Supplementary materials contain Supplementary Figures S1-5 and Supplementary Tables S1-4.

Supplementary Figures

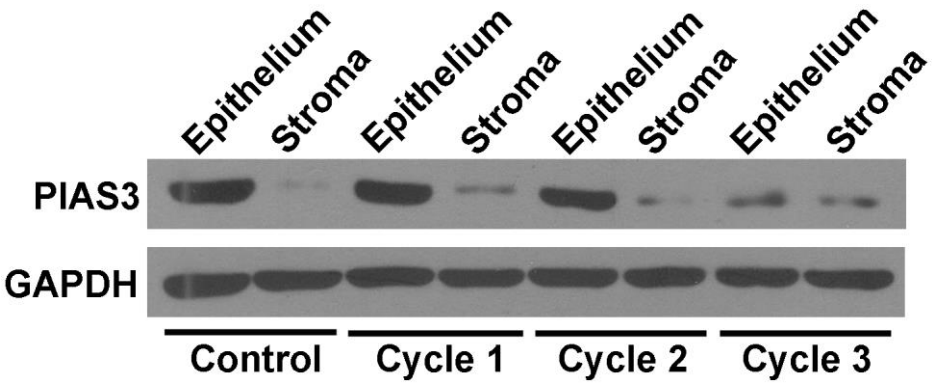

Figure S1. The protein levels of PIAS3 in epithelium and stroma of colons were determined by western blotting after every DSS/ddH2O cycle. The western blotting data shown are representative of three individual analyses.

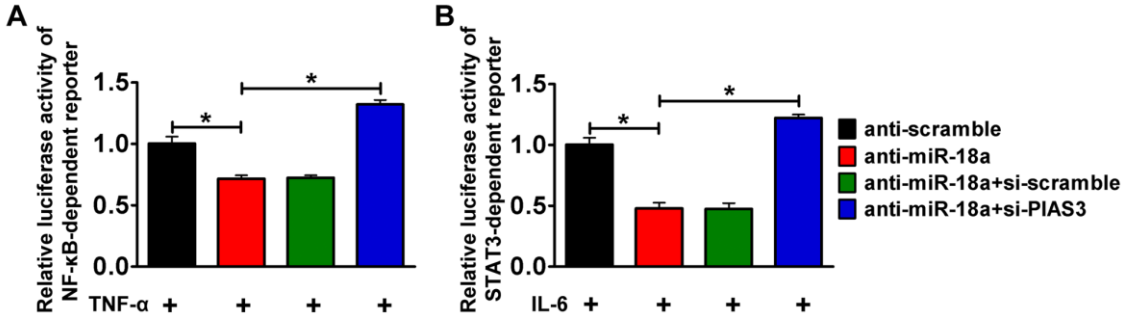

Figure S2. miR-18a enhanced NF-κB and STAT3 activity by downregulating PIAS3 expression. (A) Stably transfected Caco-2 cells carrying an NF-κB-dependent luciferase reporter were co-transfected with 50 nmol anti-miR-18a and 50 nmol PIAS3 siRNA for 48 h and then stimulated with 20 ng/ml TNF-α for another 12 h. **(B)** The stably transfected Caco-2 cells carrying a STAT3-dependent luciferase reporter were co-transfected with 50 nmol anti-miR-18a and 50 nmol PIAS3 siRNA for 48 h and then stimulated with 50 ng/ml IL-6 for another 12 h. Finally, the cells were harvested and submitted to a luciferase activity assay. Five samples were analyzed per condition, and the experiments were performed in triplicate. Values are expressed as

the mean ± SEM. *P<0.05; One-way ANOVA with post-hoc Bonferroni correction.

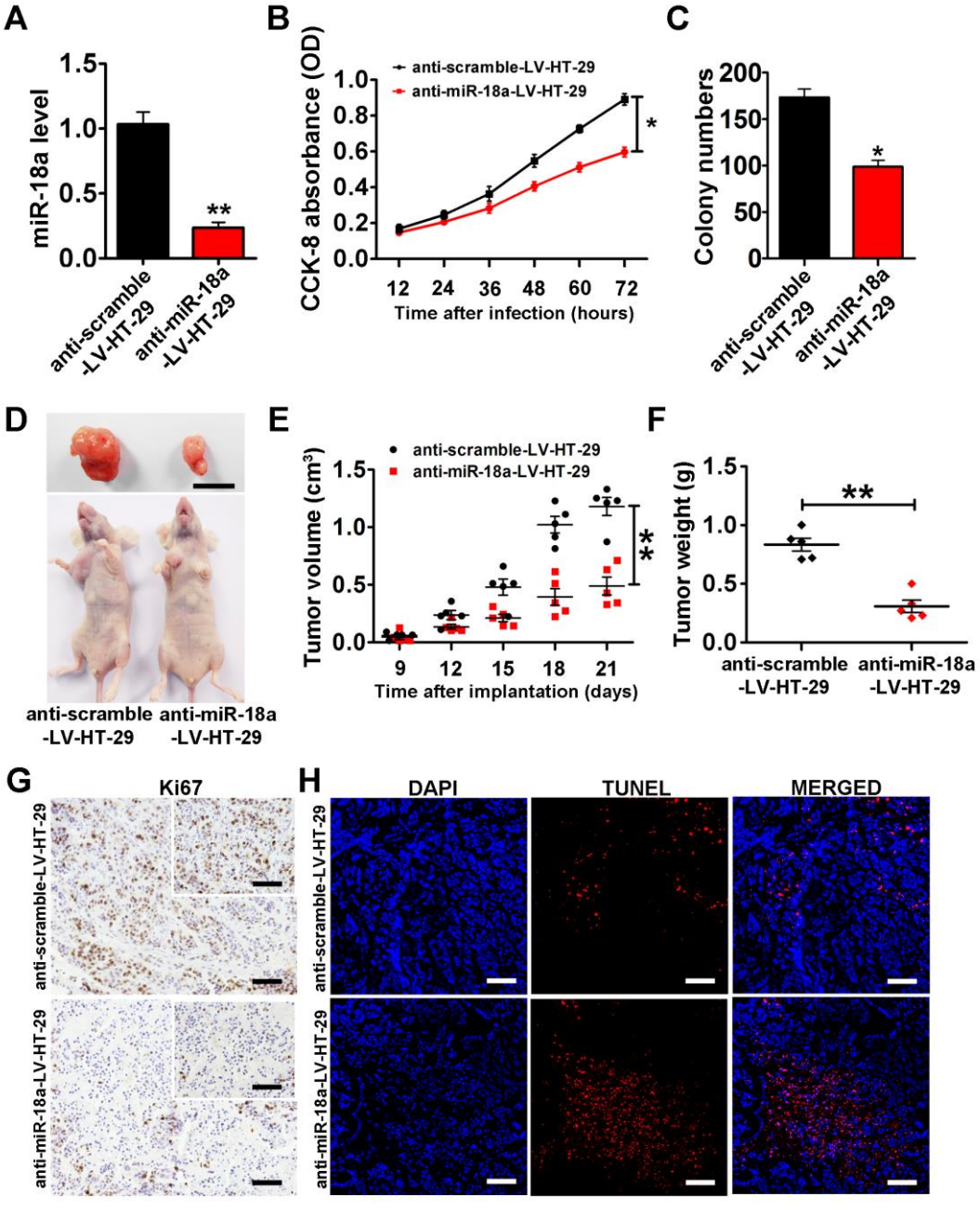

Figure S3. The effect of anti-miR-18 on CRC cell proliferation and apoptosis. (A) miR-18a levels in stably transfected anti-miR-18a-LV-HT-29 cells. **(B)** Cell proliferation was assayed in anti-miR-18a-LV-HT-29 cells using a CCK-8. **(C)** A colony formation assay was performed using anti-miR-18a-LV-HT-29 cells, and colony numbers were counted 21 days after cell seeding. **(D)** A representative image of tumors from tumor-bearing mice at 21 days post-implantation with

anti-miR-18a-LV-HT-29 cells. Scale bar = 1 cm. **(E)** The volumes of the xenograft tumors in the nude mice determined at 21 days post-implantation. **(F)** The weights of the xenograft tumors in the nude mice measured at 21 days post-implantation. **(G)** Immunohistochemical staining of Ki67 and (H) a TUNEL assay were performed in tumor tissues from nude mice at 21 days post-implantation with anti-miR-18a-LV-HT-29 cells (magnification: 200x, scale bar = 50 μm; insert magnification: 400x, scale bar = 50 μm). For cell experiments, five samples were analyzed per condition, and the experiments were performed in triplicate. A total of 5 mice were examined per group; values are expressed as the mean ± SEM. **P*<0.05 and ***P*<0.01; two-tailed Student's *t*-test.

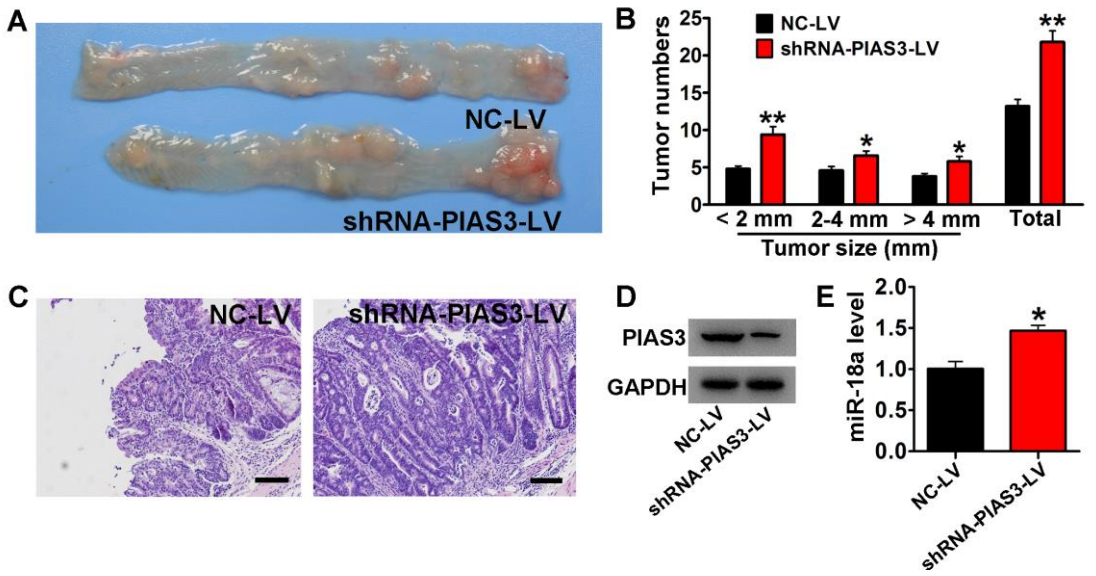

Figure S4. Knockdown of colonic PIAS3 further promoted CAC progression. shRNA-PIAS3-LV was intracolonically administered into AOM-treated C57BL/6J mice at the beginning of each DSS/ddH_2O cycle. **(A)** Images of the colons harvested from AOM-DSS-induced mice after three treatments with shRNA-PIAS3-LV. **(B)** Tumor numbers were counted and tumor sizes were determined using a caliper (> 2 mm) or a dissection microscope (< 2 mm). **(C)** Colon sections from AOM-DSS-induced mice that received three shRNA-PIAS3-LV treatments were

examined by H&E staining (magnification: 100x, scale bar = 100 μm). **(D)** The expression levels of PIAS3 and **(E)** miR-18a levels in colon tissues from CAC mice following shRNA-PIAS3-LV treatments. The western blotting data shown are representative of three individual analyses. A total of 5 mice were examined per group; values are expressed as the mean ± SEM. **P*<0.05 and ***P*<0.01; two-tailed Student's *t*-test.

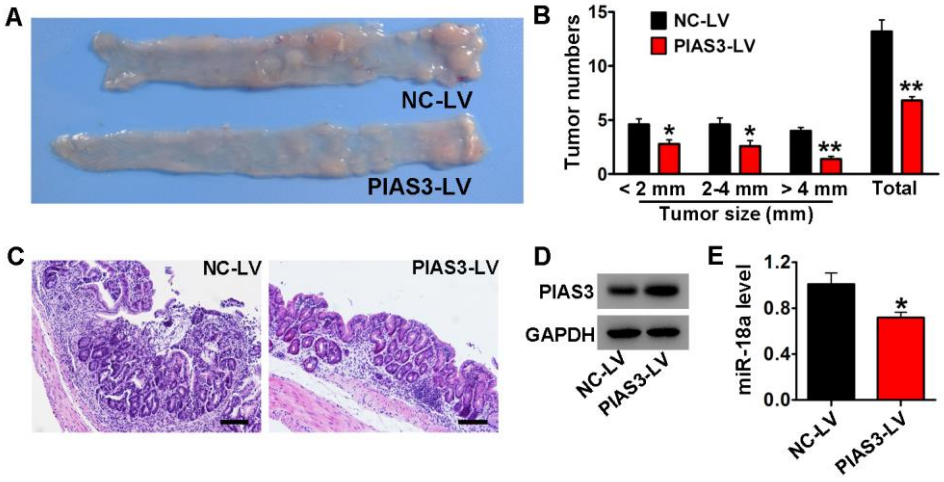

Figure S5. Overexpression of colonic PIAS3 inhibited tumor growth in the advanced stages of CAC mice. PIAS3-LV were intracolonically administered into AOM-treated C57BL/6J mice on day 52 and day 56 during the third DSS/ddH2O cycle. **(A)** Images of the colons harvested from AOM-DSS-induced mice after treatments with PIAS3-LV. **(B)** Tumor numbers were counted, and tumor sizes were determined using a caliper (> 2 mm) or a dissection microscope (< 2 mm). **(C)** Colon sections from AOM-DSS-induced mice that received PIAS3-LV treatments were examined by H&E staining (magnification: 100x, scale bar = 100 μm). **(D)** The expression levels of PIAS3 and **(E)** miR-18a levels in colon tissues from CAC mice following PIAS3-LV treatments. The western blotting data shown are representative of three individual analyses. **P*<0.05 and ***P*<0.01; two-tailed Student's *t*-test.

Supplementary Tables

Table S1. The primer, siRNA and shRNA sequences used in this study.

Gene	Sequence (5’ to 3’)
miR-18a Forward	ACACTCCAGCTGGGTAAGGTGCATCTAGTG
Reverse	CTCAACTGGTGTCGTGGAGTCGGCAATTCA GTTGAGTATCTGCA
URP	TGGTGTCGTGGAGTCG
U6 Forward	CTCGCTTCGGCAGCACA
U6 Reverse	AACGCTTCACGAATTTGCGT
hsm-C13orf25 Forward	CAGTAAAGGTAAGGAGAGCTCAATCTG
Reverse	CATACAACCACTAAGCTAAAGAATAATCTGA
hsm-PIAS3 Forward	TGTCACCATGAAACCATTGC
Reverse	AGGTAAAGTGCGCTTCCTCA
hsm-β-actin Forward	GACCTCTATGCCAACACAGTGC
Reverse	GTACTCCTGCTTGCTGATCCAC
hsm-RelA Forward	GGGAAGGAACGCTGTCAGAG
Reverse	TAGCCTCAGGGTACTCCATCA
mus-IL-6 Forward	CAAAGCCAGAGTCCTTCAGAG
Reverse	GCCACTCCTTCTGTGACTCC
mus-IL-1β Forward	CCCAACTGGTACATCAGCACCTC
Reverse	GACACGGATTCCATGGTGAAGTC
mus-TNF-α Forward	ACCACGCTCTTCTGTCTACT
Reverse	AGGAGGTTGACTTTCTCCTG
mus-Bcl-2 Forward	TAGAGAGATGCGAGGAACCGATG
Reverse	ATAAGCAATCCCAGGGTCTGTCC

mus-Bcl-xL	Forward	GGCAACCCATCCTGGCACCT
	Reverse	AGCGCTCCTGGCCTTTCCG
mus-Cox-2	Forward	TGGGTGTGAAGGGAAATAAGG
	Reverse	CATCATATTTGAGCCTTGGGG
mus-c-Myc	Forward	GCTCTCCATCCTATGTTGCGG
	Reverse	TCCAAGTAACTCGGTCATCATCT
mus-CyclinD1	Forward	CCCAACAACTTCCTCTCCT
	Reverse	TCCAGAAGGGCTTCAATCTG
mus-PIAS3	Forward	GGACGTGTCCTGTGTGTGACAA
	Reverse	ATCTCATCACAATCCGAACAGGAA
mus-β-actin	Forward	GGTGTGATGGTGGGAATGGG
	Reverse	ACGGTTGGCCTTAGGGTTCAG
PIAS3 siRNA	sense	GACAGAGAGUCAGCACUAUUU
	antisense	AUAGUGCUGACUCUCUGUCUU
PIAS3 shRNA		CCGGGCTGACATCCAAGGTTTAGATCTCGAG
		A TCTAAACCTTGGATGTCAGCTTTTTG

Table S2. The clinicopathological features of the CAC patients in this study.

Clinicopathological feature	Number (n)	Proportion (%)
All cases	5	100
Age (years)		
≤45	3	60
>45	2	40
Gender		
Female	3	60
Male	2	40
Differentiation		
Well–moderately differentiated	4	80
Poorly differentiated	1	20

Table S3. The clinicopathological features of the CRC patients in this study.

Clinicopathological feature	Number (n)	Proportion (%)
All cases	46	100
Age (years)		
≤60	22	47.8
>60	24	52.2
Gender		
Female	15	32.6
Male	31	67.4
Tumor location		
Colon	19	41.3
Rectum	27	58.7
TNM stage		
T1	1	2.2
T2	12	26.1
T3	30	65.2
T4	3	6.5
Differentiation		
Well differentiated	7	15.2
Moderately differentiated	32	69.6
Poorly differentiated	7	15.2

Table S4. The antibodies used in this study.

Antibody	Poly/monoclonal	Manufacturer	Dilution
P-STAT3	Monoclonal	Cell Signaling Technology Inc. (#9145)	1:2,000
STAT3	Monoclonal	Cell Signaling Technology Inc. (#8232)	1:1,000
PIAS3	Polyclonal	Cell Signaling Technology Inc. (#4164)	1:1,000 (WB)
PIAS3	Polyclonal	Abgent (AP1245a)	1:100 (IHC)
HRP-conjugated goat anti-rabbit IgG	Monoclonal	Jackson ImmunoResearch (111-005-003)	1:2,000
HRP-conjugated anti-GAPDH	Monoclonal	KangChen Bio-tech Inc.(KG-5G5)	1:10,000
Ki67	Polyclonal	Abcam (ab21700)	1:100
Biotinylated goat anti-rabbit IgG	Monoclonal	Boster Biological technology (11F12B)	1:100
α-SMA	Monoclonal	Cell Signaling Technology Inc （#19245）	1:1,000
Pan-Keratin	Monoclonal	Cell Signaling Technology Inc.（#4545）	1:1,000