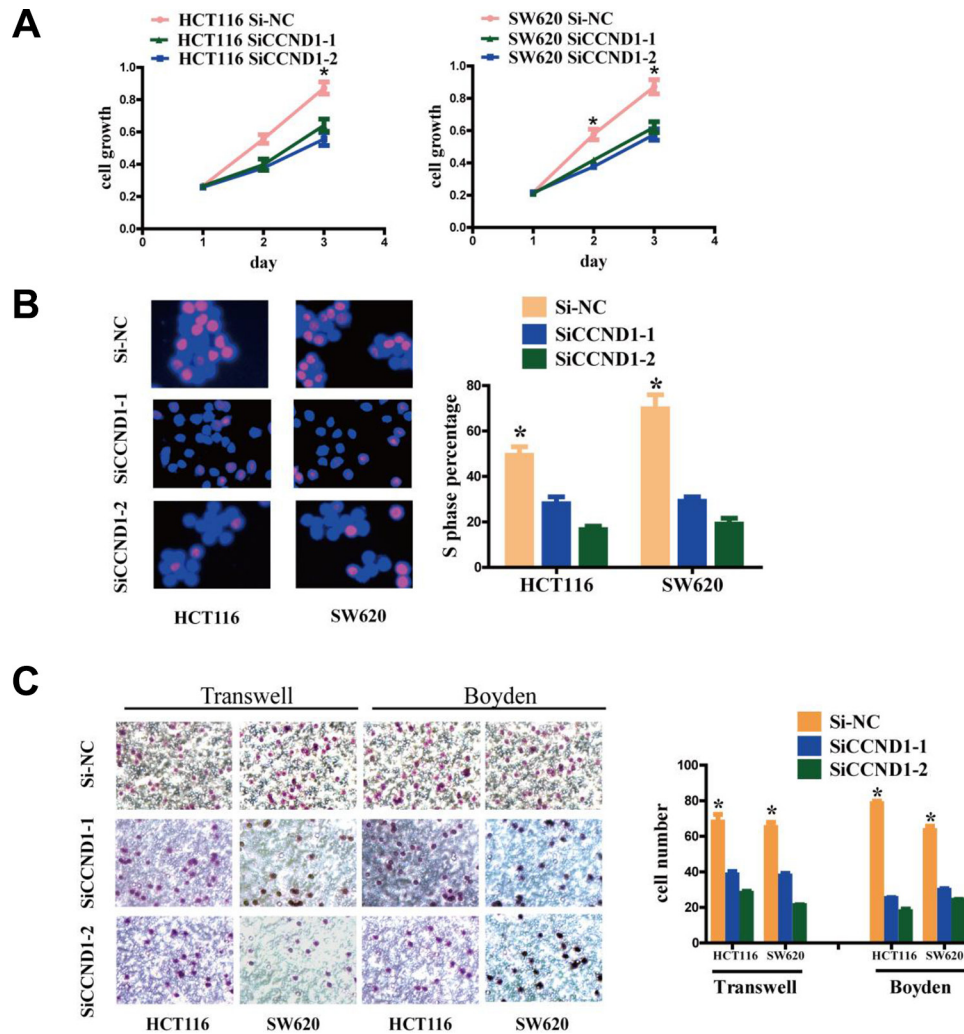
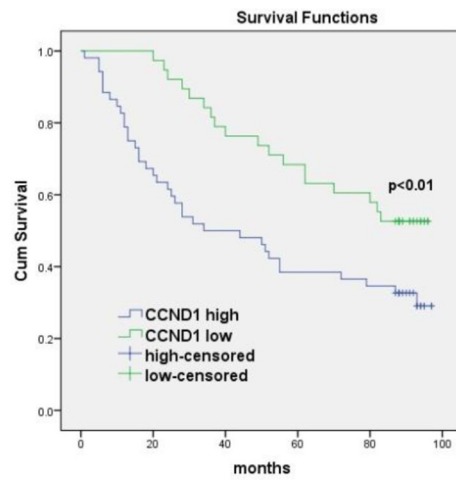
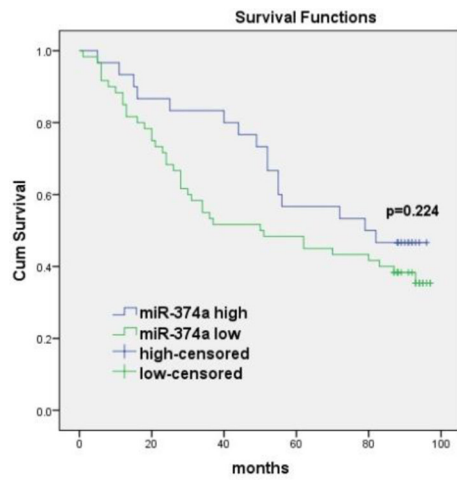


microRNA-374a suppresses colon cancer progression by directly reducing CCND1 to inactivate the PI3K/AKT pathway

Supplementary Materials



Supplementary Figure S1: The effect of CCND1 silencing on proliferation, invasion and migration. (A–C) HCT116 and SW620 cells were transfected with two sequences of si-CCND1. Cell growth and cell cycle were measured by MTT assays (A) and Edu assays (B). Invasion and migration were measured by Transwell and Boyden Chamber assays (C).



Supplementary Figure S2: Respective survival curve of miR-374a expression (left) and CCND1 expression (right) in CRC tissues.

Supplementary Table S1: Correlation between the clinical pathological factors and expression of miR-374a in CRC specimens

Clinical parameter	<i>n</i>	High expression (<i>n</i> %)	Low expression (<i>n</i> %)	χ^2 value	<i>P</i> value
gender					
male	48	17 (35.4)	31 (64.6)	0.201	0.654
female	42	13 (31)	29 (69)		
age (year)					
> 65	52	18 (34.6)	34 (65.4)	0.091	0.763
≤ 65	38	12 (31.6)	26 (68.4)		
T stage					
T1–T2	15	3 (20)	12 (80)	1.44	0.23
T3–T4	75	27 (36)	48 (64)		
N stage					
N ₀	58	19 (32.8)	39 (67.2)	0.024	0.876
N1–N2	32	11 (34.4)	21 (65.6)		
Grade					
I–II	48	18 (37.5)	30 (62.5)	0.804	0.37
III–IV	42	12 (28.6)	30 (71.4)		

Supplementary Table S2: Correlation between the clinical pathological factors and expression of CCND1 in CRC specimens

Clinical parameter	<i>n</i>	High expression (<i>n</i> %)	Low expression (<i>n</i> %)	χ^2 value	<i>P</i> value
gender					
male	48	24 (50)	24 (50)	2.551	0.110
female	42	28 (66.7)	14 (33.3)		
age (year)					
> 65	52	31 (59.6)	21 (40.4)	0.170	0.680
≤ 65	38	21 (55.3)	17 (44.7)		
T stage					
T ₁ –T ₂	15	3 (20)	12 (80)	10.53	0.001
T ₃ –T ₄	75	49 (65.3)	26 (34.7)		
N stage					
N ₀	58	30 (51.7)	28 (48.3)	2.450	0.117
N ₁ –N ₂	32	22 (68.8)	10 (31.2)		
Grade					
I–II	48	29 (60.4)	19 (39.6)	0.294	0.588
III–IV	42	23 (54.8)	19 (45.2)		

Supplementary Table S3: Sequences of miR-374a and U6

		Sequence
miR-374a	Sense	5' CGGCGGTTATAATACAACCTG3'
	Antisense	----
U6	Sense	5' CTCGCTTCGGCAGCACATATA3'
	Antisense	----

Supplementary Table S4: Primer sequences for CCND1 and ARF5

		Sequence
CCND1	Sense	5'GACGGCCGAGAAGCTGTGCA 3'
	Antisense	5' GCCACCATGGAGGGCGGATT 3'
ARF5	Sense	5' ATCTGTTTCACAGTCTGGGACG3'
	Antisense	5' CCTGCTTGTTGGCAAATACC3'

Supplementary Table S5: Sequences of miR-374a mimics and negative control, miR-374a inhibitors and inhibitors negative control

Gene	Sequence	
miR-374a mimics	Sense	5' UUAUAAUACAACCUGAUAAGUG3'
	Antisense	5' CUUAUCAGGUUGUAUUUAAUU3'
Negative control	Sense	5' UUCUCCGAACGUGUCACGUTT3'
	Antisense	5' ACGUGACACGUUCGGAGAATT3'
miR-374a inhibitors		5' CACUUAUCAGGUUGUAUUUAA3'
Inhibitors negative control		5' CAGUACUUUUGUGUAGUACAA3'

Supplementary Table S6: SiRNA sequences of CCND1

		Sequence
CCND1-1	Sense	5' UGGAAUAGCUUCUGGAAUU dTdT 3'
	Antisense	3' dTdA ACCUUAUCGAAGACCUUAA 5'
CCND1-2	Sense	5' CAAGCUCAAGUGGAACCUG dTdT 3'
	Antisense	3'dTdT GUUCGAGUUCACCUUGGAC 5'

Supplementary Table S7: Primer sequences for CCND1 3' UTR

		Sequence
CCND1 3' UTR	Sense	5' ATCGCTCGAGACAAAGGAGGCGTCTCGG 3'
	Antisense	5' ATCGCGCCGCTAGGAGTGGGACAGGTGGC 3'