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Supplemental Information

circCAMSAP1 Promotes Tumor Growth in Colorectal Cancer via the miR-328-5p/E2F1 Axis

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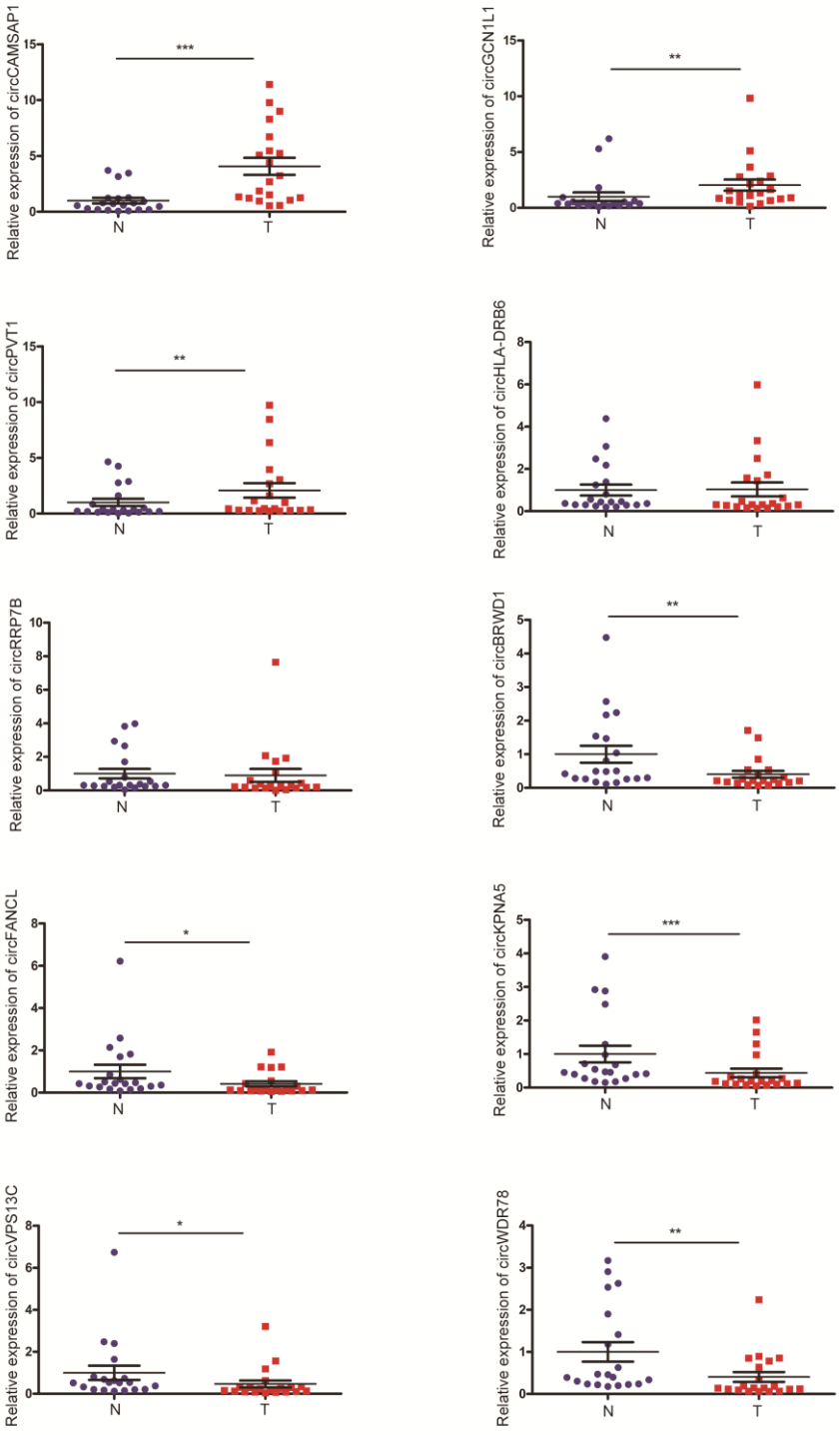
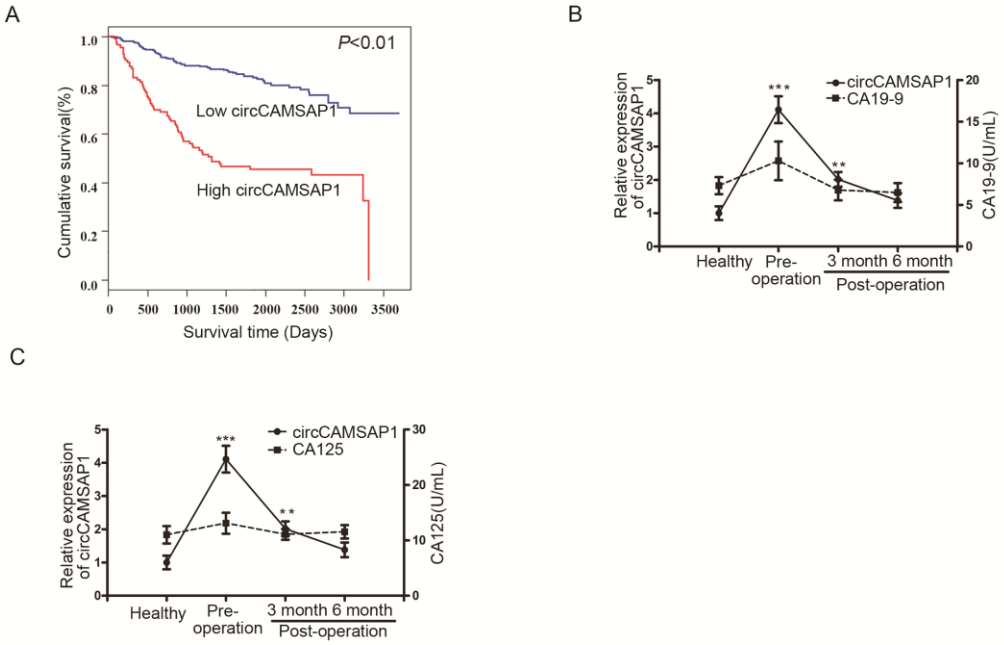
Supplemental Figures and Legends



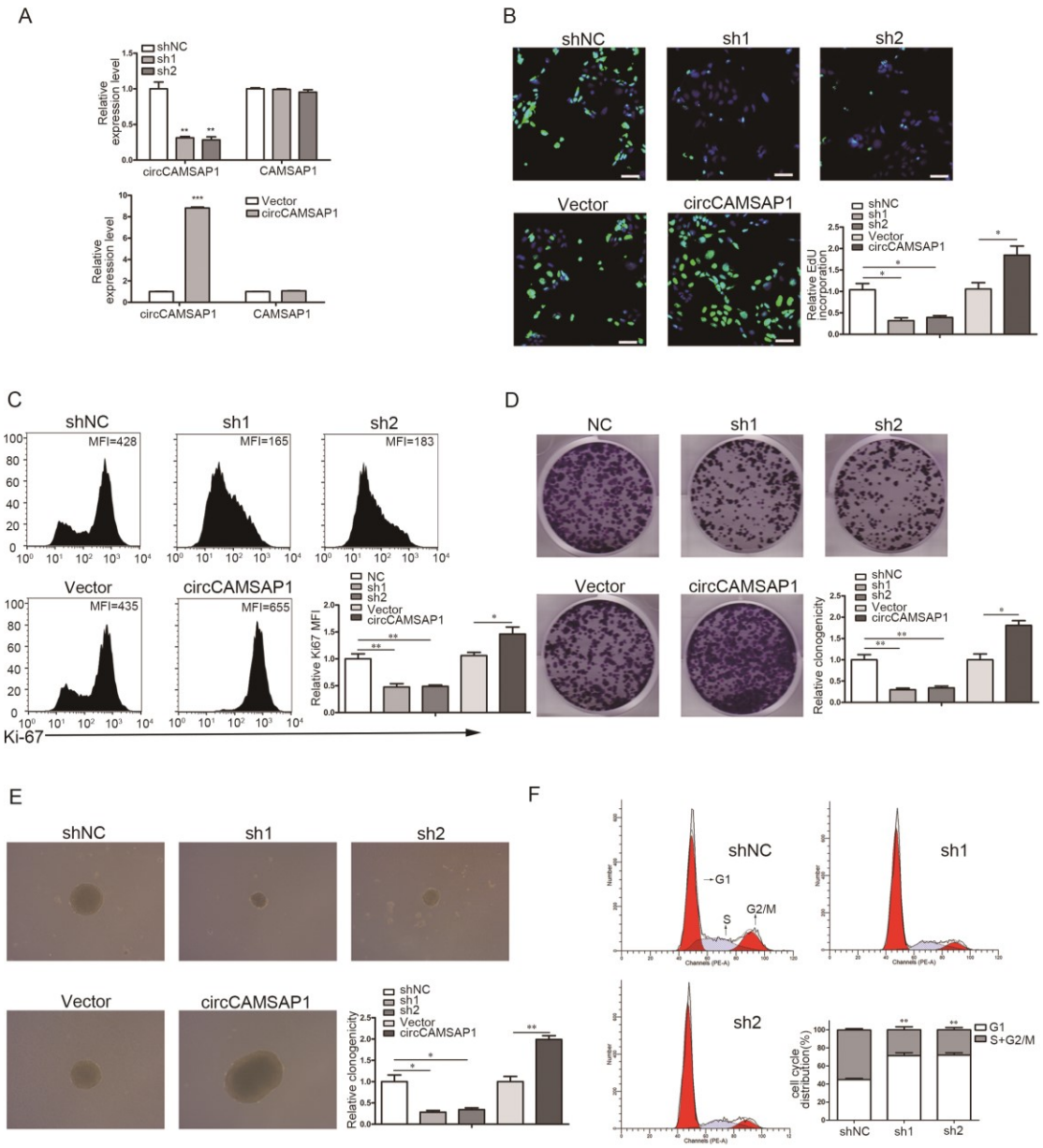
Supplemental Figure S1, related to Figure 1, CircRNA expression in CRC

Expression of candidate circRNAs in 20 pairs of CRC tissues compared with the matched non-tumor tissues. N, non-tumor tissues; T, tumor tissues. ***p<0.01, *** p<0.001 by Student's t-test. Error bars indicate S.E.M.*



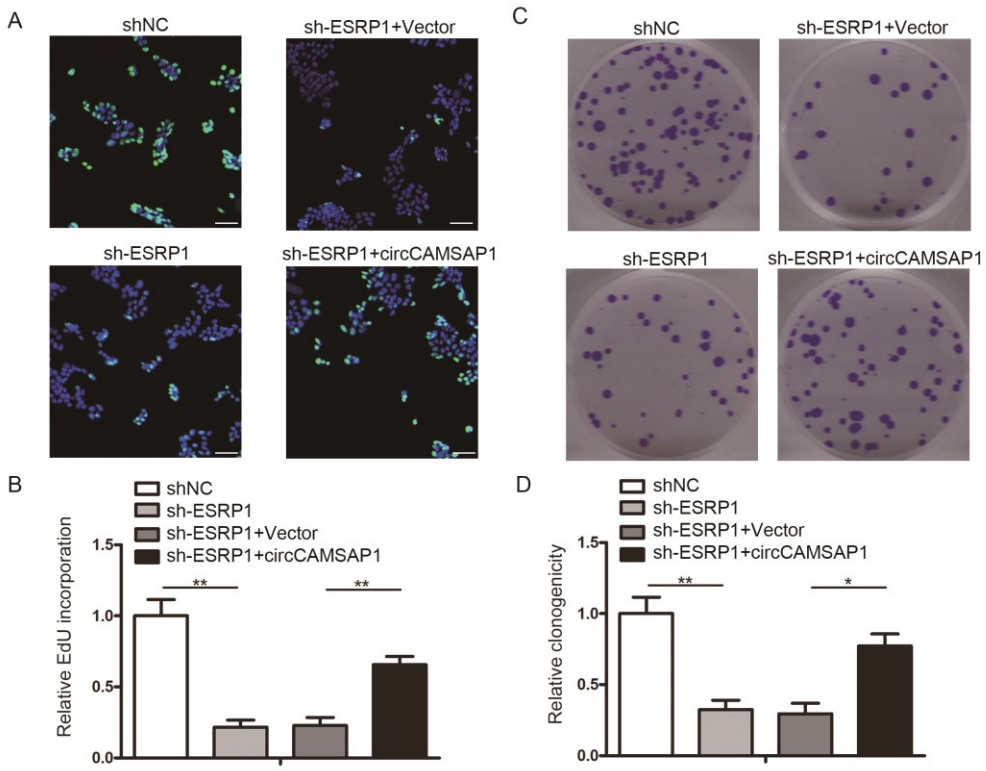
Supplemental Figure S2, related to Figure 2, The expression of circCAMSAP1 in CRC tissue and serum.

A. Kaplan-Meier analysis of the correlation between circCAMSAP1 expression and disease-free survival. Kaplan-Meier survival curves for CRC patients with high (n=113) and low (n=311) expression of circCAMSAP1, determined by ISH. The optimal survival cut point was determined by X-Tile statistical software. B and C. The solid line showed the expression of circCAMSAP1 in the serum of healthy people (n=20) and CRC patients (n=20) pre- and post-operation, validated by RT-ddPCR. The dashed line showed the levels of CA19-9 (B) and CA125 (C) in the serum of healthy people (n=20) and CRC patients pre- and post-operation (n=20).



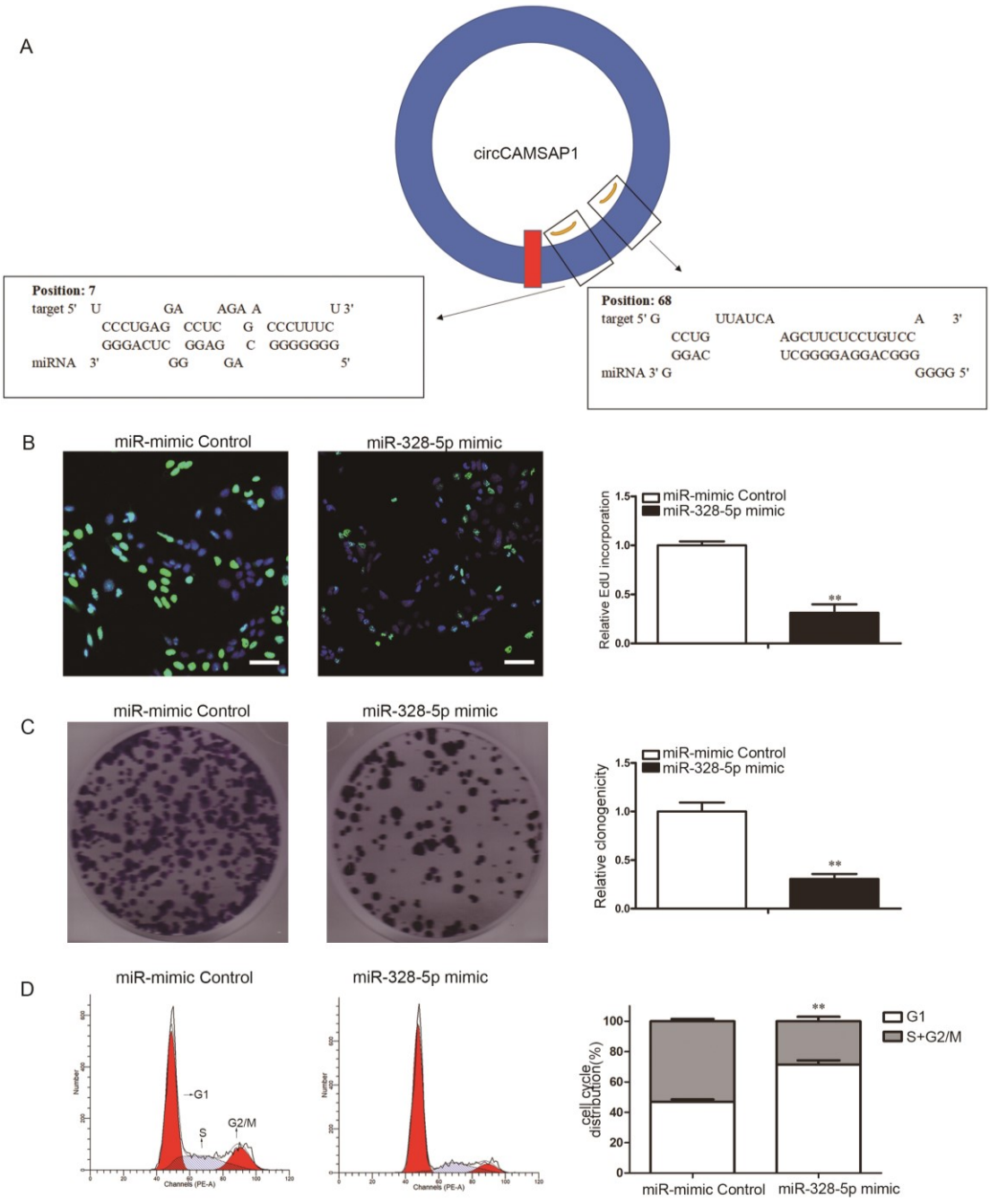
Supplemental Figure S3, related to Figure 3, CircCAMSAP1 promotes the proliferation of CRC cells.

A. qRT-PCR analysis of circCAMSAP1 and CAMSAP1 RNA expression of circCAMSAP1 knockdown or overexpression stable cell lines. B-E. The proliferation of DLD1 cells with circCAMSAP1 knockdown or overexpression shown by the EdU (B), FACS analysis of Ki-67 staining (C), plate colony formation (D) and soft agar colony formation (E) assays. Scale bar, 50 um. F. FACS cell cycle analysis of DLD1 cells with circCAMSAP1 knockdown, compared to shNC-treated DLD1 cells, using PI DNA staining. **p<0.05, **p<0.01, *** p<0.001 by Student's t-test. Error bars indicate S.D.*



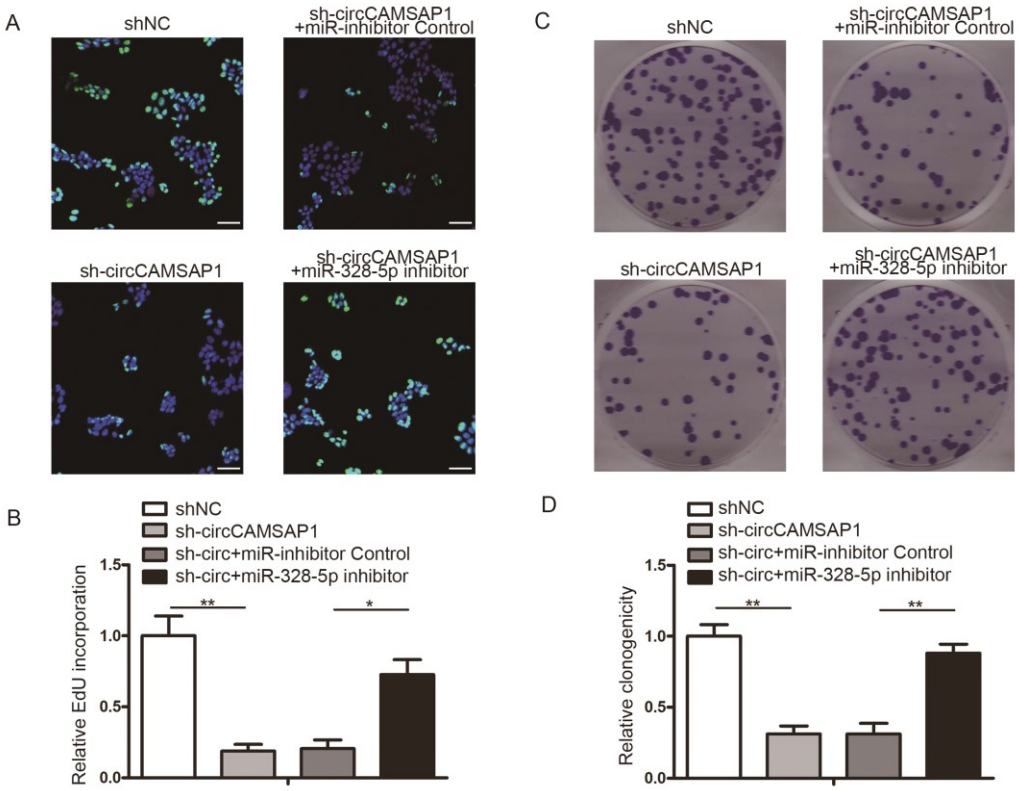
Supplemental Figure S4. Related to Figure 4, Overexpression of circCAMSAP1 rescues the proliferation-inhibitory effect of ESRP1 knockdown in DLD1 cells.

The proliferation of ESRP1-silenced DLD1 cells transfected with or without circCAMSAP1 overexpression vector were assessed by EdU (A-B) and plate colony formation (C-D) assays. Scale bar, 50 um. **p<0.05, ** p<0.01 by Student's t-test. Error bars indicate S.D.*



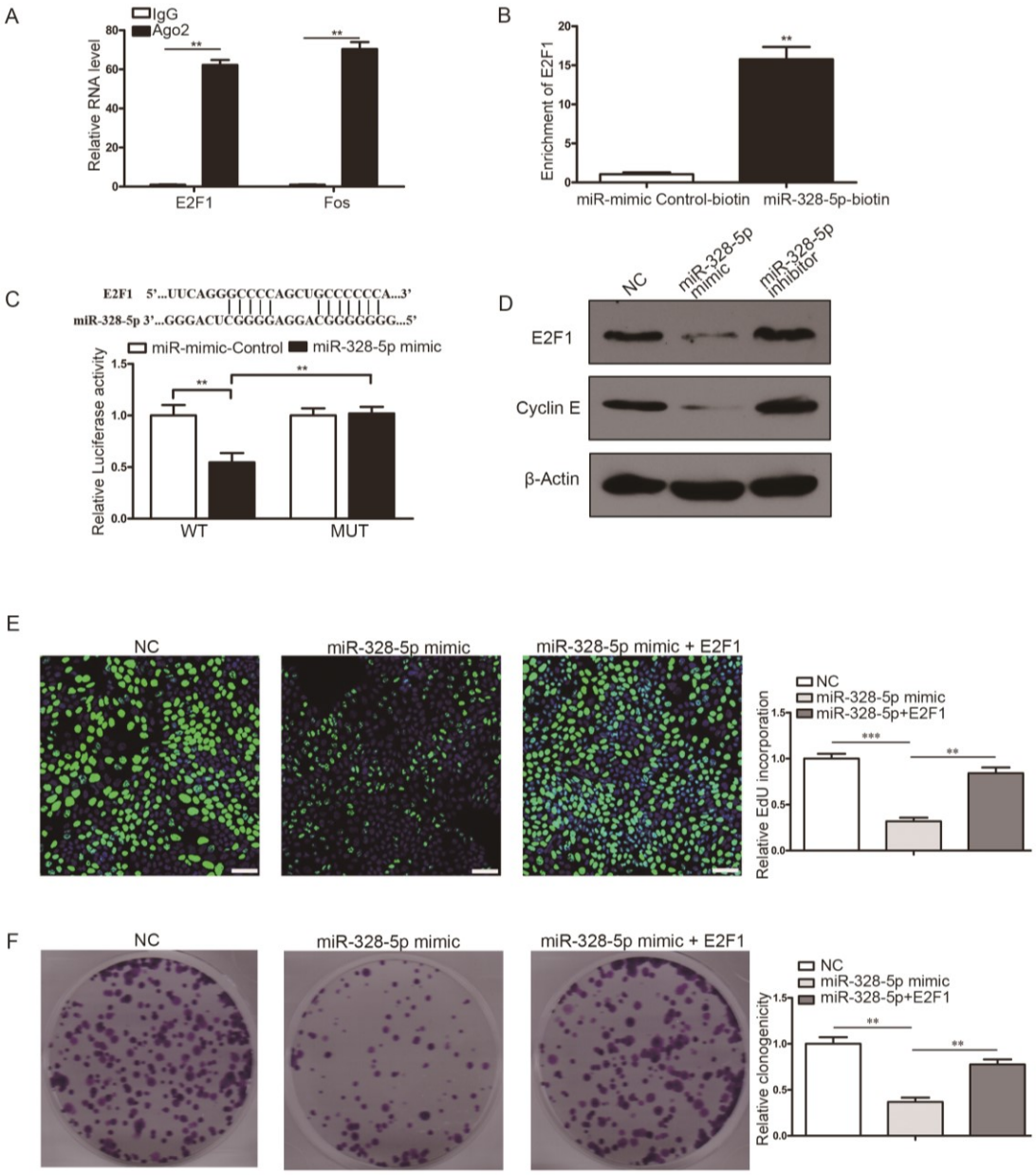
Supplemental Figure S5. Related to Figure 5, Biological functions of miR-328-5p.

A. Schematic diagram showing the putative binding sites of the miR-328-5p associated with circCAMSAP1. B and C. The proliferation of HCT15 cells transfected with miR-328-5p mimics, as shown by EdU (B), and plate colony formation (C) assays. Scale bar, 50 um. D. FACS cell cycle analysis of HCT15 cells transfected with miR-328-5p mimics. **p<0.01, *** p<0.001 by Student's t-test. Error bars indicate S.D.*



Supplemental Figure S6, related to the Figure 5, miR-328-5p inhibitor partially abolishes the effects of circCAMSAP1 silence on cell growth of DLD1 cells.

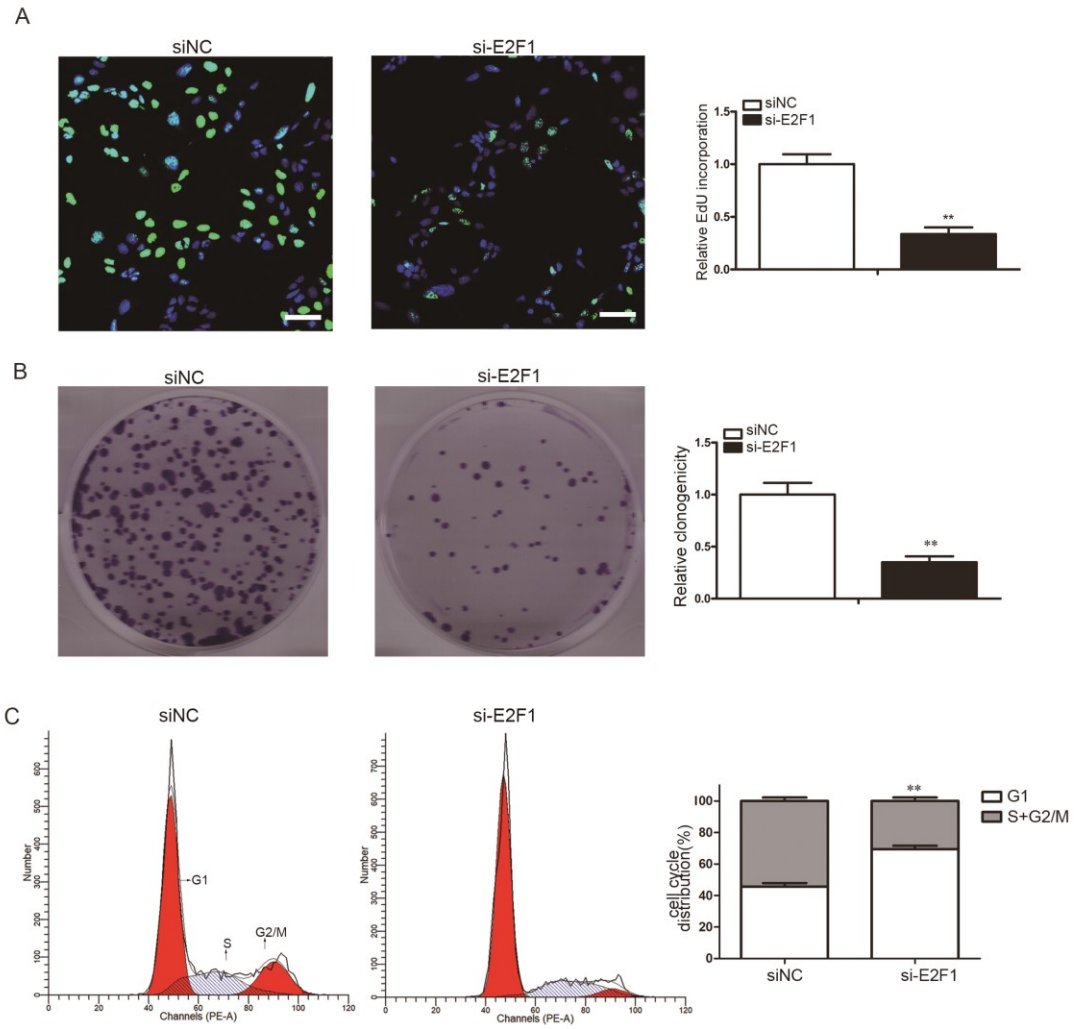
The proliferation of sh1-mediated circCAMSAP1-silenced DLD1 cells transfected with or without miR-328-5p inhibitor were assessed by EdU (A-B) and plate colony formation (C-D) assays. Scale bar, 50 um. **p<0.05, ** p<0.01 by Student's t-test. Error bars indicate S.D.*



Supplemental Figure S7, related to the Figure 5, E2F1 was a direct target of miR-328-5p.

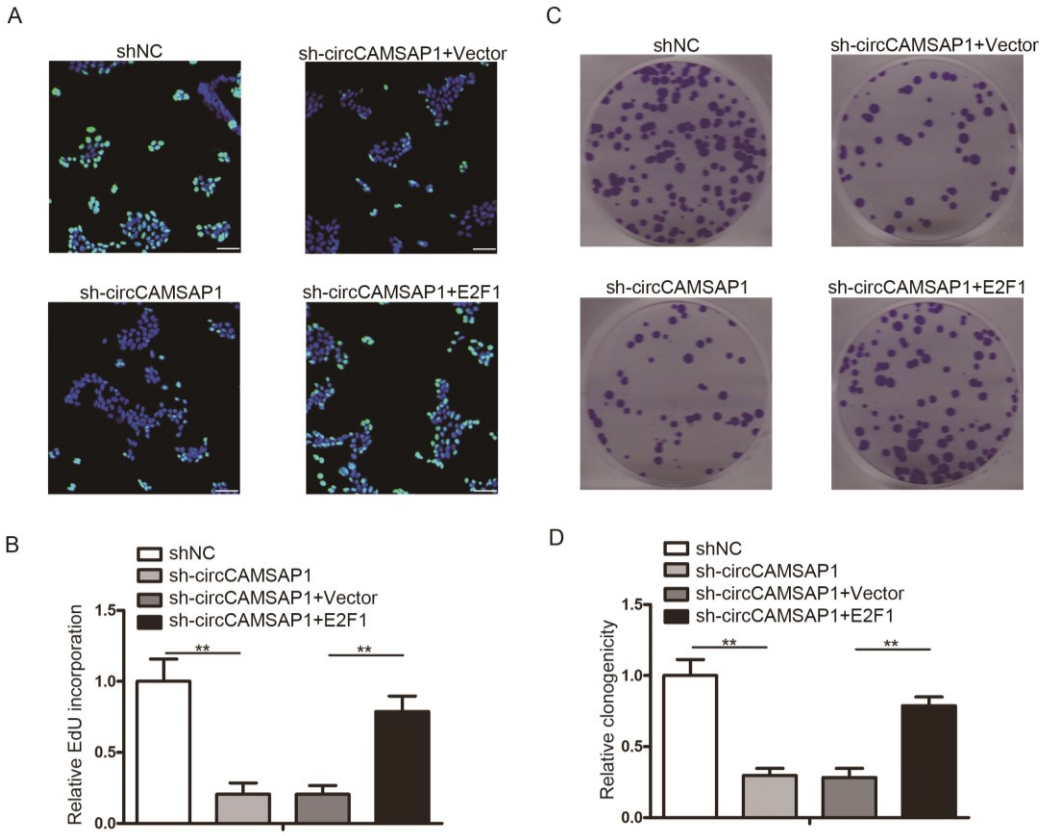
A. RIP assay using an antibody against Ago2, followed by qRT-PCR, followed by detection of circCAMSAP1 and Fos (positive control). B. MiRNA biotin pulldown assay using biotin-coupled miR-328-5p, followed by qRT-PCR. C. The luciferase activities of HCT15 co-transfected with wild-type (WT) or mutant (MUT) E2F1 luciferase reporter vector and miR-328-5p mimics or control miR-mimics. D. The expression level of E2F1 and Cyclin E after transfected with miR-328-5p mimics and inhibitor were measured by western blot. E, F. The proliferation of HCT15 cells transfected with miR-328-5p mimics or co-transfected with miR-328-5p mimics and E2F1 overexpression vector were measure by EdU (E) and plate colony formation (F) assays. ***p<0.01, **** p<0.001 by Student's t-test.*

Error bars indicate S.D.



Supplemental Figure S8, related to the Figure 5, biological functions of E2F1.

A and B. The proliferation of HCT15 cells after E2F1 silence, as shown by EdU (A), and plate colony formation (B) assays. Scale bar, 50 um. C. Silence of E2F1 induces G1 phase cell cycle arrest. ***p<0.01, *** p<0.001 by Student's t-test. Error bars indicate S.D.*



Supplemental Figure S9, related to the Figure 6, the E2F1 overexpression partially abolishes the effects of circCAMSAP1 silence on cell growth of DLD1 cells.

The proliferation of sh1 mediated circCAMSAP1-silenced DLD1 cells transfected with or without E2F1 overexpression vector were assess by assessed by EdU (A-B) and plate colony formation (C-D) assays. Scale bar, 50 um. ** *p<0.01 by Student's t-test. Error bars indicate S.D.*

Supplemental Tables

Supplemental Table S1. Correlation between expression of circCAMSAP1 and patients' clinicopathological variables in CRC patients

Variables	All cases(N=424)	circCAMSAP1 expression (%)		
		Low (n=311)	High (n=113)	P-value
Age (years)				0.062
≤60	218	151(69.3)	67(30.7)	
>60	206	160(77.7)	46(22.3)	

Gender				0.912
Male	238	174(73.1)	64(26.9)	
Female	186	137(73.7)	49(26.3)	
T stage				<0.001
T1/T2	80	77(96.2)	3(3.8)	
T3/T4	344	234(68.5)	110(32.0)	
N stage				<0.001
N0	248	207(83.5)	41(16.5)	
N1/N2	176	104(59.1)	72(40.9)	
M stage				<0.001
M0	389	304(78.1)	85(21.9)	
M1	35	7(20.0)	28(80.0)	
TNM stage				<0.001
I/II	232	204(87.9)	28(12.1)	
III/IV	192	107(55.7)	85(45.3)	
CEA(ng/mL)				<0.001
<5	279	222(79.6)	57(20.4)	
≧5	145	89(61.4)	56(38.6)	
CA19-9(U/mL)				<0.001
<37.5	318	248(78.0)	70(22.0)	
≧37.5	106	63(59.4)	43(40.6)	

Supplemental Table S2. Primers and RNA sequences used in this study

Name	Sequence	Application
circCAMSAP1-forward	GTGTCAAGCGCTTCTCAACG	qRT-PCR
circCAMSAP1-reverse	GCTGGACAGGAGAAGCTTGA	qRT-PCR
circPVT1-forward	CCGACTCTTCCTGGTGAAGC	qRT-PCR

circPVT1- reverse	CAGCGTTATTCCCCAGACCA	qRT-PCR
circGCN1L1-forward	CTGGATGCTTTGGGACGAGTT	qRT-PCR
circGCN1L1-reverse	ATTGACCAGAGCCTCTCAGC	qRT-PCR
circHLA-DRB6-forward	CACAACTACGGGGTTGGTGA	qRT-PCR
circHLA-DRB6-reverse	CTATGGAGACCACCGCAGC	qRT-PCR
circRRP7B-forward	AGCCCAAAGGAGTCAAGGTC	qRT-PCR
circRRP7B- reverse	ACCCCTCCCGCAGTACTAAC	qRT-PCR
circKPNA5-forward	CAACAGCTAACAGCAACACAGA	qRT-PCR
circKPNA5-reverse	ACATTTCTGCGTTTGAACAACTG	qRT-PCR
circWDR78-forward	AGGGCAGTTTACAAGGTCAGT	qRT-PCR
circWDR78-reverse	AGACTTCTTTGGTTGTGTGGC	qRT-PCR
circBRDW1-forward	AGAGGTTCAAGCAACGGCA	qRT-PCR
circBRDW1-reverse	CATCATGGACTCTCCTCCTTCC	qRT-PCR
circFANCL-forward	GCTGTATGCACTACCTCCTCC	qRT-PCR
circFANCL-reverse	TCAGGCAACACTATCCTAAGGTG	qRT-PCR
circVPS13C-forward	AAGCACAGGCAGTTACTCAAG	qRT-PCR
circVPS13C-reverse	TTCCAGTAGGCGCTAAGACT	qRT-PCR
Linear-CAMSAP1-forward	GGGCGCTTTACTGCTACT	qRT-PCR

Linear-CAMSAP1-reverse	ACAGGTGGTTGTGGATTGT	qRT-PCR
Pre-CAMSAP1-a-forward	GATGCTGCCTGGTCCAAAAA	qRT-PCR
Pre-CAMSAP1-a-reverse	CCCAGCATCCTCTTTCTGACAT	qRT-PCR
Pre-CAMSAP1-b-forward	TGCCCTGCCTAGATGTGGTG	qRT-PCR
Pre-CAMSAP1-b-reverse	CAGACAAGGTGTGCTGCTCT	qRT-PCR
RPS6KL1-forward	GCAGATTCGCAACAGGGTG	qRT-PCR
RPS6KL1-reverse	ACGCCATTCTGATAGTGGTTGA	qRT-PCR
COPZ2-forward	CGGTTGCAGGAACCTTCCC	qRT-PCR
COPZ2-reverse	TGCTGTTCTTGTAGACGATGGT	qRT-PCR
GUSB-forward	GACACGCTAGAGCATGAGGG	qRT-PCR
GUSB-reverse	GGGTGAGTGTGTTGTTGATGG	qRT-PCR
GAPDH-forward	CGCTCTCTGCTCCTCCTGTTC	qRT-PCR
GAPDH-reverse	ATCCGTTGACTCCGACCTTCAC	qRT-PCR
E2F1-forward	CCATGGGTGGTCAGATGGTG	qRT-PCR
E2F1-reverse	ACATCAGTGAAGGTCCCCCA	qRT-PCR
si-circCAMSAP1#1	TCAACAAGATAACATCCCT	siRNA target site
si-circCAMSAP1#2	GGATCAACAAGATAACATC	siRNA target site
si-circPVT1	GCTGGGCTTGAGGCCTGATCT	siRNA target site

si-circGCN1L1	AAGCCAGGGGCTGATGGAACT	siRNA target site
si-circKPNA5	GCTGCTTTCTAAAGATGCCAT	siRNA target site
si-circWDR78	AGTTTCACAGGAACAATGCCA	siRNA target site
si-circBRDW1	AAGTATCAGGATGATAGTGAT	siRNA target site
si-circFANCL	GTTGGGATAAGGAAGAGACTT	siRNA target site
si-circVPS13C	GAAATTCAGACTGCAAATGAA	siRNA target site
si-E2F1	GGCCCGATCGATGTTTTCC	siRNA target site
si-ESRP1#1	GGACAGCATTGCCCTATTA	siRNA target site
si-ESRP1#2	CAGTGAGCAATGAACTGAA	siRNA target site
si-ESRP2#1	GGGTAAGCGATACATTGAA	siRNA target site
si-ESRP2#2	GGGAAGTCAAGACAATGGT	siRNA target site
si-QKI#1	GGGACCTATTGTTCAGTTA	siRNA target site
si-QKI#2	GCTGCTCCAAGGATCATTA	siRNA target site
si-NOVA1#1	GGTTCTCATACCTAGTTAT	siRNA target site
si-NOVA1#2	GGTGCAAGGATACAGATCT	siRNA target site
si-NOVA2#1	CCAAGTCCAAAGACTTCTA	siRNA target site
si-NOVA2#2	GCATCCAGATCTCCAAGAA	siRNA target site
si-MEX3A#1	GTGTTTCCCTTCACTCTCT	siRNA target site

si-MEX3A#2	CTAGTGAAGACACGTACAA	siRNA target site
si-MEX3B#1	GGCTCCTTAAAGAAACGCT	siRNA target site
si-MEX3B#2	GCGAAGACCAATACTTACA	siRNA target site
si-CELF1#1	TTTGGCTGCACTAGCTGCT	siRNA target site
si-CELF1#2	GAGCCAACCTGTTCATCTA	siRNA target site
si-RBM47#1	CACGGTGGCTCCAAACGTTCA	siRNA target site
si-RBM47#2	GGATCTCTCCTTAAGCCAACA	siRNA target site
circCAMSAP1 probe	CAGGGATGTTATCTTGTTGATCCAGAAC	FISH, ISH, RNA pull down
Pre-CAMSAP1 probe-1	ACTTCCTATCTCACTCCTCCCTAATGACCTAATTCAGT CTCATGGCTAGAAATACC	RNA pull down
Pre-CAMSAP1 probe-2	TGGACCAGGCAGCATCAAACTCTTGACATGAACCACG CTACTTAATCTTGCCACTG	RNA pull down
Pre-CAMSAP1 probe-3	GAGCACCGGCCTGGCTCTGCATGTGCAAGCTAACTAT CAGTAACTCCAGAACCATC	RNA pull down
Pre-CAMSAP1 probe-4	CAGCAACAGCCTGAAAAGTCACTTACAAATGCACAGC AAATGAGAAACGTGACCAA	RNA pull down
miR-328-5p probe	CCCTGAGCCCCTCCTGCCCCC	FISH
Biotin-miR-3116 mimic sense	UGCCUGGAACAUAGUAGGGACU	RNA pull down
Biotin-miR-3116 mimic anti-sense	AGUCCCUACUAUGUUCCAGGCA	RNA pull down
Biotin-miR-328-5p mimic sense	GGGGGGGCAGGAGGGGCUCAGGG	RNA pull down

Biotin-miR-328-5p mimic anti-sense	CCCTGAGCCCCTCCTGCCCCCCC	RNA pull down

Supplemental Table S3. MiRNA microarray after circRNA pull down

	Fold change	ForeGround		ForeGround-BackGround		Normalized	
Name	circ vs. NC	NC	circ	NC	circ	NC	circ
hsa-miR-3116	4.97	579.50	2813.00	527.50	2766.00	2.41	11.97
hsa-miR-328-5p	4.32	3406.00	15329.00	3357.00	15284.00	15.33	66.16
hsa-miR-323b-5p	3.53	3583.50	13180.00	3530.50	13132.50	16.12	56.85
hsa-miR-550a-3-5p/hsa-miR-550a-5p	3.32	1182.00	4006.00	1131.50	3960.50	5.17	17.15
hsa-miR-4500	2.94	4347.00	13369.00	4296.50	13322.50	19.62	57.67
hsa-miR-3664-3p	2.91	1873.00	5641.00	1820.50	5593.00	8.31	24.21
hsa-miR-4728-5p	2.82	1404.50	4073.00	1354.00	4027.00	6.18	17.43
hsa-miR-625-5p	2.80	2915.00	8496.50	2864.50	8453.00	13.08	36.59
hsa-miR-378g	2.65	1676.50	4600.00	1627.50	4556.00	7.43	19.72
hsa-miR-3182	2.61	6128.00	16755.00	6078.00	16710.50	27.75	72.34
hsa-miR-4270	2.60	2093.00	5649.50	2044.50	5606.00	9.34	24.27
hsa-miR-4447	2.60	1636.00	4388.50	1584.00	4341.00	7.23	18.79
hsa-miR-431-5p	2.56	1576.50	4171.00	1528.00	4126.50	6.98	17.86
hsa-miR-410-5p	2.55	2274.00	6035.50	2223.00	5990.50	10.15	25.93
hsa-miR-4459	2.48	1027.00	2597.50	977.00	2553.00	4.46	11.05
hsa-let-7a-5p	2.36	5956.50	14777.00	5907.00	14733.00	26.97	63.78
hsa-miR-4534	2.33	2603.00	6314.00	2552.50	6267.00	11.66	27.13
hsa-miR-98-5p	2.32	8259.50	20107.00	8205.50	20058.50	37.47	86.83

hsa-let-7c-5p	2.29	3311.50	7938.50	3263.00	7895.00	14.90	34.18
hsa-miR-149-3p	2.20	3457.50	7968.00	3409.50	7924.50	15.57	34.31
hsa-miR-3650	2.17	2346.00	5300.00	2297.00	5256.00	10.49	22.75
hsa-miR-4444	2.08	1340.50	2874.00	1292.00	2831.00	5.90	12.26
hsa-miR-204-3p	2.08	3690.00	8014.50	3638.50	7967.50	16.61	34.49
hsa-miR-4299	2.07	1291.50	2762.00	1242.00	2718.00	5.67	11.77

Supplemental Table S4. Whole transcriptome deep sequencing in circCAMSAP1 silencing cell line

Name	Gene ID	sh-circCAMSAP1	NC	Fold Change
GPR75-ASB3	100302652	0	2.41	0.00
CORO7-PAM16	100529144	0	1.74	0.00
TNFAIP8L2-SCNM1	100534012	0	1.32	0.00
C21orf33	8209	0	1.21	0.00
OR2A42	402317	0	0.85	0.00
LOC107984152	107984152	0	0.68	0.00
GSTT2	2953	0	0.67	0.00
TICAM2	353376	0	0.66	0.00
CKMT1B	1159	0	0.49	0.00
PRODH	5625	0	0.49	0.00
LOC102724994	102724994	0	0.42	0.00
TBC1D3C	414060	0	0.38	0.00
ZNF559-ZNF177	100529215	0	0.34	0.00
ANKRD20A2	441430	0	0.32	0.00
HSPE1-MOB4	100529241	0	0.3	0.00
LOC107986354	107986354	0	0.23	0.00
POTEF	728378	0	0.22	0.00
CDIP1	29965	0.02	0.35	0.06
TBC1D3H	729877	0.21	2.56	0.08
U2AF1L5	102724594	1.45	14.69	0.10
LOC105371591	105371591	0.07	0.7	0.10
FBXW10	10517	0.07	0.48	0.15
LOC107985728	107985728	0.06	0.39	0.15
TAS2R14	50840	0.22	1.3	0.17

FAM198B	51313	0.07	0.34	0.21
ATOH8	84913	0.38	1.8	0.21
ATP5MF-PTCD1	100526740	0.84	3.67	0.23
POC1B-GALNT4	100528030	0.23	0.97	0.24
LOC107986352	107986352	0.39	1.62	0.24
EGR2	1959	1.03	4.04	0.25
MSMP	692094	1.06	4.08	0.26
LOC107987464	107987464	0.2	0.76	0.26
DND1	373863	0.38	1.43	0.27
FZD8	8325	0.27	0.99	0.27
TTC34	100287898	0.11	0.39	0.28
LOC105373102	105373102	1.77	6.17	0.29
CDH7	1005	0.55	1.91	0.29
TSPAN8	7103	2.84	9.71	0.29
SAMD11	148398	0.44	1.43	0.31
SLC30A3	7781	0.29	0.94	0.31
EVX1	2128	0.83	2.63	0.32
CXCL3	2921	1.64	5.17	0.32
GRIN1	2902	0.33	1.03	0.32
KLHL14	57565	0.31	0.95	0.33
HOXB8	3218	15.61	46.82	0.33
NFATC1	4772	0.2	0.59	0.34
CYP1B1	1545	1.13	3.33	0.34
LFNG	3955	0.61	1.79	0.34
HMOX1	3162	47.23	138.55	0.34
RASD1	51655	0.57	1.66	0.34
EGR3	1960	0.73	2.12	0.34
NDP	4693	0.5	1.43	0.35
URGCP-MRPS24	100534592	1.61	4.59	0.35
PSD4	23550	1.8	5.11	0.35
PIGR	5284	0.68	1.92	0.35
DGCR8	54487	3.63	10.02	0.36
LSMEM1	286006	0.9	2.47	0.36
CXCL1	2919	2.74	7.51	0.36
HOXB4	3214	8.81	24.09	0.37
LOC105371191	105371191	0.93	2.52	0.37
HOXB5	3215	8.9	24.06	0.37
CCNE2	9134	1.4	3.75	0.37
CTGF	1490	3.35	8.94	0.37
HOXB3	3213	23.18	61.77	0.38
EME2	197342	3.69	9.66	0.38
TRIM73	375593	2.15	5.62	0.38

CRB2	286204	0.93	2.41	0.39
MYO7B	4648	0.22	0.57	0.39
FNTB	2342	1.86	4.79	0.39
CLDN2	9075	17.9	45.66	0.39
EBF4	57593	1.83	4.66	0.39
RPS6KL1	83694	0.81	2.03	0.40
RASL11A	387496	1.69	4.23	0.40
GGT1	2678	6.99	17.49	0.40
FOS	2353	20.87	51.63	0.40
FOSB	2354	2.85	7.04	0.40
ARMCX5-GPRASP2	100528062	0.41	1.01	0.41
CLDN5	7122	0.79	1.94	0.41
BEND3	57673	1.04	2.55	0.41
E2F1	1869	5.37	13.09	0.41
ELAC1	55520	1.05	2.51	0.42
CDH17	1015	5.73	13.59	0.42
FBP1	2203	3.51	8.29	0.42
HOXD4	3233	2.04	4.81	0.42
FSIP2	401024	0.14	0.33	0.42
MFSD3	113655	11.29	26.5	0.43
FOXN4	121643	0.6	1.39	0.43
PITX2	5308	12.01	27.38	0.44
TMEM178B	100507421	0.81	1.84	0.44
BHLHA15	168620	5.72	12.99	0.44
TSPOAP1	9256	1.41	3.19	0.44
SLC25A13	10165	4.42	9.95	0.44
EGR1	1958	32.57	72.82	0.45
C5	727	0.85	1.89	0.45
PRMT6	55170	3.32	7.35	0.45
FGFBP1	9982	4.14	9.1	0.45
EPDR1	54749	8.72	19.1	0.46
COPZ2	51226	2.12	4.63	0.46
LOC107984110	107984110	1.13	2.46	0.46
HOXB6	3216	52.75	113.31	0.47
ASCL2	430	27.31	58.54	0.47
FIBCD1	84929	1.29	2.75	0.47
LOC102723996	102723996	2.39	5.09	0.47
CDX2	1045	38.97	82.55	0.47
DLX1	1745	0.86	1.82	0.47
MCIDAS	345643	1.81	3.8	0.48
CHPF2	54480	10.23	21.4	0.48
CRIP2	1397	2.89	6.04	0.48
PANK1	53354	3.23	6.73	0.48

NFKBIZ	64332	3.4	7.07	0.48
CBWD6	644019	2.74	5.69	0.48
CALCA	796	4.69	9.64	0.49
FAM86C1	55199	3.9	8.01	0.49
SRSF2	6427	40.67	83.48	0.49
FAM222A	84915	1.17	2.4	0.49
WDR77	79084	8.94	18.28	0.49
SYK	6850	3.69	7.54	0.49
AUTS2	26053	0.95	1.94	0.49
PTPRG	5793	4.02	8.19	0.49
NPY4R	5540	2.83	5.74	0.49
CD83	9308	3.59	7.25	0.50
HTR1D	3352	0.95	1.91	0.50
IL20RA	53832	2.27	4.56	0.50
TBX3	6926	19.16	38.33	0.50
RGPD1	400966	0.41	0.82	0.50
LURAP1L	286343	3.46	1.73	2.00
LTB4R2	56413	3.62	1.81	2.00
CXorf40A	91966	8.29	4.12	2.01
SELENOM	140606	43.54	21.63	2.01
H1F0	3005	244.39	121.25	2.02
ITGAX	3687	2.51	1.24	2.02
WNK4	65266	1.56	0.77	2.03
ABCA12	26154	2.29	1.13	2.03
APOBEC3B	9582	88.84	43.79	2.03
RORC	6097	3.42	1.68	2.04
CABYR	26256	5.44	2.67	2.04
PRPH	5630	4.94	2.42	2.04
RNF112	7732	0.84	0.41	2.05
EFNA3	1944	21.43	10.4	2.06
IL15	3600	2.73	1.32	2.07
C1QL1	10882	5.59	2.7	2.07
MT1E	4493	283.01	136.28	2.08
NR1D1	9572	47.39	22.76	2.08
CCDC107	203260	15.06	7.2	2.09
LGALS1	3956	273.41	130.12	2.10
BNIP3L	665	91.22	43.36	2.10
UGT1A6	54578	8.25	3.92	2.10
RAB3A	5864	13.37	6.3	2.12
ELF3	1999	90.66	42.69	2.12
KCNN4	3783	3.57	1.68	2.13
C4orf3	401152	52.24	24.48	2.13
LOC102724770	102724770	15.14	7.09	2.14
LOC101927789	101927789	2.62	1.22	2.15

LDHA	3939	658.94	306.34	2.15
ECE2	9718	15.92	7.4	2.15
THEMIS2	9473	5.62	2.61	2.15
FAM162A	26355	113.16	52.09	2.17
YPEL4	219539	2.61	1.2	2.18
GPI	2821	313.96	144.05	2.18
GBE1	2632	20.42	9.35	2.18
HK2	3099	80.87	36.83	2.20
FUT11	170384	15.2	6.91	2.20
PGK1	5230	419.53	190.01	2.21
DKK1	22943	12.57	5.67	2.22
PADI2	11240	1.52	0.68	2.24
KRT15	3866	10.04	4.45	2.26
DHDH	27294	8.54	3.78	2.26
AHNAK2	113146	4.93	2.18	2.26
SLC16A3	9123	93.73	41.28	2.27
PTCD1	26024	6.64	2.91	2.28
CD74	972	5.75	2.5	2.30
PFKFB3	5209	32.3	13.99	2.31
PER1	5187	28.41	12.25	2.32
ENO2	2026	98.25	41.19	2.39
NOS3	4846	1.56	0.65	2.40
CDA	978	24.41	10.15	2.40
IQCN	80726	1.72	0.71	2.42
JMJD7-PLA2G4B	8681	5.34	2.19	2.44
PRF1	5551	3.68	1.49	2.47
CYP4F3	4051	3.42	1.37	2.50
MT2A	4502	810.14	323.67	2.50
ALDOA	226	2801.2	1108.74	2.53
PPFIA4	8497	1.73	0.68	2.54
TMEM40	55287	7.25	2.82	2.57
CLIP4	79745	1.81	0.7	2.59
P4HA1	5033	129.44	49.94	2.59
APOL3	80833	2.04	0.78	2.62
SH3D21	79729	16.24	6.18	2.63
SLCO3A1	28232	1.5	0.57	2.63
SLC2A14	144195	1.3	0.49	2.65
NPAS1	4861	3.38	1.26	2.68
MAT1A	4143	0.8	0.29	2.76
CA9	768	128.81	46.63	2.76
KRTAP3-1	83896	9.85	3.51	2.81
PFKFB4	5210	24.49	8.67	2.82
LOC105374299	105374299	13.5	4.76	2.84

ANGPTL4	51129	40.6	14.01	2.90
SLC2A1	6513	211.69	73.01	2.90
GAL3ST1	9514	3.09	1.06	2.92
MYH15	22989	0.35	0.12	2.92
KRT81	3887	2.11	0.72	2.93
CYP1A1	1543	6.19	2.05	3.02
IFI27	3429	9.38	3.04	3.09
ACAP1	9744	1.59	0.51	3.12
SDS	10993	13.52	4.32	3.13
SAA2	6289	26.53	8.23	3.22
CD68	968	18.73	5.77	3.25
ASB9	140462	1.34	0.41	3.27
DUOXA1	90527	0.63	0.19	3.32
ADM	133	32.63	9.83	3.32
PADI1	29943	0.67	0.2	3.35
HILPDA	29923	73.63	21.67	3.40
LYPD3	27076	5.37	1.56	3.44
MT1X	4501	109.71	30.34	3.62
LOC101928422	101928422	1.24	0.34	3.65
SAA1	6288	90.03	24.54	3.67
NDRG1	10397	318.6	85.47	3.73
SLC2A3	6515	342.92	91.62	3.74
FGD2	221472	1.06	0.28	3.79
BHLHE40	8553	60.46	15.82	3.82
SH2D3C	10044	1.6	0.41	3.90
ZBED2	79413	0.82	0.19	4.32
ASIC4	55515	1.8	0.41	4.39
STC1	6781	0.93	0.21	4.43
AKR1C3	8644	4.98	1.1	4.53
ANKRD37	353322	107.29	23.18	4.63
DACT3	147906	0.89	0.19	4.68
APLN	8862	1.18	0.24	4.92
AZGP1	563	2.9	0.56	5.18
ALDOC	230	19.23	3.35	5.74
PHOSPHO2-KLHL23	100526832	2.73	0.36	7.58
IGFL1	374918	3.76	0.47	8.00
SERF1A	8293	1.51	0.17	8.88
EDN2	1907	15.69	1.55	10.12
HGD	3081	0.6	0.04	15.00
DCT	1638	0.18	0	Inf
GPRASP2	114928	0.21	0	Inf
LOC102723623	102723623	0.22	0	Inf
TBC1D3I	102724862	0.42	0	Inf

LOC107984153	107984153	0.49	0	Inf
SNAP91	9892	0.65	0	Inf
HLA-DRB1	3123	0.81	0	Inf
LOC107985109	107985109	0.87	0	Inf
LOC101927345	101927345	2.77	0.03	92.33
RNF103-CHMP3	100526767	1.13	0	Inf
LOC107986353	107986353	1.15	0	Inf
LOC107987477	107987477	1.39	0	Inf
FSBP	100861412	1.96	0	Inf

Supplemental Table S5. Up-regulated genes in CRC tissues

Supplemental Methods

RNA isolation, qRT-PCR and ddPCR

According to the manufacturer's protocol, total RNA from cells and tissues were extracted using TRIzol (Invitrogen, CA, USA). The nuclear and cytoplasmic fractions were isolated by NE-PER™ Nuclear and Cytoplasmic Extraction Reagents (Thermo Scientific). The cell-free RNA was extracted and purified from serum using miRNeasy Serum/Plasma Kit (Qiagen, Helden, Germany) based on the manufacturer's protocol. For mRNA and circRNA, total RNAs were reversely transcripted using reverse transcription kit (Takara, Otsu, Japan). For miRNA, total RNAs were reversely transcripted via RiboBio reverse transcription kit (Guangzhou, China). For miRNA, the expression was determined by stem-loop primer SYBR Green quantitative real time-PCR (RiboBio, Guangzhou, China), and U6 was used as reference gene. For circRNA and mRNA of cells and tissues, quantification was performed by a SYBR Green PCR Kit (Takara, Otsu, Japan), and GAPDH and GUSB were served as the reference genes for cells and tissues respectively. The $2^{-\Delta\Delta CT}$ method was applied to calculate relative expression. For circRNA of serum, the ddPCR was constructed on the Nacia Crystal System (Stilla Technologies, France) using ddPCR EvaGreen (PexBio, Beijing, China) and qPCR ThoughMix (PexBio, Beijing, China) and its expression was compared using absolute copy number. The primer sequences were shown in Table S2.

RNase R treatment

RNase R (Epicentre Technologies, Madison, WI, USA) was used to assess the stability of circRNA. Total RNA (2 μg) was mixed with 0.6ul 10 × RNase R Reaction Buffer and 0.2 μl RNase R or

DEPC-treated water (control group). The samples were then incubated at 37 °C for 15 min[1]. The expression levels of circCAMSAP1 and linear CAMSAP1 were detected by qRT-PCR. GAPDH in the control group was used as an internal control[2,3].

Actinomycin D assay

For the half-life of circRNA assessment, the gene transcription was blocked by adding 2mg/mL Actinomycin D (Sigma-Aldrich, St. Louis, MO, USA) to the cell culture medium[4]. DMSO was used as a negative control. Cells were harvested at 0, 4, 8, 12, 24h and the stability of circCAMSAP1 and linear CAMSAP1 was analyzed by qRT-PCR[5].

Oligonucleotide transfection

SiRNAs, miRNA-328-5p mimics and inhibitors were purified and synthesized by RiboBio (Guanzhou, China) or Gene-Pharma (Shanghai, China). Transfection was performed using Lipofectamine RNAiMAX Reagent (Thermo Fisher Scientific, Massachusetts, USA). The RNA sequences used were listed in Table S2.

Plasmids construction and stable transfection

To generate the circCAMSAP1 overexpression construct, the full-length circCAMSAP1 cDNA was cloned into pLO-ciR (Geneseed Biotech, Guangzhou, China). For circCAMSAP1 minigene reporters, full-length of human circCAMSAP1 along with 0.8 kb endogenous 5′-flanking intron and 0.8 kb 3′ -flanking intron with or without ESRP1-binding site mutation was subcloned into the pCDH-CMV-GFP vector (Geenseed Biotech, Guangzhou, China). For E2F1 and ESRP1-expressing vectors, the full-length ORF sequences of these three genes were respectively subcloned into the pCDH-CMV-MCS-EF1-Puro vectors. For construction of sh-circCAMSAP1 and sh-ESRP1, si-circCAMSAP1 and si-ESRP1 sequences were cloned into pLKO.1 vectors. All constructs were verified by sequencing. For stable transfection, lentiviral containing above vectors was generated in HEK293T cells. After infected with lentivirus, all cell lines were selected with 1-2 ug/mL puromycin.

Whole transcriptome deep sequencing

Total RNA from HCT15 cells with or without circCAMSAP1 silencing was isolated by RNeasy Mini Kit (Qiagen, Germany) and subsequently tested by an Agilent 2200 Bioanalyzer for quality control. According to the manufacturer's protocol, the sequencing library of each RNA sample was prepared and then the expression levels of genes were quantified (BGI, Shenzhen, China). Differential expression genes for RNA-sequencing were analyzed using the R/Bioconductor software package

(limma).

Tissue microarray (TMA) and *in situ* hybridization (ISH)

424 samples of CRC FFPE tissue were used to construct TMA as previously described[6]. Digoxin-labeled circCAMSAP1 probes were synthesized by Sangon Biotech (Shanghai, China). The expression level of circCAMSAP1 in TMA was detected by digoxin-labeled circCAMSAP1 probes using ISH Detection Kit (BosterBio, Pleasanton, CA). Briefly, the sections were dewaxed and rehydrated, after which the sections were digested with pepsin, hybridized with the digoxin-labeled circCAMSAP1 probes at 37°C overnight. Then the sections were incubated overnight at 4°C with anti-digoxin antibody, after which the sections were stained with nitro blue tetrazolium/5-bromo-4-chloro-3-indolylphosphate. The expression of circCAMSAP1 were quantitated as follow: Proportion score: 1-100 (1%-100%); Intensity score: 0 (negative); 1 (weak); 2 (intermediate); 3 (strong). Total score= Proportion score × Intensity score[7].

Luciferase reporter assay

The circCAMSAP1 or E2F1 3' UTR sequences containing WT or mutant miR-328-5p binding sites were synthesized and inserted into pMir-Report (Ambion, Austin, TX, USA) luciferase reporter vector, respectively. CRC cells were co-transfected with the luciferase reporter constructs, miR-328-5p mimics, and Renilla luciferase construct (Promega, Madison, WI, USA) and incubated for 24 hours. Then the luciferase activities were measured by the dual-luciferase reporter assay kit (Promega, Madison, WI, USA). The specific activity was expressed as the relative activity ratio of firefly to Renilla luciferase.

Biotin-miRNA pull-down

As previously mentioned[8], HCT15 cells were transfected with biotin-labeled miR-328-5p mimics, miR-3116 mimics or control biotin-mimics at a concentration of 20 nM, after which the cells were incubated for 48 hours. The cells were collected, fixed with 1% formaldehyde for 30 min and lysed in Co-IP buffer. The mixture was sonicated at high amplitude for 30 cycles of 30 secs (on/off) pulses. Then RNA was pulled down after incubating the cell lysate with streptavidin-coated magnetic beads (Life Technologies, CA, USA) for 30 min at 37°C. Finally, RNA in the solution was extracted followed by qRT-PCR.

Pre-CAMSAP1 pull-down

Biotin-labeled probes targeting the flanked intron regions of circCAMSAP1 were synthesized by Sangon Biotech (Shanghai, China). The biotin-labeled probes were then used for pull-down as

mentioned previously, with some modifications[8]. In brief, 1×10^7 cells were cross-linked by 1% formaldehyde for 30 min and then lysed in Co-IP buffer. The mixture was sonicated at high amplitude for 30 cycles of 30 secs (on/off) pulses. The cell lysis was incubated with Pre-CAMSAP1 probe-streptavidin beads (Life Technologies, CA, USA) mixture overnight at 37 °C, washed for 5 times and eluted using biotin elution buffer[9]. The interacting proteins were precipitated by acetone. Finally, the obtained proteins were used for Western Blot or Mass Spectrometry.

Cell proliferation, cell cycle and apoptosis assays

Cell proliferation was examined using cell counting, EdU assay and FACS analysis of Ki-67 staining. For cell counting, 5×10^4 cells were cultured in 10% FBS/DMEM and the cell number was counted by Coulter Counter (Beckman Coulter, Brea, CA, USA) every other day. For EdU assay, the experiments were performed using a Cell-Light EdU DNA Cell Proliferation Kit (Beyotime, Haimen, China). The EdU-positive cells were imaged and counted. For FACS analysis of Ki-67 staining, cells (2×10^5) were cultured in 10% FBS/DMEM medium in culture dishes and maintained at 37°C for 12 h. After cell attachment, the medium was replaced with serum-free DMEM medium. After 5 days, the cells were collected, followed by Ki-67 intracellular staining using mouse-anti-human Ki-67-PE staining set (eBioscience) following manufacture's instructions. Mean fluorescence intensity (MFI) were used to measure the expression of Ki-67. For plate colony formation, 500 cells were plated into 6-well plates and cultured for two weeks. Then the colonies were fixed with methanol and stained with 0.1% crystal violet for 15 minutes at room temperature. Cell colonies were counted and imaged. For soft agar colony formation assay, 500 cells were mixed in 0.3% low melting agarose with DMEM/10% FBS, and plated on 0.66% agarose-coated 6-well plates. Colony formation was monitored weekly. After 4 weeks, colonies were examined and photographed. For cell cycle analysis, cells were resuspended in cold phosphate buffered saline (PBS) and incubated in ice-cold 70% ethanol for 3h and resuspended in propidium iodide (PI) master mix (40 mg/ml PI and 100 mg/ml RNase in PBS) and then incubated at 37°C for 10 minutes before analysis with flow cytometry. Cell apoptosis was assessed using the Annexin V-FITC/ PI Apoptosis Detection Kit (BD Biosciences #556547) according to the manufacturer's instruction. The cell apoptosis data were analyzed by Flowjo V10 software (Tree Star, San Francisco, CA, USA).

Supplemental References

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