# Quercetin inhibits the tumorigenesis of colorectal cancer cells through downregulation of hsa_circ_0006990

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**Primary research**

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## Abstract

# Background

This study was intended to investigate the function of Quercetin in chemoresistant colorectal cancer (CRC) cells. In addition, this research aimed to explore the mechanism by which Quercetin regulates the malignant behavior of CRC cells.

# Methods

To induce THP-1 cells into M2 tumor-associated macrophages (M2-TAMs), THP-1 cells were stimulated by PMA and IL-4. MDC staining was used to investigate the autophagy in M2-TAMs. Meanwhile, cell proliferation was tested by colony formation assay. In addition, wound healing and transwell assay were performed to detect the cell migration and invasion, respectively. Dual luciferase assay was used to investigate the correlation between hsa_circ_0006990 and miR-132-3p/miR-532-3p. Furthermore, mRNA and protein levels were detected by RT-qPCR and western blot, respectively.

# Results

Quercetin suppressed autophagy of M2-TAMs. In addition, M2-TAMs significantly inhibited the apoptosis and promoted the proliferation of CRC cells, while this phenomenon was reversed by Quercetin. Meanwhile, the expression of hsa_circ_0006990 in CRC cells was decreased by M2-TAMs, while Quercetin reversed this phenomenon. Furthermore, overexpression of hsa_circ_0006990 significantly reversed the anti-tumor effect of Quercetin on CRC.

# Conclusion

Quercetin inhibited the tumorigenesis of colorectal cancer cells through downregulation of hsa_circ_0006990. Thus, our study might shed new lights on exploring the new strategies against CRC.

## Introduction

Colorectal cancer (CRC) ranks third among the most commonly diagnosed malignant tumors in females [1]. Genetic mutations, inflammation-pertinent malfunctions and bad habits could contribute to the progression of CRC [2, 3]. Despite great efforts have been made for the treatment of CRC, the outcomes remain not ideal [4]. Therefore, it is urgent to explore new strategies against CRC.

Quercetin is a natural bioflavonoid compound extracted from hippophase rhamnoides, and it could significantly inhibit the progression of multiple cancers. For example, Mohammed HA et al found Quercetin could induce the apoptosis and inhibit the invasion of breast cancer cells [5]; Soofiyani SR et al indicated that Quercetin could act as a novel agent for the treatment of lymphoma [6]. Meanwhile, Quercetin could

inhibit the growth of CRC cells [7]. However, the detailed mechanism by which Quercetin regulates the progression of CRC remains largely unknown.

Tumor associated macrophages (TAMs) stands up 30%-50% of total cells in tumor tissues [8, 9], and it TAMs could promote the metastasis and lymphangiogenesis of malignant tumors through recruiting abundant monocytes into tumor matrix [10, 11]. For instance, TAMs-secreted epidermal growth factor (EGF), PDGF, TGF-β and basic fibroblast growth factor (bFGF) were expected to increase the growth and invasion of tumor cells [12]. More importantly, TAMs might exhibit the anti-tumor function by preventing macrophages from converting into type II TAMs (M2-TAMs) [13–15]. Meanwhile, it has been reported that inhibition of autophagy could inhibit the polarization of M2-TAMs [16], and autophagy of TAMs could affect the biological activity of CRC cells [17]. Thus, TAM autophagy might affect the progression of CRC by regulation of TAM polarization [18]. Nevertheless, the detailed correlation between Quercetin and TAM autophagy in the progression of CRC remains unclear.

Circular RNAs (circRNAs) are endogenous RNAs which have stable closed structure [19]. In addition, it has been reported that circRNAs could modulate the cellular process (cell proliferation, apoptosis, autophagy, et al) [20, 21]. Meanwhile, circRNAs could regulate the growth of CRC cells through sponging miRNAs [22, 23]. For example, hsa_circ_0026344 positively regulated the deterioration of CRC through sponging miR-21/miR-31 [24]. However, circRNAs that involved in Quercetin-mediated CRC progression need to be further explored.

Based on the above backgrounds, we sought to investigate the correlation among Quercetin, TAMs and circRNAs in the progression of CRC. We hope this study would supply a novel strategy for the treatment of CRC.

## Materials And Methods

# Cell culture

THP-1 cells were obtained from ATCC (USA). Cells were cultured in an incubator at 37 ℃ and 5% $CO_2$. THP-1 cells were maintained in RPMI 1640 medium containing 10% fetal bovine serum (FBS) and 0.05 mmol/L β-mercaptoethanol. CRC cell lines (HCT116 and Lovo) were purchased from Shanghai Cell Bank of Chinese Academy of Sciences. They were seeded in RPMI-1640 medium supplemented with 10% FBS. CRC cells were cultured in the condition of 37°C and 5% $CO_2$.

# Induction and collection of M2-TAMs

THP-1 cells were seeded in 6-well plates at the density of $1\times10^6$/ml. After treated with 100 ng/mL PMA for 6 h, THP-1 cells were added with 20 ng/mL IL-4 for another 18 h. After that, THP-1 cells were cultured with 2 ml fresh serum-free medium for 24 h. Then, the supernatant was collected to be frozen at -80 ℃ for later use. The expressions of CD68, CD163 and CD206 were identified by flow cytometry.

# Treatment of M2-TAMs with quercetin

Quercetin (Sigma, USA) was dissolved in dimethyl sulfoxide (DMSO), and then stored at -20 ℃ for later use. Subsequently, M2-TAMs were treated with Quercetin.

# Co-culture of M2-TAMs and CRC cell lines

CRC cells at the logarithmic growth phase were seeded into 6-well culture plates at the density of $1\times10^5$/ml, and M2-TAMs were seeded into the upper transwell chamber (semipermeable membrane pore size: 0.4 μm, model: 3450, Costar) at the density of $2\times10^5$/ml. Then, CRC cells were treated with the chamber. After 48 h of incubation, total RNAs and proteins were extracted from CRC cells.

# Monodansylcardeverine (MDC) staining

MDC staining was performed to determine autophagic vesicle (AV) of M2-TAMs according to the previous reference [25].

# Cell transfection

CRC cells were seeded into 6-well plates. When the confluence reached 40%, cells were transfected by si-has_circ_0006990/NC, miR-132-3p inhibitor/mimic, miR-342-3p inhibitor/mimic or miR-NC for 48 h using Lipofectamine 2000.

# Colony formation assay

CRC cells were seeded into 6-well plates for 12 days. After that, cells were fixed with 4% paraformaldehyde for 15 min and stained with Giemsa solution for 20 min after rinsed with PBS. Finally, the data was calculated.

# TUNEL assay

Cells were washed and permeabilized. Then, TUNEL reaction mixtures (50 μl) were used to incubate the cells for 60 min with no light. After that, peroxidase (POD, 50 μl) was used to incubate the slides for 30 min at 37°C. Then, cells were rinsed with PBS, and then diaminobenzidine (DAB, 50 μl) substrate solution was used to incubate the cells for 10 min. Finally, the expression of apoptotic cells was observed under an optical microscope.

# Wound healing assay

CRC cell lines ($1.0\times10^5$/ml) were seeded overnight. Then, cells were underlined perpendicular to the cell culture plate with a small pipette head. After washing with PBS 3 times, serum-free medium was used for further culture, and the scratch widths at 0 and 24 h were recorded under an optical microscope.

# Transwell invasion assay

Matrigel (100 μl) was used to pre-treat the upper chamber. CRC cells ($1.0\times10^6$ cells per chamber) were seeded into the upper chamber in medium (1% FBS). In addition, the lower chamber was supplemented with RPMI1640 (10% FBS). Subsequently, the chamber was rinsed and fixed at 4°C. Then, crystal violet (0.1%) was used to stain the chamber for 20 minutes. The data was observed under a microscope after the chamber was washed.

# Western blot

RIPA was applied to extract protein from cell lines. BCA kit was applied to quantify the total protein. SDS-PAGE (10%) was applied to separate the proteins (40 μg per lane), and then proteins were transferred onto PVDF membranes. Subsequently, the membranes were incubated overnight at 4˚C with primary antibodies targeted against: Bcl-2 (1:1,000), Bax (1:1,000), MUC13 (1:1,000), E2F1 (1:1,000) and GAPDH (1:1,000) after blocked with skimmed milk (5%) for 1 h. Following primary incubation, HRP-conjugated secondary antibodies (1:5,000) were used to incubate the membranes for 1 h. ECL kit was used to visualize the protein bands. GAPDH was regarded as internal control. The densitometry analysis was performed by using IPP 6.0 (Image-Pro Plus 6.0).

# Real time polymerase chain reaction (RT-qPCR)

TRIzol® reagent was applied to extract total RNA. PrimeScript RT reagent kit was used in reverse transcription. Then, SYBR premix Ex Taq II kit (Takara) was used in RT-qPCR, and Real-Time qPCRs were used three times: 2 minutes at 94°C, followed by 35 cycles (94°C for 30 s and 55°C for 45 s). Primers of circRNAs and miRNAs were listed in Table 2 and Table 3. The data were quantified using $2^{-\Delta\Delta Ct}$ method. GAPDH was regarded as internal control.

Table 2
**Primers of colorectal cancer-relevant circRNAs.**

| CircRNAs | Primers | |
|---|---|---|
| | Sense | Anti-sense |
| hsa_circ_0096088 | 5'-TGGAGGAGCCCAGGTATAAA-3' | 5'-TCCATGTCGGGATCTTCTTC-3' |
| hsa_circ_0006990 | 5'-TGAAATGCCCAATGAAAATG3' | 5'-GGCGAGGTGCTGTAGTCTTC-3' |
| hsa_circ_0004380 | 5'-GAATGCGGGAGTGATTGAGT-3' | 5'-TCTTAGCACGTCCGATCTCA-3' |
| hsa_circ_0001946 | 5'-CATGTCTTCCAACGTCTCCA-3' | 5'-CTGGAAGACCCGGAGTTGT-3' |

Table 3
**Primers of hsa_circ_0006990-sponged miRNAs.**

| MiRNAs | Primers | |
|---|---|---|
| | Sense | Anti-sense |
| hsa-miR-3611 | 5'-GCGGCGGTTGTGAAGAAAGAAA-3' | 5'-ATCCAGTGCAGGGTCCGAGG-3' |
| hsa-miR-377-3p | 5'-GCGGCGGATCACACACAAAGGC-3' | 5'-ATCCAGTGCAGGGTCCGAGG-3' |
| hsa-miR-342-3p | 5'-GCGGCGGTCTCACACAGAAATC-3' | 5'-ATCCAGTGCAGGGTCCGAGG-3' |
| hsa-miR-132-3p | 5'-GCGGCGGTAACAGTCTACAGCC-3' | 5'-ATCCAGTGCAGGGTCCGAGG-3' |

# Dual luciferase reporter gene assay

Hsa_circ_0006990 containing the binding sites of miR-132-3p/miR-342-3p was cloned into the pmirGLO vectors for establishment of hsa_circ_0006990 (WT/MT). Hsa_circ_0006990 (WT/MT) was transfected into CRC cells with miR-132-3p/miR-342-3p/NC mimics using Lipofectamine 2000. Dual-Glo Luciferase Assay System was used to analyze the result.

3'-UTR of E2F1/MUC13 containing the putative binding sites of miR-342-3p/miR-132-3p were obtained from Beyotime (Shanghai, China), then were cloned into the pmirGLO vectors to construct wild type/mutant type vectors E2F1/MUC13 (WT/MT). E2F1/MUC13 (WT/MT) was transfected into cells using Lipofectamine 2000 (Thermo Fisher Scientific). Dual-Glo Luciferase Assay System was used to analyze the result.

## Statistical analyses

Three independent experiments were performed in each group. In addition, the mean ± standard deviation (SD) was used to express all data. The comparisons between two groups were analyzed using Student's t-test, and the differences between multiple groups (more than 2 groups) were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test (Graphpad Prism7). $P<0.05$ indicates a significant change.

# Results

## Quercetin suppressed the polarization of M2-TAMs via inhibiting the autoiphagy

In order to induce THP-1 cells into M2-TAMs, THP-1 cells were treated with PMA and IL-4. As revealed in Figure 1A, cells exhibited more pseudopods and round-shaped after 48 h of treatment. (Figure 1A). This phenomenon was in consistent with the feature of M2-TAMs [26]. Furthermore, the percentage of CD68, CD163 and CD206 in THP-1 cells was significantly increased by PMA and IL-4 (Figure 1B). These data indicated that THP-1 cells were successfully induced into M2-TAMs.

Additionally, the data of MDC staining revealed that autophagy of M2-TAMs was promoted by rapamycin, while it was obviously inhibited by bafilomycina1 and quercetin (Figure 1C). Meanwhile, bafilomycin A1 and Quercetin notably decreased the percentage of CD163 and CD206 (Figure 1D). Taken together, Quercetin suppressed autophagy of M2-TAMs and induced the differentiation into M1-TAMs.

## Quercetin significantly reverses M2-TAMs-induced proliferation of CRC cells via inhibiting the autophagy

CRC cells were co-cultured with M2-TAMs. Then, colony formation assay was performed. The data revealed that proliferation of CRC cells was significantly promoted by M2-TAMs, which was further increased by rapamycin (Figure 2A). In contrast, M2-TAMs-induced CRC cell proliferation was significantly reversed in the presence of Bafilomycin A1 or Quercetin (Figure 2A). Moreover, Quercetin or Bafilomycin A1 greatly induced the apoptosis of M2-TAMs-treated CRC cells (Figure 2B). Meanwhile, M2-TAMs significantly inhibited the level of Bax and upregulated the expression of Bcl-2 in CRC cells, while this phenomenon was greatly

restored by Bafilomycin A1 or Quercetin (Figure 2C and 2D). In summary, Quercetin significantly reversed M2-TAMs-induced proliferation of CRC cells via inhibiting the autophagy.

# Quercetin significantly restores M2-TAMs-induced migration and invasion of CRC cells via inhibiting the autophagy

To investigate the cell migration, wound healing assay was used. As shown in Figure 3A, M2-TAMs significantly promoted the migration of CRC cells, while the effect of M2-TAMs was obviously reversed by Bafilomycin A1 or Quercetin. Consistently, M2-TAMs-indcued CRC cell invasion was notably inhibited by Bafilomycin A1 or Quercetin (Figure 3B). To sum up, Quercetin significantly restored M2-TAMs-induced migration and invasion of CRC cells via inhibiting the autophagy.

# Quercetin could regulate hsa_circ_0006990 in M2-TAMs-induced CRC cells

The differentially expressed circRNAs in CRC were presented in Table 1 and Supplementary Figure 1A. Among these differentially expressed circRNAs, hsa_circ_0006990 was reported to regulate the tumorigenesis of CRC [27]. Thus, hsa_circ_0006990 was selected in our research. Furthermore, downstream miRNAs of hsa_circ_0006990, as predicted by bioinformatics tool (https://circinteractome.nia.nih.gov/rna_binding_protein.html), were also measured in CRC cells (Supplementary Figure 1B). It was indicated that miR-132-3p and miR-342-3p were both downregulated in CRC cells co-cultured with M2-TAMs. In addition, quercetin and Bafilomycin A1 reversed the effect of M2-TAMs on miR-132-3p and miR-342-3p levels (Supplementary Figure 1B). Based on the above results, miR-132-3p and miR-342-3p were selected in our study.

Table 1
**Screening of oncogenic circRNAs in CRC**

| CircRNAs | Gene symbol | Position* | Genomic length* | Spliced length* | Expression change in CRC | Reference |
|---|---|---|---|---|---|---|
| hsa_circ_0096088 | MACROD1 | chr11:63918710-63919865 | 1155 | 219 | ↑ | [PMID: 32564659] |
| hsa_circ_0006990 | VAPA | chr18:9931806-9937063 | 5257 | 338 | ↑ | [PMID: 32564659] |
| hsa_circ_0004380 | TRAPPC9 | chr8:141407718-141415797 | 8079 | 248 | ↑ | [PMID: 32564659] |
| hsa_circ_0001946 | CDR1 | chrX:139865339-139866824 | 1485 | 1485 | ↑ | [PMID: 32508871] |
| *CircBase (http://circrna.org/). | | | | | | |

The level of miR-132-3p/miR-342-3p in CRC cells was significantly upregulated by miR-132-3p/miR-342-3p mimics but inhibited by miR-132-3p/ miR-342-3p inhibitor (Supplementary Figure 2A and 2B). Meanwhile,

the luciferase activity in WT-hsa_circ_0006990 was significantly reduced by miR-132-3p/miR-342-3p mimics (Supplementary Figure 2C and 2D). Furthermore, the expression of miR-132-3p or miR-342-3p in CRC cells was negatively regulated by hsa_circ_0006990 (Supplementary Figure 2E and 2F).

# Quercetin inhibited the proliferation and invasion of CRC cells via mediation of hsa_circ_0006990

To investigate the effect of Quercetin on hsa_circ_0006990 expression, RT-qPCR was performed. As demonstrated in Figure 4A, Quercetin significantly inhibited the level of hsa_circ_0006990 in CRC cells. In addition, the inhibitory effect of Quercetin on CRC cell proliferation was significantly rescued by pcDNA3.1-hsa_circ_0006990 (Figure 4B). Consistently, overexpression of hsa_circ_0006990 significantly reversed Quercetin-induced cell apoptosis (Figure 4C). Furthermore, the migration and invasion of CRC cells was significantly inhibited by Quercetin, which was obviously restored by pcDNA3.1-hsa_circ_0006990 (Figure 5A and 5B). To sum up, Quercetin inhibited the proliferation and invasion of CRC cells via mediation of hsa_circ_0006990.

# MiR-342-3p directly targets E2F1 in CRC cells

To explore the downstream mRNA of miR-342-3p, targetscan was used. As revealed in Figure 6A, miR-342-3p had binding sites with E2F1, and the luciferase activity in WT-E2F1 was notably decreased by miR-342-3p mimics (Figure 6B). Meanwhile, miR-342-3p could negatively regulate the level of E2F1 in CRC cells (Figure 6C). Furthermore, the effect of hsa_circ_0006990 silencing on E2F1 level was markedly reversed by miR-342-3p inhibitor (Figure 6D). Altogether, MiR-342-3p directly targets E2F1 in CRC cells.

# MiR-132-3p directly targets MUC13 in CRC cells

To explore the downstream mRNA of miR-132-3p, targetscan was used. As revealed in Figure 7A, miR-132-3p had binding sites with MUC13, and the luciferase activity in WT-MUC13 was notably decreased by miR-132-3p mimics (Figure 7B). Meanwhile, miR-132-3p could negatively regulate the level of MUC13 in CRC cells (Figure 7C). Furthermore, the effect of hsa_circ_0006990 silencing on MUC13 level was markedly reversed by miR-132-3p inhibitor (Figure 7D). Altogether, MiR-132-3p directly targets MUC13 in CRC cells.

## Discussion

Quercetin has been reported to inhibit the progression of malignant tumors (including breast cancer, ovarian cancer and gastric cancer) [28–30]. Consistently, we found Quercetin could inhibit the progression of CRC. Moreover, this research revealed Quercetin could inhibit the M2 polarization of macrophages, and it could inhibit the autophagy. Thus, this study firstly explored the function of Quercetin in M2-TAMs and autophagy during the progression of CRC, suggesting that Quercetin could act as an inhibitor in M2-TAMs.

It has been confirmed that M2-TAMs could play a vital role in tumor microenvironment [31, 32]. For instance, cytokines generated by M2-TAMs (including TNF-α and TGF-ß) could induce the angiogenesis in breast cancer [33]; the activation of M2-TAMs could lead to the poor prognosis among patients with tumors

(lymphoma, breast cancer, et al) [34, 35]. Consistently, our data found that Quercetin could inhibit the tumorigenesis of CRC via inhibiting the polarization of M2 macrophages. On the other hand, it has been reported that autophagy activation could lead to the polarization of macrophages [36, 37]. Our research indicated that autophagy could induce the polarization of M2 macrophages, and Quercetin could inhibit the autophagy in M2 macrophages. Thus, it could be suggested that Quercetin could inhibit the polarization of M2 macrophages via inhibiting the autophagy. Notably, the mechanism by which Quercetin regulates the autophagy remains unclear, and it is needed to be further explored in future.

This study found that hsa_circ_0006990 was upregulated in CRC cells co-cultured with M2-TAMs. It has been indicated that hsa_circ_0006990 might acts as a promoter in CRC [27]. Thus, our data was in consistent to this previous study. In addition, our study firstly explored the relation between Quercetin and hsa_circ_0006990 in CRC, suggesting that Quercetin could inhibit the tumorigenesis of CRC via downregulation of hsa_circ_0006990. Meanwhile, the detailed correlation among hsa_circ_0006990, M2-TAMs and autophagy remains further investigating.

MiR-342-3p and miR-132-3p were found to be sponged by hsa_circ_0006990 in this study. Thus, our research firstly explored the relation between hsa_circ_0006990 and miR-342-3p/miR-132-3p in CRC. In addition, miR□342□3p/E2F1 and miR-132/MUC13 were also found to be involved in Quercetin-mediated CRC progression. It has been reported that miR□342□3p could inhibit the progression of lung cancer [38], prostate cancer [39], osteosarcoma [40], glioma [41] and CRC [42, 43]. In addition, downregulation E2F1 was reported to enhance the sensitivity of CRC cells to oxaliplatin treatment [44]. Based on these backgrounds, it could be concluded that Quercetin significantly inhibited the tumorigenesis of CRC via mediation of hsa_circ_0006990/miR-342-3p/E2F1 axis. On the other hand, miR-132-3p was reported to act as an inhibitor in cancer progression. In detail, miR-132-3p could inhibit the progression of lung cancer [45], glioma [46], pituitary tumor [47], CRC [48] and bladder cancer [49]. More importantly, high expressed miR-132-3p could sensitize CRC cells to adriamycin treatment [50]. Besides, MUC13 was identified to be the downstream of miR-132, and it was confirmed to be upregulated in CRC tissues [51]. Thereby, our data confirmed that Quercetin notably suppressed the progression of CRC via mediation of hsa_circ_0006990/miR-132-3p/MUC13 axis

In conclusion, Quercetin could inhibit the tumorigenesis of CRC via inhibiting the polarization of M2 macrophages and downregulating hsa_circ_0006990. Thus, our study might shed new lights on exploring the new methods against CRC.

# Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Availability of data and material

All data generated or analyzed during this study are included in this article.

### Competing interests

The authors declare that they have no competing interests.


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### Authors' contributions

BC, HX and LW made major contributions to the conception, design and manuscript drafting of this study. TW, SW, HY, GW, MX and XH were responsible for data acquisition, data analysis, data interpretation and manuscript revision. WX, RZ and WD made substantial contributions to conception and design of the study and revised the manuscript. All authors agreed to be accountable for all aspects of the work. All authors read and approved the final manuscript.


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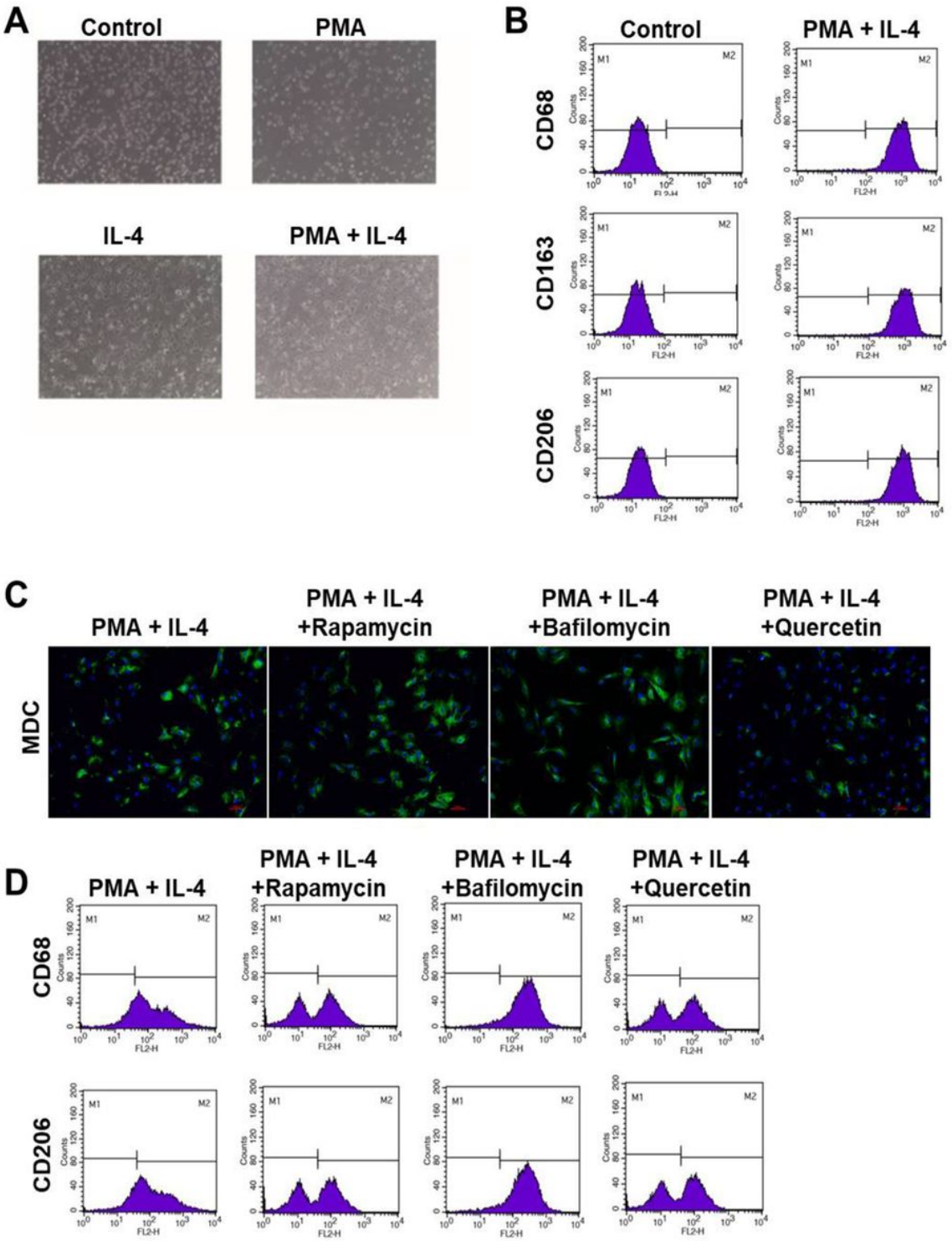
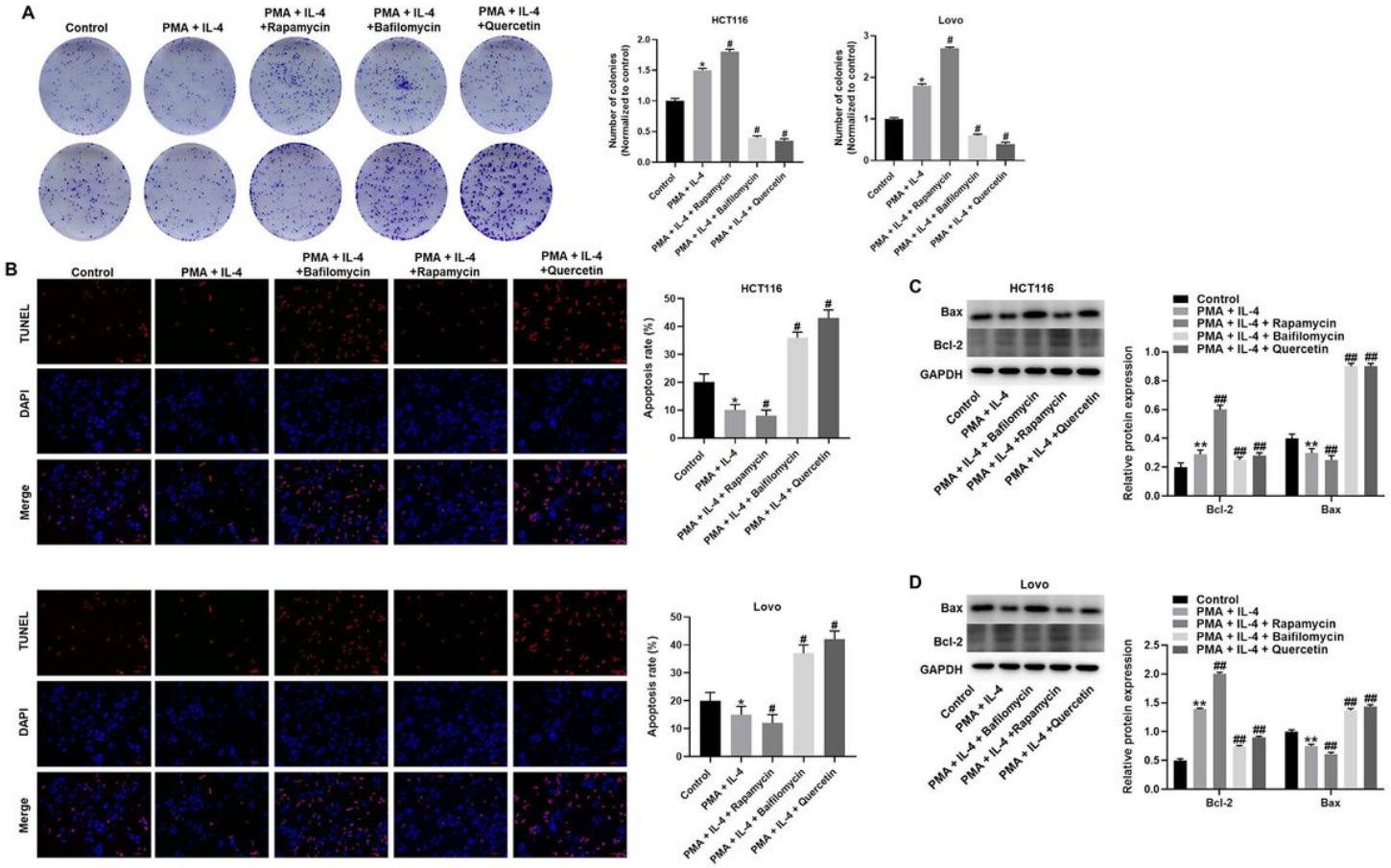
# Figures



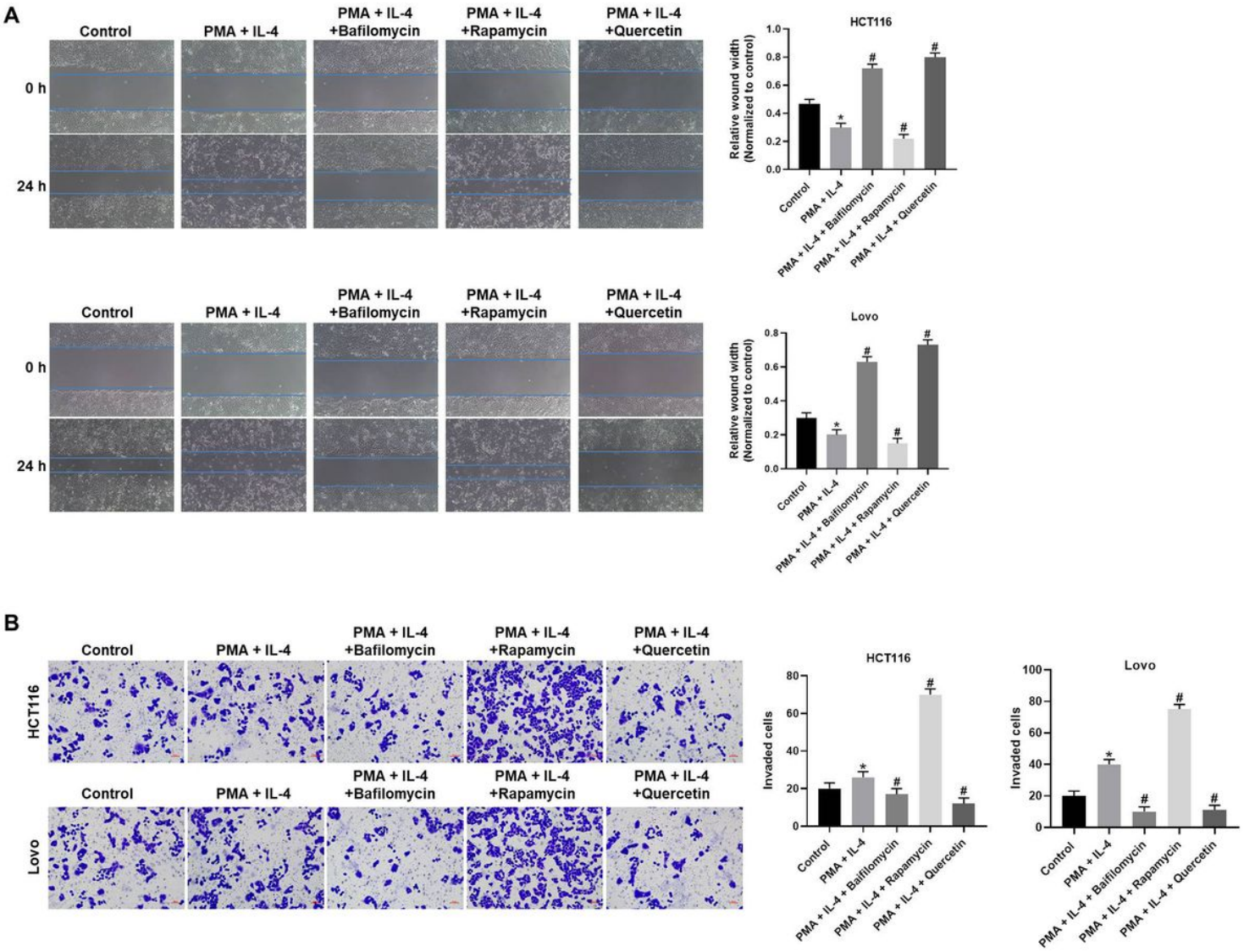

## Figure 1

Quercetin suppressed the polarization of M2-TAMs via inhibiting the autoiphagy. (A) THP-1 cells were treated with PMA and IL-4. THP-1 cells were observed by a light microscope. (B) The percentage of CD68, CD163 and CD206 in THP-1 cells were determined by flow cytometry. (C) M2-TAMs were treated with Rapamycin, Bafilomycin A1 (Bafilomycin) or Quercetin. The autophagy of M2-TAMs was observed by MDC staining. (D) The percentage of CD68 and CD206 in M2-TAMs was measured by flow cytometry.



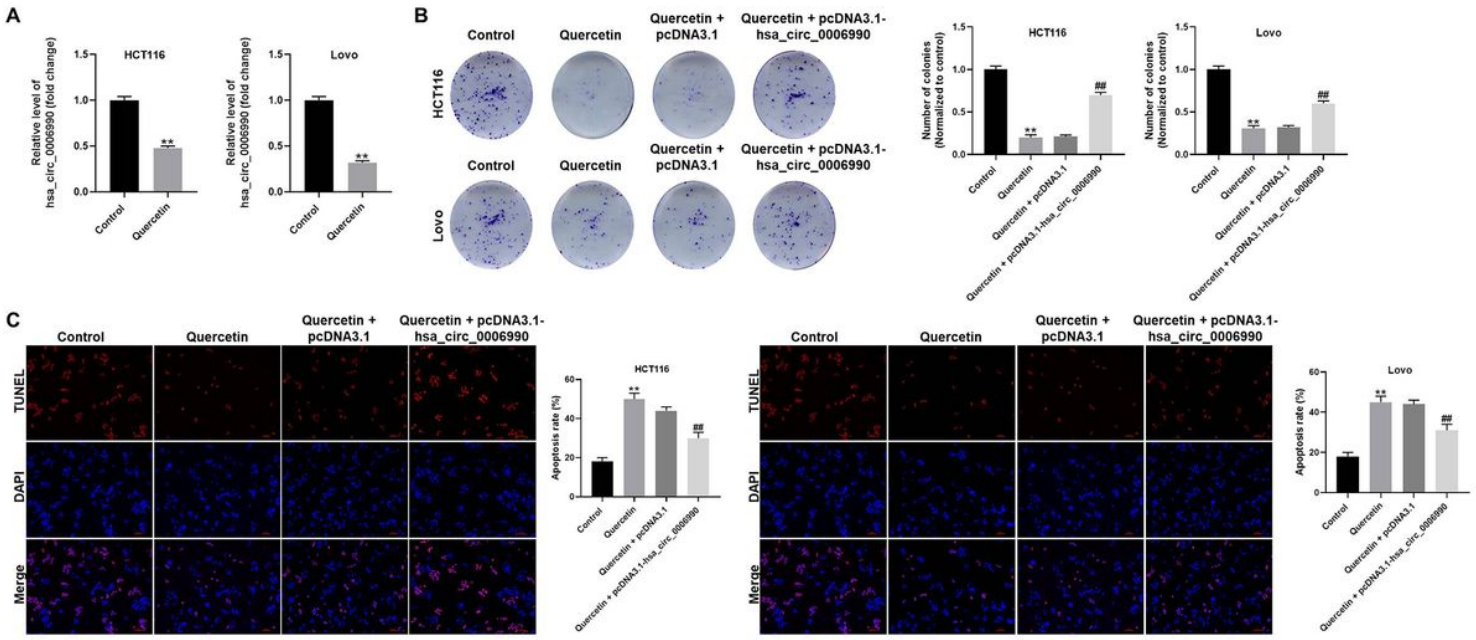

## Figure 2

Quercetin significantly reverses M2-TAMs-induced proliferation of CRC cells via inhibiting the autophagy. THP-1 cells were treated with PMA and IL-4. Then, CRC cells were co-cultured with M2-TAMs (THP-1 cells treated with PMA and IL-4). (A) The proliferation of CRC cells was measured by colony formation assay. (B) The apoptosis of CRC cells was tested by TUNEL staining. (C, D) The protein levels of Bax and Bcl-2 in CRC cells were investigated by western blot. GAPDH was used for normalization. *P<0.05, **P<0.01 compared to control. #P<0.05, ##P<0.01 compared to PMA + IL-4.



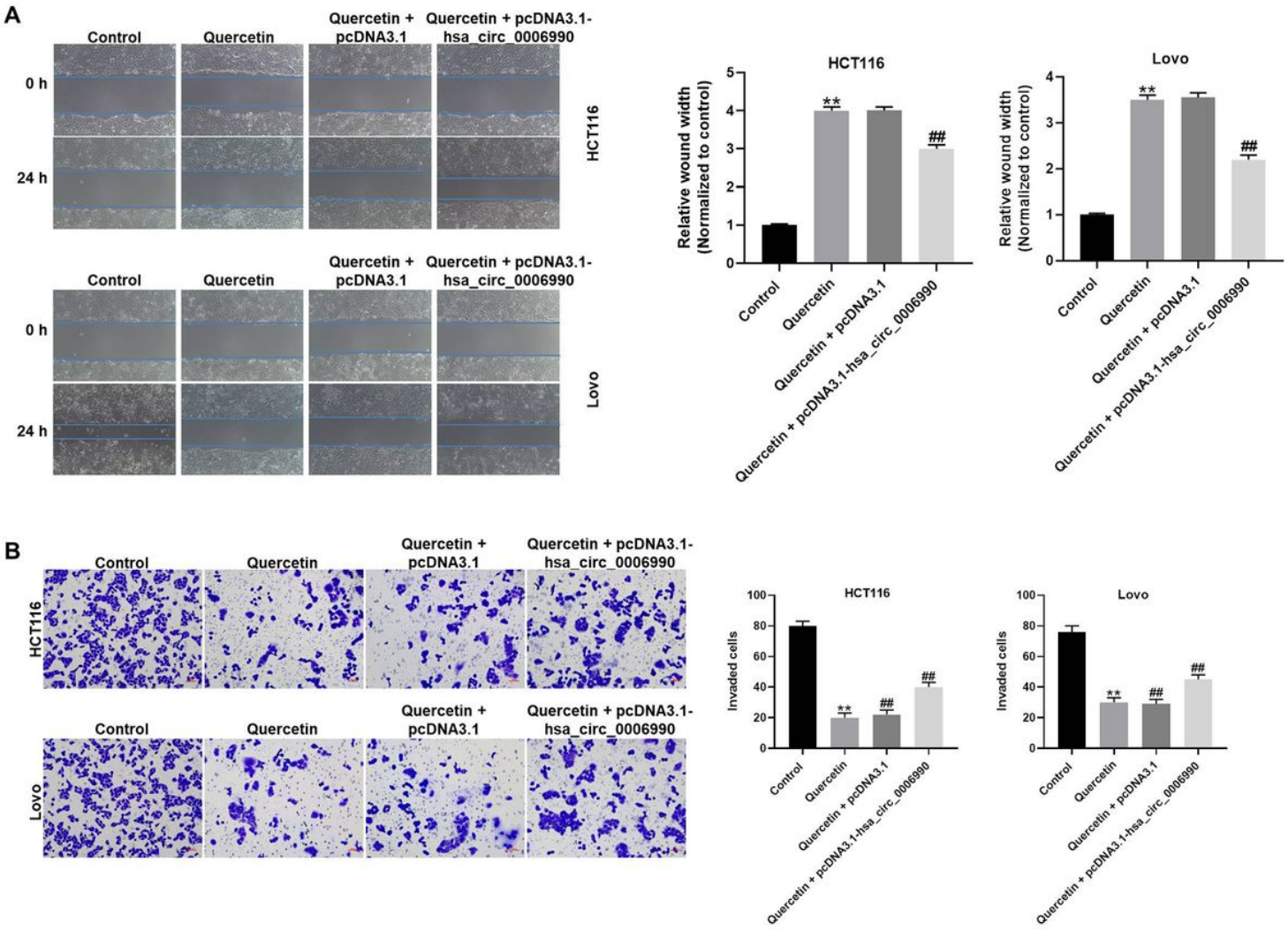

## Figure 3

Quercetin significantly reverses M2-TAMs-induced CRC cell invasion via inhibiting the autophagy. (A) The migration of CRC cells was tested by wound healing assay. (B) The invasion of CRC cells was measured by transwell assay. *P<0.05 compared to control. #P<0.05 compared to PMA + IL-4.



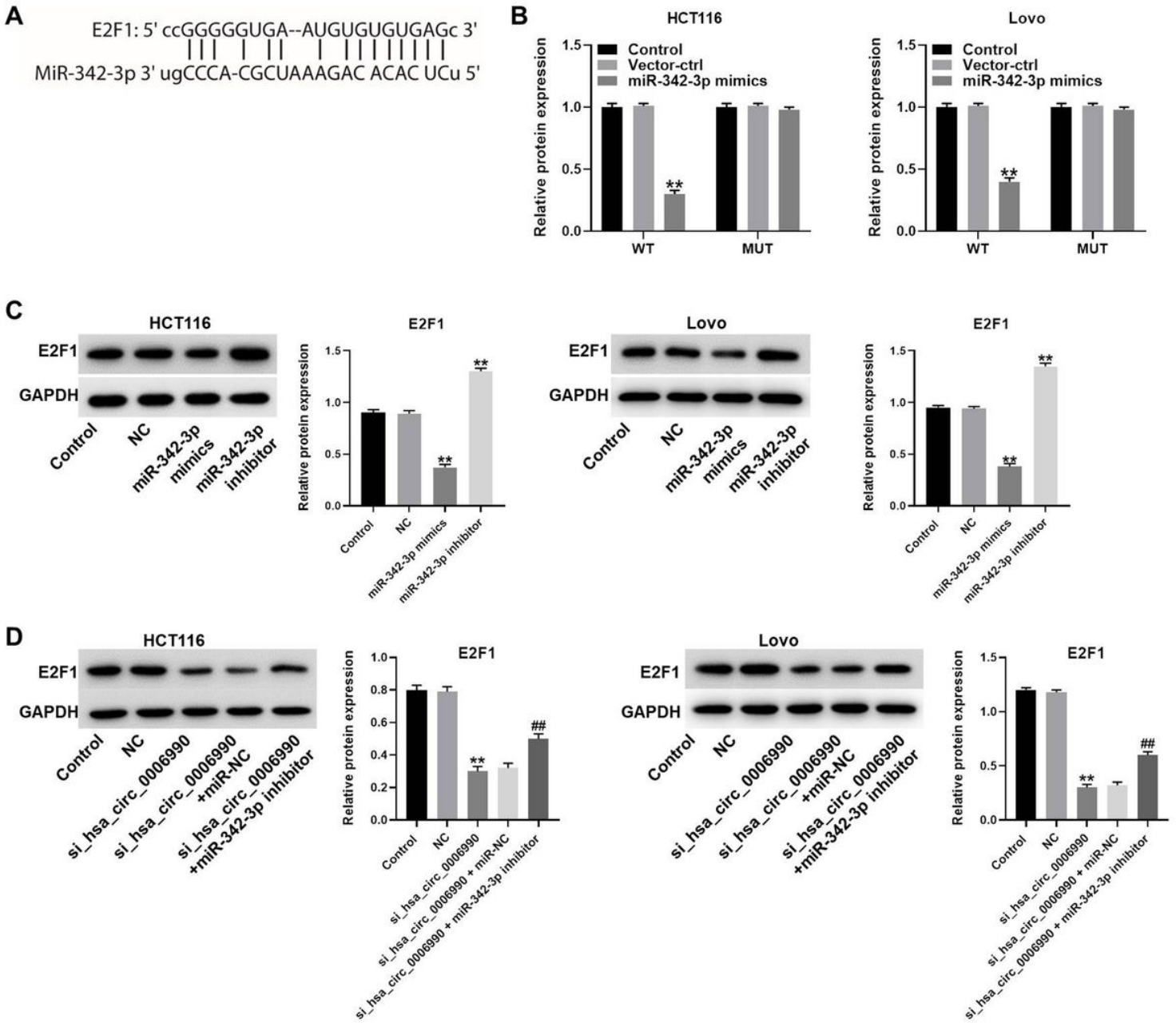

## Figure 4

Quercetin inhibited the proliferation of CRC cells via mediation of hsa_circ_0006990. (A) CRC cells were treated with Quercetin. Then, the expression of hsa_circ_0006990 in CRC cells was investigated by RT-qPCR. (B) The proliferation of CRC cells was tested by colony formation assay. (C) The apoptosis of CRC cells was tested by TUNEL staining. **P<0.01 compared to control. ##P<0.01 compared to Quercetin.



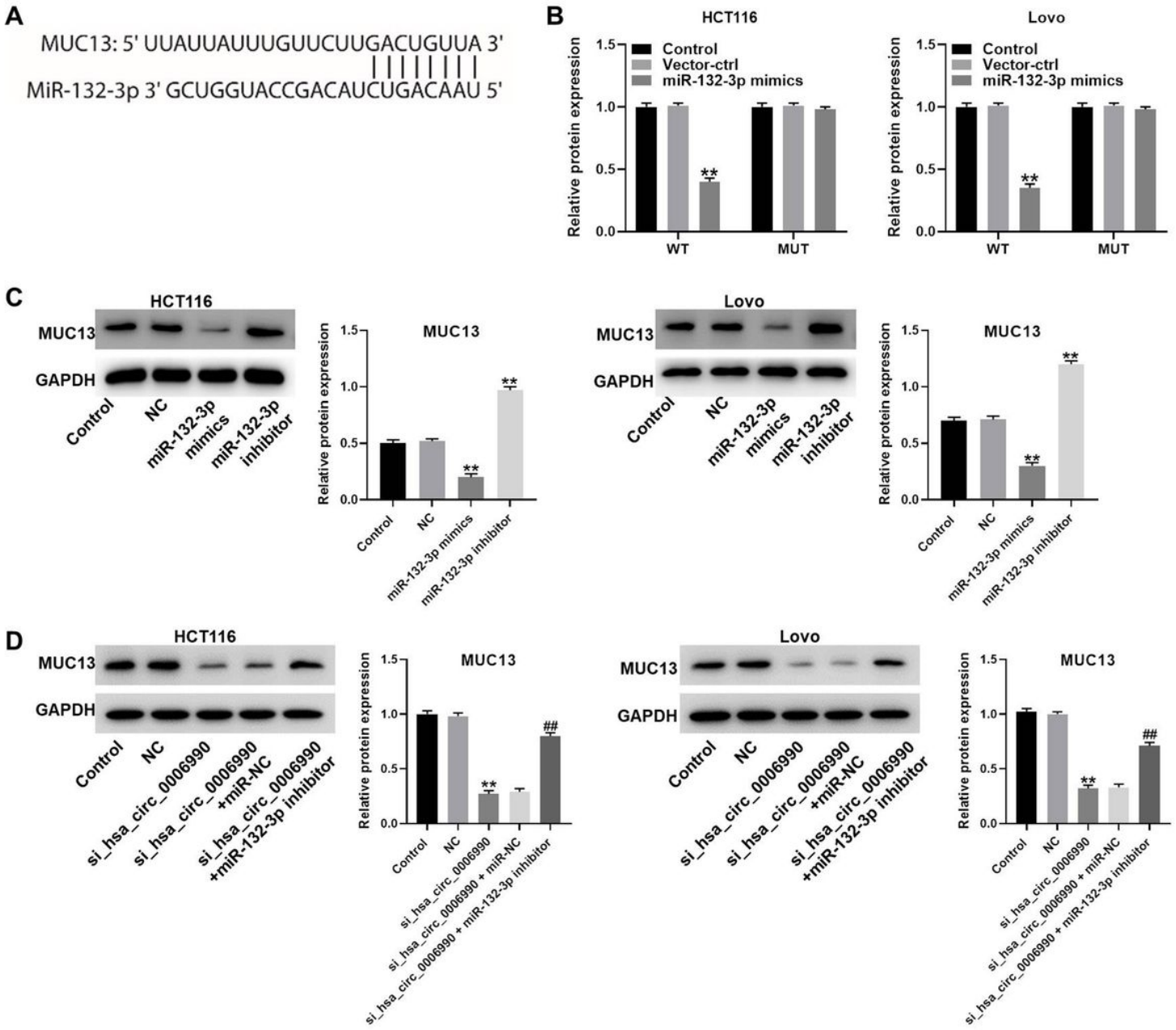

## Figure 5

Quercetin inhibited the migration and invasion of CRC cells via mediation of hsa_circ_0006990. (A) The migration of CRC cells was tested by wound healing assay. (B) The invasion of CRC cells was tested by transwell assay. **P<0.05 compared to control. ##P<0.01 compared to Quercetin.




## Figure 6

MiR-342-3p directly targets E2F1 in CRC cells. (A) The downstream target of miR-342-3p was predicted by targetscan. (B) The relative luciferase activity in WT/MUT-E2F1 was tested by dual luciferase assay. (C) CRC cells were transfected with miR-342-3p mimics/inhibitor. Then, the protein level of E2F1 in CRC cells was investigated by western blot. GAPDH was used for normalization. (D) CRC cells were treated with NC, si-hsa_circ_0006990 or si-hsa_circ_0006990 + miR-342-3p inhibitor. Then, the protein level of E2F1 in CRC cells was investigated by western blot. GAPDH was used for normalization. **P<0.01 compared to control. ##P<0.01 compared to si-hsa_circ_0006990.




## Figure 7

MiR-132-3p directly targets MUC13 in CRC cells. (A) The downstream target of miR-132-3p was predicted by targetscan. (B) The relative luciferase activity in WT/MUT-MUC13 was tested by dual luciferase assay. (C) CRC cells were transfected with miR-132-3p mimics/inhibitor. Then, the protein level of MUC13 in CRC cells was investigated by western blot. GAPDH was used for normalization. (D) CRC cells were treated with NC, si-hsa_circ_0006990 or si-hsa_circ_0006990 + miR-132-3p inhibitor. Then, the protein level of MUC13 in CRC cells was investigated by western blot. GAPDH was used for normalization. **P<0.01 compared to control. ##P<0.01 compared to si-hsa_circ_0006990.

# Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigure1.jpg
- SupplementaryFigure2.jpg