Hepatitis B virus evasion from cGAS sensing in human hepatocytes

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SUPPORTING INFORMATION

Supplementary Experimental Procedures

Detection of cGAS protein expression using two independent antibodies. HepG2-NTCP cells were transfected with a siRNA targeting *MB21D1* expression (sicGAS) or with a non-targeting siRNA control (siCtrl). Three days after transfection, cells were lysed and cGAS protein expression was assessed as described in the Experimental Procedures section using two rabbit polyclonal anti-cGAS antibodies (HPA031700, Sigma & NBP1-86761, Novus Biologicals). Given the proximity of the bands, another western blot was run in parallel for the detection of β-actin as a control. Molecular weights were assessed using the Precision Plus Protein™ Standards molecular weight marker (Bio-Rad).

Detection of HBV DNA by PCR and qPCR. DNA was extracted using QiaAMP DNA MiniKit (Qiagen) following manufacturer's instructions. The presence of HBV DNA was confirmed by PCR using the following primers (expected band size: 148 bp) (1) : forward primer 5'-CACCTCGCCTAATCATC-3', reverse primer 5'-GGAAAGAAGTCAGAAGGCA-3'. For qPCR quantification of HBV DNA, The presence of HBV DNA was confirmed by PCR and quantified by qPCR using the following primers and probe (1) : forward primer 5'-CACCTCGCCTAATCATC-3', reverse primer 5'-GGAAAGAAGTCAGAAGGCA-3'; TaqMan probe 5'-[6FAM]-TGGAGGCTTCAACAGTAGGACATGAAC-[BHQ1]-3'. Copy number of HBV was determined using a standard curve.

Detection of NTCP expression by flow cytometry. HepG2 cells, HepG2-NTCP-Ctrl_ORF cells, and HepG2-NTCP-cGAS_OE cells were treated with the AF647-labelled pres1 peptide for one hour at 37°C as described (1). Cells were then fixed with 2% paraformaldehyde for 20 minutes at room temperature. NTCP expression was then quantified by flow cytometry using MacsQuant instrument (Miltenyi).

Supplementary Figures

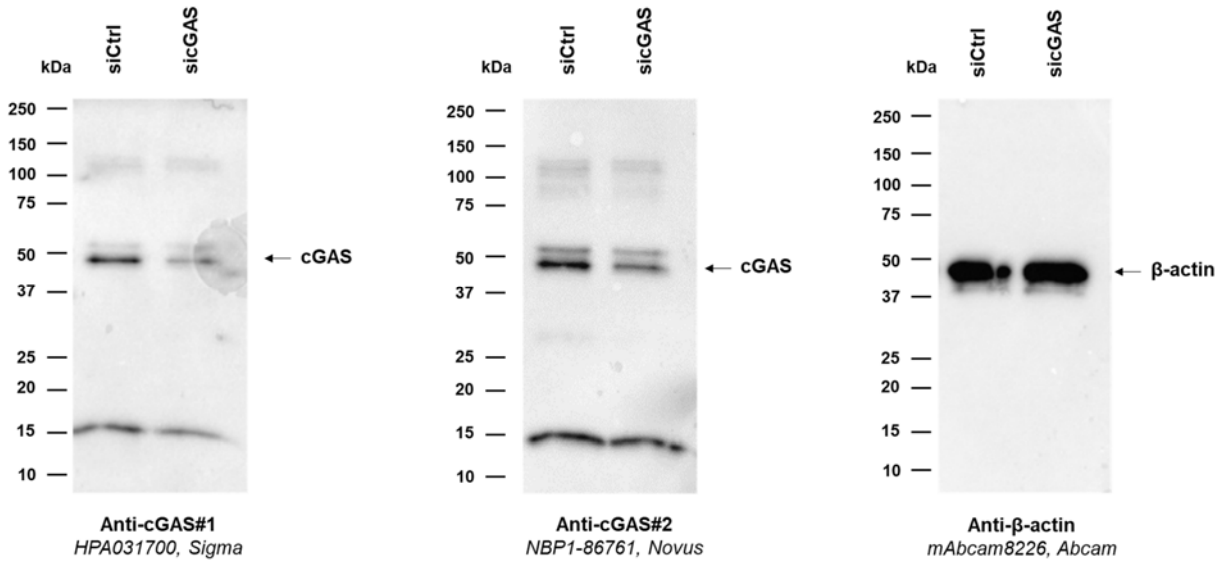

Figure S1. Detection of cGAS protein in HepG2-NTCP cells (related to Figure. 1). HepG2-NTCP cells were transfected for three days with a siRNA targeting *MB21D1* expression (sicGAS) or with a non-targeting siRNA control (siCtrl). Cells were then lysed and cGAS protein expression was assessed using two rabbit polyclonal anti-cGAS antibodies (HPA031700, Sigma, used in the main manuscript & NBP1-86761, Novus Biologicals). β-actin expression was assessed as a control. One representative experiment is shown.

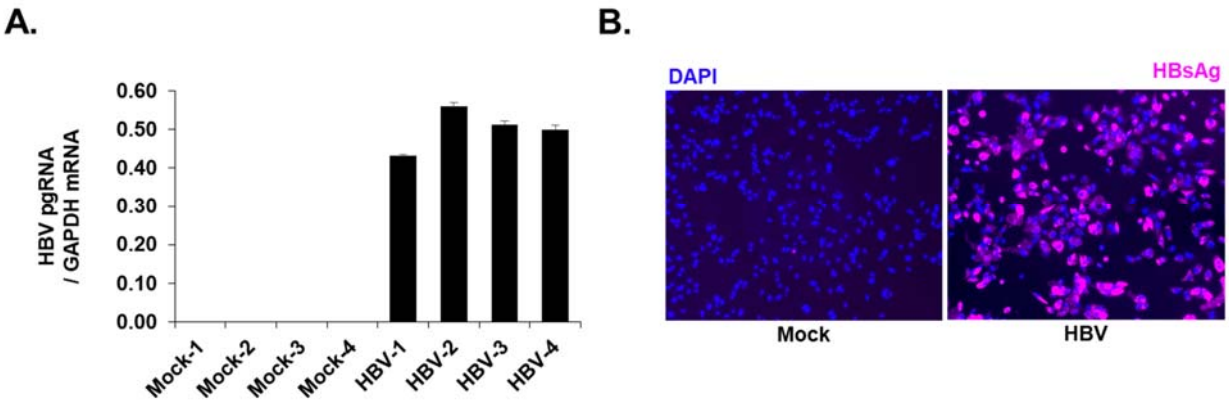

Figure S2. Analysis of HBV infection in HBV time course samples with quantification of HBV pregenomic RNA and HBsAg expression (related to Figure 2). HepG2-NTCP cells were infected with HBV as described in Experimental Procedures. 10 days after infection, total RNA was extracted and HBV infection was assessed by quantification of HBV pgRNA as described in Methods. Results are expressed as means ± SD HBV pgRNA / GAPDH mRNA from four independent experiments performed in duplicate (corresponding to the four experiments shown in Figure 2A). Alternatively, cells were infected for 10 days in HBV, and HBV infection was assessed by immunofluorescence using an anti-HBsAg antibody. One representative experiment is shown.

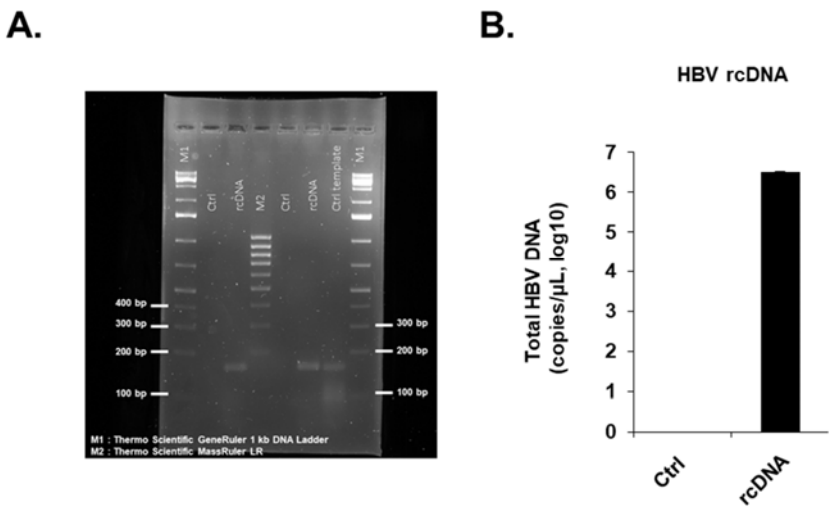

Figure S3. Analysis and quantification of HBV DNA extracted from HBV infectious particles by PCR (related to Figure 4). A-B. HBV genomic DNA (rcDNA) was extracted from cell culture-derived HBV virions. Extraction from naive HepG2-NTCP control supernatants without virus was used as a control (Ctrl). HBV DNA standard preparation used as a template for the calculation of HBV DNA concentration was used as a positive control (Ctrl template). The presence of HBV DNA was controlled by PCR (expected band size: 148 base pairs [bp]) (A) and quantified by qPCR (B). Two independent experiments (A) and one experiment performed in triplicate (B) are shown. C. Quantification of total HBV DNA in transfected or infected cells. HepG2-NTCP were infected with HBV or transfected with rcDNA. Three days after transfection/infection, DNA was extracted and total HBV DNA was quantified by qPCR.

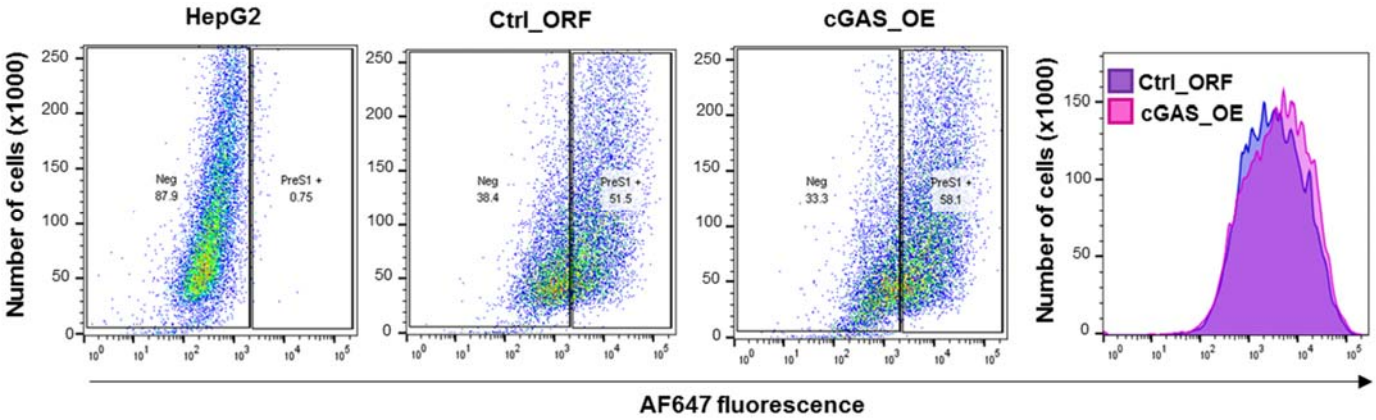

Figure S4. PreS1-binding/NTCP cell surface expression is independent on cGAS expression. HepG2 cells, HepG2-NTCP-Ctrl_ORF cells, and HepG2-NTCP-cGAS_OE cells were treated with the AF647-labelled preS1 peptide for one hour at 37°C. PreS1 binding corresponding to NTCP expression was quantified by flow cytometry.

Supplementary Tables

Tables S1: Specific probes used for the detection of HBV and Mitochondrial DNA (2).

Target	Name	Sequence
HBV	HBV-F1	TAGCGCCTCATTTTGTGGGT
	HBV-R1	CTTCCTGTCTGGCGATTGGT
	HBV-F2	TAGGACCCCTGCTCGTGTTA
	HBV-R2	CCGTCCGAAGGTTTGGTACA
	HBV-F3	ATGTGGTATTGGGGGCCAAG
	HBV-R3	GGTTGCGTCAGCAAACACTT
	HBV-F4	TGGAACCTTTTCGGCTCCTC
	HBV-R4	GGGAGTCCGCGTAAAGAGAG
	HBV-F6	TACTGCACTCAGGCAAGCAA
	HBV-R6	TGCGAATCCACACTCCGAAA
	HBV-F8	AGACGAAGGTCTCAATCGCC
	HBV-R8	ACCCACAAAATGAGGCGCTA
Mitochondrial DNA	Fw_huND1	CCCTACTTCTAACCTCCCTGTTCTTAT
	Rw_huND1	CATAGGAGGTGTATGAGTTGGTCGTA
	Fw_huND5	ATTTTATTTCTCCAACATACTCGGATT
	Rw_huND5	GGGCAGGTTTTGGCTCGTA
	Fw_huATP6	CATTTACACCAACCACCCAACTATC
	Rw-huATP6	CGAAAGCCTATAATCACTGTGCC

Tables S2: Specific probes of the IAR gene set for multiplexed gene profiling analysis.

Gene	Accession Number	Target Sequence	Note
ATP5B	NM_001686.3	GAAATTCTGGTGACTGGTATCAAGGTTGTCGATCTGCTA GCTCCCTATGCCAAGGGTGGCAAAATTGGGCTTTTTGGT GGTGCTGGAGTTGGCAAGACTG	HG
BAG6	NM_001199698.1	CATTGATCACGGGGCTAGAAGAGTATGTGCGGGAGAGTT TTTCCTTGGTGCAGGTTCAGCCAGGTGTGGACATCATCC GGACAAACCTGGAATTTCTCCA	HG
NDUFA2	NM_001185012.1	ATGGGCTAGGCTTTAGGGTCCGCGGTTGGTCAGACCGG AGCACTTGGCCTGAAGACCTGGAATTGGCGACTTCGATA TTAACAAGGATGGCGGCGGCCGC	HG
ARL9	NM_206919.1	CAGATATCCATGAAGCTTTGGCATTATCTGAAGTGGGAAA TGACAGGAAGATGTTCTTGTTTGGAACCTACCTGACTAAG AATGGCTCAGAGATACCCTC	Schoggins
CASP1	NM_001223.3	TGGAGACATCCCACAATGGGCTCTGTTTTTATTGGAAGA CTCATTGAACATATGCAAGAATATGCCTGTTCCTGTGATG TGGAGGAAATTTTCCGCAAGG	Schoggins
CXCL16	NM_001100812.1	CCATGGGTTCAGGAATTGATGAGCTGTCTTGATCTCAAA GAATGTGGACATGCTTACTCGGGGATTGTGGCCCACCAG AAGCATTTACTTCCTACCAGCC	Schoggins
CXCL8	NM_000584.2	ACAGCAGAGCACACAAGCTTCTAGGACAAGAGCCAGGAA GAAACCACCGGAAGGAACCATCTCACTGTGTGTAAACAT GACTTCCAAGCTGGCCGTGGCT	Schoggins
HERC5	NM_016323.2	TGGGCTGCTGTTTACTTTCGGTGCTGGAAAACATGGGCA ACTTGGTCATAATTCAACACAGAATGAGCTAAGACCCTGT TTGGTGGCTGAGCTTGTTGGG	Schoggins

HERC6	NM_001165136.1	TCCATCACCCAGATTTATACTTAGAGTCAGACGAAGTCGC CTGGTTAAAGATGCTCTGCGTCAATTAAGTCAAGCTGAA GCTACTGACTTCTGCAAAGTA	Schoggins
HLA-B	NM_005514.6	CCCTGAGATGGGAGCCGTCTTCCCAGTCCACCGTCCCC ATCGTGGGCATTGTTGCTGGCCTGGCTGTCCTAGCAGTT GTGGTCATCGGAGCTGTGGTCGC	Schoggins
HLA-H	NR_001434.3	GAGCGGGAGGGGCCGGAGTATTGGGACCGGAACACACA GATCTGCAAGGCCCAAGCACGGACTGAACGAGAGAACC TGCGGATCGCGCTCCGCTACTACA	Schoggins
IFI35	NM_005533.3	TGCCCTCTGCTTGCGGGCTCTGCTCTGATCACCTTTGAT GACCCCAAAGTGGCTGAGCAGGTGCTGCAACAAAAGGA GCACACGATCAACATGGAGGAGT	Schoggins
IFI44	NM_006417.4	GATGAAAGAAAGATAAAAGGGGTCATTGAGCTCAGGAAG AGCTTACTGTCTGCCTTGAGAACTTATGAACCATATGGAT CCCTGGTTCAACAAATACGAA	Schoggins
IFIH1	NM_022168.2	GCTTGGGAGAACCCTCTCCCTTCTCTGAGAAAGAAAGAT GTCGAATGGGTATTCCACAGACGAGAATTTCCGCTATCT CATCTCGTGCTTCAGGGCCAGG	Schoggins
IFIT3	NM_001031683.2	CGCCTGCTAAGGGATGCCCCTTCAGGCATAGGCAGTATT TTCCTGTCAGCATCTGAGCTTGAGGATGGTAGTGAGGAA ATGGGCCAGGGCGCAGTCAGCT	Schoggins
ISG20	NM_002201.5	AGCCCGCCGAGGGCTGCCCCGCCTGGCTGTGTCAGACT GAAGCCCCATCCAGCCCGTTCCGCAGGGACTAGAGGCT TTCGGCTTTTTGGGACAGCAACTA	Schoggins
LRRC17	NM_001031692.1	CAGCACAACCAGATCAAAGTCTTGACGGAGGAAGTGTTC ATTTACACACCTCTCTTGAGCTACCTGCGTCTTTATGACA ACCCCTGGCACTGTACTTGTG	Schoggins
MX1	NM_002462.2	GCCTTTAATCAGGACATCACTGCTCTCATGCAAGGAGAG GAAACTGTAGGGGAGGAAGACATTCGGCTGTTTACCAGA CTCCGACACGAGTTCCACAAAT	Schoggins
OAS2	NM_016817.2	TGAAAAACAATTTCGAGATCCAGAAGTCCCTTGATGGGTT CACCATCCAGGTGTTCACAAAAAATCAGAGAATCTCTTTC GAGGTGCTGGCCGCCTTCAA	Schoggins
OASL	NM_198213.1	GGCGTTTCTGAGCTGTTTCCACAGCTTCCAGGAGGCAGC CAAGCATCACAAAGATGTTCTGAGGCTGATATGGAAAAC CATGTGGCAAAGCCAGGACCTG	Schoggins
PLCG2	NM_002661.2	GCTTGAAAATCTTACACCAGGAAGCGATGAATGCGTCCA CGCCCACCATTATCGAGAGTTGGCTGAGAAAGCAGATAT ATTCTGTGGATCAAACCAGAAG	Schoggins
PSMB8	NM_004159.4	ACTCACAGAGACAGCTATTCTGGAGGCGTTGTCAATATG TACCACATGAAGGAAGATGGTTGGGTGAAAGTAGAAAGT ACAGATGTCAGTGACCTGCTGC	Schoggins
PSMB9	NM_002800.4	TCAGGTATATGGAACCCTGGGAGGAATGCTGACTCGACA GCCTTTTGCCATTGGTGGCTCCGGCAGCACCTTTATCTA TGGTTATGTGGATGCAGCATAT	Schoggins
RARRES3	NM_004585.3	CTGACCCTCGTGCCCTGTCTCAGGCGTTCTCTAGATCCT TTCCTCTGTTTCCCTCTCTCGCTGGCAAAAGTATGATCTA ATTGAAACAAGACTGAAGGAT	Schoggins
SLC15A3	NM_016582.1	GCCGCTTCTTCAACTGGTTTTACTGGAGCATCAACCTGG GTGCTGTGCTGTCGCTGCTGGTGGTGGCGTTTATTCAGC AGAACATCAGCTTCCTGCTGGG	Schoggins
TNFRSF1B	NM_001066.2	CCCAGCTGAAGGGAGCACTGGCGACTTCGCTCTTCCAGT TGGACTGATTGTGGGTGTGACAGCCTTGGGTCTACTAAT AATAGGAGTGGTGAACTGTGTC	Schoggins
UBA7	NM_003335.2	GCGGGAGGATGGGTCCCTGGAGATTGGAGACACAACAA CTTTCTCTCGGTACTTGCGTGGTGGGGCTATCACTGAAG TCAAGAGACCCAAGACTGTGAGA	Schoggins
UBE2L6	NM_004223.3	TGTTTCAAAACCACTTGCCATCCTGTTAGATTGCCAGTTC CTGGGACCAGGCCTCAGACTGTGAAGTATATATCCTCCA GCATTCAGTCCAGGGGGAGCC	Schoggins
ZC3HAV1	NM_020119.3	CTCCTTCTTCACATCGTAGAAACATGGCATATAGGGCTAG AAGCAAGAGTAGAGATCGGTTCTTTCAGGGCAGCCAAGA ATTTCTTGCGTCTGCTTCAGC	Schoggins
ZMYND15	NM_032265.1	CCTCAGAGCGGCCGACAACTGCATGTCCTGGTACTGCAA TGCCTTCATCTTCCACCTGGTTTACAAGCCTGCTCAAGG GAGCGGGGCCCGCCCGGCGCCC	Schoggins

PMAIP1	NM_021127.2	CTAGTGTTTTTGCCGAAGATTACCGCTGGCCTACTGTGA AGGGAGATGACCTGTGATTAGACTGGGCGGCTGGGGAG AAACAGTTCAGTGCATTGTTGTT	Schoggins
GBP4	NM_052941.4	TTCTACAAGATATGCCATGGGCCTTTTCACAGGGGACAC AGGCTTCTTAAAACAACCCGGCTTCCTCACCCTATGTCCT TTATTTACAAAGCTGTGCTCC	Schoggins
TMEM173	NM_198282.1	CTGGCATGGTCATATTACATCGGATATCTGCGGCTGATC CTGCCAGAGCTCCAGGCCCGGATTCGAACTTACAATCAG CATTACAACAACCTGCTACGGG	STING
IFI16	NM_005531.1	ACGACTGAACACAATCAACTGTGAGGAAGGAGATAAACT GAAACTCACCAGCTTTGAATTGGCACCGAAAAGTGGGAA TACCGGGGAGTTGAGATCTGTA	
IFNB1	NM_002176.2	ACAGACTTACAGGTTACCTCCGAAACTGAAGATCTCCTA GCCTGTGCCTCTGGGACTGGACAATTGCTTCAAGCATTC TTCAACCAGCAGATGCTGTTTA	
IRF3	NM_001571.5	TCATGGCCCCAGGACCAGCCGTGGACCAAGAGGCTCGT GATGGTCAAGGTTGTGCCCACGTGCCTCAGGGCCTTGG TAGAAATGGCCCGGGTAGGGGGTG	
IRF7	NM_001572.3	CGCAGCGTGAGGGTGTGTCTTCCCTGGATAGCAGCAGC CTCAGCCTCTGCCTGTCCAGCGCCAACAGCCTCTATGAC GACATCGAGTGCTTCCTTATGGA	
STAT1	NM_139266.1	ACAGTGGTTAGAAAAGCAAGACTGGGAGCACGCTGCCAA TGATGTTTCATTTGCCACCATCCGTTTTCATGACCTCCTG TCACAGCTGGATGATCAATAT	
TBK1	NM_013254.2	ACCAGTCTTCAGGATATCGACAGCAGATTATCTCCAGGT GGATCACTGGCAGACGCATGGGCACATCAAGAAGGCAC TCATCCGAAAGACAGAAATGTAG	

HG: Housekeeping genes
Schoggins: cGAS-related genes described by Shoggins et al., (3)

Supplementary References

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2. Lucifora J, Salvetti A, Marniquet X, Mailly L, Testoni B, Fusil F, Inchauspe A, et al. Detection of the hepatitis B virus (HBV) covalently-closed-circular DNA (cccDNA) in mice transduced with a recombinant AAV-HBV vector. Antiviral Res 2017;145:14-19.
3. Schoggins JW, MacDuff DA, Imanaka N, Gainey MD, Shrestha B, Eitson JL, Mar KB, et al. Pan-viral specificity of IFN-induced genes reveals new roles for cGAS in innate immunity. Nature 2014;505:691-695.