Supplementary Information

Molecular insights into Spindlin1-HBx interplay and its impact on HBV transcription from cccDNA minichromosome

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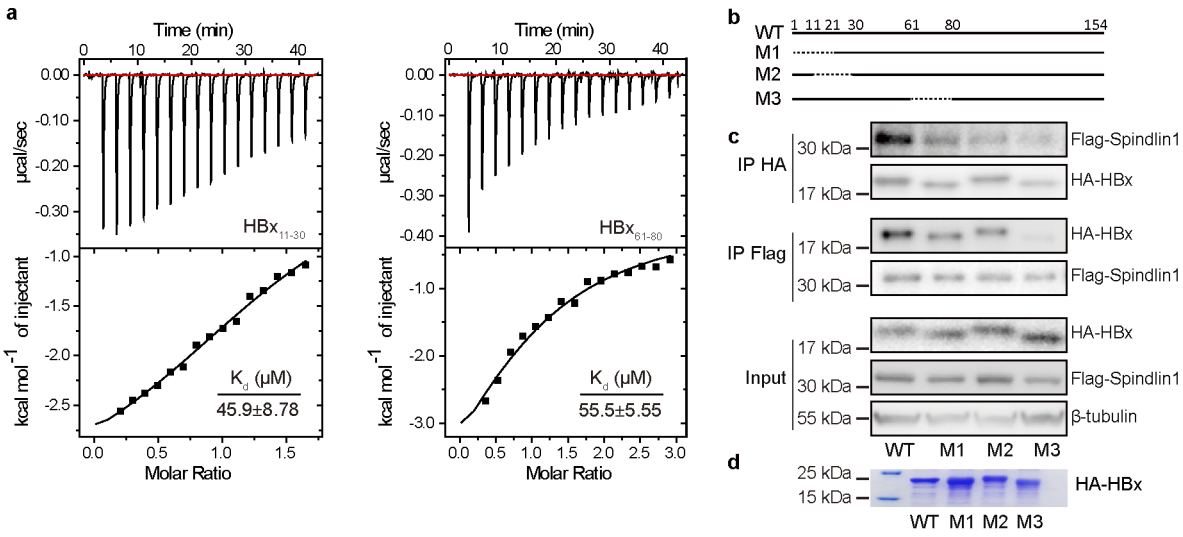
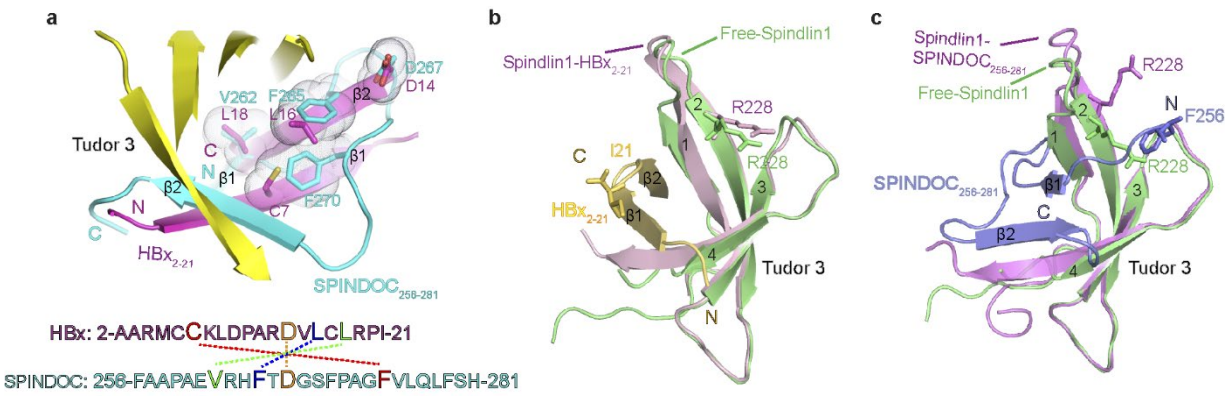
This Supplementary Information file contains:

Supplementary Figures 1-5

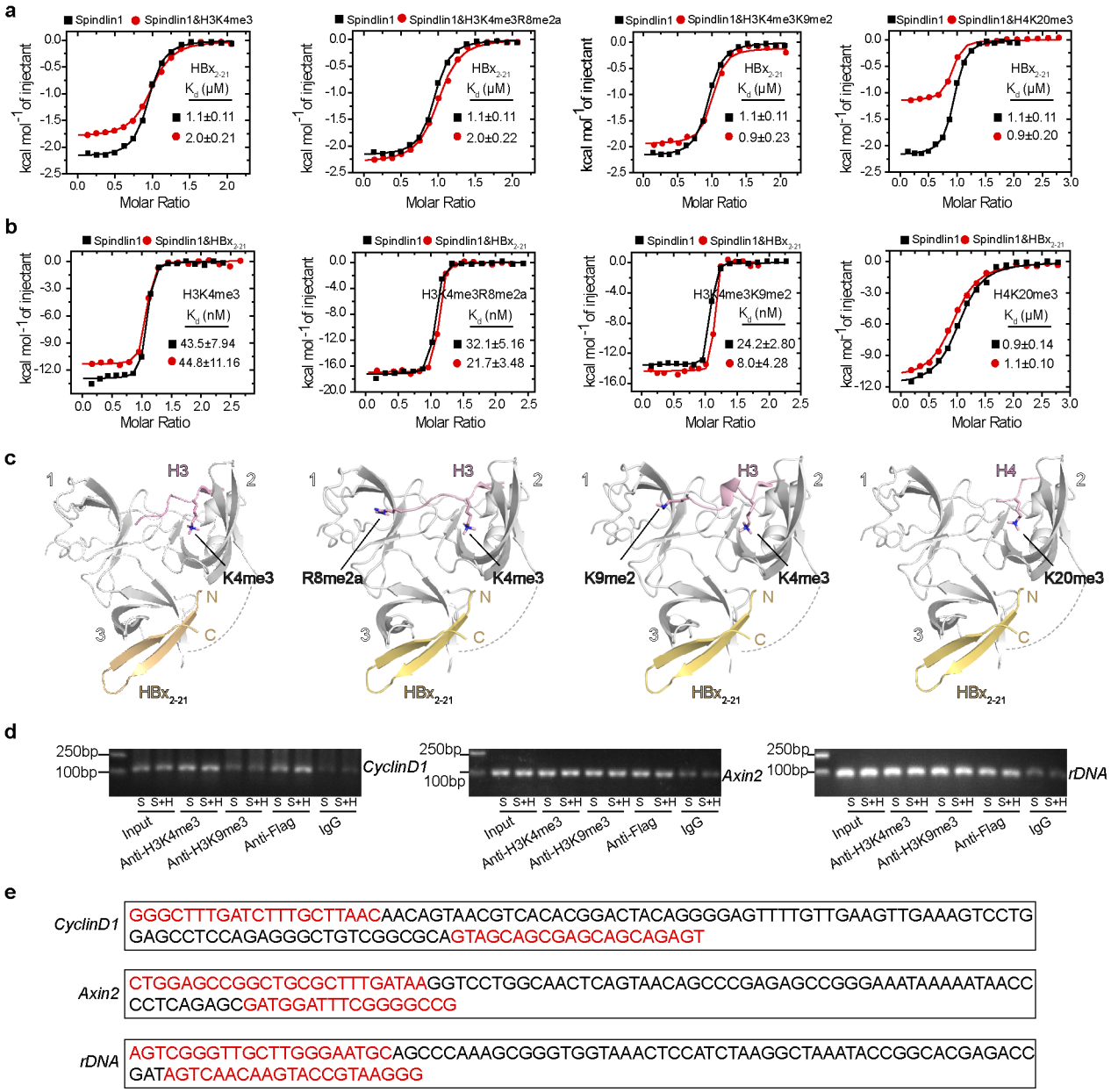
Supplementary Tables 1-4



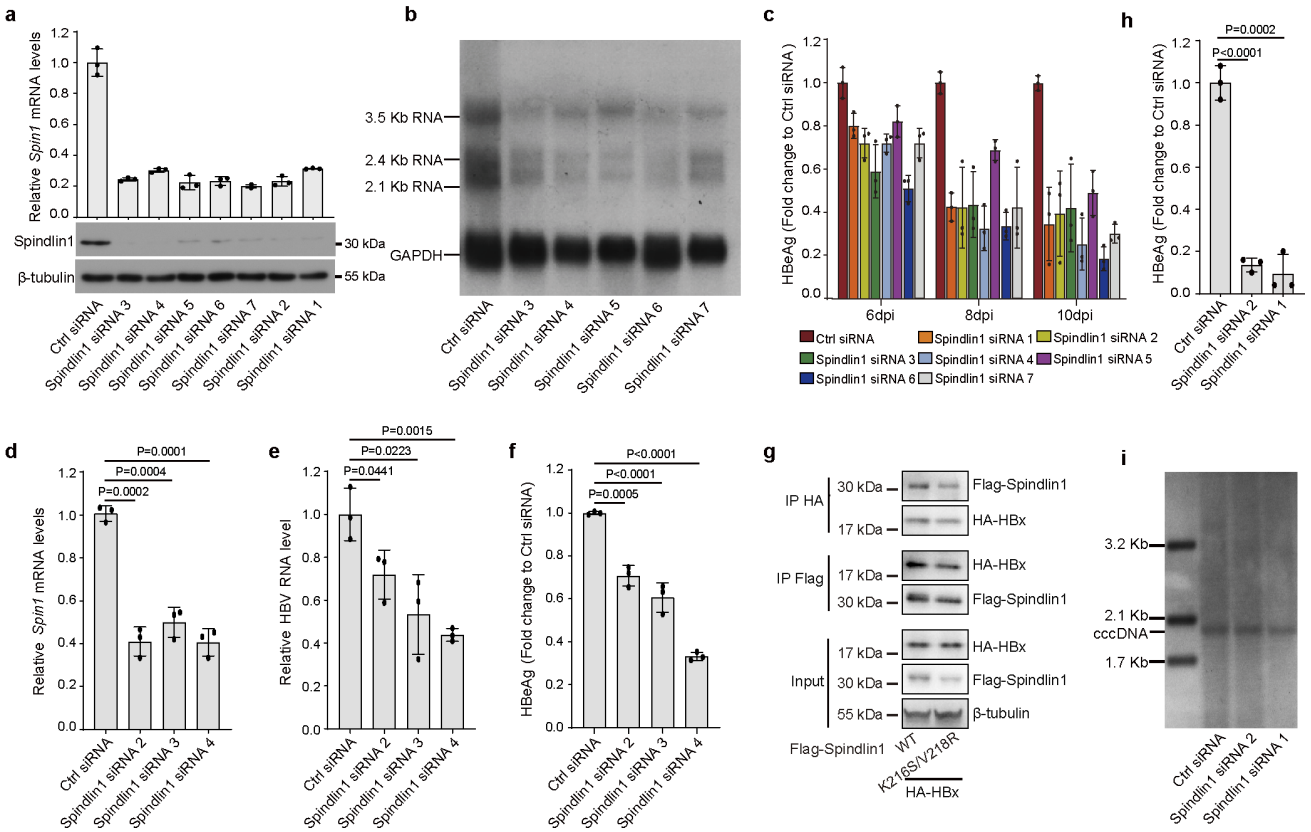
Supplementary Figure 1. Spindlin1 interacts with HBx. (a) ITC fitting curves of HBx_{11-30} and HBx_{61-80} peptides titrated to human $Spindlin1_{50–262}$ protein. (b) Determination of the region of HBx interacting with Spindlin1. Schematic representation of the full-length WT and mutant HBx protein. (c) HEK 293 T cells were co-transfected with Flag-Spindlin1 and HA-tagged WT or mutant HBx containing a substitution of 20 consecutive amino acids by alanine (M1=HBx_{2-21} mutant, M2=HBx_{11-30} mutant and M3=HBx_{61-80} mutant). Cellular extracts were immunoprecipitated with anti-Flag M2 agarose beads and anti-HA antibodies and analyzed by the indicated antibodies using WB. β-tubulin was measured and analyzed as an input control. n = 3 independent experiments. (d) SDS-PAGE gel showing the band positions of the four purified proteins: WT, M1, M2 and M3. n = 2 independent experiments. Source data are provided as a Source Data file.



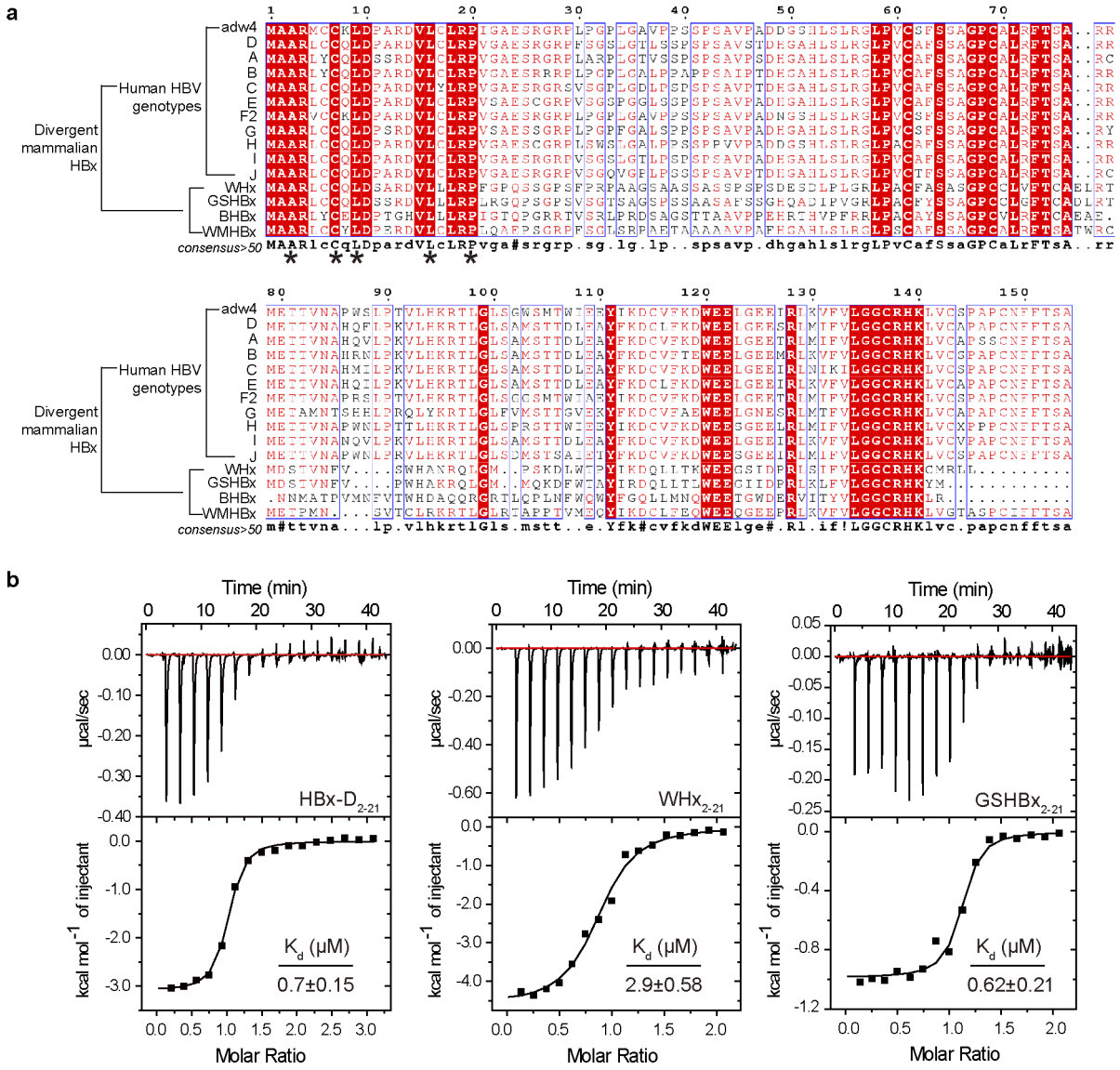
Supplementary Figure 2. Structural comparison of Spindlin1-HBx$_{2\text{-}21}$ complex with Spindlin1-SPINDOC$_{256\text{-}281}$ complex. (a) Both HBx$_{2\text{-}21}$ (light magenta) and SPINDOC$_{256\text{-}281}$ (cyan) complete the β-barrel fold of Spindlin1 Tudor 3 (yellow) with similar hydrophobic core formation and β-sheet formation. (b-c) Structural alignment of free Spindlin1 Tudor 3 (green) (PDB ID 2NS2) with Spindlin1-HBx$_{2\text{-}21}$ complex (b, pink-yellow) or Spindlin1-SPINDOC$_{256\text{-}281}$ (c, violet-slate, PDB ID 7E9M).



Supplementary Figure 3. HBx engagement is compatible with histone binding by Spindlin1. (a) ITC fitting curves of HBx_{2-21} peptide titrated to free $Spindlin1_{50–262}$ protein or $Spindlin1_{50–262}$ protein incubated with H3K4me3, H3 “K4me3-R8me2a”, H3 “K4me3-K9me2” or H4K20me3 peptide. (b) ITC fitting curves of H3K4me3, H3 “K4me3-R8me2a”, H3 “K4me3-K9me2” or H4K20me3 peptide titrated to free $Spindlin1_{50–262}$ protein or $Spindlin1_{50–262}$ protein incubated with HBx_{2-21} peptide. (c) Cartoon representation of structural comparison of Spindlin1-HBx_{2-21} with Spindlin1-H3K4me3 (PDB ID 4MZG), Spindlin1-H3 “K4me3-R8me2a” (PDB ID 4MZF), Spindlin1-H3 “K4me3-K9me2” (PDB ID 7BU9) or Spindlin1-H4K20me3 (PDB ID 5Y5W). (d-e) DNA gel (d) and representative Sanger sequencing data (e) of ChIP-qPCR results in HEK 293 T cells, which were transfected with Flag-Spindlin1 (S) or Flag-Spindlin1 plus HBx (S+H). The bases marked in red represent the corresponding primer sequences used for qPCR analyses. n = 3 independent experiments. Source data are provided as a Source Data file.



Supplementary Figure 4. Spindlin1 is required for HBV transcription. (a-c) We chose another five Spindlin1 siRNAs (3-7) to confirm the function of Spindlin1-HBx interaction in HBV transcription. (a) Detection of Spindlin1 knockdown efficiency in HBV infected HepG2-NTCP cells by WB and RT-qPCR analysis (Spindlin1 siRNAs versus Ctrl siRNA, *P*-values are 0.0001, 0.0002, 0.0002, 0.0001, 0.0001, 0.0001 and 0.0002, respectively from Spindlin1 siRNA 3 to 7, 2, 1). (b-c) (b) NB analysis of HBV transcription in HBV infected HepG2-NTCP cells, which were transfected with Ctrl siRNA or Spindlin1 siRNAs at 3dpi and collected at 10dpi for NB analysis. The corresponding culture medium were collected every two days for HBeAg analysis by ELISA (c). Spindlin1 siRNAs versus Ctrl siRNA, *P*-values are 0.0199, 0.0080, 0.0077, 0.0043, 0.0377, 0.0008 and 0.0080 at 6dpi, 0.0003, 0.0066, 0.0037, 0.0005, 0.0014, 0.0001 and 0.0066 at 8dpi, 0.0114, 0.0065, 0.0081, 0.0005, 0.0013, <0.0001 and <0.0001 at 10dpi, respectively from Spindlin1 siRNA 1 to 7. (d) Detection of Spindlin1 knockdown efficiency in HBV infected primary human hepatocytes (PHHs). (e-f) PHHs were transfected with Ctrl siRNA or Spindlin1 siRNAs (2, 3 and 4 tested here) for 16h and then infected with HBV. At 11dpi, cells and culture medium were harvest and HBV RNA levels and HBeAg levels were analyzed by RT-qPCR (e) and ELISA (f), respectively. (g) Co-IP assay assessing the interaction between HBx and Spindlin1 mutant in Huh7 cells. Cells were co-transfected with HA-tagged HBx and WT or K216S/V218R double mutant Flag-Spindlin1. After 48h, cellular extracts were immunoprecipitated with anti-Flag and anti-HA antibodies and analyzed using WB. β-tubulin was measured and analyzed as an input control. (h-i) HepG2-NTCP cells were transfected with Ctrl siRNA or Spindlin1 siRNAs for 48h and then infected with HBV virus. At 3dpi, culture medium and cells were harvested and HBeAg and HBV cccDNA levels were analyzed by ELISA (h) and Southern blot (i), respectively. (a, c-f, h) Data represent the mean±SD (n = 3 independent experiments). *P*-values between the groups were calculated with an unpaired two tailed *t* test. Source data are provided as a Source Data file.



Supplementary Figure 5. Conservation of Spindlin1-HBx interaction in mammals. (a) Alignment of HBx protein sequences from various human HBV subtypes and mammals. * Key residues involved in Spindlin1 engagement. (b) ITC fitting curves of HBx-D$_{2-21}$, WHx$_{2-21}$ and GSHBx$_{2-21}$ peptides titrated to Spindlin1$_{50–262}$ protein. WHx: woodchuck HBx; GSHBx: ground squirrel HBx; BHBx: bat HBx; WMHBx: wooly monkey HBx.

Supplementary Table 1. List of peptides used in this study.

Peptide name	Sequences
HBx_{2-21}	AARMCCKLDPARDVLCLRPI-NH2
HBx_{11-30}	Ac-PARDVLCLRPIGAESRGRPL-NH2
HBx_{21-40}	Ac-IGAESRGRPLPGPLGAVPPS-NH2
HBx_{31-50}	Ac-PGPLGAVPPSSPSAVPADDG-NH2
HBx_{41-60}	Ac-SPSAVPADDGSHLSLRGLPV-NH2
HBx_{51-70}	Ac-SHLSLRGLPVCSFSSAGPCA-NH2
HBx_{61-80}	Ac-CSFSSAGPCALRFTSARRME-NH2
HBx_{71-90}	Ac-LRFTSARRMETTVNAPWSLP-NH2
HBx_{81-100}	Ac-TTVNAPWSLPTVLHKRTLGL-NH2
HBx_{91-110}	Ac-TVLHKRTLGLSGWSMTWIEE-NH2
$HBx_{101-120}$	Ac-SGWSMTWIEEYIKDCVFKDW-NH2
$HBx_{111-130}$	Ac-YIKDCVFKDWEELGEEIRLK-NH2
$HBx_{121-140}$	Ac-EELGEEIRLKVFVLGGCRHK-NH2
$HBx_{131-154}$	Ac-VFVLGGCRHKLVCSPAPCNFFTSA
$H3_{1-10}$K4me3	ARTK(me3)QTARKS-NH2
$H3_{1-20}$K4me3	ARTK(me3)QTARKSTGGKAPRKQL-NH2
$H3_{1-20}$K4me3R8me2a	ARTK(me3)QTAR(me2a)KSTGGKAPRKQL-NH2
$H3_{1-15}$K4me3K9me2	ARTK(me3)QTARK(me2)STGGKA-NH2
$H3_{1-15}$K4me3K9me3	ARTK(me3)QTARK(me3)STGGKA
$H3_{1-20}$K4me3K9me3	ARTK(me3)QTARK(me3)STGGKAPRKQL
$H4_{11-30}$K20me3	Ac-GKGGAKRHRK(me3)VLRDNIQGIT-NH2
HBx-D_{2-21}	AARLCCQLDPARDVLCLRPV-NH2
WHx	AARLCCQLDSARDVLLLRPF-NH2
GSHBx	AARLCCQLDSSRDVLLLRPL-NH2

Supplementary Table 2. Summary of thermodynamic parameters from isothermal titration calorimetry binding assays.

Protein	Peptide name	Δ H (kal/mol)	Δ S (cal/mol/deg)	K (M^{-1})	N
Spindlin1$_{50-262}$	HBx$_{2-21}$	-2183±19.1	20	9.14E5±9.40E4	0.9±0.005
	HBX$_{11-30}$	-3496±197.8	8.2	2.26E4±4.32E3	1.2±0.029
	HBx$_{61-80}$	-6319±252.7	-1.7	1.82E4±1.82E3	1±0.000
	HBx-D$_{2-21}$	-3096±42.71	18.0	1.56E6±2.43E5	0.951±0.009
	FWHx	-4600±130.4	9.96	3.54E5±7.02E4	0.9±0.018
	GSHBx	-987.3±23.4	25.3	1.83E6±6.32E5	1.1±0.017
	HBx$_{2-21}$V15R	-854.0±51.42	19.4	7.36E4±1.78E4	1.0±0.049
	HBx$_{2-21}$L18R	-840.2±102.4	17.7	3.11E4±9.95E3	1.1±0.083
	HBx$_{2-21}$ V15E/C17R	N.D.	N.D.	N.D.	N.D.
	HBx$_{2-21}$AAAAA	N.D.	N.D.	N.D.	N.D.
	H3$_{1-20}$K4me3	-12980±106.6	-9.75	2.38E7±4.35E6	1.0±0.005
	H3$_{1-20}$K4me3R8me2a	-17280±115.8	-23.6	3.20E7±5.15E6	1.0±0.004
	H3$_{1-15}$K4me3K9me2	-14960±61.22	-15.3	4.18E7±4.82E6	1.0±0.002
	H3$_{1-20}$K4me3K9me3	-13500±85.75	-9.0	8.53E7±2.90E7	1.0±0.003
	H4$_{11-30}$K20me3	-12920±254.2	-12.3	1.1E6±1.6E5	1.0±0.016
Spindlin1$_{50-262}$ K216S/V218R		N.D.	N.D.	N.D.	N.D.
Spindlin1$_{50-262}$ V232R	HBx$_{2-21}$	N.D.	N.D.	N.D.	N.D.
Spindlin1$_{50-262}$ I245R		N.D.	N.D.	N.D.	N.D.
Spindlin1$_{50-262}$&H3$_{1-20}$K4me3		-1803±19.54	20.1	5.07E5±5.28E4	1.0±0.007
Spindlin1$_{50-262}$&H3$_{1-20}$K4me3R8me2a		-2330±26.26	18.3	4.98E5±5.37E4	1.0±0.008
Spindlin1$_{50-262}$&H3$_{1-15}$K4me3K9me2	HBx$_{2-21}$	-1913±34.77	21.5	1.24E6±3.23E5	1.0±0.016
Spindlin1$_{50-262}$&H3$_{1-20}$K4me3K9me3		-1371±34.55	21.5	5.13E5±1.27E5	1.0±0.018
Spindlin1$_{50-262}$&H4$_{11-30}$K20me3		-1145±17.93	23.9	1.17E6±2.58E5	0.8±0.009
Spindlin1$_{50-262}$&HBx$_{2-21}$	H3$_{1-20}$K4me3	-11440±112.1	-4.62	2.38E7±5.93E6	1.0±0.005
	H3$_{1-20}$K4me3R8me2a	-17040±96.9	-22.0	4.74E7±7.62E6	1.0±0.003
	H3$_{1-15}$K4me3K9me2	-14860±139.6	-12.1	1.74E8±9.28E7	1.0±0.004
	H4$_{11-30}$K20me3	-15790±47.9	-14.3	2.80E8±6.75E7	1.0±0.001
	H4$_{11-30}$K20me3	-11220±165.1	-10.3	9.43E5±8.44E4	0.9±0.010

N.D. not detectable

Supplementary Table 3. Primer sequences used in this study (for HBV primers the positions are indicated relative to EcoRI site).

Primer ID	Sequences	Experiment
β-actin_F	CGTCACCAACTGGGACGACA	RT-qPCR
β-actin_R	CTTCTCGCGGTTGGCCTTGG	RT-qPCR
Spindlin1_F	ACCCCATTCGGAAAGACACC	RT-qPCR
Spindlin1_R	CCATTCCCCTCTTTCCACCC	RT-qPCR
HBV_F	TCACCAGCACCATGCAAC	RT-qPCR
HBV_R	AAGCCACCCAAGGCACAG	RT-qPCR
Cyclin D1_F	CCGTCCATGCGGAAGATC	RT-qPCR
Cyclin D1_R	ATGGCCAGCGGGAAGAC	RT-qPCR
Axin2_F	AGTGTGAGGTCCACGGAAAC	RT-qPCR
Axin2_R	CTTCACACTGCGATGCATTT	RT-qPCR
Pre-rRNA_F	TGTCAGGCGTTCTCGTCTC	RT-qPCR
Pre-rRNA_R	AGCACGACGTCACCACATC	RT-qPCR
HBV cccDNA_F	GTGCACTTCGCTTCACCTCT (Positions 1579-1598)	ChIP-qPCR
HBV cccDNA_R	AGCTTGGAGGCTTGAACAGT (Positions 1859-1878)	ChIP-qPCR
rDNA_F	AGTCGGGTTGCTTGGGAATGC	ChIP-qPCR
rDNA_R	CCCTTACGGTACTTGTTGACT	ChIP-qPCR
Axin2_F	CTGGAGCCGGCTGCGCTTTGATAA	ChIP-qPCR
Axin2_R	CGGCCCCGAAATCCATCGCTCTGA	ChIP-qPCR
Cyclin D1_F	GGGCTTTGATCTTTGCTTAAC	ChIP-qPCR
Cyclin D1_R	ACTCTGCTGCTCGCTGCTAC	ChIP-qPCR

Supplementary Table 4. Sequences of siRNAs used in this study.

siRNA name	Sequences (5'-3')
Ctrl siRNA sense	UUCUCCGAACGUGUCACGUTT
Ctrl siRNA antisense	ACGUGACACGUUCGGAGAATT
Spindlin1 siRNA 1 sense	GCAAAGCAGUGGAACAUAUTT
Spindlin1 siRNA 1 antisense	AUAUGUUCCACUGCUUUGCTT
Spindlin1 siRNA 2 sense	GCAUUAUGCCUGAUUCCAATT
Spindlin1 siRNA 2 antisense	UUGGAAUCAGGCAUAAUGCTT
Spindlin1 siRNA 3 sense	GGAAUAUGCCAAAGAAGAUTT
Spindlin1 siRNA 3 antisense	AUCUUCUUUGGCAUAUUCCTT
Spindlin1 siRNA 4 sense	GCACCUGUCAUGAACACAUTT
Spindlin1 siRNA 4 antisense	AUGUGUUCAUGACAGGUGCTT
Spindlin1 siRNA 5 sense	GCACACUUGGCAGACACAATT
Spindlin1 siRNA 5 antisense	UUGUGUCUGCCAAGUGUGCTT
Spindlin1 siRNA 6 sense	GGACCAGGUGCCUGUAAAUTT
Spindlin1 siRNA 6 antisense	AUUUACAGGCACCUGGUCCTT
Spindlin1 siRNA 7 sense	CCCUGUUACCCAGUGGAAATT
Spindlin1 siRNA 7 antisense	UUUCCACUGGGUAACAGGGTT