

Figure S1. HepAD38 cells were grown with tetracycline (Tet⁺), then the cells were incubated with medium with tetracycline (Tet⁺) or medium without tetracycline (Tet⁻, induction of HBV replication) at the indicated times. The cells were analyzed by immunoblot with the indicated antibodies (**A**). To determine the replication rate of HBV, the supernatant was collected per every 12 h as indicated (0-12 h, 12-24 h, 24-36 h, 36-48 h and 60-72 h), and were subjected to real-time PCR for analysis of HBV DNA (**B**).

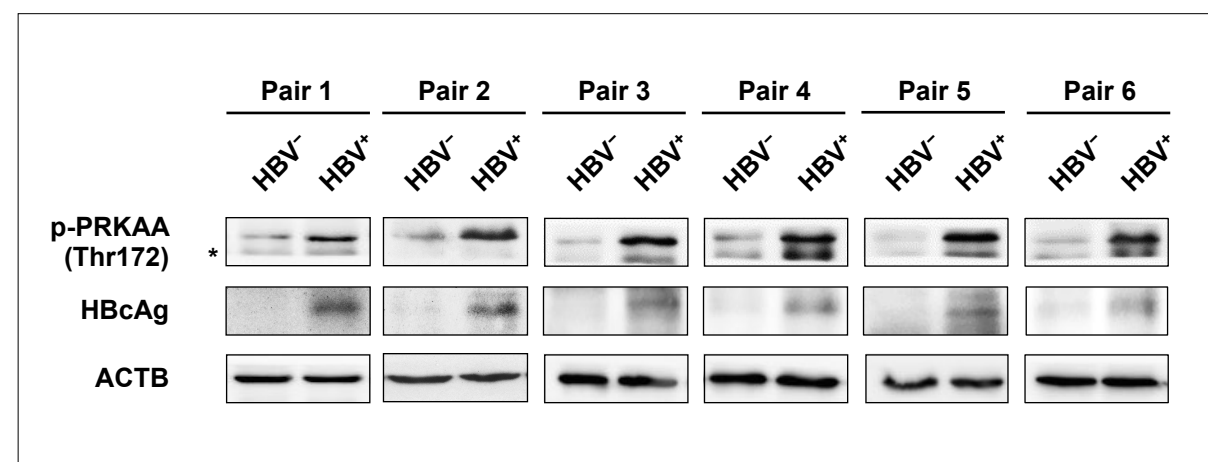


Figure S2. HBV-infected liver tissues have higher levels of PRKAA phosphorylation. The phosphorylation levels of PRKAA (Thr172) in HBV-infected (HBV⁺) and HBV noninfected (HBV⁻) liver samples were analyzed by immunoblotting and a representative blot is shown. *, nonspecific band.

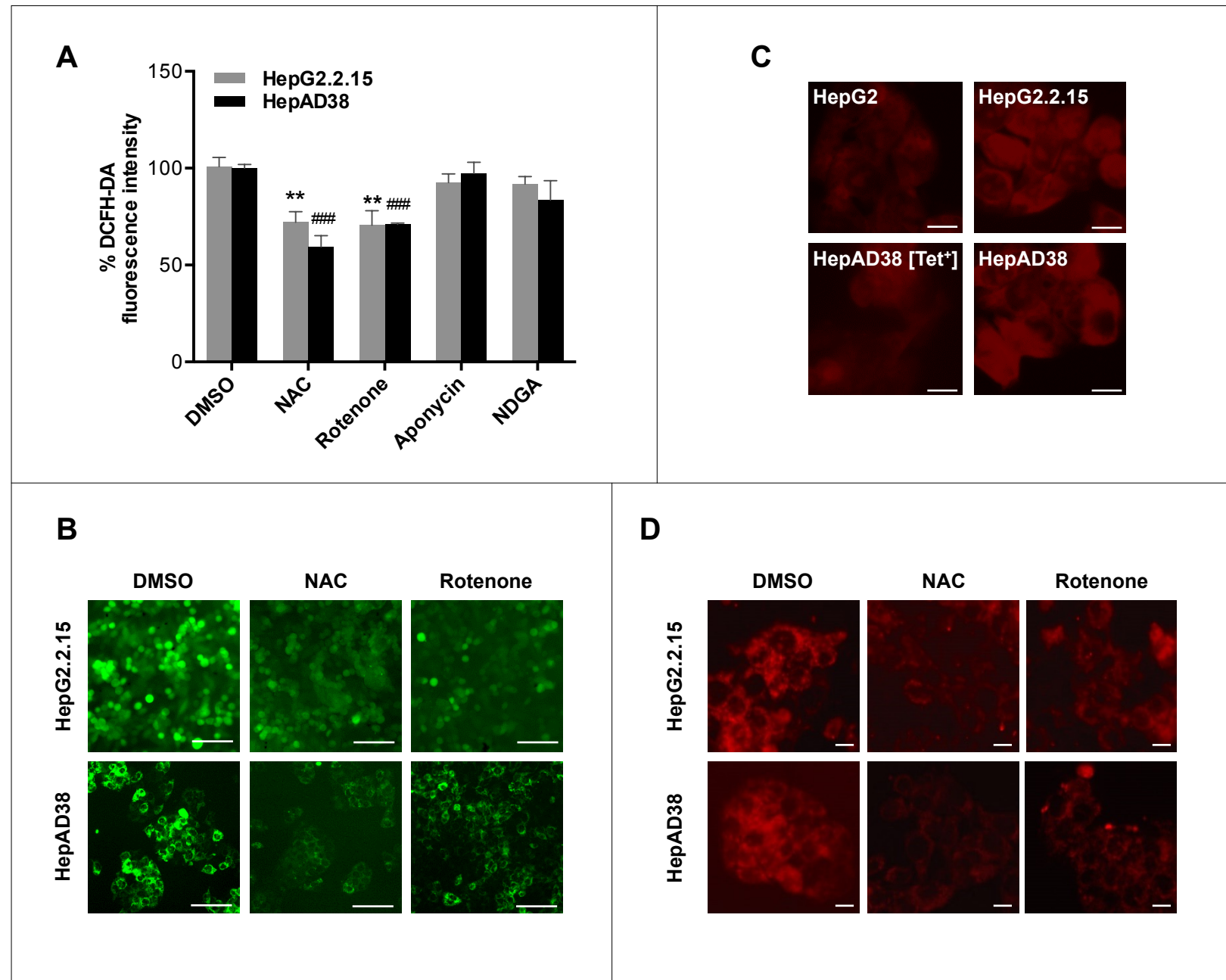


Figure S3. HBV replication induces the production of mitochondrial ROS. **(A)** HepG2.2.15 or HepAD38 cells were treated with NAC (10 mM), aponycin (10 μ M), NDGA (10 μ M) and rotenone (10 μ M) for 30 min, respectively. The ROS level was monitored with an oxidant-sensitive fluorescent probe, DCFH-DA, and results were given as mean \pm SD. **, $p < 0.01$ (in HepG2.2.15); ###, $p < 0.001$ (in HepAD38). **(B)** HepG2.2.15 or HepAD38 cells were treated with NAC (10 mM) or rotenone (10 μ M) for 30 min. The cellular ROS were stained with DCFH-DA and visualized using Thermo Scientific Cellomics HCS imaging platform. Scale bar: 100 μ m. **(C)** The mitochondrial ROS were stained with MitoSOX and visualized using Leica microscopy. Scale bar: 20 μ m. **(D)** HepG2.2.15 or HepAD38 cells were treated with NAC (10 mM) or rotenone (10 μ M) for 30 min. The mitochondrial ROS were labeled with MitoSOX and visualized using Thermo Scientific Cellomics HCS imaging platform. Scale bar: 20 μ m.

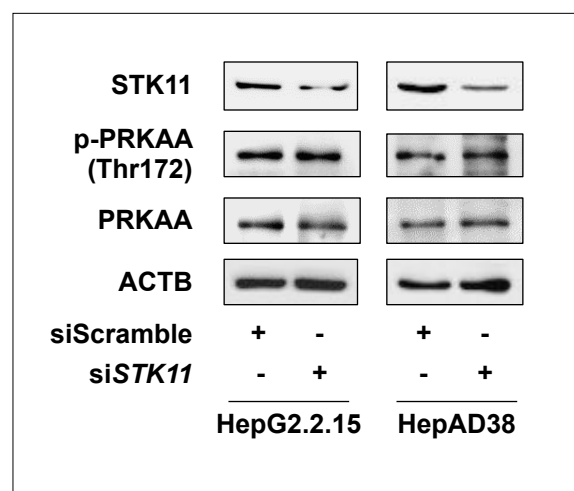


Figure S4. STK11 is not involved in the activation of PRKAA in HBV-producing cells. HepG2.2.15 or HepAD38 cells were transfected with siSTK11 or siRNA pools to a universal control (siScramble) for 48 h. Immunoblot analysis of total protein extracts from siRNA-transfected cells with the indicated antibody, respectively.

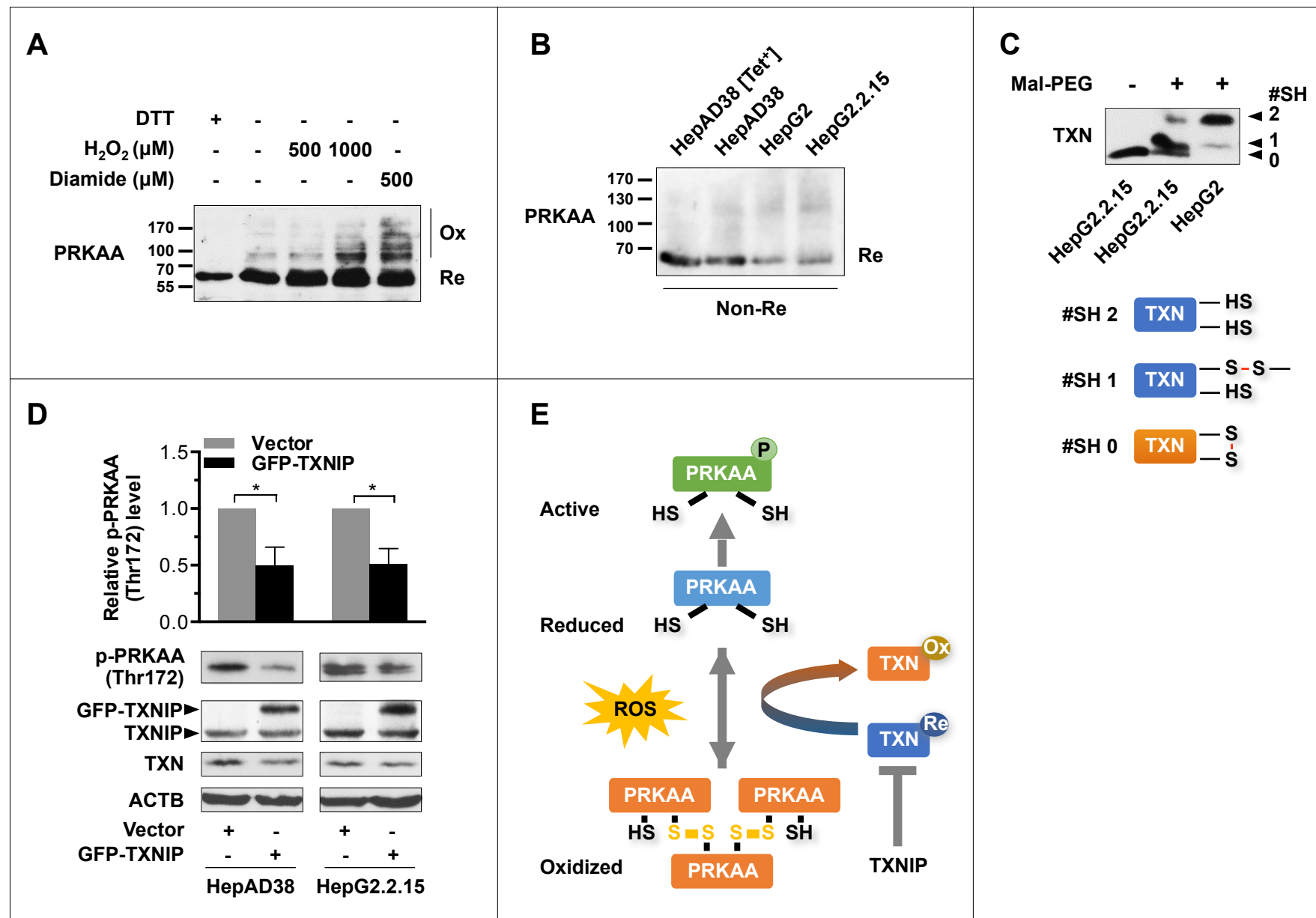


Figure S5. TXN/TXNIP is involved in ROS-mediated activation of PRKAA. (A) HepG2 cells were treated with H₂O₂ or diamide for 30 min and were analyzed by immunoblot with PRKAA antibody under reducing (dithiothreitol, DTT) or nonreducing conditions. (B) Cells were analyzed by immunoblot with PRKAA antibody as indicated under nonreducing conditions. (C) Cell lysates were incubated with or without Mal-PEG (1 mM) for 4 h before immunoblot analysis with the anti-TXN antibody. #SH indicates the apparent number of TXN sulfhydryls modified by Mal-PEG. (D) HepG2.2.15 or HepAD38 cells were transfected with control vector or pLVX-puro-TXNIP plasmid, followed by immunoblot analysis with antibodies as indicated. (E) A scheme depicting redox regulation of PRKAA by TXNIP. In response to oxidative stress, PRKAA forms oxidative aggregates through intermolecular disulfide bonds, which prevents its phosphorylation. TXN acts as an indispensable cofactor, allowing PRKAA activation by suppressing oxidation and aggregation of PRKAA, which could be reversed by TXNIP, a binding partner and inhibitor of TXN. Re, reduced; Ox, oxidized; Non-Re, nonreducing SDS-PAGE.

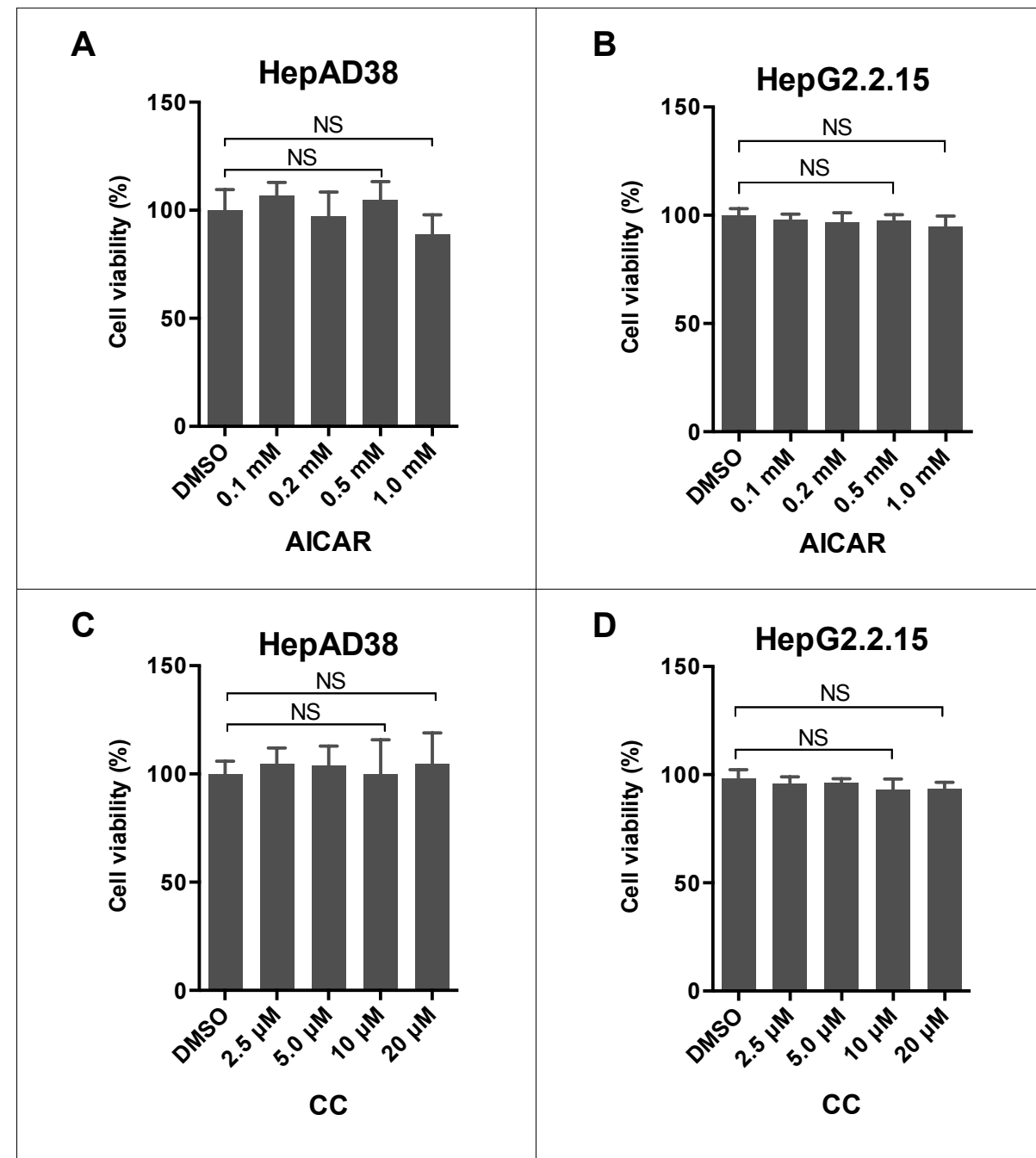


Figure S6. CC or AICAR have no obvious effect on cell viability. MTT assay-based cell viability analysis of cells treated with DMSO (0.1%), AICAR (**A**, **B**), or compound C (**C**, **D**), with the concentration as indicated for 24 h. The values obtained from control samples were set at 100%. Values are means \pm SD. $n=5$ per group. NS, nonsignificant by Student's t-test.

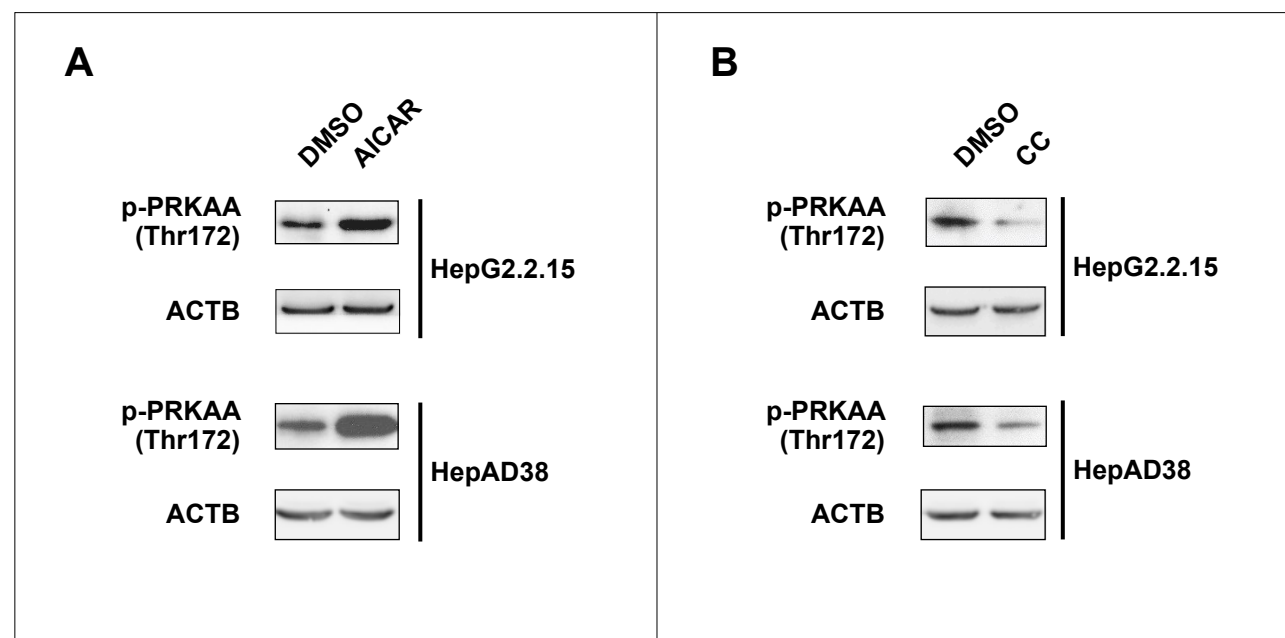


Figure S7. PRKAA agonist or inhibitor alters the phosphorylation levels of PRKAA. Immunoblot analysis of total protein extracts from HepG2.2.15 or HepAD38 cells treated with DMSO, 1 mM AICAR (**A**) or 10 μ M compound C (**B**) for 24 h.

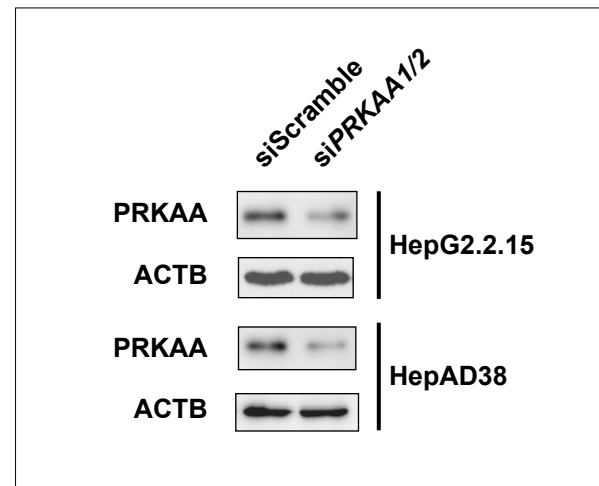


Figure S8. Validation of siRNAs against *PRKAA*. HepG2.2.15 or HepAD38 cells were transfected with si*PRKAA1/2* or siRNA pools to a universal control (siScramble) for 48 h. Immunoblot analysis of total protein extracts from siRNA pools-transfected with the indicated antibody.

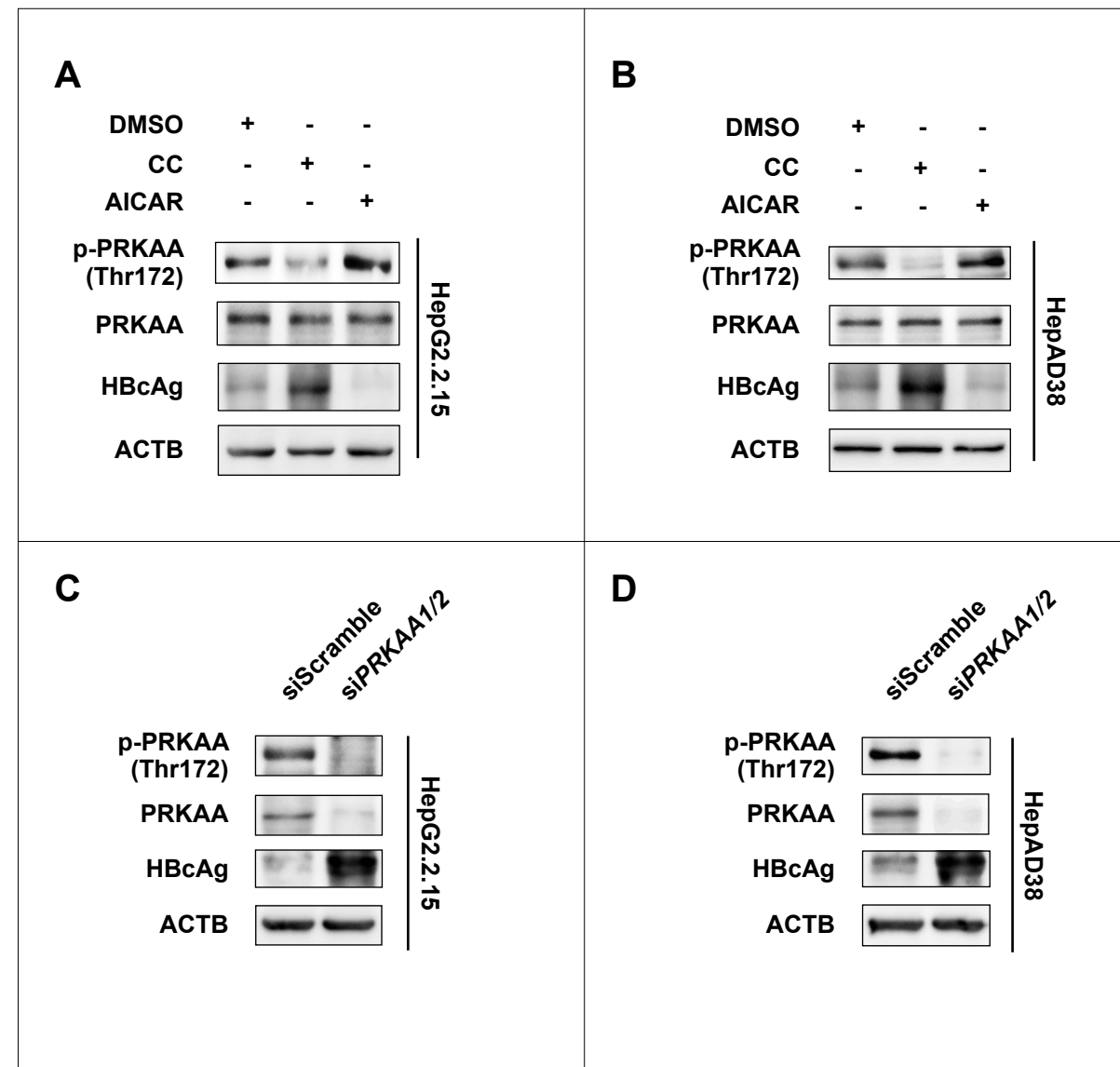


Figure S9. PRKAA alters the expression levels of HBcAg. HepG2.2.15 and HepAD38 cells were treated with DMSO, 1 mM AICAR or 10 μ M compound C for 24 h (**A**, **B**), or transfected with siScramble and siPRKAA1/2 for 48 h (**C**, **D**). The cell lysates were analyzed by immunoblot using the indicated antibodies.

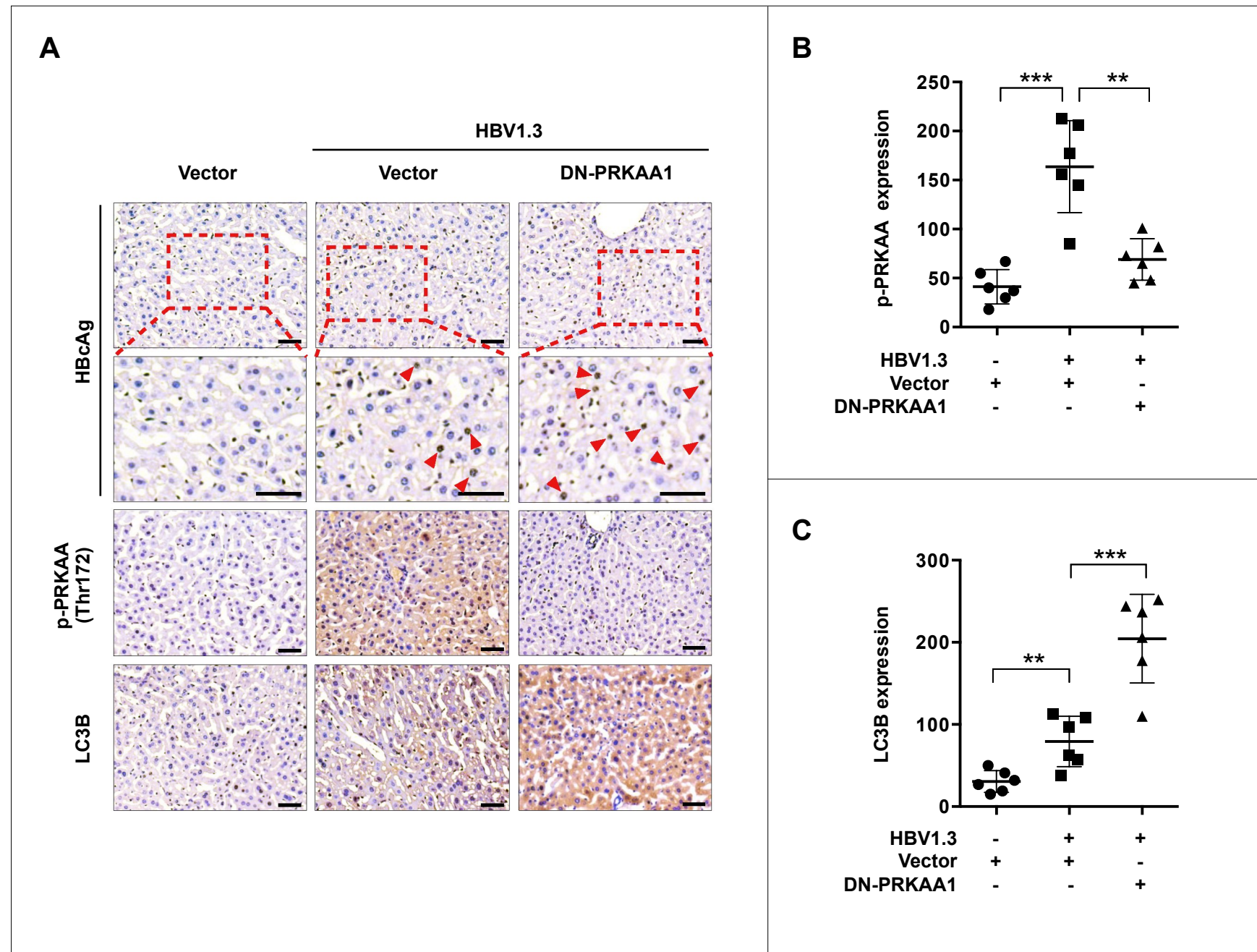


Figure S10. The expression levels of PRKAA and LC3B in the liver tissues of HBV-producing mice. (A) BALB/c mice were hydrodynamically injected with vector, HBV1.3 and vector, HBV1.3 and/or DN-PRKAA1. The expression levels of HBCAg, phosphorylated (p)-PRKAA (Thr172) and LC3B in the liver tissues were determined by immunohistochemical staining (A), and analyzed as described in Materials and Methods (B, C). Data are presented as mean \pm SD (n=6); **, $p < 0.01$; ***, $p < 0.001$. Red arrow indicates the HBCAg-positive liver cells. Scale bar: 20 μ m.

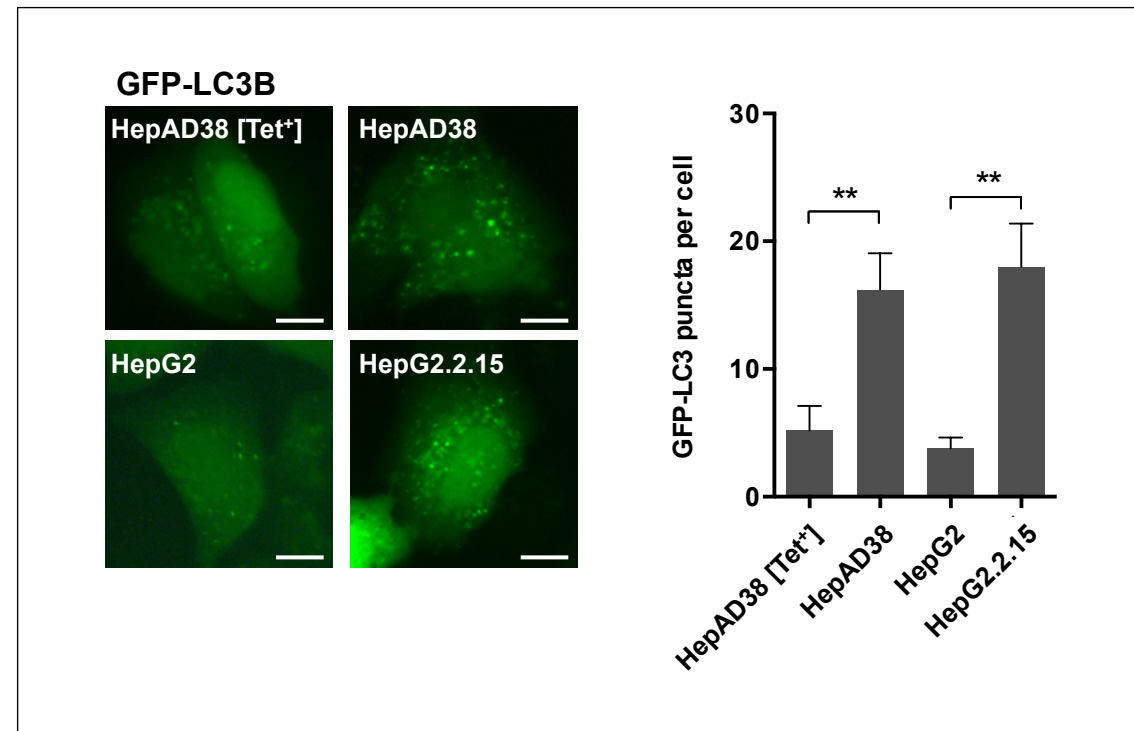


Figure S11. HBV replication promotes the accumulation of GFP-LC3B puncta. HepG2, HepAD38 [Tet⁺], HepG2.2.15 and HepAD38 cells were transfected with a plasmid encoding GFP-LC3B and examined by fluorescence microscopy. Scale bar: 10 μ m. The results are given as mean \pm SD. **, $p < 0.01$.

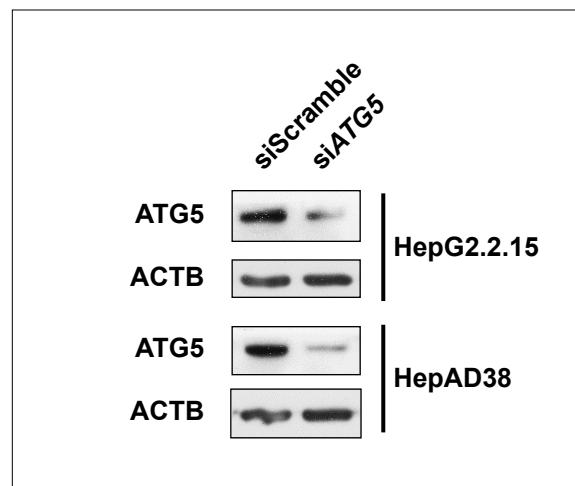


Figure S12. Validation of siRNAs against *ATG5*. HepG2.2.15 or HepAD38 cells were transfected with si*ATG5* or siRNA pools to a universal control (siScramble) for 48 h. Immunoblot analysis of total protein extracts from siRNA pools transfected with the indicated antibody.

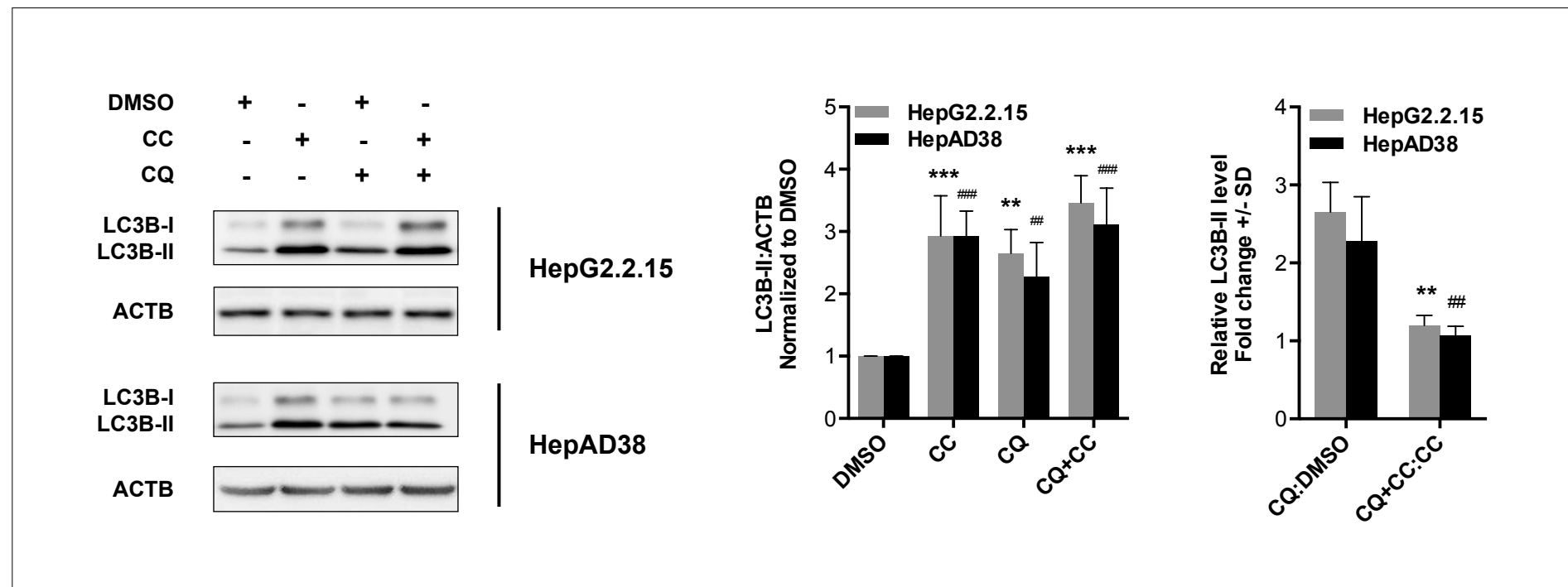


Figure S13. PRKAA is required for the degradation of autophagosomes. Immunoblot analysis of total protein extracts from cells treated with DMSO (0.1%), or CC (10 μ M) in the absence or presence of chloroquine (CQ; 10 μ M) for 24 h. Relative intensity of LC3B-II was quantified by normalization to ACTB by ImageJ software. Values are means \pm standard error (n=3). **, $p < 0.01$; ***, $p < 0.001$ (in HepG2.2.15); #, $p < 0.01$; ###, $p < 0.001$ (in HepAD38).

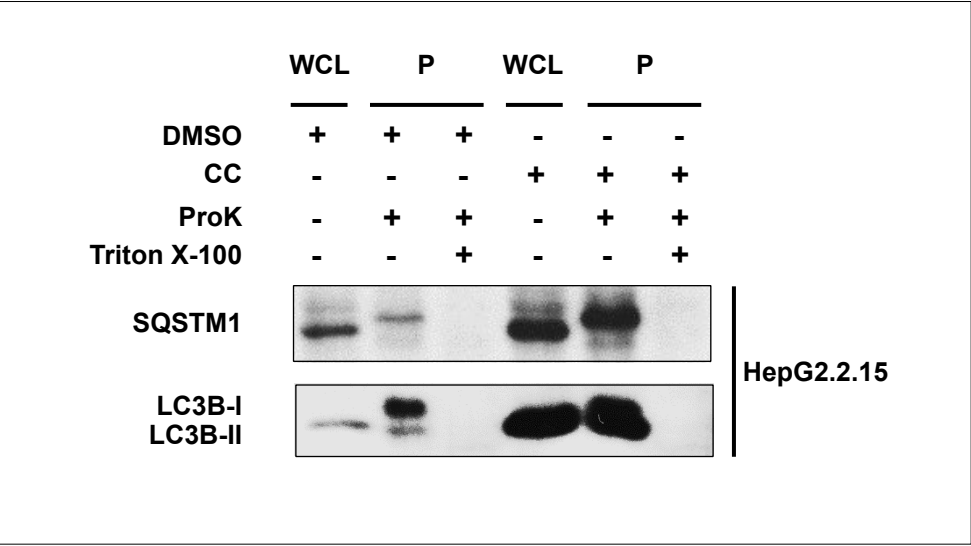


Figure S14. Sequestration of SQSTM1 within completed autophagosomes. HepG2.2.15 cells were incubated with DMSO and CC (10 μ M) for 24 h, followed by a proteinase K (ProK) protection assay for SQSTM1. WCL, whole cell lysate; P, pellet fraction.

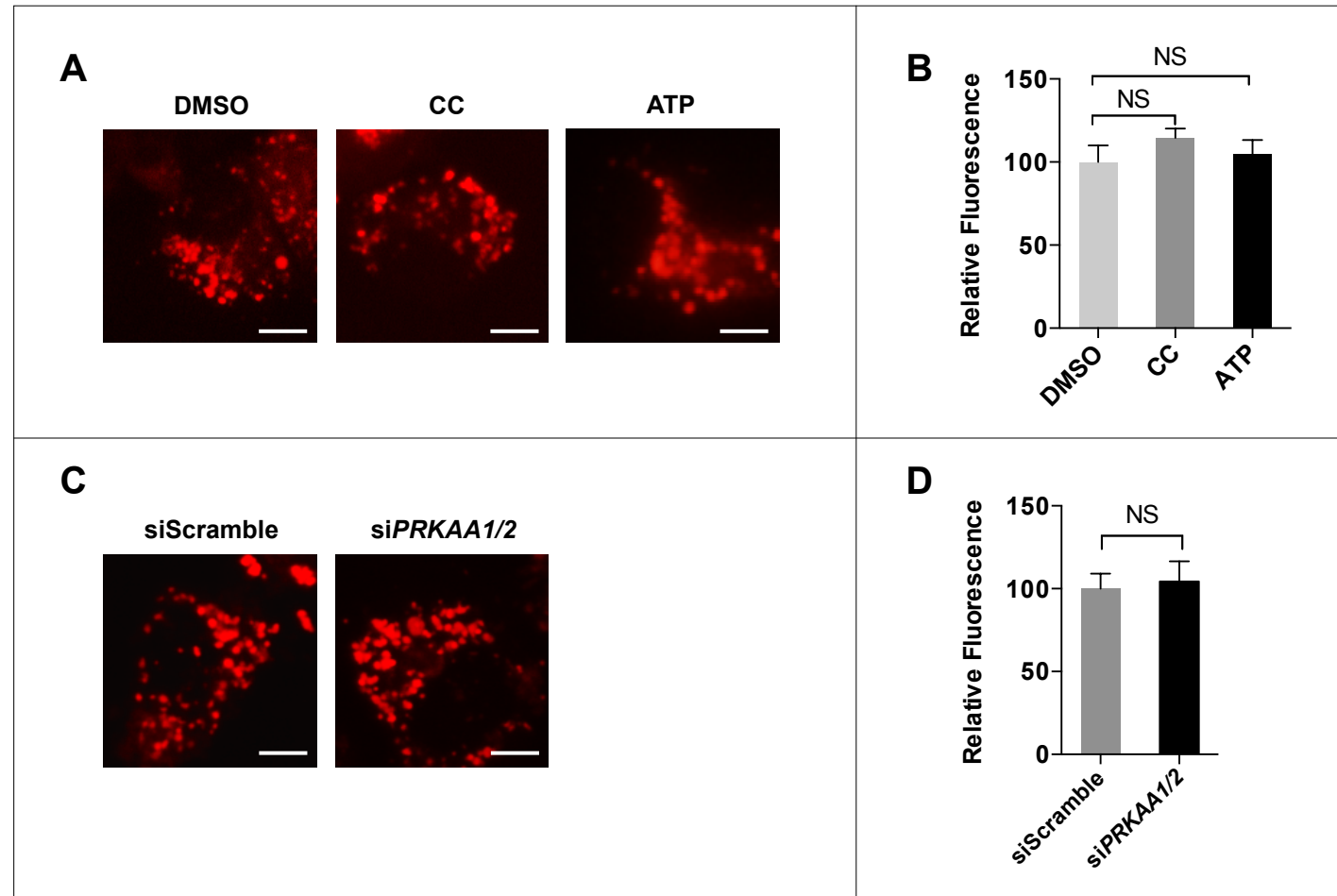


Figure S15. Effect of PRKAA on the quantity of lysosomes. (**A, B**) HepAD38 cells were treated with DMSO, CC (10 μ M), or 5'-ATP- Na_2 (0.25 mM) for 24 h. (**C, D**) HepAD38 cells were transfected with siScramble, or siPRKAA1/2 for 48 h. Lysosomes were stained with LysoTracker Red and visualized using Thermo Scientific Cellomics HCS imaging platform for automated image capture and analysis (**B, D**). Values are means \pm standard error (n=3). NS, nonsignificant by Student's t-test. Scale bar: 10 μ m.

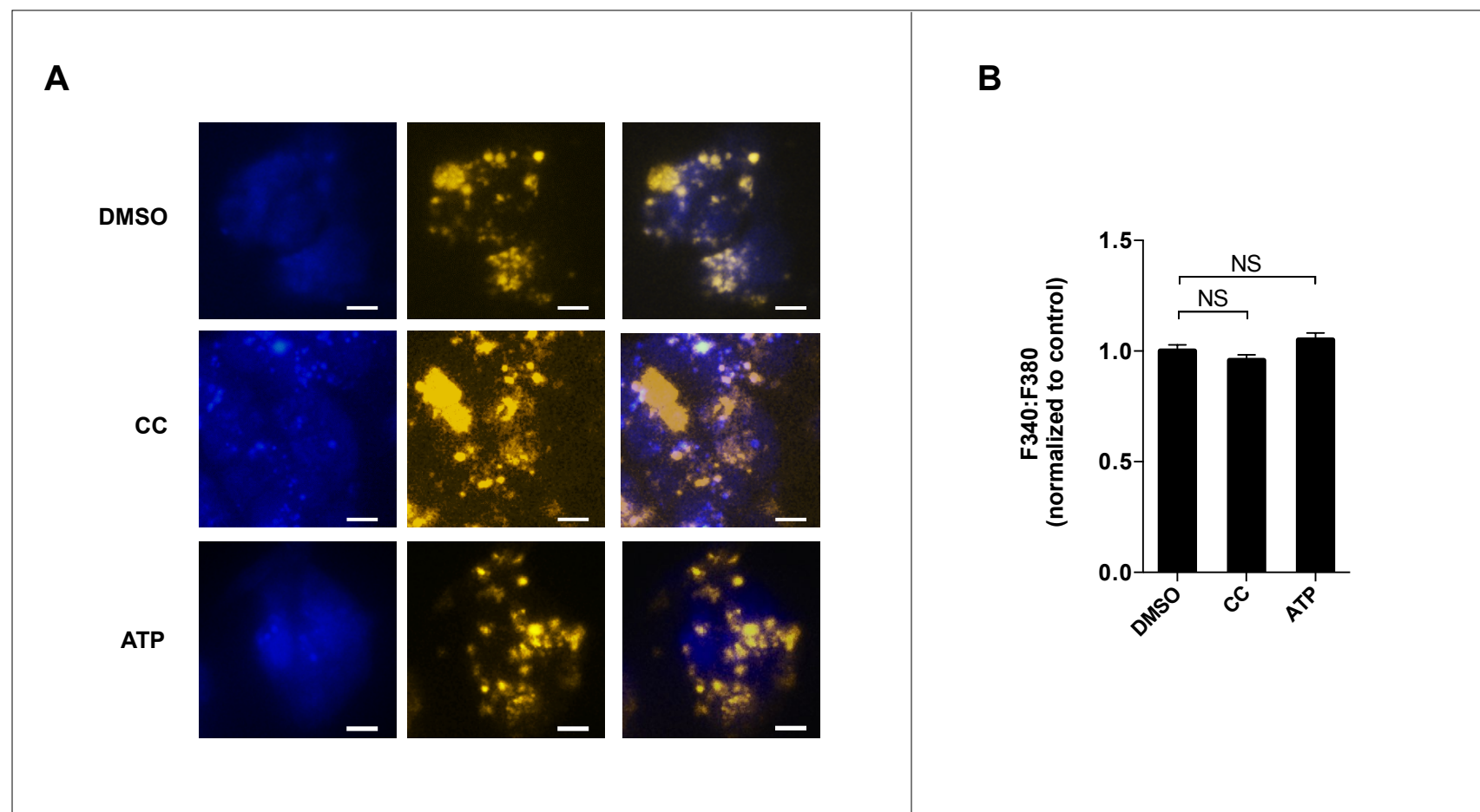


Figure S16. Effect of PRKAA on the acidification ability of lysosomes. HepAD38 cells were treated with DMSO, CC (10 μ M) and 5'-ATP-Na₂ (ATP, 0.25 mM) for 24 h. The cells were incubated with the lysosomal pH indicator dye for 5 min at room temperature, Imaging was performed with a Leica DM2500 microscope with LAS-AF imaging software (**A**), and the fluorescence was measured with VarioskanFlash (**B**). Values are means \pm standard error (n=3). NS, nonsignificant by Student's t-test. Scale bar: 20 μ m.

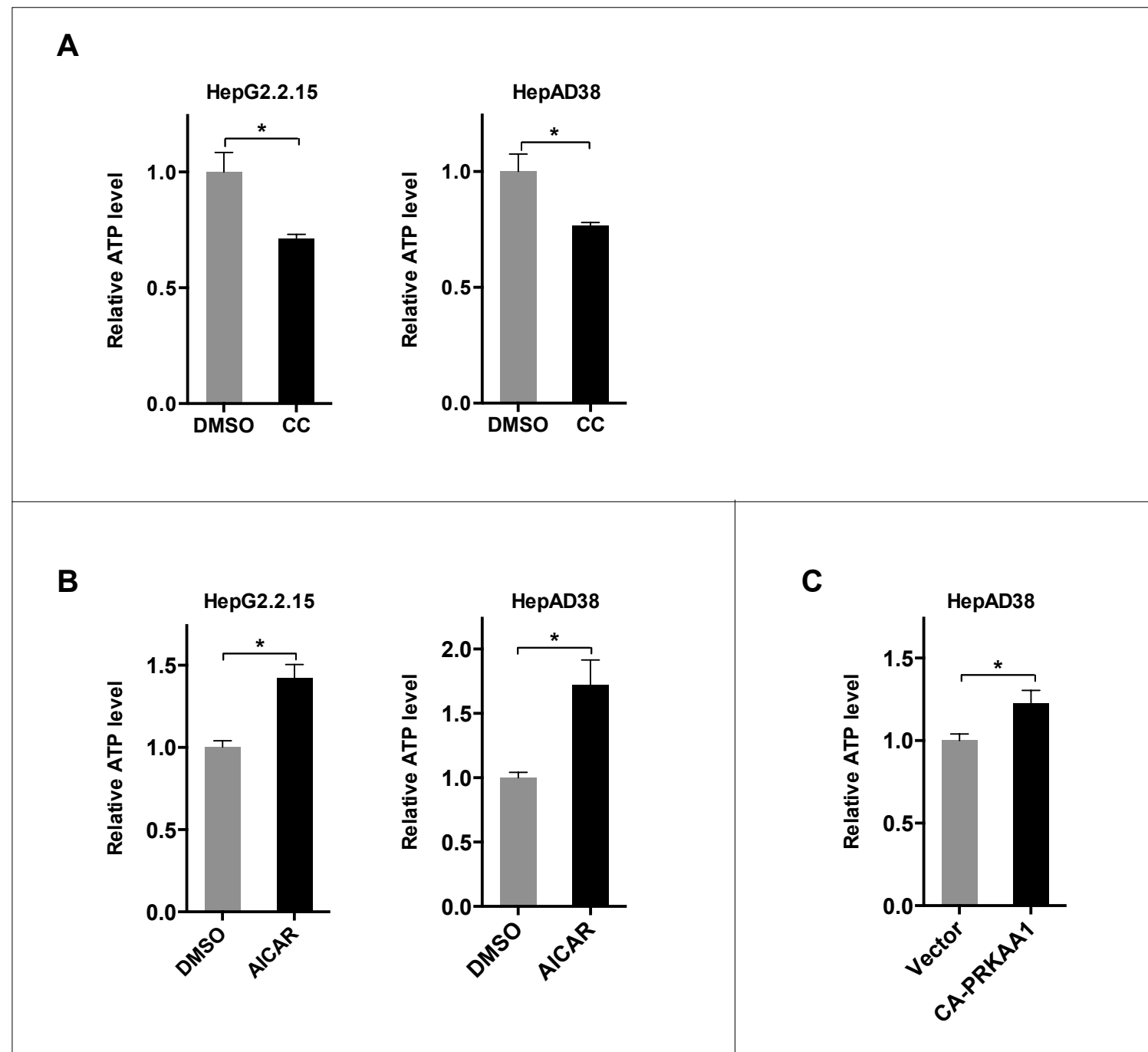


Figure S17. PRKAA activation elevates the cellular ATP level. **(A)** HepG2.2.15 and HepAD38 cells were incubated with DMSO or CC (10 μ M) for 24 h. Cellular ATP levels were determined and normalized to protein content. **(B)** HepG2.2.15 and HepAD38 cells were incubated with DMSO or AICAR (1 mM) for 24 h. ATP levels were normalized to protein content. ATP concentration of DMSO-treated cells was set to 1.0. **(C)** HepAD38 cells were transfected with vector or CA-PRKAA1 plasmids for 48 h. ATP levels were normalized to protein content. ATP concentration of DMSO-treated cells was set to 1. Values are means \pm SD, $n=3$ per group. *, $p < 0.05$.