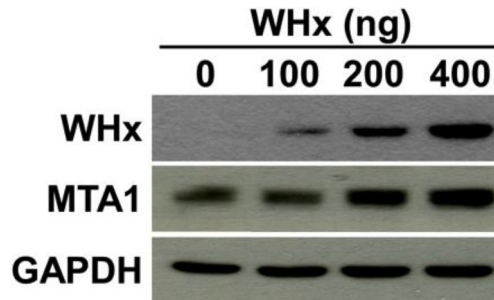
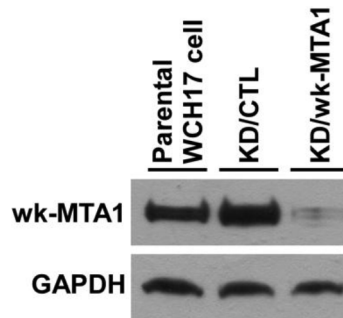


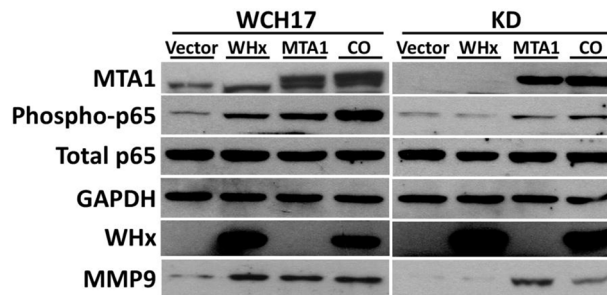
Supplementary Figure S2: Presence of wk-MTA1 spliced variant wk-MTA1dE4 was examined in woodchuck autopsy samples. The expression levels of wk-MTA1-FL and wk-MTA1dE4 were determined using conventional RT-PCR with primer pairs covering the spliced region of wk-MTA1dE4 (MTA1, +40 and MTA1, -477) in liver tissues from one WHV-resolved woodchuck (designated as 4619 liver) and 4 woodchucks with paired tumor and nontumor tissues. DDX5 was used as the internal control.



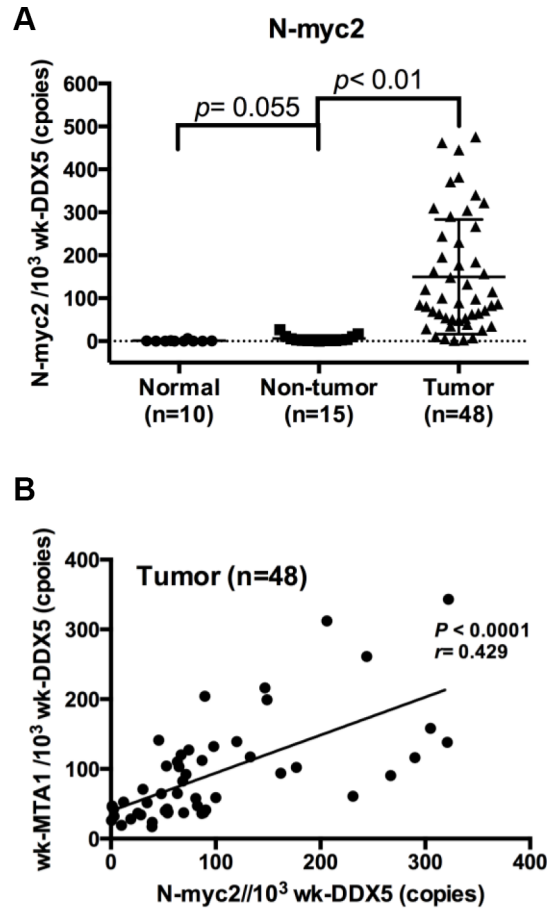
Supplementary Figure S3: WHx dose-dependently upregulates wk-MTA1 expression in WCH17 cells. Western blotting of WHx and wk-MTA1 in WCH17 cells transfected with either control vector (0) or WHx-expressing plasmids at different concentrations (100, 200, and 400 ng/transfection). GAPDH was used as the loading control.



Supplementary Figure S4: Wk-MTA1 protein was significantly decreased in KD/wk-MTA1 cells but not in KD/CTL cells. WCH17 cells (designated as parental cells) were transfected with wk-MTA1-targeting shRNA (sh-MTA1-1) and control shRNA (sh-m-MTA1). Puromycin-resistant cells stably expressing shMTA1-1 and sh-mMTA1 were designated as KD/wk-MTA1 and KD/CTL (control designated as CTL). Cells were lysed, and the expression level of wk-MTA1 was examined using Western blotting.

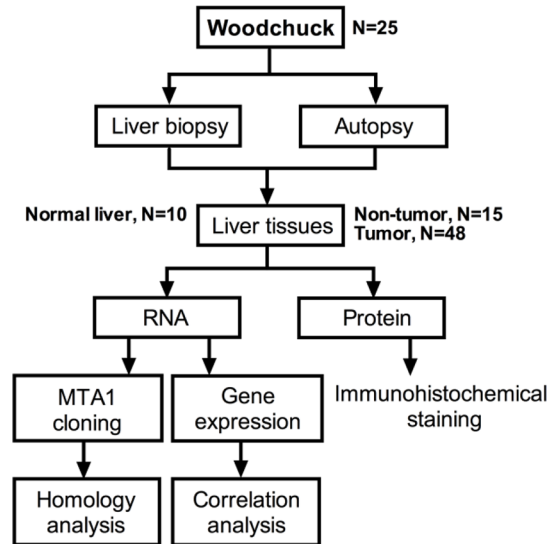


Supplementary Figure S5: Wk-MTA1 is essential for WHx-mediated NF-κB signaling. The expression levels of wk-MTA1, phosphorylated p65, total p65, WHx, and MMP9 were assessed in WCH17 and KD/wk-MTA1 WCH17 (designated as KD) cells by western blot. GAPDH served as a loading control. CO: cells cotransfected with WHx and wk-MTA1-expressing plasmids.

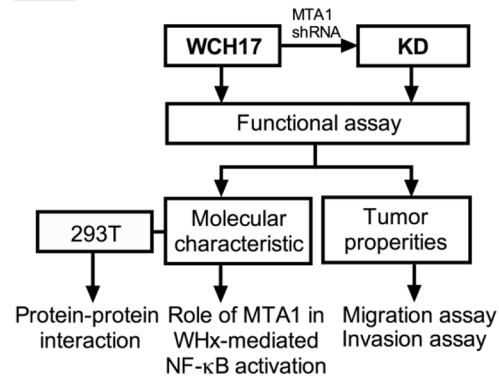


Supplementary Figure S6: N-myc2 was upregulated in woodchuck HCC tissues and positively correlated with the levels of wk-MTA1. (A) The expression of N-myc2 was detected using qRT-PCR with primer pairs (N-myc2, +780 and N-myc2, -1368) in normal and nontumor and tumor tissues. (B) A positive correlation was observed between wk-MTA1 expression and N-myc2 expression in woodchuck tumor liver samples ($n = 48$). The statistical significance and correlation coefficient r was determined using Pearson correlation. The expression of each target gene was normalized to 10^3 wk-DDX5 mRNA. The results are presented as mean \pm SD.

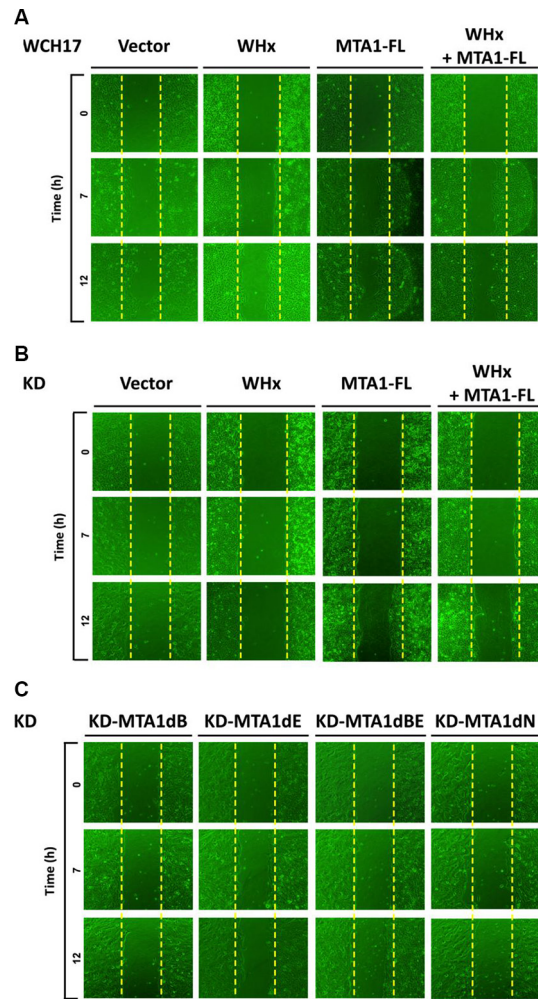
Animal model



in vitro

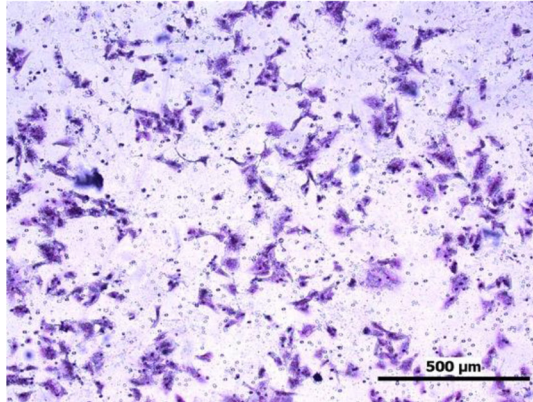


Supplementary Figure S7: Flowchart of the experimental approaches used in this study. Liver biopsies from 10 WHV-resolved woodchucks were designated as normal liver tissues. All paired tumor and nontumor tissues were obtained from 15 woodchucks. Four of the woodchucks had a single tumor, whereas the remaining woodchucks had multiple tumors.

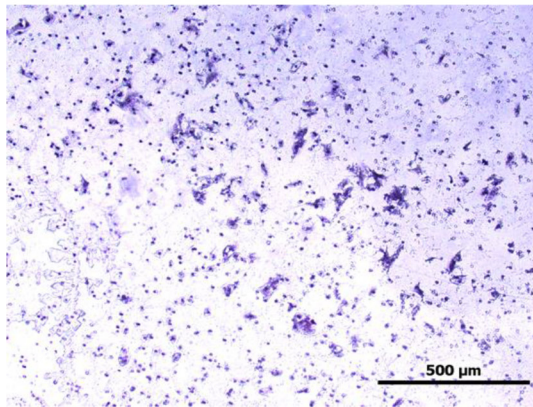


Supplementary Figure S8: Wound healing assay was employed to determine the migration ability of WCH17 and MTA1-knocked down cells. (A) WCH17 and (B) KD cells were transfected with an empty vector, WHx, and wk-MTA1 expression plasmids. (C) KD cells were transfected with wk-MTA1 expressing plasmids with different domain deletions. Cells were monitored and photographed at 7 and 12 hours after incubation. Original magnification: $\times 200$.

A WCH17



B KD



Supplementary Figure S9: Knockdown of wk-MTA1 expression reduces cell invasion ability. The invasiveness of WCH17 and KD cells was determined using the transwell assay. The invaded (A) WCH17 and (B) KD cells were photographed at 22 hours after plating. Original magnification: $\times 400$.

Supplementary Table S1: Primers and probes used for cloning, Northern blotting, RT-PCR, and qRT-PCR analysis

Primer		Priming site ²	Oligonucleotide sequence (5'→3')
3'RACE Outer ¹	Rv		GCG AGC ACA GAA TTA ATA CGA CT
5'RACE Outer ¹	Fw		GCT GAT GGC GAT GAA TGA ACA CTG
5'RACE Inner ¹	Fw		CGC GGA TCC GAA CAC TGC GTT TGC TGG CTT TGA TGA AA
MTA1-F	Fw	1320→1346	GCC RGG ACC AAA CCG CAR TAA CAT GAG ³
MTA1-R	Rv	1727→1710	GGA GCC ATT GTT GAT GAC C
MTA1-F2	Fw	-15→3	GCC GCC GGC CCG GAC <u>ATG</u>
MTA1-FC	Fw	1→12	GGA ATT CCG ACA <u>TGG</u> CCG CCA AC
MTA1-RC	Rv	2148→2135	GCT CTA GAG <u>CCT</u> AGT CCT CRA TGA ³
MTA1 +392	Fw	392→414	TCA ACG AGA CCG AGT CGC TCA AG
MTA1 -910	Rv	910→888	CAT ATT TTT CCA GGG CTT CCT C
18 s rRNA F	Fw		CCC TGT AAT TGG AAT GAG TCC A
18 s rRNA R	Rv		ATC TGA TCG TCT TCG AAC CTC C
MTA1 +392	Fw	392→414	TCA ACG AGA CCG AGT CGC TCA AG
MTA1 -910	Rv	910→888	CAT ATT TTT CCA GGG CTT CCT C
18 s rRNA F	Fw		CCC TGT AAT TGG AAT GAG TCC A
18 s rRNA R	Rv		ATC TGA TCG TCT TCG AAC CTC C
MTA1-FQ	Fw	1177→1194	TGC GAG AGC TGT TAC ACC
MTA1-RQ	Rv	1244→1227	RCG ACA CTG CAT GTT AGG ³
DDX5-F	Fw		CTT GTC CTT GAT GAA GCA GA
DDX5-R	Rv		AGT CGC ACT CCA CAT TAG
MTA1-FL-F	Fw	194→212	TGT CAG TCT GCT ATA AGG C
MTA1dE4-F	Fw	178→190, 242→246	GAC AAG CAY GCA AGG GAA ³
MTA1 -421	Rv	421→405	GGT AGG ACT TGA GCG AC
MTA1 +40	Fw	40→62	TTC GAG AAC TCC TCC AGC AAC CC
MTA1 -477	Rv	477→455	GGT CTT CTG CTG TGG GTC GTA GA
N-myc2 +780	Fw		GTT GAC GAG GAG GAA GAT GA
N-myc2 -948	Rv		GCA TAG TTG TGC TGC TGG TA

Fw: forward primer; Rv: reverse primer; EcoRI and XbaI endonuclease digestion sites are shown in bold. Start and stop codons are indicated by underlined and double underlined characters, respectively.

1: The primer was obtained from the FirstChoice RLM-RACE kit.

2: The position of the primers relative to our published *Marmota monax* MTA1 cDNA sequence (Accession number: JX891465.1).

3: Degenerate primer (symbol R = A or G nucleotide, symbol Y = C or T nucleotide).

Supplementary Table S2: Details of PCR amplification programs for molecular cloning

Resulted region	Strategy	PCR program			
		Step	Temperature (°C)	Time	No. of Cycle
3' RLM -RACE 1320 ↓ 2629	RACE-PCR	Initial Denature	95	3 min	1
		Denature Annealing Extension	95 57 72	30 sec 30 sec 30 sec	35
		Final extension	72	7 min	1
5' RLM -RACE 1072 ↓ 1727	Slowdown PCR ⁺	Initial Denature	98	3 min	1
		Denature Annealing Extension	98 66→52 [#] 68	30 sec 30 sec 30 sec	45
		Denature Annealing Extension	98 57 68	30 sec 30 sec 30 sec	15*
		Final extension	68	7 min	1
wk-MTA1	1 ↓ 1244	Slowdown PCR ⁺	As same as 5' RLM-RACE		

All cycles were run in a generally reduced ramp rate at 2.5°C and especially a small cooling rate for reaching annealing temperature at 1.5°C.

[#]The annealing temperature is gradually decreased during the cycling process.

⁺A well-established PCR method, which allows the successful PCR-amplification of extremely GC-rich (> 83%) DNA targets.

*Additional cycles.