

# MicroRNA-29a Inhibits the Occurrence of Endometrial Carcinoma by Regulating mTOR Signal Pathway Through STAT3

**Yaling Wu**

Tianjin Medical University General Hospital

**Huihui Li**

Tianjin Medical University General Hospital

**Xue Li**

Tianjin Medical University General Hospital

**Jin Luo**

Shanxi Provincial Peoples Hospital

**Yingmei Wang** (✉ [wangyingmei@tmu.edu.cn](mailto:wangyingmei@tmu.edu.cn))

Shanxi Provincial Peoples Hospital <https://orcid.org/0000-0001-9883-5054>

---

## Research

**Keywords:** STAT3, apoptosis, miR-29a, endometrial carcinoma, signal pathway

**Posted Date:** March 22nd, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-313495/v1>

**License:**   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

# Abstract

**Background:** As a tumor suppressor, miR-29a is involved in the progression of multiple tumors. Its specific role in human endometrial carcinoma (EC) cells remains unclear.

**Methods:** The cells with the overexpression and knockdown of miR-29a were constructed, their effects on EC cell lines were detected, and the binding sites of miR-29a and STAT3 were analyzed. Cell viability was detected by CCK-8 and colony formation assay. The apoptosis was detected by flow cytometry.

**Results:** miR-29a inhibitors increased the survival rate of EC cells, thus inhibiting the apoptosis. miR-29a inhibitors can also promote AKT/mTOR signal pathway. miR-29a mimics lead to diametrically opposite results. The data set analyses showed that STAT3 may be the downstream target of miR-29a. miR-29a inhibited the expression of STAT3 in EC cells. STAT3 promoted cell viability and inhibited apoptosis by activating AKT/mTOR signal pathway. miR-29a mimics can block the proliferation of EC cells induced by STAT3 overexpression and the activation of AKT/mTOR signal pathway.

**Conclusions:** miR-29a inhibits cell growth by targeting STAT3 and inhibiting AKT/mTOR signaling pathway. miR-29a/STAT3/AKT/mTOR may become a new target axis of clinical treatment.

## Background

Endometrial cancer (EC) is the fourth most common malignant tumor in women [1]. In 2018, there were an estimated 63230 new cases and 11350 deaths in the United States [2]. According to the clinicopathological features and pathogenesis, EC is divided into estrogen-dependent type (type I) and non-estrogen-dependent type (type II) [3]. Several studies have defined IHC and/or mutation maps to help distinguish endometrial cancer subtypes and further classify risk categories [4–5]. It is reported that the 5-year relative survival rate of endometrial cancer is about 82%. The mortality rate of endometrial cancer increased by about 2% per year from 2010 to 2015 [6]. There are many factors affecting its prognosis, including tumor differentiation, stage, lymph node metastasis and so on, but there is still a lack of effective treatment for advanced EC [7]. All these observations suggested that it was necessary to have a more comprehensive understanding of the molecular genetics of EC. Therefore, elucidating the molecular mechanism of the occurrence and development of EC is of great significance for the new diagnosis, prognosis and / or treatment strategy of the disease.

MicroRNA (miRNAs) are small non-coding RNA molecules with about 22 nucleotides. They bind to the 3'-untranslated region (3'-UTR) of the corresponding mRNA targets and play an important role in gene regulation in animals and plants, resulting in mRNAs decay and translation inhibition [8]. miRNAs are involved in a variety of biological processes, including cancer proliferation, metastasis and other characteristics, and regulate up to 1/3 of human genes at the post-transcriptional level [9]. Although previous studies have found that the differential expression of miRNAs may be related to the occurrence of EC [10–11], the role and possible mechanism of miRNAs in EC is still largely unknown. At present, only a few miRNAs, such as miR-145 [12], miR-181c [13], miR-200a, miR-200b [14], miR-218 [15] and miR-449a

[16], have been studied in the pathogenesis of EC. Little is known about the role of miR-29a in endometrial carcinoma.

In this study, we studied the role of miR-29a in the proliferation of EC cells through overexpression and down-regulation of miR-29a. Subsequently, the molecular mechanism of its action was discussed, and STAT3 was identified as the direct functional target of miR-29a. In addition, we also down-regulated STAT3 to detect the role of STAT3 in the proliferation of EC cells. Our results provided an evidence for the regulation mechanism of miR-29a/STAT3 axis in EC for the first time. Therefore, this study provides a potential candidate target for the treatment of EC.

## Methods And Methods

### Cell lines and transfection

Human EC cell line HHUC was purchased from the Cell Resource Center of Shanghai Institute of Biosciences, Chinese Academy of Sciences. EC cells were stored in DMEM with 10% fetal bovine serum at 37 °C and in 5% CO<sub>2</sub>. miR-29a mimics or inhibitors, STAT3 plasmids (overexpressed STAT3 and STAT3-OE) or shRNA (knockdown of STAT3 and STAT3-KD) were transfected into 6-well plates. Liposome 2000 (Thermo Fisher Science) with untreated HHUC were used as the negative control (NC). miR-29a mimics, miR-29a inhibitors, STAT3 plasmids (pcDNA3.1-STAT3) and STAT3-siRNA were all purchased from Ribobio (RiboBio Co.Ltd., China).

### Cell viability

The activity of EC cells was detected by CCK-8 and colony formation assay. In CCK-8 assay, 1000 cells per well were transfected and inoculated into 96-well plate. 10 microliters of CCK-8 reagent (Promega) was added to the target well every 24 hours. The optical density value (OD value) was measured on a spectrophotometer. In the clone formation assay, 1000 cells/well were transfected and inoculated into 6-well plates. After 2 weeks of culture, the cells were fixed with 4% paraformaldehyde for 15 min and stained with Giemsa for 15 min and observed under microscope.

### Real-time RT-PCR

24 hours after transfection, total RNA was extracted by TRIzol reagent (American Invitgen) and reversely transcribed into cDNA using HiFiScript cDNA synthesis kit (Cowin Biotech Co.Ltd, China).

MiRNA was extracted with miRNA purification kit (China Coven Biotechnology Co., Ltd, China) and reversely transcribed into cDNA with miRNA cDNA synthesis kit (China Coven Biotechnology Co., Ltd, China). Using UltraSYBR mixture and miRNA qPCR test kit (China Coven Biotechnology Co., Ltd, China), qPCR was carried out according to the manufacturer's plan. The relative expression of genes was calculated by  $2^{-\Delta\Delta CT}$  method.

### Western Blot

48 hours after transfection, total cell proteins were extracted with RIPA buffer. Protein concentration was determined by BCA method. Each group of proteins (20 µg) was loaded and separated by SDS-PAGE. The protein was then transferred to the PVDF membrane and sealed in 5% skim milk for 1 hour, incubated with the primary antibody at room temperature for 1 hour, and then incubated with the secondary antibody at room temperature for 1 hour. CECL Western Blot Kit (Cowin Biotech Co.Ltd.,China) was used for substrate luminescence. GAPDH was used as the internal control. The protein level was analyzed by ImageJ software.

## Flow cytometry analysis

24 hours after transfection, the cell concentration was adjusted to  $1 \times 10^6$ /mL with Annexin V binding buffer. According to the manufacturer's plan, Annexin V-FITC-PI apoptosis detection kit (Sigma, USA) was used to detect apoptosis. The percentage of apoptotic cells was analyzed by Flowjo software. Annexin channels and PI channels were set on the horizontal axis and vertical axis respectively, and double negative cells were selected. In the double negative cell grid, all the elements can be obtained by setting the horizontal axis and vertical axis to FCS and SSC, inversely selected fragment gate respectively. The horizontal and vertical axes were set to the Annexin channel and the PI channel, respectively, and the cross door was to drawn to determine the cell scale.

## Statistical analyses

All data were expressed as mean  $\pm$  standard deviation. The statistical significance between the two groups was analyzed by single factor analysis of variance (One-way ANOVA). All the experiments were carried out independently for 3 times.  $P < 0.05$  indicates statistically significant differences.

## Results

### Inhibitory effect of miR-29a on the viability of EC cells

In order to study the effect of miR-29a on the activity of EC cells, miR-29a inhibitors (miR-29a specific siRNA) or mimics were transfected into EC cell lines, with non-specific siRNA or mimics as negative controls. From the qPCR results, miR-29a inhibitors significantly inhibited miR-29a levels compared with inhibitor controls, while miR-29a mimics significantly increased miR-29a levels compared with simulated controls (Fig. 1A). The survival rate of cells in each group was detected by CCK8 and clone formation assay. As shown in Fig. 1B, compared with the inhibitors control group, the OD value of miR-29a inhibitor transfection group increased significantly at 24 h, 48 h and 72 h after transfection, and decreased 72 h after miR-29a mimetic transfection compared with the mimics control group. The clone formation assay provided consistent results. Compared with the inhibitor control group, the colony number of miR-29a inhibitor was significantly increased, while the colony number of miR-29a mimic was significantly lower than that of the mimic control group (Fig. 1C). These results suggested that miR-29a inhibited the viability of EC cells.

#### miR-29a induced apoptosis in EC cells.

24 hours after transfection, apoptosis was analyzed by flow cytometry. As shown in Fig. 1D, the percentage of apoptotic cells decreased after transfection of miR-29a inhibitor compared with the inhibitor control group, while the percentage of apoptotic cells increased after transfection of miR-29a mimics compared with the mimics control group. It suggested that miR-29a could enhance apoptosis of EC cells. Previous studies have confirmed that miR-29a inhibits the progression of hepatocellular carcinoma by negatively regulating PTEN expression and activating Akt/mTOR signaling pathway[17]. In this study, we also explored the progress of miR-29a influencing EC through AKT/mTOR pathway. As shown in Fig. 2, miR-29a inhibitors significantly increased the phosphorylation and P70 levels of p-AKT/AKT and p-mTOR; miR-29a inhibitors also decreased the levels of p53, Bax and C-caspase3, while increased the levels of T-caspase-3 and Bcl-2. miR-29a simulated the opposite effect. The results showed that miR-29a inhibited the growth of EC cells by inhibiting the activation of AKT/mTOR signal pathway.

### **miR-29a inhibited the expression of STAT3 by binding to the 3' untranslated region (3' untranslated region) of STAT3.**

In order to further study the action mechanism of miR-29a, we analyzed the binding sites of miR-29a on TargetScan data sets. The results showed that there were two binding sites between the 3' region of STAT3 and miR-29a (Fig. 3A). Then, we examined the regulatory effect of miR-29a on STAT3 expression. As shown in Figs. 3B and C, the expression of STAT3 increased significantly after transfection of miR-29a inhibitor, while the expression of STAT3 decreased significantly after transfection of miR-29a mimic, indicating that miR-29a inhibited the expression of STAT3 in EC cells. It was speculated that miR-29a can inhibit the growth of tumor cells by targeting STAT3 in EC cells.

### **STAT3 promoted the viability and inhibited apoptosis of EC cells.**

In order to study the specific effect of STAT3 on EC cells, EC cells was transfected with shRNA or high expression plasma to construct STAT3 gene knockdown and overexpression cell lines. The results of qPCR and western blot showed that miR-29a inhibitors could promote the expression of STAT3, while miR-29a mimics could inhibit the expression of STAT3 (Fig. 4A and Fig. B). The cell viability was detected by CCK8 and clone formation assay. Compared with NC group, the OD value of EC cells in STAT3-OE group increased and that of EC cells decreased in STAT3-KD group (Fig. 4C). Similar results were observed in clone formation assay. Compared with NC cells, the number of colonies in STAT3-OE group increased, while that in STAT3-KD group decreased (Fig. 4D). Apoptosis was analyzed by flow cytometry. As shown in Fig. 4E, the percentage of apoptotic cells decreased after overexpression of STAT3, while the percentage of apoptotic cells increased when STAT3 was knocked down. It is proved that STAT3 can promote cell viability and inhibit apoptosis of EC cells.

### **STAT3 activated AKT/mTOR signal pathway in EC cells.**

Since STAT3 was the target of miR-29a, we further studied its effect on AKT/mTOR signal pathway. Western blot results showed that overexpression of STAT3 could activate the phosphorylation of p-AKT/AKT and p-mTOR, increase the expression of P70, T-caspase-3 and Bcl-2, and decrease the

expression of p53, Bax and C-Caspase3. Knockdown of STAT3 gene showed the opposite effect. Our results suggested that STAT3 may promote the growth of EC cells by activating AKT/mTOR signal pathway. (Fig. 5).

### **miR-29a/STAT3/AKT/mTOR inhibited the growth of human EC cells.**

Then, we verified the functional axis of miR-29a/STAT3/AKT/mTOR by overexpressing STAT3 and miR-29a (STAT3-OE + miR-29a mimic) in EC cells. As shown in Figs. 4 and 5, overexpression of miR-29a significantly blocked the increase of cell viability induced by overexpression of STAT3 and the inhibition of apoptosis induced by overexpression of STAT3. In addition, miR-29a mimic can reverse the effect of STAT3-OE activating AKT/mTOR signal pathway. (Fig. 5).

## **Discussion**

miRNA is a non-coding RNA, that exists in various eukaryotes with a length of about 20 nucleotides and plays a certain role in cell proliferation, apoptosis, differentiation and so on[8]. The production of miRNAs follows a standard multi-step process, starting from the nucleus to the end of the cytoplasm[18]. The fact that miRNAs plays an important role in human diseases initially comes from the high-throughput and functional study of cancer cells. The difference of miRNA expression reflects a single developmental pedigree and transformation mechanism. For example, epithelial tumors and hematopoietic malignant tumors have different miRNA expression profiles. Although its overall regulation is maladjusted, compared with normal tissues, most miRNAs is inhibited in cancer tissues, this may lead to a decrease in the ability of tumor cells to proliferate [8]. Consistent with these observations, the deletion of miRNA is conducive to cell transformation and tumorigenesis through the genetic deletion of the miRNA treatment mechanism [8–9]. This finding emphasizes that the change of miRNA is not only the result of tumorigenesis, but also plays a pathogenic role in the development of cancer. miR-29a has been proved to play an important role in a variety of human cancers. In recent years, it has been found that miR-29a can participate in the occurrence and development of tumor through multiple signal pathways. For example, miR-29a is a tumor suppressor miRNA in gliomas, which inhibits tumor stem cells and tumor growth by regulating PDGF pathway [19]. miR-29a inhibits the proliferation, migration and invasion of thyroid cancer by targeting DPP4, which may provide a new target for clinical treatment of thyroid cancer [20]. MIR-29 family regulates MTX drug resistance and apoptosis by regulating COL3A1 or MCL1. Targeting miR-29 family may provide a new strategy to overcome the cytotoxicity of high-dose MTX in osteosarcoma therapy [21]. In addition, studies have shown that miR-29a is involved in the progression of EC by targeting mRNA. For example, studies have shown that miR-29a down-regulates the expression of TPX2, inhibits the proliferation and invasion and promotes the apoptosis of EC cells. The high expression of TPX2 in primary esophageal carcinoma is associated with poor prognosis. Therefore, these biomarkers can be used as promising prognostic indicators [22], indicating that miR-29a can inhibit the progression of EC by regulating mRNA.

In order to further study the role of miR-29a in the pathogenesis of EC and its effect on the biological behavior of EC cells, synthetic miR-29a mimics and inhibitors were transfected into EC cells. The results of CCK-8 and colony formation assay showed that miR-29a could inhibit the viability of EC cells. Flow cytometry analysis showed that miR-29a promoted apoptosis of EC cells. Therefore, we speculate that miR-29a may play an anticancer effect by regulating the activity and apoptosis of EC cells. In order to elucidate the mechanism of miR-29a inhibiting tumor progression, the key proteins of AKT/mTOR signal pathway were detected by western blot. miR-29a can inhibit the phosphorylation of AKT and mTOR and regulate the expression of downstream proteins involved in the survival and apoptosis of EC cells.

miRNAs can affect the occurrence and development of tumors by targeting oncogenes or tumor suppressor genes. At present, it has been found that miR-29a has multiple target genes, which are distributed in different signal pathways. Through the analysis of the online target gene prediction software TargetScan, it is found that STAT3 may be the downstream target gene of miR-29a. QPCR and Western blot analysis showed that miR-29a inhibited the expression of STAT3 in EC cells. We further constructed STAT3 gene knockdown and overexpression cell lines. Cell function analysis showed that STAT3 promoted the activity of EC cells and inhibited apoptosis. In addition, miR-29a simulator can block the cell growth induced by overexpression of STAT3, suggesting that miR-29a/STAT3/AKT/mTOR has a functional axis in the process of EC.

STAT3 is a kind of transcription factor, which is located in the 17q21 region of chromosome. It is a DNA-binding protease that responds to the stimulation of epidermal growth factor. Phosphorylation of STAT3 protein will cause its dimerization, translocation to the nucleus, and lead to DNA binding. Therefore, it can regulate cell proliferation, differentiation and apoptosis[23]. More and more studies have shown that miRNA can negatively regulate STAT3 and affect tumor progression. For example, studies have shown that miR-124 can inhibit the progression of breast cancer by inhibiting the level of STAT3 [24]. In addition, miR-125a targets STAT3 to inhibit the growth, invasion and metastasis of cervical cancer [25]. Interestingly, STAT3 is activated and highly expressed in some human diseases, including EC [26]. In addition, studies have shown that long non-coding RNA NEAT1 promotes the progression of invasive EC through genes related to STAT3 and tumor microenvironment regulated by miR-361 [24]. This suggests that miR-361 affects the progression of EC by regulating STAT3. Our study demonstrated that STAT3 promotes the growth and metastasis of human EC cells. The results showed that miR-29a could inhibit the expression of STAT3, and then inhibit the activity of Akt/mTOR signal pathway, thus reduce the level of P70 and the activity of T-caspase-3, and finally lead to apoptosis and inhibit cell activity. To sum up, we confirmed that miR-29a inhibits cell viability and promotes apoptosis by targeting STAT3 and AKT/mTOR signaling pathways. miR-29a/STAT3/AKT/mTOR may become a new target of clinical treatment strategy.

## Conclusions

miR-29a inhibits cell growth by targeting STAT3 and inhibiting AKT/mTOR signaling pathway. miR-29a/STAT3/AKT/mTOR may become a new target axis of clinical treatment.

# Declarations

## Acknowledgements

We wish to thank the people who helped us during the writing of this paper and any other reviewer whose valuable instructions and suggestions on the thesis as well as his careful reading of the manuscript.

## Authors' Contributions

All authors participated in the design, interpretation of the studies and analysis of the data and review of the manuscript; YMW, YLW and HHL conducted the experiments, XL and JL supplied critical reagents, YLW and HHL wrote the manuscript, and XL contributed.

## Funding

Supported by Key Project of Natural Science Foundation of Tianjin Science and Technology Bureau (20JCZDJC00330).

## Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

## Competing interests

All authors declare no competing financial interests.

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

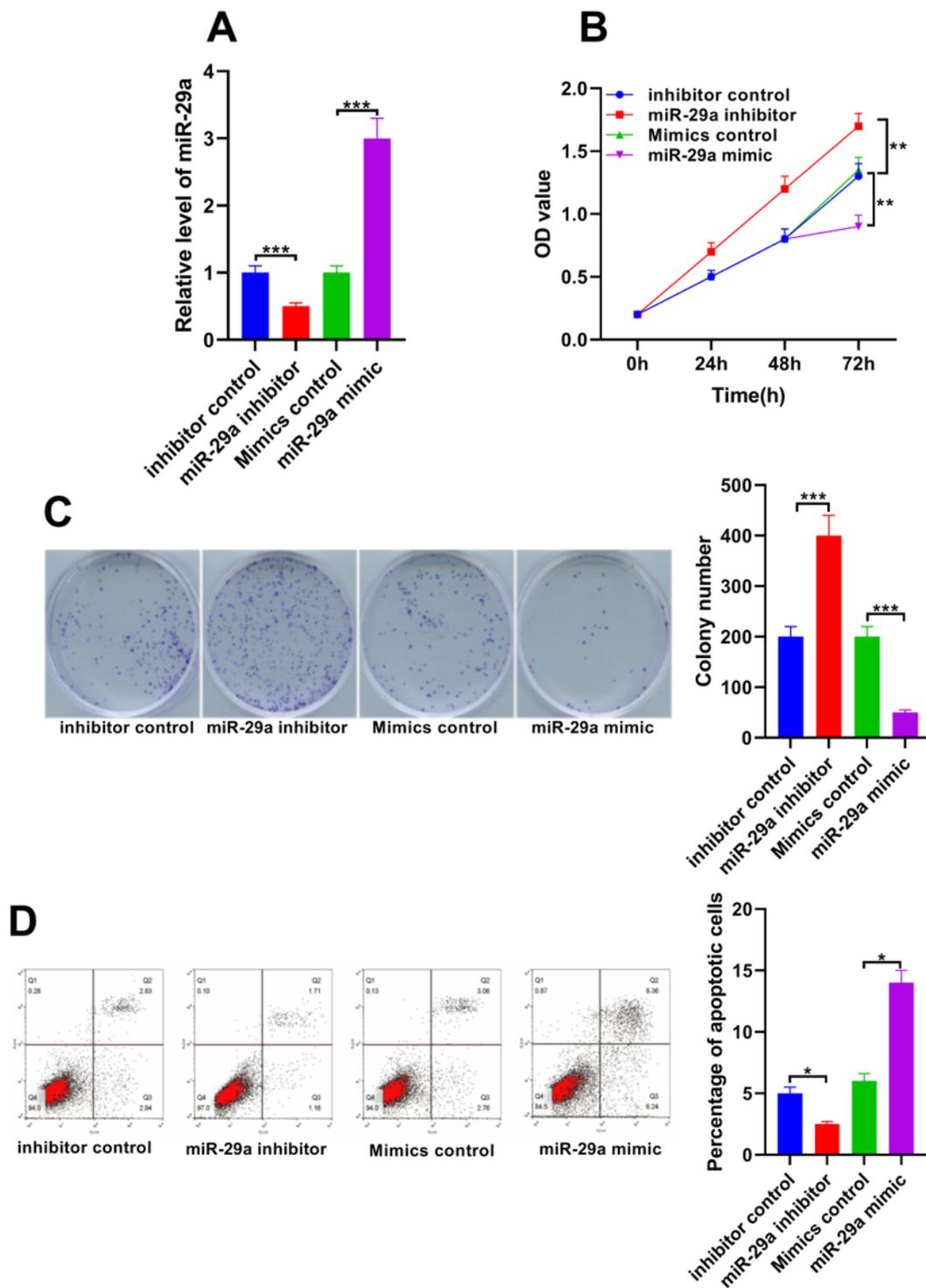
# References

1. Sorosky JI. Endometrial cancer. *Obstet Gynecol.* 2012;120:383–97.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. *CA Cancer J Clin.* 2018;68:7–30.
3. Bokhman JV. Two pathogenetic types of endometrial carcinoma. *Gynecol Oncol.* 1999;15:10–7.
4. McConechy MK, Ding J, Senz J, Yang W, Melnyk N, Tone AA, Prentice LM, Wiegand KC, McAlpine JN, Shah SP, Lee CH, Goodfellow PJ, Gilks CB, Huntsman DG. Ovarian and endometrial endometrioid carcinomas have distinct CTNNB1 and PTEN mutation profiles. *Mod Pathol.* 2014;27:128–34.

5. Hoang LN, Lee YS, Karnezis AN, Tessier-Cloutier B, Almandani N, Coatham M, Gilks CB, Soslow RA, Stewart CJ, Köbel M, Lee CH. Immunophenotypic features of dedifferentiated endometrial carcinoma-insights from BRG1/INI1-deficient tumours. *Histopathology*. 2016;69:560–9.
6. Siegel RL, Miller KD, Jemal A. Cancer Statistics. 2017. *CA Cancer J Clin*. 2017;67:7–30.
7. Boren T, Xiong Y, Hakam A, Wenham R, Apte S, Wei Z, Kamath S, Chen DT, Dressman H, Lancaster JM. MicroRNAs and their target messenger RNAs associated with endometrial carcinogenesis. *Gynecol Oncol*. 2008;110:206–15.
8. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*. 2004;116:281–97.
9. Esquela-Kerscher A, Slack FJ. Oncomirs – microRNAs with a role in cancer. *Nat Rev Cancer*. 2006;6:259–69.
10. Sianou A, Galyfos G, Moragianni D, Andromidas P, Kaparos G, Baka S, Kouskouni E. The role of microRNAs in the pathogenesis of endometrial cancer: a systematic review. *Arch Gynecol Obstet*. 2015;292:271–82.
11. Yanokura M, Banno K, Iida M, Irie H, Umene K, Masuda K, Kobayashi Y, Tominaga E, Aoki D. MicroRNAs in endometrial cancer: recent advances and potential clinical applications. *EXCLI J*. 2015;14:190–8.
12. Chang L, Yuan Z, Shi H, Bian Y, Guo R. miR-145 targets the SOX11 3'UTR to suppress endometrial cancer growth. *Am J Cancer Res*. 2017;7:2305–17.
13. Devor EJ, Miecznikowski J, Schickling BM, Gonzalez-Bosquet J, Lankes HA, Thaker P, Argenta PA, Pearl ML, Zweizig SL, Mannel RS, Brown A, Ramirez NC, Ioffe OB, Park KJ, Creasman WT, Birrer MJ, Mutch D, Leslie KK. Dysregulation of miR-181c expression influences recurrence of endometrial endometrioid adenocarcinoma by modulating NOTCH2 expression: an NRG oncology/gynecologic oncology group study. *Gynecol Oncol*. 2017;147:648–53.
14. Wu Q, Lu RL, Li JX, Rong LJ. MiR-200a and miR-200b target PTEN to regulate the endometrial cancer cell growth in vitro. *Asian Pac J Trop Med*. 2017;10:498–502.
15. Ran X, Yang J, Liu C, Zhou P, Xiao L, Zhang K. MiR-218 inhibits HMGB1-mediated autophagy in endometrial carcinoma cells during chemotherapy. *Int J Clin Exp Pathol*. 2015;8:6617–26.
16. Ye W, Xue J, Zhang Q, Li F, Zhang W, Chen H, Huang Y, Zheng F. MiR-449a functions as a tumor suppressor in endometrial cancer by targeting CDC25A. *Oncol Rep*. 2014;32:1193–9.
17. Song Q, Zhang H, He J, Kong H, Tao R, Huang Y, Yu H, Zhang Z, Huang Z, Wei L, Liu C, Wang L, Ning Q, Huang J. Long non-coding RNA LINC00473 acts as a microRNA-29a-3p sponge to promote hepatocellular carcinoma development by activating Robo1-dependent PI3K/AKT/mTOR signaling pathway. *Ther Adv Med Oncol*. 2020;12:1–21.
18. Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR, Golub TR. MicroRNA expression profiles classify human cancers. *Nature*. 2005;435:834–38.
19. Yanzhi Yang S, Dodbele T, Park. Rainer Glass, Krishna Bhat, Erik P Sulman, Ying Zhang, Roger Abounader. MicroRNA-29a inhibits glioblastoma stem cells and tumor growth by regulating the

- PDGF pathway. *J Neurooncol.* 2019;145(1):23–34.
20. Wang Y, Han J, Lv Y, Zhang G. miR-29a inhibits proliferation, invasion, and migration of papillary thyroid cancer by targeting DPP4. *Onco Targets Ther.* 2019;12:4225–33.
  21. Xu W, Li Z, Zhu X, Xu R, Xu Y. miR-29 Family Inhibits Resistance to Methotrexate and Promotes Cell Apoptosis by Targeting COL3A1 and MCL1 in Osteosarcoma. *Med Sci Monit.* 2018;24:8812–21.
  22. Jiang T, Sui D, You D, Yao S, Zhang L, Wang Y, Zhao J, Zhang Y. miR-29a-5p inhibits proliferation and invasion and induces apoptosis in endometrial carcinoma via targeting TPX2. *Cell Cycle.* 2018;17(10):1268–78.
  23. Xiong A, Yang Z, Shen Y, Zhou J, Shen Q. Transcription Factor STAT3 as a Novel Molecular Target for Cancer Prevention. *Cancers (Basel).* 2014;6(2):926–57.
  24. Pang Y, Wu J, Li X, Wang C, Wang M, Liu J, Yang G. NEAT1/miR-124/STAT3 feedback loop promotes breast cancer progression. *Int J Oncol.* 2019;55(3):745–54.
  25. Fan Z, Cui H, Xu X, Lin Z, Zhang X, Kang L, Han B, Meng J, Yan Z, Yan X, Jiao S. MiR-125a suppresses tumor growth, invasion and metastasis in cervical cancer by targeting STAT3. *Oncotarget.* 2015;6(28):25266–80.
  26. Dong P, Xiong Y, Yue J, Xu D, Ihira K, Konno Y, Kobayashi N, Todo Y, Watari H. Long noncoding RNA NEAT1 drives aggressive endometrial cancer progression via miR-361-regulated networks involving STAT3 and tumor microenvironment-related genes. *J Exp Clin Cancer Res.* 2019;38(1):295.

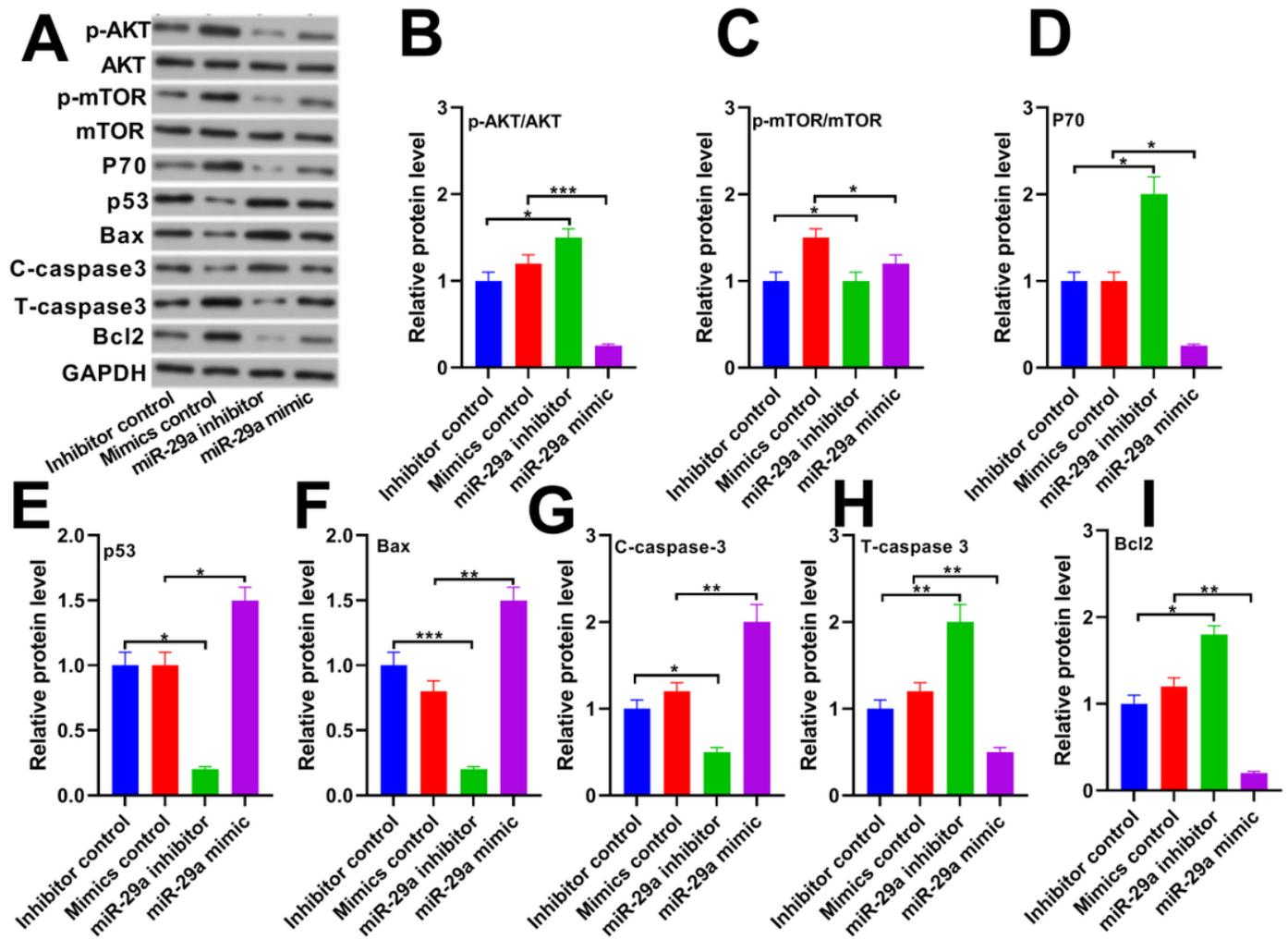
## Figures



**Figure 1**

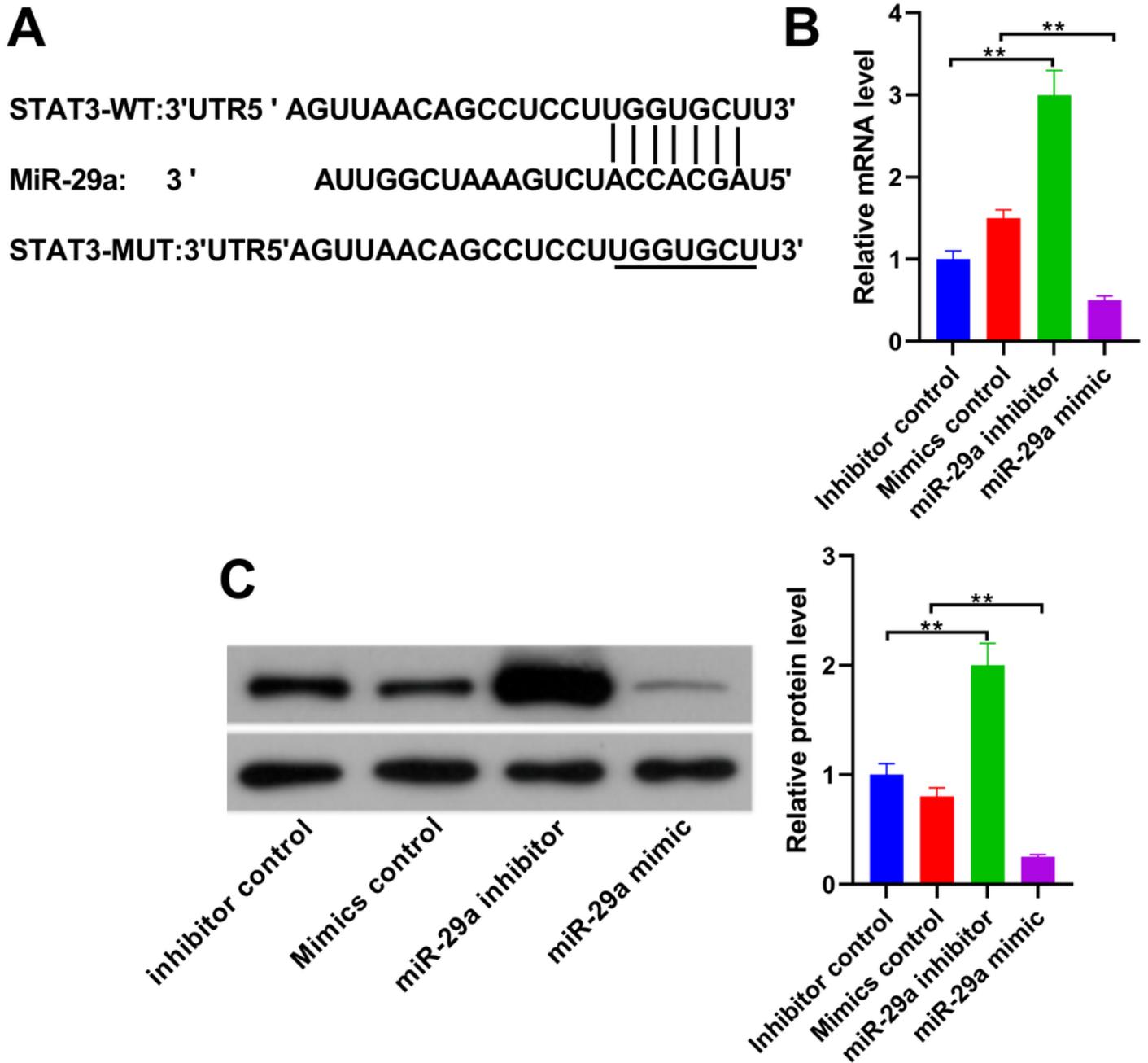
miR-29a blocks the proliferation and induces apoptosis of EC cells. (A) miR-29a mimics or inhibitors were transfected into HHUC cells, and non-specific siRNA or mimics were used as controls. The expression of miR-29a was detected by QPCR. GAPDH was used as the internal control gene. (B) The proliferation of EC cells was detected by CCK8 colorimetry. The OD value was measured at 450 nm. (C) Colony formation

assay further confirmed the proliferation of EC cells. After 2 weeks of culture, pictures were taken. (D) 24 hours after transfection, apoptosis was detected by flow cytometry. \* Compared with NC, P < 0.05.



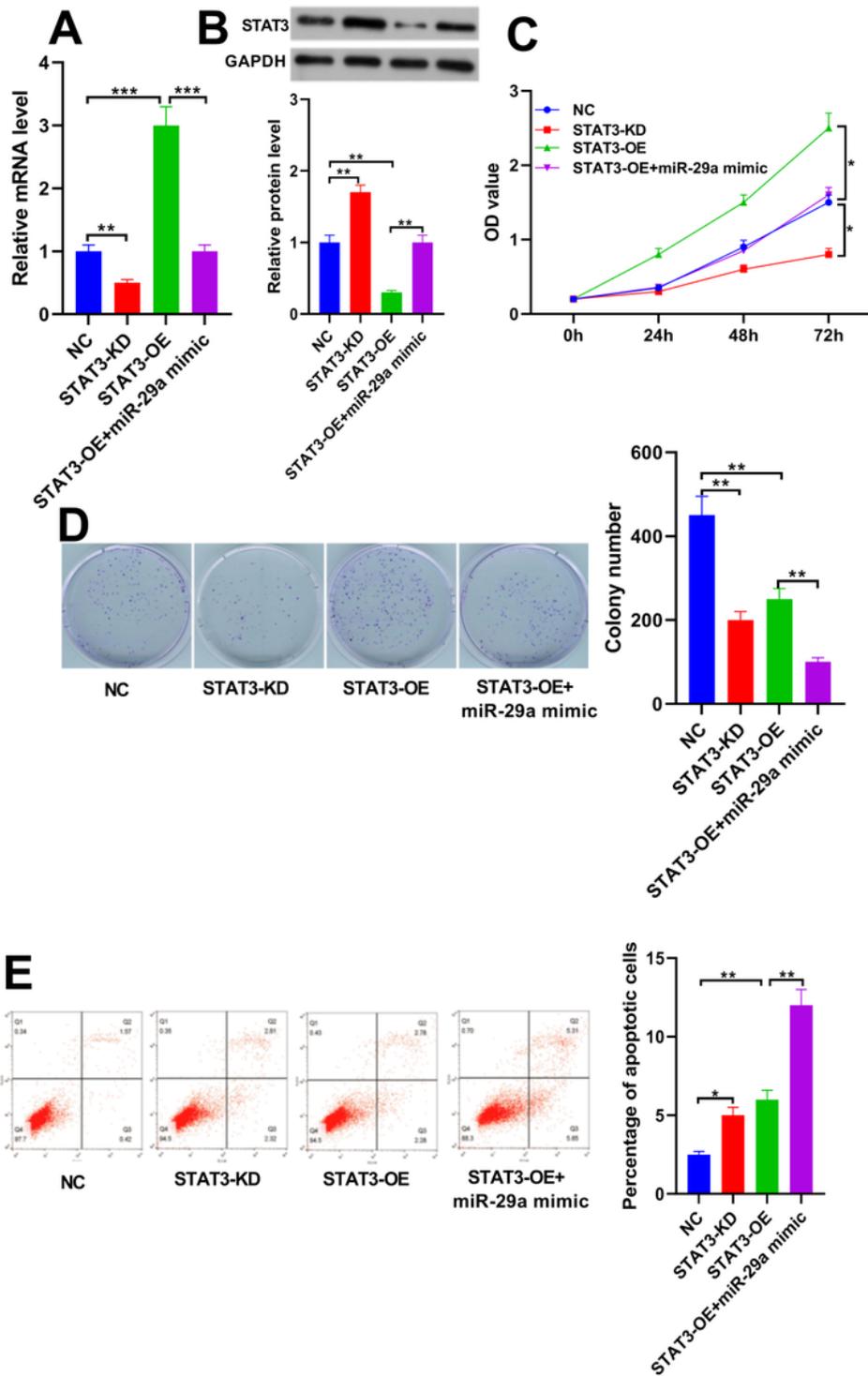
**Figure 2**

miR-29a inhibits the activation of AKT/mTOR signal pathway. The protein levels of genes related to AKT/mTOR signal pathway were detected by western blot. Histogram showed the expression of the protein, including p-AKT/AKT (B), p-mTOR/mTOR (C), P70 (D), p53 (E), Bax (F), C-Caspase3 (G), T-caspase-3 (H) and Bcl-2 (I). GAPDH was used as the internal references. \* P < 0.05.



**Figure 3**

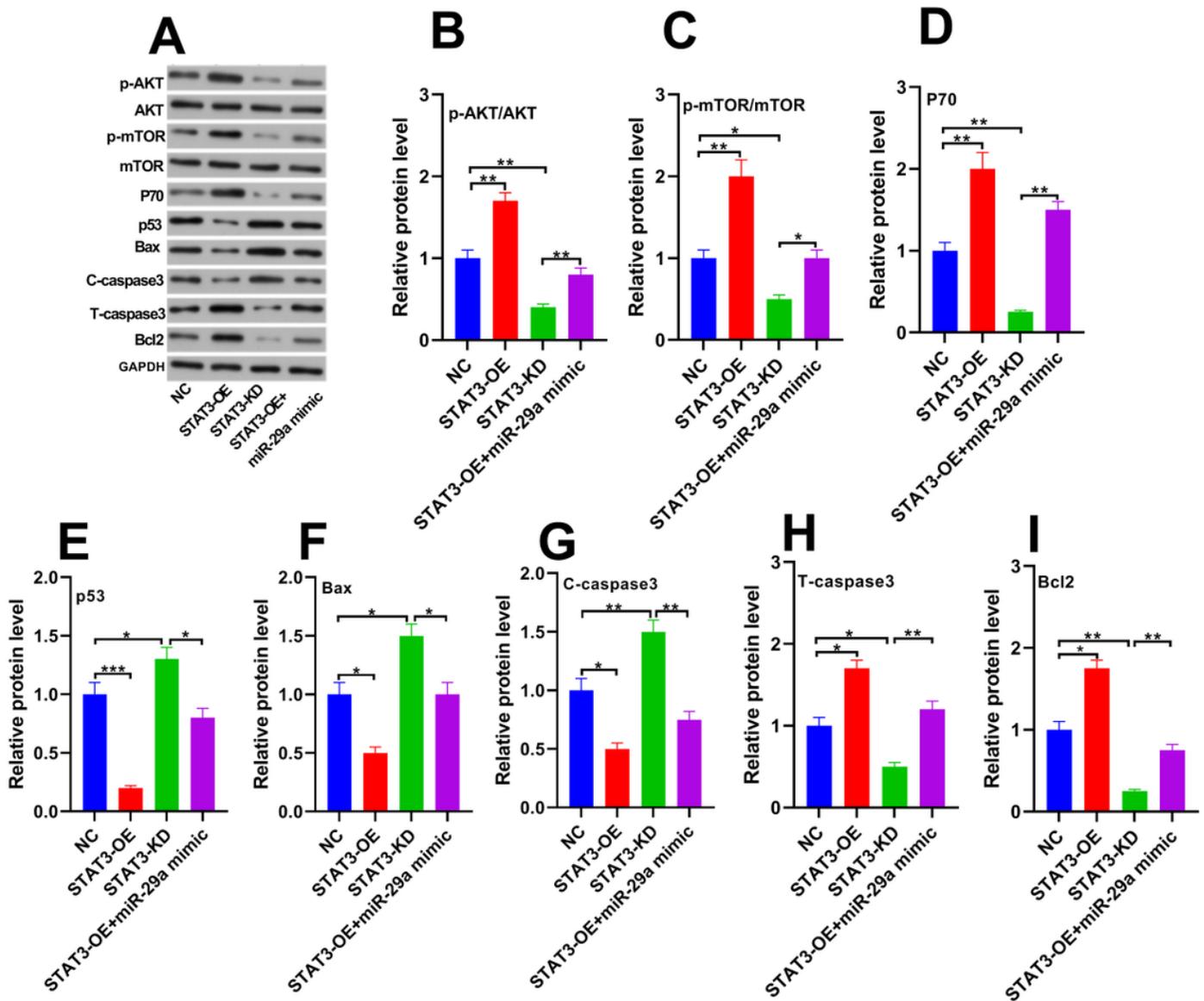
miR-29a inhibits the expression of STAT3 by binding to the 3' non-coding region of STAT3. (A) The binding sites of miR218-5p and STAT33 noncoding regions were analyzed on TargetScan data sets. (B) The mRNA level of STAT3 was detected by qPCR. (C) The level of STAT3 protein was detected by Western blot. \* P < 0.05.



**Figure 4**

STAT3 promoted the proliferation of visual EC cells and inhibits apoptosis. (A) STAT3 shRNA or overexpression plasmid was transfected into EC cells, and untreated EC cells were used as control (negative control, NC). The expression of miR-29a was detected by qPCR. GAPDH was used as the internal control gene. (B) The level of STAT3 protein was detected by western blot. (C) The proliferation of EC cells was detected by CCK8 colorimetry. The OD value was measured at 450 nm. (D) Clone formation

assay further confirmed the proliferation of EC cells. After 2 weeks of culture, pictures were taken. (E) 24 hours after transfection, apoptosis was detected by flow cytometry. \* P < 0.05.



**Figure 5**

STAT3 activated AKT/mTOR signal pathway in EC cells. (A) The protein levels of genes related to AKT/mTOR signal pathway were detected by western blot. Histogram showed the expression of the protein, including p-AKT/AKT (B), p-mTOR/mTOR (C), P70 (D), p53 (E), Bax (F), C-Caspase3 (G), T-caspase-3 (H) and Bcl-2 (I). GAPDH was used as the internal references. \* P < 0.05.