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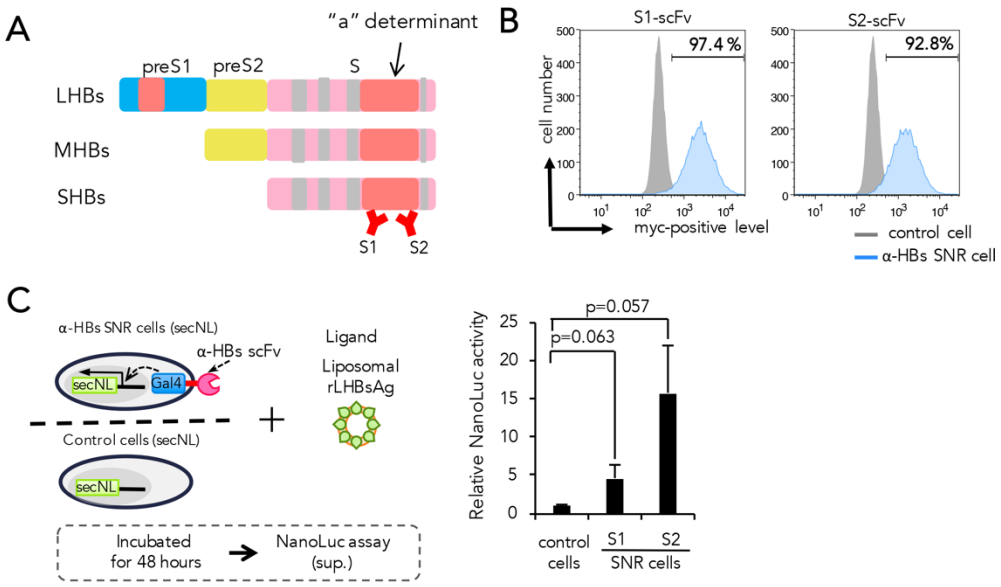
Supplemental Information

Engineering Cellular Biosensors with Customizable Antiviral Responses Targeting Hepatitis B Virus

Satoko Matsunaga, Sundararaj S. Jeremiah, Kei Miyakawa, Daisuke Kurotaki, Sayaka Shizukuishi, Koichi Watashi, Hironori Nishitsuji, Hirokazu Kimura, Tomohiko Tamura, Naoki Yamamoto, Kunitada Shimotohno, Takaji Wakita, and Akihide Ryo

SUPPLEMENTAL FIGURES

Figure S1. Generation and evaluation of single chain variable fragments (scFvs) against HBs protein. Related to figure 1.

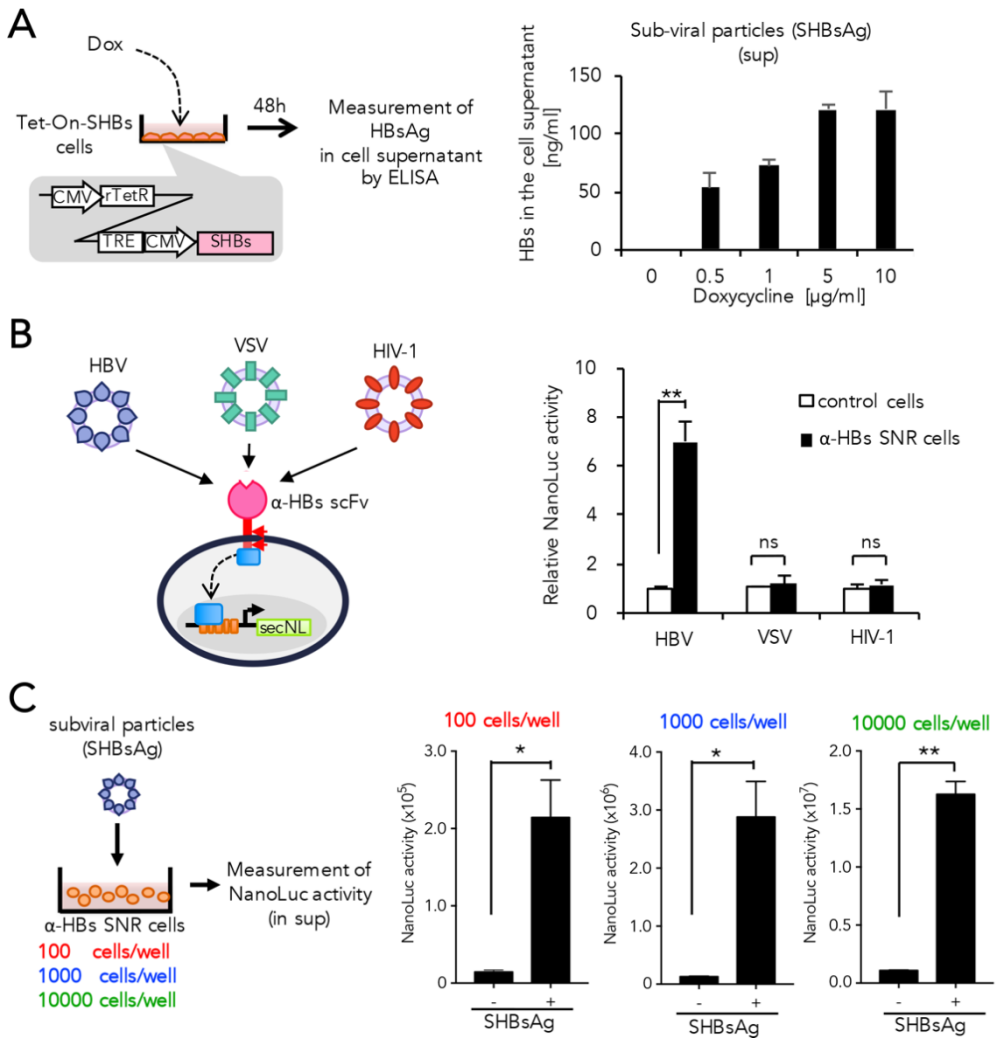


(A) Schematic diagram of recognizing region of two scFvs against HBs proteins. S1 and S2 scFvs recognize the "a" determinant region of SHBs protein.

(B) Expression level of two α-HBs scFv SNRs in Jurkat T cells. Detection of each surface α-HBs SNR on constructed cells by flow cytometry using anti-myc antibody.

(C) α-HBs SNR cells expressed secreted NanoLuc luciferase (secNL) in response to liposomal recombinant LHBs (rLHBs) protein. Control or α-HBs SNR cells and 5 μg/ml rLHBs protein were incubated at 37 ^{o}C and the NanoLuc activity in the cell supernatant was measured after 48 hours. Control cells indicate Jurkat T cells harboring only secNL reporter gene without α-HBs SNR. Each value is normalized to those obtained from without rLHBsAg and represents the mean ± standard deviation (SD) of three independent experiments. p-values were calculated using two-tailed unpaired *t*-test.

Figure S2. Generation of Tet-ON-SHBs cells and evaluation of α-HBs SNR cell specificity. Related to figure 1.



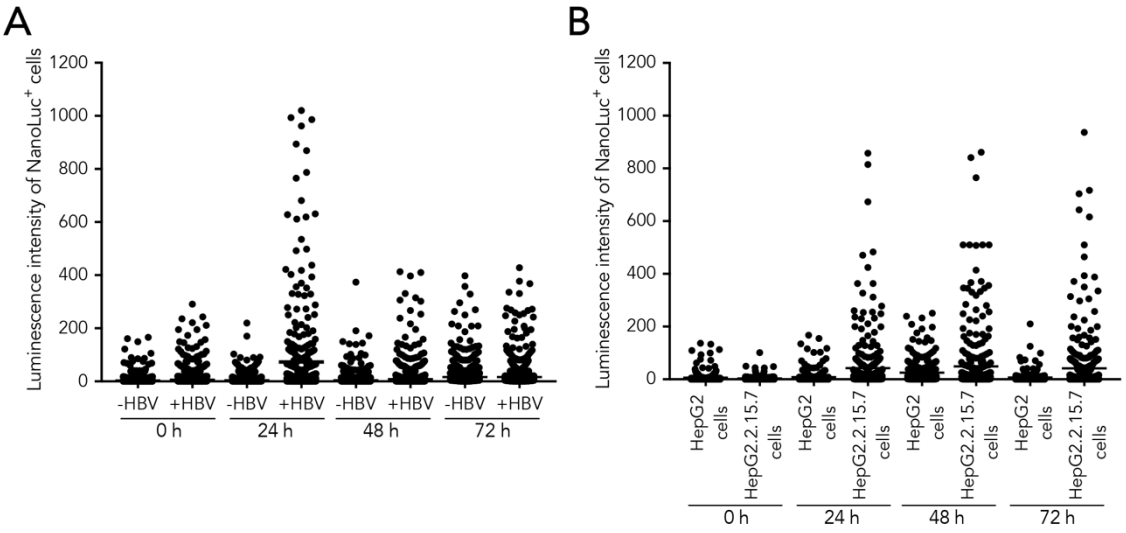
(A) Generating Tet-ON-SHBs cells that secrete HBs protein. Tet-ON-SHBs cells generated by transducing with a retroviral vector encoding the SHBs gene fused to a tetracycline-responsive element (TRE) in to HepG2 Tet-ON parental cell line. The cells were stimulated with 5μg/ml doxycycline (Dox) for 48 hours and then the HBs protein in a cell supernatant was measured by HBs ELISA kit. The data shown represents the mean ± standard deviation (SD) of three independent experiments.

(B) α-HBs SNR cells exclusively sensed HBV particles but not VSVg or HIV-1 Env-pseudotype viruses. Each viral particle was mixed with α-HBs SNR cells and incubated at 37 ^{o}C for 48 hours followed by measurement of NanoLuc activity. Control

cells indicate Jurkat T cells harboring only secNL reporter gene without α-HBs SNR. Each value is normalized to those obtained from without HBV or envelope deficient pseudoparticle and represents the mean ± SD of three independent experiments. ***p*: < 0.01, ns: not significant.

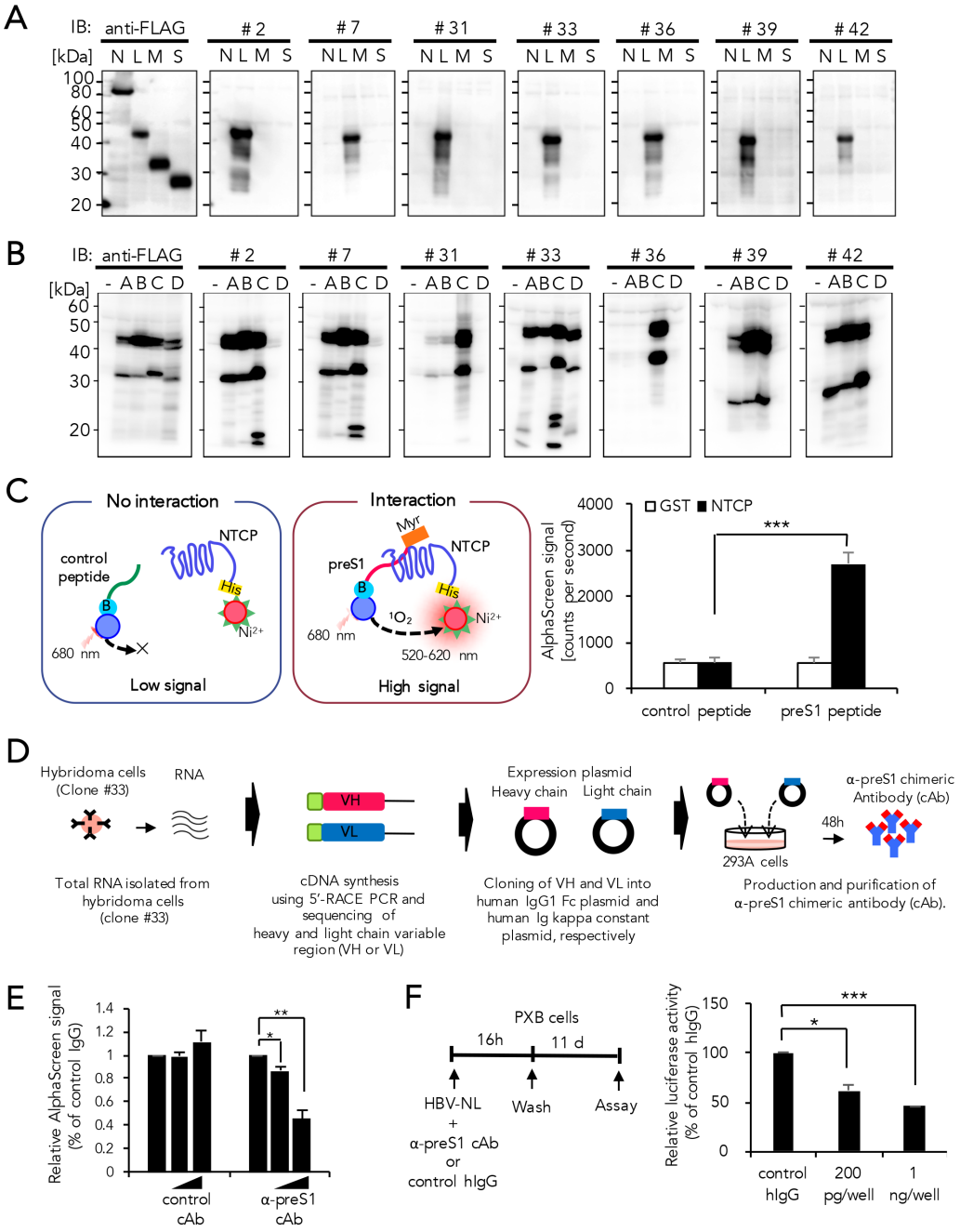
(C) α-HBs SNR cells sensed HBsAg at low cell density. Each cell number of α-HBs SNR cells was incubated with or without 33 ng/ml HBsAg at 37 ^{o}C for 48 hours followed by measurement of NanoLuc Activity. The presented data represents the mean ± SD of three independent experiments. **p*: <0.05, ***p*: < 0.01.

Figure S3. Single-cell analysis of α-HBs SNR cells exposed to HBV particles by bioluminescence imaging. Related to figure 2C and 2D.



(A,B) Luminescence intensity of α-HBs SNR cells exposed to HBV particles (A, corresponding to figure 2C) or co-cultured with HepG2.2.15.7 cells (B, corresponding to figure 2D). α-HBs SNR cells were recovered and transferred a multi-well glass bottom dish. After substrate addition, images were captured using an LV200 microscope. Exposure time was 1 second for NanoLuc. About 300 ~ 400 cells were counted per sample including non-luminescent and luminescent cells and the luminescence intensity of NanoLuc positive cells was calculated using imageJ software. Each dot represents a single cell.

Figure S4. Selection of α-HBs monoclonal antibodies by immunoblot and generation and characterization of α-HBs mouse-human chimeric antibodies. Related to figure 4 and 5.



(A) All generated α-HBs monoclonal antibodies recognized LHBs protein. Using recombinant HBs (genotype C) proteins produced by wheat germ protein production, immunoblot was performed with generated antibodies. N: Negative control protein

(HBV polymerase), L: LHBs protein, M: MHBs protein, S: SHBs protein.

(B) # 33 mAb detected LHBs of genotype A, B, C and D. Using LHBs-3xFLAG-over expression cell lysate derived from each genotype, immunoblot was performed with indicated antibodies. -: un-transfected cell lysate, A: genotype Ae-us, B: genotype Bj-JPN56, C: genotype C-JPNAT, D: genotype D-IND60.

(C) Development of preS1 peptide - NTCP interaction assay using AlphaScreen platform. Biotinylated preS1 peptide or non-specific peptide (control peptide) binds to streptavidin on the donor beads with an extremely high specificity and affinity. The nickel chelate acceptor beads are combined with His-tagged GST (as negative control protein) or NTCP. Interaction between preS1 peptide and preS1 forms a large complex with two kinds of beads through the antibody and streptavidin. Upon excitation at 680 nm, singlet oxygen molecules were produced from the donor beads, which reacted with the acceptor beads, resulting in light emission that was measured between 520 and 620 nm. The data shown represents the mean ± standard deviation (SD) of three independent experiments. ***p: < 0.001.

(D) Schematic diagram of generating α-preS1 (#33) chimeric antibody. RNA isolated from hybridoma cells producing α-preS1 (#33) mAb and converted to cDNA using 5'-RACE PCR. Identified heavy and light chain variable region sequences (VH or VL) were cloned to human IgG1 Fc vector or human Ig Kappa constant vector, respectively. After 48 hours post-transfection, secreted α-preS1 (#33) chimeric antibody (cAb) in a cell supernatant was purified.

(E) Inhibition of preS1 domain and NTCP interaction by α-preS1 cAb using AlphaScreen assay. AlphaScreen signal of biotinylated preS1 peptide and NTCP interaction was decreased in a dose-dependent manner by the addition of α-preS1 cAb. Each value is normalized to those obtained from control IgG and represents the mean ± SD of three independent experiments. *p: < 0.05, **p: < 0.01.

(F) α-preS1 cAb inhibits HBV infection. PXB cells were infected with HBV-NL in the presence of α-preS1 cAb or negative control mAb. Viral infectivity was determined by NanoLuc activity of infected cell lysates. Values obtained in control IgG were set as 100% and the presented data represents the mean ± SD of three independent experiments. *p: < 0.05, **p: < 0.01.

Transparent Methods

Single chain variable fragment (scFv) against HBs protein

The heavy and light chain sequences of two scFvs against "a" determinant region within S domain of HBs protein were obtained from a previously published sequence (GenBank accession number: S1_AF410257/AF410258 and S2_AB027447/AB027448) (Maeda et al., 2005). The scFvs sequences of heavy chain-linker-light chain were synthesized as codon-optimized genes. The cDNA and amino acid sequences of the two scFvs are shown below.

Name	Binding Region	GeneBank No.		scFv sequence_VH-(gggs)4-VL
S1	SHBs	AF410257 and AF410258	DNA	CAAGTTCAGCTGCAGCAGTCCGGCACCGAGATGGTGAGGCCCGGCACATCCGTGAA GGTGAGCTGTAAGGCCAGCGGCTACCCCTTTACCAACCATTTAATCGAGTGGGTGAA GCAGAGACCCGGACAAGGTCCCGAATGGATCGGCGTGATCAATCCCGGCTCTGGAG GCACCAACTACAACGAGAAGTTCAAGGGCAAGGCCACTTTAACCGCCGATAAGTCCA GCAGCACCGCCTACATGCAGCTGAACTCTTTAACCTCCGACGACAGCGCCGTGTACT TCTGCGCCATCATGACCACCTTTTTAGGCGAGGGCTACGCCATGGACTACTGGGGAC AAGGTACCAGCGTCACCGTGTCTAGCGGCGGCGGCGGCAGCGGTGGCGGAGGCTC TGGAGGAGGAGGAAGCGGAGGTGGAGGCAGCGACATCGTCATGTCCCAGTCCCCC AGCTCTTTAGCTGTGAGCGTGGGCGAGAAGGTGACCATGAGCTGCAAGAGCTCCCA GTCTTTACTGTACAGCGGCAACCAGAAGAACTATTTAGCTTGGTACCAGCAGAAGCCC GGACAGAGCCCCGAGTTATTAATCTACTGGGCCTCCACAAGGGAATCCGGCGTGCCC GATCGTTTTACCGGCAGCGGTTCTGGCACCGACTTCACTTTAACCATCAGCAGCGTGA AGGCCGAGGATTTAGCCGTGTATTACTGCCAGCAGTACTACTCCTATAGGACCTTCGG CGGAGGTACCAAGCTGGAGATCAAGAGGGCCGACGCT
			Amino Acid	QVQLQQSGTEMVRPGTSVKVSCKASGYPFTNHLIEWVKQRPGQGPEWIGVINPGSGGTNY NEKFKGKATLTADKSSSTAYMQLNSLTSDDSAVYFCAIMTTFLGEGYAMDYWGQGTSVTVSS ggggsggggsgggsggggsDIVMSQSPSSLAVSVGEKVTMSCKSSQSLLYSGNQKNYLAWYQ QKPGQSPELLIYWASTRESGVPDRFTGSGSGTDFTLTISSVKAEDLAVYYCQQYYSYRTFGGG TKLEIKRADA
S2	"a" determinant of SHBs	AB027447 and AB027448	DNA	CAAGTTCAGCTGCAAGAATGGGGACCCGGTCTGGCCAGACCCAGCGGAACTTTATCT TTAACATGCAGCGTGTCCGGCGGCAGCATCAGCGGAACCAGCCACTACTGGGGCTG GATCAGACAGCCCCCCGGCAAAGGCCTCGAATGGATCGGCAGCATCTACTTCTCCGG CGCCACACACTACAACCCCTCCCTCAAGTCTCGTGTGACCATCAACGTGGACACCAG CAACAACCAGTTCTCTTTAAATCTGAGGAGCATGACCGCCGCCGACACAGCCGTGTA CTACTGCACTCGTCTGATCAAGGACTACAACGGCTGGGCTTTATTCCAGCAGTACTAT TTCATGGACGTGTGGGGCAAGGGCACAACTGTGACCGTGAGCTCTGGAGGAGGCGG ATCTGGAGGAGGAGGAAGCGGAGGAGGAGGTAGCGGAGGTGGCGGAAGCCAGAG CGTGCTGACCCAGCCTCCTAGCGTGAGCGGCGCTCCCGGTCAGAGGATCAGCATCA GCTGCATCGGCAGCAGCTCCAACATCGGCGCCGGCTACGACGTCCAGTGGTACCAG CAGCTGCCCGGAACAGCCCCCAAGCTGCTGATCTTCGTGAACATCAATCGTCCCAGC GGCGTGCCCGATAGGTTTTCCGGCAGCAAGTCCGGCACCTCCGCTTCTTTAGACATTA CTGGTTTACAAGCTGAGGACGAGGCCGACTACTACTGCCAGTCCTACGACTCCTCTTT ATCCGGCTACGTGTTCGGCAGCGGCACCACAGTGACTGTGCTG
			Amino Acid	QVQLQEWGPGLARPSGTLSLTCSVSGGSISGTSHYWGWIRQPPGKGLEWIGSIYFSGATHYN PSLKSRVTINVDTSNNQFSLNLRSMTAADTAVYYCTRLIKDYNGWALFQQYYFMDVWGKGTT VTVSSggggsggggsggggsggggsQSVLTQPPSVSGAPGQRISISCIGSSSNIGAGYDVQWYQQ LPGTAPKLLIFVNINRPSGVPDRFSGSKSGTSASLDITGLQAEDEADYYCQSYDSSLSGYVFGS GTTVTVL

Vector construction

SynNotch receptor vector was purchased from addgene (plasmid #79125) (Morsut et al., 2016). We inserted two restriction enzyme sites, BstZII and BamHI, to easily customize a part of scFv region in the original synNotch vector (cDNA sequence below). PCR-amplified scFv insert DNA was cloning into a linearized SynNotch vector digested by BstZII and BamHI using In-Fusion cloning technology (TaKaRa Bio) according to the manufacturer's instructions (Fu et al., 2014).

Gene name	DNA sequence	
SynNotch receptor	ATGGCGCTCCCTGTCACCGCACTGCTTCTTCCGCTGGCACTGCTGCTGCACGCTGCA CGGCCTGAGCAAAAACTTATCTCTGAAGAGGACCTCGTATAC ----- scFv ORF ----- GGATCCATCCTGGACTACAGCTTCACAGGTGGCGCTGGGCGCGACATTCCCCCACC GCAGATTGAGGAGGCCTGTGAGCTGCCTGAGTGCCAGGTGGATGCAGGCAATAAGG TCTGCAACCTGCAGTGTAATAATCACGCATGTGGCTGGGATGGTGGCGACTGCTCCC TCAACTTCAATGACCCCTGGAAGAACTGCACGCAGTCTCTACAGTGCTGGAAGTATTT TAGCGACGGCCACTGTGACAGCCAGTGCAACTCGGCCGGCTGCCTCTTTGATGGCTT CGACTGCCAGCTCACCGAGGGACAGTGCAACCCCCTGTATGACCAGTACTGCAAGG ACCACTTCAGTGATGGCCACTGCGACCAGGGCTGTAACAGTGCCGAATGTGAGTGG GATGGCCTAGACTGTGCTGAGCATGTACCCGAGCGGCTGGCAGCCGGCACCCTGGT GCTGGTGGTGCTGCTTCCACCCGACCAGCTACGGAACAACTCCTTCCACTTTCTGCG GGAGCTCAGCCACGTGCTGCACACCAACGTGGTCTTCAAGCGTGATGCGCAAGGCC AGCAGATGATCTTCCCGTACTATGGCCACGAGGAAGAGCTGCGCAAGCACCCAATCA AGCGCTCTACAGTGGGTTGGGCCACCTCTTCACTGCTTCCTGGTACCAGTGGTGGGC GCCAGCGCAGGGAGCTGGACCCCATGGACATCCGTGGCTCCATTGTCTACCTGGAG ATCGACAACCGGCAATGTGTGCAGTCATCCTCGCAGTGCTTCCAGAGTGCCACCGAT GTGGCTGCCTTCCTAGGTGCTCTTGCGTCACTTGGCAGCCTCAATATTCCTTACAAGA TTGAGGCCGTGAAGAGTGAGCCGGTGGAGCCTCCGCTGCCCTCGCAGCTGCACCTC ATGTACGTGGCAGCGGCCGCCTTCGTGCTCCTGTTCTTTGTGGGCTGTGGGGTGCTG CTGTCCCGCAAGCGCCGGCGGATGAAGCTGCTGAGCAGCATCGAGCAGGCCTGTGA CATCTGCCGGCTGAAGAAACTGAAGTGCAGCAAAGAAAAGCCCAAGTGCGCCAAGT GCCTGAAGAACAACTGGGAGTGCCGGTACAGCCCCAAGACCAAGAGAAGCCCCCTG ACCAGAGCCCACCTGACCGAGGTGGAAAGCCGGCTGGAAAGACTGGAACAGCTGTT TCTGCTGATCTTCCCACGCGAGGACCTGGACATGATCCTGAAGATGGACAGCCTGCA GGACATCAAGGCCCTGCTGACCGGCCTGTTCGTGCAGGACAACGTGAACAAGGACG CCGTGACCGACAGACTGGCCAGCGTGGAAACCGACATGCCCCTGACCCTGCGGCA GCACAGAATCAGCGCCACCAGCAGCAGCGAGGAAAGCAGCAACAAGGGCCAGCGG CAGCTGACAGTGTCTGCTGCTGCAGGCGGAAGCGGAGGCTCTGGCGGATCTGATGC CCTGGACGACTTCGACCTGGATATGCTGGGCAGCGACGCCCTGGATGATTTTGATCT GGACATGCTGGGATCTGACGCTCTGGACGATTTCGATCTCGACATGTTGGGATCAGA TGCACTGGATGACTTTGACCTGGACATGCTCGGATCATGA	signal peptide-myctag-BstZ17I-scFv ORF-BamHI-Notch core seq-Gal4-VP64 sequence (original vector: addegene #79125)

Response vector including Gal4-VP64 binding region, pGreenFire-Gal4 which express copGFP reporter gene followed by the self-cleaving T2A peptide and the firefly luciferase gene under the control of Gal4-specific transcription response element and constitutive puromycin gene as a marker, was purchased from system biosciences (plasmid #TR017PA-P). We designed the output response genes (secreted NanoLuc luciferase, human IFNB1-HiBiT and neutralizing chimeric antibody) were cloned into PCR-amplified linear pGreenFire-Gal4 vector using In-Fusion cloning technology. The cDNA sequences of secreted NanoLuc luciferase, human IFNB1-HiBiT and neutralizing chimeric antibody are presented below.

Gene name	DNA sequence	
secreated NanoLuc luciferase	ATGAACTCCTTCTCCACAAGCGCCTTCGGTCCAGTTGCCTTCTCCCTGGGCCTGCTCCTG GTGTTGCCTGCTGCCTTCCCTGCCCCAGTCTTCACACTCGAAGATTTCGTTGGGGACTG GCGACAGACAGCCGGCTACAACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGT TTGTTTCAGAATCTCGGGGTGTCCGTAACTCCGATCCAAAGGATTGTCCTGAGCGGTGA AAATGGGCTGAAGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGGCGACC AAATGGGCCAGATCGAAAAAATTTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTA AGGTGATCCTGCACTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGATCGA CTATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTGTAA CAGGGACCCTGTGGAACGGCAACAAAATTATCGACGAGCGCCTGATCAACCCCGACGG CTCCCTGCTGTTCCGAGTAACCATCAACGGAGTGACCGGCTGGCGGCTGTGCGAACGC ATTCTGGCGTAG	GeneBank accession No. JQ437372 (Promega)
Human Interferon beta1 -HiBiT	ATAAGTGTTTACTGCAGATCGCTTTACTGCTGTGTTTCTCCACCACCGCTTTAAGCATGAG CTACAATTTATTAGGATTTTTACAGAGAAGCTCCAACTTCCAGTGCCAGAAGTTATTATGG CAGCTGAATGGAAGACTGGAGTACTGTTTAAAGGATCGTATGAACTTCGACATTCCCGA GGAGATCAAGCAGCTGCAGCAGTTCCAGAAGGAGGACGCTGCTTTAACCATCTATGAG ATGCTGCAGAACATCTTTGCCATCTTTCGTCAAGATAGCAGCTCCACCGGCTGGAACGA GACAATCGTGGAGAATTTACTCGCCAACGTGTACCACCAGATCAACCATTTAAAGACCG TGCTGGAGGAGAAGCTGGAGAAGGAGGACTTCACTCGTGGCAAGCTGATGAGCTCTTT ACATTTAAAGAGGTACTACGGTCGTATTTTACACTATTTAAAGGCCAAGGAGTACTCCCA CTGCGCTTGGACCATCGTGAGGGTGGAGATTTTAAGGAACTTCTACTTCATCAATCGTCT CACCGGCTATTTAAGGAACtctggagctagtgctggaGTGAGCGGCTGGCGGCTGTTCAAGAA GATTAGCTAG	IFNB1: artificial gene (original sequence: UniProtKB-P01574) HiBiT: artificial gene (original sequence by Promega) lowercase letters: linker sequence
#33 cAb sequence in a single-frame	atggaatgtaactggatacttcctttttattctgtcggtaacttcaggggtctattcaGAGGTGCAGCTTGTTGAGTCT GGTGGAGGATTGGTGCAGCCTAAAGGATCATTGAAACTCTCATGTGCCGCCTCTGGTTT CACCTTCAATACCTATGCCATGCACTGGGTCTGCCAGGCTCCAGGAAAGGGTTTGAAAT GGGTTGCTCGCATAAGAAGTAAAAGTAATAATTATGCAACATATTATGCCGATTCAGTGA AAGACAGATTCATCATCTCCAGAGATGATTCACAAAGCATGCTCTATCTGCAAATGAACA ACCTGAAAACTGAGGACACAGCCATGTATTACTGTGTGAGAGAGGGCAACGGCTTTGA CTACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCAGCTAGCACCAAGGGCCCATCG GTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCT GCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCT GACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCA GCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGT GAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGAC AAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCT TCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACA TGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGG ACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCA CGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGA GTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCA AAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGG AGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGA CATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCC TCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGA GCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAA CCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAAaggaagagaaggACCGGTGA GGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAGAATCCCGGCCCTTCCGGT atggtgtccacttctcagctccttggacttttgctttctggacttcagcctccagatgtgacattgtgatgtcacagtctccatcct ccctagctgtgtcagttggagagaaggttactatgagctgcaagtccagtcagagccttttatatagtagcaatcaaaagaact acttggcctggtaccagcagaaaccagggcagtcctcctaaactgctgatttcctgggcatccactaggggaatctgggggtccct gatcgcttcacaggcagtggatctgggacagatttcagtctcaccatcagcagtgtgaaggctgaagacctggcagtttatta ctgtcagcaatattatagctatccgtacacgttcggaggggggaccaagctggaaataaaacggggctgatgctcgtaccggt ggctgcaccatctgtcttcatcttcccgccatctgatgagcagttgaaatctggaactgcctctgttgtgtgcctgctgaataact tctatcccagaggaggccaaagtacagtggaaggtggataacgccctccaatcgggtaactcccaggagagtgtcacagag caggacagcaaggacagcacctacagcctcagcagcaccctgacgctgagcaaagcagactacgagaaacacaaagtct acgcctgcgaagtcacccatcagggcctgagctcgcccgtcacaaagagcttcaacaggggagagtgttag	Signal peptide (L) – #33 heavy chain sequence (C) – furin cleavage site (L) - T2A sequence (C)- #33 light chain sequence (L) L: Lowercase letters C: Capital letters

The heavy chain and light chain expression vectors of chimeric antibody, pFUSE-CHIg-hG1 and pFUSE2-CLIg-hk, respectively, were purchased from invivogen (Takebe et al., 1988; Yu and Russell, 2001). The identified sequence of heavy and light chain variable regions was inserted to each vector digested by EcoRI-NheI and NcoI-BsiWI, respectively, using In-Fusion technology. Single frame of #33 chimeric

antibody was amplified by PCR and cloning into PCR-amplified linear pGreeFire-Gal4 vector using In-Fusion technology. Primers used for cloning are shown below.

		Sequence
Heavy chain of α-preS1 (#33) mAb	DNA	gaggtgcagcttgttgagtctggtggaggattggtgcagcctaaaggatcattgaaactctcatgtgccgcctctggtttcaccttcaatac ctatgccatgcactgggtctgccaggctccaggaaagggtttgaaatgggttgctcgcataagaagtaaaagtaataattatgcaacatatt atgccgattcagtgaaagacagattcatcatctccagagatgattcacaaagcatgctctatctgcaaatgaacaacctgaaaactgaggac acagccatgtattactgtgtgagagagggcaacggctttgactactggggccaaggcaccactctcacagtctcctca
	Amino Acid	EVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMHWVCQAPGKGLKWVARIRSKSNNYATYYADSVKDR FIISRDDSQSMLYLQMNNLKTEDTAMYYCVREGNGFDYWGQGTTLTVSS
Light chain of α-preS1 (#33) mAb	DNA	gacattgtgatgtcacagtctccatcctccctagctgtgtcagttggagagaaggttactatgagctgcaagtccagtcagagccttttatat agtagcaatcaaaagaactacttggcctggtaccagcagaaaccagggcagtctcctaaactgctgatttcctgggcatccactagggaat ctggggtccctgatcgcttcacaggcagtggatctgggacagatttcagtctcaccatcagcagtgtgaaggctgaagacctggcagttt attactgtcagcaatattatagctatccgtacacgttcggaggggggaccaagctggaaataaaacgggctgatgct
	Amino Acid	DIVMSQSPSSLAVSVGEKVTMSCKSSQSLLYSSNQKNYLAWYQQKPGQSPKLLISWASTRESGVPDRFTG SGSGTDFSLTISSVKAEDLAVYYCQQYYSYPYTFGGGTKLEIKRADA

Produced vector name	Primer name	Primer seq (5'- seqence -3')
SynNotch vector	BstZ17I-S1scFv-Syn-F	GAGGACCTCGTATACCAAGTTCAGCTGCAG
	BamHI-S1scFv-Syn-R	GTCCAGGATGGATCCAGCGTCGGCCCTCTTG
	BstZ17I-S2scFv-Syn-F	GAGGACCTCGTATACCAAGTTCAGCTGCAAG
	BamHI-S2scFv-Syn-R	GTCCAGGATGGATCCCAGCACAGTCACTG
Reporter vector for secNL gene	pGF1-F2	CTCGAGTCGACTCGACAATCAACC
	pGF1-R2	CATGGTGGCGGATCCAACGAATG
secNL gene into reportor gene	IF-secNL-F	TGGATCCGCCACCATGAACTCCTTCTCCACAA
	IF-secNL-R	TCGAGTCGACTCGAGCTACGCCAGAATGCG
Reporter vector for IFNB1 gene	Gal4-BamHI-R	GGATCCAACGAATGTCGAGAGGCTGG
	T1-Hi-F	TCTGGAGCTAGTGCTGGAGTGAGC
IFNB1 into reportor gene	BamHI-hIFNB1-F	TCGAGTCGACTCGAGCTACGCCAGAATGCG
	T1-hIFNB1-R	AGCACTAGCTCCAGAGTTCCTTAAATAGCCGG
#33 chimeric antibody vector	HBs vH F	GATCACCGGTGAATTCACCATGGTGTTGGGGCTG
	HBs vH R	GGCCCTTGGTGCTAGCTGAGGAGACTGTGAG
	HBs vL F	CACCGGTCACCATGGACCATGTTACTGCTGC
	HBs vL R	TGCAGCCACCGTACGAGCATCAGCCCG
single frame sequence of #33 chimeric antibody into pGreenFire-Gal4	GF1-33hG-F	ACATTCGTTGGATCCACCATGGAATGTAACTGG
	Furin-33H-R	CCTTCTCTTCCTTTTACCCGGAGACAGGG
	T2A-F	AAAAGGAAGAGAAGGACCGGTGAGGGCAGAGG
	T2A-R	AGAAGTGGACACCATACCGGAAGGGCC
	33L-F	ATGGTGTCCACTTCTCAGCTCC
	GF1-33hk-R	TCGAGTCGACTCGAGCTAACACTCTCCCC

Cell culture

Jurkat cells were cultured in RPMI containing 10% (V/V) fetal bovine serum (FBS; Gibco-BRL). HepG2 cells were maintained on collagen-coated dishes with DMEM containing 10% (V/V) FBS. HepG2.2.15.7 cells were maintained on collagen-coated dishes with DMEM/F-12, GlutaMAX (Life Technologies) supplemented with 10% (V/V) FBS, 10mM HEPES and 5 μg/ml insulin. To generate

HepG2 Tet-ON-SHBs cells, a HepG2 Tet-ON Advanced Cell (Clontech) parental cell line was transduced with a retroviral vector encoding the SHBs gene fused to a tetracycline-responsive element, then was selected with puromycin (1 μg/ml), and cultured with DMEM (Wako) supplemented with 10% FBS. Unless otherwise indicated, Tet-ON-SHBs cells were treated with 5μg/ml doxycycline (Sigma-Aldrich) for 24 hours before experiments. HepG2 Tet-ON-NTCP (HepG2-iNTCP) cells were cultured as previously reported (Miyakawa et al., 2018). Primary human hepatocytes (PXB cells) and the specific medium were purchased from PhoenixBio.

Production of lentiviral vectors and synNotch cells

SynNotch cells were established using lentivirus vector system (Morsut et al., 2016). In brief, lentivirus was produced by co-transfecting either response plasmid or synNotch plasmid and vectors encoding packaging proteins (pCMV-VSVg and pHIV-GagPol) using Lipofectamine 3000 reagent (Invitrogen) in lintiX-293A cells (TaKaRa Bio) plated in 6 cm dish at approximately 70% confluence. Viral supernatants were collected 2 days after transfection, by filtering through 0.45 μm pore sized membrane filter and were used for transduction immediately or stored at -80 ^{o}C for later use.

For producing synNotch cells, the initial step was the incorporation of each reporter vector into Jurkat cells. For lentiviral transduction, viral supernatant including each response plasmid at MOI of ~50 was added directly to ~ 1x 10^{6} cells Jurkat cells seeded in 12 well plates. Viral media was replaced with sterile growth medium 24 hours post-infection and cultured further for 2 days. The cultured cells were subsequently selected with 1 μg/ml puromycin. Viral supernatant including each synNotch plasmid were concentrated approximately 15 times using Lenti-X concentrator (TaKaRa Bio) according to the manufacturer's instructions (Ginsberg et al., 2015). The concentrated viral supernatant at MOI of ~100 was mixed ViroMag (OZ Biosciences) and then added directly to ~ 5x 10^{5} reporter gene-transduced Jurkat cells plated in 12-well plates. They

were cultured for 24 hour using the recommended magnetic plate (OZ Biosciences) and viral media was replaced with sterile growth medium.

For single-cell clonal population establishment, α-HBs SNR cells that responded to express GFP were seeded $1x10^7$ cells in the 25 cm^2 flask and stimulated with 70 ng/ml SHBsAg for 24 hours. The population of GFP-positive cells was then sorted using a FACS ARIA II. Subsequently, single cells were isolated as 24 clones from GFP-positive pool cells using limited dilution method. Among them, the clone that showed better ON/OFF response was selected to perform further tests using fluorescence imaging and flow cytometry.

Flow cytometry

To determine the surface expression level of SynNotch receptor, α-HBs SNR cells were incubated with anti-myc antibody (clone 9E10, ThermoFisher) in PBS containing 2% FBS and 0.02% azide. Cells were then stained with PE-conjugated secondary antibody (#12-4010-82, invitrogen). GFP-positive α-HBs SNR cells stimulated with HBV particle were fixed with 4% formalin. Flow cytometry was performed using a FACSCanto II instrument (BD Biosciences), and data were analyzed with FlowJo software (TreeStar).

Validation of synNotch function

α-HBs SNR cells were seeded at a concentration of $1x10^4$ cells/well in a 96-well plate and then recombinant LHBs protein (Beacle. Inc.) or HBsAg or HBV particles were added and incubated for 48 hours at 37 ^oC, 5% CO_2. In case of co-culture with Tet-ON-SHBs cells or HepG2.2.15.7 cells, 3×10^4 cells or 3×10^5cells target cells were plated in each well of a 96- or 12-well collagen-coated plate with $1x10^4$ or 5×10^5 cells α-HBs SNR cells and then cells were co-cultured for 48 hours. For detecting synNotch cells' activation, secreted Nano-Luciferase (secNL) or IFNB1-HiBiT in the cell supernatant was measured using Nano-Glo luciferase assay system or Nano-Glo HiBiT

Extracellular Detection System (Promega), respectively, according to the manufacturer's instructions. The secreted chimeric antibodies (α-preS1 ncAb) were measured by biotinylated preS1 peptide using AlphaScreen assay. Briefly, cellular supernatant containing α-preS1 ncAb was incubated with biotinylated preS1 peptide in the 384-well OptiPlate (PerkinElmer) for 30 min at 26 ^{o}C and then added to proteinG acceptor beads and Streptavidin-coated donor beads. After incubation for 1min at 26^{o}C, AlphaScreen signal was analyzed by the AlphaScreen detection program with Envision (Perkin Elmer). Image of GFP-positive cells were captured using a luminescence microscopy (KEYENCE).

Imaging and image analysis

NanoLuc luciferase images at the single-cell level were obtained using the LUMINOVIEW LV200 imaging system (Olympus) as described previously (Horibe et al., 2014; Ogoh et al., 2014). Briefly, after stimulation with HBV particles or without stimulation, synNotch cells were recovered and re-suspended with PBS. In case of co-cultured experiment, cells were treated with trypsin for 2 min and only α-HBs SNR cells were recovered by gentle pipetting. The cells were then transferred to a multi-well glass-bottom dish. Images were taken using a 20x objective lens with 1 second exposures after adding the furimazine substrate (Promega) at 50-fold dilution. Data analysis was performed using ImageJ software (Hartig, 2013). All images were changed to 8-bit grayscale, subtracted background noise and then threshold was defined (Otsu mode). A total of 300-400 cells were counted for each sample including luminescent and non-luminescent cells. Using phase contrast image, the luminescence intensity of each bioluminescent image was defined as follows: (area integrated intensity) x (mean grey value). Data was analyzed and graphed using GraphPad Prism software (GraphPad Software Inc.).

Generation of α-HBs monoclonal antibody

Immunization of BALB/c mice with full-length recombinant LHBs protein (Beacle. Inc.) and generation of hybridoma cells producing α-HBs antibody were performed as previously described (Matsunaga et al., 2014; Yamaoka et al., 2016). Epitope identification of the generated antibodies was performed by immunoblot analysis using recombinant HBs proteins produced by wheat germ cell-free protein system (CellFree Sciences) or LHBs-3x FLAG-overexpression cell lysate. To identify the variable region of heavy and light chain, antibody's mRNA was extracted from hybridoma cells using RNeasy kit (QIAGEN). Then cDNA synthesis was performed using SMARTer RACE 5'/3' Kit (TaKaRa) according to manufacturer's instructions and then sequenced the variable region. Reverse transcription primers were designed for the constant regions of heavy and light (κ) chain based on the sequence of mouse IgG classes registered in the NCBI nucleotide database; heavy chain; 5'-gattacgccaagcttGGAAATAGCCCTTGACC-3', light chain (κ); 5'-gattacgccaagcttGGCACCTCCAGATGTTAAC-3' (under line; vector specific sequence).

Protein-protein interaction using AlphaScreen

Inhibitor assay of preS1 peptide and NTCP interaction was established according to previous reports (Saso et al., 2018; Watashi et al., 2014). Briefly, recombinant NTCP was synthesized using wheat cell-free protein system. 70 nM biotinylated preS1 peptide was incubated with serially diluted antibody at 26 °C for 10 min followed by the addition of 0.1μl of recombinant NTCP protein in a 384-well OptiPlate and the mixtures were then incubated at 26 °C for 1 hour. Then the Nickel Chelate acceptor beads and streptavidin-coated donor beads were added to the 384-well plate. After further 1 hour incubation at 26 °C, AlphaScreen signal was analyzed by the AlphaScreen detection program with Envision (Perkin Elmer) as described previously (Eglen et al., 2008).

HBV preparation and infection

Wild-type HBV and HBV reporter viruses (HBV-NL) were prepared as previously reported (Miyakawa et al., 2018; Nishitsuji et al., 2015). Briefly, wild-type HBV and HBV-NL were derived from the supernatants of HepG2.2.15.7 cells and concentrated approximately 100 times using a PEG Virus precipitation kit (Bio Vision). Cells were infected with wild-type HBV at a concentration of 500 genome equivalents per cell in the presence of 4% PEG8000 for 16 hours. Alternatively, cells in a 48- or 98-well plate were inoculated with 1 μl or 0.4 μl of HBV in the presence of 4% PEG8000 for 16 hours. HBV-infected cells were cultured in fresh medium for an additional 5-6 days and their infectivity was determined by vDNA quantification in culture supernatants, as previously described (Miyakawa et al., 2015). The infectivity of HBV-NL was quantified using the Nano-Glo Luciferase System (Promega) according to the manufacturer's instructions.

Quantification of HBs proteins (HBsAg)

HBs antigen derived from HepG2 Tet-On-SHBs cells or HepG2.2.15.7 cells was quantified by enzyme-linked immunosorbent assay (ELISA; Alpha Diagnostic Intl Inc.) as described previously (Miyakawa et al., 2018). HBsAg amount was calculated with standard curve using control HBsAg according to the manufacturer's instructions.

Gene expression analysis

Messenger RNA extraction and subsequent cDNA synthesis was performed using ISOGEN (NIPPON GENE) and ReverTra Ace (Toyobo), respectively, according to their corresponding manufacturer's instructions. Gene expression was analyzed by qPCR using SYBR Premix Ex Taq II (TaKaRa). The primer pairs used were 5'-GGCTGTTTACCAGACTCCGACA -3' and 5'-CACAAAGCCTGGCAGCTCTCTA -3' for Mx1; 5'-CTCTGAGCATCCTGGTGAGGAA -3' and 5'-AAGGTCAGCCAGAACAGGTCGT -3' for ISG15.

Statistical analyses

All data were analyzed for statistical significances using unpaired t-test. Difference was assessed with two-side with a significance level of 0.05. Calculation of half maximal effective value (Figure 2B) was performed using GraphPad Prism software (GraphPad Software Inc.).

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