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RESEARCH ARTICLE

MIR-128 Targets GAREM to Regulate the ERK/MAPK Signaling Pathway and Alleviate Yak Endometritis

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ABSTRACT

Endometritis is a serious postpartum inflammatory disease that poses a significant threat to the reproductive health of cows and results in considerable economic losses. MicroRNA (miRNA) has been demonstrated to be important in the occurrence and progression of various diseases, but there is limited research on miRNA in the context of yak endometritis. Thus, this study aims to investigate whether miR-128 can influence yak endometritis by regulating the ERK/MAPK pathway via the targeting of GAREM. *In-vivo* studies indicated that endometritis could cause uterine damage in yaks and significantly decrease the expression of miR-128, while levels of TNF-α, IL-6, and IL-1β are dramatically increased. In vitro cell experiments demonstrated that the overexpression of miR-128 significantly reduced the expression of associated proteins and inflammatory factors within the MAPK pathway, whereas the inhibition of miR-128 resulted in increased inflammation. Furthermore, we identified GAREM as a target gene of miR-128. Notably, the suppression of GAREM expression significantly decreased the levels of related proteins in the MAPK signaling pathway and further caused a sharp decline in the expression of inflammatory factors. On the other hand, high GAREM expression correlated with intense inflammation. Further inhibition of the pathway with JNK pathway blockers (SP600125) and p38 pathway blockers (Adezmapimod) demonstrated that overexpression of miR-128 was able to lower the protein expression of p-ERK, GAREM expression, and the expression of inflammatory factors, indicating that miR-128 targets the ERK/MAPK signaling pathway in LPS-induced yak endometritis. In conclusion, this study demonstrates that miR-128 targets GAREM in the modulation of the ERK/MAPK signaling pathway, thus influencing yak endometritis.

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INTRODUCTION

Endometritis is a mucous or purulent inflammation caused by multiple factors, which often occur 21 days after delivery (Sheldon *et al*., 2019). Clinically, common pathogens that cause bovine endometritis include *Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli*, etc (Shaukat *et al*., 2021). Among them, *Escherichia coli is* the main cause of bovine endometritis (Wang *et al*., 2024). It infects the endometrium and disrupts the balance of the intrauterine environment by producing LPS, ultimately providing favorable conditions for other pathogens to enter the uterus and induce inflammation. Previous studies indicated that even in the absence of clinical symptoms, some patients with endometritis may cause infertility, as well as repeated implantation failures and habitual abortions (Chen *et al*., 2023). Moreover, cows with endometritis have a reduced pregnancy rate compared to that in healthy populations and require more inseminations to conceive successfully (Takimoto *et al*., 2023). Thus, endometritis may culminate in the culling of cows due to reproductive disorders, highly impacting the

cattle and dairy industries with huge economic losses. At present, the clinical treatment for endometritis is still the use of antibiotics (Cicinelli *et al*., 2021). However, the negative impacts brought about by the extensive use of antibiotics, including bacterial resistance and drug residues, are now prominent and persist (Miao *et al*., 2024). Thus, it is imperative to explore novel treatment strategies for bovine endometritis.

MicroRNA (miRNA) is a group of endogenous RNAs comprising 19 to 25 nucleotides, which exert a vital regulatory role in the host through targeting mRNA for cutting or translation inhibition (Bouzari *et al*., 2022). Currently, numerous studies involving miRNAs have demonstrated that they are closely related to multiple diseases, such as cell differentiation, proliferation, apoptosis, tumorigenesis, and virus replication (Takahashi *et al*., 2020). Additionally, recent studies on miRNAs have also revealed their important role in endometritis (Umar *et al*., 2021). For instance, Salilew-Wondim *et al*. (2016) reported differential expression of multiple miRNAs in bovine endometritis, and these differential miRNAs were closely associated with cell apoptosis and inflammatory damage. Previous studies revealed that miR-128 is significantly elevated in the gingival tissue of patients with periodontitis, where its increased expression could decrease TNF-α secretion and restrain p38 phosphorylation. Furthermore, some research has also demonstrated a significant decrease in miR-128 in patients with endometritis, indicating its potential relationship with endometritis. Notably, we also observed that miR-128 was significantly downregulated in the LPS-induced yak endometritis cell model. This evidence indicated the important relationship between miR-128 and endometritis. However, the underlying mechanism by which miR-128 participates in the regulation of yak endometritis remains unclear.

Mitogen-activated protein kinase is considered a key hub in the transduction of extracellular signals to the cell nucleus, and p38, ERK, and JNK are regarded as the three most principal members of the MAPK family (Liu *et al*., 2025). Previous studies indicated that MAPK participates in various cellular events such as differentiation, inflammation, apoptosis, protein biosynthesis, and tumorigenesis (Yu *et al*., 2024). Recent studies associated with the MAPK signaling pathways also reveal its important role in endometritis (Zhou *et al*., 2025). Grb2-associated regulator of ERK/MAPK1 is a ubiquitous multifunctional scaffold protein implicated in connecting activated receptors to multiple intracellular signaling pathways that mediate various biological processes and regulate the course of numerous diseases. It has been reported that GAREM can bind to tyrosine 453 of ERK and thus activate the regulatory function of ERK. In addition, GAREM has also been identified as a direct downstream target of miR-128, suggesting that miR-128 may target GAREM in modulating the ERK/MAPK pathway.

Yaks are closely related to local agricultural development and social civilization. However, due to the harsh environmental conditions and nutritional deficiencies, yaks frequently suffer from endometritis. Currently, most research on endometritis has been conducted in dairy cows, beef cattle, and donkeys, while studies on yak endometritis remain scarce. Thus, we assumed that miR-128 may target GAREM to regulate the ERK/MAPK signaling pathway, thereby influencing yak endometritis.

MATERIALS AND METHODS

Sample acquisition: In this study, uterine samples were collected from healthy and endometritis-afflicted yaks in Tibet, China, and then brought to the laboratory for subsequent experiments. The criteria for determining health or endometritis in yaks were determined in previous studies. The criteria for a healthy yak are the presence of clear mucous secretions emanating from the uterus and a PMN% less than 18% approximately 21 days post-delivery, without any significant symptoms arising clinically. A yak is then diagnosed with endometritis if the uterus contains purulent secretions and its PMN percentage exceeds 18% at around 21 days after delivery.

Histological observations: Tissue section preparation and staining were performed according to previous studies by Cao *et al*. (2024) and Wang *et al*. (2024).

Endometrial epithelial cells culture and identification: The culture and identification of endometrial epithelial cells were performed according to our previous study (Cao *et al*., 2024; Wang *et al*., 2024).

Sources of mimics, inhibitors, and siRNA: In the present study, we synthesized miR-128 and GAREM mimics, inhibitors, and their negative controls according to previously described procedures (Cao *et al*., 2024; Wang *et al*., 2024).

Cell transfection: Cell transfection steps were performed according to our previous studies (Cao *et al*., 2024; Wang *et al*., 2024).

Dual luciferase assay and regulation of target genes: The detailed procedures can be referred to in our previous works: Cao *et al*. (2024), Wang *et al*. (2024).

Cell viability assay and ELISA detection: The specific steps of cell viability assay and ELISA detection can be found in our previous research (Cao *et al*., 2024; Wang *et al*., 2024).

Quantitative real-time PCR analysis: Specific steps and processes of total RNA extraction, reverse transcription of RNA to cDNA, and RT-qPCR refer to the previous studies (Cao *et al*., 2024; Wang *et al*., 2024).

Western blot analysis: Western blot analysis was performed, refer to previously published (Cao *et al*., 2024; Wang *et al*., 2024). The detection of protein expression was performed by ImageJ and normalized to GAPDH protein levels.

In situ hybridization assay: The steps of the in situ hybridization assay were performed as per our previous studies (Cao *et al*., 2024; Wang *et al*., 2024).

Statistical analysis: All data are presented as mean ± standard deviation (Mean ± SD). Additionally, one-way ANOVA and

t-tests were conducted using GraphPad Prism to investigate the differences between the control and experimental groups. P<0.05 was considered statistically significant.

RESULTS

Morphological observation: As depicted in Fig. 1A, B, the surface of the uterine tissue in healthy yaks is smooth, whereas the uterine tissue of yaks with endometritis exhibits numerous congested spots. Moreover, the uterine tissue of healthy yaks exhibited no signs of bleeding, erosion, or other abnormalities on the surface of the endometrium. Conversely, the endometrium of yaks suffering from endometritis displayed a significant presence of bleeding spots and erosion. Yaks with endometritis had endometrial epithelial shedding, inflammatory cell infiltration, endometrial congestion, and abnormal curvature of the uterine glands compared with healthy yaks (Fig. 1C-H). Compared with healthy yaks, the protein (P<0.01, Fig. 1 I) and mRNA (P<0.05, Fig. 1 J) abundance of miR-128 was lower in the endometrial tissue of yaks with endometritis. Furthermore, the expressions of TNF-α, IL-6, and IL-1β were higher in the endometrial tissue of yaks with endometritis (Fig. 1K, L, M).

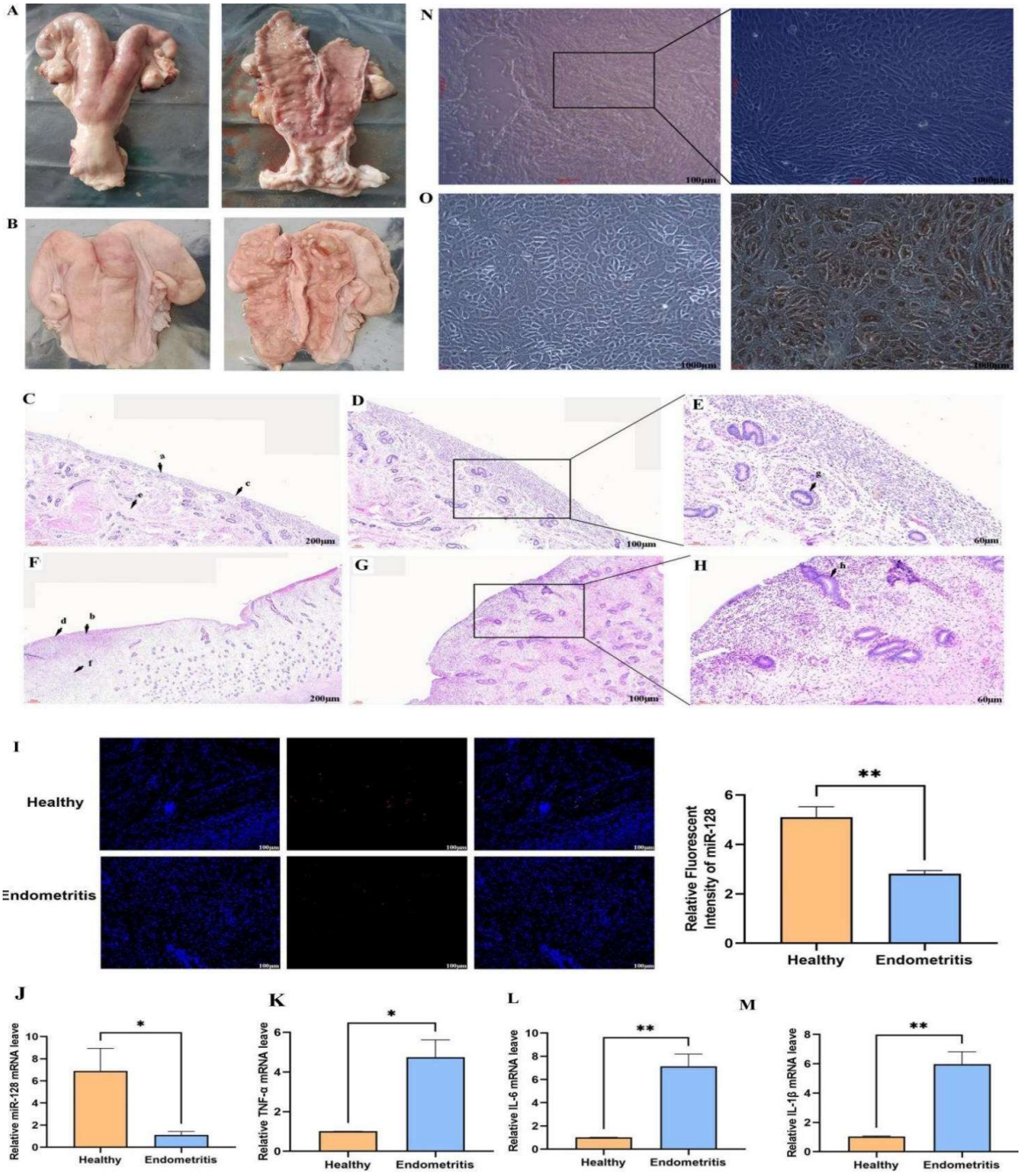

Fig. 1: Effects of endometritis on yak uterine tissue. A, B: Visual assessment of the uterus in healthy yaks and yaks with endometritis. C, D, E: Morphology of endometrial tissue of healthy yaks. F, G, H: Morphology of endometrial tissue of yaks affected by endometritis. I: In situ hybridization was used to detect the distribution of miR-128 in yak endometrial tissues. J-M: miR-128, TNF-α, IL-6, and IL-1β in yak endometrial tissues. Yak endometrial epithelial cells culture and keratin identification. N: Negative control, no color appears, scale bar is 100μm and 1000μm. O: ABC identification result of CK18, scale bar is 1000 μm. *P<0.05; **P<0.01

LPS affects endometrial epithelial cell apoptosis and cell viability: Cytokeratin 18 staining validated the purity of yak endometrial epithelial cells (Fig. 1). The apoptosis rate of endometrial epithelial cells increased significantly after LPS (5μg/mL, 10μg/mL) treatment for 6 h (Fig. 2A). Moreover, the apoptosis rate of endometrial epithelial cells also increased dramatically after LPS (3μg/mL, 5μg/mL, 10μg/mL) treatment for 12h (Fig. 2B). When the LPS treatment time reached 24h, the concentration exceeded 0.5μg/mL, which could lead to a significant increase in the apoptosis rate of endometrial epithelial cells (Fig. 2C). When LPS treatment lasted 6 hours and the concentration was 3 μg/mL, the expression levels of inflammatory factors were detected, and no significant changes in inflammatory factors were found (Fig. 2D, E).

Based on the above results, we observed that when the LPS treatment time was 12h and the treatment concentration was 1μg/mL, there was no significant effect on the cell apoptosis rate; but the inflammatory response was effectively induced.

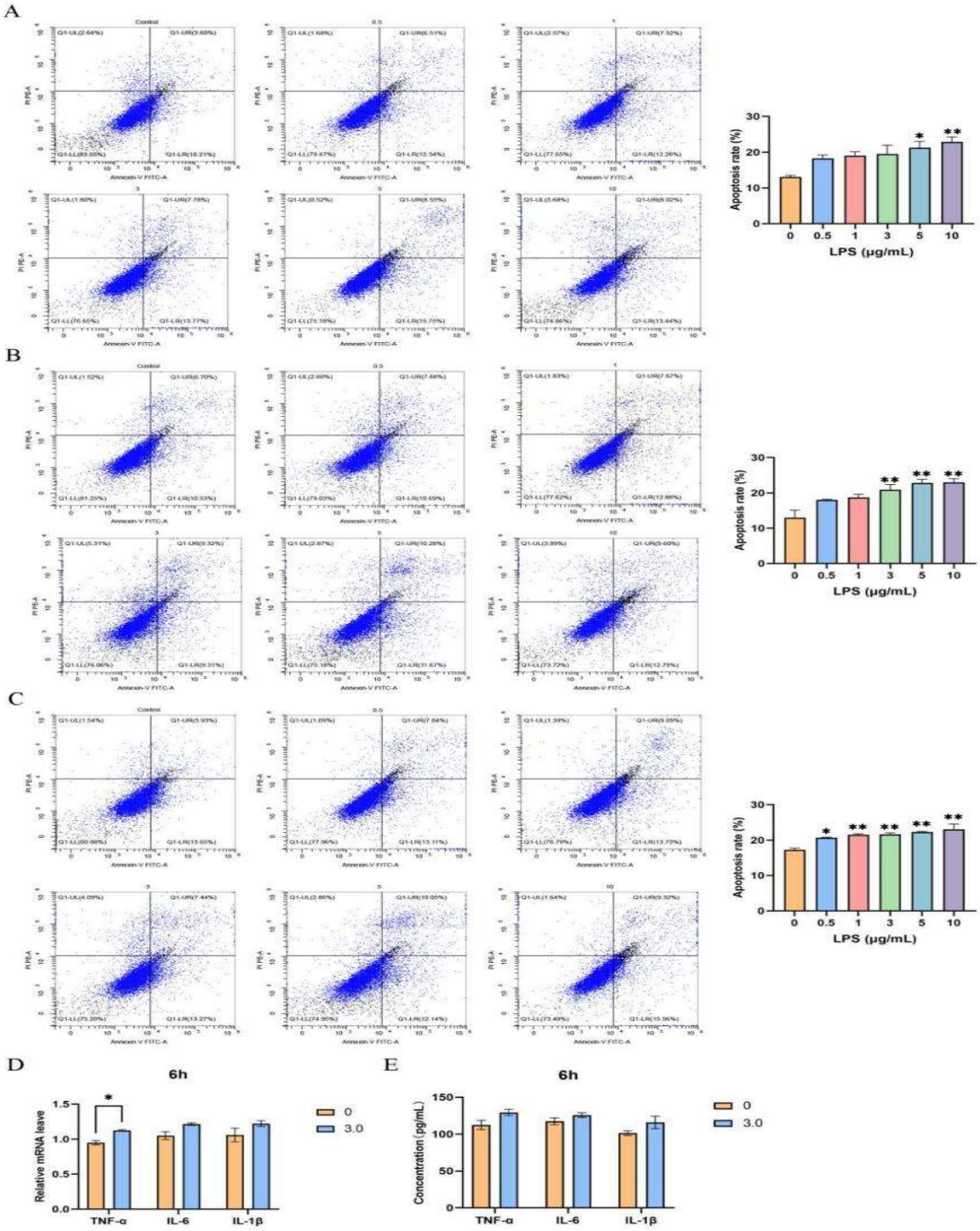

Fig. 2: LPS affects the apoptosis and inflammatory factors. A, B, and C represent the treatment groups with 0, 0.5L, 1, 3, 5, and 10 μg/mL of LPS administered for durations of 6, 12, and 24 h, respectively. D, E: Inflammatory factors expression. *P<0.05; **P<0.01

LPS induced MAPK signaling and inflammatory activation: LPS treatment resulted in a significant increase in the ratios of p-ERK/ERK, p-JNK/JNK, and p-P38/P38 in yak endometrial epithelial cells (P<0.01, Fig. 3A). The levels of TNF-α, IL-6, and IL-1β were higher in the yak endometrial epithelial cells treated with LPS (Fig. 3B, C). The fluorescence intensity and mRNA abundance of miR-128 were lower in the yak endometrial epithelial cells treated with LPS compared with the control group (Fig. 3D, E). Thus, a treatment of 1μg/mL LPS for 12 h was utilized to establish the endometritis model for the subsequent study.

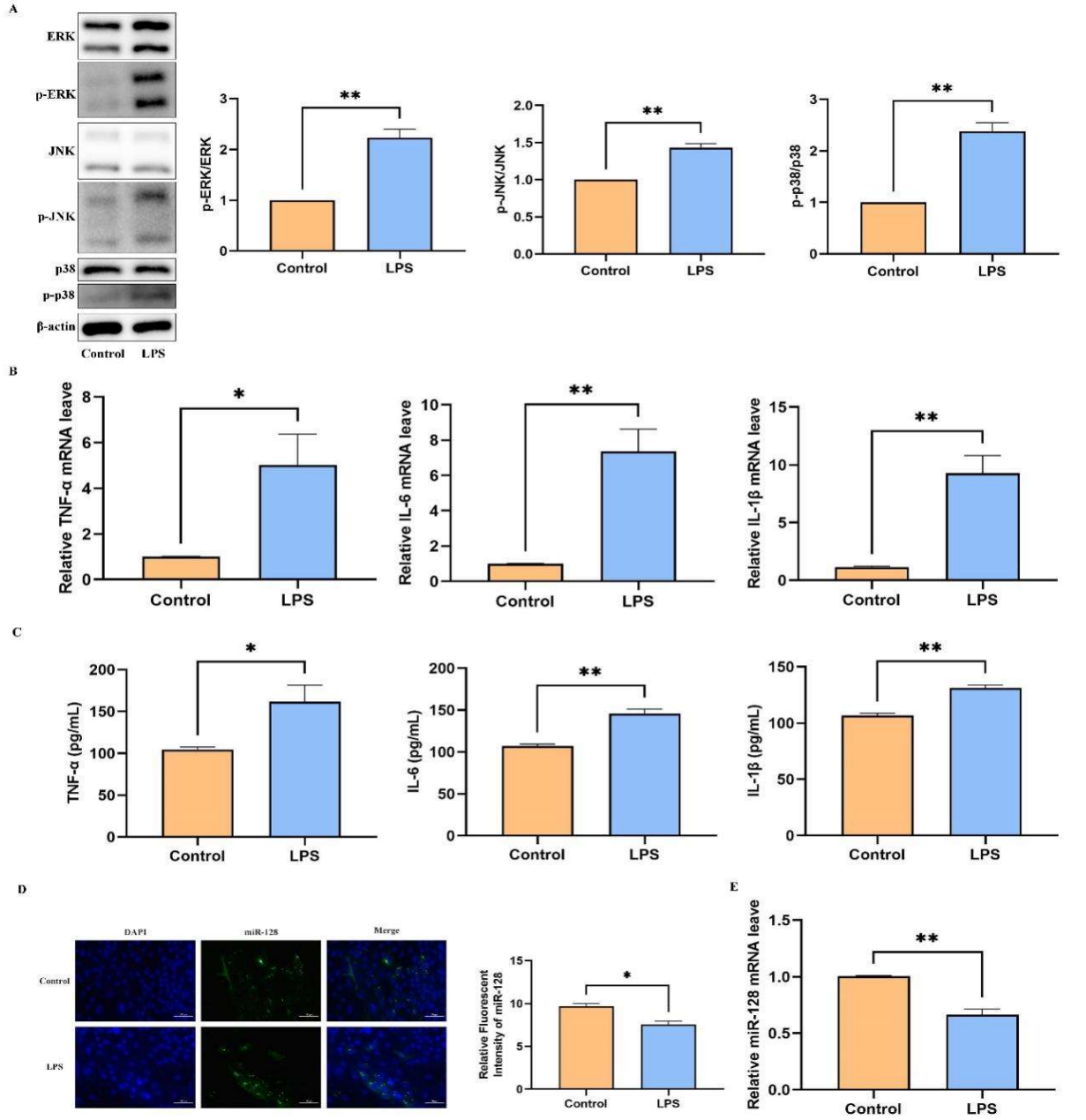

Fig. 3: 1 μg/mL LPS can activate the MAPK pathway and affect the miR-128 expression. A: Phosphorylation proteins. B, C: Inflammatory factors. D: miR-128 localization. E: miR-128 expression. *P<0.05; **P<0.01.

miR-128 is involved in LPS-induced inflammatory response: The fluorescence intensity and mRNA abundance of miR-128 were up-regulated in the overexpression of miR-128 group, while down-regulated when using inhibitors (Fig. 4A, B). Moreover, miR-128 mimics and inhibitors were transfected into endometrial epithelial cells, followed by treatment with LPS. Results showed that miR-128 can affect the expression of proteins in the MAPK pathway and downstream TNF-α, IL-6, and IL-1β. In the control group, the overexpression of miR-128 could reduce the expression of proteins p-ERK, p-JNK, p-P38, TNF-α, IL-6, and IL-1β, but without a statistical difference (P>0.05) (Fig. 5A-C). Conversely, reducing the expression of miR-128 could increase the MAPK signaling pathway-related proteins and inflammatory factors (P>0.05), but also without a significant statistical difference (P>0.05). These findings indicated that miR-128 exerts a regulatory effect on the endometrial epithelial cells.

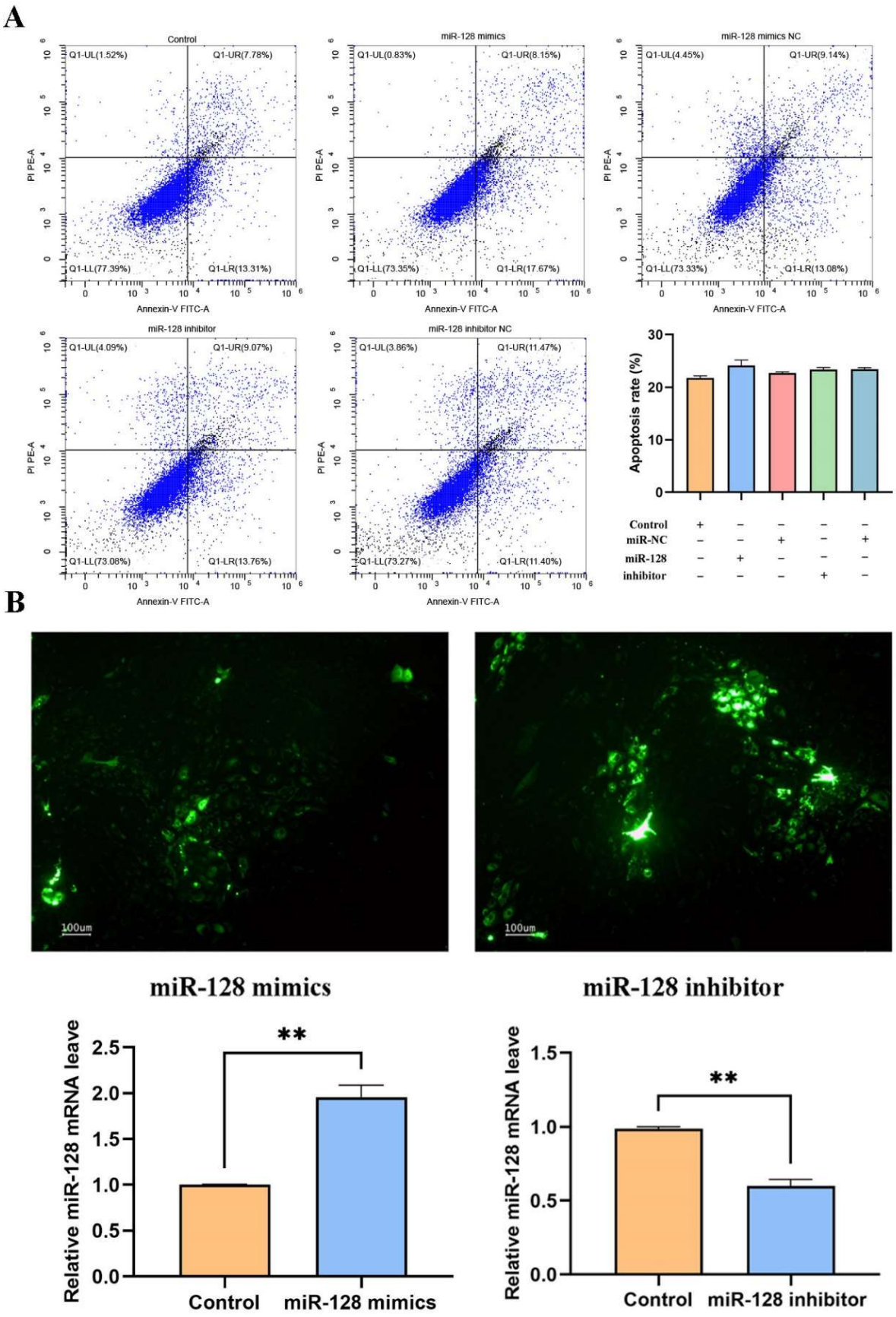

Fig. 4: Effect of miR-128 transfection on apoptosis. A: Effect of miR-128 transfection on cell apoptosis rate. B: miR-128 expression. **P<0.01.

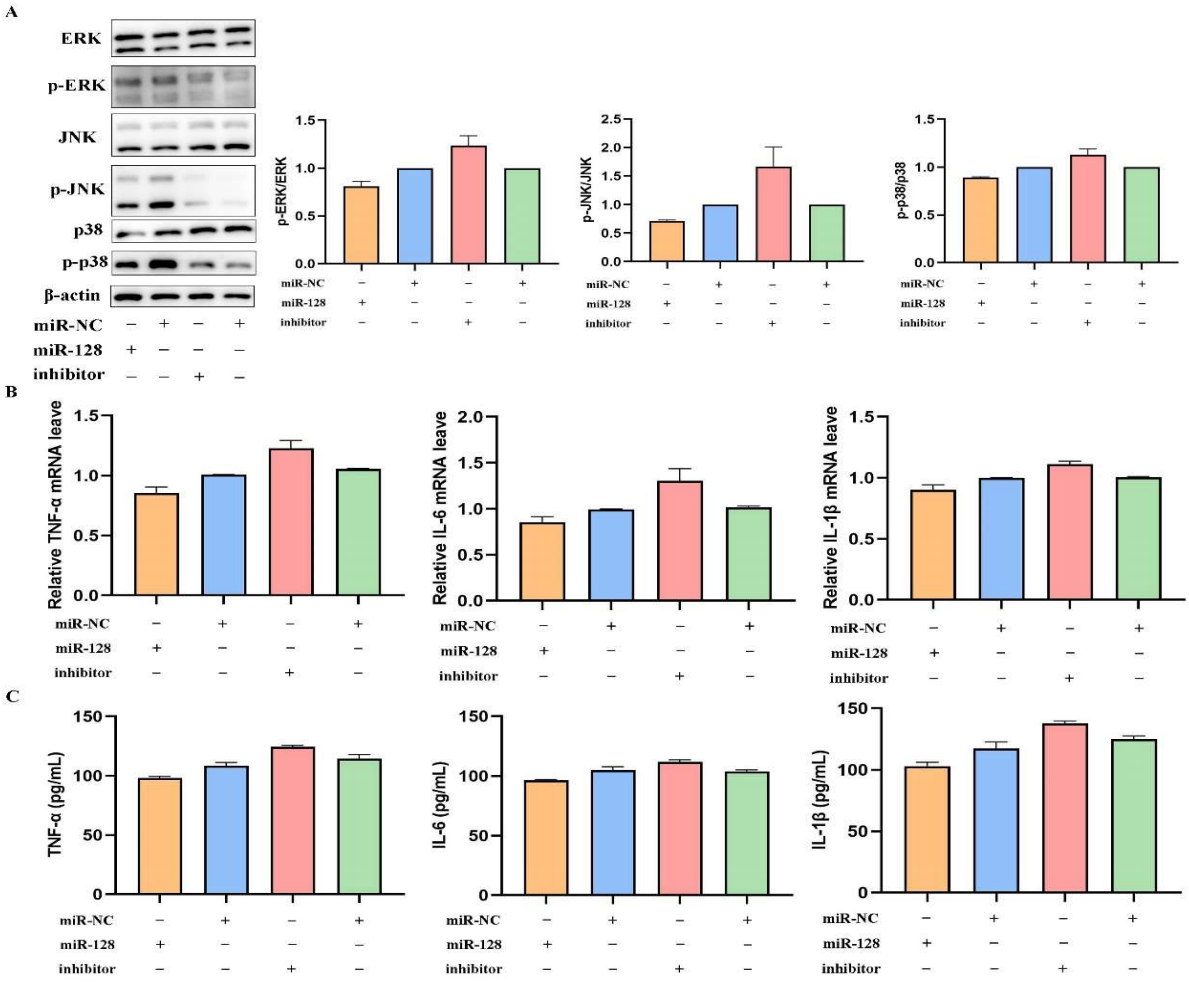

Fig. 5: Regulatory effect of miR-128 on endometrial epithelial cells. A: The regulatory effect of miR-128 on related proteins in the MAPK signaling pathway. B, C: Inflammatory factors.

After LPS treatment, the miR-128 overexpression significantly decreased the expression of related proteins in the MAPK pathway and inflammatory factors (Fig. 6A, B, C). Conversely, the downregulation of miR-128 resulted in increased inflammation. The immunofluorescence results for p-ERK corroborated the western blot findings, demonstrating that the overexpression of miR-128 reduced p-ERK expression ($P<0.01$) (Fig. 6D).

The 10μM JNK pathway blocking agent (SP600125) and Adezmapimod are used to inhibit the ERK/MAPK signaling pathways. Meanwhile, miR-128 was transfected into cells and then treated with LPS. Results from the streaming cell analysis indicated that there is no significant difference in the apoptosis rate, indicating that further study could be performed (Fig. 7A). Additionally, the excessive expression of miR-128 can significantly reduce the protein expression of p-ERK and GAREM, and the gene expression of TNF-α, IL-6, and IL-1β (Fig. 7B-D). Notably, the immunofluorescence results of p-ERK were in line with the western blot findings (Fig. 7E), indicating that miR-128 can regulate LPS-induced yak endometritis through the ERK/MAPK signaling pathway.

Prediction, verification, and regulation of miR-128 target genes: The target genes of miR-128 were predicted using the biological information website TargetScan. GAREM was identified as a candidate target gene, and the potential binding sites of miR-128 within the GAREM 3' UTR were predicted (Fig. 8A). Subsequently, wild-type and mutant dual-luciferase reporter vectors for GAREM were constructed (Fig. 8B). The two constructed plasmids were double digested with XhoI and NotI, and the results of agarose gel electrophoresis confirmed that fragments of the expected size were obtained (Fig. 8C). Combined with the sequencing results, these findings indicate that the plasmid construction was successful (Fig. 8D). Dual-luciferase activity was then assessed, revealing that, compared to the mimics NC + GAREM wild-type reporter plasmid group, the miR-128 mimics + GAREM wild-type reporter plasmid group significantly inhibited the dual-luciferase activity of the wild-type plasmid ($P<0.01$), thereby confirming that GAREM is a target gene of miR-128 (Fig. 8E). Furthermore, when miR-128 was overexpressed, the expression level of GAREM protein was significantly reduced ($P<0.01$). Conversely, a reduction in miR-128 expression led to a significant increase in the expression level of GAREM protein ($P<0.01$), with consistent gene expression levels observed, indicating that miR-128 and GAREM expression are negatively regulated (Fig. 8F).

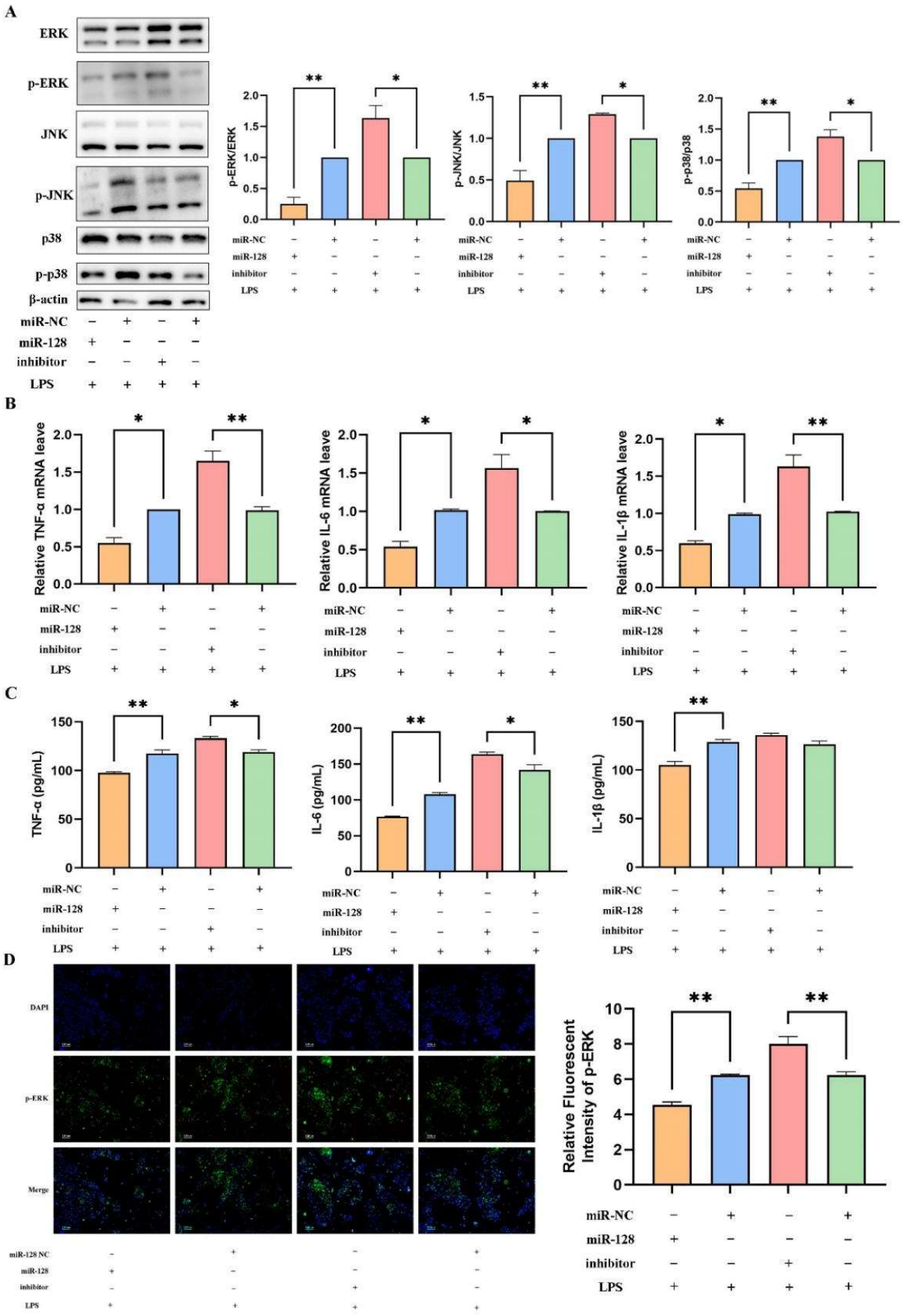

Fig. 6: miR-128 regulates LPS-induced yak endometritis. A: Proteins in the MAPK signaling pathway. B, C: Inflammatory factors. D: Immunofluorescence expression results of p-ERK. *P<0.05; **P<0.01

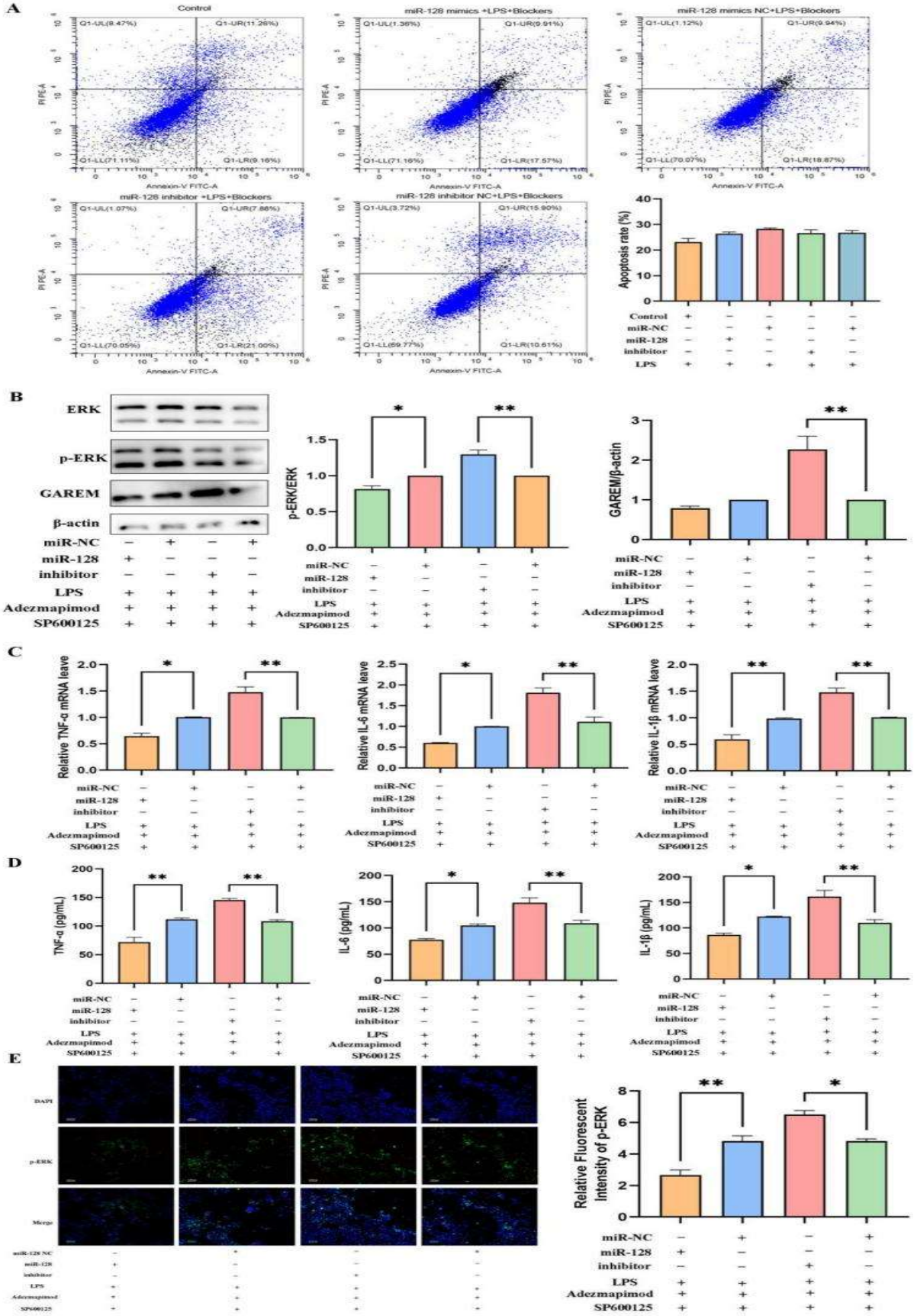

Fig. 7: miR-128 alleviates endometritis in yaks by mediating the ERK/MAPK signaling pathway. A: Results of the cell apoptosis rate following the transfection of miR-128 and its associated blockers. B: The regulatory effect of miR-128 on the p-ERK protein. C, D: The impact of miR-128 on the gene and protein expression levels of inflammatory factors. E: Immunofluorescence results indicating the expression of p-ERK. *P<0.05; **P<0.01.

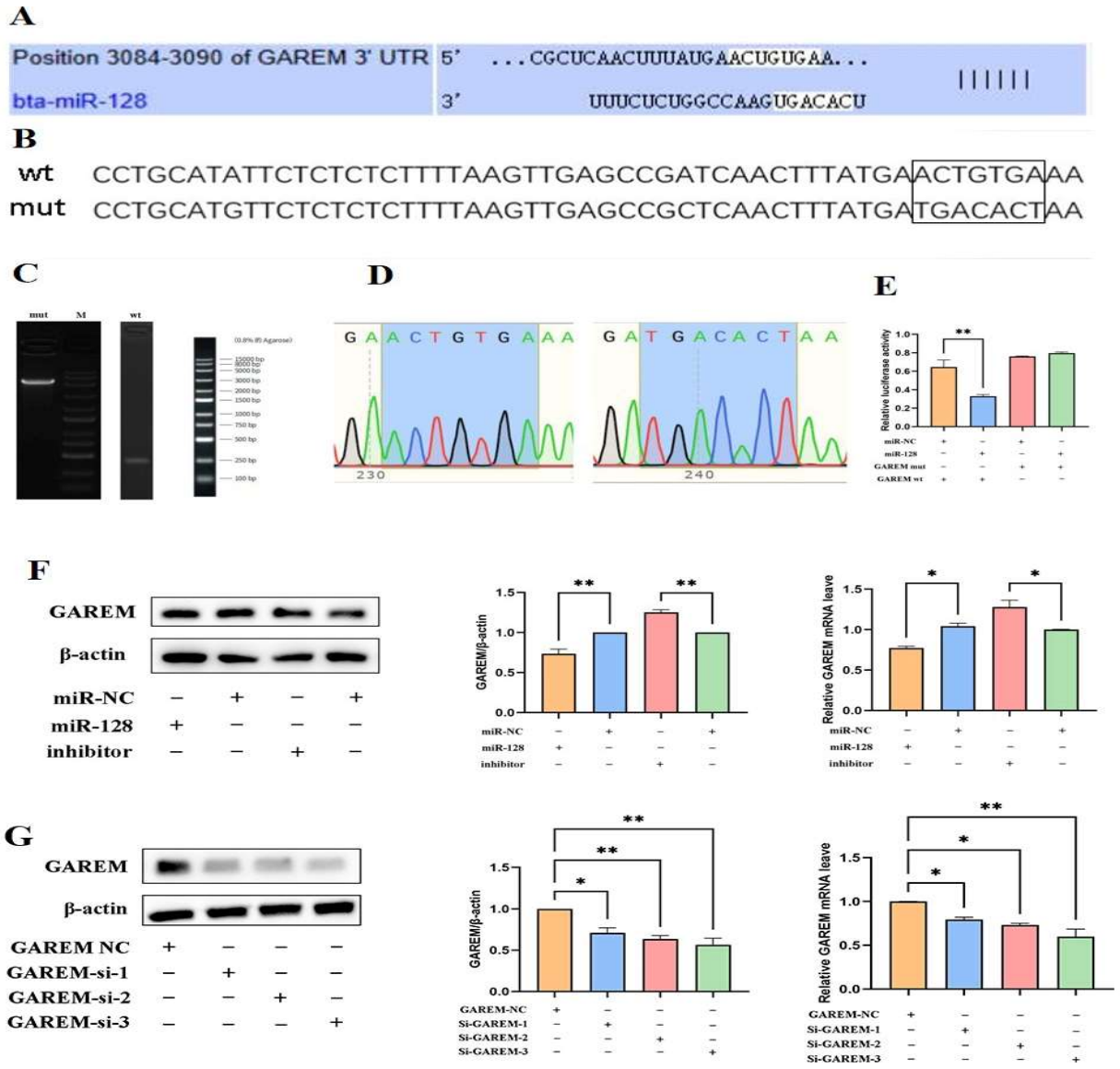

Fig. 8: Prediction, verification and regulation of miR-128 target genes. A: Prediction of the binding sites of miR-128 and GAREM. The white areas are the predicted binding sites. B: Schematic diagram of the wild-type (wt) and mutant (mut) dual-luciferase reporter vector structures. C: Results of double restriction enzyme digestion of wild-type (wt) and mutant (mut) dual-luciferase plasmids. D: Sequencing results of wild-type and mutant plasmid partial sequences. The blue part is the predicted target sequence region. E: Results of dual luciferase. F: GAREM expression. G: Screening of target gene GAREM interference sequences. *P<0.05; **P<0.01.

Silencing of GAREM reduces LPS-induced inflammatory response: Compared with GAREM NC, the mRNA and protein abundance of GAREM were significantly reduced in the GAREM-si-3 group; thus, GAREM-si-3 was chosen for subsequent experiments (Fig. 8G). Flow cytometry results indicated that the overexpression of GAREM and interference sequence transfection of GAREM into endometrial epithelial cells did not significantly affect the apoptosis rate, suggesting subsequent experiments could be performed (Fig. 9A). Without LPS treatment, the interference of GAREM or enhancing GAREM expression did not affect the protein expression of p-ERK, p-JNK, p-P38, and the expression of related inflammatory factors (Fig. 9B, C, D). After LPS treatment, GAREM interference significantly reduced the expression of related proteins in the MAPK pathway and the levels of inflammatory factors (P<0.01) (Fig. 10A, B, C). Conversely, the overexpression of GAREM exacerbated inflammation. Notably, the immunofluorescence results for p-ERK protein were consistent with the Western blot findings, indicating that GAREM interference resulted in a significant decrease in p-ERK levels (P<0.01) (Fig. 10D).

miR-128 targets GAREM to regulate LPS-induced endometritis in yaks: The co-transfection of miR-128 mimics and GAREM mimics did not affect cell apoptosis (Fig. 11A). However, the expression of MAPK pathway-related proteins, inflammatory factors, and GAREM in the co-transfection group of miR-128 and GAREM overexpression was significantly decreased as compared to NC group (Fig. 11B, C, D). The immunofluorescence detection results of p-ERK were consistent with those obtained from western blot (Fig. 11E). Furthermore, miR-128 overexpression can also significantly reduce the expression of MAPK pathway-related proteins and inflammatory factors (P<0.01).

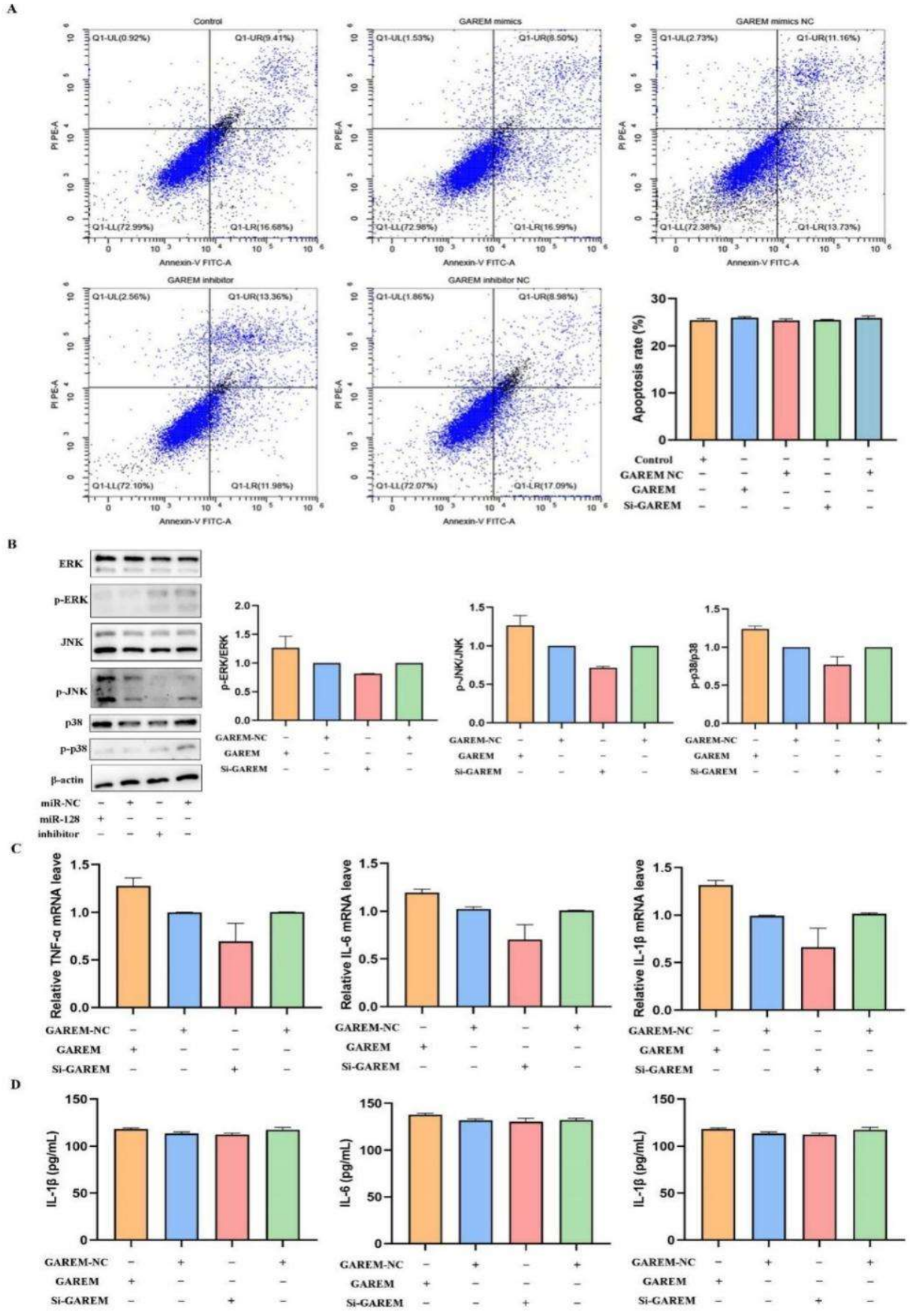

Fig. 9: Effects of target gene GAREM transfection on apoptosis of endometrial epithelial cells and its regulatory effect on the cells themselves. A: The rate of cell apoptosis following GAREM transfection. B: Proteins in the MAPK pathway. C, D: Inflammatory factors.

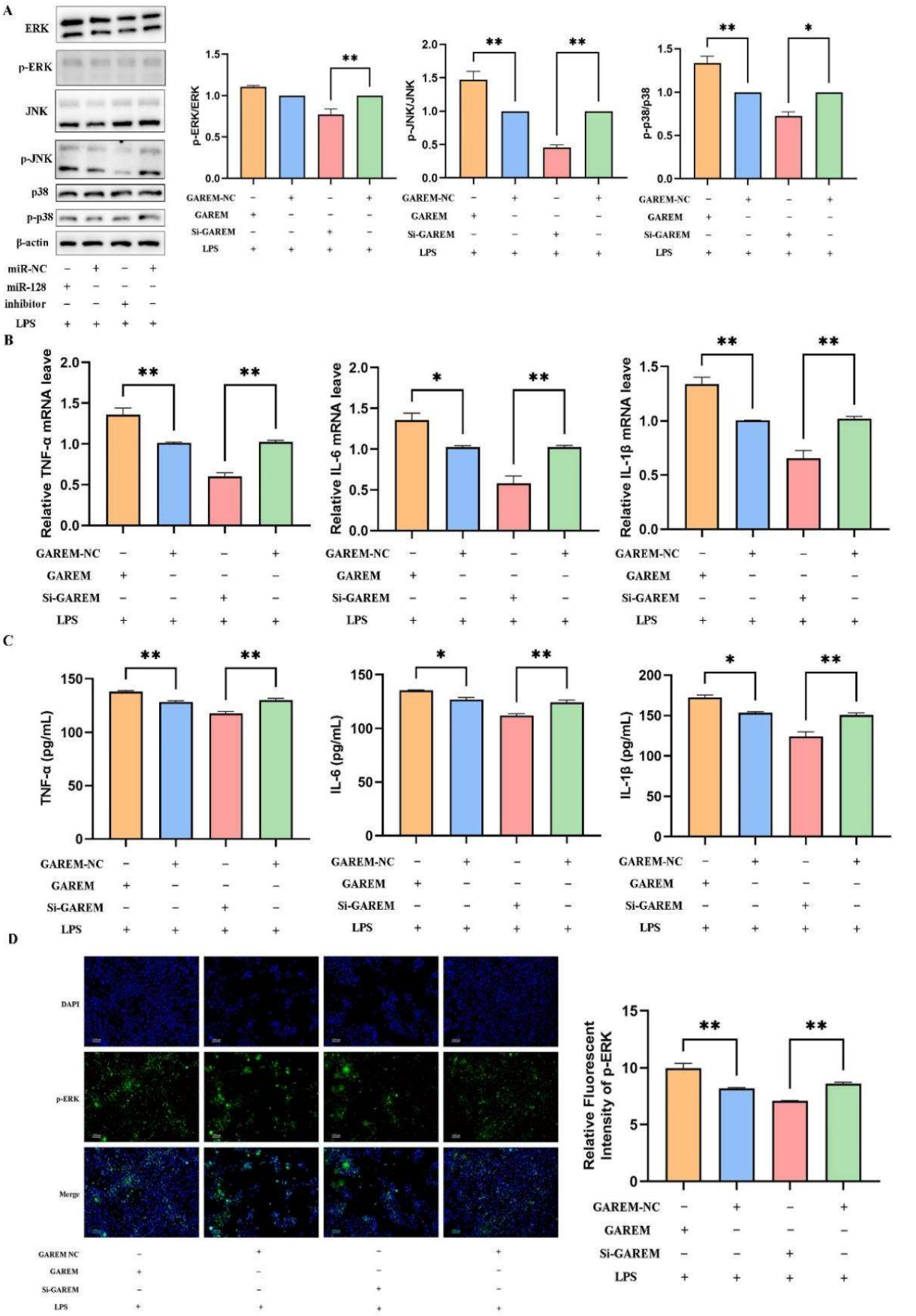

Fig. 10: Regulatory effect of target genes on LPS-induced endometritis in yaks. A: The regulatory effect of GAREM on associated proteins within the MAPK signaling pathway. B, C: Inflammatory factors. D: Results of immunofluorescence analysis for p-ERK expression. *P<0.05; **P<0.01.

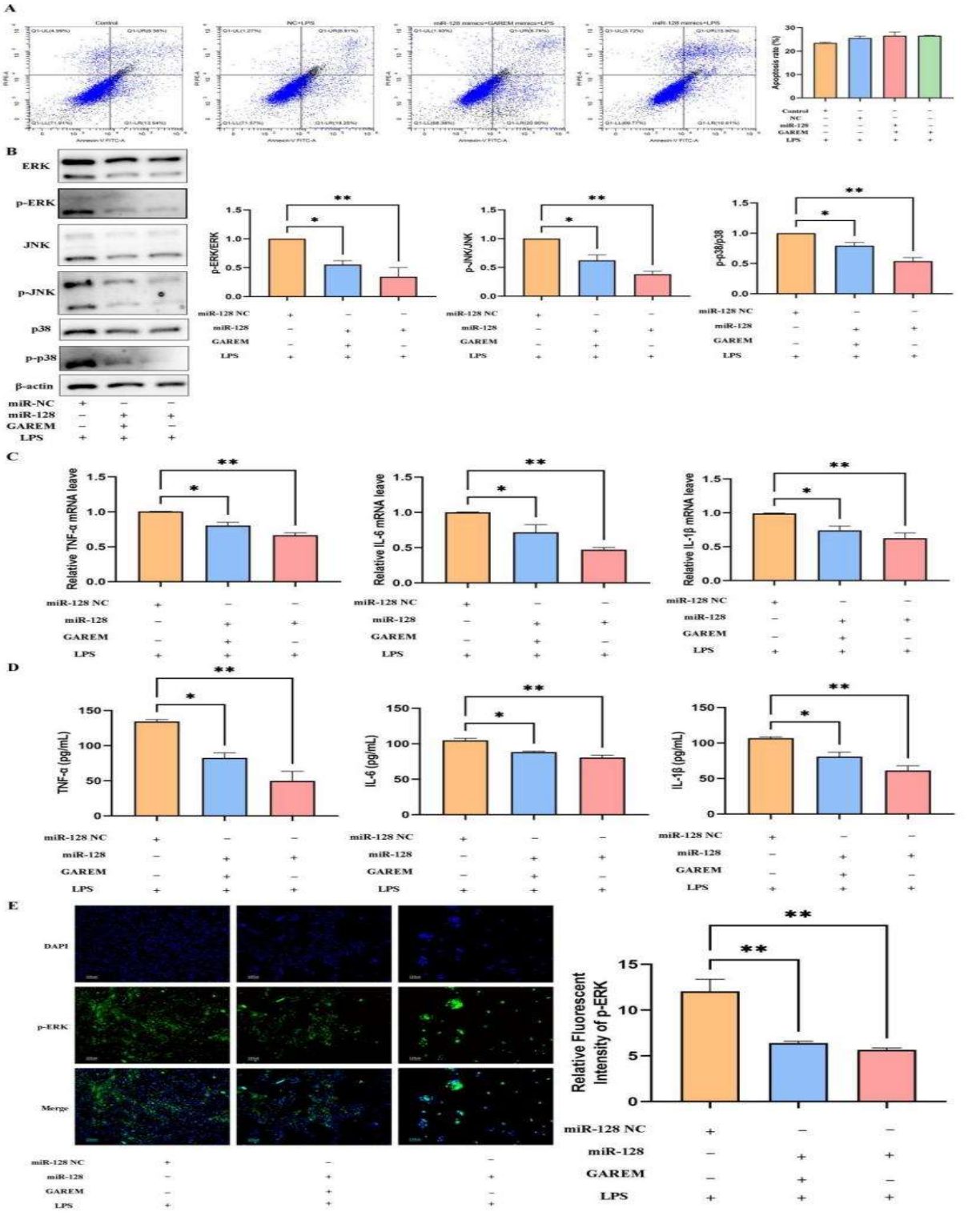

Fig. 11: Sequential transfection of miR-128 and its target gene GAREM reduces inflammation. A: Cell apoptosis rate after target gene and miR-128 transfection. B: Regulatory effect on MAPK-related proteins after target gene and miR-128 transfection. C, D: Inflammatory factors. E: Immunofluorescence expression results of p-ERK. *P<0.05; **P<0.01.

Co-transfection of the GAREM interference sequence and the miR-128 inhibitor did not significantly affect the apoptosis (P>0.05) (Fig. 12A). However, the levels of MAPK pathway-related proteins, inflammatory factors, and GAREM in the co-transfection group were significantly increased as compared to the NC group (Fig. 12B, C, D). Additionally, the immunofluorescence detection results of p-ERK were in line with the Western blot detection (Fig. 12E). However, interference with GAREM alone resulted in a significant reduction in the expression of MAPK pathway-related proteins and inflammatory factors (P<0.05), which were lower than those observed in the co-transfection group. These results suggest that miR-128 regulates LPS-induced yak endometritis through GAREM.

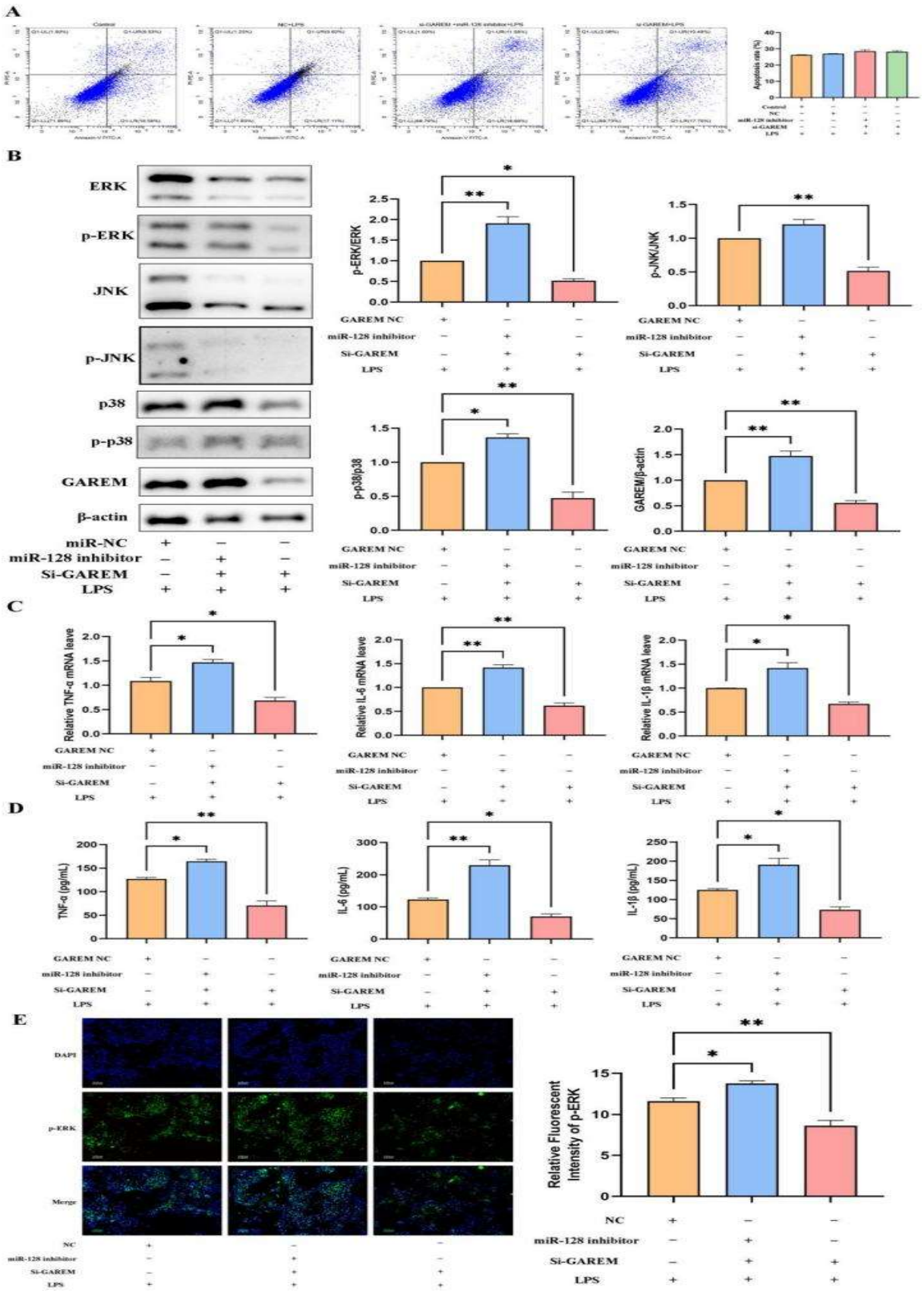

Fig. 12: Sequential transfection of target genes and miR-128 enhances inflammatory responses. A: Results of the cell apoptosis rate following the transfection of target genes and miR-128. B: The regulatory effects of target genes and miR-128 on MAPK-related proteins. C: The impact of target genes and miR-128 on the mRNA expression levels of inflammatory factors. D: The protein expression levels of inflammatory factors after the transfection of target genes and miR-128. E: Immunofluorescence results for the expression of p-ERK. *P<0.05; **P<0.01.

DISCUSSION

Endometritis, caused by microbial infection during or after childbirth in cows, is a common contributor to infertility in dairy cattle (Dickson *et al*., 2020). Yaks are native to the high-altitude hypoxic environment and have strong adaptability to such environmental conditions (Wu *et al*., 2022). Moreover, yaks have great importance for herders as they provide milk, meat, and fuel in the plateau, with closely related to agricultural development and social civilization. Moreover, endometritis is a common gynecological disease in yaks, causing miscarriage and seriously affecting the economic benefits. At present, traditional treatment still involves the administration of antibiotics (Zhang *et al*., 2022). However, the large-scale misuse of antibiotics can lead to bacterial resistance and may threaten food safety and human health. miRNAs can regulate the development of diseases, including tumors, obesity, inflammatory diseases, and bovine endometritis (Xing *et al*., 2021; Li *et al*., 2024). Therefore, miRNAs could be used as a promising therapeutic target for yak endometritis.

miRNAs modulate protein synthesis, thereby promoting degradation of target mRNAs or inhibiting their translation. It is through these means that miRNAs are an active regulator of multiple cellular processes, including cell differentiation, development, senescence, and neurodegeneration. miR-128 is a single-stranded small RNA molecule, which has been shown to hold critical responsibilities in apoptosis, cancer, and inflammation. For example, Ma *et al*. (2019) indicated that MyD88 expression was increased during acute pneumonia, while the expression of miR-128 was decreased. However, miR-128 caused a significant decrease in the phosphorylation of IκBα and p65 and the secretion of inflammatory cytokines, suggesting that miR-128 plays an important role in the alleviation of pneumonia. Additionally, Ren *et al*. (2021) indicated that miR-128 inhibited the expression of inflammatory cytokines by regulating the NF-κB signaling pathway mediated by TAB2 in order to avoid excessive inflammation. Recently, miR-128 has been demonstrated to play key roles in endometritis of dairy cows. Salilew-Wondim *et al*. (2016) used high-throughput sequencing to identify differentially expressed miRNAs in LPS-treated bovine endometritis samples and found that the miR-128 level was reduced. We also found a dramatic reduction in miR-128 expression in yak uterine tissues during endometritis. Moreover, we also constructed the yak endometritis cell model and further detected miR-128 expression after LPS induction. Results indicated that the level of miR-128 was significantly down-regulated, further demonstrating the key role that miR-128 plays in LPS-induced yak endometritis.

Excessive inflammation may disrupt immune homeostasis and lead to chronic inflammation or autoimmune diseases. Moreover, overproduction of pro-inflammatory mediators is harmful and promotes several inflammatory diseases. Previous studies indicated that LPS-treated bovine endometrial epithelial cells present an inflammatory response. In the current study, it was noted that the upregulation of IL-6, IL-1β, and TNF-α occurred in yak endometrial epithelial cells treated with LPS, indicating the inflammatory response. However, miR-128 repressed the expression of the above inflammatory factors, which suggested that miR-128 might alleviate endometritis in yaks.

MAPKs can be activated by multiple extracellular stimulation signals (Kim et al., 2019). After the activation of MAPK, extracellular signals are transmitted to the cell nucleus and regulate the activity of transcription factors with changes in the expression of related genes, inducing various cellular responses. Previous studies indicated that MAPK plays a crucial role in stress and inflammation, and is often implicated in inflammatory diseases, diabetes, and cancer (Yang *et al*., 2023). For example, cortisol could restrain the phosphorylation level of ERK1/2, JNK, and p38 in goat endometrium, which suppresses the activity of the MAPK pathways and produces anti-inflammatory effects (Cui *et al*., 2020). Similarly, Lv *et al*. (2025) indicated that high concentrations of non-esterified fatty acids can cause damage to endometrial epithelial cells in dairy cows, which is manifested as decreases in cell viability, increases in concentration of inflammatory factors, and lipid accumulation. Further exploration of mechanisms revealed that the elevation of the phosphorylation levels of JNK, ERK, and p38 and activation of the MAPK signaling pathway are closely related to non-esterified fatty acid-induced damage in dairy cows endometrial epithelial cells (Lv *et al*., 2025). However, isorhamnetin can inhibit the activation of the MAPK pathway in endometrial epithelial cells induced by non-esterified fatty acids through reducing the phosphorylation of JNK, ERK, and p38, thereby alleviating the damage caused by non-esterified fatty acids to dairy cow endometrial epithelial cells (Lv *et al*., 2025). Therefore, the activity control of the MAPK signaling pathway is important for the alleviation of inflammatory responses. Increased phosphorylation levels of ERK1/2, JNK, and p38 have been demonstrated to be closely related to the expression levels of pro-inflammatory factors (Chen *et al*., 2025). Similarly, we also observed that LPS treatment promoted the phosphorylation of ERK1/2, JNK, and p38 in yak endometrial epithelial cells. These results indicated that the activation of the MAPK signaling pathway was involved in the pathogenesis of yak endometritis. However, miR-128 could inhibit the phosphorylation of ERK1/2, JNK, and p38 induced by LPS, indicating that the alleviation effect of miR-128 on yak endometritis might be mediated

Previous studies demonstrated that GAREM could regulate multiple cellular biological functions through the activation of ERK signal transduction, indicating its important role in the MAPK signaling pathway. For example, GAREM has been identified to activate the MAPK/ERK signaling pathway, thus affecting the progression of gastric cancer. However, miR-128 can inhibit the development of gastric cancer, and the possible mechanism can be due to the fact that miR-128 inhibits the activation of the MAPK signaling pathway induced by GAREM expression. We have also confirmed that miR-128 is a direct downstream target of GAREM. Moreover, knocking down GAREM expression can inhibit MAPK pathway activation and alleviate inflammatory responses, demonstrating that GAREM should be closely associated with the MAPK pathway. Therefore, miR-128 may target GAREM to regulate the MAPK signaling pathway and affect endometritis in yaks.

Conclusions: In conclusion, the miR-128 overexpression suppresses GAREM expression, leading to a reduction in the levels of related proteins and their phosphorylation within the MAPK signaling pathway. This cascade ultimately decreases the level of proinflammatory factors, thereby alleviating yak endometritis. This study also provides new ideas and perspectives for exploring the pathogenesis and treatment of yak endometritis. However, some limitations of this study need to be noted, such as the lack of positive drug control and the absence of animal experiments.

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