Lnc-UCID promotes G1/S transition and hepatoma growth by preventing DHX9-mediated CDK6 downregulation

Yun-Long Wang, Jin-Yu Liu, Jin-E Yang, Xiao-Man Yu, Zhan-Li Chen, Ya-Jing Chen, Ming Kuang, Ying Zhu and Shi-Mei Zhuang

Inventory of supplementary data

Supplementary Materials and Methods

Analysis of gene expression

Real-time quantitative polymerase chain reaction (qPCR) assays were performed to evaluate the RNA levels. For mRNA and lncRNA analysis, total RNA was extracted using TRIzol reagent (Invitrogen) and reverse-transcribed using M-MLV reverse transcriptase (M1701, Promega, Madison, WI, USA). qPCR was performed on a LightCycler 480 (Roche Diagnostics, Germany) using 2×SYBR Green qPCR Master Mix (B21202, Bimake, Houston, TX, USA). For microRNA analysis, reverse-transcription and qPCR were conducted using miDETECT A Track™ miRNA qRT-PCR Starter Kit (Ribobio, Guangzhou, China). All reactions were performed in duplicate. The cycle threshold (Ct) values differed by less than 0.5 between duplicate wells. The relative expression levels of the target genes were normalized to that of internal control genes, which yielded a $2^{-\Delta Ct}$ value. U6 and GAPDH were used as the reference genes for the relative expression levels in tissues and cell lines, respectively.

Western blotting was performed to determine the protein levels. The antibodies used included the following: mouse polyclonal antibody against GAPDH (BM1623, Boster, Wuhan, China); mouse monoclonal antibodies against pRb (cat. 9309, Cell Signaling Technology, CST, Beverly, MA, USA), CDK6 (cat. 3136, CST) and α-tubulin (BM1452, Boster); rabbit polyclonal antibodies against phospho-Ser780 of pRb (cat. 9307, CST), DHX9 (EPP12284, Elabscience, Wuhan, China), lamin A/C (D120927, BBI, Shanghai, China), cyclin E1 (ENT1176, Elabscience), cyclin E2 (cat. 4132, CST), p15 (ab53034, Abcam, Cambridge, MA, USA), and E2F1 (cat. 3742, CST); and rabbit monoclonal antibodies against cyclin D1 (ab134175, Abcam,

Cambridge, MA, USA), cyclin D2 (cat. 3741, CST), cyclin A2 (ab181591, Abcam), CDK4 (ab199728, Abcam), and p16 (ab51243, Abcam).

Vector Construction

The following expression vectors were used: pc3-puro-UCID, pc3-puro-UCID-Δcore, pc3-gab-UCID, pc3-gab-CDK6 and pCDH-Flag-DHX9. The plasmids pc3-puro-UCID and pc3-puro-UCID-Δcore were produced by respectively inserting full-length lnc-UCID and mutant lnc-UCID with a 850-1030-nt deletion (Supplementary Figure 4C) into the *Eco*RI/*Xba*I sites of pc3-puro. pc3-gab-UCID and pc3-gab-CDK6 were generated by respectively cloning full-length lnc-UCID and the coding sequence of CDK6 into the *Eco*RI/*Xba*I sites of pc3-gab. The backbone plasmids pc3-puro and pc3-gab were produced based on the pcDNA3.0 vector (Invitrogen, Carlsbad, CA, USA) in which the *neomycin* open reading frame was replaced with an expression cassette for the *puromycin-resistance* gene or *enhanced green fluorescent protein (EGFP)* gene (21), respectively. To create pCDH-Flag-DHX9, C-terminal Flag-tagged DHX9 was PCR-amplified from pUC19-hDHX9 (HG17921-U, Sino Biological Inc., Beijing, China) and inserted into the *Nde*I/*Xho*I sites of pCDH-CMV-MCS-EF1-copGFP (pCDH, System Biosciences, Palo Alto, CA, USA), which contains a copGFP expression cassette.

To verify the function of the DHX9 binding element (DBE) in the 3’UTR of CDK6, luciferase reporter vectors, named psi-CDK6-DBE1 and psi-CDK6-DBE2, were generated by cloning the 1928–4104 (designated as DBE1) or 8983–11193 (DBE2) nucleotides of CDK6 mRNA (NM_001259) into the *Xho*I/*Not*I sites downstream of the stop codon of *Renilla* luciferase in psiCHECK2 (Promega), which contains *Renilla* luciferase as the primary reporter and *Firefly* luciferase as the control reporter.

For miR-148a target validation, luciferase reporter vectors, named psi-UCID-WT and psi-UCID-MUT (Supplementary Figure 10), were generated by cloning wild-type lnc-UCID sequence or mutated lnc-UCID sequence in the predicted miR-148a-binding site into the *Xho*I/*Not*I sites downstream of the stop codon of *Renilla* luciferase in psiCHECK2.

Cell counting assay

Cell counting assay was used to evaluate cell growth. For loss-of-function assays, HepG2 (4.5×10^4), QGY-7703 (1×10^4) and SF (3×10^4) cells were transfected with the indicated siRNAs and cultured for 4 days before cell counting. For gain-of-function assays, QGY-7703 cells were transfected twice at 24-hour intervals with the indicated plasmids. Twenty-four hours after the last transfection, 1×10^4 cells were reseeded in a 24-well plate and cultured for 4 days before cell counting.

Cell cycle analysis

For loss-of-function studies, all cell cycle analyses were performed using a detergent-containing hypotonic solution (Krishan's reagent: 0.05 mg/mL propidium iodide, 0.1% Na-citrate, 0.02 mg/mL ribonuclease A, 0.3% NP-40) and fluorescence-activated cell sorting (FACS) (Gallios, Beckman Coulter, Miami, FL, USA) as previously described (21). Briefly, HepG2 and SF cells were transfected with the indicated siRNA duplex for 36 hours and then treated with 50 ng/ml nocodazole for 12 (HepG2) or 18 (SF) hours. The cells were resuspended in Krishan's reagent, incubated at 37°C for 30 minutes and subjected to FACS analysis.

For gain-of-function studies, the DNA content of live cells was stained with Hoechst 33342 solution and then analysed by FACS. Briefly, QGY-7703 cells were transfected with 0.5 μg pc3-gab, pc3-gab-UCID, or pc3-gab-CDK6 for 40 hours,

cultured with nocodazole (50 ng/mL) for an additional 9 hours and then deprived of serum for 10 minutes, followed by incubation with a Hoechst 33342 solution (15 μg/mL) for 20 minutes. After that, the cells were resuspended in PBS containing 5% FBS and subjected to FACS analysis. EGFP-expressing cells were gated for the cell cycle analyses.

BrdU incorporation assay

DNA replication was examined by BrdU incorporation assay. Twelve hours after the last transfection or reseeding, cells were cultured in serum-free medium for 48 (HepG2 and SF) or 36 (QGY-7703) hours and then grown in 15% FBS-containing medium for 16 (HepG2), 18 (SF) or 6 (QGY-7703) hours, followed by labelling with 40 μM BrdU (Sigma) for 2 hours. After incubation in a fixative solution (15 mM glycine at pH 2.0 and 70% ethanol) at room temperature (RT) for 1 hour, the cells were permeabilized in a trypsin solution (0.05% trypsin and 0.05% $CaCl_2$ in 1× PBS) at 37°C for 7 minutes. The fixed cells were incubated with 4 M hydrochloric acid for 15 minutes, pre-blocked with goat serum for 1 hour, and then incubated with a rat anti-BrdU antibody (Santa Cruz) at RT for 2 hours, followed by incubation with goat anti-rat IgG (H+L) (Alexa Fluor® 488 Conjugate) (cat. 4416, CST) at RT for 1 hour. The nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI, Sigma). The BrdU incorporation rate is expressed as the percentage of BrdU-stained cells/total number of cells. At least 500 cells were counted for each sample.

Polysome analysis

HepG2 cells (2×10^7) were transfected with the indicated siRNA duplex for 48 hours and then incubated with 100 μg/mL cycloheximide (cat. 2112, CST) for 10 minutes. The cells were mixed with lysis buffer, which contained 5 mM Tris-HCl at pH 7.5, 2.5

mM $MgCl_2$, 1.5 mM KCl, 0.5% Triton X-100, 0.5% sodium deoxycholate, 2 mM DTT, 100 μg/mL cycloheximide, 200 U/mL RNasin ribonuclease inhibitor (Promega) and a protease inhibitor cocktail (Roche), and incubated on ice for 20 minutes. The cell lysates were centrifuged at 9,000×g and 4°C for 30 minutes to remove the cell debris; then, the samples were layered onto an 11-mL 10%–50% sucrose gradient and centrifuged at 35000 rpm and 4°C for 120 minutes in a Beckman SW41-Ti rotor. The gradients were fractionated and analysed at an absorbance of 254 nm. The collected fractions were subjected to qPCR analysis.

RNA pulldown assay

RNA pulldown assays were conducted as follows: 1) The template DNA for *in vitro* transcription was produced by PCR, which introduced a T7 promoter to the targeting sequence. Biotin-labelled lnc-UCID and its antisense RNA were transcribed *in vitro* from the corresponding PCR product using T7 RNA polymerase (Roche, Mannheim, Germany) and Biotin RNA Labeling Mix (Roche); then, this product was purified and renatured with Annealing Buffer for RNA Oligos (Beyotime, Shanghai, China) and heating at 90°C for 2 minutes, followed by incubation on ice for 2 minutes and at RT for 20 minutes. 2) HepG2 cells (1×10^7) were lysed with 1 mL IP-lysis buffer (25 mM Tris-HCl at pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% NP-40, 5% glycerol, 500 U/mL RNasin Ribonuclease Inhibitor and protease inhibitor cocktail) at 4°C for 30 minutes. The supernatant was collected by centrifugation (13,000×g, 4°C, 10 minutes) and mixed with the renatured biotin-labelled RNA (3 μg) by rotation at RT for 1 hour; then, 30 μL streptavidin Dynabeads (Invitrogen) was added, and the sample was rotated at RT for another 30 minutes. The beads were washed with IP-lysis buffer 5 times and then digested with 20 μg RNase A (Fermentas) at 37°C for 15 minutes to release the RNA-binding proteins. 3) The retrieved proteins were boiled in 1× SDS

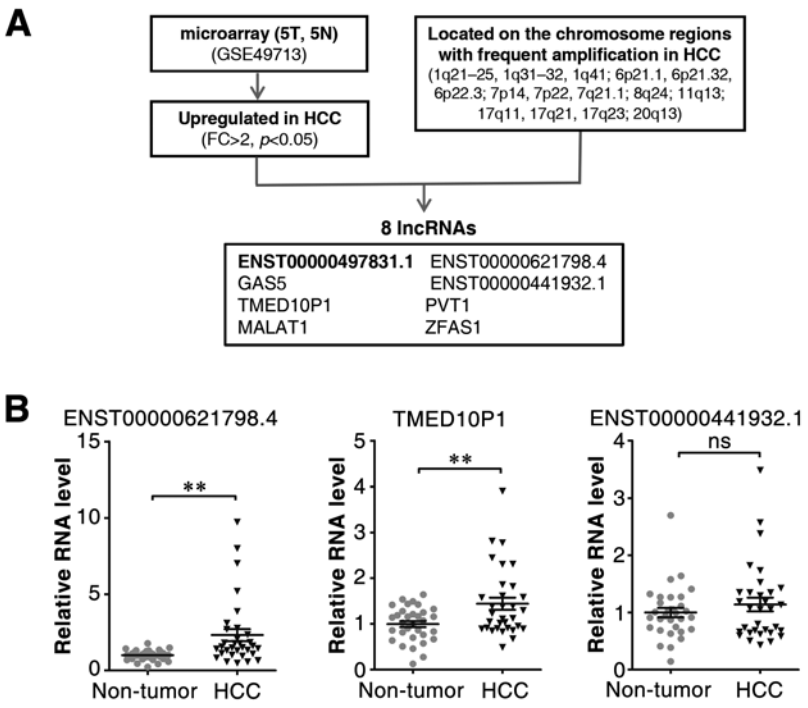
buffer (50 mM Tris-HCl at pH 6.8, 2% SDS, 0.01% bromophenol blue, 10% glycerol and 1% 2-mercaptoethanol), resolved on a sodium dodecyl sulfate-polyacrylamide gel, and subjected to silver staining. The specific band was excised and analysed by mass spectrometry (Beijing Protein Innovation, Beijing, China).

RIP assay

HepG2 cells (1×10^7) were washed with 1× PBS 3 times, cross-linked with 0.5% formaldehyde at RT for 10 minutes, incubated with 250 mM glycine at RT for another 5 minutes and then washed with 10 mL ice-cold 1×PBS 3 times. The pellets were collected by centrifugation at 400×g for 5 minutes, resuspended in 1 mL IP-lysis buffer and sonicated gently; then, the samples were centrifuged at 13,000×g and 4°C for 10 minutes. The supernatants were collected and incubated with 4 μg DHX9 antibody (EPP12284, Elabscience) or isotype-matched control IgG (A7016, Beyotime) at 4°C with gentle rotation for 4 hours, followed by the addition of 40 μl Dynabeads G (Invitrogen) and further incubation at 4°C for 2 hours. The beads were washed with a high-salt IP-lysis buffer (25 mM Tris-HCl at pH 7.4, 500 mM NaCl, 1 mM EDTA, 1% NP-40 and 5% glycerol) 5 times and then resuspended in 50 μL 100 mM glycine (pH 3.0) to release the immunoprecipitated complex. To reverse the formaldehyde crosslinking, 5 μL 1 M Tris-HCl at pH 8.0 and 5 μg proteinase K were added to the immunoprecipitates, which were then incubated sequentially at 55℃ for 1 hour and 70°C for 45 minutes. RNA was extracted by TRIzol reagent (Invitrogen) and detected by qPCR.

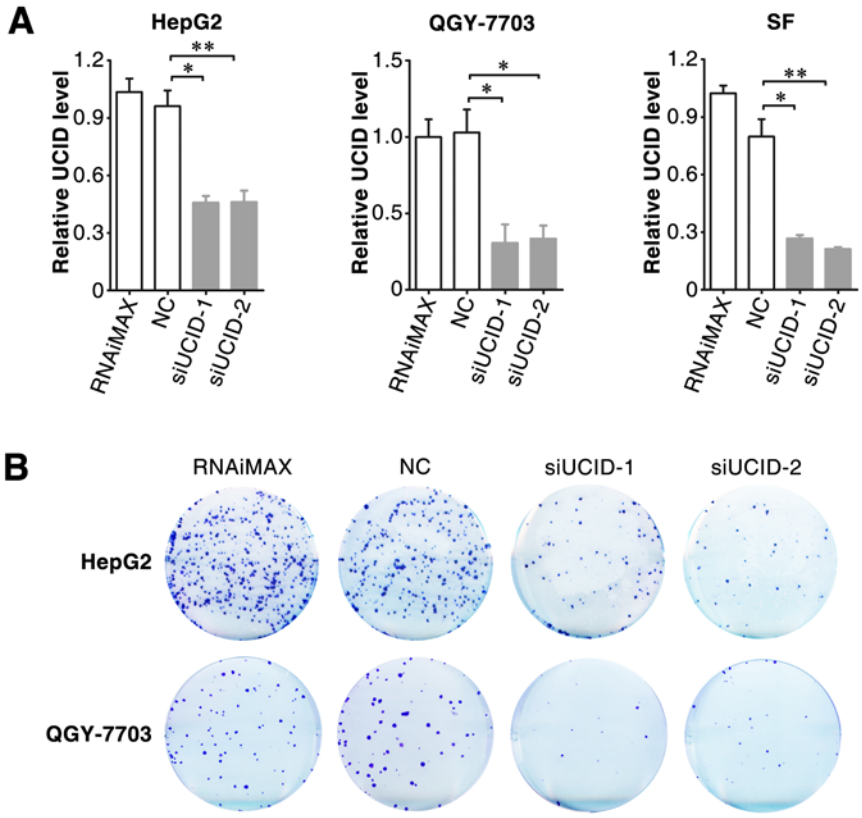
Supplementary Figures and legends

Supplementary Figure 1



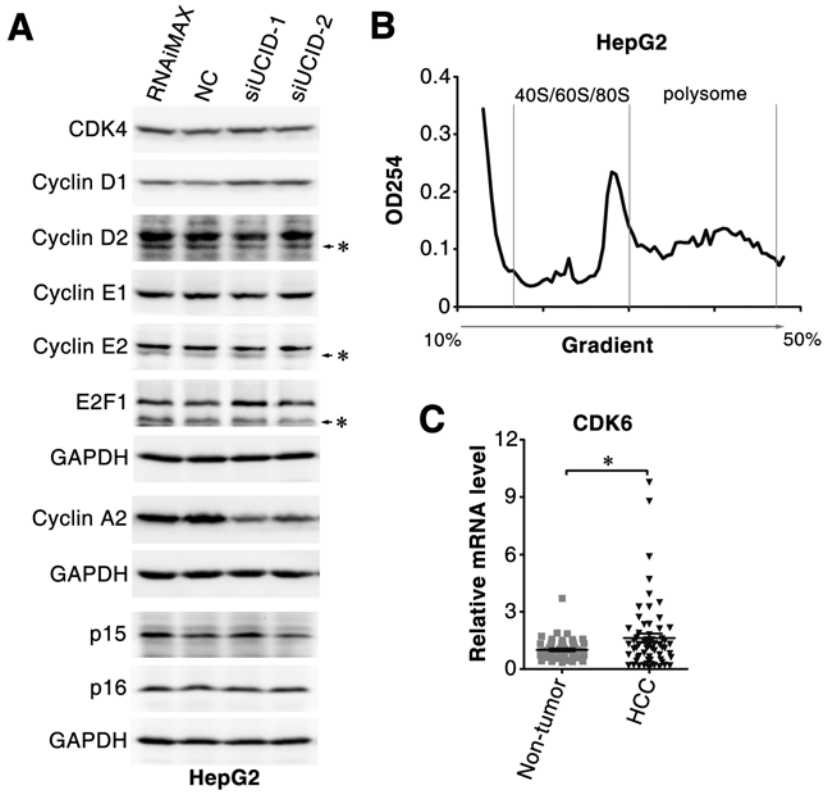
Supplementary Figure 1. The screening for oncogenic lncRNAs. (A) The screening workflow for candidate lncRNAs. Microarray data of five paired HCC and adjacent non-tumour liver tissues were derived from National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO accession number GSE49713). The chromosome regions with frequent amplification in HCC include 1q21–25, 1q31–32, 1q41, 6p21.1, 6p21.32, 6p22.3, 7p14, 7p22, 7q21.1, 8q24, 11q13, 17q11, 17q21, 17q23 and 20q13. (B) The expression of ENST00000621798.4, TMED10P1 and ENST00000411932.1 in HCC tissues. The level of lncRNAs were detected by qPCR in 32 paired HCC and non-tumor liver tissues. **, $p < 0.01$; ns, no significance.

Supplementary Figure 2



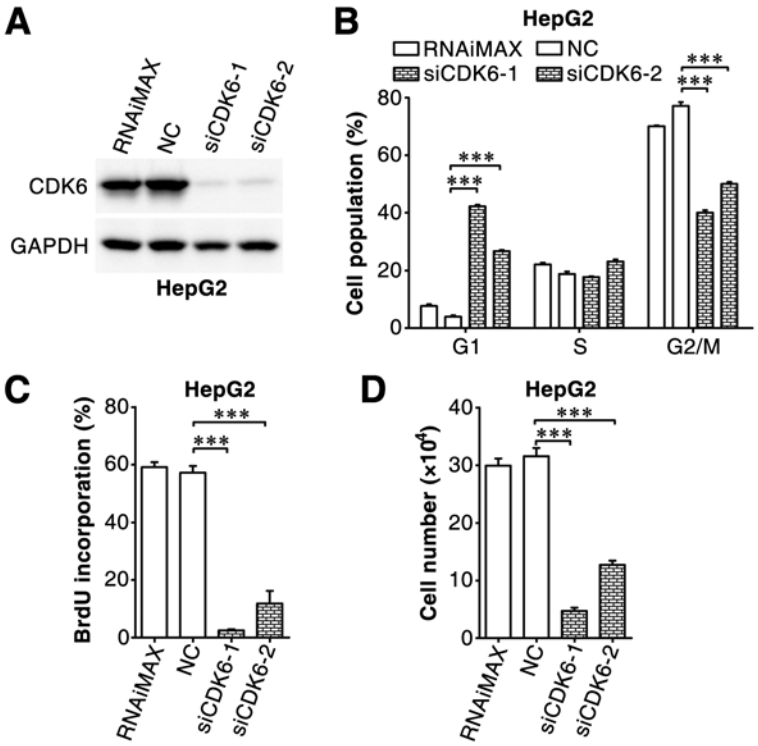
Supplementary Figure 2. Effect of lnc-UCID knockdown on hepatoma cell growth. (A) Knockdown of endogenous lnc-UCID by siUCID. (B) Lnc-UCID knockdown suppressed the colony formation of hepatoma cells; representative images of Figure 1E. RNAiMAX, cells exposed to Lipofectamine RNAiMAX but not RNA duplexes. NC, cells transfected with the negative control RNA duplex. siUCID-1 and siUCID-2, cells transfected with siRNA targeting lnc-UCID. *, $P < 0.05$; **, $P < 0.01$.

Supplementary Figure 3



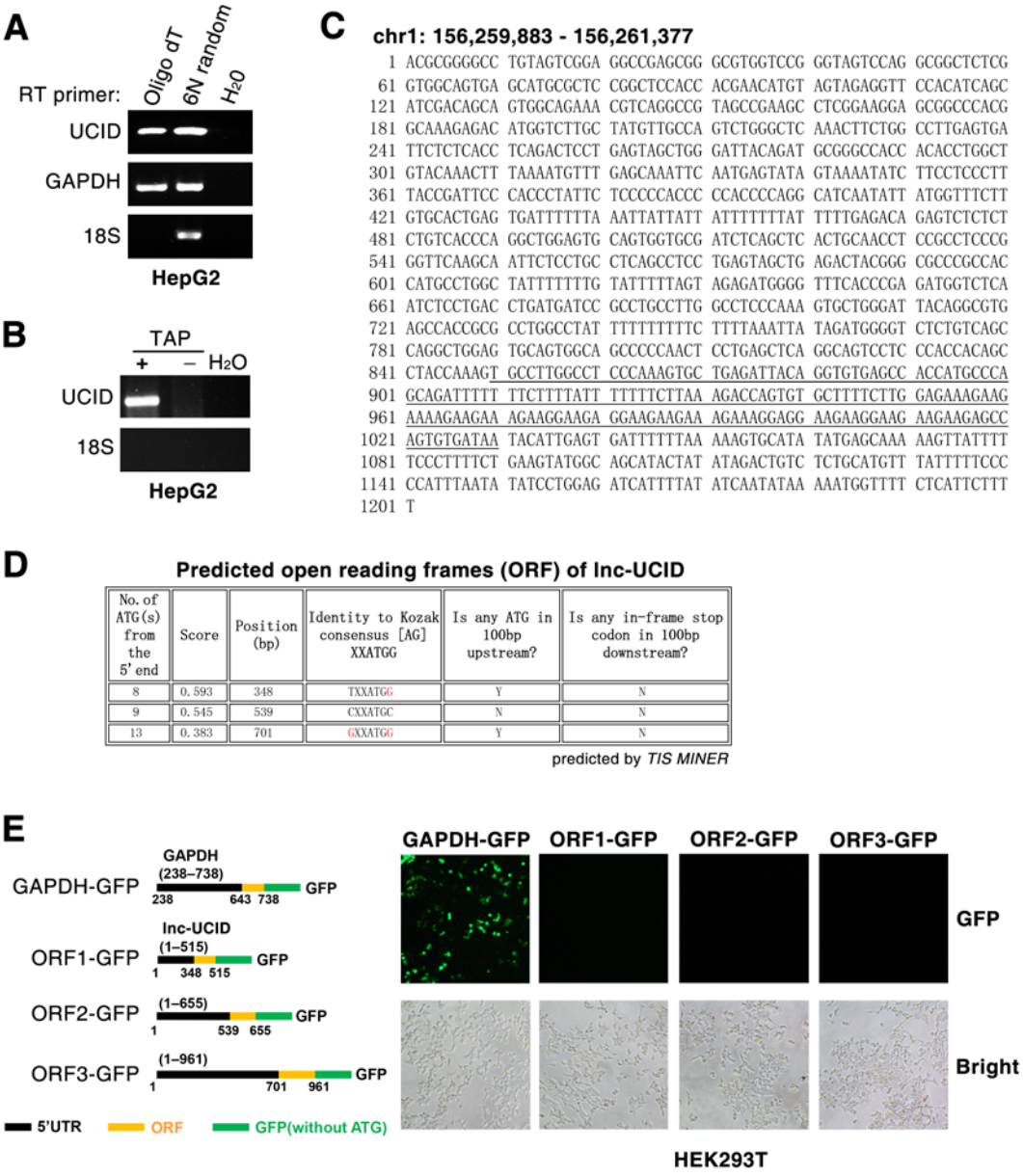
Supplementary Figure 3. Detection on the key regulators of pRb phosphorylation. (A) The impact of siUCID on the protein levels of key regulators of pRb phosphorylation. HepG2 cells were exposed to RNAiMAX or transfected with NC or siUCID for 48 hours before Western blotting. Arrow indicates a nonspecific band. (B) Polysome fraction assays were performed with a 10%–50% sucrose gradient. The gradients were fractionated and measured for absorbance at 254 nm (OD254). (C) The mRNA levels of CDK6 were increased in HCC tissues. The expression of CDK6 in 62 paired HCC and non-tumour liver tissues was assessed by qPCR. *, $P < 0.05$.

Supplementary Figure 4



Supplementary Figure 4. CDK6 silencing increases G1 population and inhibits DNA replication and cell growth. (A) CDK6 was silenced by siCDK6. (B-D) CDK6 silencing increased G1 population (B), reduced DNA replication (C) and inhibited cell growth (D) in HepG2 cells. ***, $P < 0.001$.

Supplementary Figure 5



Supplementary Figure 5. Characterization of the lnc-UCID transcript. (A) The lnc-UCID transcript was polyadenylated. RNA from HepG2 cells was reverse-transcribed into cDNA with oligo dT or random 6-mer (6N random) primers and then subjected to PCR analysis. GAPDH and 18S rRNA were used as control genes with and without a poly(A) structure, respectively. (B) The lnc-UCID transcript had a 5' cap structure. RNA was treated with alkaline phosphatase, incubated without (-) or with (+) tobacco acid phosphatase (TAP), linked with an RNA adaptor,

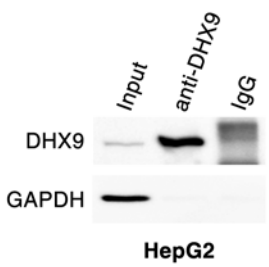
reverse-transcribed into cDNA and subjected to PCR analysis. 18S rRNA was used as a control for the non-5’ cap structure RNA. (C) The sequence of the lnc-UCID transcript. The 850-1030-nt of lnc-UCID are underlined. (D) The putative open reading frames of lnc-UCID were predicted by the *TIS MINER* website (http://dnafsminer.bic.nus.edu.sg/Tis.html). (E) The lnc-UCID transcript had no protein-coding capacity. The top three predicted ORFs, including their 5’-UTR sequence, were fused in frame with GFP (without ATG), cloned into a pc3-puro plasmid and then transfected into HEK293T cells. A GAPDH-GFP fusion plasmid was used as a positive control.

Supplementary Figure 6

A

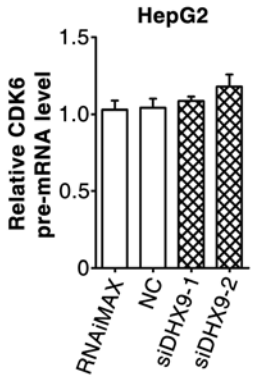
Gene symbol	Score	Mass (kD)
DHX9	1116	142
HNRNPH1	135	49
GNAI2	107	41
ALDOA	54	40
ICE1	28	250
HSPAB	21	71

B



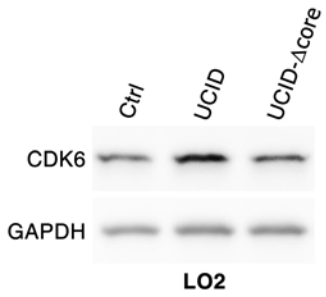
Supplementary Figure 6. Identification of lnc-UCID-associated proteins. (A) List of lnc-UCID-associated proteins identified by RNA pulldown and MS analyses in HepG2 cells. (B) The DHX9 antibody had a high immunoprecipitation efficiency. HepG2 cells were subjected to IP assays with a DHX9 antibody or isotype-matched control IgG.

Supplementary Figure 7



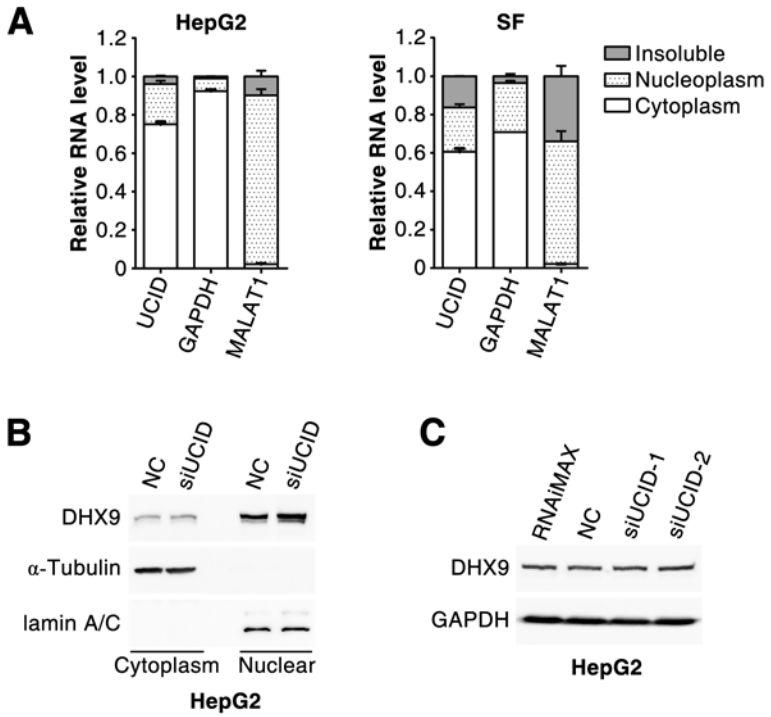
Supplementary Figure 7. DHX9 knockdown has no impact on the level of CDK6 precursor mRNA. HepG2 cells were treated with RNAiMAX or transfected with NC or siDHX9 for 48 hours before qPCR analysis for CDK6 pre-mRNA.

Supplementary Figure 8



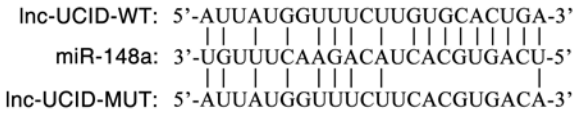
Supplementary Figure 8. The lnc-UCID-induced upregulation of CDK6 is attenuated when its 850-1030-nt sequence was deleted. LO2 cells were transfected with the indicated plasmid for 24 hours, cultured with puromycin for 48 hours and then subjected to Western blotting. Ctrl, pc3-puro was used as a negative control. UCID, pc3-puro-lnc-UCID containing the full length lnc-UCID. UCID-Δcore, pc3-puro-lnc-UCID-Δcore containing mutant lnc-UCID with the 850-1030-nt deletion.

Supplementary Figure 9



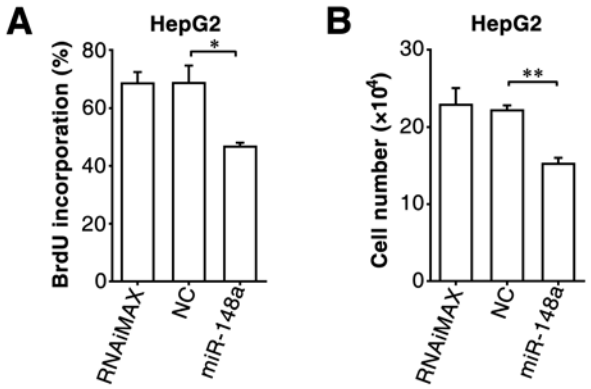
Supplementary Figure 9. Effect of lnc-UCID knockdown on the localization and expression of DHX9 protein. (A) Lnc-UCID is localized in both the cytoplasm and nucleus. The cytoplasm and nuclei of HepG2 and SF cells were fractioned and then subjected to RNA isolation and qPCR analysis. (B) Lnc-UCID knockdown did not affect the subcellular localization of DHX9 protein. α-Tubulin and lamin A/C were used as markers for cytoplasmic and nuclear proteins, respectively. (C) Lnc-UCID knockdown did not affect DHX9 protein levels. For (B-C), HepG2 cells were transfected with NC or siUCID for 48 hours before further analysis. α-Tubulin and lamin A/C were used as markers for cytoplasmic and nuclear proteins, respectively.

Supplementary Figure 10



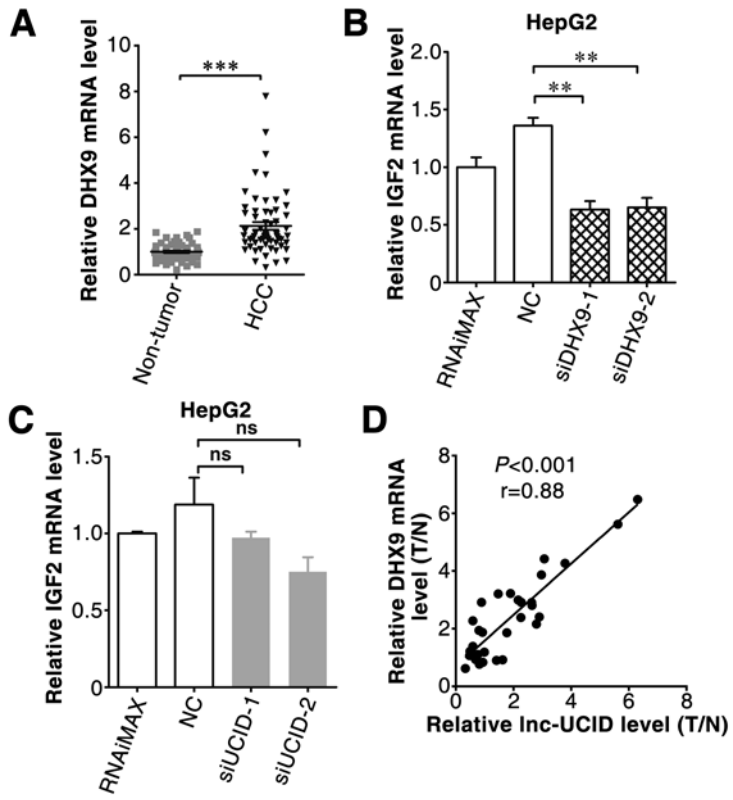
Supplementary Figure 10. miR-148a and its putative binding sequences in lnc-UCID. miR-148a sequence and the wild-type and mutant lnc-UCID sequence are shown. Mutations were generated in the complementary site that binds to the seed region of miR-148a.

Supplementary Figure 11



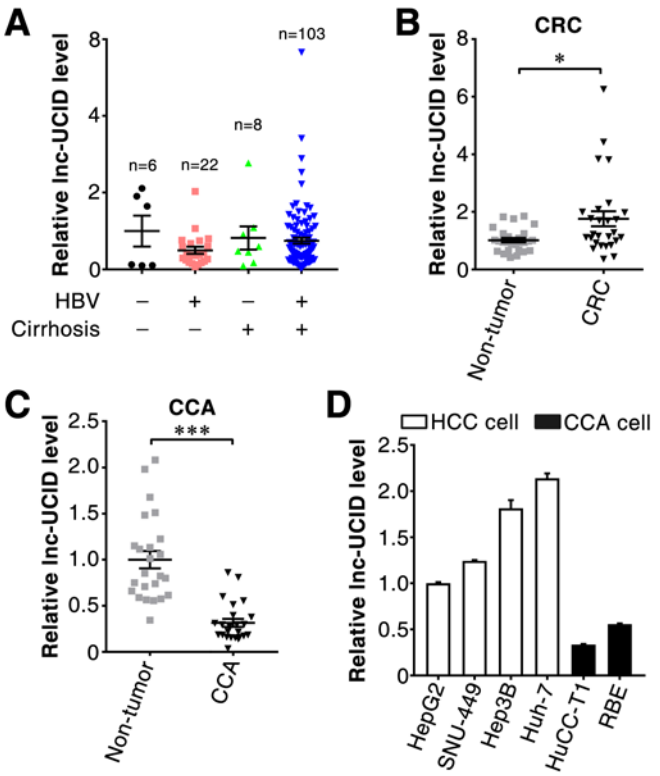
Supplementary Figure 11. Expression of miR-148a inhibits DNA replication and cell growth. DNA replication (A) and cell growth (B) were evaluated. *, $P < 0.05$; **, $P < 0.01$.

Supplementary Figure 12



Supplementary Figure 12. Expression of DHX9 in HCC tissues and its impact on IGF2 expression. (A) The mRNA level of DHX9 were upregulated in HCC tissues. The mRNA level of DHX9 were analyzed in 62 paired HCC and adjacent non-tumor tissues by qPCR. (B) DHX9 knockdown reduced IGF2 level. (C) Lnc-UCID knockdown did not affect IGF2 expression. HepG2 cells were exposed to Lipofectamine RNAiMAX or transfected with NC, siDHX9 (B) or siUCID (C) for 48 hours before qPCR analysis. Error bars represent mean ± SEM from three independent experiments, the differences between groups were analyzed using an unpaired t-test. (D) The expression of lnc-UCID was positively correlated with DHX9 level in HCC tissues. The DHX9 mRNA and lnc-UCID level were analyzed by qPCR in 30 paired HCC and adjacent non-tumor tissues. **, $P < 0.01$; ***, $P < 0.001$; ns, not significant.

Supplementary Figure 13



Supplementary Figure 13. The level of lnc-UCID in HCC, CRC and CCA tissues. (A) The lnc-UCID expression of HCC patients with different HBV and cirrhosis status. No statistical differences in lnc-UCID level were observed between any two groups. (B) lnc-UCID was upregulated in CRC tissues. The expression of lnc-UCID was analyzed in 27 CRC and their adjacent normal colon tissues by qPCR. CRC, colorectal cancer. (C) lnc-UCID was downregulated in CCA tissues. The expression of lnc-UCID was analyzed in 24 CCA and their adjacent non-tumor liver tissues by qPCR. (D) The expression of lnc-UCID in HCC and CCA cell lines was analyzed by qPCR. *, $P < 0.05$; ***, $P < 0.001$.

Supplementary Table 1. Univariate and multivariate analysis of factors associated with recurrence-free survival.

Clinical Variables	Case Number	HR (95% CI) [a]	*P* [a]
Univariate analysis			
lnc-UCID (High vs Low) [b]	85/54	2.309 (1.262-4.224)	**0.007**
Gender (M vs F)	116/23	1.884 (0.808-4.395)	0.143
Age-yr (>50 vs ≤50)	68/71	0.845 (0.502-1.422)	0.527
HBV (+ vs -) [c]	125/14	1.719 (0.622-4.755)	0.296
Cirrhosis (+ vs -)	111/28	1.383 (0.678-2.821)	0.372
AFP (≥400 vs <400 ng/mL)	48/91	0.883 (0.505-1.543)	0.761
Edmondson grade (>II vs I– II)	50/89	2.072(1.230-3.490)	**0.006**
Tumor size (>5 vs ≤5 cm)	73/66	1.343 (0.794-2.273)	0.271
Tumor number (>1 vs 1)	28/111	1.975 (1.119-3.485)	**0.019**
Portal vein tumor thrombus (+ vs -) [c]	9/130	3.051 (1.378-6.756)	**0.006**
TNM stage (II/III/IV vs I)	66/73	2.322 (1.347-4.003)	**0.002**
Multivariate analysis			
lnc-UCID (High vs Low) [b]	54/85	2.052 (1.114-3.781)	**0.021**
Edmondson grade (>II vs I– II)	50/89	1.854 (1.094-3.145)	**0.022**
TNM stage (II/III/IV vs I)	66/73	1.932 (1.107-3.373)	**0.020**

[a] HR (hazard ratio) and *P* values were calculated using univariate or multivariate Cox proportional hazards regression; 95% CI, 95% confidence interval.

[b] lnc-UCID level was examined in 139 HCC tissues by qPCR and normalized to U6 level. The 39th percentile value of the examined samples was chosen as the cut-off point to separate lnc-UCID-low from lnc-UCID-high expression groups.

[c] -, absence; +, presence.

Supplementary Table 2. Sequences of DNA and RNA oligonucleotides

Name	Sense Strand/Sense Primer (5' - 3')	Antisense Strand/Antisense Primer (5' - 3')
siRNA duplexes		
siUCID-1	GAGCAAAUUCAAUGAGUAUdTdT	AUACUCAUUGAAUUUGCUCdTdT
siUCID-2	CUUCUGGCCUUGAGUGAUUdTdT	AAUCACUCAAGGCCAGAAGdTdT
siDHX9-1	GAGCCAACUUGAAGGAUUAdTdT	UAAUCCUUCAAGUUGGCUCdCdT
siDHX9-2	CGACAAUGGAAGCGGAUAUdTdT	AUAUCCGCUUCCAUUGUCGdTdA
siCDK6-1	GAACAGACAGAGAAACCAAdTdT	UUGGUUUCUCUGUCUGUUCdGdT
siCDK6-2	CUGGAAAGGUGCAAAGAAAdTdT	UUUCUUUGCACCUUUCCAGdGdT
NC	UUCUCCGAACGUGUCACGUdTdT	ACGUGACACGUUCGGAGAAdTdT
Primers for qPCR		
lnc-UCID	CGGCCCACGGCAAAGAGA	TTGTACAGCCAGGTGTGGTG
CDK6	TGCACAGTGTCACGAACAGA	ACCTCGGAGAAGCTGAAACA
pre-CDK6	AGAGACCCTGCCAGTGATAAAGT	CTGGGAGTCCAATCACGCTAC
IGF2	CGTTGAGGAGTGCTGTTTCC	GGACTGCTTCCAGGTGTCAT
MALAT1	TCGTTTGCCTCAGACAGGTA	GGAAGGGGTCAGGAGAAAGTG
GAPDH	GAGTCAACGGATTTGGTCGT	GACAAGCTTCCCGTTCTCAG

Supplementary Table 2. Sequences of DNA and RNA oligonucleotides (Continued)

Name	Sense Strand/Sense Primer (5' - 3')	Antisense Strand/Antisense Primer (5' - 3')
CCNA2	TTATTGCTGGAGCTGCCTTT	CTCTGGTGGGTTGAGGAGAG
CDC6	AAGCTGTCTCGGGCATTGAA	TGCCTTGCTTTGGTGGAGAA
MCM3	AGTTCGTCCCAAAGTCGTCC	CCTGGATGGTGATGGTCTGG
TK1	TGCTCAGTACAAGTGCCTGG	TCGTCGATGCCTATGACAGC
DHFR	CACAAGGAGCTCATTTTCTTTCC	AGTTTAAGATGGCCTGGGTGA
DHX9	CAAAGCAATGCTGCCAGAGA	AGAGCCAGATGTGGAGGAAGAG
Primers for RACE		
3’RACE		
3’RACE-adaptor	ATGGCAGCAAGGTGATCACTAAAGTGATATCCTTTTTTTTTTTTTTTVN	
3’RACE-adaptor-primer-inner/outer		ATGGCAGCAAGGTGATCACTAAA
Nest PCR-inner	GACTCCTGAGTAGCTGGGATT	
Nest PCR-outer	CGGCCCACGGCAAAGAGA	
5’RACE		
Nest PCR-inner	AATCGGTAAAGGGAGGAAGA	
Nest PCR-outer	GAATAGGGTGGGAATCGGTA	

Supplementary Table 2. Sequences of DNA and RNA oligonucleotides (Continued)

Name	Sense Strand/Sense Primer (5' - 3')	Antisense Strand/Antisense Primer (5' - 3')
5’RACE-adaptor-primer-inner		CGCGGATCCACAGCCTACTGATGATCAGTCGATG
5’RACE-adaptor-primer-outer		CATGGCTACATGCTGACAGCCTA
Primers for 5’cap detection		
lnc-UCID	CATGGCTACATGCTGACAGCCTA	TTGTACAGCCAGGTGTGGTG
18S	CATGGCTACATGCTGACAGCCTA	TGGTTTTGATCTGATAAATGCAC
Primers for poly(A) detection		
lnc-UCID	CGGCCCACGGCAAAGAGA	TTGTACAGCCAGGTGTGGTG
GAPDH	GAGTCAACGGATTTGGTCGT	GACAAGCTTCCCGTTCTCAG
18S	CTCTTAGCTGAGTGTCCCGC	GAACCGCGGTCCTATTCCAT
Primers for cloning (restriction enzyme sites are underlined)		
lnc-UCID	AGAGAATTCGCAGTGAGCATGCGCTCCGG	TGATCTAGAAAAAGAATGAGAAAACCATTTTTATATTGATATAAAATGATCTCCA
lnc-UCID -Δcore	CAAAGTGCAAGAAGAGCCAGTGTGATAA	CTCTTCTTGCACTTTGGGAGGCCAAGGC
DHX9	GCGAATTCGAATTTAAATCGGCCACCATGGGTGACGTTAAAAATTTTCT	GGCCGCGAAGGATCTTTACTTATCGTCGTCATCCTTGTAATCATAGCCGCCACCTCCTC
CDK6-DBE1	ATCCTCGAGCAGGCATTTGTTCACTGAAGG	ATCGCGGCCGCGCAAGACTGCTTGTGTTGGA

Supplementary Table 2. Sequences of DNA and RNA oligonucleotides (Continued)

Name	Sense Strand/Sense Primer (5' - 3')	Antisense Strand/Antisense Primer (5' - 3')
CDK6-DBE2	ATCCTCGAGTTCGTCTTCAGTTGGCAAAA	ATCGCGGCCGCGAGAAAGTTTTAACCAGGCCCTA
lnc-UCID-ORF1	TCAGAATTCGCAGTGAGCATGCGCTCCGG	TCACTCGAGGCTACTCAGGAGGCTGAGGC
lnc-UCID-ORF2	TCAGAATTCGCAGTGAGCATGCGCTCCGG	TCACTCGAGCGCCTGTAATCCCAGCACTT
lnc-UCID-ORF3	TCAGAATTCGCAGTGAGCATGCGCTCCGG	TCACTCGAGCACTGGCTCTTCTTCTTCCTT
GFP	TCACTCGAGGTGAGCAAGGGCGAGGAGCT	TCAGGGCCCTTACTTGTACAGCTCGTCCATGCC
GAPDH	TCAGAATTCCTGTTTCATCCAAGCGTGTAA	TCACTCGAGGTTAAAAGCAGCCCTGGTGA
Primers for RIP		
CDK6 primer set		
CDS	TGCACAGTGTCACGAACAGA	ACCTCGGAGAAGCTGAAACA
3’UTR-1	CAGGCATTTGTTCACTGAAGG	TTTTTCTCCTTTTTGCTGTGTG
3’UTR-2	AGTGCCTTGTGCTCACTCCT	TCAGAAAGCACAGGTGGCTA
3’UTR-3	GAATGAAAGGGCATCTCAGC	GCAAGACTGCTTGTGTTGGA
3’UTR-4	TGGCCATTATTGTTCCATTG	AAGCCTGCTTTTGGAAAATGT
3’UTR-5	TGCAATTCACAACCCAATTT	TCCCCAGAAAGGCTACTTGA
3’UTR-6	ACCCTCTCTGCTGCTTTCAA	TGGTATTTTAGGGCTCTGTTTCA

Supplementary Table 2. Sequences of DNA and RNA oligonucleotides (Continued)

Name	Sense Strand/Sense Primer (5' - 3')	Antisense Strand/Antisense Primer (5' - 3')
3’UTR-7	GGCGCTAAGCTCAGTTTTTG	TGAGAGCTTTTGCCAACTGA
3’UTR-8	CTAAGCCCCCAAATAAGCTG	CCACCTGGCACAGTTTCTAA
3’UTR-9	TCATCTAGGCTGTGTGAACCA	GGTCCATGATGTGGAGGAAG
3’UTR-10	TGTCATTCAAGGACCACCAA	CACTGCAGTTCTCCCTTTCC
lnc-UCID	GACCAGTGTGCTTTTCTTGGA	TGGGGGAAAAATAAACATGC
GAPDH	GAGTCAACGGATTTGGTCGT	GACAAGCTTCCCGTTCTCAG
Primers for *in vitro* transcription (T7 promoter sequences are underlined)		
lnc-UCID		
Full length	ATTAATACGACTCACTATAGGGACGCGGGGCCTGTAGTCG	AAAAGAATGAGAAAACCATTTTTATATTGAT
1–900 nt	ATTAATACGACTCACTATAGGGACGCGGGGCCTGTAGTCG	AGCCAGGTGTGGTGGCCCGC
1–600 nt	ATTAATACGACTCACTATAGGGACGCGGGGCCTGTAGTCG	GTGGCGGGCGCCCGTAGT
1–300 nt	ATTAATACGACTCACTATAGGGACGCGGGGCCTGTAGTCG	TGGGCATGGTGGCTCACACC
850–1201 nt	ATTAATACGACTCACTATAGGGAGCCTTGGCCTCCCAAAGTGC	AAAAGAATGAGAAAACCATTTTTATATTGAT
850–1030 nt	ATTAATACGACTCACTATAGGGAGCCTTGGCCTCCCAAAGTGC	TTATCACACTGGCTCTTCTT
antisense lnc-UCID	ACGCGGGGCCTGTAGTCG	ATTAATACGACTCACTATAGGGAAAAGAATGAGAAAACCATTTTTATATTGAT

Supplementary Table 2. Sequences of DNA and RNA oligonucleotides (Continued)

Name	Sense Strand/Sense Primer (5' - 3')	Antisense Strand/Antisense Primer (5' - 3')
CDK6-3'UTR		
CDK6-DBE1	ATTAATACGACTCACTATAGGGCAGGCATTTGTTCACTGAAGG	GCAAGACTGCTTGTGTTGGA
CDK6-non-DBE	ATTAATACGACTCACTATAGGGTCATTTAGCCCCAGGTGACTTAC	CTGTTCACAGATGAGGACTGCC
Primers for DNA copy number		
lnc-UCID	TCCACATCAGCATCGACAGC	TGCATCTGCCTCCCCTATCTA
β-actin	GGACTTCGAGCAAGAGATGG	AGGAAGGAAGGCTGGAAGAG

Supplementary Table 3. The list of candidate lncRNAs[a]

Probe ID	FC[b]	GENCODE v24[c]	Chromosome
AS000035328	3.38	**ENST00000497831.1**	1q21-25
AS000035329	2.99		
AS000035284	2.33	ENST00000621798.4	
AS000035373	3.34	GAS5	
AS000032403	3.12		
AS000033338	2.51	ENST00000441932.1	1q31-32
AS000005618	2.83	PVT1	8q24
AS000032465	2.55	TMED10P1	
AS000035932	2.08	MALAT1	11q13
AS000032670	4.19	ZFAS1	20q13
AS000032669	3.74		

[a] The microarray data was obtained from GEO database with accession number GSE49713.

[b] FC, fold change. The ratio of HCC relative to that of adjacent noncancerous tissue.

[c] GENCODE v24, the v24 version of GENCODE annotation for the human genome.