**Supplemental Material**

**Supplemental materials and methods**

***Detection of GPT/ALT and GOT1/AST***

Serum GPT/ALT and GOT1/AST were measured by fully automated biochemical analysis (BIOSINO, China), according to the manufacturer’s instructions.

**Supplementary Figures**



**Figure S1.** GlcN promotes HBV replication and HBsAg expression in Huh7 cells.Huh7 cells transfected with HBV plasmid pSM2 were treated with 0, 1, 2, or 5 mM GlcN for 48 h. (**A**) HBsAg and HBeAg secreted in culture supernatants and intracellular HBsAg from cell lysates were analyzed by CMIA. (**B**) Encapsidated HBV replicative intermediates were detected by Southern blotting. S:CO, signal to cutoff ratio; RC, relaxed circular DNA; SS, single-stranded DNA. \**P* < 0.05; \*\**P* < 0.01; ns, not significant.



**Figure S2.** GlcN promotes HBsAg expression in HBV-transfected cells. HepG2 cells transfected with HBV plasmid pSM2 (**A**) or Huh7 cells transfected with HBV plasmid pHBV1.3 (**B**) or pHBV1.2 (**C**) were treated with 5 mM GlcN for 48 h. HBsAg and HBeAg secreted in culture supernatants and intracellular HBsAg from cell lysates were analyzed by CMIA. S:CO, signal to cutoff ratio. \**P* < 0.05; \*\**P* < 0.01; ns, not significant.



**Figure S3.** GlcN does not significantly increase HBV promoter activity.Luciferase reporters containing the *HBV* promoter regions pSP1, pSP2, pCP, and pXP were cotransfected into Huh7 cells. Next, the transfected cells were treated with 5 mM GlcN for 24 h. At 48 h after transfection, firefly and *Renilla* luciferase activities were analyzed using a Dual-Glo luciferase reporter assay. The relative luciferase expression was calculated as a fold-change and was normalized to control.



**Figure S4.** UDP-GlcNAc does not promote HBV production. HepG2.2.15 cells were treated with 20 µM UDP-GlcNAc for 48 h. HBsAg and HBeAg from culture supernatants and intracellular HBsAg from cell lysates were quantified by chemiluminescence immunoassay (CMIA). S:CO, signal to cutoff ratio. \**P* < 0.05; \*\**P* < 0.01; ns, not significant.



**Figure S5.** GlcN increases the number of autophagosomes. (**A**) Huh7 cells were transfected with GFP-LC3 plasmid and then treated with 5 mM GlcN for 48 h. Scale bar: 5 μm. (**B, C**) Huh7 cells transfected with HBV plasmid pSM2 were treated with 5 mM GlcN for 48 h. (**B**) Cells were fixed and incubated with primary antibody horse anti-HBsAg and rabbit anti-LC3, followed by staining with Alexa Fluor 488-conjugated anti-rabbit and Alexa Fluor 594-conjugated anti-horse secondary antibody IgG, respectively. Finally, the cells were imaged by confocal microscopy. Scale bar: 5 μm. (**C**) LC3, SQSTM1, and HBcAg expression were analyzed by western blotting, using ACTB as a loading control. LC3-II:ACTB ratios were quantified by densitometry. \**P* < 0.05; \*\**P* < 0.01; ns, not significant.



**Figure S6.** GlcN does not affect autophagosome-lysosome fusion. HepG2.2.15 cells were treated with 5 mM GlcN or 5 µM CID1067700 (CID) for 24 h. Then, the cells were fixed and incubated with mouse anti-LC3 and rabbit anti-LAMP1 antibodies, followed by staining with Alexa Fluor 488-conjugated anti-rabbit and Alexa Fluor 594-conjugated anti-mouse secondary antibody IgG, respectively. Colocalization of LC3 and LAMP1 was imaged by confocal microscopy. Scale bar: 5 μm.



**Figure S7.** GlcN inhibits MTORC1 signaling.(**A**) HepG2.2.15 cells were treated with 5 mM GlcN and harvested at 48 h after treatment. Western blot analysis was conducted to detect total and phosphorylated forms of MTOR, RPS6KB1, and ULK1 proteins, using ACTB as a loading control. The p-MTOR:ACTB ratio was quantified by densitometry. (**B**) HepG2.2.15 cells were incubated with 5 mM GlcN and 10 mM 3-MA, 2 µM rapamycin (Rapa), or control medium for 48 h. HBsAg and HBeAg secreted in culture supernatants were analyzed by CMIA. S:CO, signal to cutoff ratio. \**P* < 0.05; \*\**P* < 0.01; ns, not significant.



**Figure S8.** GlcN affects the clearance of serum HBeAg and liver damage in an HBV hydrodynamic injection mouse model.(**A**) Mouse serum samples were collected at the indicated time points. Serum HBeAg was analyzed by CMIA. Positivity for HBeAg was defined as ≥ 1. (**B**) GPT/ALT and GOT1/AST levels were determined at the indicated time points by fully automated biochemical analysis.

**Table S1.** List of siRNAs used in this study

|  |  |  |
| --- | --- | --- |
| **Name** | **Product Name** | **Company** |
| siNC | Allstars Negative Control siRNA | Qiagen |
| si*UAP1* | Hs\_UAP1\_5 FlexiTube siRNA | Qiagen |
| si*GNPNAT1* | Hs\_GNPNAT1\_4 FlexiTube siRNA | Qiagen |
| si*RRAGA* | Hs\_RRAGA\_6 FlexiTube siRNA | Qiagen |