

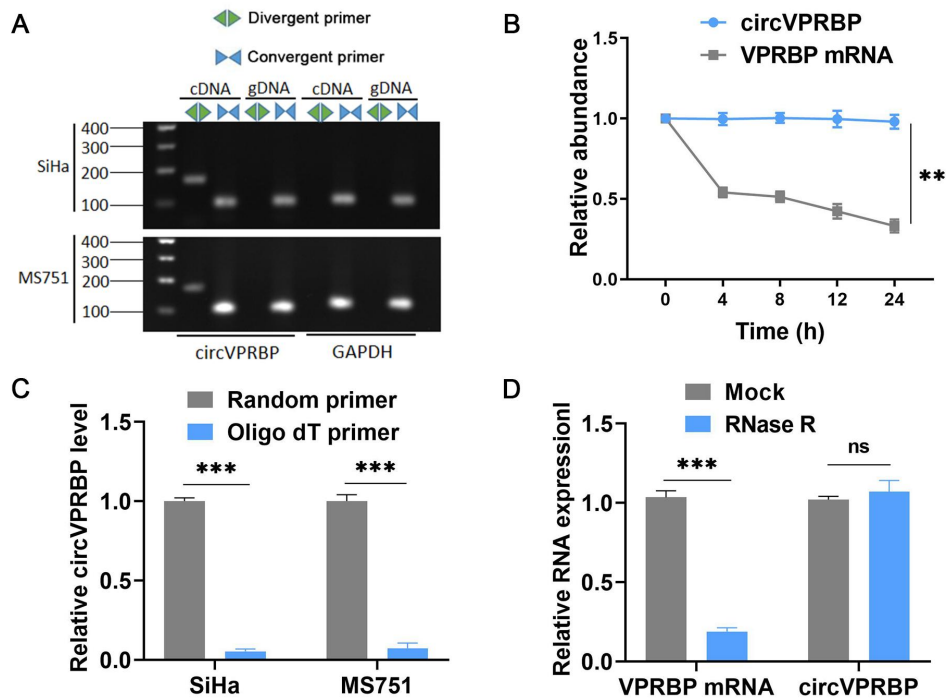


Figure S1. Characterization of circVPRBP in cervical cancer cells

A PCR analysis for circVPRBP and its linear counterpart in cDNA and genomic DNA in SiHa and MS751 cell lines. **B** RT-qPCR analysis for the abundance of circVPRBP and VPRBP mRNA treated with Actinomycin D at the indicated points. **C** Random and oligo dT primers were used to analyze the abundance of circVPRBP in reverse transcription experiments. **D** RT-qPCR analysis for the expression levels of circVPRBP and VPRBP mRNA treated with RNase R. Each experiment was performed at least three times independently. ns, no significant; ** $P < 0.01$; *** $P < 0.001$.

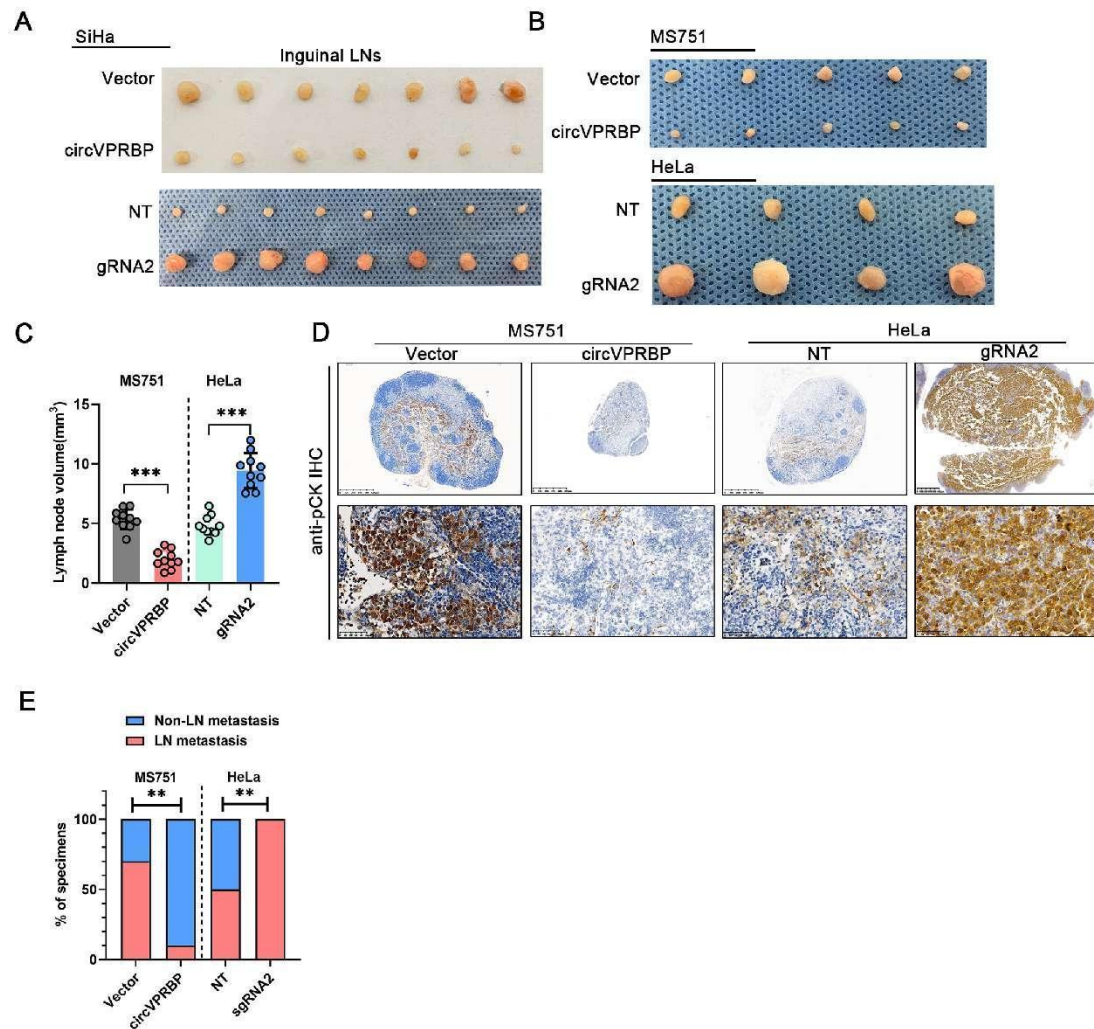


Figure S2. circVPRBP inhibits CCa LN metastasis in vivo

A and **B** Representative images of metastatic LNs in indicated groups. **C** Quantification of the lymph node volume in different groups. **D** Representative images of immunostaining of pan-cytokeratin of popliteal LNs. **E** Quantification of the rate of LN metastasis in the indicated groups. **P < 0.01; ***P < 0.001.

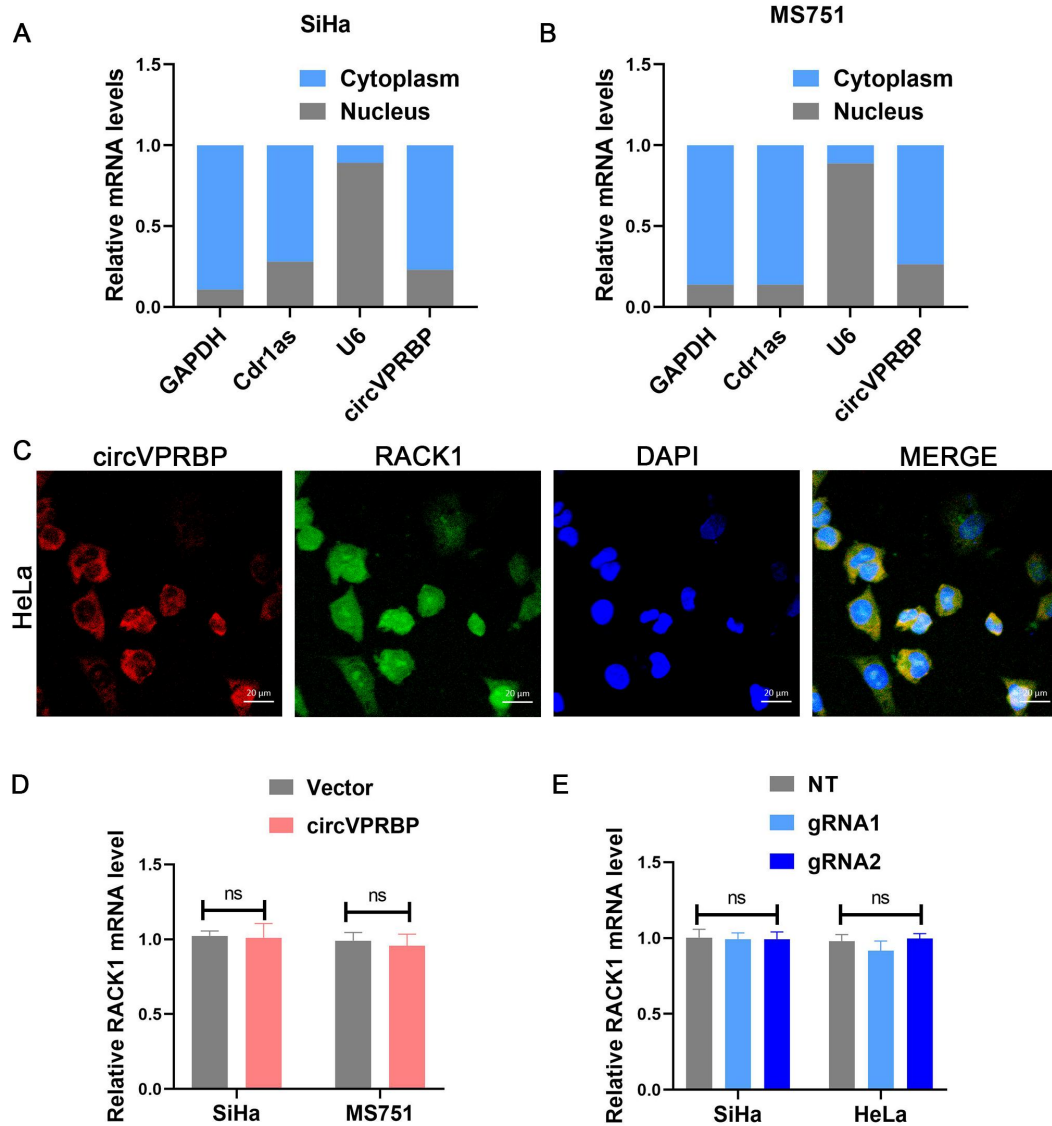


Figure S3. **A, B** Cytoplasmic and nuclear mRNA fractionation experiments in SiHa and MS751 cells showing that circVPRBP was localized mainly in the cytoplasm. GAPDH, Cdr1as, and U6 were applied as positive controls in the cytoplasm and nucleus, respectively. **C** Fluorescence colocalization assay showed that circVPRBP and RACK1 protein colocalize in the cytoplasm of HeLa cells. **D, E** mRNA levels of RACK1 in indicated CCa cells. ns, no significant.

Table S1. Correlation between circVPRBP expression with clinicopathologic characteristics of patients in cervical cancer

Characteristics	Total 94	circVPRBP expression		<i>P</i> -value
		Low	High	
Age (years)				0.104
< 42	40	20	20	
≥ 42	54	36	18	
FIGO stage				0.583
I (Ia2+Ib1+Ib2)	72	44	28	
II (IIa1+IIa2)	22	12	10	
Tumor size (cm)				0.023
≤ 4	73	39	34	
> 4	21	17	4	
Pathologic types				0.291
Squamous cell carcinoma	73	45	28	
Adenocarcinoma	16	7	9	
Adenosquamous carcinoma	5	4	1	
Differentiation				0.357
Well	6	3	3	
Moderate	40	21	19	
Poor	48	32	16	
Stromal invasion				0.270
< 1/2	58	32	26	
≥ 1/2	36	24	12	
LVSI				0.020
Positive	15	13	2	
Negative	79	43	36	
LNM				0.014
Positive	19	16	3	
Negative	75	40	35	
Vaginal invasion				0.270
Positive	3	3	0	
Negative	91	53	38	
Parametrial invasion				0.513
Positive	2	2	0	
Negative	92	54	38	

FIGO: the International Federation of Gynecology and Obstetrics; LVSI, lymphovascular space invasion; LNM, lymph node metastasis.

Table S2. Univariate Cox's proportional hazards model analysis of disease-free survival and overall survival

Variables	Total 94	OS	DFS
		<i>P</i> -value	<i>P</i> -value
Age (years)		0.186	0.255
< 42	40		
≥ 42	54		
FIGO stage		0.917	0.882
I (Ia2+Ib1+Ib2)	72		
II (IIa1+IIa2)	22		
Tumor size (cm)		0.003	0.005
≤ 4	73		
> 4	21		
Pathologic types		0.781	0.640
Squamous cell carcinoma	73		
Adenocarcinoma	16		
Adenosquamous carcinoma	5		
Differentiation		0.657	0.351
Well	6		
Moderate	40		
Poor	48		
Stromal invasion		0.034	0.008
< 1/2	58		
≥ 1/2	36		
LVSI		0.021	0.017
Positive	15		
Negative	79		
LNM		0.004	0.002
Positive	19		
Negative	75		
Vaginal invasion		0.418	0.524
Positive	3		
Negative	91		
Parametrial invasion		0.511	0.462
Positive	2		
Negative	92		
circVPRBP		0.014	0.010
Low	56		
High	38		

FIGO: the International Federation of Gynecology and Obstetrics; LVSI, lymphovascular space invasion; LNM, lymph node metastasis.

Table S3. Multivariate Cox's proportional hazards model analysis of disease-free survival and overall survival in cervical cancer patients

Variables	Overall survival		Disease-free survival	
	HR (95% CI)	<i>P</i>	HR (95% CI)	<i>P</i>
Tumor size (cm)		0.002		0.002
≤ 4 (reference)	1		1	
> 4	4.888 (1.798-13.293)		4.345 (1.752-10.777)	
LVSI		0.015		0.004
Negative (reference)	1		1	
Positive	3.456 (1.271-9.398)		3.927 (1.559-9.890)	
LNM		0.011		0.010
Negative (reference)	1		1	
Positive	3.510 (1.327-9.285)		3.101 (1.305-7.369)	
circVPRBP		0.027		0.024
Low (reference)	1		1	
High	0.181 (0.040-0.825)		0.238 (0.068-0.829)	

HR, hazard ratio; 95% CI, 95% confidence interval; LNM, lymph node metastasis; LVSI, lymphovascular space invasion; For the stepwise multivariate analysis, forward LR method was used to select significant variables. Variables entered for multivariate analysis were the following: tumor size, Stromal invasion, LNM, LVSI, circVPRBP.

Table S4. Antibodies used in the study

Target protein	Antibody	Application in analysis
AKT	CST, #4691S	WB (1:1000)
p-AKT	CST, #4060S	WB (1:1000)
FAK	Proteintech, #12636-1-AP	WB (1:1000)
pFAK	CST, #3283S	WB (1:1000)
Galectin-1	Abcam, #ab108389	WB (1:10000)
RL2	Abcam, #ab2739	WB (1:1000)
OGT	Abcam, #ab177941	WB (1:1000)
GST	Proteintech, #66001-2-Ig	WB (1:6000)
HA	Abcam, #ab18181	WB (1:1000)
Flag	Sigma-Aldrich, #F3165	WB(1:5000); IP (1:2000)
GAPDH	Proteintech, #10494-1-AP	WB (1:10000)
HRP-conjugated Affinipure Goat Anti-Rabbit IgG (H+L)	Proteintech, #SA00001-2	WB (1:10000)
HRP-conjugated Affinipure Goat Anti-Mouse IgG (H+L)	Proteintech, #SA00001-1	WB (1:10000)
Pan-cytokeratin	CST, #4545S	IHC (1:400)
LYVE-1	Abcam, #ab14917	IHC (1:200)
RACK1	SantaCruz, #sc-17754	WB(1:500); IP (2 µg per 200 µg of total protein)
RACK1	Proteintech, #27592-1-AP	IHC and IF (1:800)

Table S5. Sequences of primers, gRNAs and probes used in the study

Primer for PCR, RT-qPCR and RIP	
CircVPRBP (divergent)	F 5'-GGAGACATTGCCCACATTCC-3'
	R 5'-CTCCTCCTGTCCACTAAACAC-3'
CircVPRBP (convergent)	F 5'-GCGGATGCTTTGATAGGCACCT-3'
	R 5'-ACAGCAGGTGAAGCCACTCTCA-3'
RACK1	F 5'-ACCATCATCATGTGGAACTGAC-3'
	R 5'-GTGCCCCGTTGTGAGATCCC-3'
Galectin-1	F 5'-TCGCCAGCAACCTGAATCTC-3'
	R 5'-GCACGAAGCTCTTAGCGTCA-3'
GAPDH	F 5'-AGAAGGCTGGGGCTCATTG-3'
	R 5'-AGGGGCCATCCACAGTCTTC-3'
CDR1as	F 5'-ACGTCTCCAGTGTGCTGA-3'
	R 5'-CTTGACACAGGTGCCATC-3'
GAPDH (divergent)	F 5'-TGTACCATCAATAAAGTACCCTGTG-3'
	R 5'-AAATCCGTTGACTCCGACCT-3'
U6	F 5'-ACAGATCTGTCCGGTGTGGCAC-3'
	R 5'-GGCCCCGGATTATCCGACATTC-3'
gRNAs for circVPRBP and ISH probe	
NT	5'-GCAGGGTTTCCCAGTCACGACGTTGTAAA-3
gRNA1	5'-AAATAGGACGGAATGTGGGCAAT-3
gRNA2	5'-GAAATAGGACGGAATGTGGGCAA-3
gRNA3	5'-AATAGGACGGAATGTGGGCAATG-3
Digoxigenin-labeled circVPRBP probe for ISH	5'-AAATAGGACGGAATGTGGGCAATGTCTCCTTTTG-3'
Cy3-labeled circVPRBP probe for FISH	5'-ATAGGACGGAATGTGGGCAATGT- 3'
Primers for <i>in vitro</i> transcription	
circVPRBP(1-367)	F 5'-TAATACGACTCACTATAGGGATTCCGTCCTATTTCAAGTG-3'
	R 5'-GTGGGCAATGTCTCCTTTTGTGC-3'
circVPRBP(1-122)	F 5'-TAATACGACTCACTATAGGGATTCCGTCCTATTTCAAGTG-3'
	R 5'-AGAGCTTCAGCTGCCCTGTGC-3'
circVPRBP(122-182)	F 5'-TAATACGACTCACTATAGGGTATAATGTGTTTAGTGGACAGG-3'
	R 5'-GTGTGATGGCTGAGTTGTG-3'
circVPRBP(1-182)	F 5'-TAATACGACTCACTATAGGGATTCCGTCCTATTTCAAGTG-3'
	R 5'-GTGTGATGGCTGAGTTGTG-3'
circVPRBP(182-367)	F 5'-TAATACGACTCACTATAGGGCATCTTGAACCTTCCAGGG-3'
	R 5'-GTGGGCAATGTCTCCTTTTGTGC-3'
circVPRBP(antisense)	F 5'-TAATACGACTCACTATAGGGGTGGGCAATGTCTCCTTTTGTGC-3'
	R 5'-ATTCCGTCCTATTTCAAGTGTCC-3'

Table S6. circVPRBP-interacting proteins identified by mass spectrometry analysis.

number	protein	mass
1	sp Q96E11 RRFM_HUMAN	29373
2	sp P61604 CH10_HUMAN	10925
3	sp P06702 S10A9_HUMAN	13291
4	sp Q9UI30 TR112_HUMAN	14304
5	sp P62318 SMD3_HUMAN	14021
6	sp Q04837 SSBP_HUMAN	17249
7	sp Q8NAV1 PR38A_HUMAN	37681
8	sp P41208 CETN2_HUMAN	19726
9	sp Q14320 FA50A_HUMAN	40216
10	sp P49411 EFTU_HUMAN	49852
11	sp P63244 RACK1_HUMAN	35511
12	sp P82979 SARNP_HUMAN	23713
13	sp O95843 GUC1C_HUMAN	23864
14	sp P06576 ATPB_HUMAN	56525
15	sp Q15056 IF4H_HUMAN	27425
16	sp P13984 T2FB_HUMAN	28420
17	sp Q13112 CAF1B_HUMAN	61910
18	sp Q8WXX5 DNJC9_HUMAN	30062
19	sp P35232 PHB_HUMAN	29843
20	sp Q7Z7K6 CENPV_HUMAN	30383
21	sp P52907 CAZA1_HUMAN	33073
22	sp Q8IVM0 CCD50_HUMAN	35914
23	sp O95400 CD2B2_HUMAN	37737
24	sp Q9H6T3 RPAP3_HUMAN	75957
25	sp P12429 ANXA3_HUMAN	36524
26	sp Q9UBP9 GULP1_HUMAN	34925
27	sp Q96EI5 TCAL4_HUMAN	24746
28	sp Q8IZL9 CDK20_HUMAN	38898
29	sp Q9NVD7 PARVA_HUMAN	42274
30	sp P12277 KCRB_HUMAN	42902
31	sp Q6IN84 MRM1_HUMAN	39070
32	sp Q99706 KI2L4_HUMAN	41974
33	sp Q9BXN1 ASPN_HUMAN	43788
34	sp Q15084 PDIA6_HUMAN	48490
35	sp Q7L4I2 RSRC2_HUMAN	50586
36	sp Q2TAY7 SMU1_HUMAN	58134
37	sp Q02413 DSG1_HUMAN	114702
38	sp P12268 IMDH2_HUMAN	56226
39	sp Q9UBS0 KS6B2_HUMAN	53821
40	sp Q16630 CPSF6_HUMAN	59344
41	sp Q8N884 CGAS_HUMAN	59462
42	sp P36915 GNL1_HUMAN	69132
43	sp Q07065 CKAP4_HUMAN	66097
44	sp P49368 TCPG_HUMAN	61066
45	sp P04040 CATA_HUMAN	59947
46	sp Q9H6Z4 RANB3_HUMAN	60515
47	sp O15460 P4HA2_HUMAN	61263
48	sp O60684 IMA7_HUMAN	60733
49	sp Q96KC8 DNJC1_HUMAN	64185
50	sp P61764 STXB1_HUMAN	67925
51	sp P19338 NUCL_HUMAN	76625
52	sp Q8IXB1 DJC10_HUMAN	92333
53	sp Q08J23 NSUN2_HUMAN	87214
54	sp P14923 PLAK_HUMAN	82434
55	sp Q3YEC7 RABL6_HUMAN	79785
56	sp Q9Y4R7 TTLL3_HUMAN	88612
57	sp Q08554 DSC1_HUMAN	101406
58	sp Q9H6N6 MYH16_HUMAN	128439
59	sp Q14566 MCM6_HUMAN	93801
60	sp O75150 BRE1B_HUMAN	114350
61	sp Q5BJF6 ODFP2_HUMAN	96140
62	sp Q9UKJ3 GPTC8_HUMAN	165010
63	sp Q96HA7 TONSL_HUMAN	153001
64	sp Q9BX26 SYCP2_HUMAN	177239
65	sp P51805 PLXA3_HUMAN	211049
66	sp P35579 MYH9_HUMAN	227646
67	sp Q07283 TRHY_HUMAN	254233
68	sp Q5H9R4 ARMX4_HUMAN	237416