

Supplementary Figure legends

Figure S1. *Circ7379* is abundant in normal colorectal tissues and cells. Related to Figure 1.

(A) Microarray analysis showing the expression of *circ7379* and some other known downregulated circRNAs in 3 normal colorectal tissues.

(B) qRT-PCR showing the expression of *circ7379* and some other known downregulated circRNAs in a normal colon cell line (FHC).

The data are shown as the mean \pm SD. The *P* values were determined by a two-tailed unpaired Student's *t* test or one-way ANOVA; **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001.

Figure S2. Verification of the existence and circularization of *circ7379*. Related to Figure 2.

(A) Schematic representation of the design of divergent and convergent primers for *circ7379* using circPrimer software.

Figure S3. *DHX9* regulates the biogenesis of *circ7379*. Related to Figure 3.

(A) Identification of highly matched RCMs in the upstream sequence and downstream sequence of *circ7379* using BLAST.

(B) The plasmid vector map for the *circ7379* expression vector (GV367).

(C) qRT-PCR showing the significant knockdown of *DHX9* and *ADAR* in CRC cell lines by the transfection of corresponding siRNAs.

(D) qRT-PCR showing the expression of *circ7379* in CRC cell lines under control conditions (si-NC) or upon *ADAR* knockdown (si-ADAR).

(E) qRT-PCR showing the significant overexpression of *DHX9* in CRC cells by the transfection of vectors.

The data are shown as the mean \pm SD. The *P* values were determined by a two-tailed unpaired Student's *t* test; **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001.

Figure S4. Silencing *circ7379* promotes the growth and metastasis of CRC cells in vitro and in vivo. Related to Figure 4.

(A) qRT-PCR showing the significant overexpression of *circ7379* in CRC cell lines by the transfection of circRNA-specific lentiviral plasmid.

(B) qRT-PCR showing the significant knockdown of *circ7379* in CRC cell lines by the transfection of siRNAs specifically targeting the BSJ sites of *circ7379*.

(C) CCK-8 assay showing the proliferation ability of CRC cell lines under control conditions (si-NC) or upon *circ7379* knockdown (si-*circ7379*).

(D) Plate clone formation assay showing the clone formation ability of CRC cell lines under control conditions (si-NC) or upon *circ7379* knockdown (si-*circ7379*).

(E) Transwell assay showing the migration and invasion abilities of CRC cell lines under control conditions (si-NC) or upon *circ7379* knockdown (si-*circ7379*). Scale bar, 100 μ m.

(F) In vivo xenograft models showing the tumorigenesis ability of CRC cells under control conditions (si-NC) or upon *circ7379* knockdown (si-*circ7379*). Top, images of tumors in mice in each group (n=5 mice/group). Bottom (left), tumor growth curves in mice in each group. Bottom (right), tumor weights in mice in each group.

(G) In vivo pulmonary metastasis models showing the metastatic ability of CRC cells under control conditions (si-NC) or upon *circ7379* knockdown (si-*circ7379*). Top, incidences of lung metastases

in mice in each group (n=10 mice/group). Bottom (left), representative lung and representative H&E staining of lung metastatic lesions (black arrow). Scale bar, 200 μ m. Bottom (right), the number of metastatic nodules formed in the lungs of mice in each group.

The data are shown as the mean \pm SD. The *P* values were determined by a two-tailed unpaired Student's *t* test (A, F and G), one-way ANOVA (B, D and F), two-way ANOVA (C and F), or chi-square test (G); **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001.

Figure S5. Screening for the target genes of *circ7379*. Related to Figure 5.

(A) Volcano plot showing the expression profiles of SW480-vector cells and SW480-*circ7379* cells. The following conditions were applied to screen differences: |Fold Change| >2, *P*<0.05. The red points in the plot indicate significantly upregulated genes, and the blue points indicate significantly downregulated genes.

(B) GO analyses of potential genes regulated by *circ7379*.

(C) qRT-PCR showing the expression of selected genes in CRC cells after the transfection of the control vector or *circ7379* overexpression vector.

(D) qRT-PCR showing the expression of selected genes in CRC cells under control conditions (si-NC) or upon *circ7379* knockdown (si-*circ7379*).

(E) qRT-PCR showing the expression of *RUNX1* mRNA in 20 pairs of CRC tissues and adjacent normal tissues.

(F) qRT-PCR showing the expression of *RUNX1* mRNA in a normal colon cell line (FHC) and a series of CRC cell lines (HT29, HCT116, SW480, and SW620).

(G) Representative Western blot of *RUNX1* protein in 4 pairs of CRC tissues and adjacent normal tissues.

(H) Representative Western blot of *RUNX1* protein in a normal colon cell line (FHC) and a series of CRC cell lines (HT29, HCT116, SW480, and SW620).

(I) *RUNX1* expression was negatively correlated with *circ7379* expression in CRC tissues and cells.

(J) CCK-8 assay showing the proliferation ability of CRC cells under control conditions (si-NC) or upon *circ7379* knockdown (si-*circ7379*) or cotransfection of si-*circ7379* + si-*RUNX1*.

(K) Transwell assay showing the migration and invasion abilities of CRC cells under control conditions (si-NC) or upon *circ7379* knockdown (si-*circ7379*) or cotransfection of si-*circ7379* + si-*RUNX1*. Scale bar, 100 μ m.

(L) Immunohistochemistry (IHC) showing the expression of *RUNX1* protein in xenografts in mice in each group. Scale bar, 50 μ m (left), 20 μ m (right).

(M) The incidences of lung metastases in mice in each group (n=10 mice/group). ^a Vector vs. *Circ7379*, ^b *Circ7379* vs. *Circ7379*+*RUNX1*.

(N) Immunohistochemistry (IHC) showing the expression of *RUNX1* protein in lung metastatic lesions in mice in each group. Scale bar, 100 μ m (left), 20 μ m (right).

The data are shown as the mean \pm SD. The *P* values were determined by a two-tailed paired (E) or unpaired Student's *t* test (C, D and K), one-way (F) or two-way ANOVA (J), Pearson correlation analysis (I), or chi-square test (M); **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001.

Figure S6. Screening for potential proteins interacting with *circ7379*. Related to Figure 6.

(A) qRT-PCR showing the enrichment of *circ7379*, *ciRS-7* (positive control), and *circNDUFB2* (negative control) in a representative anti-AGO2 RIP assay of CRC cells. IgG was used as a control.

(B) qRT-PCR showing the enrichment of *miRNAs* upon *circ7379* pull-down in CRC cell lysates.
(C) Screening for potential proteins interacting with *circ7379* using the catRAPID online database.
(D) Screening for potential proteins interacting with *circ7379* using the RNA-Protein Interaction Prediction (RPISeq) online website.
(E) qRT-PCR showing the expression of *RUNX1* mRNA in CRC cells under control conditions (si-NC) or upon *KSRP* knockdown (si-*KSRP*), *LN28B* knockdown (si-*LN28B*), or *ELAV1* knockdown (si-*ELAV1*).
The data are shown as the mean \pm SD. The *P* values were determined by a two-tailed unpaired Student's *t* test; **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001.

Figure S7. Prediction of the interacting motif and domain between *circ7379* and KSRP. Related to Figure 7.

(A) Prediction of the binding motif of *circ7379* with KSRP using the MEME database.
(B) Prediction of the binding motif of *circ7379* with KSRP using the catRAPID database.
(C) qRT-PCR showing the significant overexpression of *circ7379* fragments in CRC cells after the transfection of the corresponding vectors.
(D) Schematic representation of a consensus recognition element for KSRP.
(E) Prediction of the binding motif of *circ7379* with KSRP using the RNA-Binding Protein DataBase (RBPDB).
(F) Schematic representation of the four RNA-binding KH domains of KSRP.
(G) Prediction of the binding domain of KSRP with *circ7379* using the catRAPID database.
(H) Representative Western blot of Flag-tagged truncation mutants of KSRP in CRC cells.
The data are shown as the mean \pm SD. The *P* values were determined by a two-tailed unpaired Student's *t* test; **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001.

Figure S8. *Circ7379* and KSRP collaboratively modulate *pri-miR-320a* and *pre-miR-320a* processing. Related to Figure 8.

(A) qRT-PCR showing the expression of *miR-320a* and *miR-1276* in CRC cells after the transfection with the control vector or *KSRP* vector.
(B) qRT-PCR showing the expression of *miR-320a* and *miR-1276* in CRC cells after the transfection with the control vector or *circ7379* vector.
(C) Potential interacting sequence between *circ7379* and *pri-miR-320a* and between *circ7379* and *pre-miR-320a* shown by BLAST.
(D) qRT-PCR showing the enrichment of *pri-miR-320a* and *pre-miR-320a* upon *circ7379* pull-down in CRC cells under control conditions (si-NC) or upon *KSRP* knockdown (si-*KSRP*).
The data are shown as the mean \pm SD. The *P* values were determined by a two-tailed unpaired Student's *t* test; **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001.

Figure S9. *miR-320a* targets *RUNX1* and inhibits its expression. Related to Figure 9.

(A) qRT-PCR showing the significant overexpression of *miR-320a* in CRC cell lines by the transfection of *miR-320a* mimics.
(B) qRT-PCR showing the significant knockdown of *miR-320a* in CRC cell lines by the transfection of *miR-320a* inhibitors.
(C) qRT-PCR showing the expression of *RUNX1* mRNA in CRC cell lines after the transfection of

control mimics or *miR-320a* mimics.

(D) Representative Western blot of RUNX1 protein in CRC cell lines after the transfection of control mimics or *miR-320a* mimics.

(E) qRT-PCR showing the expression of *RUNX1* mRNA in CRC cell lines after the transfection of control inhibitors or *miR-320a* inhibitors.

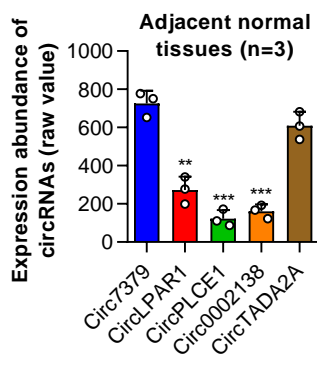
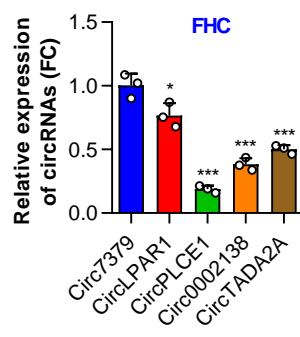
(F) Representative Western blot of RUNX1 protein in CRC cell lines after the transfection of control inhibitors or *miR-320a* inhibitors.

(G) Prediction of the binding sites of *RUNX1* 3'UTR with *miR-320a* using the TargetScan website.

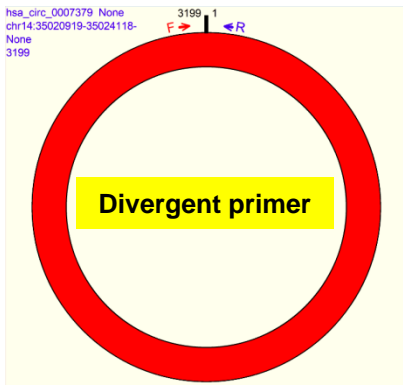
(H) Schematic representation of the construction of the WT or MUT *RUNX1* 3'UTR luciferase reporter vector.

(I) Transwell assay showing the migration and invasion abilities of CRC cells after the transfection of the control vector, *circ7379* vector, or *miR-320a* inhibitors or cotransfection of *circ7379* vector + *miR-320a* inhibitors.

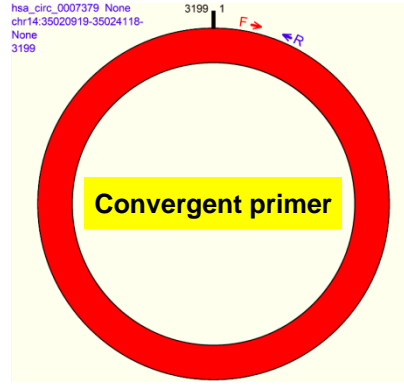
The data are shown as the mean \pm SD. The *P* values were determined by a two-tailed unpaired Student's *t* test or one-way ANOVA (I); **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001.

A**B**

A



Amplification length: 149 bp



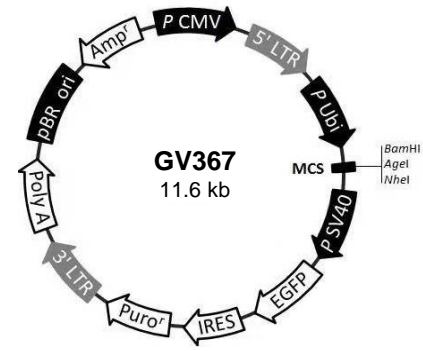
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A

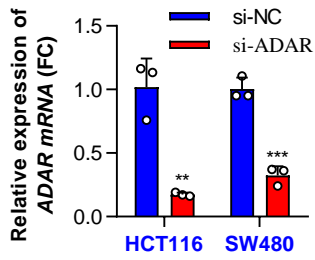
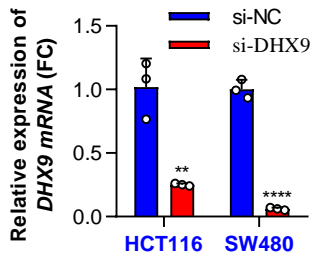
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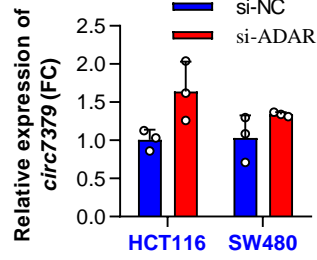
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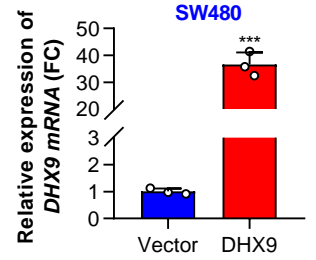
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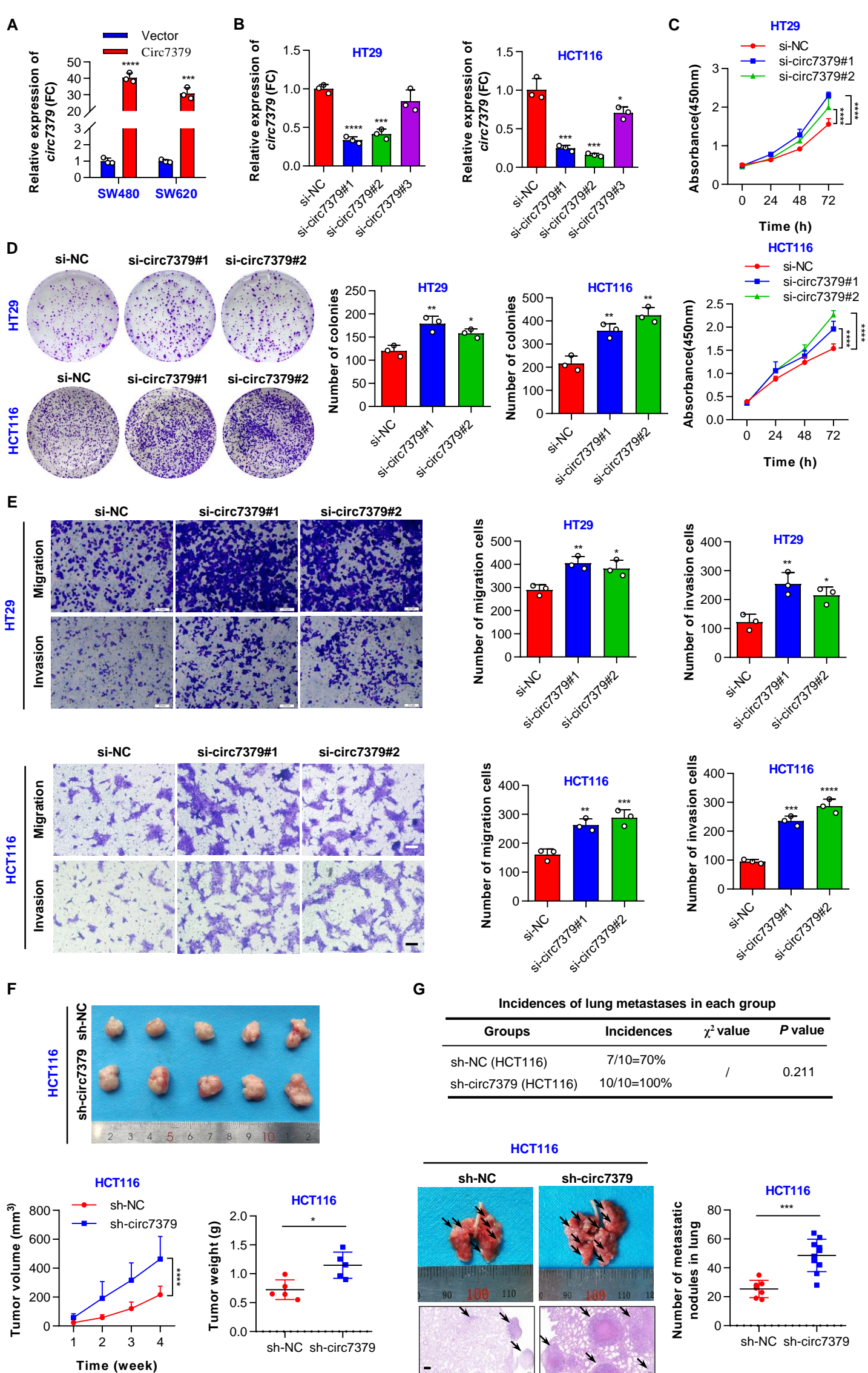


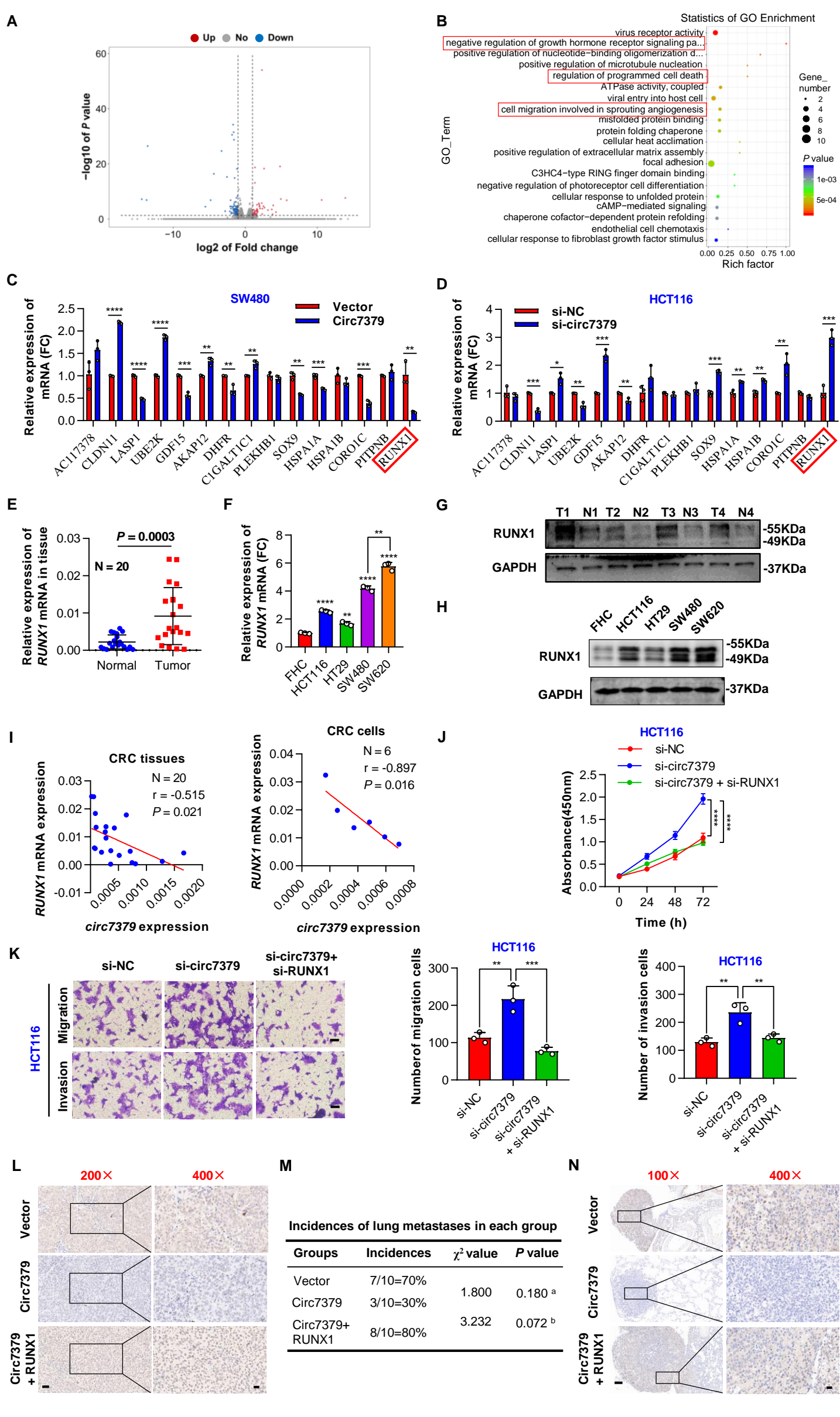
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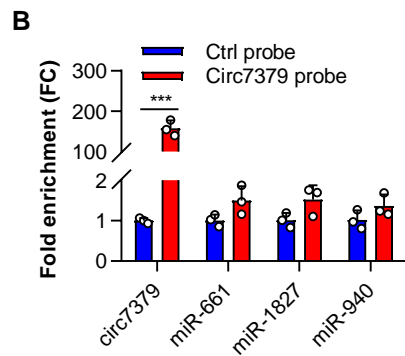
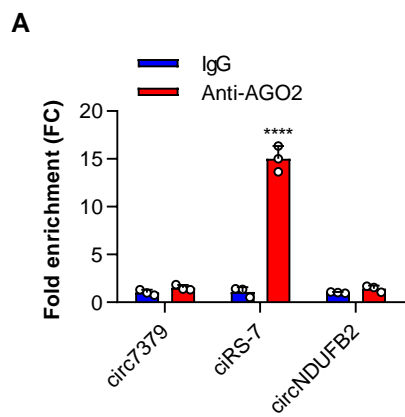


E









C

#	Protein ID	RNA ID	Z-score?	Discriminative Power (%)?	Interaction Strength (%)?	Domain?	Motif?	Ranking?
2	FUBP2_HUMAN	hsa_circ_000737_1_907-1069	0.61	92	98	yes	yes	
9	LN28B_HUMAN	hsa_circ_000737_1_882-1079	0.41	85	100	yes	yes	
7	FUBP2_HUMAN	hsa_circ_000737_1_902-1072	0.33	81	92	yes	yes	
1	FUBP2_HUMAN	hsa_circ_000737_1_7-142	0.10	67	92	yes	yes	
45	ELAV1_HUMAN	hsa_circ_000737_1_882-1079	0.04	63	89	yes	yes	
46	PCBP1_HUMAN	hsa_circ_000737_1_882-1079	0.03	63	92	yes	yes	
55	HNRPD_HUMAN	hsa_circ_000737_1_882-1079	-0.02	59	89	yes	yes	
27	SFPQ_HUMAN	hsa_circ_000737_1_7-142	-0.18	45	79	yes	yes	
3	LN28B_HUMAN	hsa_circ_000737_1_3035-3200	-0.46	22	75	yes	yes	
53	SFPQ_HUMAN	hsa_circ_000737_1_3091-3195	-0.48	22	65	yes	yes	

D

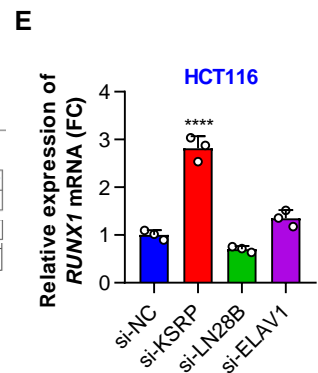
RNA-protein Interaction Prediction (RPISeq)

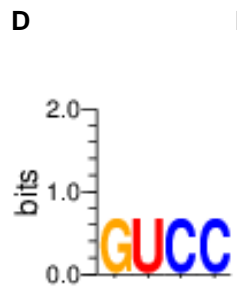
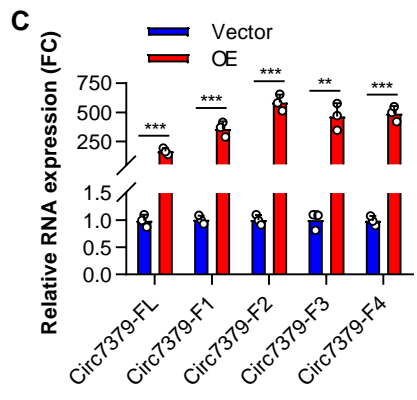
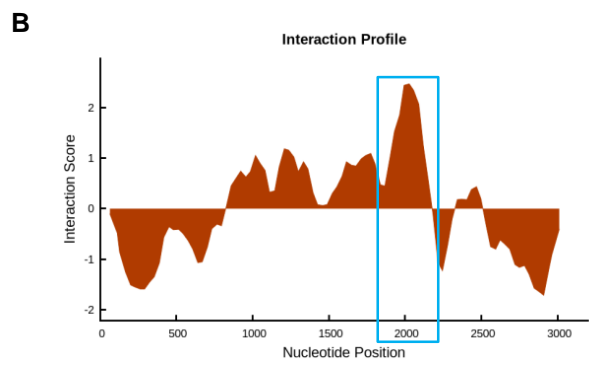
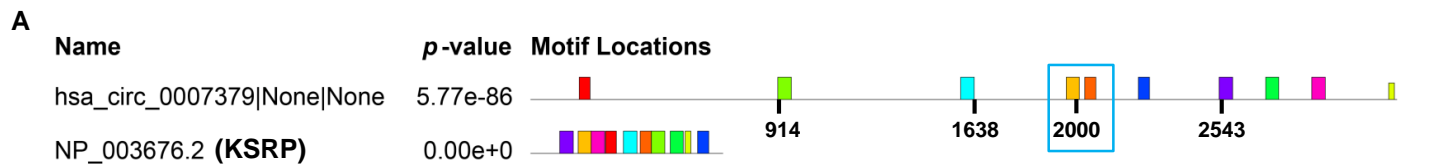
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Results

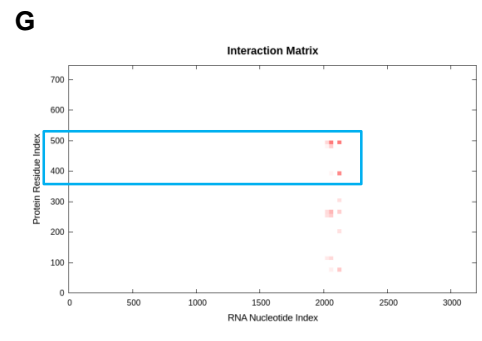
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>NP_001004317.1 protein lin-28 homolog B [Homo sap...]	0.75	0.791
>NP_001410.2 ELAV-like protein 1 [Homo sapiens]	0.75	0.839





E

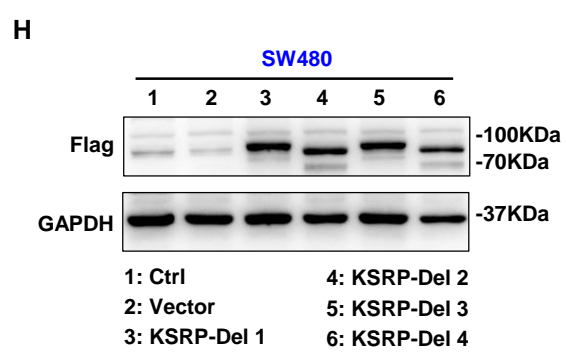
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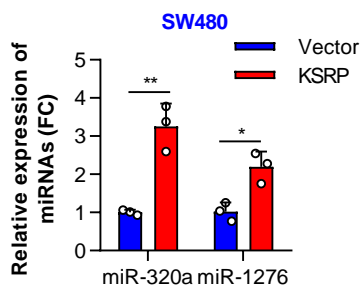
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Domains and Repeats

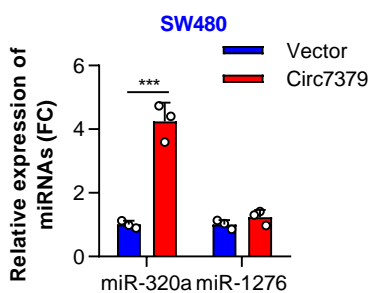
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Domain ¹	424 - 491	KH 4	PROSITE-ProRule annotation	Add BLAST	68
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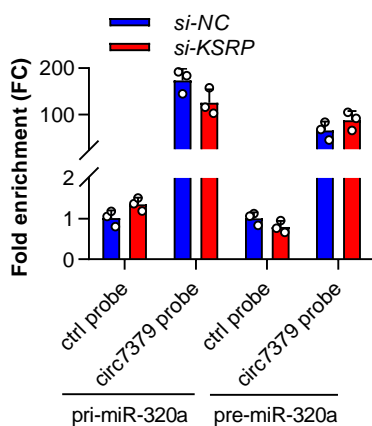
A



B



D



C

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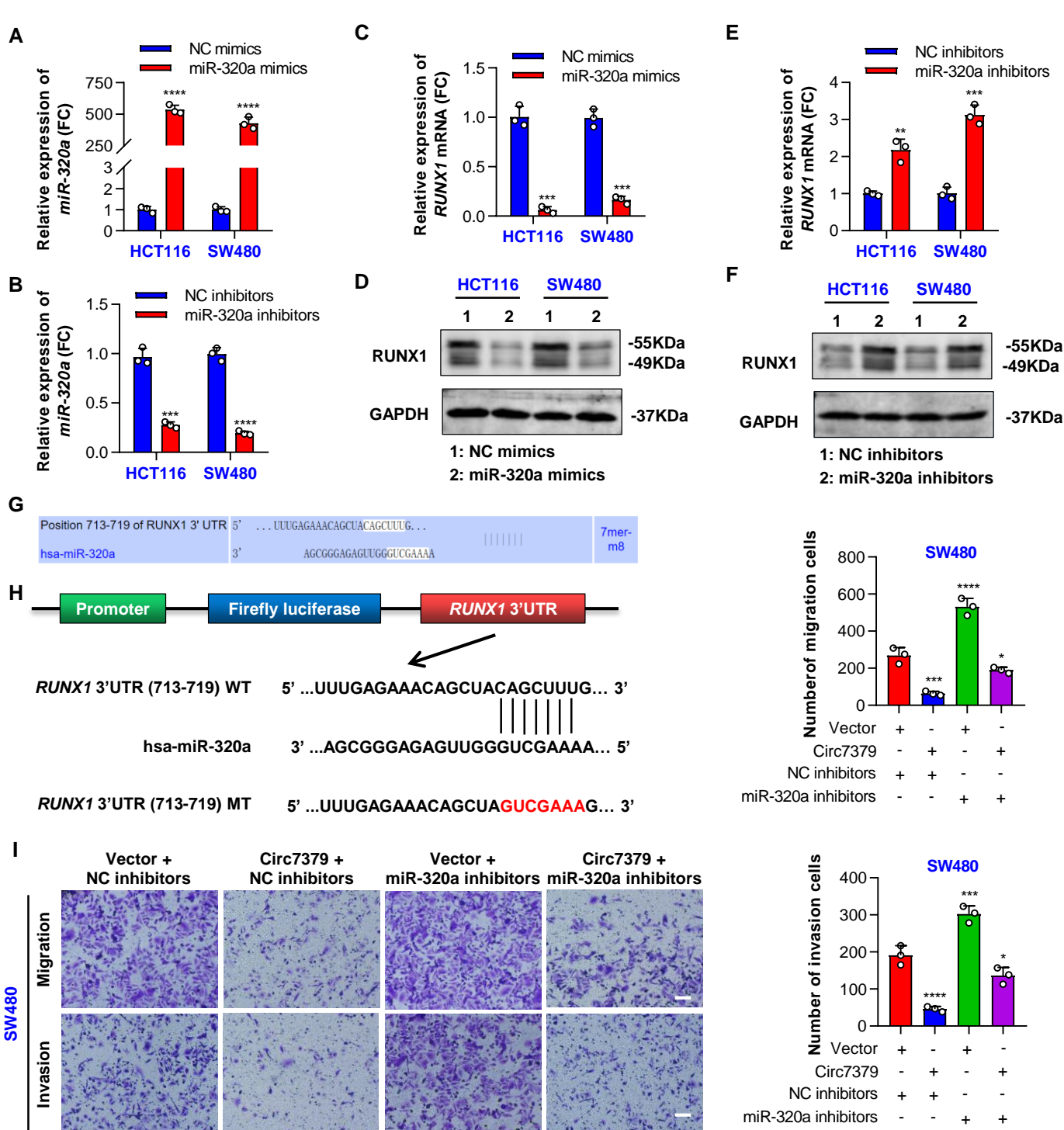
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<i>pri-miR-320a</i> Sbjct	150	CTGGGTGGTCCCGGGAGTCTCGCCACGTGG	121

Range 11: 128 to 137 [Graphics](#)

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<i>pre-miR-320a</i> Sbjct	71	ATCCTTTT	64



Supplementary Tables

Table S1 Primers used in this article

Gene names	Forward primers (5'→3')	Reverse primers (5'→3')
<i>hsa_circ_007379</i> divergent primer	AGGCATACAAGTACCAACTAGG	CCCAGGAGCTCGAGCAAA
<i>hsa_circ_007379</i> convergent primer	GGAGGATCCACTAGTCCACAC	GTGCTTACCCCCAACTTGCC
<i>hsa_circ_103908</i>	GCGACTGCTACAGGGACCAT	TGTGACATCACAGACCCATTCTT
<i>hsa_circ_406549</i>	GCACCAGACCTAGTCTTTAATGA CA	TGTTGACCGAGGGTTCTTTTG
<i>hsa_circ_405468</i>	CGTCCCTTGTTTCAGGTATCCA	AAACTCTTTGGGAAGGAGCAAC
<i>hsa_circ_071127</i>	AATGTATCAAGCGATGGAGACC	GCCTGAGAACTTGACCCCA
<i>hsa_circ_100686</i>	ACGGTACTGTGACCTGACTGG	TTGGATAGCCTTCAATGAGCC
<i>hsa_circ_001736</i>	TGCCTCCTGATGCACTTATCA	TGTAGTAGCACTGCCCTCTCTTT
<i>hsa_circ_405619</i>	ATGAATGAAACATACCCACCCA	GCAGGTCTCAGGCTTCAGTTTG
<i>hsa_circ_002534</i>	AAACCATTAGGAACCTGGACTGT	TCACAGCCACATCTTCAAAGG
<i>hsa_circ_007081</i>	ATGAAACATACCCACCCATCTG	TGGACCACAAAACAGCAAAGT
<i>hsa_circ_0101697</i>	CCGACAGTTCGGTTTATAGCC	CTCGAGCAAATGGTATTAAGTGC
<i>GAPDH</i>	TGCACCACCAACTGCTTAGC	GGCATGGACTGTGGTCATGAG
<i>circLPAR1</i>	TGTTACACCACCTACAACCAC	GAGAAGCTGTGTACCTGATGC
<i>circPLCE1</i>	AGCCCCACTCTACACCAACC	TTCATGCCGCCTTTGATCCG
<i>circ_0002138</i>	AGACACTCTGTGCTTTATGGC	CCATTACATACCTTCCACA
<i>circTADA2A</i>	TGTGCACCAAGACCAAGGAG	AGGAAAATCTGAAGTAGTGA
<i>DHX9</i>	GCCAATTTCTGGCCAAAGCA	CGAGGCTCAATGGGGAGTTT
<i>ADAR</i>	CGAGAATCCCAAACAAGGAA	CTGGATTCCACAGGGATTGT
<i>RUNX1</i>	TGAGCTGAGAAATGCTACCGC	ACTTCGACCGACAAACCTGAG
<i>KSRP</i>	CCGCTTACTACGGACAGACCC	CCCCAAACAGAACAAAATGGA
<i>hsa-miR-320a</i>	AGGGCTAAAAGCTGGGTTGA	CAGTGCGTGTCTGTGGAGT
<i>hsa-miR-1276</i>	TAAAGAGCCCTGTGGAGACAG	CTCAACTGGTGTCTGTGGAGC
<i>U6</i>	CTCGCTTCGGCAGCACATATACT	ACGCTTACGAATTTGCGTGTG

Table S2 siRNAs used in this article

siRNAs	Target sequences
<i>si-circ7379#1</i>	GAGUGCAGAUGAUGAGAAA-dTdT
<i>si-circ7379#2</i>	GCAGAUGAUGAGAAAUCAC-dTdT
<i>si-circ7379#3</i>	CCAGAGUGCAGAUGAUGAGAAAUCA-dTdT
<i>si-DHX9</i>	GAGCCAACUUGAAGGAUUA-dTdT
<i>si-ADAR</i>	CGCAGAGUCCUCACCUGU-dTdT
<i>si-RUNX1</i>	CCUCGAAGACAUCGGCAGAAA-dTdT
<i>si-KSRP</i>	GAUCAACCGGAGAGCAAGA-dTdT

Table S3 The top 10 downregulated circRNAs in our circRNA microarray

circRNA names	Type	Gene symbol	Position	Spliced length (nt)	FC (abs)	P value
<i>hsa_circRNA_103908</i>	exonic	<i>EDIL3</i>	chr5	486	6.94	0.0173
<i>hsa_circRNA_007379</i>	intergenic	/	chr14	3199	5.39	0.0250
<i>hsa_circRNA_406549</i>	exonic	<i>NR3C2</i>	chr4	496	4.54	0.0070
<i>hsa_circRNA_405468</i>	intronic	<i>MT2A</i>	chr16	303	4.22	0.0037
<i>hsa_circRNA_071127</i>	exonic	<i>NR3C2</i>	chr4	1759	4.10	0.0456
<i>hsa_circRNA_100686</i>	exonic	<i>ATRNL1</i>	chr10	536	3.61	0.0457
<i>hsa_circRNA_001736</i>	exonic	<i>KMT2E</i>	chr7	633	3.41	0.0409
<i>hsa_circRNA_405619</i>	intronic	<i>PRKCA</i>	chr17	1131	3.39	0.0390
<i>hsa_circRNA_002534</i>	exonic	<i>ZNF823</i>	chr19	188	3.29	0.0190
<i>hsa_circRNA_007081</i>	sense	<i>PRKCA</i>	chr17	18266	3.25	0.0138
overlapping						

Table S4 Correlation between *circ7379* expression level and clinicopathological features of colorectal cancer patients.

Clinicopathological features	Total (n=55)	<i>Circ7379</i> expression ^a		χ^2	P value ^b
		Low (n=28)	High (n=27)		
Gender					
Male	33	19	14	1.211	0.226
Female	22	9	13		
Age (years)					
≤60	31	15	16	0.425	0.671
>60	24	13	11		
Tumor location					
Colon	34	20	14	1.494	0.135
Rectum	21	8	13		
Tumor size (cm)					
≤5	41	17	24	2.088	0.037
>5	14	11	3		
Differentiation					
Well-moderate	41	18	23	1.469	0.142
Poor	14	10	4		
Invasion depth					
T1-2	7	0	7	/	0.004
T3-4	48	28	20		
Lymph metastasis					
N0	26	10	16	1.748	0.080
N1-2	29	18	11		
Distant metastasis					
M0	50	24	26	0.896	0.371
M1	5	4	1		
TNM stage ^c					
I-II	25	9	16	2.019	0.044
III-IV	30	19	11		

^a Using median expression level of *hsa_circ_0007379* as cutoff.

^b Two-sided Chi-squared test or Chi-square with Yates' correction or Fisher's exact test.

^c TNM stage system according to AJCC 8th classification.

Table S5 Prediction of miRNA-binding sites in circ7379 using TargetSan algorithms.

miRNA ID	Number of binding sites	Site type	context+ score	context+ score percentile
<i>hsa-miR-1273</i>	3	7mer-m8	-0.171, -0.154, -0.032	73, 68, 2
<i>hsa-miR-149</i>	3	7mer-m8	-0.132, -0.006, 0.034	79, 34, 12
<i>hsa-miR-548c-3p</i>	3	7mer-1a	0.158, 0.185, 0.257	79, 71, 14
<i>hsa-miR-658</i>	3	7mer-m8	-0.110, -0.096, -0.076	55, 40, 22
<i>hsa-miR-661</i>	4	7mer-m8	-0.222, -0.065, -0.046, 0.029	96, 69, 65, 8
<i>hsa-miR-665</i>	3	7mer-m8	-0.223, -0.173, 0.014	95, 92, 36
<i>hsa-miR-1827</i>	4	8mer-1a	-0.238, -0.131, -0.029, 0.022	96, 90, 73, 47
<i>hsa-miR-940</i>	4	7mer-m8	-0.133, -0.117, -0.027, 0.001	91, 89, 72, 55

Table S6 Assessment of coding potential in circRNAs using circBank online database.

circRNA ID	Host gene symbol	ORF_size	Coding probability	PMID
<i>hsa_circ_0007379</i>	/	291	0.0280	/
<i>hsa_circ_0006156</i>	<i>FNDC3B</i>	657	0.9744	32241279
<i>hsa_circ_0006401</i>	<i>COL6A3</i>	597	0.9780	33947841
<i>hsa_circ_0000943</i>	<i>ARHGAP35</i>	3870	1.0000	34258149
<i>hsa_circ_0000615</i>	<i>ZNF609</i>	753	0.9968	28344082
<i>hsa_circ_0001451</i>	<i>FBXW7</i>	582	0.9803	28903484
<i>hsa_circ_0001649</i>	<i>SHPRH</i>	441	0.6435	29343848