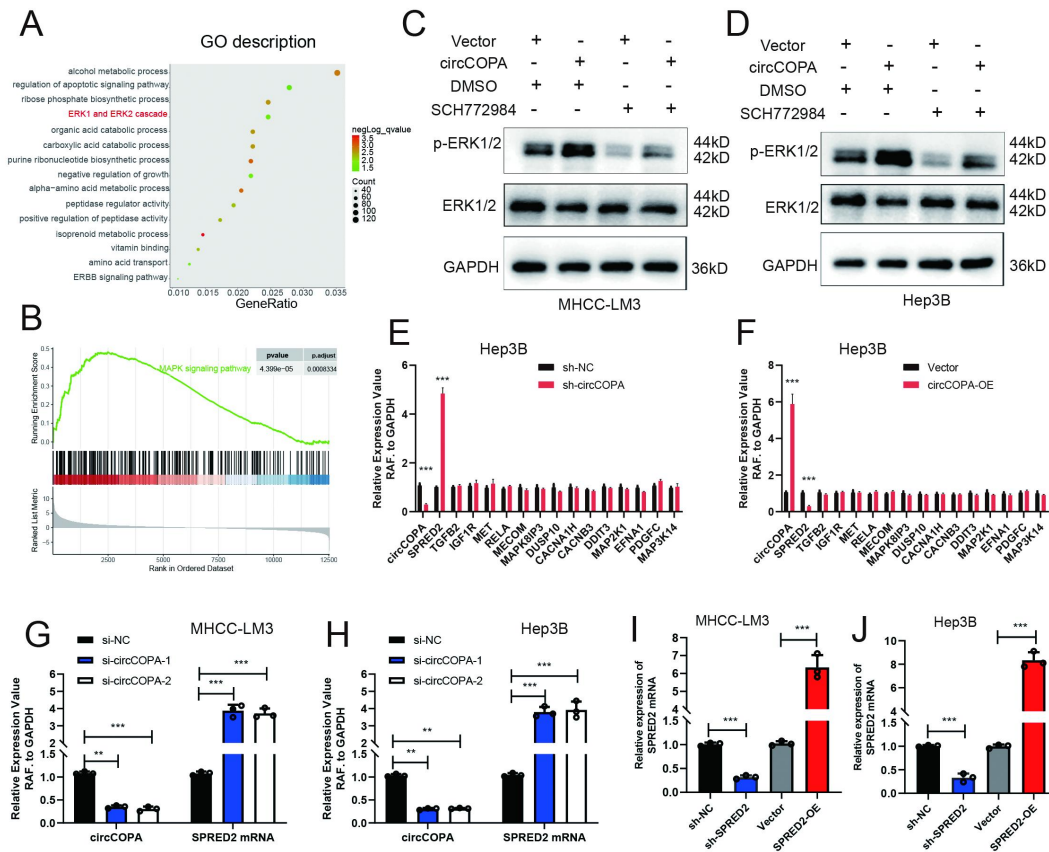


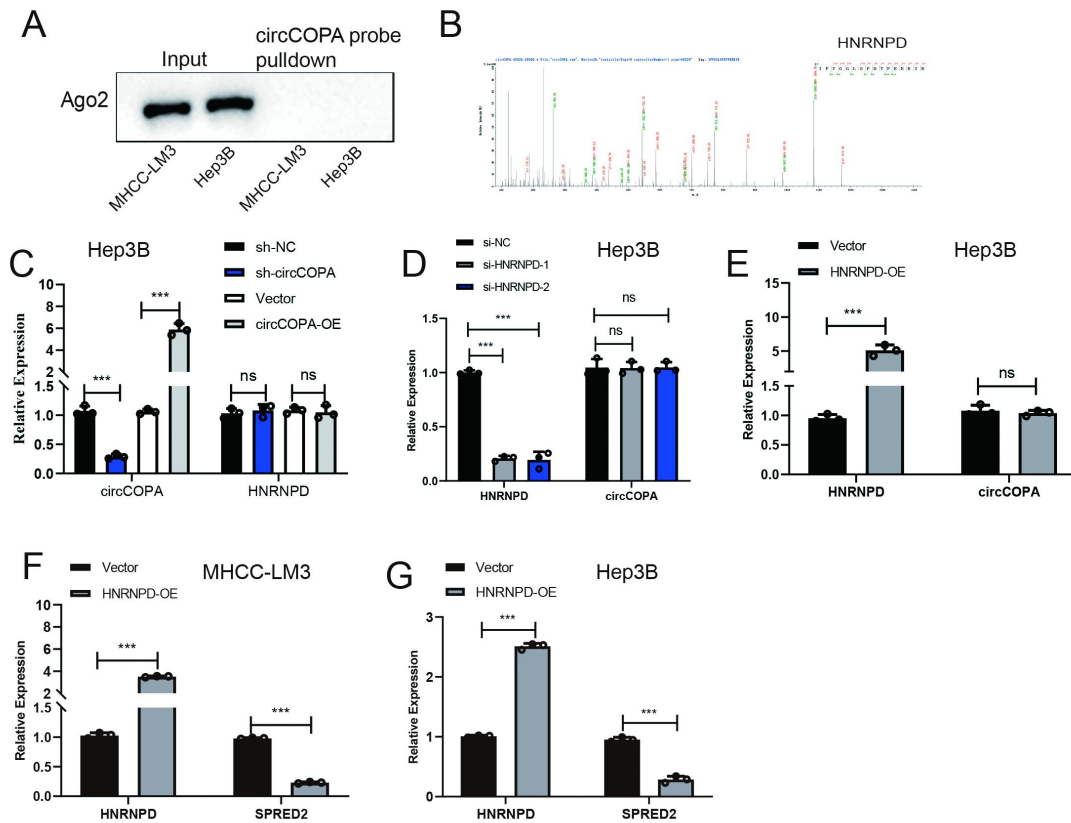
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2 **Fig. S1 Identification and characterization of circCOPA in HCC.** (A) Volcano
 3 plot showing circCOPA expression in the GSE94508 dataset. (B) Volcano plot
 4 showing circCOPA expression in the GSE155949 dataset. (C) COPA expression
 5 levels in liver hepatocellular carcinoma (LIHC) based on TCGA data (tumor tissues, n
 6 = 369; adjacent non-tumor tissues, n = 160). (D) Overall survive analysis of HCC
 7 patients stratified by COPA expression levels based on TCGA data. (F) Disease-free
 8 survive analysis of HCC patients stratified by COPA expression levels based on
 9 TCGA data.



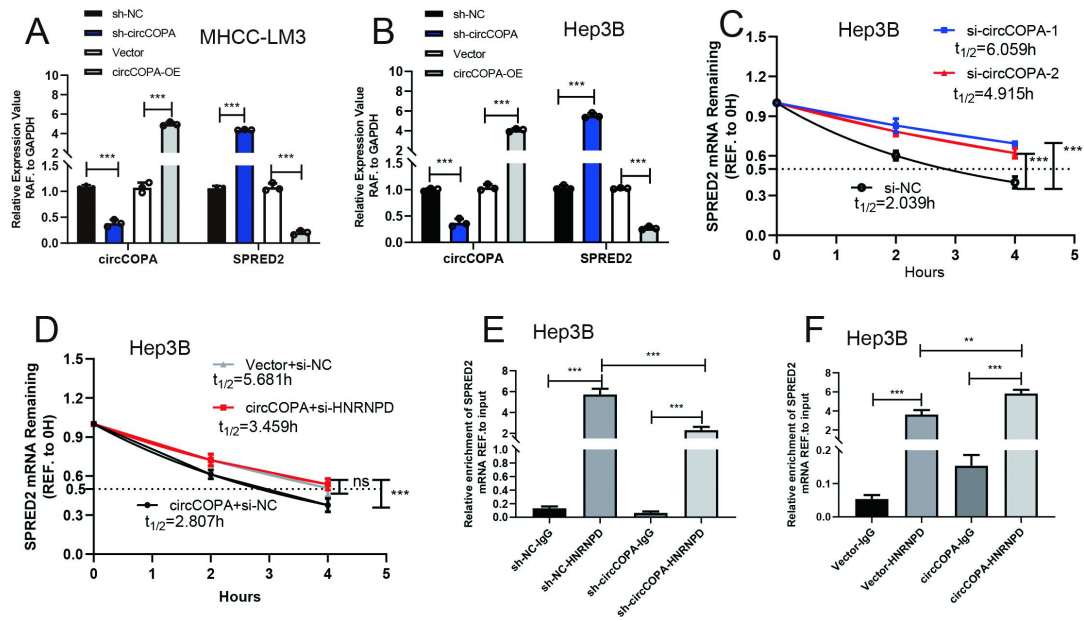
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11 **Fig. S2 circCOPA activates the ERK/MAPK pathway by downregulating**
 12 **SPRED2.** (A) Gene Ontology (GO) enrichment analysis of differentially expressed
 13 genes identified from RNA-seq data. (B) GSEA plot showing activation of MAPK
 14 signaling pathway in Hep3B cells with circCOPA overexpression. (C,D) Western blot
 15 analysis of total ERK1/2, and phosphorylated ERK1/2 (p-ERK1/2) levels in
 16 MHCC-LM3 and Hep3B cells. (E,F) qRT-PCR analysis of the 15 candidate genes in
 17 Hep3B cells following circCOPA knockdown or overexpression. (G,H) qRT-PCR
 18 analysis of SPRED2 mRNA expression levels following circCOPA knockdown in
 19 MHCC-LM3 and Hep3B cells. (I,J) qRT-PCR validation of stable SPRED2
 20 knockdown and overexpression efficiency in MHCC-LM3 and Hep3B cells. Data are
 21 presented as mean±SD of three independent experiments. ** $p < 0.01$, *** $p < 0.001$.



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23 **Fig. S3 circCOPA directly interacts with HNRNP D protein in HCC cells.** (A)
 24 Western blot analysis of Ago2 protein levels following RNA pulldown using
 25 circCOPA-specific probes in MHCC-LM3 and Hep3B cells. (B) Mass spectrometry
 26 identification of HNRNP D from circCOPA pulldown samples. (C) qRT-PCR analysis
 27 of HNRNP D mRNA following stable circCOPA knockdown or overexpression in
 28 Hep3B cells. (D, E) qRT-PCR analysis of circCOPA following HNRNP D knockdown
 29 or overexpression in Hep3B cells. (F, G) qRT-PCR analysis of SPRED2 mRNA levels
 30 following HNRNP D overexpression in MHCC-LM3 and Hep3B cells. Data are
 31 presented as mean±SD of three independent experiments. *** p <0.001.



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33 **Fig. S4 circCOPA promotes SPRED2 mRNA decay in an HNRNPD-dependent**
 34 **manner.** (A,B) qRT-PCR analysis of SPRED2 mRNA expression levels after stable
 35 circCOPA knockdown or overexpression in MHCC-LM3 and Hep3B cells. (C)
 36 SPRED2 mRNA stability was assessed by qRT-PCR at specific time points after
 37 actinomycin D treatment (10 μ g/mL) in Hep3B cells with circCOPA knockdown. (D)
 38 SPRED2 mRNA stability was measured by qRT-PCR at specific time points following
 39 actinomycin D treatment (10 μ g/mL) in Hep3B cells with circCOPA overexpression
 40 alone or in combination with HNRNPD knockdown. (E,F) HNRNPD RIP assays
 41 were performed in Hep3B cells with circCOPA knockdown or overexpression,
 42 followed by qRT-PCR analysis of SPRED2 mRNA enrichment. Data are presented as
 43 mean \pm SD of three independent experiments. ** p <0.01, *** p <0.001.

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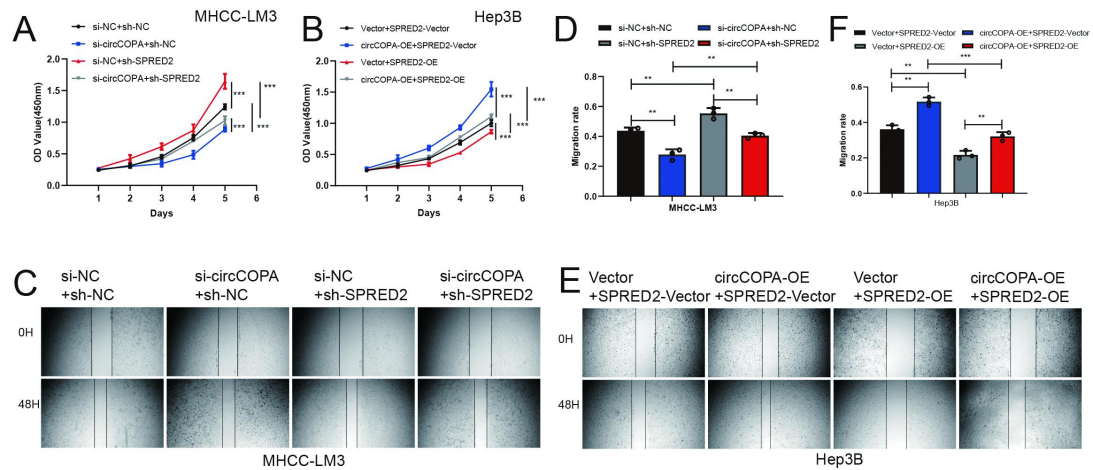
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52 **Fig. S5 The circCOPA-SPRED2 axis drives HCC malignancy.** (A,B) Rescue
 53 experiments based on CCK-8 assays were performed in circCOPA and
 54 SPRED2-modulated MHCC-LM3 or Hep3B cells. (C,D) Rescue experiments based
 55 on wound-healing assays were performed in circCOPA and SPRED2-modulated
 56 MHCC-LM3 cells. (E,F) Rescue experiments based on wound-healing assays were
 57 performed in circCOPA and SPRED2-modulated Hep3B cells. Data are presented as
 58 mean±SD of three independent experiments. ** $p < 0.01$, *** $p < 0.001$.

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84 **Supplementary Table S1.** The correlation between circCOPA expression and HCC
 85 patients' clinicopathological characteristics.

Characteristics		circCOPA Expression		P value
		High (40)	Low (40)	
Gender	Male	23	26	0.491
	Female	17	14	
Age(Years)	<60	22	19	0.502
	≥60	18	21	
Liver cirrhosis	Yes	18	23	0.263
	No	22	17	
AFP	≤200ng/ml	12	15	0.478
	>200ng/ml	28	25	
HbsAg	Positive	28	21	0.108
	Negative	12	19	
Tumor Size	<5cm	8	21	0.002**
	≥5cm	32	19	
Tumor Stage	I	11	23	0.007**
	II-III	29	17	
Edmondson grade	I-II	10	21	0.012*
	III-IV	30	19	

86 *P<0.05, **P<0.01

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110 **Supplementary Table S2.** Primer sequence of qRT-PCR used in this study

Gene	Forward Primer	Reverse Primer
circCOPA	GGCAGTGGAGCCATATCTCTG	CTACCCCAAAGCCTCCAAGTT
GAPDH	TCCAAAATCAAGTGGGGCGA	AAATGAGCCCCAGCCTTCTC
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT
COPA	TATGCTGGCACAGGCAATCT	ATCACAATGGCGTGTTTGGC
Divergent	ATCGTGGAGTAAACTGGGCTG	TCTTCTGTGGGGTGGAACTG
Convergent	CAGTTCCACCCACAGAAGA	GAAATATCCCAAACGCGCACA
SPRED2	GCCACCTCCACGACTCATAAC	GGTAATCCTCGTACCCCGTC
HNRNPD	GCGTGGGTCTGCTTTATTACC	TTGCTGATATTGTTCCCTTCGACA

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Supplementary Table S2. Sequence of sh/si-RNAs used in this study

si-circCOPA-1	UCUGGCGCAUGAAUGUGUGTT
si-circCOPA-2	CUGGCGCAUGAAUGUGUGUTT
si-circCOPA-3	UGGCGCAUGAAUGUGUGUUTT
si-circCOPA-NC	ACGTGCCACATGGTCGACGT
sh-circCOPA-1	TTCATATGACCAGTGGACATGAAA
sh-circCOPA-2	TATGACCAGTGGACATGAAAGATCA
sh-circCOPA-NC	ACGTCTGCCACATGGTCGACGT
si-HNRNPD-1	AAUGUUGGUCUUAGUAAAUGU
si-HNRNPD-2	GUUGUAGACUGCACUCUGA
si-HNRNPD-NC	ACGTGCCAGGTCCTGACGT
sh-SPRED2-1	TGCTGTTGACAGTGAGCGACTCAGAAGAGAGCAACCTTAG TGAAGCCACAGATGTA
sh-SPRED2-2	TGCTGTTGACAGTGAGCGAGCATGCTCTATCACTGTATGTAGT GAAGCCACAGATGTA
sh-SPRED2-NC	ACGTGCCACCTATCACTGTATGATGGTCGACGT
circCOPA-probe	TGGACTGCTTCTTGCTCTGTTGTTC -/3bio/
circCOPA-NC-probe	GAACAACAGAGCAAGAAGCAGTCCA- /3bio/
circCOPA-probe(FISH)	/5CY3/-GAACAACAGAGCAAGAAGCAGTCCA

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Supplementary Table S3. Antibodies used in this study

Antibody	Source	Cat no.
HNRNPD (WB)	ABclonal	Cat#A15679
HNRNPD (RIP)	ABclonal	Cat#A15679
HNRNPD (IF)	ABclonal	Cat#A15679
Anti-JNK	Abcam	ab199380
Anti-p-JNK	Abcam	ab215208
Anti-p38	Abcam	ab170099
Anti-Phospho-p38	Abcam	ab195049
Anti-ERK1/2	Abcam	ab184699
Anti-Phospho-ERK1/2	Cell Signaling Technology	4370
Anti-MEK1/2	Proteintech	11049-1-AP
Anti-Phospho-MEK1/2	Abclonal	AP0209
Anti-GAPDH	Servicebio	GB15004-100
Anti-SPRED2	Proteintech	24091-1-AP
Flag-tag (WB)	Sigma-Aldrich	Cat#F3165
Ki67 (IHC)	Servicebio	Cat#GB111499
Anti-IgG Control	Proteintech	30000-0-AP