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Supplemental Information

circDCUN1D4 suppresses tumor metastasis and glycolysis in lung adenocarcinoma by stabilizing TXNIP expression

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Supplementary Materials and Methods

Actinomycin D and RNase R treatment

Cells were planted into six-well plates. Up to 60% confluency after 24 h, cells were treated with 5μg/ml Actinomycin D at indicated time points.

Total RNA (2μg) was incubated with 3 U/μg of RNase R (Sigma) for 15 min at 37 °C. After treatment with Actinomycin D or RNase R, the RNA expression levels of circDCUN1D4 and other mRNAs were analyzed by qRT-PCR.

Transwell migration and invasion assay

4×10^4 cells, suspended in medium without FBS, were seeded into transwell chambers (Corning), with or without Matrigel (Sigma-Aldrich) coating. The lower chamber contained medium with 10% FBS as chemokine. Twenty-four hours later, the migratory or invasive cells on the lower surface of the chamber were photographed and counted in 10 random microscopic fields after crystal violet staining.

Wound-healing assay

The transfected cells were cultured in 6-well plates. After the cells reached 90% confluence, a standard 200μl pipette tip was subsequently utilized to scratch linear wounds. In addition, the cell monolayers were cultivated in FBS-free medium. After scratching, the images of the wound closure were captured at 0, and 24h.

Real-time cell analysis

The CIM-plate16 contains 16 wells, each a modified Boyden chamber, which can be used independently but simultaneously to measure cell migration in real-time through 8μm pores of a polyethylene terephthalate membrane on to gold electrodes on the

underside of the membrane using the xCELLigence system (ACEA Biosciences, USA). Experiments were set up according to the manufacturer's instructions with the membrane uncoated (migration) or coated with growth-factor-reduced-matrigel (invasion) (BD BioSciences, UK) (20 µl 1:40 diluted matrigel per well on the upper surface). Cell index (electrical impedance) was monitored every 30 min for the duration of the experiment. Traces show the average of quadruplicate wells.

RNA-Fluorescence in situ hybridization assay and Fluorescence immunocytochemical staining

RNA-Fluorescence in situ hybridization (FISH) assays were performed using a RNA-FISH kit (GenePharma, China) according to the manufacturer's instructions. Cy3-labeled antisense probe was synthesized by GenePharma company (Suzhou, China) against circDCUN1D4. The sequence is listed in Table S2. In briefly, A549 cells were fixed with 4% paraformaldehyde. After pre-hybridization with 1× PBS/0.5% Triton X-100, cells were blocked and hybridized in hybridization buffer with Cy3-labeled antisense probe at 37 °C overnight. Then cells were incubated with specific antibodies for HuR (ab200342, 1:200 dilution) at 4 °C overnight. Cells were treated with tyramide-conjugated goat anti-rabbit IgG (Invitrogen 1:10000 dilution) and DAPI (300 nmol/L) staining. The images were photographed under a Nikon A1Si Laser Scanning Confocal Microscope (Nikon Instruments Inc, Japan).

Luciferase reporter assay

Human HuR luciferase reporter was constructed according to the previous article[2]. The TXNIP binding sites of circDCUN1D4 were analyzed by StarBase

(http://starbase.sysu.edu.cn/index.php). The different fragment sequences were synthesized and then inserted into the psiCHECK-2 vector (Promega). All vectors were verified by sequencing and luciferase activity was assessed using the Dual Luciferase Assay Kit (Promega) according to the manufacturer's instructions. The sequences were provided in Table S2

Microarray assay

The Agilent human mRNA array was designed with 8 identical arrays per slide (8 × 60 K format), with each array containing probes interrogating approximately 27958 Entrez Gene RNAs and 7419 long intergenic noncoding RNA. The array also contains 1280 Agilent control probes. Total RNA containing small RNA was extracted from cells by using TRIzol reagent (Invitrogen) according to the manufacturer's protocol. The purity and concentration of RNA were determined from optical density 260/280 readings using a spectrophotometer (NanoDrop ND-1000). RNA integrity was determined by 1% formaldehyde denaturing gel electrophoresis.

Tissue microarray and in situ hybridization assay

Tissue microarray (TMA) was obtained from Outdo Biotech Co. Ltd. (Shanghai, China). Ninety-two pairs of LUAC tissues and their paired peripheral normal lung tissues were used to construct the TMA. Special biotin labelled probe against circDCUN1D4 was synthesized by GenePharma company (Suzhou, China). The sequence is provided in Table S2. After incubating with the biotin labelled probe, the TMA was then stained with DAB and hematoxylin, dehydrated and covered. The TMA staining scores were evaluated by two independent observers blinded to the clinicopathological data. The

staining scores were based on two indicators: the proportion of positively stained cells and the staining intensity. The proportion of positively stained cells was evaluated with five scoring levels: 0, <10%; 1, 10–25%; 2, 25–50%; 3, 50–75%; and 4, >75%. The staining intensity was scored with the following point system: 0 (no staining), 1 (yellow), 2 (yellow-brown) and 3 (dark brown). The products of the above two indicators were considered the total score.

Files uploaded separately in Excel Format:

Additional Dataset 1. Fold enrichment of 152 circRNA in HuR CLIP.

Additional Dataset 2. 661 genes altered by circDCUN1D4.

Supplementary Figures

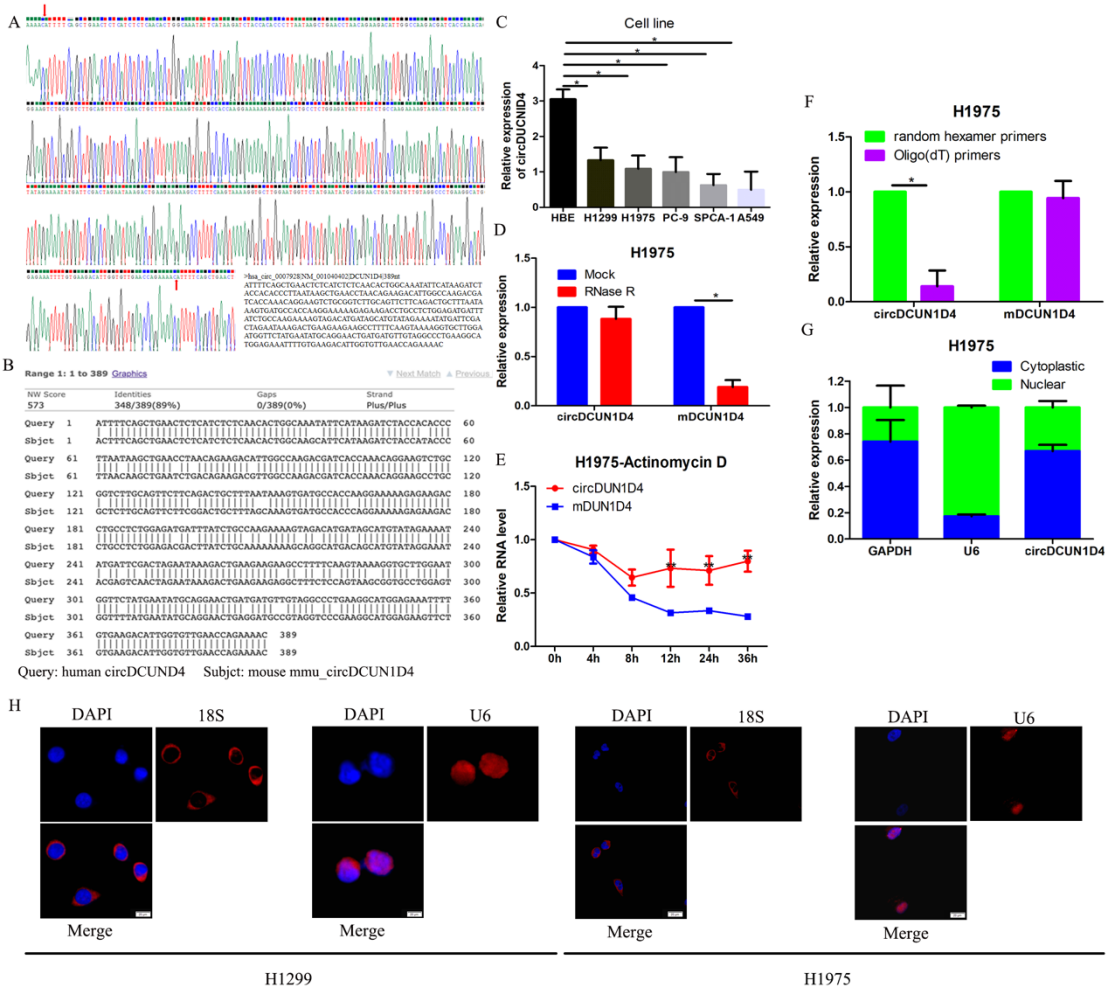

Fig. S1 Characterization of circDCUN1D4. A The full length of circDCUN1D4. **B** Conservative analysis of circDCUN1D4 between humans and mice. **C** qRT-PCR assay detected the expression of circDCUN1D4 in a variety of LUAD cell lines and a normal lung cell line HBE. (mean ± SD, n = 4) **D** qRT-PCR for the abundance of circDCUN1D4 and DCUN1D4 in H1975 cells treated with RNase R compared with the mock group. (mean ± SD, n = 4) **E** qRT-PCR for the abundance of circDCUN1D4 and DCUN1D4 in H975 cells treated with Actinomycin D at the indicated time point. (mean ± SD, n = 4) **F** Random hexamer or oligo (dT)18 primers were used in the reverse transcription experiment. The relative RNA levels were analyzed by qRT-PCR and normalized to the value using random hexamer primers. (mean ± SD, n = 4) **G** qRT-PCR for the distribution of circDCUN1D4, GAPDH, and U6 in the cytoplasmic and nuclear fractions of H1975 cells. **H** The 18S and U6 were applied as positive control for

cytoplasm and nucleus respectively in RNA-FISH assay. Student's t test and analysis of variance compared the difference in C, D, and E. *P < 0.05 vs. HBE, mock, mDCUN1D4.

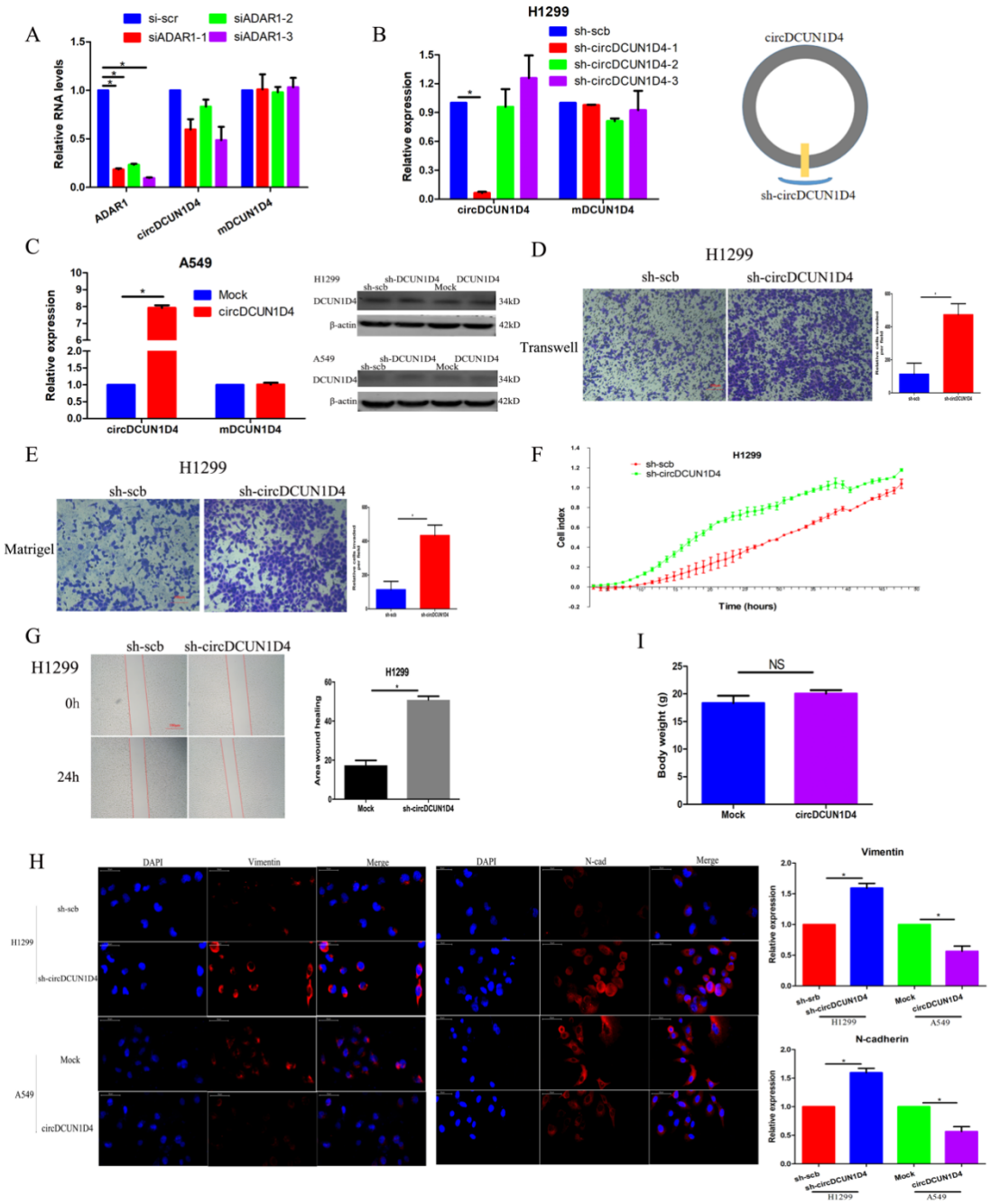

Fig. S2 Effects of circDCUN1D4 on the invasion of cancer cells. A qRT-PCR assay showing the transcript levels of EIF4A3, QKI, and ADAR1 in cancer cells stably transfected with si-scb, or siEIF4A3, or siQKI, or siADAR1, respectively. (mean ± SD, n = 4) **B** qRT-PCR and western blot assays showing the transcript and protein levels of DCUNID4 in cancer cells stably transfected with mock, circDCUN1D4, sh-Scb, or sh-DCUN1D4. (mean ± SD, n = 4)**C-F** Representative images and quantification of transwell (C), matrigel (D), C-plate (E) and wounding healing (F) assays showing the invasion of H1299 cells stably transfected with sh-scb or sh-DCU1ND4 (mean ± SD,

n=4). Scale bar: 100 μm. **G** Representative images (left panel) and quantification (right panel) of immunofluorescence staining assay showing the expression of Vimentin or N-cadherin of A549 and H1299 cells stably transfected with mock, circDCUN1D4, scramble shRNA (sh-scb), or sh-circDCUN1D4 (mean ± SD, n=4). **H** The body weight of mice after tail vein injection of A549 cells stably transfected with mock and circDCUN1D4 (n=4 for each group). Student's t test and analysis of variance compared the difference in A-G. *P < 0.05 vs. si-scb, mock, sh-scb.

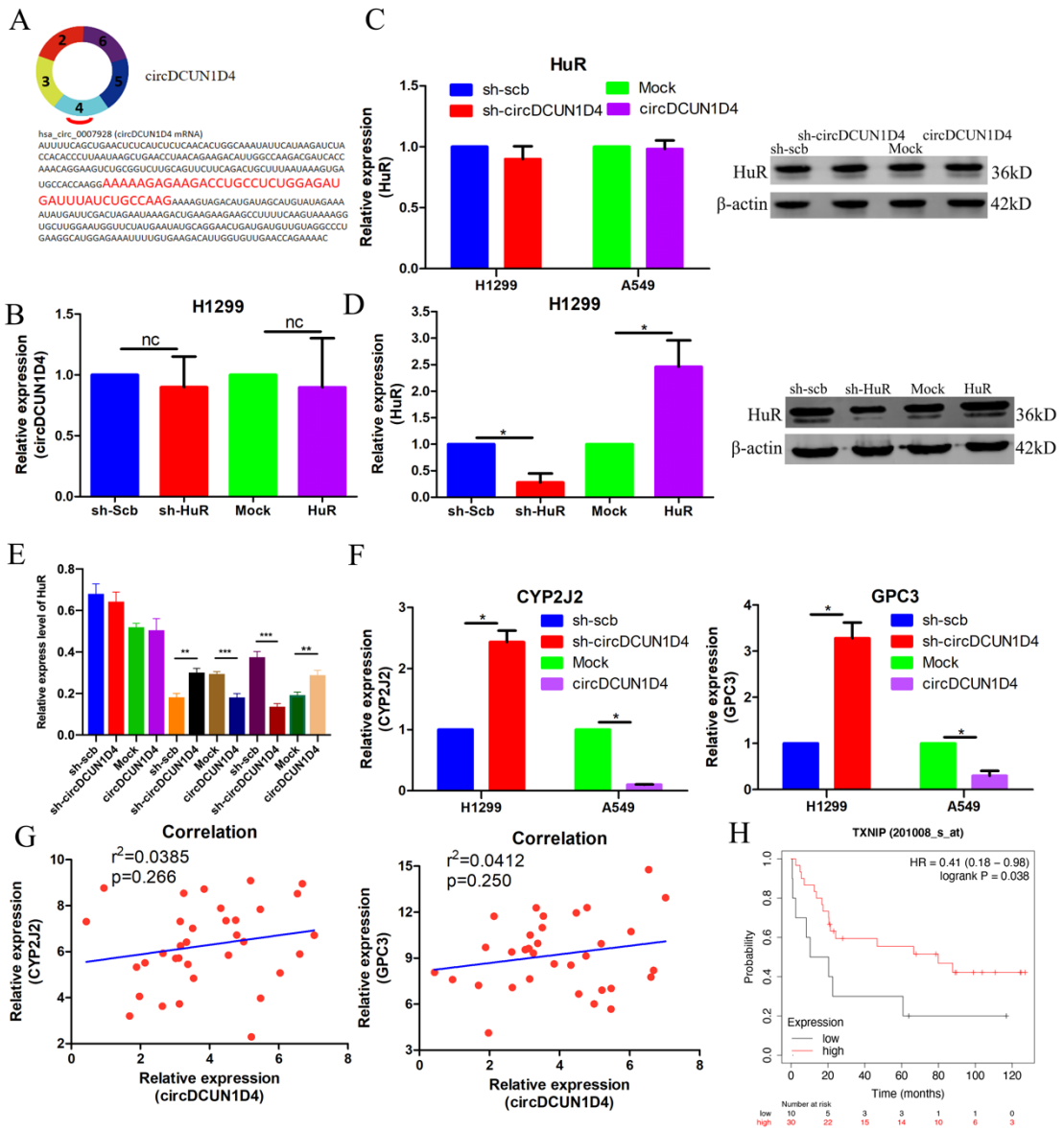

Fig. S3 Effects of circDCUN1D4 on the expression of HuR, CYP2J2 or GPC3 in cancer cells. A The sequences of circDCUN1D4 interacts with HuR protein. **B** qRT-PCR assay showing the expression of circDCUN1D4 in cancer cells stably transfected with mock, HuR, sh-scb, or sh-HuR. (mean ± SD, n=4) **C** qRT-PCR and western blot assays showing the transcript and protein levels of HuR in cancer cells stably transfected with mock, circDCUN1D4, sh-Scb, or sh-DCUN1D4. (mean ± SD, n=4) **D** qRT-PCR and western blot assays showing the transcript and protein levels of HuR in cancer cells stably transfected with mock, HuR, sh-scb, or sh-HuR. (mean ± SD, n=4) **E** The quantification of Western blot indicating the expression of HuR in total lysates

or subcellular fractions of H1299 cells stably transfected with mock, circDCUN1D4, sh-scb, or sh-circDCUN1D4 vectors. **F** qRT-PCR detection of CYP2J2 or GPC3 in cancer cells stably transfected with mock, circDCUN1D4, scramble shRNA (sh-scb), or sh-circDCUN1D4 (mean ± SD, n=4). **G** The correlation between the relative expression of CYP2J2 or GPC3 and circDCUN1D4 in 34 LUAD tissues. **H** Kaplan-Meier curves indicating the overall (OS) survival of TXNIP in lung cancer cases derived from Kaplam-Meier Plotter. Student's t test and analysis of variance compared the difference in A-F. ***P<0.001, **P<0.01, *P < 0.05 vs. mock, sh-scb.

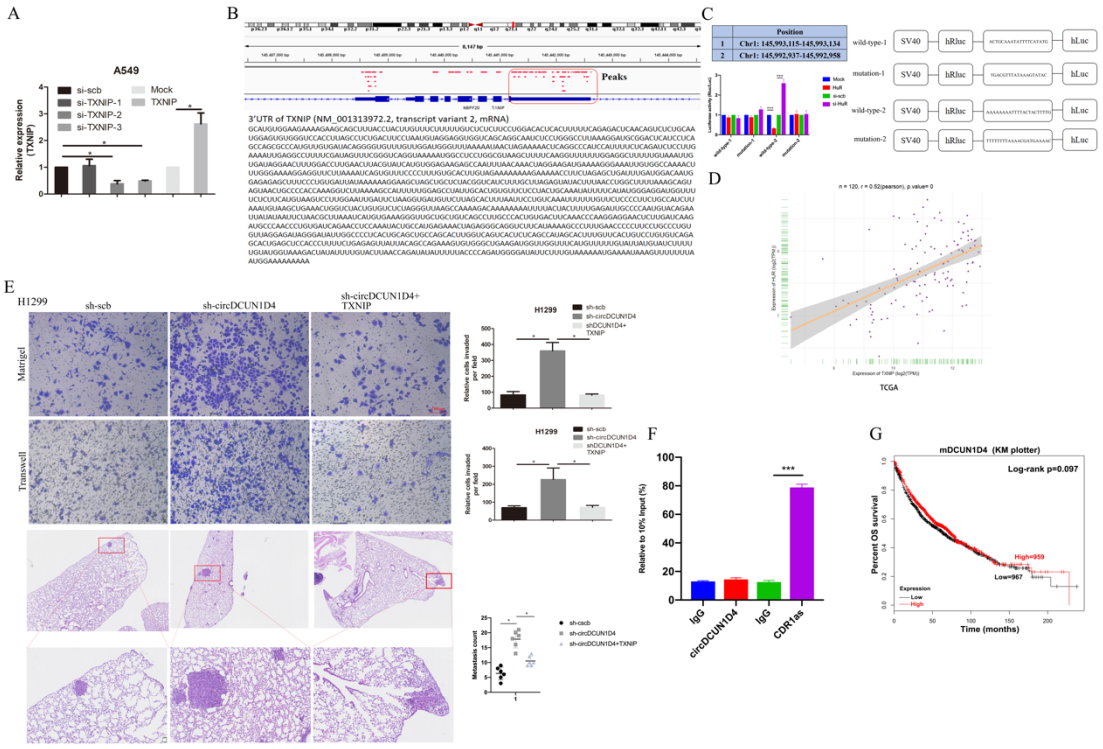

Fig. S4 circDCUN1D4 suppresses metastasis depend on TXNIP pathway. A qRT-PCR assay showing the expression of TXNIP in cancer cells stably transfected with Mock, TXNIP, si-scb, si-TXNIP-1, si-TXNIP-2, and si-TXNIP-3. (mean ± SD, n=4). **B** IGV showing the peaks localized in 3'UTP of TXNIP which interacts with HuR protein. **C** Dual-luciferase assay indicating the interacted region between 3'UTR region of TXNIP and HuR in H1299 cells stably transfected with mock, HuR, si-scb, or si-HuR (mean ± SD, n=4). **D** The expression level of TXNIP was significantly positive relative to the expression level of HuR. **E** Representative images (left panel) and quantification (right panel) of transwell, matrigel, and *in vivo* assays showing the invasion of H1299 cells stably transfected with sh-scb, sh-circDCUN1D4 or sh-circDCUN1D4 plus TXNIP (mean ± SD, n=4). Scale bar: 100 μm. **F** RIP assay showing that the RNA binding protein Ago2 could not interacts with circDCUN1D4. **G** Kaplan-Meier curves indicating the overall (OS) survival of DCUN1D4 in lung cancer cases derived from Kaplam-Meier Plotter. Student's t test and analysis of variance compared the difference in A, E and F. ***P<0.001, *P < 0.05 vs. mock, sh-scb, sh-circDCUN1D4, AND IgG. Log-rank test for survival comparison in G.

Supplementary Tables

Supplementary Table S1 Primer sets used for RT-PCR, qRT-PCR, and RIP

Primer set	Primers	Sequence	Application
hsa_circ_0001212	Forward	5’ -GTGAAGCGAGACAGTGGTGA-3’	qRT-PCR
	Reverse	5’ -ACCACAACCAGCTGACAAAA-3’	
hsa_circ_0002343	Forward	5’ -GTGGTGTGCAGGTGAACAAG-3’	qRT-PCR
	Reverse	5’ -ACAACACGCCAACTACCACA-3’	
hsa_circ_0007386	Forward	5’ -CCCGGACAGCTATGAAACTC-3’	qRT-PCR
	Reverse	5’ -AGGAGCATAACCCTCGATCA-3’	
hsa_circ_0007928 (circDCUN1D4)	Forward	5'- GTTGAACCAGAAAACATTTTC-3	qRT-PCR, RIP RT-PCR
	Reverse	5'- AGACTTCCTGTTTGGTGATC -3'	
hsa_circ_0012265	Forward	5'-GGTTCCTAAGCCTGTACCACC -3'	qRT-PCR
	Reverse	5'-GCTGGTGCCAAGAATCTCCT-3'	
mDCUN1D4	Forward	5'-TGCAGGCAGTGGACAAGAAA-3'	qRT-PCR RT-PCR
	Reverse	5'-GGATCTTGCTGACCACGACA-3'	
HuR	Forward	5'-ATGTTCTCTCGGTTTGGGCG-3'	qRT-PCR
	Reverse	5'-GTTCTGGTTGGGGTTGGCTG-3'	
18S	Forward	5’ -CGAACGTCTGCCCTATCAACTT-3’	qRT-PCR
	Reverse	5’ -ACCCGTGGTCACCATGGTA-3’	
U6	Forward	5’ -CTCGCTTCGGCAGCACA-3’	qRT-PCR
	Reverse	5’ -AACGCTTCACGAATTTGCG-3’	
GAPDH	Forward	5’ -AGAAGGCTGGGGCTCATTTG-3’	qRT-PCR RT-PCR
	Reverse	5’ -AGGGGCCATCCACAGTCTTC-3’	
DHX9	Forward	5’ -TGCCTCCAAGAAAGTCCA-3’	qRT-PCR
	Reverse	5’ -TCCGCTTCCATTGTCGTAT-3’	
EIF4A3	Forward	5’ -GGGGCATCTACGCTTACGG-3’	qRT-PCR
	Reverse	5’ -GCGATGACATCTCTCCCTTTGA-3’	
QKI	Forward	5’ -CAAACGGAACTCCTCACCC-3’	qRT-PCR
	Reverse	5’ -GCCACCGCACCTAATACAC-3’	
ADAR1	Forward	5’ -CGAGAA TCCCAAACAAGGAA-3’	qRT-PCR
	Reverse	5’ -CTGGATTCCACAGGGATTGT-3’	
TXNIP	Forward	5’ -TGTGTGAAGTTACTCGTGTCAAA-3’	qRT-PCR
	Reverse	5’ -GCAGGTACTCCGAAGTCTGT-3’	
CYP2J2	Forward	5’ -TCCATCCTCGGACTCTCCTAC-3’	qRT-PCR
	Reverse	5’ -GTCACCAAGCTCCAAGCTAAAA-3’	
GPC3	Forward	5’ -CAGTAAGGACTGTGGCCGAAT-3’	qRT-PCR
	Reverse	5’ -AGCAGTACGTTCTCCATGTCAT-3’	
pre-TXNIP	Forward	5’ -AGAAGTTGACTTAGACGGATTGCT-3’	qRT-PCR

	Reverse	5’ -ACAGACTATTTCAGTTCAGGTAAGA-3’	
TXNIP-1	Forward	5’ -ACGCTTCTTCTGGAAGACCA-3’	qRT-PCR
	Reverse	5’ -AGGGGTATTGACATCCACCA-3’	
TXNIP-2	Forward	5’ -GCAAGCCTAATGGCTACTCG-3’	qRT-PCR
	Reverse	5’ -AGGGGTATTGACATCCACCA-3’	
3’ UTR (TXNIP)	Forward	5’ -GAGTGTGGGTCCACCTTAGC-3’	qRT-PCR
	Reverse	5’ -AGGGGTATTGACATCCACCA-3’	
BEX2	Forward	5’ -AAAGAGGAACGAGCGTTAAACA(-3’	qRT-PCR
	Reverse	5’ -TCACTAACATTCAAAGGTAGGGC-3’	
DDX6	Forward	5’ -ATGGGTCTGTCCAGTCAAAATG-3’	qRT-PCR
	Reverse	5’ -GGTGGTCATACTCTGTGCTTG-3’	
FBXW2	Forward	5’ -TTCTTTCTCTGACGGACTTGC-3’	qRT-PCR
	Reverse	5’ -GCTTGAGGAGAGTCTCTAGGTT-3’	
FXR1	Forward	5’ -GAGAGAAGATTTAATGGGCCTGG-3’	qRT-PCR
	Reverse	5’ -GCTCAATGGCGGTAACTCCA-3’	
HK2	Forward	5’ -GAGCCACCACTCACCCTACT-3’	qRT-PCR
	Reverse	5’ -CCAGGCATTCGGCAATGTG-3’	
IFI6	Forward	5’ -GGTCTGCGATCCTGAATGGG-3’	qRT-PCR
	Reverse	5’ -TCACTATCGAGATACTTGTGGGT-3’	
LOX	Forward	5’ -CGGCGGAGGAAAACTGTCT-3’	qRT-PCR
	Reverse	5’ -TCGGCTGGGTAAGAAATCTGA-3’	
MGAT5	Forward	5’ -CTTCACTCCGTGGAAGTTGTC-3’	qRT-PCR
	Reverse	5’ -TGGATGGTAAAGTGCAGAAGC-3’	
PCDH9	Forward	5’ -CTGCTCTGATTGCCTGTTTAAGG-3’	qRT-PCR
	Reverse	5’ -ACCAGTCTGTAGACAAGGCTG-3’	
PLIN2	Forward	5’ -ATGGCATCCGTTGCAGTTGAT-3’	qRT-PCR
	Reverse	5’ -GGACATGAGGTCATACGTGGAG-3’	
PML	Forward	5’ -CGCCCTGGATAACGTCTTTTT-3’	qRT-PCR
	Reverse	5’ -CTCGCACTCAAAGCACCAGA-3’	
SMAD2	Forward	5’ -CGTCCATCTTGCCATTCACG-3’	qRT-PCR
	Reverse	5’ -CTCAAGCTCATCTAATCGTCCTG-3’	
STXBP4	Forward	5’ -CCTTGGCCTGAAGGTACTAGG-3’	qRT-PCR
	Reverse	5’ -AGCAGATTCTAACCTCAACTTGG-3’	
ZNF680	Forward	5’ -TGGGTGCTCCAGCCTTACT-3’	qRT-PCR
	Reverse	5’ -GCAGGCCAGTTAAAAACATTGC-3’	
UQCRB	Forward	5’ -GGTAAGCAGGCCGTTTCAG-3’	qRT-PCR
	Reverse	5’ -AGGTCCAGTGCCCTCTTAATG-3’	
SMC2	Forward	5’ -ACAACACCAGAGTACAGGATCT-3’	qRT-PCR
	Reverse	5’ -CGGCCCTGCATGATGAGAA-3’	
DCUN1D4-intron2	Forward	5’ -CAGATGCAGGGTGTACAAGG-3’	qRT-PCR
	Reverse	5’ -TCAGTAAACAGAGGGGCAACT-3’	

DCUN1D4-intron6	Forward	5’ - CTTCGTGCCCCTCAGTAGTT-3’	qRT-PCR
	Reverse	5’ - CATACCATTGCACTCCAGCC-3’	

Supplementary Table S2 Oligonucleotide sets used for constructs, guide DNA, and probe

Oligo Set	Sequences
pcDNA3.1(+)-circDCUN1D4 (positive)	5’-tactaatgacttttttttatacttcagATTTTCAGCTGAACTCTCAT-3’(sense)
	5’-gcctaattcttttccttgcttcttacGTTTTCTGGTTCAACACCAA-3’ (antisense)
sh-circDCUN1D4-1	5'-CACCGCAGAAAACATTTTCAGCTGAATTCAAGAGATTCAGCTGAAAATGTTTTCTGTTTTTTG-3' (sense)
	5'-GATCCAAAAAACAGAAAACATTTTCAGCTGAATCTCTTGAATTCAGCTGAAAATGTTTTCTGC-3' (antisense)
sh-circDCUN1D4-2	5'-CACCGCAAACATTTTCAGCTGAACTCAAGAGAAGUUCAGCUGAAAAUGTTTTCTTTTTTG-3' (sense)
	5'-GATCCAAAAAACAAACATTTTCAGCTGAACTCTCTTGAAAGTTCAGCTGAAAATGTTTTC-3' (antisense)
sh-circDCUN1D4-3	5'-CACCGCCCAGAAAACATTTTCAGCTTCAAGAGAAGCTGAAAATGTTTCTGGTTTTTTG-3' (sense)
	5'-GATCCAAAAAACCCAGAAAACATTTTCAGCTCTCTTGAAAGCUGAAAATGTTTCTGGC-3' (antisense)
sh-scb	5'-CACCGCTTCTCCGAACGTGTCACGTTTCAAGAGAACGTGACACGTTCGGAGAATTTTTTG-3' (sense)
	5'-GATCCAAAAAACTTCTCCGAACGTGTCACGTTTCAAGAGAACGTGACACGTTCGGAGAAC-3' (antisense)
sh-HuR	5’-CACCGCCCATCACAGTGAAGTTTGCATTCAAGAGATGCAAACTTCACTGTGATGGGTTTTTTG-3’ (sense)
	5’-GATCCAAAAAACCCATCACAGTGAAGTTTGCATCTCTTGAATGCAAACTTCACTGTGATGGGC-3’ (antisense);
psiCHECK2-HuR-Rluc	5'-TCGAGTGGATACCGTTCGTTGTTAACCGTTGATAGCCTTGGTTTTTAAGCCGTATATGGCTGTAAATAATTGC-3' (sense)
	5'-GGCCGCAATTATTTACAGCCATATACGGCTTAAAAACCAAGGCTATCAACGGTTAACAACGAACGGTATCCAC-3' (antisense)
pcDNA3.1(+)-Flag-HuR	5'-CCGGAATTCATGTCTAATGGTTATGAAGACC-3' (sense);
	5'-CCGCTCGAGTTATTTGTGGGACTTGTTGGTT-3' (antisense)

pcDNA3.1(+)-Flag-HuR(ΔRRM3)	5'-CCGGAATTCATGTCTAATGGTTATGAAGACC-3' (sense);
	5'-CCGCTCGAGCCAGCCGGAGGAGGCGTTTCCT-3' (antisense)
pcDNA3.1(+)-Flag- HuR (RRM2 + Hinge)	5'-CCGGAATTCATCAAAGACGCCAACTTGTACA-3' (sense);
	5'-CCGCTCGAGCCAGCCGGAGGAGGCGTTTCCT-3' (antisense)
pcDNA3.1(+)-Flag- HuR (Hinge)	5'-CCGGAATTCAACAAAAACGTGGCACTCCTCT-3' (sense);
	5'-CCGCTCGAGCCAGCCGGAGGAGGCGTTTCCT-3' (antisense)
psiCHECK2-TXNIP-WT-Rluc	GCATGTGGAAGAAAAGAAGCAGCTTTACCTACTTGTTTCTTTTTGTCTCTCTTC CTGGACACTCACTTTTTCAGAGACTCAACAGTCTCTGCAATGGAGTGTGGGTCC ACCTTAGCCTCTGACTTCCTAATGTAGGAGGTGGTCAGCAGGCAATCTCCTGGG CCTTAAAGGATGCGGACTCATCCTCAGCCAGCGCCCATGTTGTGATACAGGGGT GTTTGTTGGATGGGTTTAAAAATAACTAGAAAAACTCAGGCCCATCCATTTTCT CAGATCTCCTTGAAAATTGAGGCCTTTTCGATAGTTTCGGGTCAGGTAAAAATG GCCTCCTGGCGTAAGCTTTTCAAGGTTTTTTGGAGGCTTTTTGTAAATTGTGAT AGGAACTTTGGACCTTGAACTTACGTATCATGTGGAGAAGAGCCAATTTAACAA ACTAGGAAGATGAAAAGGGAAATTGTGGCCAAAACTTTGGGAAAAGGAGGTTCT TAAAATCAGTGTTTCCCCTTTGTGCACTTGTAGAAAAAAAAGAAAAACCTTCTA GAGCTGATTTGATGGACAATGGAGAGAGCTTTCCCTGTGATTATAAAAAAGGAA GCTAGCTGCTCTACGGTCATCTTTGCTTAGAGTATACTTTAACCTGGCTTTTAA AGCAGTAGTAACTGCCCCACCAAAGGTCTTAAAAGCCATTTTTGGAGCCTATTG CACTGTGTTCTCCTACTGCAAATATTTTCATATGGGAGGATGGTTTTCTCTTCA TGTAAGTCCTTGGAATTGATTCTAAGGTGATGTTCTTAGCACTTTAATTCCTGT CAAATTTTTTGTTCTCCCCTTCTGCCATCTTAAATGTAAGCTGAAACTGGTCTA CTGTGTCTCTAGGGTTAAGCCAAAAGACAAAAAAAATTTTACTACTTTTGAGAT TGCCCCAATGTACAGAATTATATAATTCTAACGCTTAAATCATGTGAAAGGGTT GCTGCTGTCAGCCTTGCCCACTGTGACTTCAAACCCAAGGAGGAACTCTTGATC AAGATGCCCAACCCTGTGATCAGAACCTCCAAATACTGCCATGAGAAACTAGAG GGCAGGTCTTCATAAAAGCCCTTTGAACCCCCTTCCTGCCCTGTGTTAGGAGAT AGGGATATTGGCCCCTCACTGCAGCTGCCAGCACTTGGTCAGTCACTCTCAGCC ATAGCACTTTGTTCACTGTCCTGTGTCAGAGCACTGAGCTCCACCCTTTTCTGA GAGTTATTACAGCCAGAAAGTGTGGGCTGAAGATGGTTGGTTTCATGTTTTTGT ATTATGTATCTTTTTGTATGGTAAAGACTATATTTTGTACTTAACCAGATATAT TTTTACCCCAGATGGGGATATTCTTTGTAAAAAATGAAAATAAAGTTTTTTTAA TGGAAAAAAAAA

psiCHECK2-TXNIP-Mut-Rluc	GCATGTGGAAGAAAAGAAGCAGCTTTACCTACTTGTTTCTTTTTGTCTCTCTTC CTGGACACTCACTTTTTCAGAGACTCAACAGTCTCTGCAATGGAGTGTGGGTCC ACCTTAGCCTCTGACTTCCTAATGTAGGAGGTGGTCAGCAGGCAATCTCCTGGG CCTTAAAGGATGCGGACTCATCCTCAGCCAGCGCCCATGTTGTGATACAGGGGT GTTTGTTGGATGGGTTTAAAAATAACTAGAAAAACTCAGGCCCATCCATTTTCT CAGATCTCCTTGAAAATTGAGGCCTTTTCGATAGTTTCGGGTCAGGTAAAAATG GCCTCCTGGCGTAAGCTTTTCAAGGTTTTTTGGAGGCTTTTTGTAAATTGTGAT AGGAACTTTGGACCTTGAACTTACGTATCATGTGGAGAAGAGCCAATTTAACAA ACTAGGAAGATGAAAAGGGAAATTGTGGCCAAAACTTTGGGAAAAGGAGGTTCT TAAAATCAGTGTTTCCCCTTTGTGCACTTGTAGAAAAAAAAGAAAAACCTTCTA GAGCTGATTTGATGGACAATGGAGAGAGCTTTCCCTGTGATTATAAAAAAGGAA GCTAGCTGCTCTACGGTCATCTTTGCTTAGAGTATACTTTAACCTGGCTTTTAA AGCAGTAGTAACTGCCCCACCAAAGGTCTTAAAAGCCATTTTTGGAGCCTATTG CACTGTGTTCTCCTACTGCAAATATTTTCATATGGGAGGATGGTTTTCTCTTCA TGTAAGTCCTTGGAATTGATTCTAAGGTGATGTTCTTAGCACTTTAATTCCTGT CAAATTTTTTGTTCTCCCCTTCTGCCATCTTAAATGTAAGCTGAAACTGGTCTA CTGTGTCTCTAGGGTTAAGCCAAAAGACAAAAAAAATTTTACTACTTTTGAGAT TGCCCCAATGTACAGAATTATATAATTCTAACGCTTAAATCATGTGAAAGGGTT GCTGCTGTCAGCCTTGCCCACTGTGACTTCAAACCCAAGGAGGAACTCTTGATC AAGATGCCCAACCCTGTGATCAGAACCTCCAAATACTGCCATGAGAAACTAGAG AAACCTTTTCATAAAAGCCCTTTGAACCCCCTTCCTGCCCTGTGTTAGGAGAT AGGGATATTGGCCCCTCACTGCAGCTGCCAGCACTTGGTCAGTCACTCTCAGCC ATAGCACTTTGTTCACTGTCCTGTGTCAGAGCACTGAGCTCCACCCTTTTCTGA GAGTTATTACAGCCAGAAAGTGTGGGCTGAAGATGGTTGGTTTCATGTTTTTGT ATTATGTATCTTTTTGTATGGTAAAGACTATATTTTGTACTTAACCAGATATAT TTTTACCCCAGATGGGGATATTCTTTGTAAAAAATGAAAATAAAGTTTTTTTAA TGGAAAAAAAAA
psiCHECK2-AluJo-WT-Rluc	GCTTACATTTTTATTGCCTAATATATATGCAAACATACTTTACTTCAAGTTGAG GCTGCGCGTAGTGGCCCACACCTTTAATCCCAACATTTTGGGAGGCTGAGGCAG GAGGATCACTTGAACCCAGGAGTTCAAGACCAGCCTGGGAAACATAGAAAGACC CTGTCTCTACAAGAAAAAAAAAAAGAAAAATTAGCTGTGCATGATGGTGCACAC

psiCHECK2-AluJo-Mut-Rluc	GCTTACATTTTTATTGCCTAATATATATGCAAACATACTTTACTTCAAGTTGAG GCTGCGCGTAGTGGCCCACACCTTTAAAGGGTTGATTTTGGGAGGCTGAGGCAG GAGGATCACTTGAACCCAGGAGTTCAAGACCAGCCTGGGAAACATAGAAAGACC CTGTCTCTACAAGAAAAAAAAAAAGAAAAATTAGCTGTGCATGATGGTGCACAC
psiCHECK2-AluSc-WT-Rluc	GTAGCTGGGACTACAGGTGTGCGCCACCATGCTTGGCTAATGTTTTTTGTATTT TAGTAGAGATGGGATTTCACCATGTTGGCCAGATGTCTCTATCTCCTGACCTTG TGATCTGCCCGGCTCAGCCTCCCAAAGTGCTGGGATTATAGGCGTGAGCCACAG CACCCGGCCATGATTGCTTTTTATAATGTAAAAGCCCTAGGTATTTAT

psiCHECK2-AluSc-WT-Rluc	GTAGCTGGGACTACAGGTGTGCGCCACCATGCTTGGCTAATGTTTTTTGTATTT TAGTAGAGATGGGATTTCACCATGTTGGCCAGATGTCTCTATCTCCTGACCTTG TGATCTGCCCGGCTCAGCCAGGGTTTGTGCTGGGATTATAGGCGTGAGCCACAG CACCCGGCCATGATTGCTTTTTATAATGTAAAAGCCCTAGGTATTTAT
CircDCUN1D4 junction probe (northern blot)	5'-GTTGAGAGATGAGAGTTCAGCTGAAAATGTTTTCTGGTTCAACACCAATGTCTTC- DIG-3'
CircDCUN1D4 junction probe (pull down)	5'-GTTGAGAGATGAGAGTTCAGCTGAAAATGTTTTCTGGTTCAACACCAATGTCTTC-3'
CircDCUN1D4 Scramble probe	5'-GTGTAACACGTCTATACGCCCA-3'
CircDCUN1D4 FISH Probe	5'-GAAAACAUUUUCAGCUGAATT-3' (sense)
	5'-UUCAGCUGAAAAUGUUUUCUG-3' (antisense)
CircDCUN1D4 ISH Probe	5'-GTTGAGAGATGAGAGTTCAGCTGAAAATGTTTTCTGGTTCAACACCAATGT CTTC-3'

Supplementary Table S3 Target sequences of siRNA

Oligo Set	Target sequences
si-ADAR1-1	GCAGAGTCAGCATATATGA
si-ADAR1-2	GGCCCGAGATATAAATGCT
si-ADAR1-3	GGCTCTCCGTGTCTTGATT
si-DHX9-1	CGAACACCATTGCATGAAA
si-DHX9-2	GGACTAGTAGCAACATTGA
si-DHX9-3	GCATGGACCTCAAGAATGA
si-QKI-1	GAAGCTGGTTTAATCTATA
si-QKI-2	GACCTATTGTTCAGTTACA
si-QKI-3	GAAACCGGATGTAAAATCA
si-EIF4A3-1	CGAGCAATCAAGCAGATCA
si-EIF4A3-2	GCTGGATTACGGACAGCAT
si-EIF4A3-3	CTCTCGGTGACTACATGAA
si-TXNIP-1	CAACATCCTTCGAGTTGAA
si-TXNIP-2	ACACGCTTCTTCTGGAAGA
si-TXNIP-3	GTCTGTCTCTGCTCGAATT

Supplementary Table S4 candidate targets of circDCUN1D4

BEX2	CYP2J2	DDX6
FBXW2	FXR1	GPC3
HK2	IFI6	LOX
MGAT5	PCDH9	PLIN2
PML	SMAD2	SMC2
STXBP4	TXNIP	UQCRB
ZNF680		

Table S5 Pathological and clinical data of lung cancer cases in tissue microarray

Variables	Cases(n) (total n=92)	circDCUN1D4		P value
		Low(n)	High(n)	
Age(yes)				
≤60	38	14	24	0.731
>60	54	18	36	
Gender				
Male	51	22	29	0.062
Female	41	10	31	
Tumor size				
≤5cm	69	23	46	0.618
>5cm	23	9	14	
Lymph node invasion				
Present	41	15	26	0.008
Absent	51	17	34	
TNM stage				
I	7	3	4	0.045
II-Ⅲ	85	29	56	
EGFR mutation				
Present	22	9	13	0.495
Absent	70	23	47	

Table S6 Univariate analysis and Multivariable analysis

Parameters	Univariate analysis			Multivariable analysis		
	HR	95%CI	P value	HR	95%CI	P value
Age(>60)	2.701	1.466-4.975	0.001	2.383	1.266-4.483	0.007
Gender(Male)	1.501	0.60-1.865	0.869	—	—	—
Lymph node metastasis(Positive)	3.297	1.857-5.855	<0.0001	2.002	1.034-3.875	0.039
Pathological grading(G2-G4)	4.709	3.144-6.366	<0.0001	3.805	2.540-5.091	0.002
Tumor diameter(>5cm)	4.150	2.185-7.884	0.031	1.676	0.756-3.716	0.204
EGFR mutation(positive)	4.563	2.427-8.577	0.215	1.405	0.636-3.107	0.401
circDCUN1D4 level(low)	2.250	1.285-3.940	0.005	1.593	1.205-2.922	0.033