Supplementary Information

Supplementary Table 1. Primer sequences used for qPCR		
HSP27 (HSPB1)	Forward	5 '- AAGGATGGCGTGGTGGAGA-3'
	Reverse	5'- GGGAGGAGGAAACTTGGGTG-3'
HSP40 (DNAJB1)	Forward	5'- AGGGCTTTCGTACTGCTGA-3'
	Reverse	5'- GAGGGATCAAGTCCATCTGC-3'
HSP60 (HSPD1)	Forward	5'- TTTATTGGATGCTGCTGGTG-3'
	Reverse	5'- CTGCCTTGGGCTTCCTGTC-3'
HSP70 (HSPA1A)	Forward	5'- CCACCATTGAGGAGGTAGATTAG-3'
	Reverse	5'- CTGCATGTAGAAACCGGAAAA-3'
HSC70 (HSPA8)	Forward	5' –GGTGGTTCTACTCGTATCCCCA-3'
	Reverse	5'-AGTGACATCCAAGAGCAGCAA-3'
HSP90 (HSP90AA1)	Forward	5'- GAGATAAACCCTGACCATTCCA-3'
	Reverse	5'- TTCCAGACTGAAGCCAGAAGAC-3'
HSP105 (HSPH1)	Forward	5'- TGCCTTGGTAACTTTCAGATTC-3'
	Reverse	5' - GAAAACTACCTTGGCAGGAACA-3'
GRP78 (HSPA5)	Forward	5’- GACGGGCAAAGATGTCAGGAA-3’
	Reverse	5’- TCATAGTAGACCGGAACAGATCCA-3’
GRP94 (HSP90B1)	Forward	5’- TTTCCTATTTATGTATGGAGCAGC-3’
	Reverse	5’- TTCCCAGTCCCAGACAGTTTT-3’
PDI	Forward	5’- CAGGTGCTGTTCGTGGTGGT-3’
	Reverse	5’- TGGCTCAGGAGATAGGGCTTG-3’
PDIA3	Forward	5’- GGGTATCATCTTATTTCGTCC-3’
	Reverse	5’- TCATGTGAGGGCAGATACCAA-3’
Calreticulin	Forward	5’- CGAGCCTGCCGTCTACTTC-3'
	Reverse	5’- AACTGCACCACCAGCGTCT-3'
Calnexin	Forward	5’-TCAATTAGTGGGTTGATCTTCG-3’
	Reverse	5’- ATCAGTAGCATTCCCTCCTTTT-3’

pgRNA	Forward	5’- TGTTCAAGCCTCCAAGCT-3’
	Reverse	5’- GGAAAGAAGTCAGAAGGCAA-3’
IFN-β	Forward	5’- GACCAACAAGTGTCTCCTCCAAA -3’
	Reverse	5’- GAACTGCTGCAGCTGCTTAATC -3’
MxA	Forward	5’-CGTTAGCCGTGGTGATTTAG-3’
	Reverse	5’-ACACTGGGTTTGTGAAGGGA-3’
ISG56	Forward	5’-GTGGCATTCAAGGAGTACCTC-3’
	Reverse	5’-GCCTTCGATTCTGGATTCAGACA-3’
TRIM22	Forward	5’-ACCAAACATTCCGCATAAAC-3’
	Reverse	5’-GTCCAGCACATTCACCTCAC-3’
GAPDH	Forward	5’- GATTCCACCCATGGCAAATTCCA-3’
	Reverse	5’- TGGTGATGGGATTTCCATTGATGA-3’

Figure S1

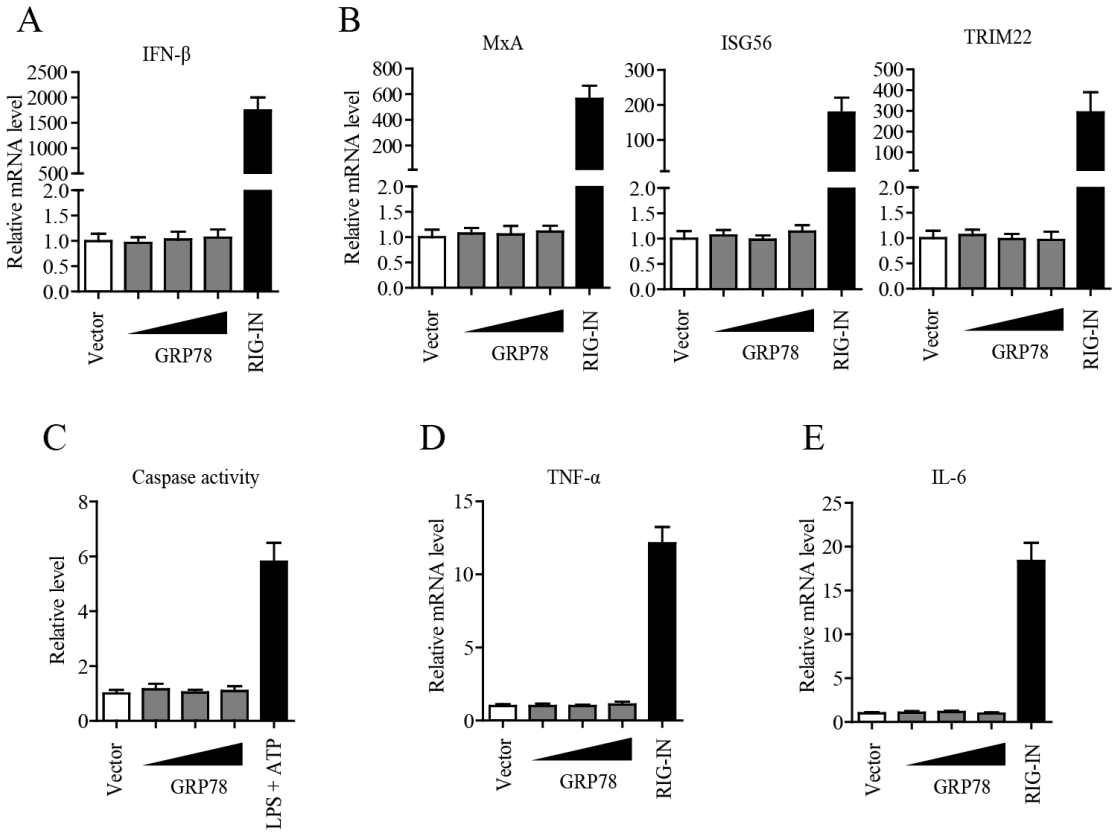

Fig. S1 GRP78 does not have significant effect on the activation of innate immune response in HepG2 cells. (A) HepG2 cells were cotransfected with pHBV1.3 and increasing amounts of GRP78 expression plasmid (pGRP78) or RIG-IN expression plasmid (pRIG-IN). 48 hours posttransfection, cells were collected and the level of IFN-β mRNA was tested by qRT-PCR. Data are mean ± SEM of 4 samples pooled from 3 independent experiments. **(B)** Cells were treated as in A, the mRNA level of MxA, ISG56 or TRIM22 was determined by qRT-PCR. Data are mean ± SEM of 4 samples pooled from 3 independent experiments. **(C)** HepG2 cells were cotransfected with pHBV1.3 and pcDNA or increasing amounts of pGRP78. 48 hours posttransfection, the caspase-1 activity was assayed by an enzymatic assay. Data are mean ± SEM of 3 samples pooled from 3 independent experiments. Cells stimulated with LPS (500 ng/ml) plus ATP (5mM) was used as a positive control for inflammasome activation. **(D and E)** HepG2 cells were treated as in A, mRNA levels of TNF-α (**D**) or IL-6 (**E**) were determined by qRT-PCR. Data are mean ± SEM of 4 samples pooled from 3 independent experiments.

Figure S2

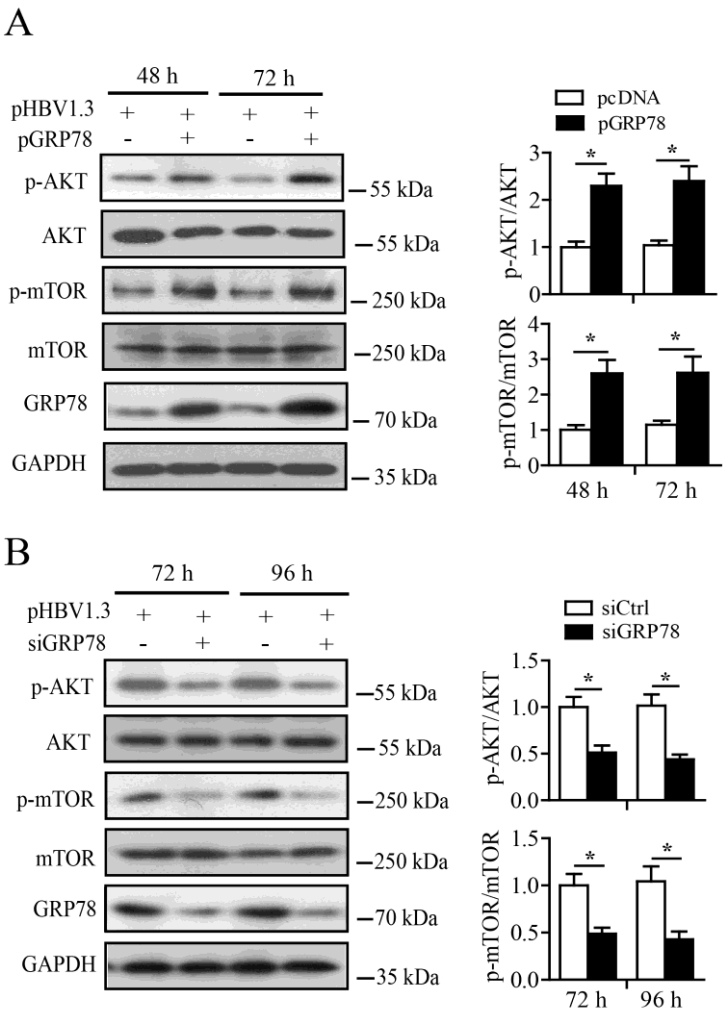

Fig. S2 GRP78 contributes to the activation of AKT/mTOR signaling in HBV-replicating Huh7 cells. (A) Huh7 cells were transfected with pHBV1.3 together with pGRP78 or pcDNA. 48 and 72 hours post-transfection, cells were subjected to western blot using antibodies against p-AKT, AKT, p-mTOR, mTOR, GRP78 and GAPDH. *Right panel:* Relative level of p-AKT to AKT or pmTOR to mTOR was examined by densitometric analysis, and the value from pcDNA-transfected cells was set at 1.0. Data are mean ± SEM of 4 samples pooled from 3 independent experiments. **P* < 0.05. **(B)** Huh7 cells were transfected with transfected with siGRP78 or siCtrl, followed by pHBV1.3 transfection. 72 and 96 hours post-transfection, cells were subjected to western blot as in a. The value from control siRNA-transfected cells was set at 1.0. Data are mean ± SEM of 4 samples pooled from 3 independent experiments. **P* < 0.05.

Figure S3

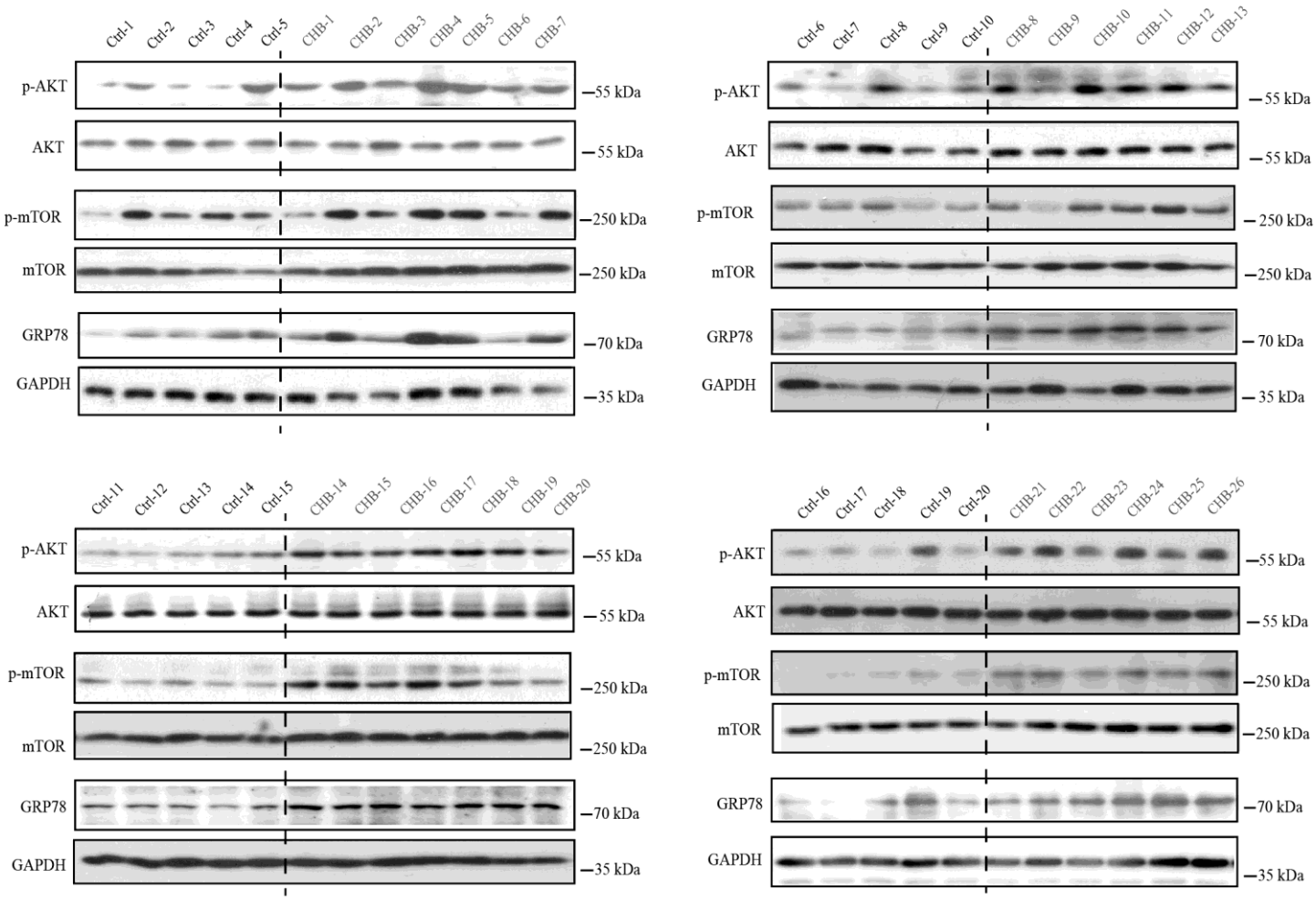

Fig. S3 The level of p-AKT, p-mTOR and GRP78 in the liver biopsies. Liver tissues from control individuals (n=20) and CHB patients (n=26) were subjected to western blot using antibodies against p-AKT, AKT, p-mTOR, mTOR and GRP78, respectively.

Figure S4

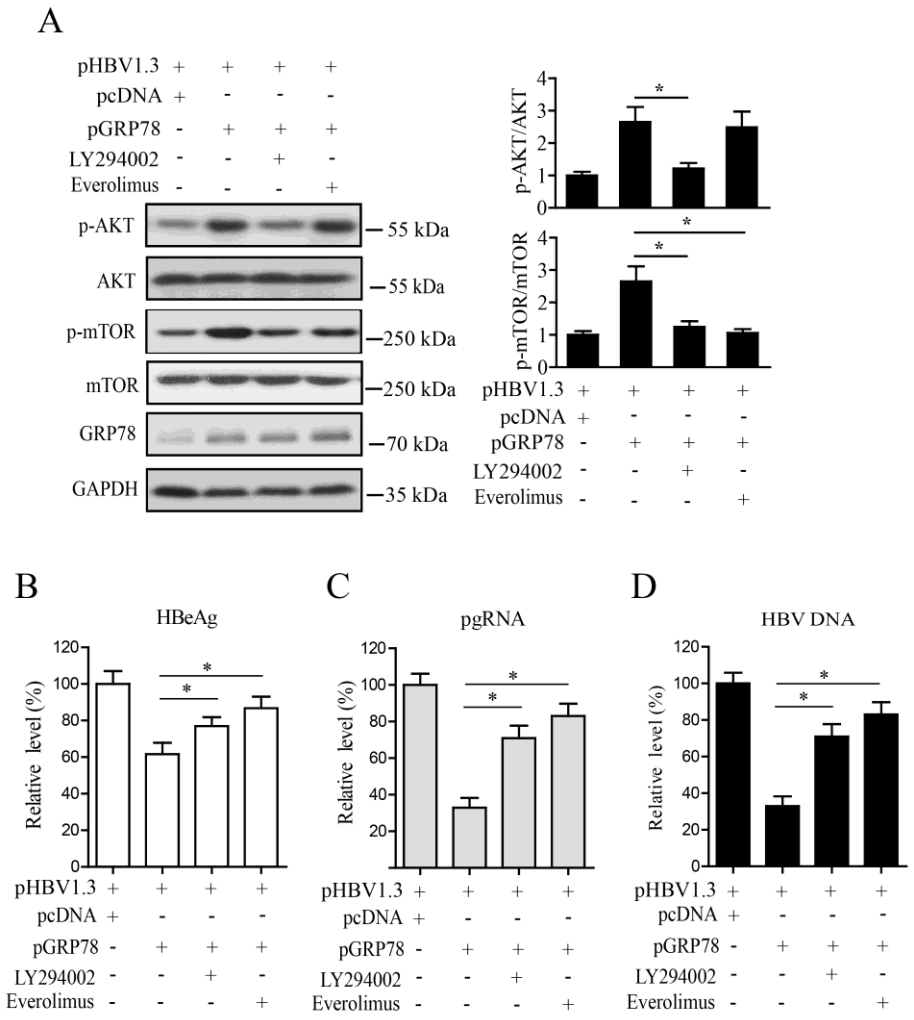

Fig. S4 AKT/mTOR signaling is involved the in GRP78-mediated inhibition of HBV transcription and replication in Huh7 cells. (A) Huh7 cells were cotransfected with pHBV1.3 and pGRP78 for 48 hours, followed by treatment with rapamycin (100 nM) or everolimus (100 nM) for another 24 hours, cells were then subjected to western blot using antibodies against p-mTOR, mTOR, p-p70S6K, p70S6K, GRP78 and GAPDH. *Right panel:* Relative level of p-mTOR to mTOR or p-p70s6K to p70s6K was examined by densitometric analysis, and the value from empty vector-transfected cells was set at 1.0. Data are mean ± SEM of 5 samples pooled from 3 independent experiments. **P* < 0.05. **(B-D)** Huh7 cells were treated as in a, levels of HBeAg (**B**), pgRNA (**C**) and HBV DNA (**D**) were determined by ELISA, qRT-PCR and qPCR, respectively. Data are mean ± SEM of 4 samples pooled from 3 independent experiments. **P* < 0.05.

Figure S5

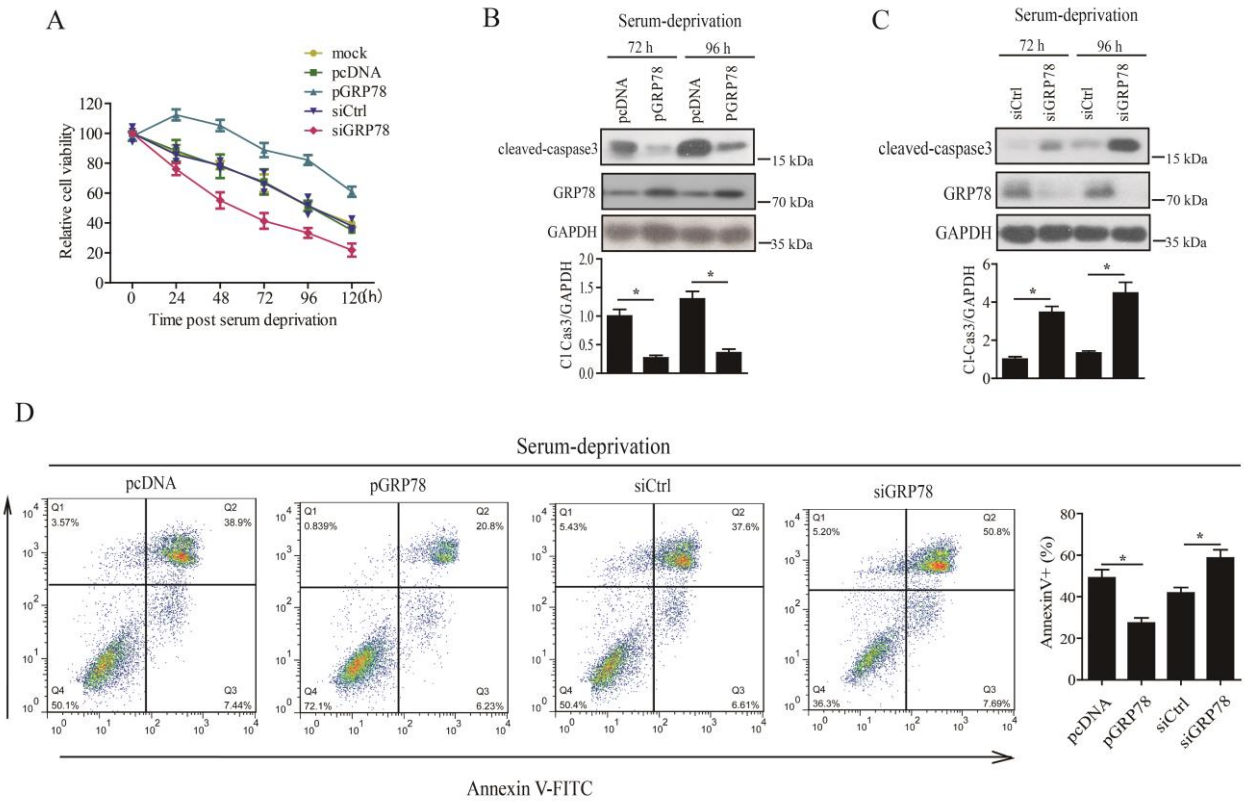

Fig. S5 GRP78 plays a protective role in HBV-replicating HepAD38 cells in response to serum deprivation. (A) GRP78 knockdown HepAD38 cells were subjected to serum starvation for 72 hours in the presence or absence of 4-PBA (5 mM), and the cell viability was then determined by CCK8 assay. Data are mean ± SEM of 5 samples pooled from 3 independent experiments. **(B)** HepAD38 cells with GRP78 overexpression were subjected to serum starvation for 72 or 96 hours, cells were then subjected to western blot using antibodies against cleaved-caspase3, GRP78 or GAPDH. *Lower panel:* Relative level of cleaved-caspase3 to GAPDH was examined by densitometric analysis, and the value from empty vector-transfected cells was set at 1.0. Data are mean ± SEM of 5 samples pooled from 3 independent experiments. **P*< 0.05. **(C)** GRP78 knockdown HepAD38 cells were subjected to serum starvation for 72 or 96 hours, cells were then subjected to western blot as in B Data are mean ± SEM of 4 samples pooled from 3 independent experiments. *Lower panel:* Relative level of cleaved-caspase3 to GAPDH was examined by densitometric analysis, and the value from siCtrl-transfected cells was set at 1.0. Data are mean ± SEM of 5 samples pooled from 3 independent experiments. **P*< 0.05. **(D)** GRP78 overexpression or knockdown cells were subjected to serum starvation for 72 hours,

and cells were then subjected to apoptosis analysis by FACS Data are mean ± SEM of 5 samples pooled from 3 independent experiments. *P< 0.05.

Figure S6

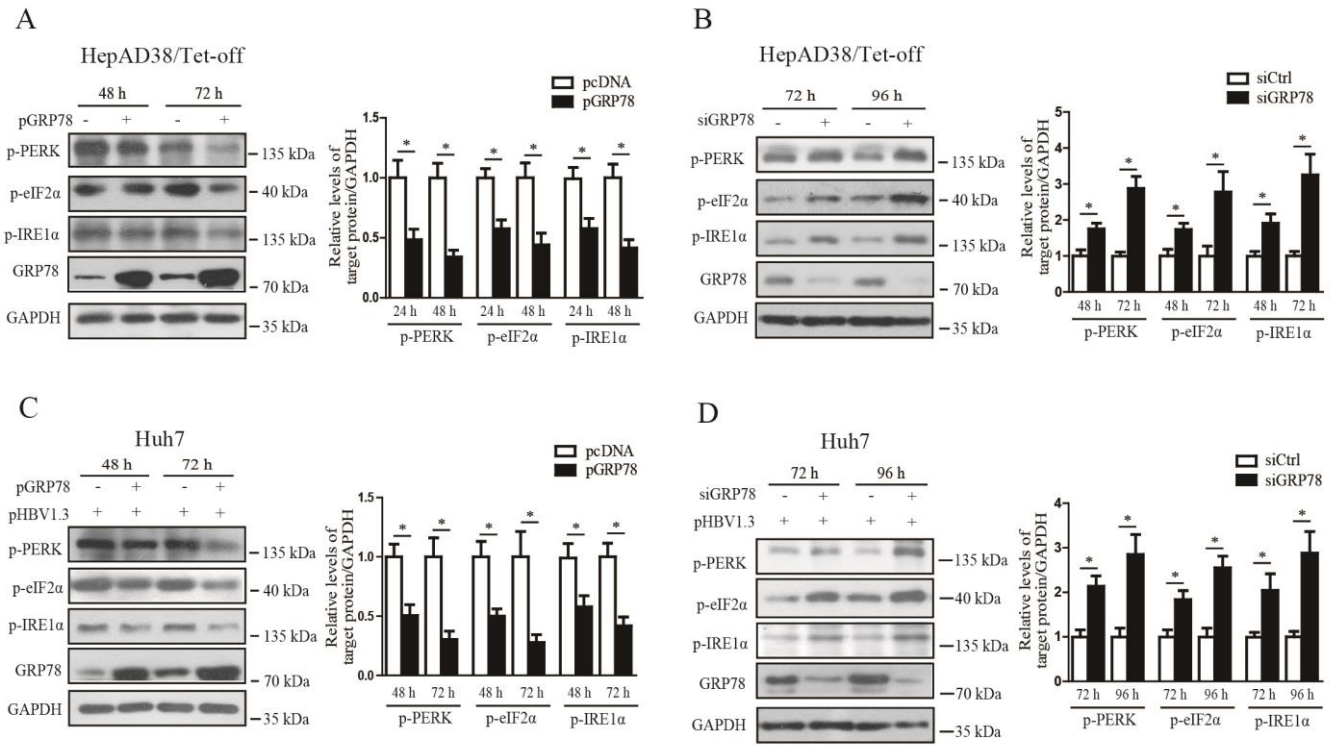

Fig. S6 GRP78 plays a crucial role in the regulation of ER stress in HBV-replicating cells. (A) HepAD38/Tet-off cells were electro-transfected with pGRP78 or pcDNA. 48 or 72 hours post-transfection, cells were subjected to western blot using antibodies against p-PERK, p-eIF2α, p-IRE1, GRP78 or GAPDH. *Right panel:* Relative level of p-PERK, p-eIF2α or p-IRE1 to GAPDH was examined by densitometric analysis, and the value from pcDNA-transfected cells was set at 1.0. Data are mean ± SEM of 4 samples pooled from 3 independent experiments. *P < 0.05. **(B)** HepAD38 cells were electro-transfected with siGRP78 or siCtrl. 72 and 96 hours post-transfection, cells were subjected to western blot as in A. **(C)** Huh7 cells were transfected with pHBV1.3 together with pGRP78 or pcDNA. 48 and 72 hours post-transfection, cells were collected and subjected to western blot as in A. **(D)** Huh7 cells were transfected with transfected with siGRP78 or siCtrl, followed by pHBV1.3 transfection. 72 and 96 hours post-transfection, cells were collected and subjected to western blot as in A.

Figure S7

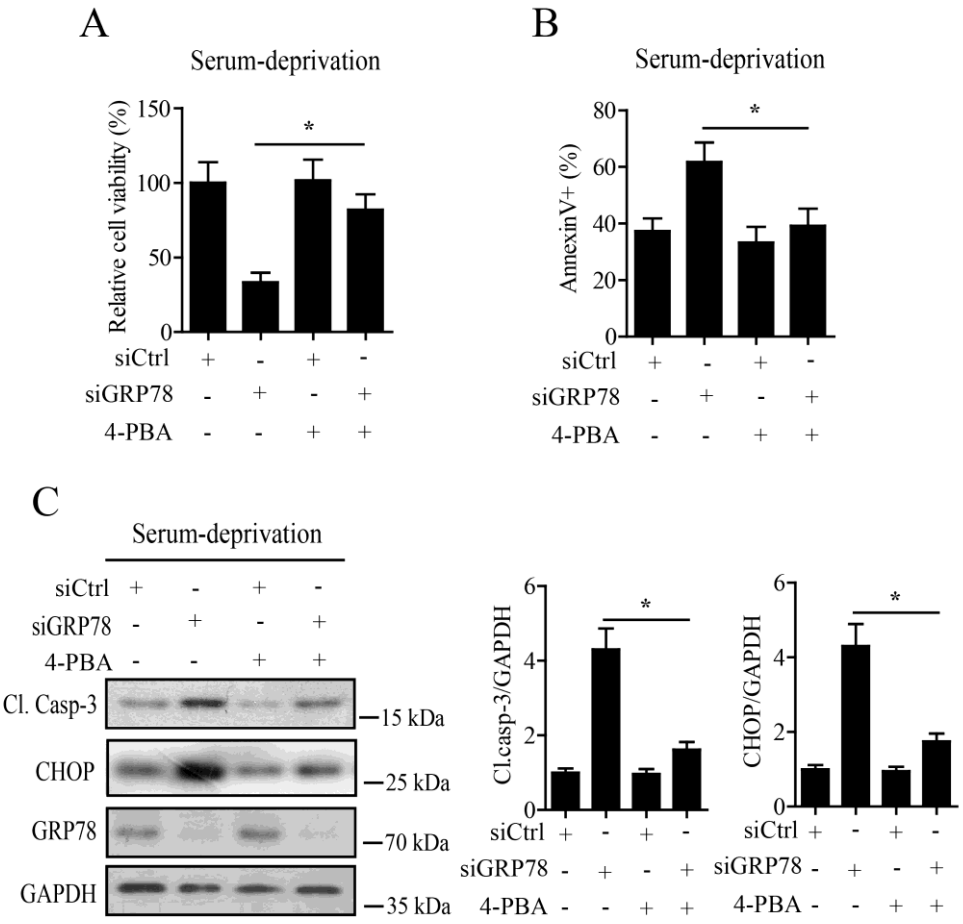

Fig. S7 Inhibition of ER stress by 4-PBA reverses serum starvation-induced cell death in GRP78-knockdown HepAD38 cells. (A) GRP78 knockdown HepAD38 cells or control cells were starved for 72 hours in the presence or absence 4-PBA (5 mM), and the cell viability was then determined by CCK8 test. Data are mean ± SEM of 5 samples pooled from 3 independent experiments. **P* < 0.05. **(B)** Cells were treated as in a, and then subjected to apoptosis analysis by FACS. Data are mean ± SEM of 5 samples pooled from 3 independent experiments. **P* < 0.05. **(C)** Cells were treated as in A, and then subjected to western blot using antibodies against cleaved-caspase3, CHOP, GRP78 or GAPDH. *Right panel:* Relative level of Cl. Casp-3 or CHOP to GAPDH was examined by densitometric analysis, and the value from control siRNA-transfected cells was set at 1.0. Data are mean ± SEM of 5 samples pooled from 3 independent experiments. **P* < 0.05.

Figure S8

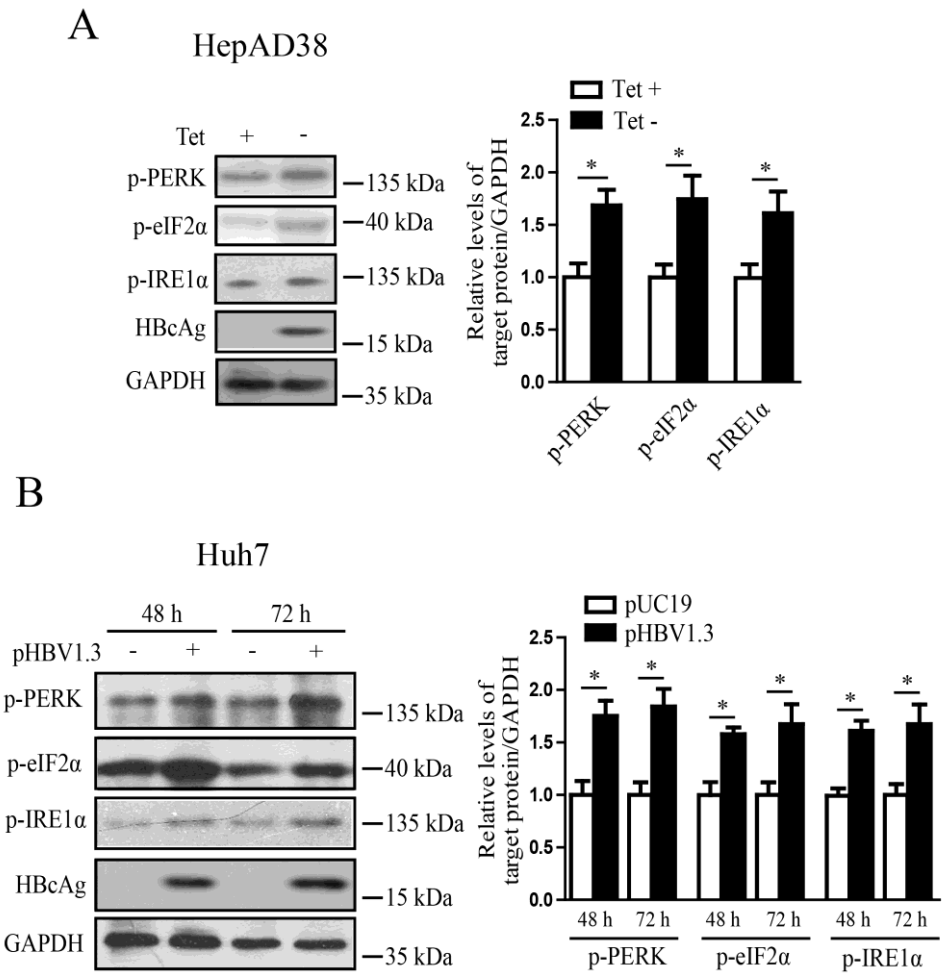

Fig. S8 HBV induces ER stress in hepatoma cells. (A) HepAD38 cells with or without tetracycline were subjected to western blot using antibodies against p-PERK, p-eIF2α, p-IRE1, HBc or GAPDH. *Right panel:* Relative level of p-PERK, p-eIF2α or p-IRE1to GAPDH was examined by densitometric analysis, and value from Tet-treated cells was set at 1.0. Data are mean ± SEM of 4 samples pooled from 3 independent experiments. **P* < 0.05. **(B)** Huh7 cells were transfected with pHBV1.3 or pUC19 for 48 and 72 hours, western blot was then performed as in a. *Right panel:* Relative level of p-PERK, p-IRE1 or p-eIF2α to GAPDH was examined by densitometric analysis, and the value from pUC19-transfected cells was set at 1.0. Data are mean ± SEM of 4 samples pooled from 3 independent experiments. **P* < 0.05.

Figure S9

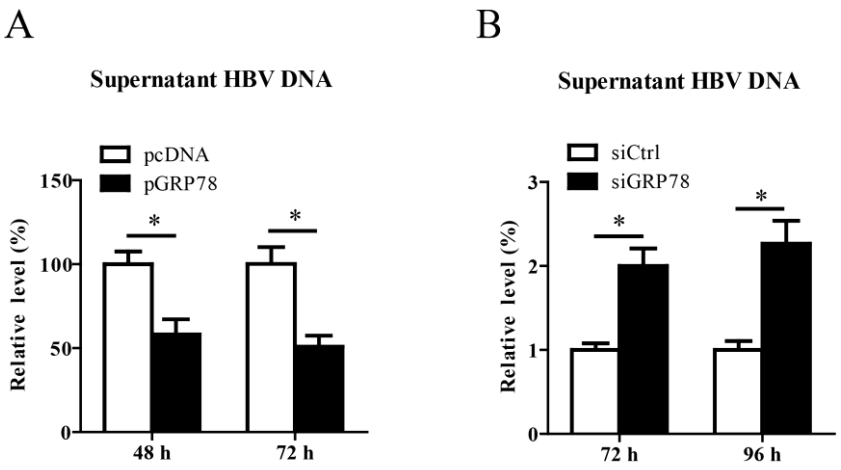

Fig. S9 The effect of GRP78 on the HBV DNA level in the culture supernatants of HepAD38 cells. (A) HepAD38 cells were electro-transfected with pGRP78 or pcDNA. 48 or 72 hours post-transfection, the cell culture supernatants were harvested and subjected to the quantification of HBV DNA by qPCR. Data are mean ± SEM of 4 samples pooled from 3 independent experiments. **(B)** HepAD38 cells were electro-transfected with GRP78-specific siRNA (siGRP78) or control siRNA (siCtrl). 72 or 96 hours post-transfection, the HBV DNA level in the cell culture supernatants was determined as in A. Data are mean ± SEM of 4 samples pooled from 3 independent experiments.