

YMTHE, Volume 30

## **Supplemental Information**

**circMRPS35 promotes malignant  
progression and cisplatin resistance  
in hepatocellular carcinoma**

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**Figure S1**

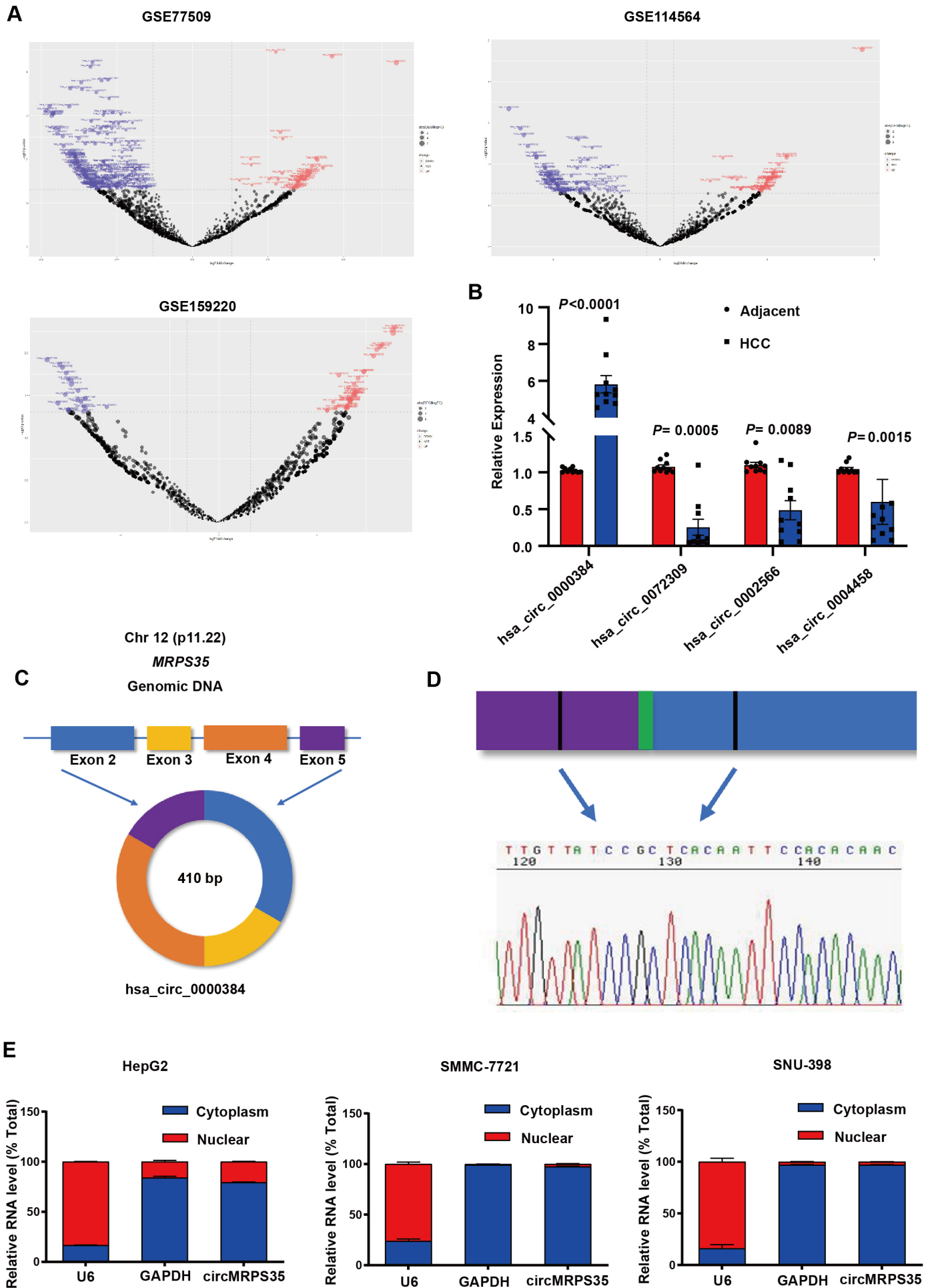
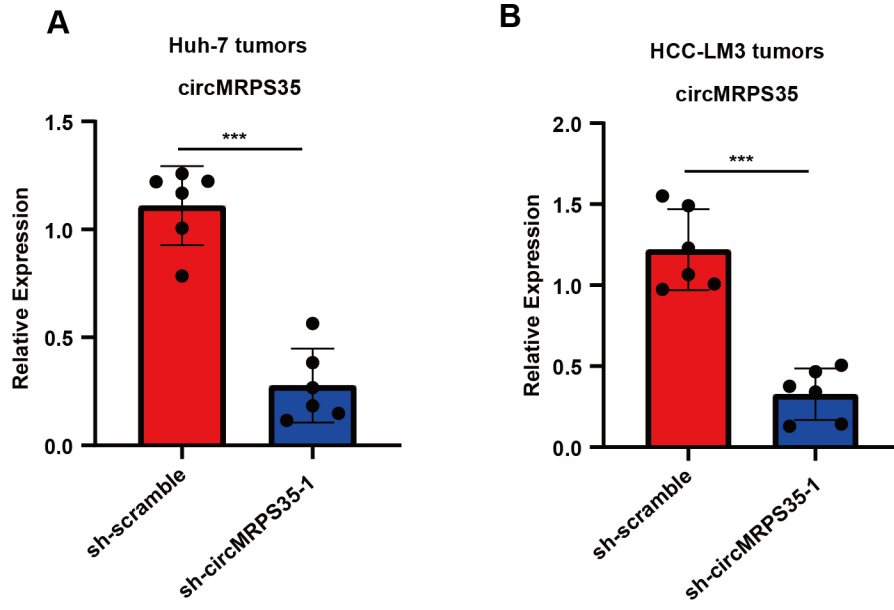
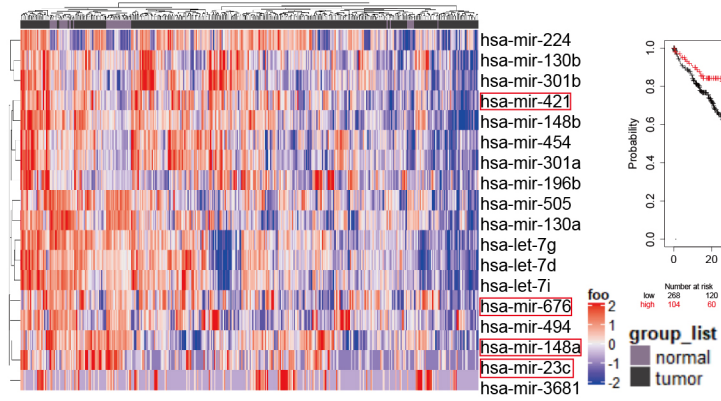


Figure S2

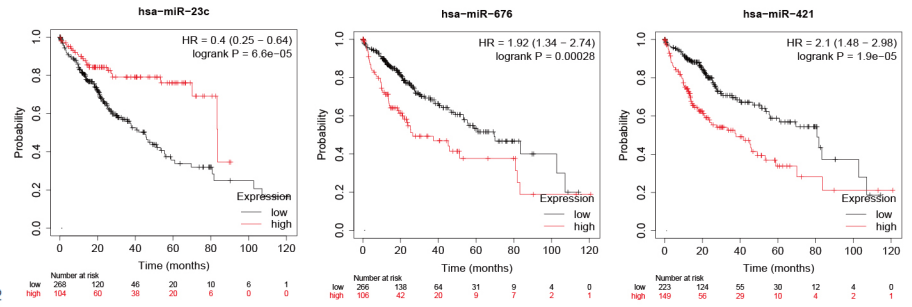


**Figure S3**

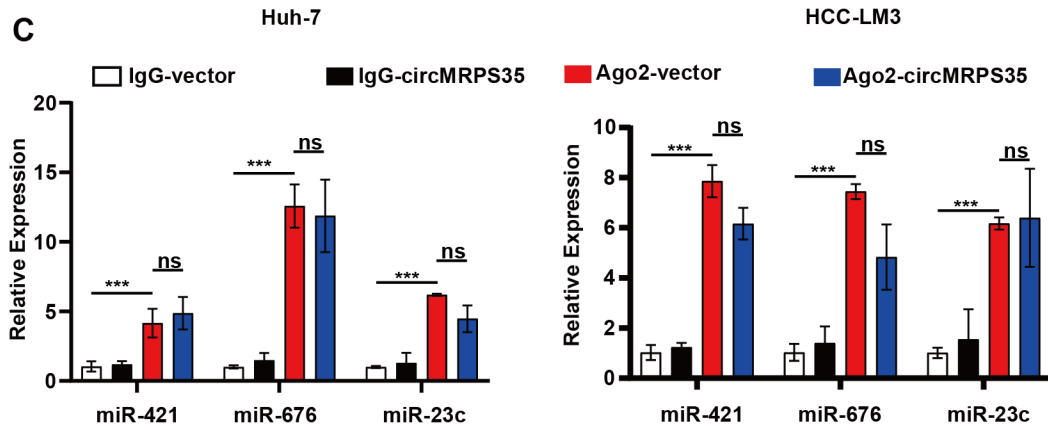
**A**



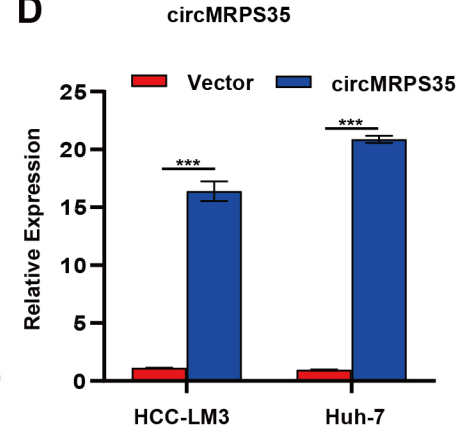
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**C**



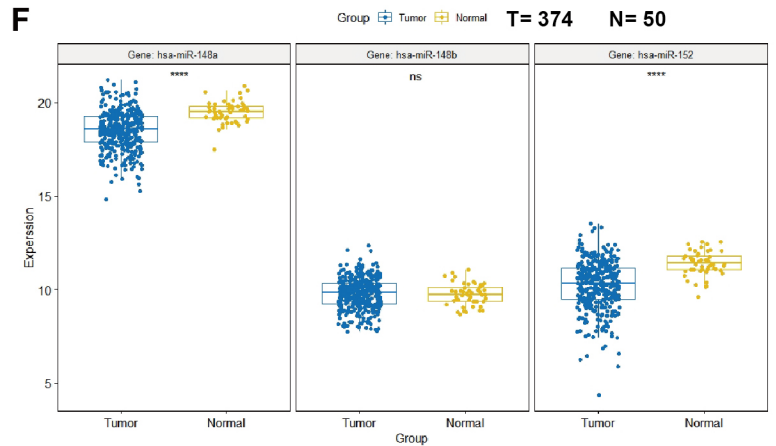
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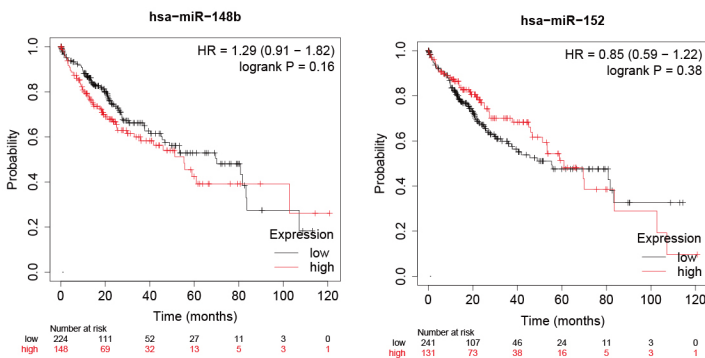
**E**

miR-148a 3' UGUUUCAAGACAUC **ACGUGACU** 5'  
 miR-148b 3' UGUUUCAAGACACU **ACGUGACU** 5'  
 miR-152 3' GGUUCAAGACAGU **ACGUGACU** 5'

**F**



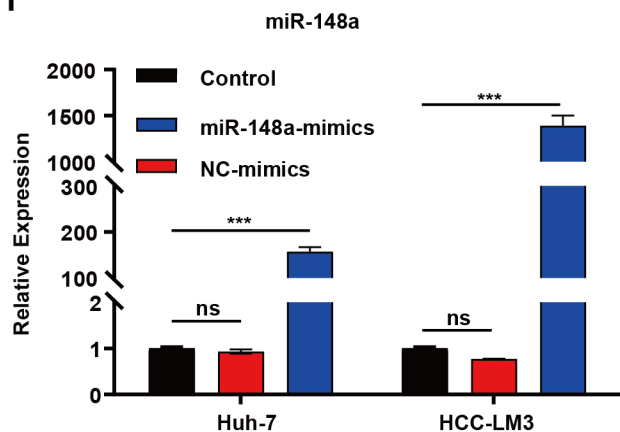
**G**



**H**

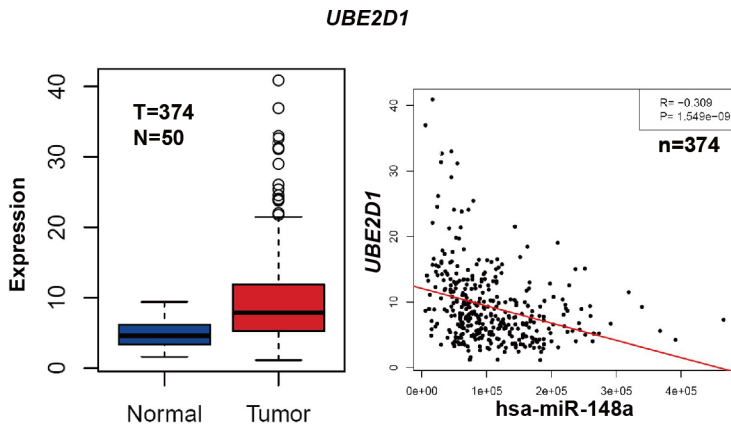
circbase ID	miRNA ID	targetscan binding site (positions)
has_circ_0000384	has-miR-148a-3p	251-256, 276-283, 294-299, 346-352

**I**

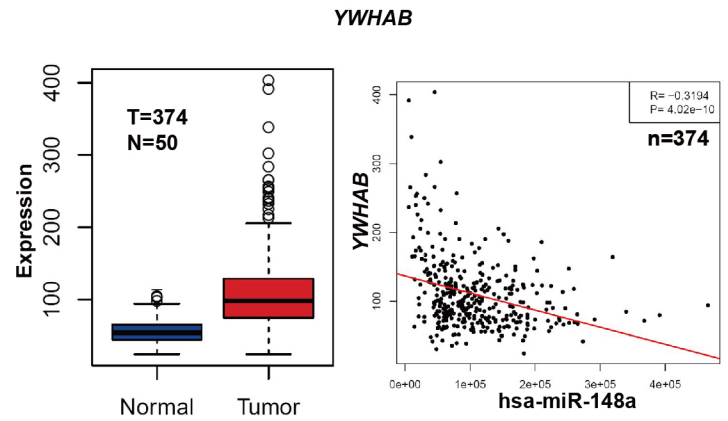


**Figure S4**

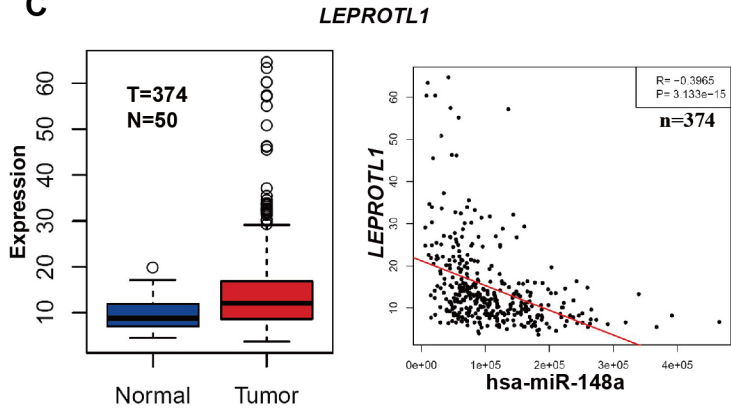
**A**



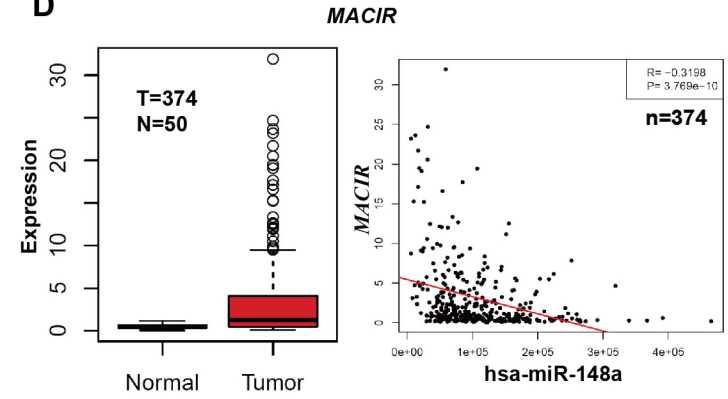
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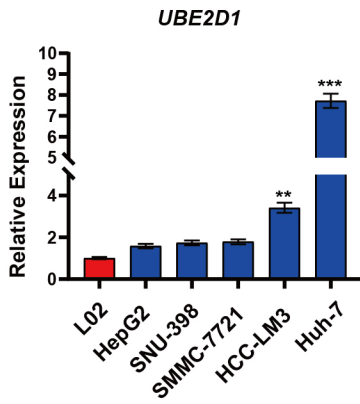
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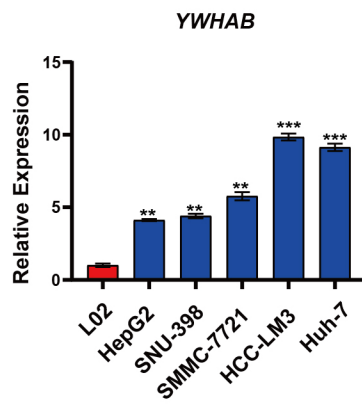
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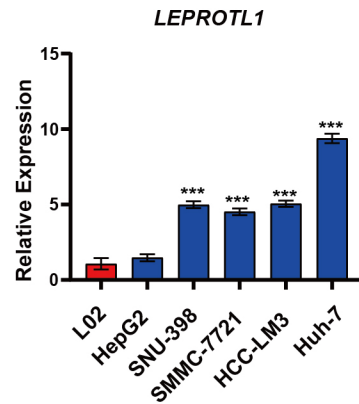
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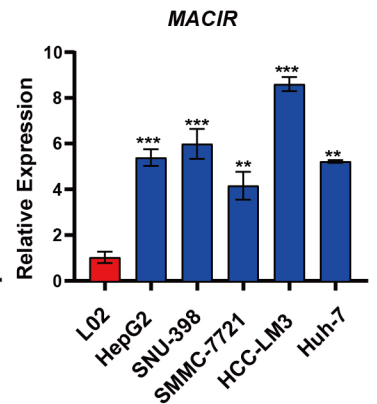
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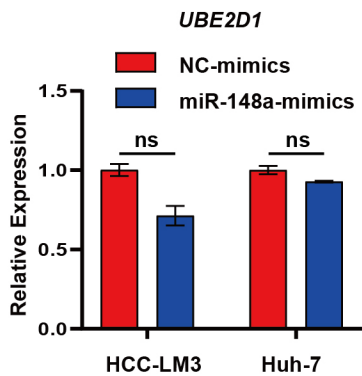
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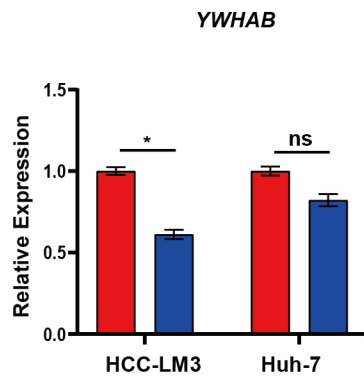
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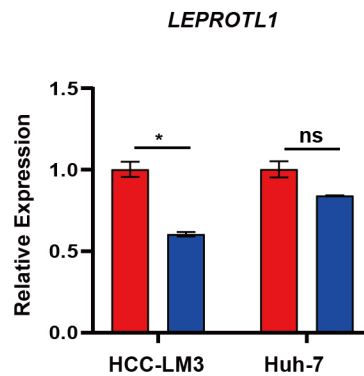
**I**



**J**



**K**



**L**

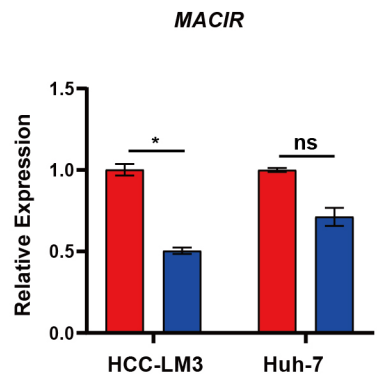
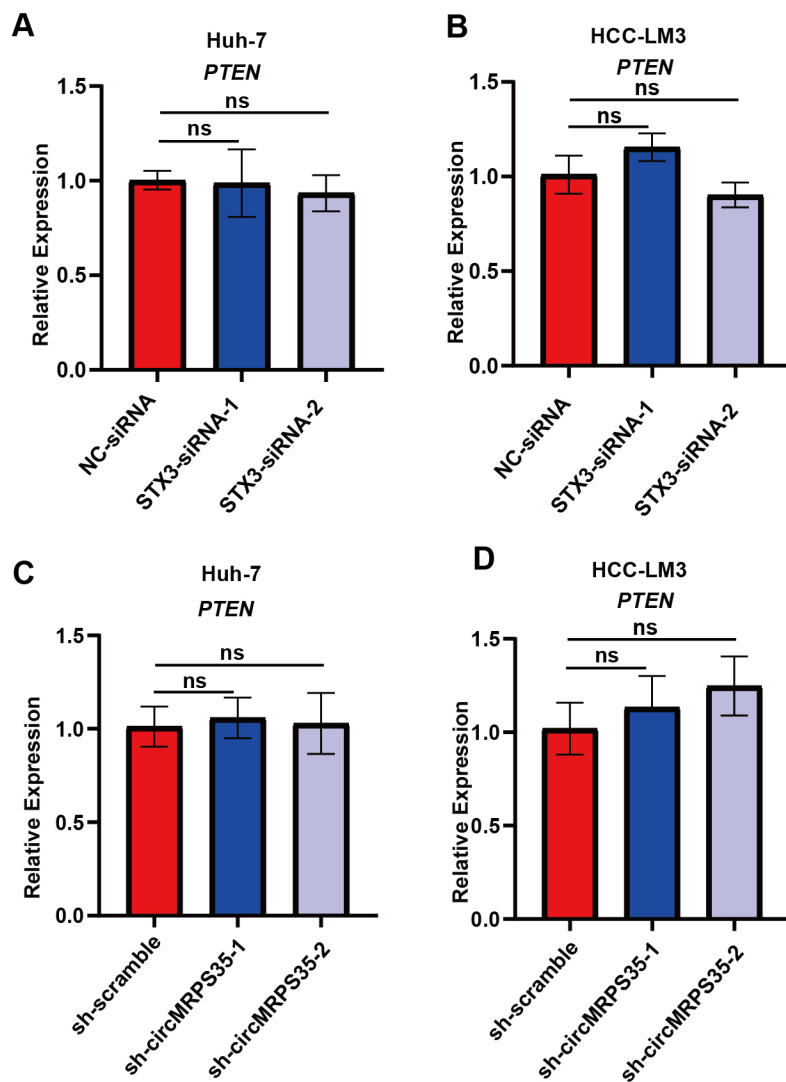
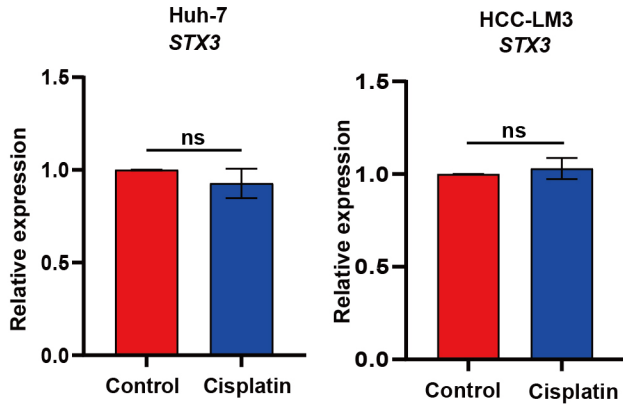


Figure S5

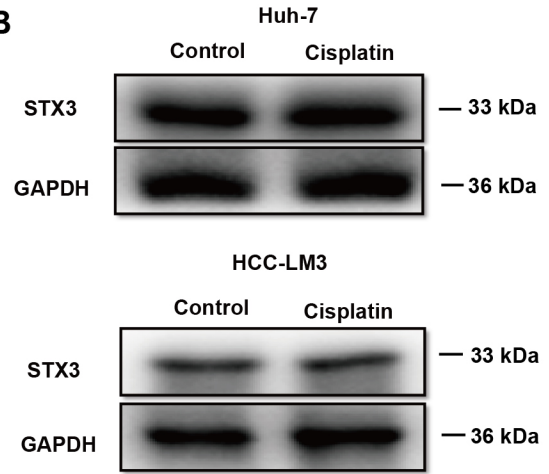


**Figure S6**

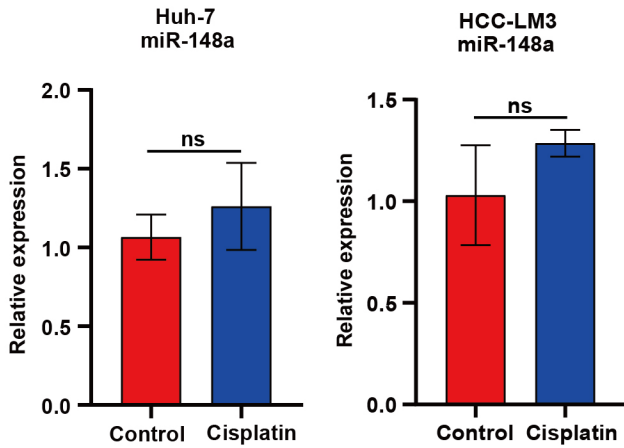
**A**



**B**



**C**



**D**

**circRNADb**  
Database for human circular RNAs

Search

**circRNADb - A Database for Human Circular RNAs**

Protein coding potential

IRES Elements	Parameter Index		
	Position (start-end)	R Score	With Pseudoknot (Y/N)
	14-158	1.907254	Y
	81-161	1.512615	Y

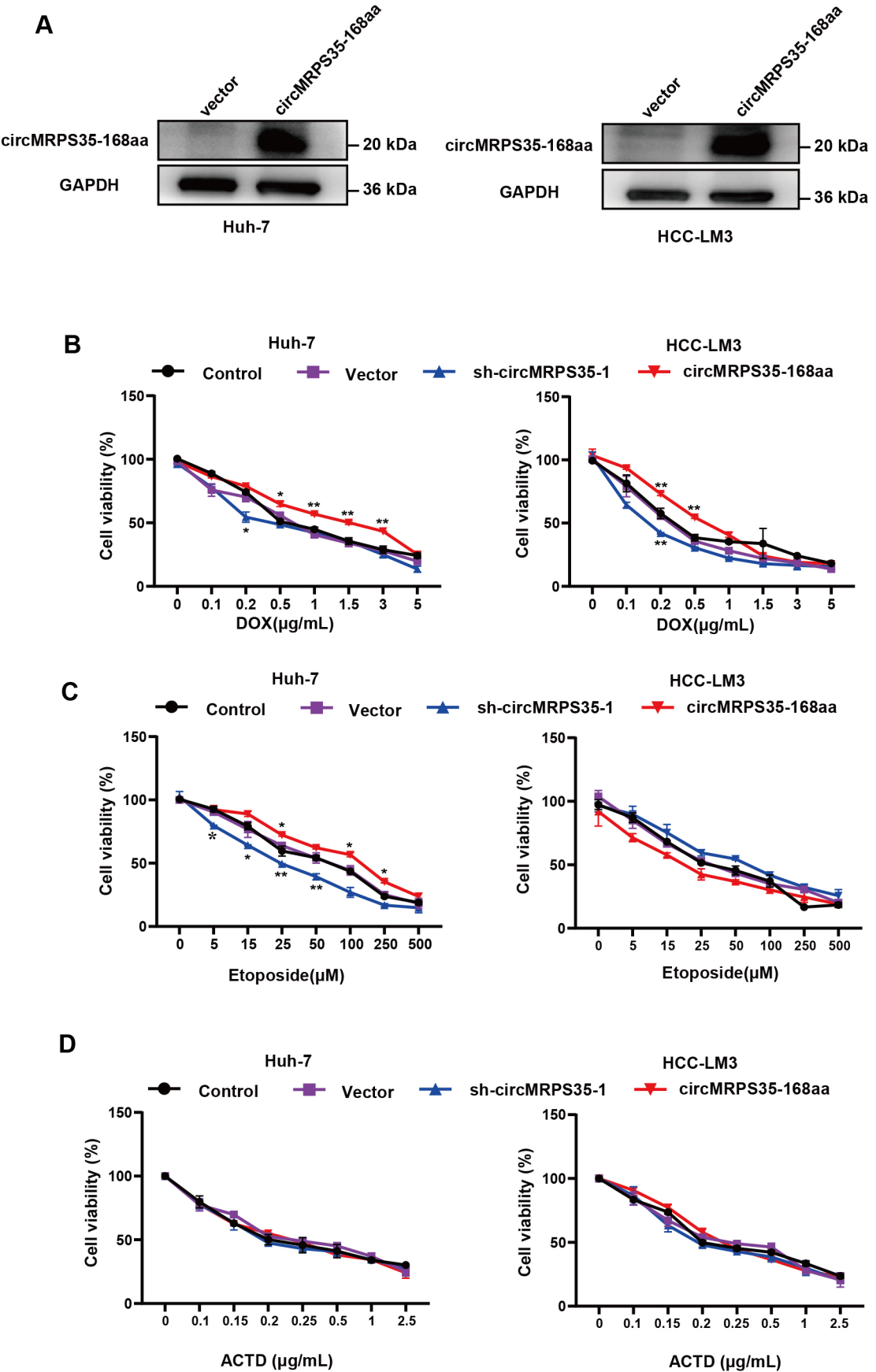
Open Reading Frame (ORF)	Start Position	End Position	Protein Length
	68	1*162	168 aa

Open Reading Frame (ORF)

MAVDQMPSPV YPVAAPFKPS AVPLPVRNGY PVKKGVPMAK EGNLELLKIP NFLHLTPVAI KKHCEALKDF CTEHPAALDS DEKCEKHFTI EIDSDYVSS GPSVRNPRAR VVVLRRKEHP ENKGQERH YLLGQRKULL TRTGLVFTQL QHHLNPLQYL FLFEWVQ\*

Note:  
(1). nr represents n rounds (n<3); (2). \* represents a stop codon.

**Figure S7**





### Supplemental Figure Legends

**Figure S1** (A) Volcano plots analysis of circRNAs in 3 RNA-seq data (GSE77509, GSE114564, GSE159220). (B) Quantitative real-time PCR analysis of the 4 candidates circRNAs in HCC tissues (n = 10) compared with non-tumor adjacent tissues. (C) Schematic representation of circMRPS35 formation. (D) The back-splice junction site of circMRPS35 was validated by Sanger sequencing. (E) Quantitative real-time PCR analysis of circMRPS35 after RNA Nucleocytoplasmic separation, U6 and GAPDH as markers of nucleus and cytoplasm in HepG2, SMMC-7721 and SNU-398 cells, respectively. Error bars represent the means  $\pm$  SEM of 3 independent experiments. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

**Figure S2** (A-B) Quantitative real-time PCR analysis of circMRPS35 expression in sh-circMRPS35-1 and sh-scramble Huh-7 and HCC-LM3 cells (n = 6). Error bars represent the means  $\pm$  SEM of 3 independent experiments. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

**Figure S3** (A) Expression heat map of 24 target miRNAs with analysis of TCGA database. (B) Kaplan-Meier analysis of the miR-23c, miR-676 and miR-421 in HCC (n = 364). (C) Quantitative real-time PCR analysis of miR-23c, miR-421, miR-676 with AGO2-RIP in Huh-7 and HCC-LM3 cells. (D) Quantitative real-time PCR analysis of circMRPS35 in circMRPS35 overexpression Huh-7 and HCC-LM3 cells. (E) The sequences of miR-148a, miR-148b and miR-152. (F) Expression of miR-148a, miR-148b and miR-152 with analysis of TCGA database (T = 374, N = 50). (G) Kaplan-Meier analysis of the miR-148b and miR-152 in HCC (n = 364). (H) Binding positions of circMRPS35 and miR-148a was showed in Targetscan database. (I) Quantitative real-time PCR analysis of miR-148a in miR-148a-mimics overexpression Huh-7 and HCC-LM3 cells. Error bars represent the means  $\pm$  SEM of 3 independent experiments. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

**Figure S4** (A-D) TCGA analysis of *UEB2D1*, *YWHAB*, *LEPROTL1* and *MAC1R* expressions in HCC tissues and correlation analysis of these genes and miR-148a expressions. (E-H) Quantitative real-time PCR assays of *UEB2D1*, *YWHAB*, *LEPROTL1* and *MAC1R* expressions in HCC cell lines compared to L02 cells. (I-L) Quantitative real-time PCR assays of *UEB2D1*, *YWHAB*, *LEPROTL1* and *MAC1R* expressions in miR-148a overexpression Huh-7 and HCC-LM3 cells. Error bars represent the means  $\pm$  SEM of 3 independent experiments. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

**Figure S5** (A-B) Quantitative real-time PCR assays of *PTEN* expression in *STX3* silenced or control Huh-7 and HCC-LM3 cells. (C-D) Quantitative real-time PCR assays of *PTEN* expression in circMRPS35 KD or control Huh-7 and HCC-LM3 cells. Error bars represent the means  $\pm$  SEM of 3 independent experiments. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

**Figure S6** (A) Quantitative real-time PCR analysis of *STX3* expression in cisplatin treatment or none-treated Huh-7 and HCC-LM3 cells. (B) Western blot analysis of *STX3* expression in cisplatin treatment or none-treated Huh-7 and HCC-LM3 cells. (C) Quantitative real-time PCR analysis of miR-148a expression in cisplatin treatment or none-treated Huh-7 and HCC-LM3 cells. (D) circRNADb database showed IRES regions and the potentially peptide translated by circMRPS35. Error bars represent the means  $\pm$  SEM of 3 independent experiments.

**Figure S7** (A) Western blot analysis of circMRPS35-168aa in circMRPS35-168aa overexpression Huh-

7 and HCC-LM3 cells, GAPDH as the control. (B-D) Cell viability assay of different circMRPS35-168aa expressed Huh-7 and HCC-LM3 cells with different concentrations of DOX, Etoposide and ACTD treatment. Error bars represent the means  $\pm$  SEM of 3 independent experiments. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

### Supplemental Table

Table S1 The List of Primers		
Gene	Forward (5'-3')	Reverse (5'-3')
circMRPS35	CCCCAGAGCACGAGTAGTAG	TGCTGCAACTGGGTAAACAC
MRPS35	GGAAAGAACACCCGGAAATGA	GTGCTGCAACTGGGTAAACAC
$\beta$ -ACTIN	AGTGTGACGTGGACATCCGCA	ATCCACATCTGCTGGAAGGTGGAC
hsa_circ_0072309	TTCCACACCGCTCAAATGTT	AGCCACTGGAAATTTGAAGCA
hsa_circ_0002566	AGCCTCACCTGATAACCTGT	AATTCCGTTGTGCAGTGTCC
hsa_circ_0004458	ACAGGAATGACGCTGGATCA	GTGTTGTTTTACCCAGCA
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT
U48	AGTGATGATGACCCAGGTA	CTCAACTGGTGTGCTGGGA
GAPDH	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG
sh-scramble	CAACAAGATGAAGAGCACCAA	TTGGTGCTCTTCATCTTGTG
sh-circMRPS35-1	GTAGTCTTAAGACGGAAAG	CTTCCGTCCTTAAGACTAC
sh-circMRPS35-2	GTCTTAAGACGGAAAGAAC	GTTCTTCCGTCCTTAAGAC
miR-148a	GCCGAGTCAGTGCCTACAG	CTCAACTGGTGTGCTGGGA
miR-421	GCCGAGATCAACAGACATTAA	CTCAACTGGTGTGCTGGGA
miR-23c	GCCGAGATCACATTGCCAGT	CTCAACTGGTGTGCTGGGA
miR-676	GCCGAGCTGTCCTAAGGTT	CTCAACTGGTGTGCTGGGA
STX3	TCGGCAGACCTTCGGATTC	TCCTCATCGGTTGTCTTTTTGC
LEPROTL1	TTTGATGCTTGGATGTGCCCT	GCCCGTTGTAAGAAAGATGGC
YWHAB	CATGAAGGCAGTCACAGAACA	CTCACGGTACTCTTTGCCAT
UBE2D1	TAGCGCATATCAAGGTGGAGT	TGGTGACCATTGTGACCTCAG
MACIR	ACCGTGTGACGGCTACCAGAT	TGTTTGGAGCGTAAGGATGGC
PTEN	TTTGAAGACCATAACCCACCAC	ATTACACCAGTTCGTCCCTTTC
NC-siRNA	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT
STX3-siRNA-1	GGAGAAGCAUUAUGAAGAAGA	UCUUCUCAAUAUGCUUCUCC
STX3-siRNA-2	GCAAGCCUCAGUGAGAUUGA	UCAAUCUCACUGAGGGCUUGC

**Table S2 The expressions of circRNAs in GSE77509**

**Table S3 The expressions of circRNAs in GSE114564**

**Table S4 The expressions of circRNAs in GSE159220**

**Table S5 The List of microRNAs**