Supplementary Materials and Methods for

ZNF638 represses the transcription of HBV closed circular DNA involving HUSH complex-mediated histone modifications of epigenetic silencing

1. Cell culture

HBV-expressing human liver cancer cell line HepAD38, HepG2.2.15, were kindly provided by Dr. Dexi Chen from Beijing You-An Hospital, and maintained in Dulbecco's modified eagle medium (DMEM, KeyGen Bio Tech) supplemented with 10% fetal bovine serum (FBS, Biological Industries).

2. Cell viability assay

The number of 5,000 cells were seeded in each well of 96-well plates. The cell viability was determined using cell counting kit (CCK8) (KeyGENBioTECH) according to the vendor's standard protocols. The plates were scanned at 450 nm for absorbance using a spectrophotometer (BioTek). Each data point was measured for the average from six duplicates. The experiments were repeated independently for 3 times.

Table S1 The siRNA sequences used in this study

Target gene	**Sense (5'-3')**	**Anti-sense (5'-3')**
ZNF638	GGAGGAAGAUGGAGAUAAUTT	AUUAUCUCCAUCUUCCUCCTT
MPP8	CAAGGUUAAGUUGCUAAUATT	UAUUAGCAACUUAACCUUGTT
TASOR	CUGGCUUGGUGACUUUAAATT	UUUAAAGUCACCAAGCCAGTT
PPHLIN1	GGCAGAAUUUACAUGAAAUTT	AUUUCAUGUAAAUUCUGCCTT
SETDB1	GACCUAUCAGGAAUGAGCATT	UGCUCAUUCCUCAUAGGUCTT
siRNA in animal experiments		

ZNF638	GmsCmsCmAmCfAmGfAfAmCfAmAvpAvp GvpAmAmAmUmGmAmAmTmTm-Galnac	UmsUfsCmAmUmUvpCmUfUfUmGm UmUmCfUmGmUfGmGmCmsTmsTm
HBV	GmsUmsGmUmGfCmAfCfUmUmCmGmCm UmUmCmAmCmAm-Galnac	UmsGfsUmGmAm(Agn)GmCfGfAmA m GmUmGfCmAfCmAmCmsUmsUm

m: 2'-O-methyl; f: 2'-fluoro; ps: phosphorothioate; vp: vinylphosphonate; gn: glycol nucleic acid

Table S2 Clinicopathological information of patient serum samples used in this study

Record number	Age	Gender	Disease	HBeAg (IU/ml)	HBV rcDNA (x 1,000 copies/ml)
11104577	45	M	HCC	>250	3,360
11300054	55	M	HCC	>250	135
11181709	54	F	HCC	>250	29.4
11152462	27	M	HCC	>250	6

Table S3 The qPCR primers used in this study

qPCR primers	**Forward**	**Reverse**
HBV rcDNA	GGAGGGATACATAGAGGTTCCTTGA	GTTGCCCGTTTGTCCTCTAATTC
HBV pgRNA	CTCCTCCAGCTTATAGACC	GTGAGTGGGCCTACAAA
Southern probe	AGACGAAGGTCTCAATCGCC	ACCCACAAAATGAGGCGCTA
HBV cccDNA	TCCCCGTGTGTGCCTTC	CCCCAAAGCCACCCAA
HBx	ATGGCTGCTAGGCTGTGC	TTAGGCAGAGGGGAAAAAGTTG
HBc	ATTCCTATGGGAGTGGGCC	AAGCCAAACAGTGGGGAAA
HBV 91-318 nt	CCTGTTCTGACTACTGCCTC	CTGCGAATTTTGGCCAAGAC
HBV 445-778 nt	CTTCTGGACTATCAAGGTAT	TGCTGTACAGACTTGGCCCC
ZNF638-human	CACACGATTTGCCTACGTCAC	TCTGCCCTTTAGGAGAGTGG
ZNF638-mice	ATGTCGAGACCCAGGTTTAATCC	TGTGGCCCCATGTTCTGATAAA
MPP8	CGATCACAAAACCAAGGAAAA	TTCTCATCTGCTTGGCACAC
TASOR	TGAAGACATTGCAGGTTTCATTC	CATCCAGGCTATCAACACCAG
SETDB1	GGTGGTCGCCAAATACAAA	GGAAGCATAGCCATCATCAA

PPHLIN1	GGTTCCAGTGTCAGTAGCAG	TCATTCTGCCGTTTGTAGGAG
HBeAG	CATGCAACTTTTTCACCTCTG	ACGCTGGATCTTCCAAATTAA
GAPDH-human	GGGAGCCAAAAGGGTCATCA	CGTGGACTGTGGTCATGAGT
GAPDH-mice	CTCTGGAAAGCTGTGGCGTGATG	ATGCCAGTGAGCTTCCCGTTCAG
HBV DNA fragment		
F1	AATTCCACAACCTTCCACCA	TCCTGATGTGATGTTCTCCA
F2	CATCAGGATTCCTAGGACCC	AGTGATTGGAGGTTGGGGAC
F3	CACCAACCTCTTGTCCTC	AGTCCAGAAGAACCAACAA
F4	ATCAAGGTATGTTGCCCGTT	GGAGAAACGGGCTGAGGCCC
F5	CCATCCCATCATCCTGGGCT	TGCTGTACAGACTTGGCCCC
F6	GCATCTTGAGTCCCTTTTTA	TTTGTTTTGTTAGGGTTTAA
F7	GAGATGGGGTTACTCTCTA	GTTTTCTAAAACATTCTTTGA
F8	TTCCTATTAACAGGCCTATT	TGCATACAAAGGCATCAACG
F9	TGTATTCAATCTAAGCAGGC	GCAAACACTTGGCACAGACC
F10	TGACGCAACCCCCACTGGCT	GCGAGCAAAACAAGCGGCTA
F11	AGCAGGTCTGGAGCAAACAT	GTGCGCCCCGTGGTCGGTCG
F12	CTCTCTTTACGCGGACTCCC	CAAGGTCGGTCGTTGACATT
F13	AGGCATACTTCAAAGACTG	TGCTGGTGCGCAGACCA
F14	CATGCAACTTTTTCACCTCTG	ACGCTGGATCTTCCAAATTAA
F15	CCAGCGTCTAGAGACCTAGT	ATTTGGTGGTCTATAAGCTG
F16	GCCCCTATCCTATCAACACT	TTAAAGACAGGTACAGTAGAAG
F17	TCCTCATTGGAAAACACCAT	TAATGATTAACTAGATGTTC
F18	CTTCCAAACTAGACACTATT	CTGGATTTGCGGTGTTTGC
F19	ATTGGGACTTCAATCCCAAC	CAAAGTTTGTAGTATGCCCT

F20	CCGCCTCCTGCCTCCACCAA	CTCAAAGGTGGAGACAGCGG