

**Hexosamine biosynthetic pathway promotes the antiviral activity of  
SAMHD1 by enhancing O-GlcNAc transferase-mediated protein  
O-GlcNAcylation**

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## Supplementary Figure Legends

### Figure S1. HBV infection enhances HBP activity and upregulates GLUT1

**expression.** (A) KEGG pathway enrichment analysis of metabolites detected using metabolomics assay in HepG2 cells infected with AdHBV1.3 or AdGFP for 72 h. (B-C) Fold change in UDP-GlcNAc (B) and glucose (C) levels in HBV1.3-infected HepG2 cells was determined using LC-MS/MS targeted metabolomics assay,  $n = 6$ . (D) Immunoblot of total O-GlcNAc from PHH cells treated for the indicated periods. (E-F) RT-qPCR quantification (E) and immunofluorescence staining (F) of GLUT1 in HepG2 cells, DAPI (blue) was used to counterstain nuclei;  $n = 3$ . Scale bar, 10  $\mu\text{m}$ . (G-H) Immunoblots of OGA, OGT, p-GFPT1, and GFPT1 from HepG2, HepAD38 (G), and HepG2-NTCP (H) cells. Data are expressed as the mean  $\pm$  SD.  $P$  values were derived from unpaired, two-tailed Student's  $t$ -test in B-C and E (\*\* $P < 0.01$ ).

### Figure S2. Role of O-GlcNAc modification in regulating HBV replication

**in HepG2 cells.** (A-C) Immunoblot of total O-GlcNAc from HBV1.3-infected HepG2 cells treated with or without GLUT1 inhibitor WZB117 (50  $\mu\text{M}$ ) (A), GFPT1 inhibitor DON (30  $\mu\text{M}$ ) (B), or OGT inhibitor ST04 (100  $\mu\text{M}$ ) (C) for 72 h. (D-F) Quantification of HBV core DNA levels in HBV1.3-infected HepG2 cells treated as indicated using qPCR,  $n = 3$ . (G) Immunoblot of total O-GlcNAc from HBV1.3-infected HepG2 cells treated with or without OGA inhibitor TMG (100  $\mu\text{M}$ ) for 72 h. (H) Quantification of HBV core DNA levels in

HBV1.3-infected HepG2 cells treated as in (G) using qPCR,  $n = 3$ . Data are expressed as the mean  $\pm$  SD.  $P$  values were derived from unpaired, two-tailed Student's  $t$ -test in D-F and H; (\*\* $P < 0.01$ , \*\*\* $P < 0.001$ ).

**Figure S3. O-GlcNAc modification regulates HBV replication in HepG2-NTCP cells and PHH cells.** (A-E) Immunoblot of total O-GlcNAc from HBV-infected cells treated as indicated. (F-J) Quantification of HBV core DNA levels in HBV-infected cells treated as indicated using qPCR,  $n = 3$ . (K-L) Immunoblot of total O-GlcNAc from HBV-infected cells treated with or without TMG (100  $\mu$ M) for 72 h. (M-N) qPCR quantification of HBV core DNA levels in HBV-infected cells treated with or without TMG,  $n = 3$ . Data are expressed as the mean  $\pm$  SD.  $P$  values were derived from unpaired, two-tailed Student's  $t$ -test in F-J and M-N (\*\* $P < 0.01$ , \*\*\* $P < 0.001$ ).

**Figure S4. Inhibition of protein O-GlcNAcylation enhances HBV replication.** (A-B) Immunoblot of total O-GlcNAc from HBV1.3-infected HepG2 cells following shRNA-mediated GFPT1 or OGT knockdown. (C-D) Quantification of HBV core DNA levels in HBV1.3-infected HepG2 cells treated as above using qPCR,  $n = 3$ . (E) Immunoblot of total O-GlcNAc from HBV1.3-infected OGA knockdown HepG2 cells. (F) Quantification of HBV core DNA levels in HBV1.3-infected HepG2 cells treated as in (E) using qPCR,  $n = 3$ . Data are expressed as the mean  $\pm$  SD.  $P$  values were derived from one-way

ANOVA in C-D and F; (\*\* $P < 0.01$ , \*\*\* $P < 0.001$ ).

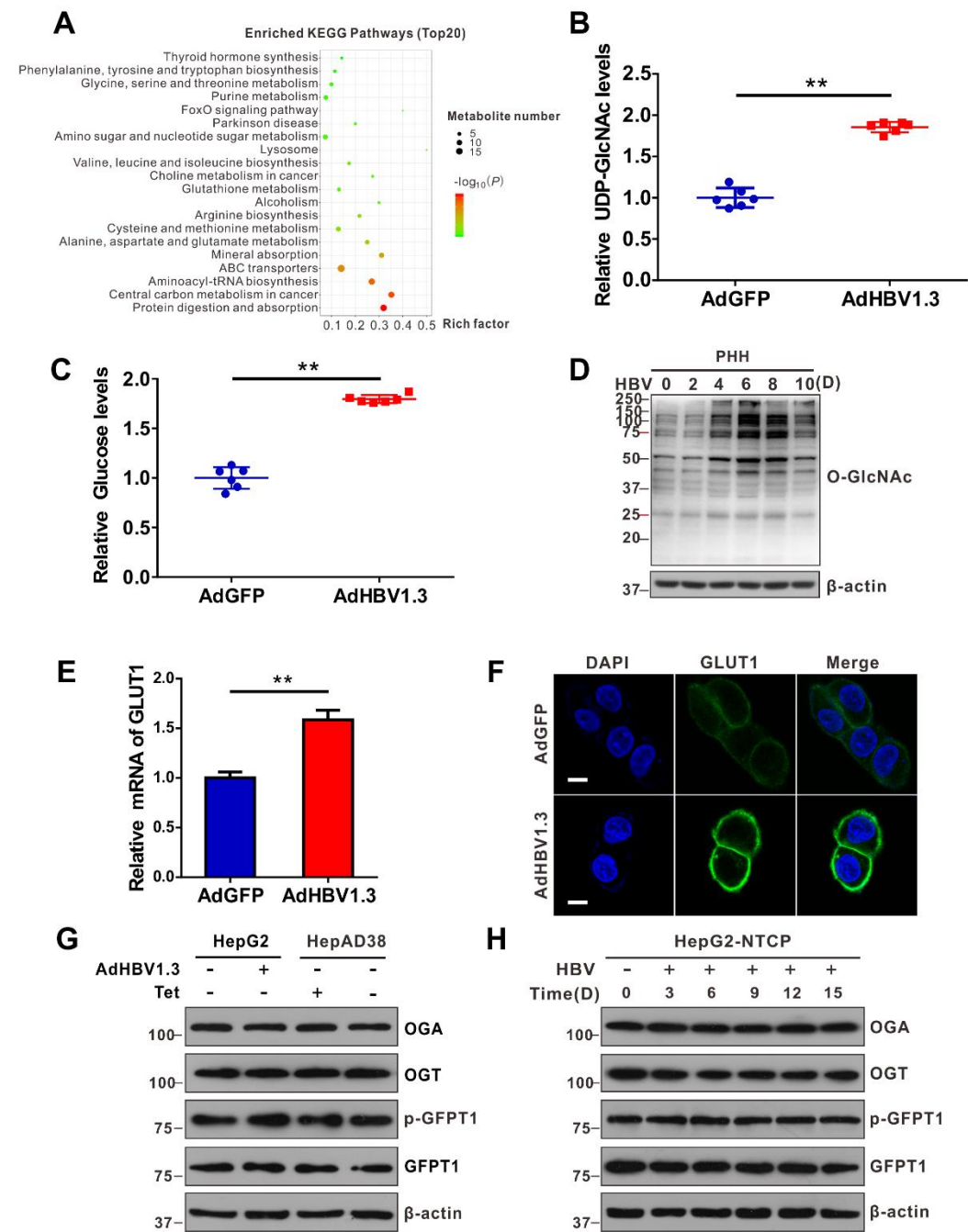
**Figure S5. HBV infection promotes SAMHD1 O-GlcNAcylation. (A)**

IP-LC-MS/MS analysis of O-GlcNAc modified proteins. HepAD38 cell lysates were immunoprecipitated using an anti-O-GlcNAc antibody. **(B)** HEK293T cells, **(C)** Tetracycline-inducible HepAD38 cells, **(D)** HepG2-NTCP cells were transfected with the Flag-SAMHD1 construct or the control vector for 48 h, and treated with 100  $\mu$ M TMG for 12 h **(B)**, Cells lysates were purified using sWGA-conjugated agarose beads and probed with an anti-Flag or anti-O-GlcNAc antibody. **(E)** PHH cells were infected with HBV for 72 h. After cell lysis, O-GlcNAc-modified proteins were purified using the sWGA-conjugated agarose beads and probed with an anti-SAMHD1 antibody. The immunoprecipitated and input proteins were probed with an anti-O-GlcNAc or anti-SAMHD1 antibody. **(F-G)** Southern blot assay in stable HBV-expressing HepAD38 and SAMHD1-KO HepAD38 cells. rc DNA, relaxed circular DNA; ds DNA, double-stranded DNA; ss DNA, single-stranded DNA. **(H)** SAMHD1-KO HepG2-NTCP cells were transfected with either empty vector, Flag-tagged SAMHD1 WT or the S93A mutant, and treated with 100  $\mu$ M PUGNAc for 12 h. Cells lysates were purified using sWGA-conjugated agarose beads and probed with an anti-Flag or anti-O-GlcNAc antibody.

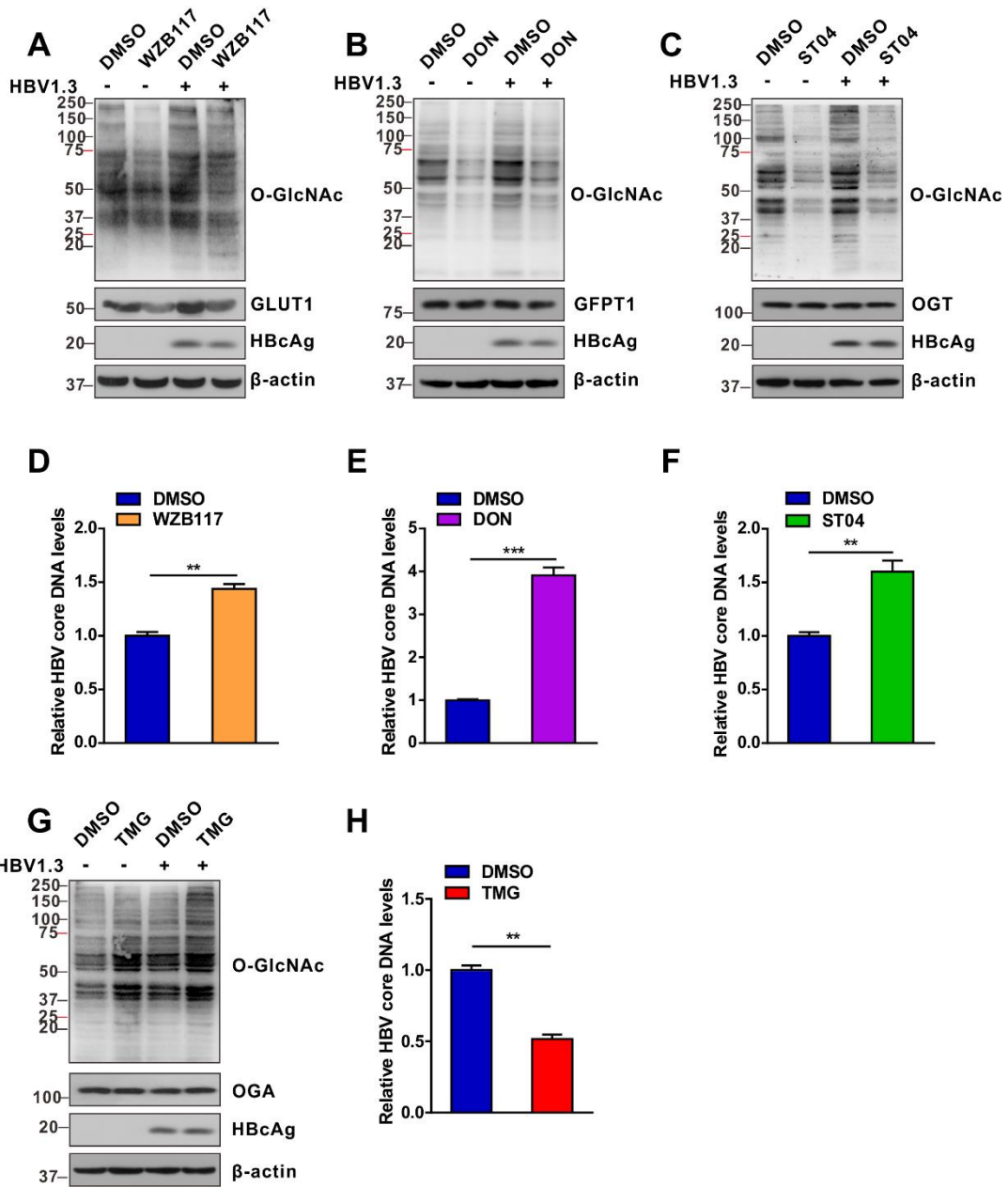
**Figure S6. SAMHD1 protein expression, purification, and dNTPase**

**activity. (A-B)** His-tagged pET28a-SAMHD1 constructs were expressed in *Escherichia coli* BL21 (DE3) cells and induced with 0.2 mM IPTG. Cell lysates were purified using Ni-NTA affinity column. The purified target protein was stained with Coomassie Brilliant Blue dye. Lane 1, molecular-mass markers; Lane 2, cell supernatant after ultrasonic crash; Lane 3, Ni-NTA affinity column flow through fraction; Lane 4-6, eluted target protein with 20 to 400 mM imidazole; Lane 7, eluted protein following hyperfiltration. **(C-D)** HPLC assay to determine SAMHD1 dNTPase activity. SAMHD1-WT (C) and SAMHD1-S93A (D) constructs were purified using Ni-NTA affinity and size-exclusion chromatography. Nucleotide hydrolysis reactions were performed in SAMHD1 buffer containing 500  $\mu$ M dNTPs and 500  $\mu$ M SAMHD1-WT, the peak height in Y-axis(mAU) refers to the maximum concentration of 'dG', X-axis(min) refers to retention time. The reactions were terminated using 10 mM EDTA. Protein was separated using an Amicon Ultra 0.5-mL 10-kDa filter and analyzed using a Venusil MP-C18 column on an HPLC system.

**Figure S1**



**Figure S2**



**Figure S3**

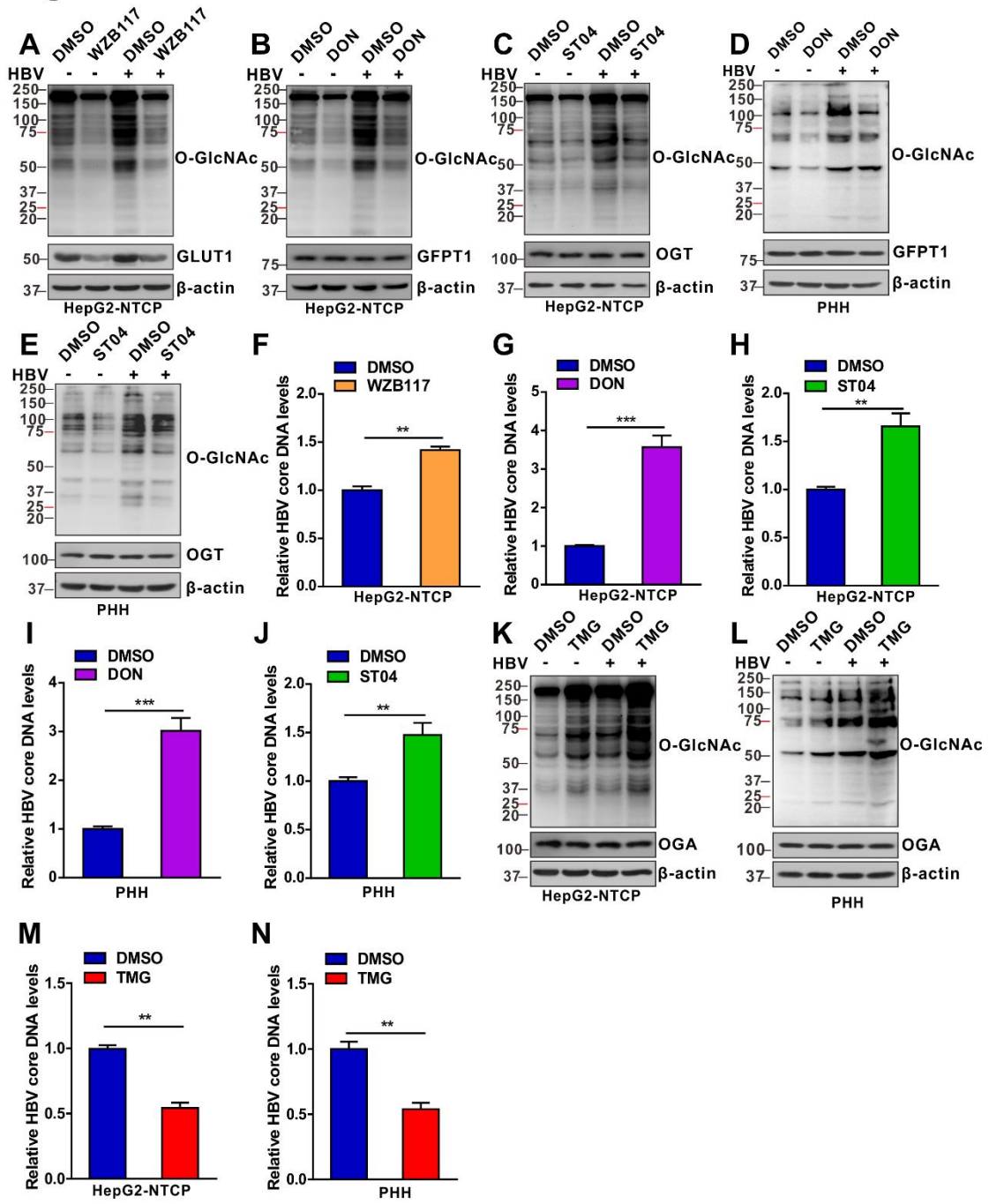
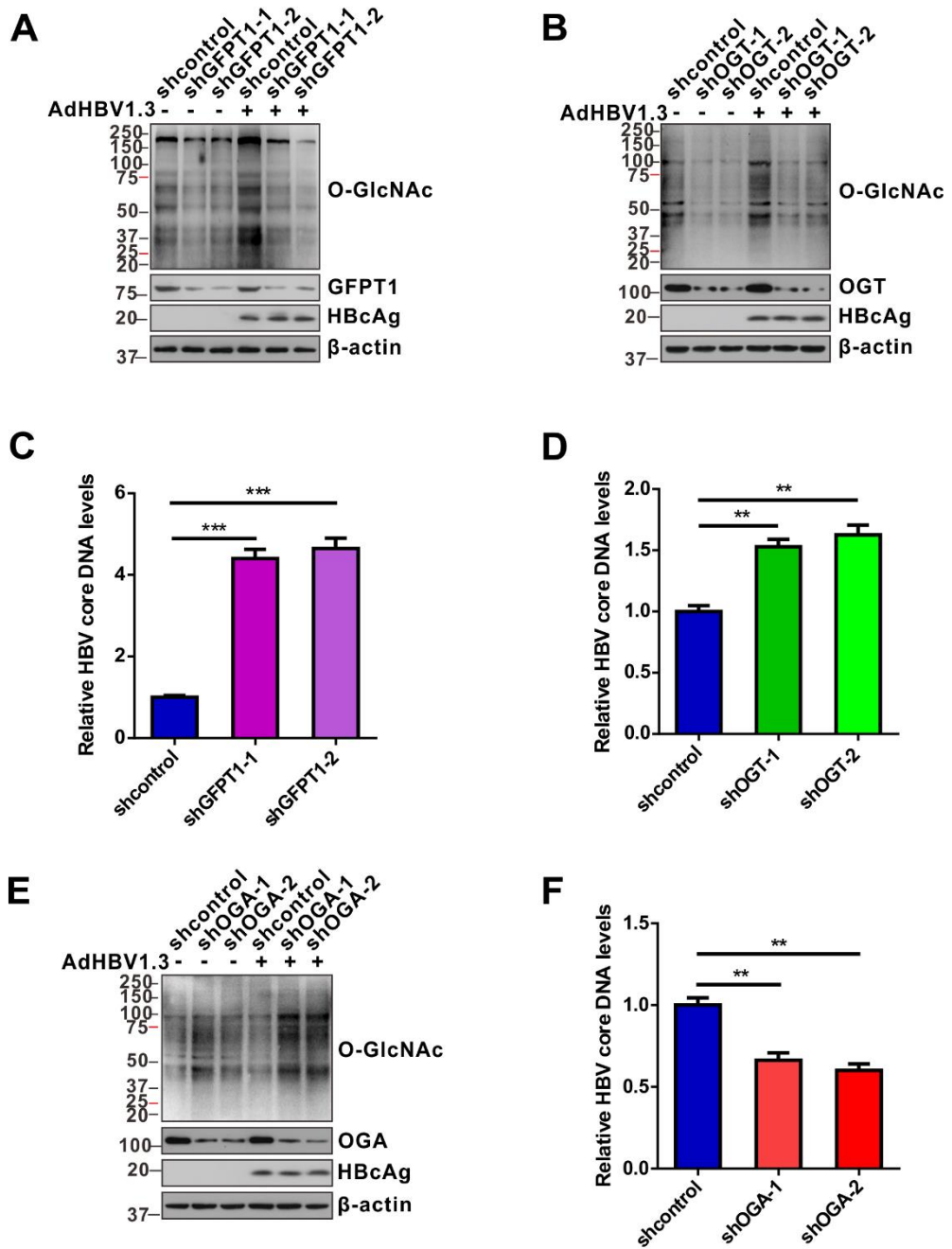
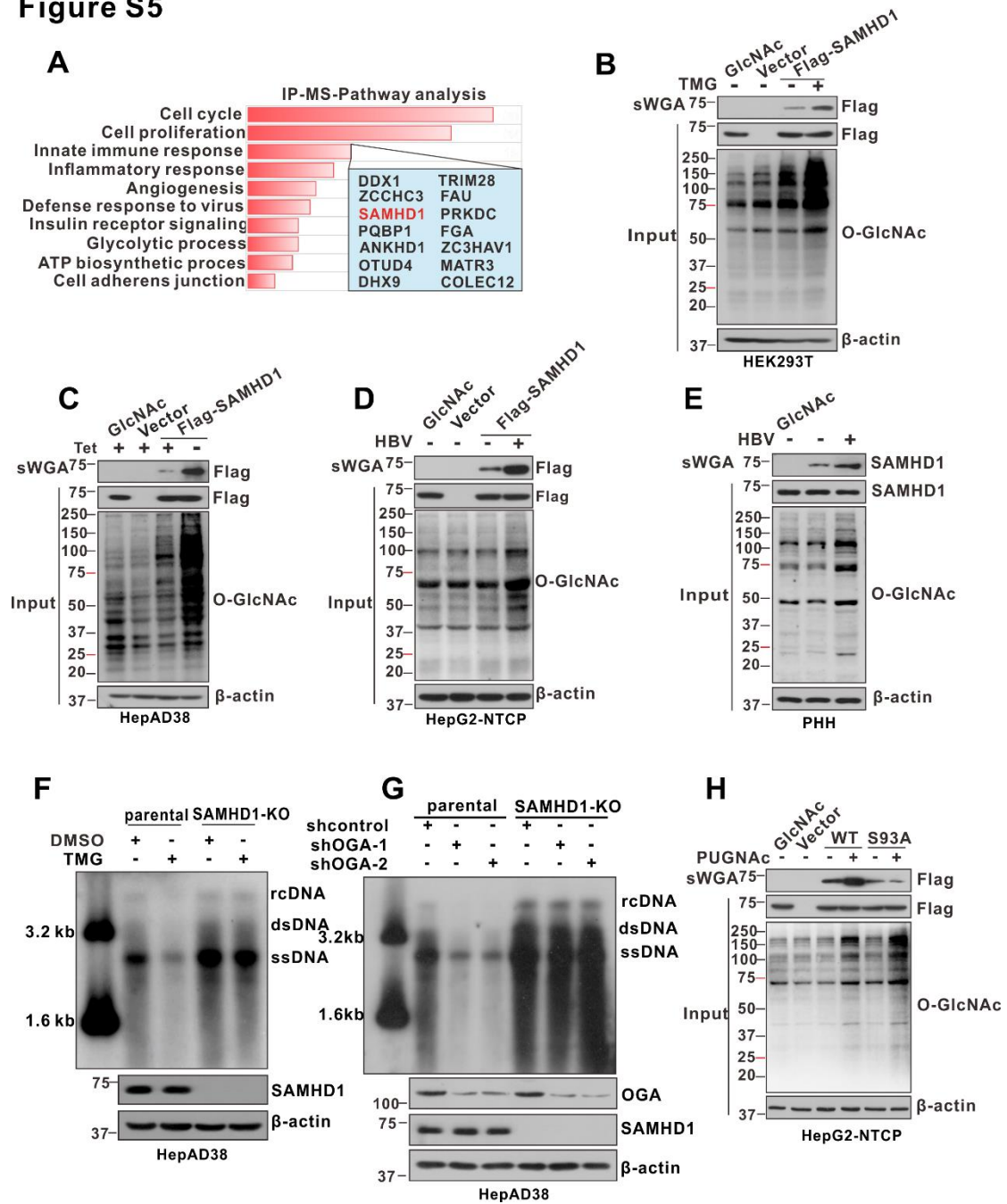




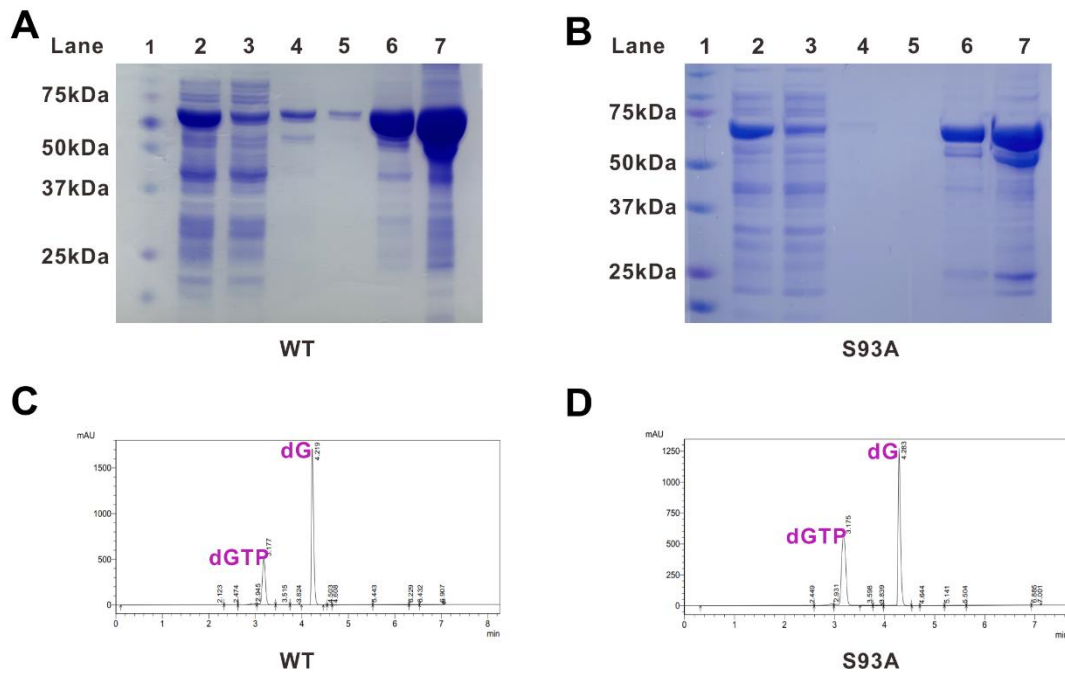
Figure S4



**Figure S5**



**Figure S6**



**Table S1. Sequence of Oligonucleotides.**

Name	Sequence (5'-3')
<b>Primer sequences for quantitative RT-PCR.</b>	
HBV	Forward: TGCGGCGTTTTATCATATTCC Reverse: ATACCTTGGTAGTCCAGAAGAACCA
GLUT1	Forward: ACCGCAACGAGGAGAACCG Reverse: CCACAAACAGCGACACGACAG
OGA	Forward: AGCAGCCTTGAGTGGTGAGC Reverse: TGGGGATTTTGATTGAGCTATG
OGT	Forward: AGAGGCAGTTCGCTTGTATCG Reverse: AGTAGGCATCAGCAAAGGTAGG
GFPT1	Forward: CCAGTCCTGTCAATAGCCACC Reverse: CACAAGTGCAAAGCACCTTC
SAMHD1	Forward: TGCCCGTGTCTGTGAAGTAG Reverse: ATTGCTGTAGAAATGCGATA
GAPDH	Forward: GAAATCCCATCACCATCTTCCAGG Reverse: GAGCCCCAGCCTTCTCCATG
<b>Primer sequences for knockdown.</b>	
shOGA	Forward1: TGGACAATTCTTTATGACATTTCAAGAGAATGTCAT AAAGAATTGTCCTTTTTTC Reverse1: TCGAGAAAAAAGGACAATTCTTTATGACATTCTCT TGAAATGTCATAAAGAATTGTCCA Forward2: TGCCTTTGTACACTGCGGAATTCAAGAGTTCCGC AGTGTACAAAGGCTTTTTTC Reverse2: TCGAGAAAAAAGCCTTTGTACACTGCGGAATCTC

	TTGAATTCCGCAGTGTACAAAGGCA
shOGT	Forward1: TGCATGTTATTTGAAAGCAATTCAAGAG ATTGCTTTCAAATAACATGCTTTTTTC Reverse1: TCGAGAAAAAAGCATGTTATTTGAAAGC AATCTCTTGAATTGCTTTCAAATAACATGCA Forward2: TGCCCTAAGTTTGAGTCCAATTCAAGAG ATTGGACTCAAACCTAGGGCTTTTTTC Reverse2: TCGAGAAAAAAGCCCTAAGTTTGAGTC CAATCTCTTGAATTGGACTCAAACCTAGGGCA
shGFTP1	Forward1: TGGAGAGAGTTATCCAACAATTCAAGAG ATTGTTGGATAACTCTCTCCTTTTTTC Reverse1: TCGAGAAAAAAGGAGAGAGTTATCCAA CAATCTCTTGAATTGTTGGATAACTCTCTCCA Forward2: TGGAAGTACTGAGCATGGATTTCAAGAG AATCCATGCTCAGTACTTCCTTTTTTC Reverse2: TCGAGAAAAAAGGAAGTACTGAGCATG GATTCTCTTGAATCCATGCTCAGTACTTCCA
<b>Primer sequences for molecular cloning.</b>	
pSEB-Flag-OGT	Forward: ACGCGTCGACACCATGGCGTCTTCCGTGGGC Reverse: CGCGGATCCTGCTGACTCAGTGACTTCAACAGG
pBud-HA-OGT	Forward: ATAGCGGCCGCACCATGGCGTCTTCCGTGGGC Reverse: CGCGGATCCTGCTGACTCAGTGACTTCAACAGG
pET28a- SAMHD1	Forward: TGCGGATCCCAGCGAGCCGATTCCGAGC Reverse: CCGCTCGAGTCACATTGGGTCATCTTTAA
pSEB-FlagSAMHD1	Forward: TGAAGATCTACCATGGGCCAGCGAGCCGATTCCG Reverse: CGCGTCCGACCATTTGGGTCATCTTTA AAAAGC
<b>Primer sequences for mutagenesis.</b>	
pSEB-Flag- SAMHD1 (S93A)	Forward: ATCTTGGAGTAAGTGCCTTGGGGGAGA Reverse: TCTCCCCCAAGGCACTTACTCCAAGAT

pSEB-Flag-SAMHD1 (T592E)	Forward: GGATGGCGATGTTATAGCCCCACTCATAGAACCTCAAAAA Reverse: TTTTTGAGGTTCTATGAGTGGGGCTATAACATCGCCATCC
pSEB-Flag-SAMHD1 (1-150)	Forward: TGAAGATCTACCATGGGCCAGCGAGCCGATTCCG Reverse: CGCGTCGACCAGCTGTTTGATGTATCGAAGAC
pSEB-Flag-SAMHD1(151-328)	Forward: TGAAGATCTACCATG GGAGGTGGTTACTATGTTTTTCC Reverse: CGCGTCGACATTATTTTGGATTCCAAGATGATG
pSEB-Flag-SAMHD1(329-626)	Forward: TGAAGATCTACCATG GGCTTTGATTACAAGCGCTTTATTA Reverse: CGCGTCGACCATTGGGTCATCTTTAAAAGC
<b>OGT, SAMHD1-targeted single gRNAs.</b>	
SAMHD1- sgRNA	Forward: CACCGCTTAGTTATATCCAGCGAT Reverse: AAACATCGCTGGATATAACTAAGC
OGT-sgRNA	Forward: CACCGCACATCGAGAATATCAGGC Reverse: AAACGCCTGATATTCTCGATGTGC
lentiCRISPR-v2	Forward: GGTTTATTACAGGGACAGCAG Reverse: ACACGACATCACTTTCCAG

**Table S2. Reagent or resource.**

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Antibodies</b>		
Mouse anti-O-GlcNAc	Abcam	Cat#ab2739
Rabbit anti-OGT	Abcam	Cat#ab177941
Rabbit anti-OGA/MGEA5	Abcam	Cat#ab124807
Rabbit anti-GFPT1/GFAT1	Abcam	Cat#ab125069
Mouse monoclonal anti-SAMHD1	Abcam	Cat#ab67820
Rabbit anti-GLUT1	Abcam	Cat#ab115730
Rabbit anti-K48	Abcam	Cat#ab140601
Goat anti-Rabbit IgG	Abcam	Cat# ab6721
Goat anti-Mouse IgG	Abcam	Cat# ab6789
Mouse monoclonal anti-FLAG M2-peroxidase (HRP)	Sigma-Aldrich	Cat#A8592
ANTI-FLAG M2 Affinity Gel	Sigma-Aldrich	Cat#A2220
Mouse anti-GAPDH	Beyotime	Cat#AF0006
Mouse monoclonal anti-HA	Thermo	Cat#26183-HRP
Mouse anti- $\beta$ -actin	ZSGB-BIO	Cat#TA-09
Rabbit polyclonal anti-HBcAg	Dako	Cat#B0586
Rabbit anti-p-GFPT1/GFAT1(Ser243)	Immuno-Biological Laboratories	Cat# JP28123
Goat anti-rabbit IgG/TRITC, secondary	ZSGB-BIO	Cat#ZF-0316
Goat anti-mouse IgG/TRITC, secondary	ZSGB-BIO	Cat#ZF-0313
Goat anti-rabbit IgG/FITC, secondary	ZSGB-BIO	Cat#ZF-0311
Goat anti-mouse IgG/ FITC, secondary	ZSGB-BIO	Cat#ZF-0312
<b>Bacterial and Virus Strains</b>		
E.coli BL21	Lab stock	
DH10B Chemically Competent E. coli	Lab stock	

pseudotyped HIV-1 single-round luciferase virus (HIV-LUC-G)	He et al., 1995
Lenticrispr-SAMHD1	This study
Lenticrispr-OGT	This study
DH10B Chemically Competent E. coli	Lab stock
AdHBV1.3	Lab stock

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### Biological Samples

Human liver tissue with chronic hepatitis B	The Second Affiliated Hospital, Chongqing Medical University.China
Human serum with chronic hepatitis B	The Second Affiliated Hospital, Chongqing Medical University.China

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### Chemicals, Peptides, and Recombinant Proteins

Polybrene	Solarbio	Cat#H8761
PNGase F	NEB	Cat#P0704S
Fetal Bovine Serum	Gibco	Cat#A3160801
Trypsin	Gibco	Cat#12605010
RPMI1640	Gibco	Cat#11875500BT
Opti-MEM™ I Reduced Serum Medium	Gibco	Cat#31985070
DMEM/F-12	Gibco	Cat#11330500BT
DMEM	Gibco	Cat#11995500BT
MEM	Hyclone	Cat#SH30024.01
Penicillin-Streptomycin Solution	HyClone	Cat#SV30010
Lipofectamine 2000	Invitrogen	Cat#11668019
DMSO	Sigma	Cat#D2660
Phorbol 12-myristate 13-acetate (PMA)	Sigma-Aldrich	Cat#P8139
PUGNAc	Sigma-Aldrich	Cat#A7229
Cycloheximide	MCE	Cat#HY-12320



ST045849	TimTec	Cat#MFCD03308174
Thiamet G	Selleck	Cat#S7213
WZB117	Selleck	Cat#S7927
UDP-GlcNAc	BBL Life Sciences	Cat#7512-17-6
6-diazo-5-oxo-L-norleucine(Don)	Sigma-Aldrich	Cat#D2141
SuperSignal West Pico PLUS chemiluminescent Substrates	Thermo Fisher Scientific	Cat#34577
DAPI	Roche	Cat# 10236276001
Trizol	Invitrogen	Cat# 15596026
SYBR Green PCR Master Mix	BioRad	Cat#172-5121

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### Critical Commercial Assays

PrimeScript RT reagent Kit	Takara	Cat# RR047A
TIANampVirus DNA/RNA Kit	TianGen	Cat#DP315
HBsAg ELISA KIT	Kehua	Cat#S10910113
HBeAg ELISA KIT	Kehua	Cat#20163400144
DIG high prime DNA labeling and detection starter kit II	Roche	Cat#11585614910
Dual-Glo® Luciferase Assay System	Promega	Cat#E2920
ALT ELISA kit	Mengbio	Cat#MBE1741
Elivision plus Polyer HRP (Mouse/Rabbit) IHC Kit	Maixin	Cat# KIT-9901
DAB kit	ZhongshanGolden Bridge	Cat# ZLI-9019
BCA protein assayKit	BEIJING DINGGUO	Cat# BCA02
Cell lysis buffer for Western and IP	Beyotime	Cat# P0013

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### Deposited Data

#### Experimental Models: Cell Lines

HEK293T	ATCC
HepG2	ATCC

HepAD38	Lab stock
HepG2-NTCP	Lab stock
THP-1	ATCC

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#### Experimental Models: Organisms/Strains

Mouse: C57BL/6	Experimental Animal Center of Chongqing Medical University
HBV-transgenic (HBV-Tg) mice	Xiamen University

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#### Recombinant DNA

LentiCrisprv2	Lab stock	
pAdTrack-TO4	Dr. T-C He, University of Chicago, USA	
pBUD-HA-OGT	This paper	
pSEB-Flag-OGT	This paper	
pcDNA3-HA-SAMHD1	This paper	
pcDNA3-HA-ubiquitin	Kamitani et al., 1997	HA-Ubiquitin, Addgene plasmid, # 18712
pSEB-Flag-SAMHD1(WT)	This paper	N/A
pSEB-Flag-SAMHD1(S93A)	This paper	N/A
pSEB-Flag-SAMHD1(T592E)	This paper	N/A
pSEB-Flag-SAMHD1(1-150)	This paper	N/A
pSEB-Flag-SAMHD1(151-328)	This paper	N/A
pSEB-Flag-SAMHD1(329-626)	This paper	N/A

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#### Software and Algorithms

GraphPad Prism	GraphPad Software	<a href="https://www.graphpad.com">https://www.graphpad.com</a> .
CorelDRAW X7	Corel Corporation	<a href="https://www.coreldraw.com/">https://www.coreldraw.com/</a>

ImageJ	Schneider et al., 2012	<a href="https://imagej.nih.gov/ij/">https://imagej.nih.gov/ij/</a>
Image pro plus	<a href="http://www.mediacy.com/imageproplus">http://www.mediacy.com/imageproplus</a>	N/A

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