Supplementary Materials

Sanger sequencing

The protein coding exons of *SAMD9L* gene were amplified to produce those overlapped DNA fragments by designed primers (Supplementary Table 1). Sanger sequencing were performed on ABI 3730xl sequencers.

Reverse transcription and polymerase chain reaction (RT-PCR)

The first strand cDNA was synthesized with 2 μg total RNA using M-MLV reverse transcriptase (Promega). Amplified products were visualized by electrophoresis on a 2% agarose gel-containing ethidium bromide. Real-time PCR was performed with Thermal Cycler Dice Real Time System and SYBR green dye (TaKaRa). The relative expression method was applied to quantitate SAMD9L mRNA levels relative to internal reference gene β-actin. All Real-time PCR reactions were performed in triplicate.

Cell proliferation assay

HCC cells were seeded at 3000 cells per well of 96-well plates in triplicate. Cell viability was measured every day using Cell Counting Kit-8 (CCK-8, Dojindo Laboratories) according to the manufacturer's instructions. Briefly, 10 μl of CCK-8 solution was added into each well of the 96-well culture plate, and then incubated for 2 h at 37 °C. The absorbance value of each well was read at 450 nm filter.

BrdU incorporation assay

Cells were incubated with 20 μM BrdU (Sigma-Aldrich) for 2 h. And then the harvested cells were denatured for 20 min at room temperature in 2 M HCl, subsequently neutralized with 0.1 M sodium borate. Fluorescein isothiocyanate (FITC)-conjugated mouse anti-BrdU monoclonal antibody (BD Pharmingen) was incubated with PBS-washed cell suspension, and FITC-conjugated mouse IgG was used as a negative control. The incubated cells were next treated with RNase and PI for 30min, and analyzed by flow cytometry with CellQuest software. For microscopic examination of DNA synthesis, cells were treated by 20 μM BrdU and fixed in 4 % paraformaldehyde for 20 min. Then cells were subject to HCl-treated denaturation and neutralization with $Na_2B_4O_7$. Cells with incorporated-BrdU were incubated with mouse anti-BrdU antibody (Santa Cruz) for 1 h at room temperature (1:500 dilution) followed by secondary mouse FITC-conjugated IgG and nuclei were simultaneously stained with 4’, 6-diamidino-2-phenylindole (DAPI).

Western blotting assay

Cells were washed with precooled phosphate-buffered saline (PBS) three times and were lysed for 30 min on ice in RIPA buffer in the presence of a protein inhibitor cocktail, then centrifuged at 12,000 rpm for 15 min at 4 °C. 40 μg from each sample was subjected to SDS-PAGE protein and then transferred onto a nitrocellulose membrane. The membrane was incubated with primary antibodies against β-catenin, β-actin, followed by incubation with an IRDye800DX-conjudated secondary antibody. The signals were detected using the Odyssey Infrared Imaging System (LI-COR Biosciences).

Supplementary Table 1. The sequence for primers, siRNAs and shRNAs used in this study

Primers for RT-PCR assay

Primers	Sequences (5'-3')
SAMD9L-F85	GCCATCAGAAGAGGAATGAGACAGC
SAMD9L-R298	GCTGCTCTTCTGGCAAGGGGC
β-actin-F	AATCGTGCGTGACATTAAGGAG
β-actin-R	ACTGTGTTGGCGTACAGGTCTT

Primers for PCR-based Sanger Sequencing of SAMD9L

SAMD9L(E5_1)-F	ttttaagagtcagcctttagcaa
SAMD9L(E5_1)-R	TGATCTCTCTGGGATCATAATCAA
SAMD9L(E5_2)-F	ACCGTCCAAAACAGAACACC
SAMD9L(E5_2)-R	TTGGCTCCCGAATACACTTC
SAMD9L(E5_3)-F	TGCCTTCATTGACCACTTCA
SAMD9L(E5_3)-R	AACTCCAACACAGCAAACCA
SAMD9L(E5_4)-F	TATGACTGGTACATTCTTGTAACAAAT
SAMD9L(E5_4)-R	TATTTAAAGTGGAAATACTGTGGTTTG
SAMD9L(E5_5)-F	CAAAGGAATGGAAAATATGTTGTG
SAMD9L(E5_5)-R	AACATGCATAGCCAGTGTGG
SAMD9L(E5_6)-F	GGGACAGTTATGAAAAGCTTAAAGA
SAMD9L(E5_6)-R	CAACATCCTGTCCTTTTAGGAT
SAMD9L(E5_7)-F	GGTGCCAAACTGAAGGAAAT

SAMD9L(E5_7)-R	CACCTTGCGCTGTCTTGTAA
SAMD9L(E5_8)-F	TGCGTATCATTCACCCTCTG
SAMD9L(E5_8)-R	TTTGCCTTTGGGATTCTTTG
SAMD9L(E5_9)-F	ATCAAATGGTGGTTGGATGG
SAMD9L(E5_9)-R	CATGGATCCAAATGACAGAAAA
SAMD9L(E5_10)-F	CCCAAAAAGAAATTGCAGAAA
SAMD9L(E5_10)-R	CGCTTGTACTGTCCCCTGA
SAMD9L(E5_11)-F	CTGGCCAGAAAATCAAGAGC
SAMD9L(E5_11)-R	gaggcaaaagcaaaatctgt

siRNAs against SAMD9L

siRNA	sense (5'-3')	antisense (5'-3')
si-257	CCGGGACAAUUAGAUAAUUdCdA	AAUUAUCUAAUUGUCCCGGdAdT
si-1213	CCGAGACUCACUGGAUAAUdTdT	AUUAUCCAGUGAGUCUCGGdTdT
si-2177	GAGUCUCCUAAACCAAUAUdTdT	AUAUUGGUUUAGGAGACUCdTdT
si-3420	GUAGGAGCAUUACUGUUAAdTdG	UUAACAGUAAUGCUCCUACdAdG
si-3558	GACGAUAUGACAUGUAUAAdCdA	UUAUACAUGUCAUAUCGUCdTdC
si-Luc	CUUACGCUGAGUACUUCGAdTdT	UCGAAGUACUCAGCGUAAGdTdT

shRNA against SAMD9L

shRNA	Forward sequence (5'-3')	Reverse sequence (5'-3')

sh1213	GATCCCCCCGAGACTCACTGGATAATttcaagagaATTATCCAGTGAGTCTCGGTTTTTGGAAA	AGCTTTTCCAAAAACCGAGACTCACTGGATAATtctcttgaaATTATCCAGTGAGTCTCGGGGG
sh3558	GATCCCCGACGATATGACATGTATAAttcaagagaTTATACATGTCATATCGTCTTTTTGGAAA	AGCTTTTCCAAAAAGACGATATGACATGTATAAtctcttgaaTTATACATGTCATATCGTCGGG
shLuc	GATCCCCCTTACGCTGAGTACTTCGAttcaagagaTCGAAGTACTCAGCGTAAGTTTTTGGAAA	AGCTTTTCCAAAAACTTACGCTGAGTACTTCGAtctcttgaaTCGAAGTACTCAGCGTAAGGGG

Supplementary Table 2. Somatic mutations of SAMD9L in liver carcinomas

Sample	Gene Name	AA Mutation	CDS Mutation	Primary Tissue	Histology	Histology Subtype 1	Pubmed Id	Somatic Status
30	SAMD9L	p.G500S	c.1498G>A	Liver	Carcinoma	Hepatocellular carcinoma	-	Confirmed Somatic
4	SAMD9L	p.D915V	c.2744A>T	Liver	Carcinoma	Hepatocellular carcinoma	-	Confirmed Somatic
A31	SAMD9L	p.L1332M	c.3994C>A	Liver	Carcinoma	Hepatocellular carcinoma	-	Confirmed Somatic
51	SAMD9L	p.K1352R	c.4055A>G	Liver	Carcinoma	Hepatocellular carcinoma	-	Confirmed Somatic
A1	SAMD9L	p.V1488I	c.4462G>A	Liver	Carcinoma	Hepatocellular carcinoma	-	Confirmed Somatic
HCC134T	SAMD9L	p.G346E	c.1037G>A	Liver	Carcinoma	NS	-	Variant of unknown origin
266T	SAMD9L	p.E40*	c.118G>T	Liver	Carcinoma	Hepatocellular	23788652	Confirmed Somatic

						carcinoma		
P55	SAMD9L	p.W495C	c.1485G>T	Liver	Carcinoma	Hepatocellular carcinoma	22922871	Confirmed Somatic
RK107_C01	SAMD9L	p.K963N	c.2889G>T	Liver	Carcinoma	NS	-	Variant of unknown origin
HCC77T	SAMD9L	p.Q1169K	c.3505C>A	Liver	Carcinoma	NS	-	Variant of unknown origin
HCC6T	SAMD9L	p.S117S	c.351T>C	Liver	Carcinoma	NS	-	Variant of unknown origin
P929	SAMD9L	p.G1554A	c.4661G>C	Liver	Carcinoma	Hepatocellular carcinoma	22922871	Confirmed Somatic
RK021_C01	SAMD9L	p.S275C	c.823A>T	Liver	Carcinoma	NS	-	Variant of unknown origin
HCC29T	SAMD9L	p.E286*	c.856G>T	Liver	Carcinoma	Hepatocellular carcinoma	-	Variant of unknown origin
HCC29T	SAMD9L	p.E286V	c.857A>T	Liver	Carcinoma	Hepatocellular carcinoma	-	Variant of unknown origin
193T	SAMD9	p.D1041fs*70	c.3120_3121ins20	Liver	Carcinoma	Hepatocellular carcinoma	23788652	Confirmed Somatic
HCC107T	SAMD9	p.Q317Q	c.951A>G	Liver	Carcinoma	NS	-	Variant of unknown origin
HCC115T	SAMD9	p.R863I	c.2588G>T	Liver	Carcinoma	NS	-	Variant of unknown origin
HCC138T	SAMD9	p.D551Y	c.1651G>T	Liver	Carcinoma	NS	-	Variant of unknown origin

RK089_C01	SAMD9	p.L619R	c.1856T>G	Liver	Carcinoma	NS	-	Variant of unknown origin

Supplementary Table 3. SAMD9L expression shown in 50 HCC specimens with clinical factors

ID	Folds	*Down-regulation	Gender	Age	HBV	HCV	AFP (µg/L)	TNM	Edmondson
1	0.01	Yes	F	59	Positive	Negative	>1210	T1N0M0	II-III
2	0.02	Yes	F	55	Positive	Negative	26.61	T2N0M0	I-II
3	0.04	Yes	F	34	Positive	Negative	>1210	T4N0M1	III-IV
4	0.12	Yes	F	61	Positive	Negative	4.66	T1N0M0	IV
5	0.15	Yes	F	37	Positive	Negative	>1210	T2N0M0	II-III
6	0.47	Yes	F	52	Positive	Negative	5.53	T1N0M0	NA
7	0.01	Yes	M	67	Positive	Negative	28.42	T1N0M0	III-IV
8	0.01	Yes	M	67	Positive	Negative	164.2	T1N0M0	III-IV
9	0.02	Yes	M	73	Positive	Negative	1.7	T1N0M0	III-IV
10	0.02	Yes	M	46	Positive	Negative	10.38	T3N0M0	III
11	0.02	Yes	M	50	Positive	Negative	827.9	T1N0M0	III
12	0.02	Yes	M	50	Positive	Negative	>1210	T4N0M1	IV
13	0.02	Yes	M	31	Positive	Negative	6.38	T1N0M0	III-IV
14	0.03	Yes	M	35	Positive	Negative	31.91	T1N0M0	III
15	0.03	Yes	M	61	Negative	Negative	1.59	T3N0M0	IV
16	0.03	Yes	M	41	Positive	Negative	>1210	T1N0M0	III

17	0.03	Yes	M	41	Positive	Negative	3.99	T1N0M0	II
18	0.05	Yes	M	60	Positive	Negative	NA	T3N0M0	II-III
19	0.06	Yes	M	45	Positive	Negative	>1210	T2N0M0	III
20	0.06	Yes	M	39	Positive	Negative	>3000	T1N0M0	IV
21	0.06	Yes	M	54	Positive	Negative	54.91	T1N0M0	IV
22	0.08	Yes	M	49	Positive	Negative	>1210	T1N0M0	III
23	0.09	Yes	M	59	Positive	Negative	10.23	T1N0M0	IV
24	0.1	Yes	M	40	Positive	Negative	36.93	T2N0M0	III
25	0.11	Yes	M	56	Positive	Negative	>1210	T2N0M0	III-IV
26	0.11	Yes	M	51	Positive	Negative	2.52	T1N0M0	I-II
27	0.12	Yes	M	57	Positive	Negative	13.69	T2N0M0	II
28	0.16	Yes	M	41	Positive	Negative	1.45	T2N0M0	II
29	0.17	Yes	M	78	Positive	Negative	1.73	T1N0M0	NA
30	0.19	Yes	M	44	Positive	Negative	332.4	T1N0M0	III
31	0.2	Yes	M	46	Positive	Negative	NA	T2N0M0	II
32	0.21	Yes	M	53	Positive	Negative	3.59	T1N0M0	II-III
33	0.24	Yes	M	68	Positive	Negative	3.28	T1N0M0	III
34	0.27	Yes	M	46	Positive	Negative	>3000	T3N0M0	III-IV
35	0.31	Yes	M	44	Negative	Negative	1064	T1N0M0	III-IV
36	0.42	Yes	M	71	Positive	Negative	5.24	T1N0M0	III
37	0.42	Yes	M	48	Positive	Negative	17.45	T2N0M0	II
38	0.43	Yes	M	70	Positive	Negative	1.53	T1N0M0	II
39	0.44	Yes	M	57	Positive	Negative	4.67	T1N0M0	IV
40	0.57	No	M	73	Positive	Negative	17.59	T1N0M0	III-IV
41	0.75	No	M	55	Positive	Negative	11.11	T1N0M0	IV

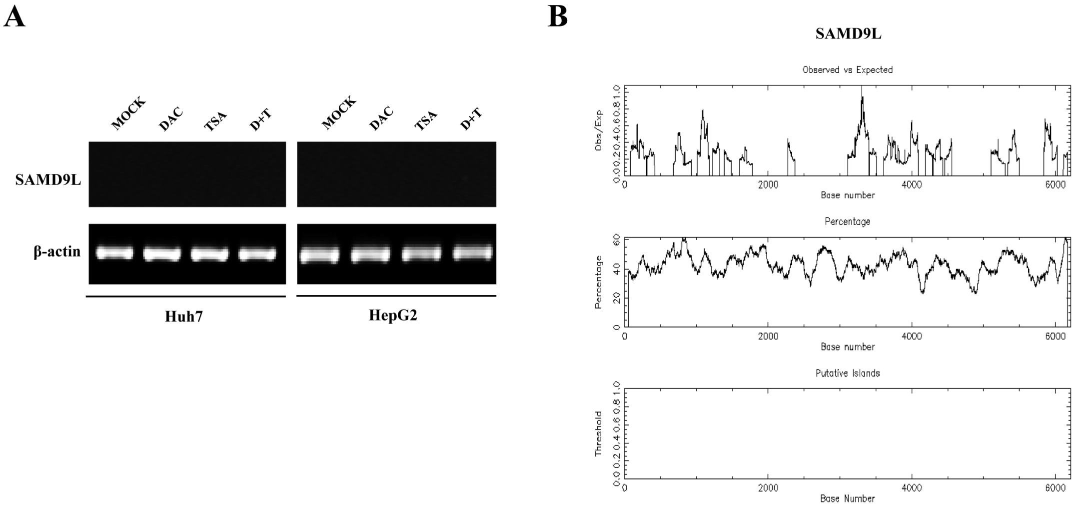
42	0.81	No	M	62	Positive	Negative	70.6	T1N0M0	NA
43	0.93	No	M	55	Positive	Negative	425.9	T1N0M0	III
44	1.02	No	M	41	Positive	Negative	3.99	T1N0M0	II
45	1.05	No	M	46	Positive	Negative	3.81	T1N0M0	III
46	1.05	No	M	55	Negative	Negative	11.47	T3N0M0	III
47	1.65	No	M	62	Positive	Negative	23.64	T1N0M0	III
48	2.15	No	M	48	Positive	Negative	211.6	T1N0M0	III
49	5.46	No	M	66	Positive	Negative	21.38	T1N0M0	III
50	8.17	No	M	55	Positive	Negative	5.55	T3N0M0	II-III

***Down-regulation of SAMD9L was designed as ≤0.5 (HCC/non-cancerous liver)**

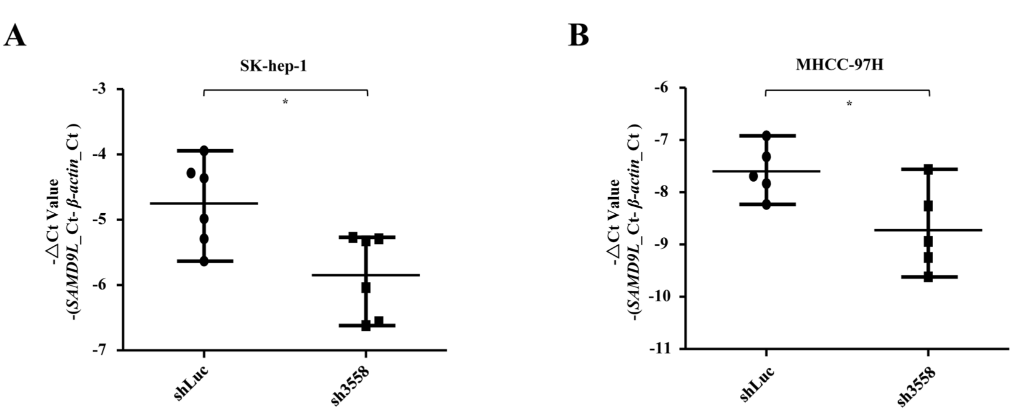
Supplementary Table 4. Correlations between SAMD9L expression and clinical factors

Clinicopathological parameters	**SAMD9L expression**		***p***
	Down-regulation	**No down-regulation**	
Gender			0.17
Male	33	11	
Female	6	0	
Age			0.3
Mean ± SD	52.21 ± 11.68	56.18 ± 9.24	
Median	51	55	
HBV			0.63

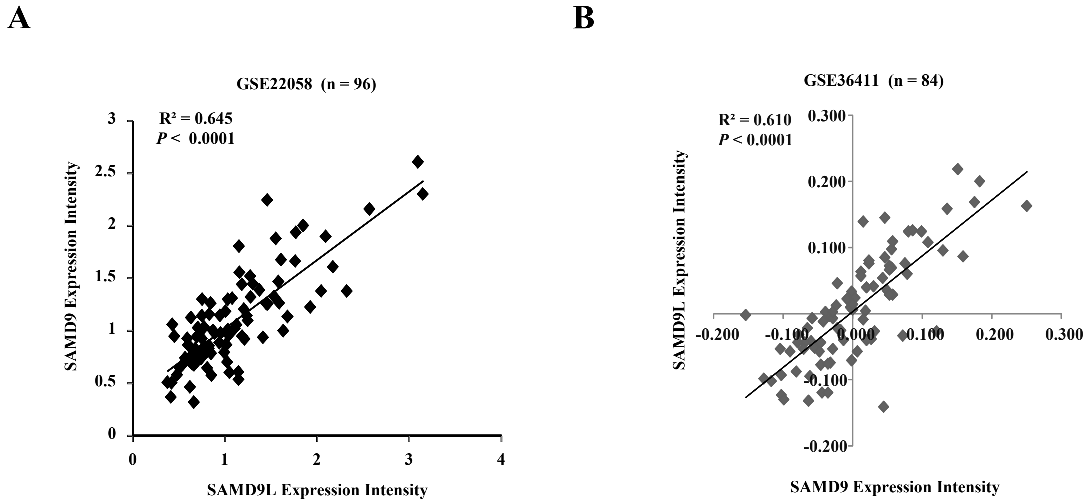
+	37	10	
-	2	1	
AFP			0.16
≤ 25	18	8	
> 25	19	3	
Tumor size (T)			0.25
T1	24	9	
T2	9	0	
T3	4	2	
T4	2	0	
Distant metastases (M)			0.44
M0	37	11	
M1	2	0	
Edmondson			0.39
I+II	8	1	
III+IV	25	8	



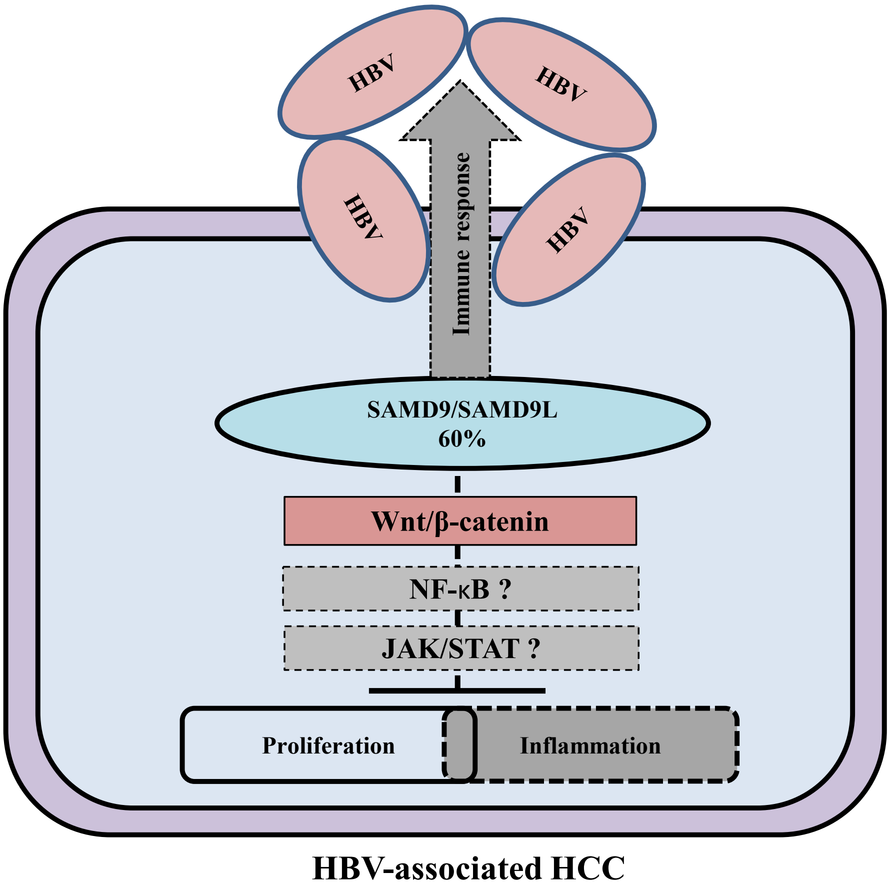
Supplementary Figure 1. (A) The SAMD9L expression was evaluated by RT-PCR in Huh7, HepG2 cells treated with no drug, DAC, TSA, or DAC plus TSA, where β-actin was used as a loading control. (B) The putative CpG islands of human SAMD9L promoter were calculated with EMBOSS Cpgplot program (http://www.ebi.ac.uk/Tools/seqstats/emboss_cpgplot/).



Supplementary Figure 2. The real-time PCR was employed to evaluate the mRNA level of SAMD9L in those xenograft tumors of SK-hep-1 (A) and MHCC-97H (B). Relative SAMD9L expression was calculated with normalized -ΔCt values, and the statistical difference between two groups was analyzed by the two-tailed Student's *t* test.



Supplementary Figure 3. The expression correlation of SAMD9 and SAMD9L in HCC tissue specimens was evaluated by analyzing 2 microarray databases (GSE22058, A; GSE 36411, B).



Supplementary Figure 4. A proposed model to demonstrate the possible mechanisms by which SAMD9/SAMD9L contribute to the development of HBV-associated HCC, where SAMD9/SAMD9L could play their physiological roles in anti-infection of hepatitis B viral and/or inhibition on inflammation response in hepatocyte, whereas their inactivation caused by genetic lesions and down-regulation could contribute to the initiation and development of HBV-associated HCC; Wnt/β-catenin JAK-STAT3 and NF-κB pathways may be implicated with the roles of SAMD9/SAMD9L in defense against HBV infection and inflammation.