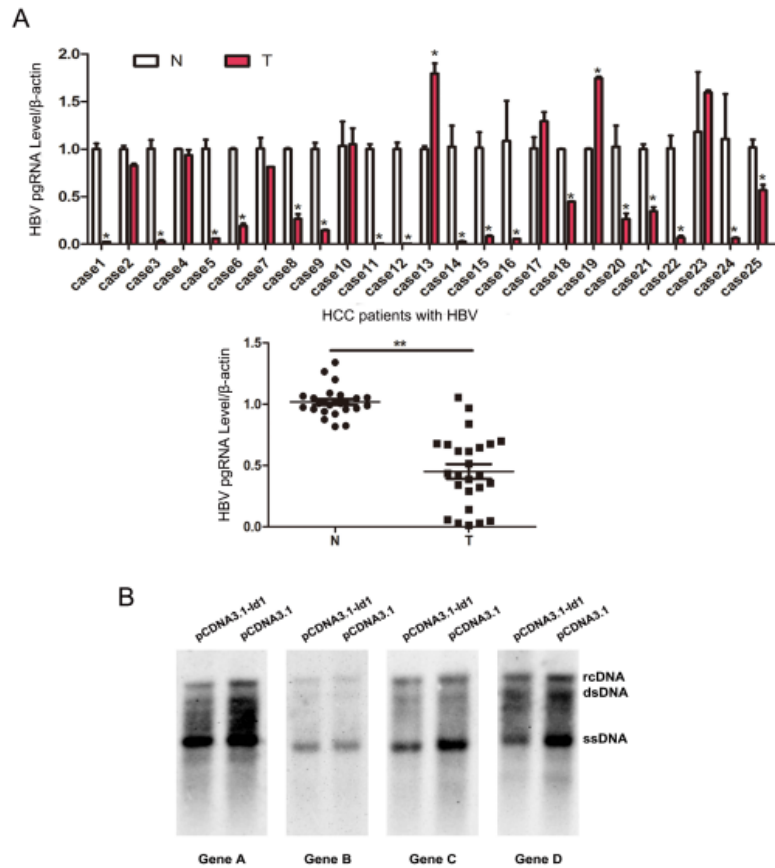


Figure S4: (A) The level of HBV pgRNA in tumor tissue (T) and adjacent non-tumor tissue (N) from 25 cases HBV-related HCC patients were tested by qPCR with β -actin used as the control gene. The bottom bar graph is the corresponding statistical figure. Data are presented as the mean \pm SE of triplicate experiments. * $P < 0.05$, ** $P < 0.01$.

(B) Detection of suppression of pcDNA3.1-Id1 on HBV1.1 genotype A/B/C/D by Southern blotting with pcDNA3.1(+) vector used as control plasmid (NC).