YMTHE, Volume *29*

Supplemental Information

ADAR1 Stimulation by IFN-α Downregulates the Expression of MAVS via RNA Editing to Regulate the Anti-HBV Response

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FIG. S1

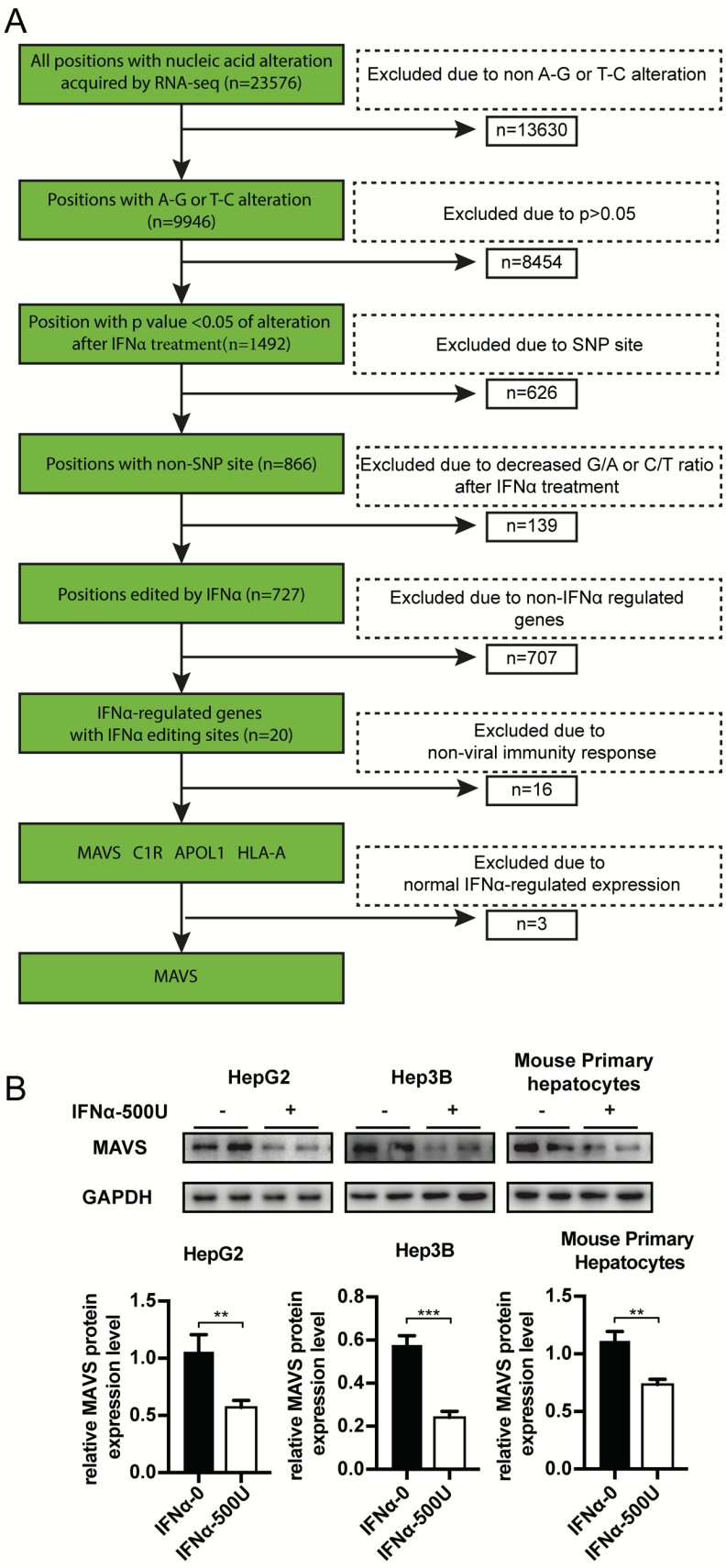

A
All positions with nucleic acid alteration acquired by RNA-seq (n=23576)
Excluded due to non A-G or T-C alteration
n=13630
Positions with A-G or T-C alteration (n=9946)
Excluded due to p>0.05
n=8454
Position with p value <0.05 of alteration after IFNα treatment(n=1492)
Excluded due to SNP site
n=626
Positions with non-SNP site (n=866)
Excluded due to decreased G/A or C/T ratio after IFNα treatment
n=139
Positions edited by IFNα (n=727)
Excluded due to non-IFNα regulated genes
n=707
IFNα-regulated genes with IFNα editing sites (n=20)
Excluded due to non-viral immunity response
n=16
MAVS C1R APOL1 HLA-A
Excluded due to normal IFNα-regulated expression
n=3
MAVS
B
HepG2
Hep3B
Mouse Primary hepatocytes
IFNα-500U
MAVS
GAPDH
relative MAVS protein expression level
IFNα-0
IFNα-500U
Mouse Primary Hepatocytes

FIG. S2

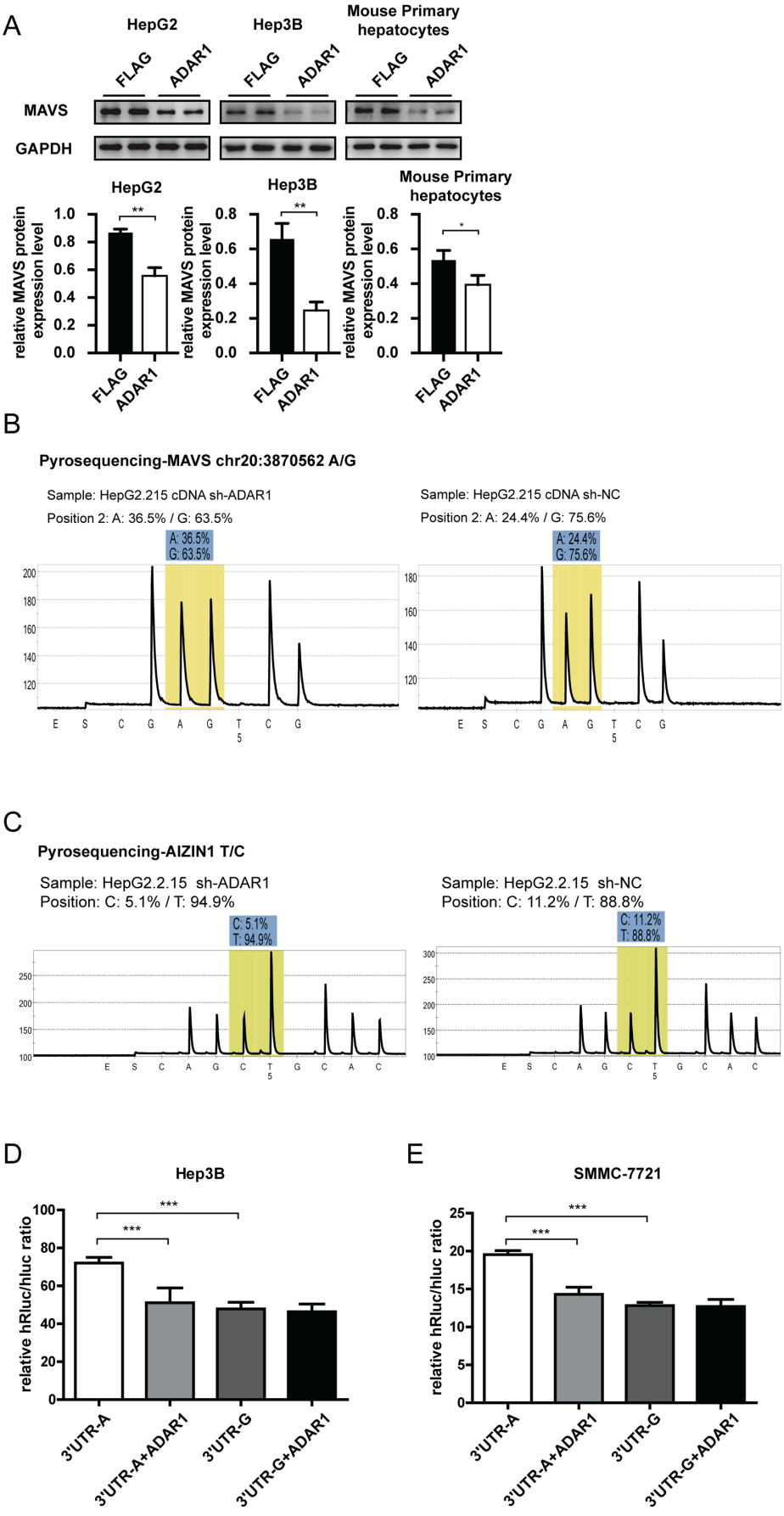

A
HepG2
Hep3B
Mouse Primary hepatocytes
FLAG
ADAR1
MAVS
GAPDH
relative MAVS protein expression level
B
Pyrosequencing-MAVS chr20:3870562 A/G
Sample: HepG2.215 cDNA sh-ADAR1
Position 2: A: 36.5% / G: 63.5%
Sample: HepG2.215 cDNA sh-NC
Position 2: A: 24.4% / G: 75.6%
C
Pyrosequencing-AIZIN1 T/C
Sample: HepG2.2.15 sh-ADAR1
Position: C: 5.1% / T: 94.9%
Sample: HepG2.2.15 sh-NC
Position: C: 11.2% / T: 88.8%
D
Hep3B
relative hRluc/hluc ratio
3'UTR-A
3'UTR-A+ADAR1
3'UTR-G
3'UTR-G+ADAR1
E
SMMC-7721

FIG. S3

A

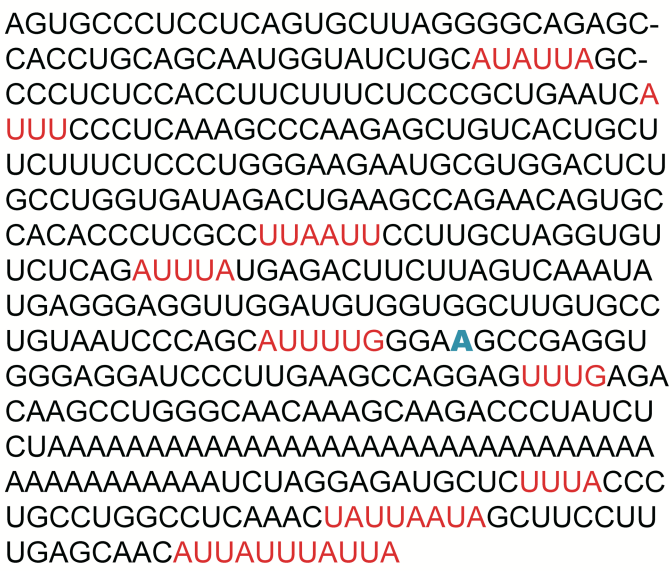

AGUGCCCUCCUCAGUGCUUAGGGGCAGAGC-
CACCUGCAGCAAUGGUAUCUGCAUAUUAGC-
CCCUCUCCACCUUCUUUCUCCCGCUGAAUCA
UUUCCCUCAAAGCCCAAGAGCUGUCACUGCU
UCUUUCUCCCUGGGAAGAAUGCGUGGACUCU
GCCUGGUGAUAGACUGAAGCCAGAACAGUGC
CACACCCUCGCCUUAAUUCCUUGCUAGGUGU
UCUCAGAUUUAUGAGACUUCUUAGUCAAAUA
UGAGGGAGGUUGGAUGUGGUGGCUUGUGCC
UGUAAUCCCAGCAUUUUGGGAAGCCGAGGU
GGGAGGAUCCCUUGAAGCCAGGAGUUUGAGA
CAAGCCUGGGCAACAAAGCAAGACCCUAUCU
CUAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAUCUAGGAGAUGCUCUUUACCC
UGCCUGGCCUCAAACUAUUAAUAGCUUCCUU
UGAGCAACAUUAUUUAUUA

FIG. S4

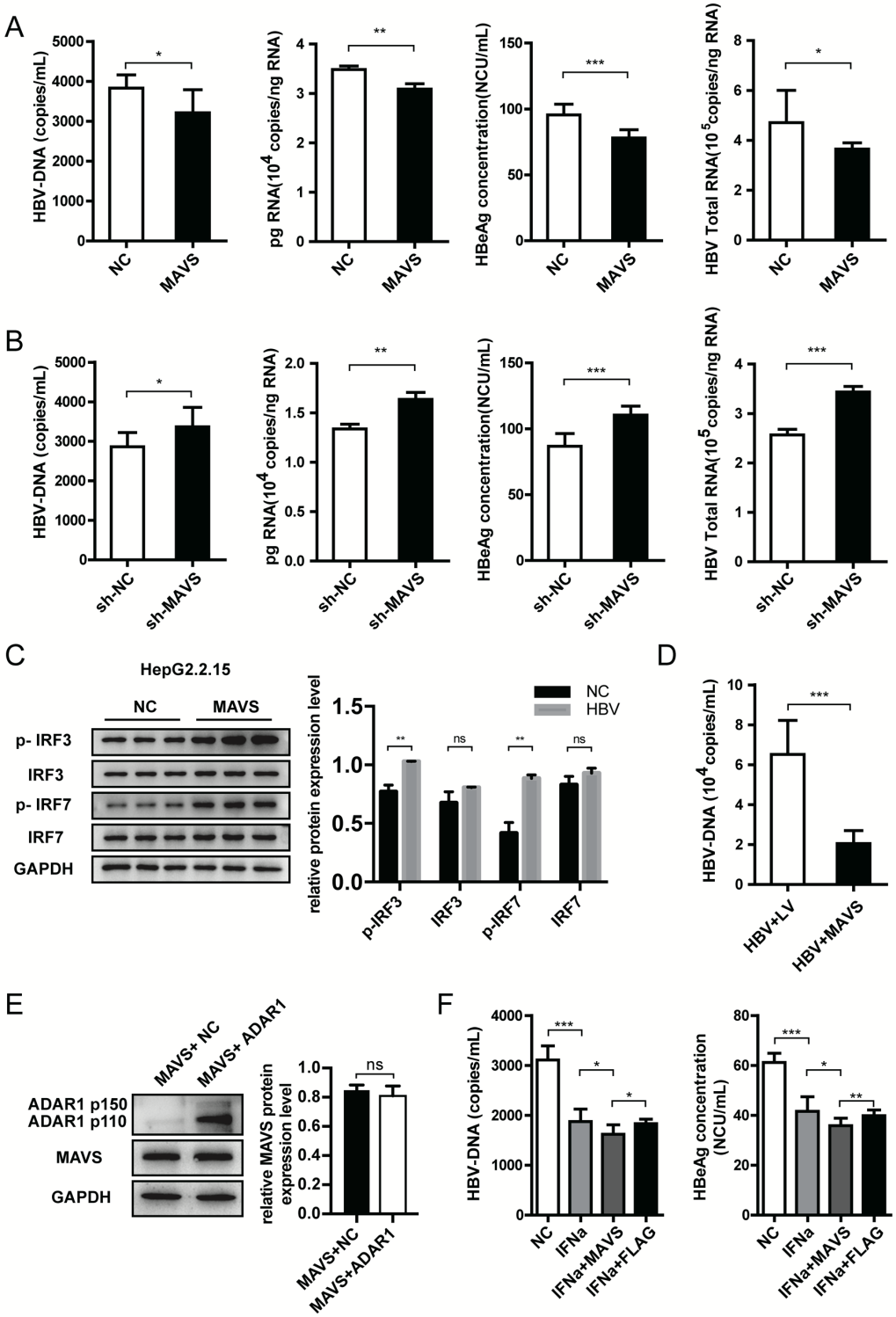

A
HBV-DNA (copies/mL)
pg RNA(10^4 copies/ng RNA)
HBeAg concentration(NCU/mL)
HBV Total RNA(10^5copies/ng RNA)
NC
MAVS
B
sh-NC
sh-MAVS
C
HepG2.2.15
NC
MAVS
p- IRF3
IRF3
p- IRF7
IRF7
GAPDH
relative protein expression level
HBV
p-IRF3
IRF3
p-IRF7
IRF7
D
HBV-DNA (10^4copies/mL)
HBV+LV
HBV+MAVS
E
MAVS+ NC
MAVS+ ADAR1
ADAR1 p150
ADAR1 p110
MAVS
GAPDH
relative MAVS protein expression level
MAVS+NC
MAVS+ADAR1
F
HBV-DNA (copies/mL)
HBeAg concentration (NCU/mL)
IFNa
IFNa+MAVS
IFNa+FLAG

FIG. S5

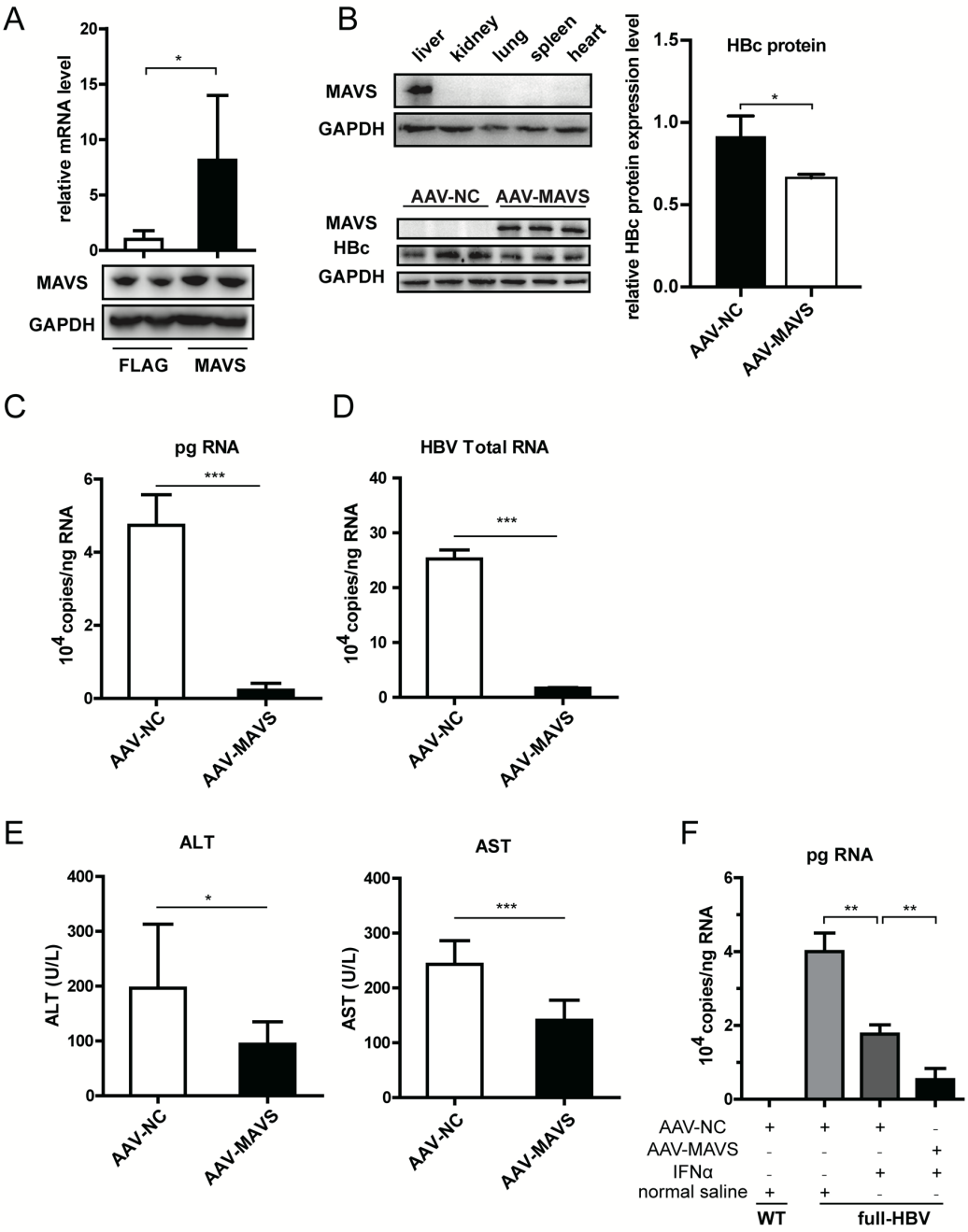
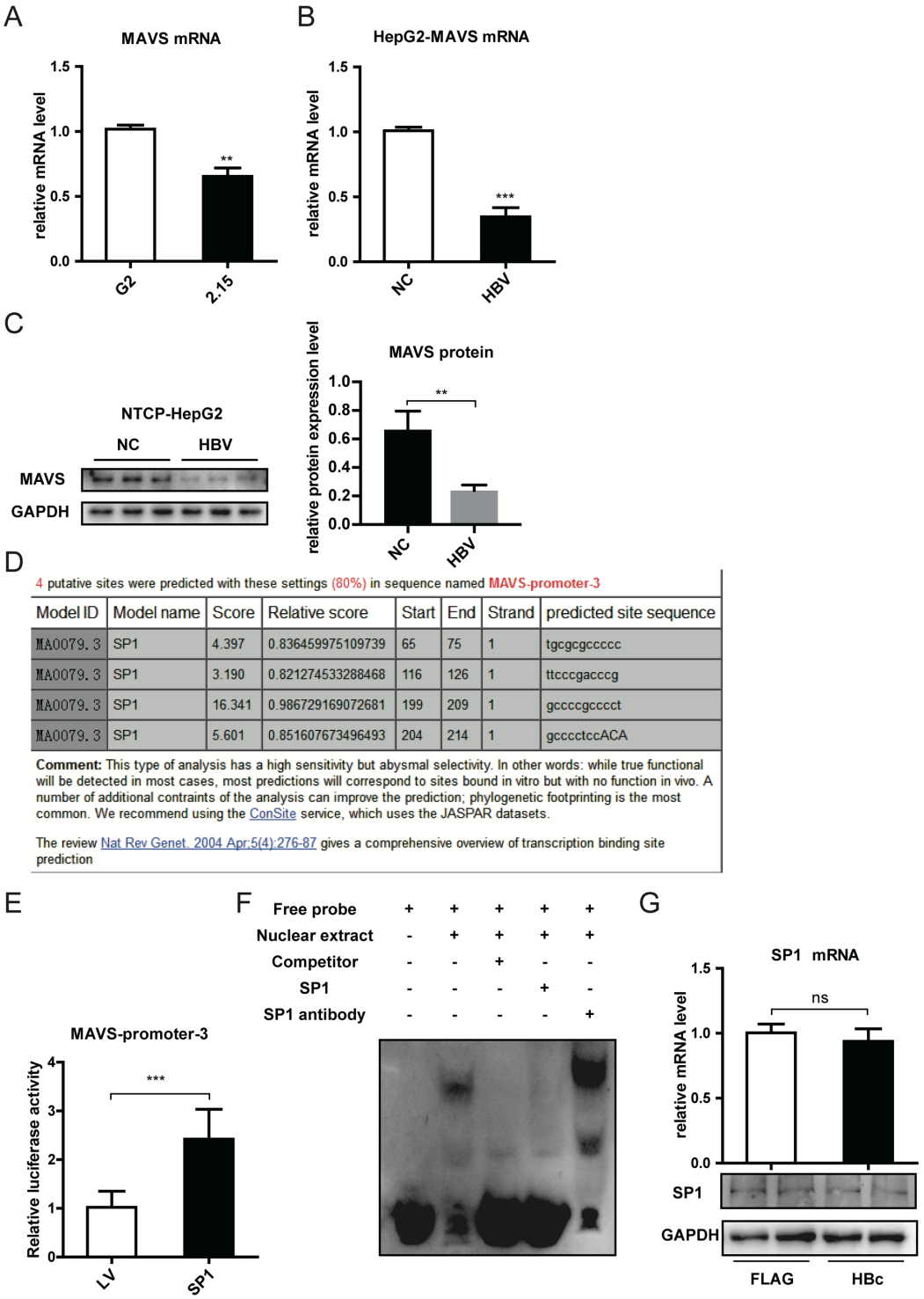

A
relative mRNA level
MAVS
GAPDH
FLAG
MAVS
B
liver
kidney
lung
spleen
heart
MAVS
GAPDH
AAV-NC
AAV-MAVS
MAVS
HBc
GAPDH
HBc protein
relative HBc protein expression level
AAV-NC
AAV-MAVS
C
pg RNA
10^4copies/ng RNA
AAV-NC
AAV-MAVS
D
HBV Total RNA
10^4copies/ng RNA
AAV-NC
AAV-MAVS
E
ALT
ALT (U/L)
AAV-NC
AAV-MAVS
AST
AST (U/L)
AAV-NC
AAV-MAVS
F
pg RNA
10^4copies/ng RNA
AAV-NC
AAV-MAVS
IFNα
normal saline
WT
full-HBV

FIG. S6


A
MAVS mRNA
relative mRNA level
1.5
1.0
0.5
0.0
**
G2
2.15
B
HepG2-MAVS mRNA
relative mRNA level
1.5
1.0
0.5
0.0
***
NC
HBV
C
NTCP-HepG2
NC
HBV
MAVS
GAPDH
MAVS protein
relative protein expression level
1.0
0.8
0.6
0.4
0.2
0.0
**
NC
HBV
D
4 putative sites were predicted with these settings (80%) in sequence named MAVS-promoter-3
Model ID | Model name | Score | Relative score | Start | End | Strand | predicted site sequence
MA0079.3 | SP1 | 4.397 | 0.836459975109739 | 65 | 75 | 1 | tgcgcgccccc
MA0079.3 | SP1 | 3.190 | 0.821274533288468 | 116 | 126 | 1 | ttcccgacccg
MA0079.3 | SP1 | 16.341 | 0.986729169072681 | 199 | 209 | 1 | gccccgcccct
MA0079.3 | SP1 | 5.601 | 0.851607673496493 | 204 | 214 | 1 | gcccctccACA
Comment: This type of analysis has a high sensitivity but abysmal selectivity. In other words: while true functional will be detected in most cases, most predictions will correspond to sites bound in vitro but with no function in vivo. A number of additional contraints of the analysis can improve the prediction; phylogenetic footprinting is the most common. We recommend using the ConSite service, which uses the JASPAR datasets.
The review Nat Rev Genet. 2004 Apr;5(4):276-87 gives a comprehensive overview of transcription binding site prediction
E
MAVS-promoter-3
Relative luciferase activity
4
3
2
1
0
***
LV
SP1
F
Free probe + + + + +
Nuclear extract - + + + +
Competitor - - + - -
SP1 - - - + -
SP1 antibody - - - - +
G
SP1 mRNA
relative mRNA level
1.5
1.0
0.5
0.0
ns
SP1
GAPDH
FLAG
HBc

Supplemental legends:

Figure S1. Analysis of high throughput sequencing in transcriptional group.

(A) Screening process of candidate gene--MAVS. (B) Western blot analysis of MAVS protein expression at different concentrations of IFN-α normalized to GAPDH in Hep3B, HepG2 and mouse primary hepatocytes. Data represent the mean ± SD of three independent experiments. *p< 0.05, ** p < 0.01, *** p <0.001.

Figure S2. ADAR1 regulated MAVS expression.

(A) Western blot analysis and quantification of MAVS protein expression in HepG2, Hep3B and mouse primary hepatocytes. HepG2, Hep3B cells were transfected with NC-FLAG, ADAR, sh-NC and shADAR1 plasmid. Lentivirus containing lv-ADAR1 or sh-ADAR1 were used to infect mouse primary hepatocytes. (B) and (C) Representative pyrosequencing results of ADAR1 overexpression, FLAG, sh-ADAR1, and sh-NC cDNAs. RNA editing rates in ADAR1, FLAG, sh-ADAR1 and sh-NC. (D) and (E) Hep3B and SMMC-7721 cells were transfected with ADAR1 or NC-FLAG plasmid and normal A allele or edited G allele reporter plasmid, respectively. Analyzed for relative luciferase activity. Data represent the mean ± SD of three independent experiments. *p< 0.05, ** p < 0.01, *** p <0.001.

Figure S3. adenosine and uridine rich elements (AREs) around the MAVS-3'UTR editing site.

The blue mark is the editing site, and the red mark is the AU enrichment area.

Figure S4. MAVS inhibits HBV replication in vitro.

(A) and (B) ELISA analysis of HBeAg in HepG2.2.15 cell supernatants and qRT-PCR determination of HBV DNA, pgRNA and total RNA levels. Cells transfected with the MAVS overexpression or knockdown plasmid. (C) Western blot analysis and quantification of Interferon regulatory Factors IRF3/IRF7 of HepG2.2.15 cells transfected with MAVS-overexpressing plasmid or the empty FLAG vector. (D) qRT-PCR determination of HBV DNA level. HepG2 Cells co-transfected with the HBV and MAVS overexpression or NC plasmid. (E) Western blot analysis and quantification of MAVS in MAVS-overexpressing HepG2.2.15 cells co-transfected with ADAR1 plasmid or not. (F) HepG2.2.15 cells were transfected with MAVS-overexpressing plasmid or the empty FLAG vector and treated with or without IFN-α. ELISA analysis of HBeAg in HepG2.2.15 cell supernatants, qRT-PCR determination of HBV DNA and pgHBV RNA levels. Data represent the mean ± SD of three independent experiments. *p < 0.05, ** p < 0.01, *** p<0.001.

Figure S5. MAVS inhibits HBV replication in vivo.

(A) Western blot analysis of MAVS protein expression in the C57BL/6J-Tg (Alb1HBV) 44Bri/J mouse liver after hydrodynamic injection with MAVS or NC-FLAG plasmid. (B) Western blot analysis of MAVS protein levels in various tissues of the full HBV Tg mouse. Western blot analysis of MAVS and HBc protein levels in HBV mouse liver after AAV-NC or AAV-MAVS injection. HBc protein level was normalized to GAPDH. (C) and (D) qPCR determined HBV pgRNA and total RNA levels in mouse hepatocytes(n=8). (E) ALT/AST assay in the serum of HBV mouse(n=8). (F) qPCR determined HBV pgRNA level in mouse hepatocytes(n=4). *p< 0.05, ** p < 0.01, *** p <0.001.

Figure S6. HBV inhibits the expression of MAVS through SP1.

(A) Detection of the MAVS mRNA level in HepG2 and HepG2.2.15 by qPCR. (B) HBV plasmid was overexpressed in HepG2 cells, and the mRNA expression of MAVS was detected by qPCR. (C) HBV virus were collected from the supernatants of HBV transfected HepG2 cells and quantified by ELISA assays, 2 $\times 10^7$ copies of genome equivalent HBV were added to 2$\times$ 10^5 cells plated in 24-well plates, western blot analysis and quantification of MAVS protein expression were performed 16 hours after infection. (D)Predicting the binding sites of SP1 used the online promoter analysis tool the JASPAR database. (E) The luciferase activity of promoter 3 was detected after overexpression of SP1 and LV-NC plasmids. (F) Electrophoretic mobility shift assay (EMSA) of Nuclear proteins extracted from HepG2.2.15 cells and biotin-labeled or unlabeled oligonucleotides probe containing MAVS promoter-3, unlabeled SP1 consensus oligonucleotides. (G) HBc plasmid was overexpressed in HepG2 cells, and the MAVS level was detected by qPCR and Western blotting. *p< 0.05, ** p < 0.01, *** p <0.001.

Table S1

Characteristics of patients

Characteristics	SVRs	NRs	*p*
Patients, n	74	166	
Age, y			
Median (range)	34.6(11,57)	31.8(10,65)	0.30[a]
Mean±SD	33.7(11.6)	31.8(9.1)	0.16[b]
Baseline ALT (U/L)			
Median (range)	158.7(12,1079)	122.0(12,1326)	0.94[a]
Mean±SD	154.8(214.6)	120.9(162.9)	0.24[b]
Baseline AST (U/L)			
Median (range)	103.3(19,467)	73.3(15,1172)	0.29[a]
Mean±SD	101.7(114.1)	73.2(118.7)	0.14[b]
Sex			0.91[c]
Male	53	116	
Female	21	50	

[a] Mann–Whitney U test.

[b] *t* test

[c] Chi-square test.

Table S2

Genotype distribution and allelic frequencies of SNPs in the *MAVS* gene

SNP, allele (1/2)	Allele, n (%)			Genotype, n (%)			
	1	2	*p*/OR(95% CI)	11	12	22	*p*
rs17857295(C/G)							
NRs (n = 162)	157(48.5)	167(51.5)	0.76	39(24.1)	79(48.8)	44(27.2)	0.70
SVRs (n = 74)	74(50)	74(50)	0.94(0.64-1.39)	21(28.4)	32(43.2)	21(28.4)	
rs2326369(C/T)							
NRs (n = 162)	246(75.9)	78(24.1)	0.36	91(56.2)	64(39.5)	7(4.3)	0.16
SVRs (n = 74)	118(79.7)	30(20.3)	0.80(0.50-1.29)	49(66.2)	20(27.0)	5(6.8)	
rs3746660(C/T)							
NRs (n = 163)	249(76.4)	77(23.6)	0.94	96(58.9)	57(35.0)	10(6.1)	0.69
SVRs (n = 73)	112(76.7)	34(23.3)	0.98(0.62-1.56)	45(61.6)	22(30.1)	6(8.2)	
rs3746661(C/G)							
NRs (n = 165)	78(23.6)	252(76.4)	0.52	8(4.8)	62(37.6)	95(57.6)	0.053
SVRs (n = 74)	31(20.9)	117(79.1)	1.17(0.73-1.87)	7(9.5)	17(23.0)	50(67.6)	
rs3746662(A/C)							
NRs (n = 164)	284(86.6)	44(13.4)	0.005	124(75.6)	36(22.0)	4(2.4)	0.02
SVRs (n = 74)	141(95.3)	7(4.7)	0.32(0.14-0.73)	67(90.5)	7(9.5)	0(0.0)	
rs3899452(C/G)							
NRs (n = 161)	103(32.0)	219(68.0)	0.54	16(9.9)	71(44.1)	74(46.0)	0.52
SVRs (n = 72)	42(29.2)	102(70.8)	1.14(0.74-1.75)	8(11.1)	26(36.1)	38(52.8)	

P values were calculated from case-control analysis by the v2 test and unadjusted for multiple testing.

Table S3

Primers sequences of p3XFLAG-CMV™-14 plasmid construction

	5’-3’
ADAR1-HindIII-F	CCCAAGCTTCACAGCGGAGTGGTAAGACCA
ADAR1-XbaI-R	CTAGTCTAGATACTGGGCAGAGATAAAAGT
MAVS-HindIII-F	CCCAAGCTTGCCACCATGCCGTTTGCTGAAG
MAVS-Kpn I-R	CGGGGTACCGCGTGCAGACGCCGCCGGTAC
MAVS-3’UTR-XhoI-F	CCGCTCGAGCGGACCAGTGCCCTCCTCAGTGCTTA
MAVS-3’UTR-NotI-R	AAGGAAAAAAGCGGCCGCAATAGGGTCTTGCTTTGTTGC
Plasmid-MAVS-3’UTR-F	GTGCTGAAGACGAGCAGTAAT
Plasmid-MAVS-3’UTR-R	AGGCTTGTCTCAAACTCCTGG

Table S4

The hairpin oligonucleotide sequence

	5’-3’
sh-ADAR1	**F:**TCGAGCCTGTGGAATCCAGTGACATTGTGCCTACTTCAAGAGA GTAGGCACAATGTCACTGGATTCCACAGGTTTTTTGGAAG **R:**GATCCTTCCAAAAAACCTGTGGAATCCAGTGACATTGTGCCTA C TCTCTTGAAGTAGGCACAATGTCACTGGATTCCACAGGC
sh-ADAR1-NC	**F:**TCGAGTTCTCCGAACGTGTCACGTTTCAAGAGAACGTGACAC GTTCGGAGAATTTTTTGGAAG **R:**GATCCTTCCAAAAAATTCTCCGAACGTGTCACGTTCTCTTGAA ACGTGACACGTTCGGAGAAC

sh-MAVS	**F:**CACCGCTGCCGCAATTTCAGCAATTTCAAGAGAATTGCTGAAA TTGCGGCAGTTTTTTG **R:**GATCCAAAAAACTGCCGCAATTTCAGCAATTCTCTTGAAATTG CTGAAATTGCGGCAGC
sh-MAVS-NC	**F:** CACCGTTCTCCGAACGTGTCACGT CAAGAGATT ACGTGACACGTTCGGAGAA TTTTTT **R:** GATCCAAAAAA TTCTCCGAACGTGTCACGT AATCTCTTG ACGTGACACGTTCGGAGAAC

Table S5

Primers sequences of RT-PCR

Gene	**Forward primer** 5'-3'	**Reversed primer** 5'-3'
ADAR1	GTCGTCAGCTTGGGAACA	CGCAGTCTGGGAGTTGTA
MAVS	CATCAGGAGCAGGACACAGA	GCTGGAAGGAGACAGATGGA
GAPDH	TCTGACTTCAACAGCGACAC	CAAATTCGTTGTCATACCAG
total -RNA	TCACCAGCACCATGCAAC	AAGCCACCCAAGGCACAG
pg-RNA	GAGTGTGGATTCGCACTCC	GAGGCGAGGGAGTTCTTCT
HBV-DNA	GAGTGTGGATTCGCACTCC	GAGGCGAGGGAGTTCTTCT
ccc-DNA	TGCACTTCGCTTCACCT	AGGGGCATTTGGTGGTC

Supplemental materials and methods

1.1 reagents and antibodies.

Tunicamycin (TM) was obtained from MilliporeSigma (cat:654380-10MG; Billerica, MA, USA), dissolved in dimethyl sulfoxide (DMSO; AMRESCO; Solon, OH, USA) and used at 5 μg/ml. Recombinant human IFN-α-2A (Fangcheng BioTech Co Ltd; Beijing, China) was added 200 units/well in 24 well plates. Phosphate-buffered saline (PBS) was purchased from TBD (cat no. PB2004Y; Tianjin, China). Lipofectamine™ 3000 was purchased from Invitrogen (cat no. L3000015; Thermo Fisher Scientific, Inc., USA). The Ultrapure RNA Kit (cat no. CW0581), FFPE DNA Kit (cat no. CW0547), Protein Extraction Kit (cat no. cw0889), SYBR Green PCR Kit (cat no. CW2601) were purchased from CWBIO (Beijing, China). Nuclear and Cytoplasmic Protein Extraction Kit (cat no. P0027) and Chemiluminescent EMSA Kit (cat no.GS009,) were purchased from Beyotime (Beijing, China). ReverTra Ace qPCR RT Master Mix was purchased from TOYOBO (cat no.FSQ-201; OSAKA, Japan). Antibodies against MAVS (polyclonal rabbit, cat no.ab189303),ADAR1(polyclonal mouse; cat no.ab88574); HBc(Anti-Hepatitis B Virus Core Antigen antibody; polyclonal mouse; cat no.ab8637); IRF3(polyclonal rabbit; cat no.DF6895); Phospho-IRF3 (Ser386; polyclonal rabbit; cat no.AF3438) IRF7(polyclonal rabbit; cat no.DF7503); Phospho-IRF7 (Ser477; polyclonal rabbit; cat no.AF8486) and GAPDH (polyclonal rabbit, cat no. CW0101) were purchased from Abcam (abcam, cambridge, UK) and CWBIO (Beijing, China). Dual-luciferase Reporter Assay System was purchased from Promega (Cat.E1960;

Madison, WI, USA).

1.2 Plasmid construction and lentivirus

1.2.1 ADAR1 and MAVS overexpression vector

The CDS of ADAR1 and MAVS were amplified by PCR using HepG2.2.15 cDNA as a template. The PCR products were digested by HindIII, XbaI or KpnI, and then ligated into p3XFLAG-CMV™-14 vector (Sigma, St. Louis, USA). PCR primers were listed in Table S1.

1.2.2 ADAR1 and MAVS interference plasmid construction

Prediction of shRNA target sequences of ADAR1 and MAVS were conducted by BLOCK-iT™ RNAi Designer website (https://rnaidesigner.lifetechnologies.com/rnaiexpress/setOption.do?designOption=shrna&pid=409507665443286793). Then we designed and synthesized the hairpin oligonucleotide sequences, annealed and inserted sh-ADAR1 into pBABE-Puro-U6 vector (Cell Biolabs, Inc, San Diego, USA) and sh-MAVS into pGPU6/GFP/Neo vector (GenePharma, Suzhou, China). The hairpin oligonucleotide sequences were listed in Table S2.

1.2.3 Dual luciferase reporter plasmid

We constructed the reporter plasmid encompassing -296 to +68 bp of MAVS 3'UTR (0 relative to the nucleotide of RNA editing site) by PCR using HepG2.2.15 genomic DNA as a template. The PCR products were digested by XhoI and NotI, and then ligated into pmiR-RB-Report™ vector (ribobio, guangzhou, China). The PCR primers were listed

in Table S1.

1.2.4 The 1.3*HBV genotype B isolate S64 on Puc18 plasmid was given by Professor Wenhui Li (National Institute of Biological Sciences, Beijing, China).

All constructs used in this study were sequenced in order to confirm their authenticity.

1.2.5 ADAR1 overexpression lentivirus

ADAR1 plasmid was co-transfected with lentiviral packaging vectors (pVSV-G and delta 8.91) into 293T cells using Lipofectamine 3000 according to the manufacturer's protocol. The supernatant containing the lentiviruses was collected and used to infect mouse primary hepatocytes.

1.3 Enzyme-linked immunosorbent assay (ELISA)

Concentration of HBsAg and HBeAg in culture supernatant of HepG2.2.15 were quantified by ELISA using commercial ELISA kits following the manufacturer's protocol (WANTAI Bio-Pharm, Beijing, China). The reporting unit was calculated by O.D 450nm minus O.D 630nm. Standard substance of HBsAg (IU/mL) and HBeAg (NCU/mL) were purchased from Beijing Controls & Standards Co., Ltd (Beijing, China). A microplate reader (Synergy H1; BioTek; Winooski, VT, USA) was used to measure absorbance at 450nm and 630nm.

1.4 Quantitative RT-PCR (qPCR)

1.4.1 The mRNA expression levels

HepG2.2.15 cells (2×10^5 per well) were seeded in 24-well plates for real time

quantitative PCR analysis. Each well of HepG2.2.15 cells were incubated with 500ηg plasmid and 1μl Lipofectamine™ 3000. cDNA was synthesized in a 10μl reaction volume using ReverTra Ace qPCR RT Master Mix following the manufacturer's instructions. The mRNA expression levels of ADAR1, MAVS, EIF2S1, OAS3 and GAPDH were measured by SYBR Green relative quantitative analysis using the Bio-Rad iQ5 Real-Time PCR Detection System (Bio-Rad; Hercules, CA, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. The detailed qPCR conditions were as follows: 95°C for 10 min, followed by 40 cycles of 92°C for 15 sec and 58°C for 1 min. The relative expression levels of genes were calculated by the $2^{-\Delta\Delta Ct}$ method.

1.4.2 Supernatant HBV-DNA expression levels

HBV DNA extraction was performed using the one-Tube Viral DNAout Kit (Tiandz, Beijing, China) according to the manufacturer's instructions. The HBV DNA was measured by SYBR Green absolute quantitative analysis using the Bio-Rad iQ5. 1.3 * S64+pUC18 plasmid was used as the quantitative standard of HBV and diluted into 5 gradients form 10^8 to 10^4 copies/ml, then formed the standard curve to extrapolate the HBV DNA quantification.

All the qRT-PCR primers were listed in Table S3.

1.5 RNA-seq analysis

HepG2.2.15 cells were treated with 500U IFN-α or negative control PBS. The total RNA of samples was extracted and the DNA was digested with DNase. Then, the library

preparation for transcriptome sequencing and data analysis were conducted by OE biotech Co., Ltd. (Shanghai, China). A total of 4 transcriptome data were uploaded to the NCBI database, numbered as SRR10298005, SRR10298006, SRR10298007, SRR10298008. GATK (Genome Analysis Toolkit) program was operated to analyze bam files acquired from HISAT2 alignment program. GCF_000001405.38_GRCh38.p12 was used as the alignment database. The minimal required read coverage were set as 1, while the final results were filtered by the following parameters: Qd < 2.0 (QualByDepth : the variant confidence divided by the unfiltered depth of non-reference samples), QUAL < 30.0 (a quality score associated with the inference of the given allele), SOR > 3.0 (StrandOddsRatio : strand bias estimated by the symmetric odds ratio test), FS >60.0 (phred-scaled p-value using Fisher's Exact Test to detect strand bias, the variation being seen on only the forward or only the reverse strand, in the reads), MQ < 40.0 (RMSMappingQuality : the Root Mean Square of the mapping quality of the reads across all samples), MQRankSum < -12.5 (MappingQualityRankSumTest : The U-based z-approximation from the Mann-Whitney Rank Sum Test for mapping qualities, reads with ref bases vs. those with the alternate allele), ReadPosRankSum (ReadPosRankSumTest : The U-based z-approximation from the Mann-Whitney Rank Sum Test for the distance from the end of the read for reads with the alternate allele).

1.6 Western blotting.

Proteins were extracted using the Mammalian Protein Extraction Kit, separated by 10%

sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred to a polyvinylidene fluoride (PVDF) membrane (cat no. ISEQ00010; Millipore; Billerica, MA, USA). After blocking with 5% non-fat powdered milk, the membranes were incubated with antibodies against ADAR1(1:1000), MAVS (1:2000), HBc(1:1000) and GAPDH (1:12000) at 4 °C overnight. The membranes were washed with Tris-buffered saline containing 0.1% Tween 20 (TBST) and incubated with the IgG (1:10000) at 37°C for 1h. Micrographs were taken using the Tanon 5200 Multi (Shanghai, China).

1.7 Dual luciferase reporter gene assay

The MAVS 3'UTR region (chr20:3870562A or G) were cloned into the Renilla luciferase gene (hRluc) downstream sites. HepG2.2.15 and Hep3B cells (2×10^5 per well) were seeded in 24-well plates, each well was transfected with chr20:3870562A or G plasmids. After 48h of incubation, cells were collected and analyzed for luciferase activity with Dual-Luciferase Reporter Assay System (Promega). Promega GloMAX 96 microplate luminometer (YuanPingHao Biotech Co.,Ltd, Beijing, China) was used to measure luciferase activity.

1.8 Immunohistochemical (IHC)

Mouse livers were fixed in 10% formaldehyde 48h, embedded in paraffin, and cut into 10-lm sections. Antigen retrieval was performed at pH 9, for 15 minutes. Sections were blocked in 5% goat serum albumin in 1*TBS 0.1% Tween 20, and the primary

antibodies HBsAg, HBxAg, HBcAg, NLRX1 and CASP1 were used, followed by secondary antibodies coupled to HRP. Counterstaining was performed with hematoxylin.

1.9 Pyrosequencing

For pyrosequencing analysis, HepG2.2.15 cells (2×10^5 per well) were seeded in 24-well plates and transfected with 500 ng plasmid using Lipofectamine™ 3000 (cat no. L3000015; Thermo Fisher Scientific, Inc., USA) according to the manufacturer's instructions. At 48 h post-transfection, cells were harvested and ADAR1 mRNA expression level was measured to confirm overexpression and knockdown of ADAR1. The cDNA samples were then pyrosequenced by Sangon Biotech Co., Ltd. (Shanghai, China) for identification of RNA editing sites.

1.10 Animal studies

Female C57BL/6J-Tg (Alb1HBV)44Bri/J mice (containing partial HBV genome including S, pre-S, and X gene, 6-8 weeks old) were purchased from Department of Laboratory Animal Science, Health Science Center, Peking University (Beijing). The partial HBV-tg mice were hydrodynamically injected via tail vein within 5-8 seconds with 20 μg of MAVS-CDS DNA or FLAG vector plasmid suspended in a volume of PBS equivalent to 8% of body weight. Plasmids were injected two times in each group and the interval between injections was five days. Five days after the injection, HBsAg level was measured in mouse serum using an ELISA assay (WANTAI Bio- Pharm,

Beijing, China). The liver tissues were dehydrated and embedded in paraffin following immunohistochemistry method. The mice were treated with 15 μg/ kg IFN-α-2A (Fangcheng BioTech Co Ltd; Beijing, China). Another female BALB/c- full-HBV-tg mice (6-8 weeks old) were purchased from Infectious Disease Center of No.458 Hospital (Guangzhou, China). The transgene mice contain 1.3 copies of HBV- genome-eq. Coding sequence of MAVS was inserted into an adeno-associated virus vector (pAV-TBG). After sequencing ensured accuracy of the vector, AAV-DJ was packaged, purified, and titrated by Vigene Biosciences (Jinan, China). AAV-DJ (8×10^{11} copies) harboring either the MAVS or the control sequence was injected through tail vein of full-HBV-tg mice. Three weeks later, the level of HBsA, HBeAg and HBV DNA were determined in every two weeks. All experimental manipulations were approved by the Animal Care and Use Committees of the Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences.

1.11 Human Subjects

Subjects were treated for 48 weeks and subsequently followed for 24 weeks to evaluate therapeutic effects. Of the initial 324 patients, 84 were excluded from analysis due to missing clinical or virologic data, lack of a validated outcome, or being absent during follow-up. Ultimately, 240 patients with PEG-IFN-α-2a therapy were retained for this study. The study was carried out in accordance with the guidelines of the Helsinki Declaration after obtaining written informed consent from all subjects. It was approved by the ethics committee of the Institute of Basic Medical Sciences, Chinese Academy

of Medical Sciences. The detection of serum HBV s antigen (HBsAg), HBV s antibody (HBsAb), HBeAg, HBV e antibody (HBeAb), HBV c antibody (HBcAb) and alanine transaminase (ALT), aspartate aminotransferase (AST) levels were measured as previously described[28]. Sustained virologic response (SVR) was defined as HBeAg seroconversion and an HBV DNA level <2000 copies/mL by the end of 48 weeks of treatment plus 24 weeks of follow-up. Paraffin embedded liver tissues of 64 HCC patients were selected from the surgical pathology files of Affiliated Hospital of Qingdao University. DNA of the paraffin-embedded tissues were extracted with FFPE DNA Kit (CWBIO, Being, China) according to the manufacturer's instruction, and were used to determine the genotypes of the patients.

All procedures were performed with informed consent and approved by the ethics committee of the hospital.

1.12 RNA immunoprecipitation (RIP)

Edited G plasmids and wild A plasmids were transfected into HepG2.2.15 cells, and cells were collected 48 days later. Cells were selectively treated with formaldehyde to fix the protein-RNA complex. The mixture was treated with RNA enzyme inhibitors. Chromatin was fragmented using an ultrasound for 15min. The HuR binding protein was immunoprecipitated with the binding RNA to wash away the unbound material. Purification of mRNA bound to HuR antibodies and agarose beads (Santa, Germany) complex. Finally, mRNA was reversely transcribed into cDNA and detected by qPCR.

1.13 Electrophoretic Mobility Shift Assay

Biotin-labeled MAVS probe and unlabeled MAVS/SP1 probe were prepared by Sangon Biotech (Shanghai, China). Nuclear proteins were extracted using Nuclear and Cytoplasmic Protein Extraction Kit (Beyotime, Beijing, China). EMSA was performed using the Chemiluminescent EMSA Kit (Beyotime, Beijing, China) according to the manufacturer's instructions.

1.14 Isolation and culture of mouse primary hepatocytes.

Primary mouse hepatocytes were isolated from livers of female full-HBV-tg mice (8 weeks old in BALB/c background). Briefly, mice were anesthetized, and their livers were perfused with 0.5 mg/ml type iv collagenase (Sigma–Aldrich), via the inferior vena cavato isolate hepatocytes. Mouse hepatocytes were cultured in RPMI-1640 containing 10% FBS, 100 units/ml penicillin, and 0.1 mg/ml streptomycin. Six to twelve hours after attachment, primary hepatocytes were infected with indicated lentivirus. Forty-eight hours after infection, cells were harvested for protein extraction and western blot assays.