BrdU incorporation assay

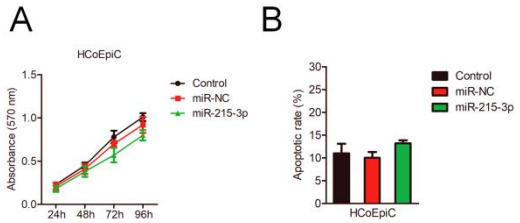
Normal colonic epithelial cell line HCoEpiC, HT-29 and SW480 cell was transfected with miR-NC or miR-215-3p. After 24 hrs post-transfection, cells were incubated with 10 μg/mL bromodeoxyuridine (BrdU) (Roche, Indianapolis, IN) for 4 hrs. The cells were then fixed in 4% paraformaldehyde for 15 minutes, incubated with 2M HCl for 30 minutes to denature genomic DNA, and neutralized by 0.1M $Na_2B_4O_7$ for 5 minutes. The cells were subsequently stained with mouse monoclonal anti-BrdU antibody (Roche, Indianapolis, IN) and AF488 anti-mouse antibody (Invitrogen, San Diego, CA). The cells were also stained with 4,6-diamidino-2-phenylindole (DAPI) (Sigma, St Louis, MO). The percentage of BrdU incorporation was determined by counting the number of BrdU-positive nuclei among DAPI-stained nuclei in three independent microscope fields.

Cell cycle assay

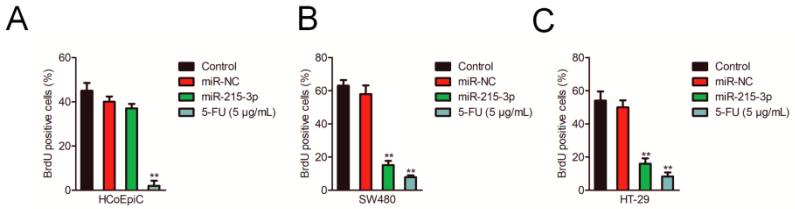
Fluorescence-activated cell sorting (FACS) analysis was used to examine the cell cycle of gastric cancer and normal cells. The CRC cancer cells (SW480 and HT-29) and normal colonic epithelial cell line HCoEpiC were transfected with miR-NC or miR-215-3p. At 24 h after transfection, the cells were collected and fixed with 70% ethanol overnight at 4°C. Then, the cells were centrifuged at 300× g for 10 min. After being washed with PBS, the cells were stained with propidium iodide solution [20 μg/ml of propidium iodide (Sigma, USA), and, 200 μg/mL of RNaseA (Sangon, China)] for 30 min at 37°C in the dark. Subsequently the cells were examined using FACS-Calibur flow cytometer (BD Biosciences, USA). Flow cytometric data were analyzed using Cell Quest Pro software (BD Biosciences, USA).

Immunohistochemistry

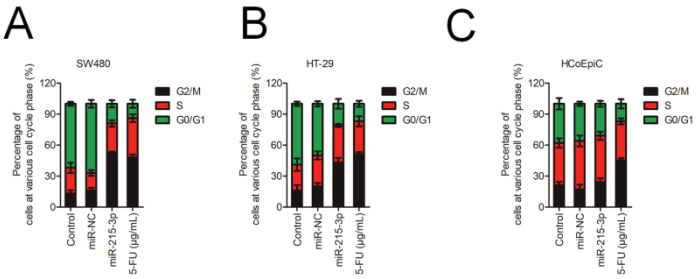
Formaldehyde-fixed, paraffin-embedded tissue samples were sectioned at 5 μm, after which the samples were deparaffinized in xylene and hydrated using graded alcohol. Antigen retrieval was performed with citrate buffer 0.01 M (pH 6.0), followed by blocking endogenous peroxidases with 3% H_2O_2 for 20 min, and blocking of non-specific binding with normal goat serum (Beijing Biosynthesis Biotechnology Co., Ltd., Beijing, China) at room temperature for 20 min. The slides were then incubated with anti-FOXM1 primary antibody (1:100; Beijing Biosynthesis Biotechnology Co., Ltd., Beijing, China) at 4°C overnight, followed by incubation with the HRP-conjugated secondary antibody (Beijing ZhongShan-Golden Bridge Biological Technology Co., Ltd., Beijing, China) for 30 min at 37°C. Detection was performed using 3,3′-diaminobenzidine for 3 min at room temperature (Beijing ZhongShan-Golden Bridge Biological Technology Co., Ltd., Beijing, China) and Harris hematoxylin for 30 sec at room temperature. Finally, a Leica Photo Microscope (Leica Microsystems GmbH, Wetzlar, Germany) was used to obtain digital images.



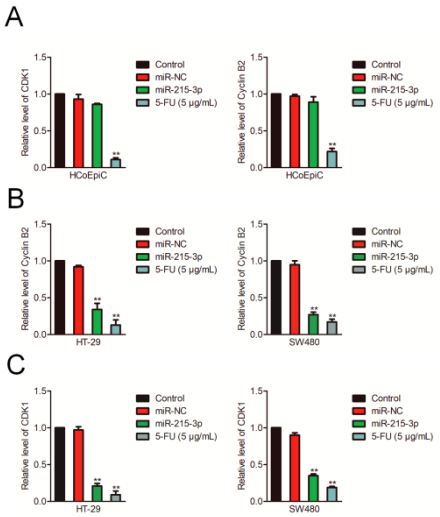
Supplementary Figure 1. HCoEpiC cells were transfected with miR-215-3p or miR-NC, respectively. The role of miR-215-3p on the proliferation of HCoEpiC cell was assessed using MTT assay.



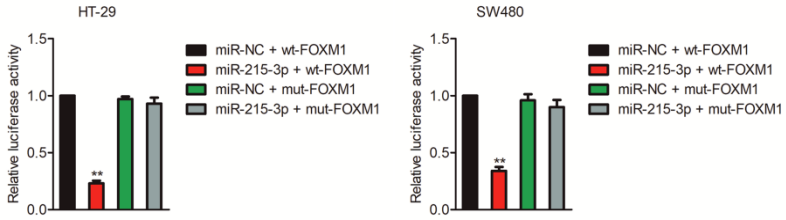
Supplementary Figure 2. BrdU incorporation assay of normal colonic epithelial cell line HCoEpiC (A), HT-29 (B) and SW480 (C) transfected with miR-NC or miR-215-3p. The bar graph represents the average percentage (%) of BrdU positive cells.



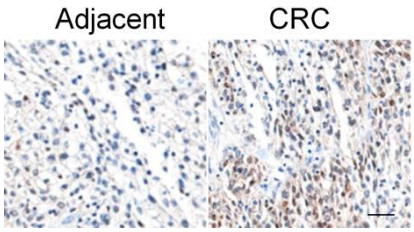
Supplementary Figure 3. Effects of miR-215-3p on the cell cycle. At 24 h after cells were transfected with the miR-215-3p or miR-NC, the cell cycle of SW480 (A), HT-29 (B) and normal colonic epithelial cell line, HCoEpiC (C) was evaluated with flow cytometry. $^{**}P<0.01$ compared to untreated cell.



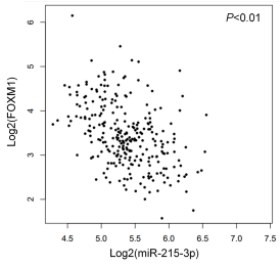
Supplementary Figure 4. Normal colonic epithelial cell line, HCoEpiC (A), HT-29 (B) or SW480 (C) was transfected with miR-NC or miR-215-3p and the levels of CDK1 and Cyclin B2 were detected by qRT-PCR assay $^{**}P<0.01$ compared to untreated cell.



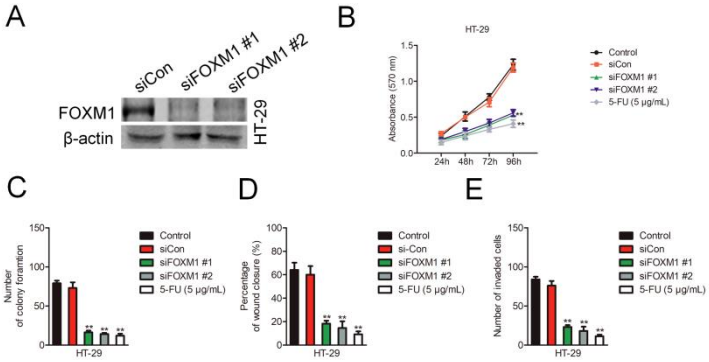
Supplementary Figure 5. Relative luciferase activities of FOXM1 wild type (wt) and mutant (mut) 3′-UTR regions were obtained by co-transfection of scrambled control miRNA or miR-215-3p precursor, and pGL3-promoter vector; and calculated as the ratio of firefly/renilla activities in the cells and normalized to those of the control. $^{**}P<0.01$ compared to miR-NC + wt-FOXM1.



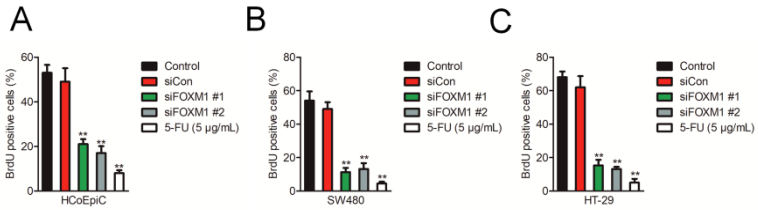
Supplementary Figure 6. The protein expression of FOXM11 in CRC tissue and adjacent tissue was detected by immunohistochemistry (IHC).



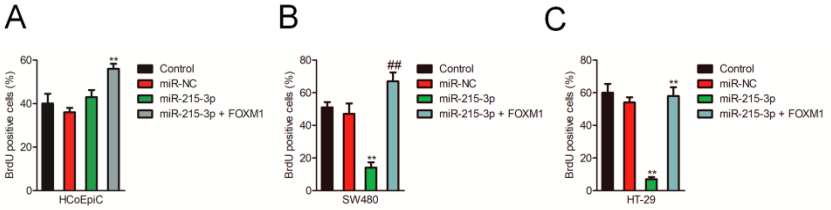
Supplementary Figure 7. Inverse correlation between miR-215-3p and FOXM1 was analyzed using the TCGA CRC cohort. Statistical analysis was performed using Pearson's correlation coefficient (P<0.01).



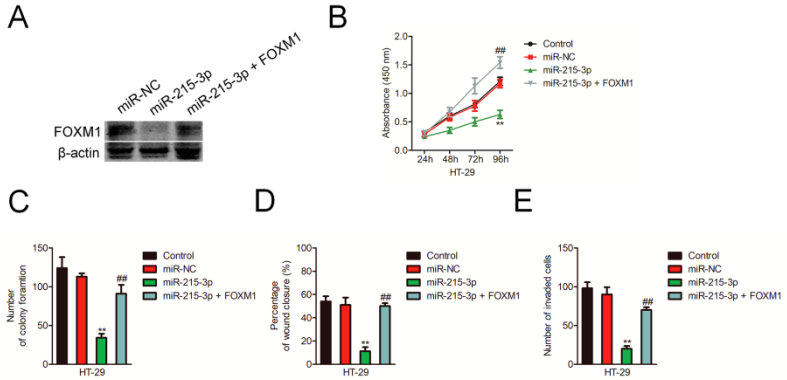
Supplementary Figure 8. Knockdown of FOXM1 inhibits the growth, migration and invasion of HT-29 cell. **A.** Detection of endogenous FOXM1 expression by western blotting after transfection of siCon, siFOXM1 #1 or siFOXM1 #2, respectively, in HT-29 cell. **B.** Effects of transfection of siFOXM1 on the proliferation of HT-29 cell. **C.** Influence of siFOXM1 on the colony formation of HT-29 cell. **D.** Wound healing migration assay of HT-29 cell transfected with siCon or siFOXM1, respectively. **E.** Transwell invasion assay of HT-29 cell transfected with siCon or siFOXM1, respectively. $^{**}P$<0.01 compared to control cell (parental HT-29 cell).



Supplementary Figure 9. BrdU incorporation assay of normal colonic epithelial cell line HCoEpiC (A), SW480 (B) and HT-29 (C) transfected with siCon or siFOXM1. The bar graph represents the average percentage (%) of BrdU positive cells.



Supplementary Figure 10. HCoEpiC, HT-29 and SW480 cells were transfected with miR-215-3p or cotransfected with miR-215-3p and pcDNA3.1 containing FOXM1. BrdU incorporation assay of normal colonic epithelial cell line HCoEpiC (A), SW480 (B) and HT-29 (C). The bar graph represents the average percentage (%) of BrdU positive cells.



Supplementary Figure 11. Re-expression of FOXM1 reverses miR-215-3p-dependent phenotypes. **A.** HT-29 cells were cotransfected with pcDNA3.1 containing FOXM1 or control vector after transfection of miR-NC or miR-215-3p. The expression of FOXM1 was assessed using western blotting assay. **B.** The proliferation of SW480 cell was assessed using the MTT assay. **C.** Colony formation assays for HT-29 cells cotransfected with pcDNA3.1 containing FOXM1 or control vector after transfection of miR-NC or miR-215-3p. **D.** Wound healing analysis of HT-29 cells co-transfected with NC or miR-215-3p mimics together with either pcDNA3.1-FOXM1 or control vector, respectively. **E.** Invasion assays of HT-29 cells co-transfected with NC or miR-215-3p mimics together with either pcDNA3.1-FOXM1 or control vector, respectively. $^{**}P<0.01$ compared to control cell (parental SW480 cell), $^{\#\#}P<0.01$ compared to HT-29 transfected with miR-215-3p.

Supplementary Table 1. Relationship of miR-215-3p and clinicopathologic features.

Variables	**MiR-215-3p**	
	High (n)	Low (n)
Age (years)		
<50	11	20
>50	7	10
Gender		
Male	18	10
Female	8	12
Tumor location		
right hemicolon	11	7

left hemicolon	13	7
rectal	4	6
Tumor size		
<4	11	6
4-6	6	13
⩾6	5	7
LNM		
Yes	11	5
No	10	22
Tumor grade		
Low	16	10
High	8	14
TNM stage		
I-II	11	7
III-IV	14	16