

Glycolytic Suppression: How PTEN Tames the Progression of Endometriosis

Jinyan Zheng¹, Shan Guan¹, Chanjuan Dai¹, Shanshan Shao¹, Meili Peng¹, Aimi Sun^{1,*}

¹Department of Obstetrics, Sanmen People's Hospital (Sanmenwan Branch of The First Affiliated Hospital of Zhejiang University), 317100 Taizhou, Zhejiang, China

*Correspondence: sunaimi@zjsmyy.com (Aimi Sun)

Submitted: 18 June 2026 Revised: 17 July 2026 Accepted: 31 July 2026 Published: 23 October 2026

Background: Endometriosis (EMs) is a common benign gynecological disorder affecting women of reproductive age, characterized by ectopic endometrial growth, invasion, and periodic bleeding. Mounting evidence indicates that metabolic reprogramming, particularly hyperactive glycolysis, critically promotes the pathological progression of EMs. Phosphatase and tensin homolog (PTEN), a pivotal tumor suppressor and endometrial housekeeping gene, is frequently downregulated in EMs; however, its precise regulatory mechanism linking glycolysis and EMs pathogenesis remains largely unexplored.

Methods: Bioinformatics analysis was applied to characterize PTEN expression and its correlation with glycolysis-associated genes in EMs. *In vitro*, PTEN was knocked down in human normal endometrial stromal cells (NESC) and overexpressed in human endometriotic epithelial cells (12Z), while both cell types were treated with the PTEN inhibitor SF1670, followed by phenotypic and glycolytic assessments. *In vivo*, an EMs mouse model was used to verify the effects of PTEN inhibition on ectopic lesion development and glycolytic metabolism by pathological and molecular detection.

Results: PTEN expression was significantly downregulated in ectopic lesions compared with normal and eutopic counterparts, and exhibited an opposite expression trend to the core glycolytic regulators glucose transporter type 1 (Glut1) and pyruvate dehydrogenase kinase 1 (PDK1). In cellular experiments, endogenous PTEN protein levels were also markedly lower in 12Z cells than in NESC cells. Functional experiments demonstrated that loss of PTEN via knockdown or pharmacological inhibition with SF1670 markedly reinforced cell proliferation, migration, invasion, and glycolytic metabolism, whereas PTEN overexpression effectively alleviated these malignant biological behaviors. In line with *in vitro* observations, *in vivo* studies further demonstrated that SF1670-mediated PTEN inhibition significantly promoted ectopic lesion growth, primarily by suppressing PTEN expression, and this effect was accompanied by enhanced glycolytic activity in lesion tissues.

Conclusion: This study reveals that PTEN expression correlates with EMs progression and is inversely associated with glycolytic activity, suggesting a potential PTEN-glycolysis association that may serve as a target for diagnosis and therapy.

Keywords: endometriosis; phosphatase and tensin homolog; glycolysis; lactate dehydrogenase; proliferation; migration; invasion

Introduction

The abnormal implantation and growth of endometrial tissue outside the uterine cavity is the hallmark of endometriosis (EMs), a common benign gynecological disease in women of reproductive age that significantly impairs patients' quality of life and is frequently accompanied by symptoms such as dysmenorrhea and infertility [1,2]. Despite continuous advances in clinical diagnosis and treatment methods for EMs [3], its pathogenesis is complex and not yet fully clarified, leading to poor efficacy and recurrent conditions in some patients. Therefore, there is an urgent need to explore new regulatory targets and intervention strategies.

The abnormal activation of glycolysis has been identified as one of the primary drivers of metabolic reprogramming during the development of EMs, which has garnered increasing attention in recent years [4,5]. As a core

metabolic feature of tumors and chronic inflammatory diseases, glycolysis can promote the proliferation, invasion, and survival of ectopic endometrial tissue by providing cells with energy and metabolic intermediates [6,7]. A previous study has shown that the expression of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3), a key glycolytic enzyme, is significantly upregulated in ectopic lesions of EMs patients, thereby directly driving the malignant phenotypic transformation of these lesions [8]. These findings suggest that the glycolytic pathway may represent a potential therapeutic target for EMs.

Phosphatase and tensin homolog (PTEN) is a tumor suppressor gene located at 10q23.3, encoding a protein of approximately 55 kDa with both lipid phosphatase and protein phosphatase activities [9,10]. It can inhibit abnormal cell proliferation and metabolic disorders by regulating signaling pathways such as Phosphatidylinositol 3-kinase (PI3K)/Protein Kinase B (AKT) [11,12,13]. As a "house-

keeping gene” of the endometrium, PTEN plays a key role in maintaining endometrial homeostasis, and its abnormal expression is closely associated with the occurrence and development of EMs [14]. A previous study has demonstrated that PTEN inactivation can enhance the survival capacity of leukemia cells by activating glycolytic metabolism [15], but whether it exerts its effects in EMs through regulating the glycolytic pathway remains unclear.

Based on the above background, this study hypothesizes that PTEN may be involved in the pathological process of EMs by regulating the glycolytic pathway. Through a combination of bioinformatics analysis, cell experiments, and animal model validation, we systematically explore the expression characteristics of PTEN in EMs tissues, investigate its regulatory effects on the biological behavior and glycolytic activity of endometrial stromal cells, and elucidate the potential interplay between PTEN and glycolytic metabolism in EMs progression, thereby providing a novel theoretical basis for the development of diagnosis and targeted therapies for EMs.

Methods

Bioinformatics Analysis

Gene Set Selection and Integration

Target-related gene sets were screened and integrated following standardized bioinformatics protocols. Lactate dehydrogenase (LDHA) pathway-associated genes (LDHAGs) were retrieved from the GeneCards database (<https://www.genecards.org/>) by searching for “LDHA pathway” and filtering genes with a Relevance score >10. Glycolysis-related genes (GLYGs) were extracted from the MSigDB database (<https://www.gsea-msigdb.org/gsea/msigdb/>) by selecting genes involved in the “KEGG_MEDICUS_REFERENCE_GLYCOLYSIS” and “KEGG_GLYCOLYSIS_GLUONEOGENESIS” pathways, with duplicates removed. LDHAGs and GLYGs were merged to form the LDHA pathway or glycolysis-related gene set (LDGLYGs).

Clinical Dataset Acquisition and PTEN Expression Correlation Analysis

Clinical transcriptomic datasets were downloaded from the Gene Expression Omnibus (GEO) database (<http://www.ncbi.nlm.nih.gov/geo/>), including GSE51981 (77 EMs patients and 34 healthy controls with disease progression information) and GSE7305 (10 EMs patients and 10 healthy controls). The Wilcoxon test was used to analyze the correlation between PTEN expression and EMs progression in GSE51981, with an adjusted p -value (p_{adj}) < 0.05 considered statistically significant.

Differential Expression Gene (DEG) Screening

Differential analysis was performed on the two datasets using the limma package (version 3.54.0, Biocon-

ductor, Seattle, WA, USA), with screening thresholds set as $|\log \text{Fold Change (FC)}| > 0.585$ and $p_{adj} < 0.05$. DEGs between the EMs and normal endometrium groups were identified in each dataset, and the overlapping upregulated and downregulated DEGs were obtained through intersecting analysis. These DEGs were further intersected with LDGLYGs to generate LDHA pathway- or glycolysis-related DEGs (LDGLY-DEGs).

Functional Enrichment Analysis and Protein-Protein Interaction (PPI) Network Construction

To identify the biological processes, cellular components, molecular activities, and important signaling pathways involved, the overlapping DEGs were submitted to functional enrichment analysis, which included Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment and Gene Ontology (GO) annotation. PPI information was obtained by submitting relevant genes to the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (<https://string-db.org/>) in order to construct the PTEN-LDGLY-DEGs interaction network. Cytoscape software (version 3.9.1, Cytoscape Consortium, San Diego, CA, USA) was used for network visualization, with node color depth and label font size positively correlated with connectivity. All bioinformatic plots were generated using R software (version 4.3.1, R Foundation for Statistical Computing, Vienna, Austria).

Clinical Sample Collection and Animal Experimental Procedures

All procedures closely followed the Declaration of Helsinki and relevant medical ethics principles, and the study was approved by the Medical Ethics Committee of Sanmen People’s Hospital (Ethics Approval Number: 2025094). Written informed consent was obtained from all patients prior to sample collection. Inclusion criteria were as follows: enrolled subjects were reproductive-aged women (19–45 years) with regular menstrual cycles in the proliferative phase; endometrial tissues collected in the proliferative phase were confirmed by pathological examination; and patients were diagnosed with ovarian EMs via laparoscopic surgery and postoperative pathological verification. Exclusion criteria included hormonal therapy or antioxidant supplementation within 3 months before sampling, concurrent pelvic inflammatory disease, adenomyosis, malignancy, pregnancy, or lactation. A total of 9 clinical samples were collected: 3 normal endometrial tissues (Normal group), 3 eutopic endometrial tissues (Eutopic group), and 3 fresh ectopic lesion tissues (Ectopic group).

The Laboratory Animal Welfare Ethics Committee of Zhejiang Laboratory Animal Center (ZJCLA-IACUC-20120024) approved the study’s animal experiments, and all protocols closely followed applicable national laws and regulations as well as the *Guidelines for the Ethical Review*

of Laboratory Animal Welfare. Twenty-four 5-week-old female virgin C57BL/6 mice weighing between 16 and 18 grams were acquired from Hangzhou Medical College in China. For a week, each mouse was acclimated to a specific pathogen-free (SPF) animal facility, where the environment was maintained at 20–26 °C with a relative humidity of 50%–60% (the humidity inside the cages was 5%–10% higher than the ambient humidity). The mice were given standard sterile feed and unrestricted access to water, and their circadian rhythm was maintained at 12 hours (h) of light and 12 h of dark. Daily observations were conducted to record their diet, activity, and health status.

Here, 5-week-old mice were chosen for modeling based on endometrial biological characteristics rather than strict chronological age matching. These mice exhibit elevated estrogen levels and proliferative endometrium that phenocopies the estrogen-dependent endometrial phenotype of fertile women aged 19–45 years. Consistently, 4–6-week-old mice are the optimal age for syngeneic endometriosis transplantation due to their superior endometrial implantation and lesion growth ability. Following one week of acclimatization, four weeks of modeling, and three weeks of intervention, all mice reached sexual maturity with regular estrous cycles at sampling, and the established ectopic lesions recapitulated the pathological features of human reproductive-age endometriosis.

Immunohistochemistry

Tissue samples were fixed in 4% paraformaldehyde (P0099, Beyotime, Shanghai, China), gradient-dehydrated, paraffin (C0175B, Beyotime, Shanghai, China)-embedded, and sectioned into 4- μ m-thick serial sections, followed by routine deparaffinization and rehydration. High-temperature, high-pressure treatment in citrate buffer (C2488, Sigma-Aldrich, St. Louis, MO, USA) was used to retrieve the antigen; sections were thereafter allowed to cool naturally and washed with phosphate-buffered saline (PBS; ST477, Beyotime, Shanghai, China). Incubation with 3% hydrogen peroxide (88597, Millipore, Burlington, MA, USA) at room temperature inhibited endogenous peroxidase activity. After blocking non-specific binding sites with goat serum, primary antibodies against PTEN (ab170941, Abcam, Cambridge, UK; 1:100 dilution), pyruvate dehydrogenase kinase 1 (PDK1; MA5-32702, Thermo Fisher, Waltham, MA, USA; 1:200 dilution), and glucose transporter type 1 (Glut1; MA5-31960, Thermo Fisher, Waltham, MA, USA; 1:200 dilution) were added, and slices were incubated overnight at 4 °C. The next day, HRP-conjugated secondary antibody (ab6721, Abcam, Cambridge, UK; 1:1000 dilution) was added for a 1-h incubation at room temperature. Slices were developed with 3,3'-diaminobenzidine (DAB) substrate (HY-W014212, MCE, Monmouth Junction, NJ, USA), counterstained with hematoxylin (C0107, Beyotime, Shanghai, China), and sections were dehydrated, cleared, and mounted. Finally, sections

were observed and scored under a light microscope (20 $\times$, 400 $\times$, Nikon Ts2R-FL, Nikon, Tokyo, Japan).

For quantitative analysis, three random fields per section were captured at 20 $\times$ magnification. The relative positive staining area was quantified using ImageJ software (version 1.53, National Institutes of Health, Bethesda, MD, USA). The percentage of positive staining area relative to the total field area was calculated, and the mean value of three fields was used as the relative positive area for each sample.

Cell Grouping and Treatment

We bought human endometriotic epithelial cells (12Z; CL-0976) and normal endometrial stromal cells (NESC; CP-H233) from Procell Life Science & Technology Co., Ltd. (Wuhan, China). All cell lines were authenticated by short tandem repeat (STR) analysis and tested negative for mycoplasma contamination. NESC were cultured in Human Endometrial Stromal Cell Complete Medium (CM-H233, Procell, Wuhan, China), while 12Z cells were maintained in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 Ham (DMEM/F12) medium (11320033, Thermo Fisher, Waltham, MA, USA) supplemented with 10% foetal bovine serum (FBS; 10100139, Thermo Fisher, Waltham, MA, USA) and 1% penicillin/streptomycin (C0222, Beyotime, Shanghai, China). When cell confluency reached 70% to 80%, further experimental treatments were performed.

Cells were divided into four groups: NESC cells included the Control group (no transfection or drug treatment), negative control small interfering RNA (siRNA) transfection group (siNC, transfected with non-targeting negative control siRNA), PTEN knockdown group (siPTEN, transfected with PTEN-specific siRNA), and PTEN inhibitor treatment group (SF1670, treated with 1 μ M PTEN inhibitor SF1670 for 24 h) (HY-15842, MCE, Monmouth Junction, NJ, USA) [16]. SF1670 was first dissolved in dimethyl sulfoxide (DMSO; HY-Y0320C, MCE, Monmouth Junction, NJ, USA) to prepare a 10 mM stock solution, which was then diluted in complete culture medium to achieve a final concentration of 1 μ M, with the final DMSO concentration not exceeding 0.01% (v/v). The corresponding control group received complete medium containing 0.01% DMSO (v/v) without SF1670. 12Z cells included the Control group (no transfection or drug treatment), empty vector transfection negative control group (NC, transfected with empty vector without target gene), PTEN overexpression group (PTEN, transfected with PTEN overexpression vector), and PTEN inhibitor treatment group (SF1670, treated with 1 μ M PTEN inhibitor SF1670 for 24 h) [16]. The solvent composition and control for the 12Z SF1670 group were identical to those described for NESC cells. PTEN-specific siRNA (siB101025113730-1-5; 5'-AGGCGCUAUGUGUAUUAUUAUTT-3') and siNC

(siN0000002-1-5; 5'-UUCUCCGAACGUGUCACGUTT-3') were obtained from RIBOBIO (Guangzhou, China). The PTEN overexpression vector (pRP [Exp]-EGFP/Puro-CAG>hPTEN [NM_001304717.5]) and NC were purchased from Yunzhou Biosciences (Guangzhou, China) Co., Ltd.

Cells were seeded in 6-well plates at 2×10^5 cells/well and cultured overnight to 70–80% confluency. For siRNA transfection, siPTEN/siNC was diluted in Opti-MEM™ (31985062, Thermo Fisher, Waltham, MA, USA) to a final siRNA concentration of 50 nM per well. 3.75 μ L Lipofectamine™ 3000 (L3000008, Thermo Fisher, Waltham, MA, USA) was diluted in 125 μ L Opti-MEM™, mixed with siRNA diluent, incubated at room temperature for 10–15 min, and dropwise added to cells. For plasmid transfection, 1.0 μ g PTEN overexpression plasmid or empty vector was diluted in Opti-MEM™ per well. 3.75 μ L Lipofectamine™ 3000 plus 5 μ L P3000™ enhancer were separately diluted in 125 μ L Opti-MEM™, combined with plasmid diluent, incubated for 10–15 min at room temperature, and dropwise added to cells. Transfection medium was replaced with complete culture medium 6 h later.

Real-time quantitative polymerase chain reaction (RT-qPCR) and Western blotting were used to detect the knock-down and overexpression efficiency of PTEN at 24 h post-transfection. Each group was transfected with the corresponding plasmid or treated with SF1670 and then cultured for 24 h under the same conditions.

Animal Grouping and Experimental Treatments

After acclimatization, the mice were randomly divided into a control group and a PTEN inhibitor (SF1670) treatment group, with 8 mice per group; an additional 8 mice were used as donors for preparing endometrial tissue grafts. A syngeneic transplantation method was used to establish the mouse EMs model [17]: donor mice were intramuscularly injected with estradiol benzoate (HY-B1192, MCE, Monmouth Junction, NJ, USA) at 3 μ g per mouse twice a week for 1 week, then anesthetized and euthanized. Specifically, anesthesia was induced using a rodent inhalation anesthesia system (R510IP, RWD Life Science, Shenzhen, China) equipped with a precision isoflurane vaporizer (0–5% concentration range, accuracy $\pm 0.2\%$), an oxygen flowmeter (0–4 L/min), and a charcoal scavenger system for waste gas absorption. Mice were placed in an induction chamber with 3% isoflurane (HY-A0134, MCE, Monmouth Junction, NJ, USA) delivered in 100% oxygen at a flow rate of 1.5 L/min. The depth of anesthesia was assessed by the loss of righting reflex and absence of pedal withdrawal reflex (confirmed within 30–60 seconds). Immediately upon induction of the surgical anesthesia plane, mice were transferred to a nose cone maintaining 1.5–2% isoflurane in oxygen, and euthanasia was performed by cervical dislocation in accordance with the IACUC-approved protocol.

The 16 recipient mice entered the intervention phase at 4 weeks post-transplantation. Following the 3-week intervention period, all mice were euthanized at the endpoint (7 weeks post-transplantation). Ectopic tissues were harvested and examined macroscopically and histologically. The overall model success rate was retrospectively confirmed as 100% (16/16), as all recipient mice were found to harbor visible ectopic lesions ($\geq 10 \text{ mm}^3$) upon necropsy, which were subsequently confirmed by H&E staining to contain endometrial glands and stromal tissue. No mouse was excluded from analysis due to failed engraftment.

Uterine horn tissues were dissected, rinsed thoroughly with normal saline (ST341, Beyotime, Shanghai, China), and cut into 1 mm^3 tissue pieces using surgical scissors, which were then mixed evenly with 0.2 mL warm normal saline. To reduce experimental bias, tissue pieces from the same donor mouse were equally divided into two parts (each part containing 5–8 tissue pieces, approximately 0.1 mL) and intraperitoneally injected into one mouse in the treatment group and one mouse in the control group, respectively. After transplantation, all recipient mice were maintained under standard housing conditions, and the model was allowed to establish for 4 weeks (28 days) before subsequent interventions. After modeling, mice in the SF1670 group were intraperitoneally injected with PTEN inhibitor SF1670 (final concentration: 3 mg/kg, injection volume: 100 μ L per mouse) twice a week [18]. SF1670 was dissolved in a vehicle prepared by mixing dimethyl sulfoxide (DMSO; HY-Y0320C, MCE, Monmouth Junction, NJ, USA) with 20% sulfobutylether- β -Cyclodextrin (SBE- β -CD; HY-17031, MCE, Monmouth Junction, NJ, USA) in normal saline at a 1:9 (v/v) ratio. For three weeks in a row, mice in the control group received injections of the same volume of the solvent. Each mouse's body weight was recorded at the conclusion of the intervention. All mice were euthanized using the methods described earlier. Ectopic tissues were collected, their weight and volume were measured, and the samples were preserved for subsequent experiments; meanwhile, peritoneal fluid was collected to measure glucose and lactate levels.

Western Blotting

Cell and tissue protein samples were prepared and quantified by bicinchoninic acid assay (P0009, Beyotime, Shanghai, China). Sodium dodecyl sulfate polyacrylamide gel electrophoresis (P0012A, Beyotime, Shanghai, China) were prepared by target protein molecular weight; denatured samples and Marker were loaded, electrophoresed at low voltage then 120 V. Polyvinylidene fluoride membranes (2215, Millipore, Burlington, MA, USA) activated with methanol (C06901102, Nanjing Reagent, Nanjing, China) for 30 s were equilibrated, assembled into transfer sandwiches, and wet-transferred at 100 V for 1 h. Membranes were rinsed, blocked with 5% nonfat milk in Tris-buffered saline with Tween-20 (TBST; 60145ES76,

Table 1. Antibodies used in this study.

Name	Catalog	Molecular weight	Dilution	Manufacturer
PTEN	ab137337	47 kDa	1/500	Abcam, Cambridge, UK
LDHA	ab52488	37 kDa	1/5000	Abcam, Cambridge, UK
PDK1	ab202468	49 kDa	1/2000	Abcam, Cambridge, UK
Glut1	ab150299	54 kDa	1/200	Abcam, Cambridge, UK
GAPDH	ab8245	36 kDa	1/1000	Abcam, Cambridge, UK
Goat anti-mouse	ab205719	—	1/2000	Abcam, Cambridge, UK
Goat anti-rabbit	ab205718	—	1/2000	Abcam, Cambridge, UK

Table 2. Gene sequence primers.

Name	Forward primer (5'-3')	Reverse primer (5'-3')
<i>PTEN</i>	TAGAGCGTGCAGATAATGACAAGGA	TGAACTGCTAGCCTCTGGATTGA
<i>GAPDH</i>	GACAGTCAGCCGCATCTTCT	GCGCCCAATACGACCAAATC

PTEN, phosphatase and tensin homolog; *GAPDH*, glyceraldehyde 3-phosphate dehydrogenase.

Yeasen, Shanghai, China), and sequentially incubated with primary and secondary antibodies (Table 1). For densitometric quantification, protein bands were visualized by ECL (P0018FS, Beyotime, Shanghai, China) and captured using the iBright CL750 imaging system (Thermo Fisher, Waltham, MA, USA). Gray-scale analysis was performed with ImageJ software (version 1.53, National Institutes of Health, Bethesda, MD, USA). Target protein expression was normalized to the corresponding GAPDH band intensity from the same lane and expressed as fold change relative to the control group. Each experiment was independently repeated three times ($n = 3$).

RT-qPCR

Total RNA was isolated with TRIzol reagent (A33250, Thermo Fisher, Waltham, MA, USA), and first-strand complementary DNA (cDNA) was synthesized using Roche's kit (04379012001, Roche Diagnostics, Mannheim, Baden-Württemberg, Germany). RT-qPCR was run on an ABI 7500 with SYBR Green Mix (4344463, Thermo Fisher, Waltham, MA, USA) in a 20 μ L system (10 μ L 2 $\times$ Mix, 0.8 μ L each primer, 2 μ L diluted cDNA, 6.4 μ L RNase-free water). Primer sequences are listed in Table 2. Relative mRNA expression was calculated using the $2^{-\Delta\Delta CT}$ method, with *glyceraldehyde 3-phosphate dehydrogenase* (*GAPDH*) serving as the internal reference [19].

Cell Counting Kit-8 (CCK-8)

For the CCK-8 cell viability assay, NESC and 12Z cells after grouping and treatment were seeded into 96-well culture plates (FCP962, Beyotime, Shanghai, China) at a density of 5×10^3 cells per well. After 24 h of incubation under standard culture conditions (37 °C, 5% CO₂), 10 μ L of CCK-8 reagent (HY-K0301, MCE, Monmouth Junction, NJ, USA) was added to each well, and the plates were further incubated at 37 °C for 2 h. The absorbance value at 450 nm was measured using a microplate reader (Varioskan

LUX, Thermo Fisher, Waltham, MA, USA), and the relative cell viability was calculated based on the absorbance data.

Colony Formation Experiment

After trypsinization and centrifugation, NESC and 12Z cells were resuspended, counted, and diluted to 1×10^3 cells/mL. 200 cells per well were seeded into 6-well plates (FCP060, Beyotime, Shanghai, China) and incubated under standard conditions until adhesion. Cells were then treated per grouping and cultured continuously for 2–3 weeks with regular medium changes. To maintain PTEN knockdown or overexpression throughout the assay period, siRNA or plasmid transfection was repeated every 72 hours until colony formation was terminated. The culture was stopped when separate clones formed. The wells were then gently rinsed two or three times with PBS, fixed with 1 mL methanol for 15 min at room temperature, and stained for 30 min with 1 mL Giemsa dye (C0133, Beyotime, Shanghai, China). Plates were air-dried after staining, rinsed with running water to remove extra dye, and visible colonies were counted under a microscope with image documentation (≥ 50 cells/clone).

Scratch Assay

Grouped and treated NESC and 12Z cells were seeded into 6-well plates. When the cells reached >90% confluency, uniform vertical scratches were generated using sterile 200 μ L pipette tips (FTIP725, Beyotime, Shanghai, China). After PBS washing twice to remove detached cells, serum-free medium was added, and cells were cultured at 37 °C with 5% CO₂ (Forma Steri-Cult, Thermo Fisher, Waltham, MA, USA). Scratches were imaged at 0 h/24 h under 100 $\times$ microscopy; migration rate was calculated by measuring scratch width to assess cell migration.

Transwell Assay

Matrigel (356234, Solarbio, Beijing, China) was diluted with serum-free medium at a ratio of 1:8 (v/v). A volume of 50 μ L per well of the diluted Matrigel solution was added to the upper chamber of Transwell inserts (FTW043, Beyotime, Shanghai, China), then incubated at 37 °C for 4–6 hours to form a gel. NESC and 12Z cells, after grouping and treatment, were resuspended in serum-free medium, adjusted to a density of 5×10^4 cells/mL, and 200 μ L of the cell suspension was added to the upper chamber. Meanwhile, 600 μ L of medium containing 10% FBS was added to the lower chamber as a chemoattractant. Cells were cultivated for 24 hours at 37 °C with 5% CO₂. The membrane was fixed with methanol for 15 min, stained with Giemsa dye (C0133, Beyotime, Shanghai, China) for 30 min, and rinsed with running water after the non-invaded upper membrane cells were removed using a cotton swab. To evaluate invasive ability, invaded lower membrane cells were counted (5 random fields/insert) and photographed under a 100 $\times$ microscope.

Detection of Glucose Consumption and Lactate Level

Cell culture medium and peritoneal fluid were collected, and the supernatants were obtained by centrifugation. According to the manufacturers' instructions for the glucose detection kit (S0202S, Beyotime, Shanghai, China) and lactate detection kit (S0208S, Beyotime, Shanghai, China), the corresponding reaction reagents were added and incubated in the dark. Absorbance at 510 nm (glucose) and 450 nm (lactate) was measured by a microplate reader. To ascertain the relative consumption and levels of glucose and lactate in cells and peritoneal fluid, their concentrations were computed using standard curves.

Hematoxylin and Eosin (HE) Staining

Ectopic tissues were fixed in 4% paraformaldehyde for 24 h, routinely dehydrated, paraffin-embedded and serially sectioned into 5- μ m-thick sections. Sections were deparaffinized and rehydrated, then stained with hematoxylin for five minutes (C0107, Beyotime, Shanghai, China). After rinsing with tap water, the sections were counterstained with eosin for two minutes (C0109, Beyotime, Shanghai, China). Finally, the sections were dehydrated, cleaned, and mounted using neutral balsam (C0173, Beyotime, Shanghai, China). To determine the extent of the lesion, pathological morphology was examined under a 400 $\times$ microscope.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism software (version 8.0, GraphPad Software, San Diego, CA, USA) [20]. Quantitative data are presented as mean $\pm$ standard deviation (SD). Comparisons between two groups were analyzed by independent samples *t*-test, while one-way analysis of variance (ANOVA) followed by

Tukey's post-hoc test was used for multiple group comparisons. $p < 0.05$ was considered statistically significant.

Results

Expression of PTEN in EMs and Characteristics of Related DEGs

Bioinformatics analysis (GSE51981) revealed that *PTEN* expression was lower in both the minimal/mild and moderate/severe endometriosis groups than in healthy controls (Fig. 1A, $p < 0.05$). Compared with healthy tissues, *PTEN* expression was decreased in mild/moderate EMs tissues (Fig. 1A, $p < 0.05$). Through differential analysis, 7344 DEGs between EMs patients and healthy controls were identified in the GSE51981 dataset, including 4298 downregulated DEGs and 3046 upregulated DEGs (Fig. 1B). In the GSE7305 dataset, a total of 3045 DEGs were identified, consisting of 1511 downregulated DEGs and 1534 upregulated DEGs (Fig. 1D). Heatmaps showed the expression patterns of different DEGs between the two groups (Fig. 1C,E). A total of 871 overlapping DEGs were identified in the two datasets, including 230 upregulated DEGs and 641 downregulated DEGs (Fig. 1F,G).

Characteristics of DEGs Enrichment in EMs and Interaction Between PTEN and Glycolysis-Related Genes

As shown in Fig. 2A, GO enrichment analysis of upregulated DEGs revealed that these genes were mainly involved in pathways such as regulation of leukocyte chemotaxis, collagen-containing extracellular matrix, and amyloid-beta binding. KEGG enrichment analysis indicated that the upregulated DEGs were primarily enriched in pathways including complement and coagulation cascades, as well as cholesterol metabolism (Fig. 2B). For downregulated DEGs, GO enrichment analysis demonstrated that these genes were mainly involved in pathways like chromosome segregation and chromosomal regions (Fig. 2C). KEGG enrichment analysis showed that the downregulated DEGs were mainly associated with pathways such as cell cycle, motor proteins, and cellular senescence (Fig. 2D).

Subsequently, we identified 198 genes related to the LDHA pathway and 39 genes associated with the glycolysis pathway. After merging these genes, we intersected them with EMs DEGs and obtained 16 LDGLY-DEGs related to either the LDHA pathway or the glycolysis pathway (Fig. 2E). The PPI network between *PTEN* and 15 LDGLY-DEGs with documented interactions was shown in Fig. 2F. One LDGLY-DEG, *NKD2*, was excluded from the network as it lacked interactions with *PTEN* or other LDGLY-DEGs in the STRING database at medium confidence. With an average node degree of 5.18 and a local clustering coefficient of 0.633, this PPI network had 16 nodes and 44 edges (Fig. 2F). *PTEN*, *CCND1*, and *IGF1* had the highest connection degrees (Fig. 2F). *PTEN* had direct asso-

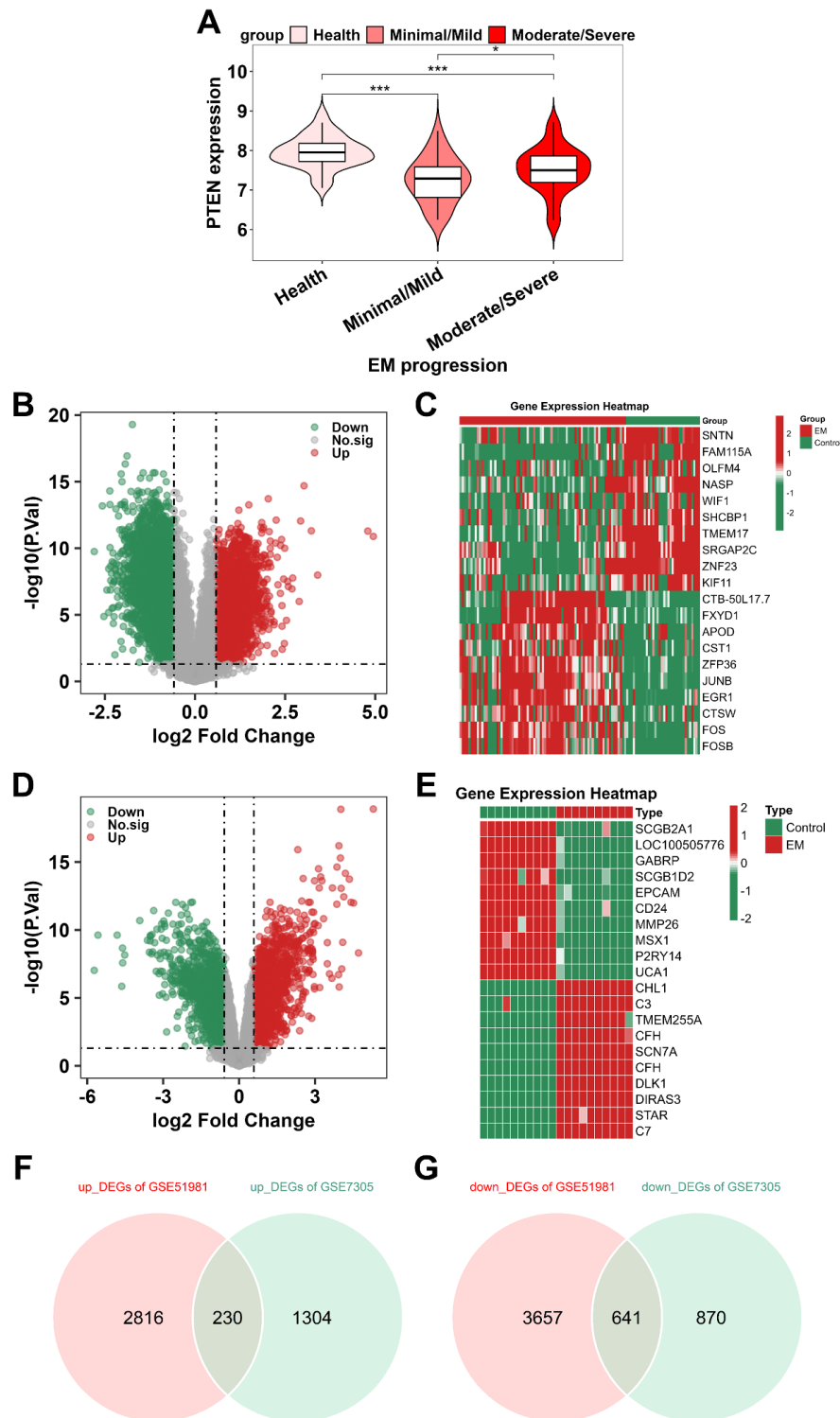


Fig. 1. Bioinformatics analysis of phosphatase and tensin homolog (*PTEN*) expression and differentially expressed genes (DEGs) in endometriosis (EMs). (A) *PTEN* expression in healthy, minimal/mild, and moderate/severe EMs endometrial tissues (Gene Expression Omnibus (GEO) datasets GSE51981). * $p < 0.05$, *** $p < 0.001$. (B,C) GSE51981 dataset (77 EMs vs 34 normal tissues): DEG volcano plot (B, red/green dots: up/downregulated DEGs) and heatmap (C, adjusted $p < 0.05$, $|\log_2 \text{fold change (FC)}| > 0.585$; green-red: low-high expression). (D,E) GSE7305 dataset (10 EMs vs 10 normal tissues): DEG volcano plot (D) and heatmap (E) (same screening criteria as B,C). (F,G) Venn diagram shows overlapping DEGs: upregulated (F, GSE51981: 3046, GSE7305: 1534, overlap: 230) and downregulated (G, GSE51981: 4298, GSE7305: 1511, overlap: 641). Fig. 1 was created using R (version 4.3.1, R Foundation for Statistical Computing, Vienna, Austria).

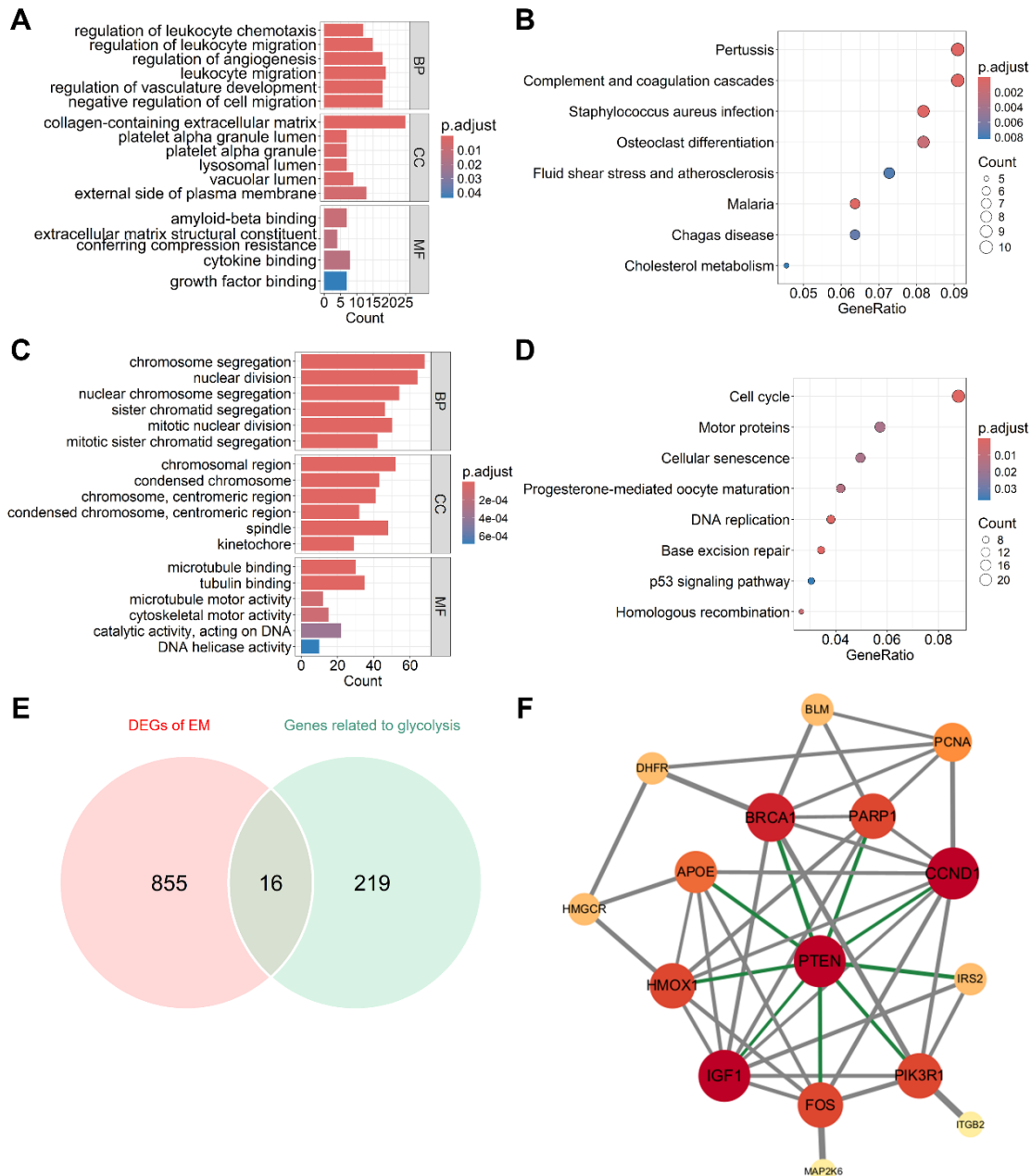


Fig. 2. Gene Ontology (GO)/Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of EMs-related DEGs and protein-protein interaction (PPI) network between *PTEN* and lactate dehydrogenase (LDHA) pathway or glycolysis-related DEGs (LDGLY-DEGs). (A,C) GO enrichment of upregulated/downregulated DEGs in EMs. (BP, biological process; CC, cellular component; MF, molecular function). (B,D) KEGG pathway enrichment of upregulated/downregulated DEGs in EMs. (E) Venn diagram identifying 16 LDGLY-DEGs by intersecting EMs DEGs, 198 LDHA pathway genes, and 39 glycolysis genes. (F) PPI network of *PTEN* and LDGLY-DEGs. Node size: connection degree. Green edges: *PTEN*-LDGLY-DEG direct interactions; gray edges: other gene interactions. Edge thickness: betweenness centrality; node color/font size: connectivity (deeper/larger = higher). Fig. 2A–E was created using R (version 4.3.1, R Foundation for Statistical Computing, Vienna, Austria); Fig. 2F was created using Cytoscape (version 3.9.1, Cytoscape Consortium, San Diego, CA, USA).

ciations with *CCND1*, *IGF1*, *BRCA1*, *PIK3R1*, *HMOX1*, *FOS*, *PARP1*, *APOE*, and *IRS2*, and indirect associations with *PCNA*, *BLM*, *DHFR*, *HMGCR*, *MAP2K6*, and *ITGB2* (Fig. 2F).

We detected the expression of *PTEN*, *PDK1*, and *Glut1* in clinical samples via immunohistochemistry. Preliminary immunohistochemical observations revealed that the expression of *PTEN* (yellow-brown) was decreased in

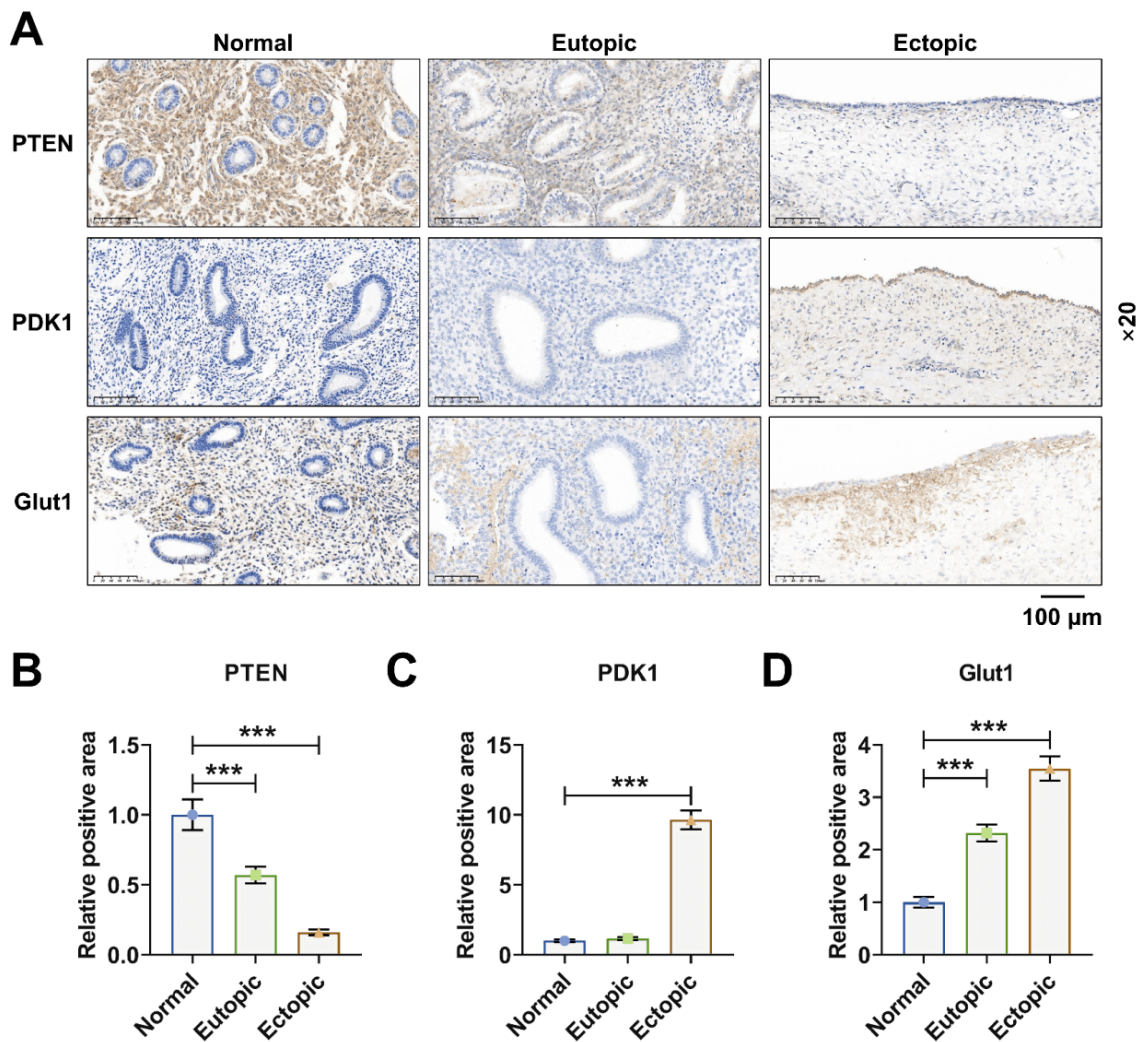


Fig. 3. Downregulation of PTEN and upregulation of pyruvate dehydrogenase kinase 1 (PDK1), glucose transporter type 1 (Glut1) in EMs tissues. (A) Immunohistochemistry staining images of PTEN, PDK1, and Glut1 in normal, eutopic, and ectopic endometrial tissues (positive signal: yellow-brown; magnification: ×20, scale bar: 100 μm). Abundant glands exist in normal/eutopic endometrium, whereas ectopic lesions inherently feature stroma-dominant tissues with sparse glands. (B–D) Quantitative analysis of the relative positive staining area for *PTEN* (B), *PDK1* (C), and *Glut1* (D) in normal, eutopic, and ectopic tissues. Normal and eutopic endometrial samples were collected from the endometrial functional layer under standard sampling. Ectopic lesion samples were obtained from laparoscopically excised ovarian or peritoneal endometriotic lesions confirmed by postoperative pathological examination. Data are presented as mean ± standard deviation (SD) (n = 3 independent experiments). *** $p < 0.001$. Statistical graphs were created using GraphPad Prism (version 8.0, GraphPad Software, San Diego, CA, USA).

ectopic tissues, while the expression patterns of key glycolysis pathway molecules, *PDK1* and *Glut1* (yellow-brown), exhibited the opposite trend, with their expression levels being higher in ectopic lesion tissues than in normal tissues (Fig. 3A). Semiquantitative analysis of the relative positive staining area supported these preliminary observations: *PTEN* staining was reduced in ectopic tissues compared to both normal and eutopic tissues ($p < 0.001$), whereas *PDK1* and *Glut1* expression was markedly elevated in ectopic lesions ($p < 0.001$), consistent with the qualitative staining patterns (Fig. 3B–D).

Regulation of Endometrial Cell Functions by PTEN Expression

Given that primary normal endometrial stromal cells exhibit high endogenous PTEN levels and 12Z cells show low endogenous PTEN expression (Fig. 4A,B, $p < 0.05$), we performed PTEN loss-of-function in NESC and gain-of-function in 12Z to evaluate bidirectional regulatory effects of PTEN on cell phenotypes and glycolysis. Further regulation of PTEN expression via transfection technology revealed that successful transfection of siPTEN into NESC cells effectively reduced PTEN protein expression, while

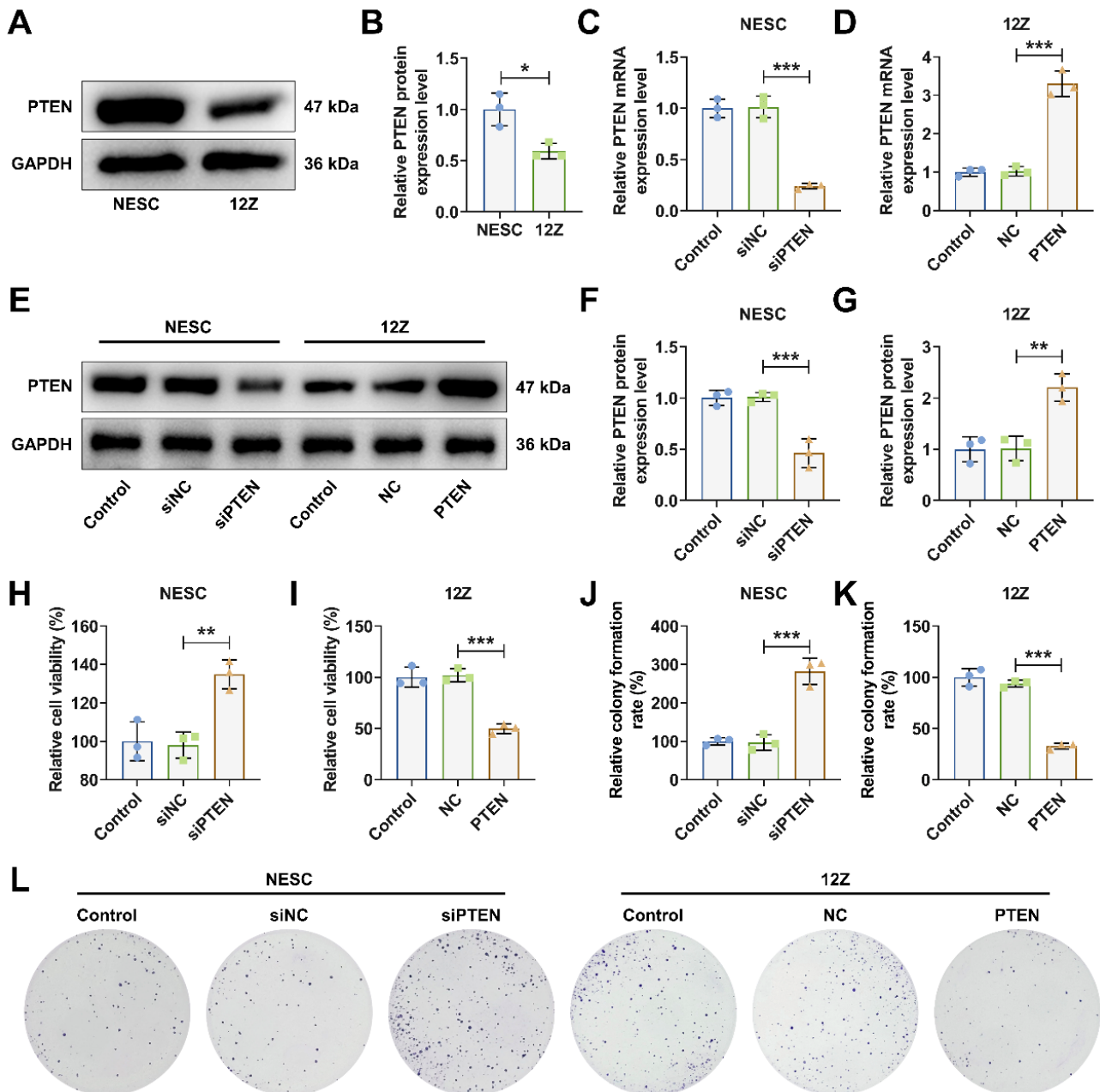


Fig. 4. PTEN regulates viability and proliferation of normal endometrial stromal cells (NESC) and human endometriotic epithelial cells (12Z). (A,B) Western blot analysis of PTEN protein expression in NESC and 12Z; glyceraldehyde 3-phosphate dehydrogenase (GAPDH) served as the internal reference. (C–G) Verification of PTEN knockdown efficiency in NESC and overexpression efficiency in 12Z via qRT-PCR (C,D) and Western blot analysis (E–G). GAPDH served as the internal reference. (H,I) Relative cell viability of each group detected by cell counting kit-8 (CCK-8) assay. (J–L) Quantification of cell proliferation in each group detected by colony formation assay. Data are presented as mean $\pm$ SD ($n = 3$ independent experiments). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Statistical graphs were created using GraphPad Prism (version 8.0, GraphPad Software, San Diego, CA, USA).

transfection of a PTEN overexpression vector into 12Z cells significantly increased PTEN levels, confirming the effectiveness of the transfection (Fig. 4C–G, $p < 0.01$). Results of cell function assays indicated that knockdown of PTEN in NESC cells enhanced cell viability and proliferation; in contrast, overexpression of PTEN in 12Z cells significantly

inhibited cell viability and proliferation (Fig. 4H–L, $p < 0.01$). Collectively, these findings demonstrate that PTEN negatively regulates the proliferation and viability of both endometrial stromal cells and ectopic endometrial epithelial cells.

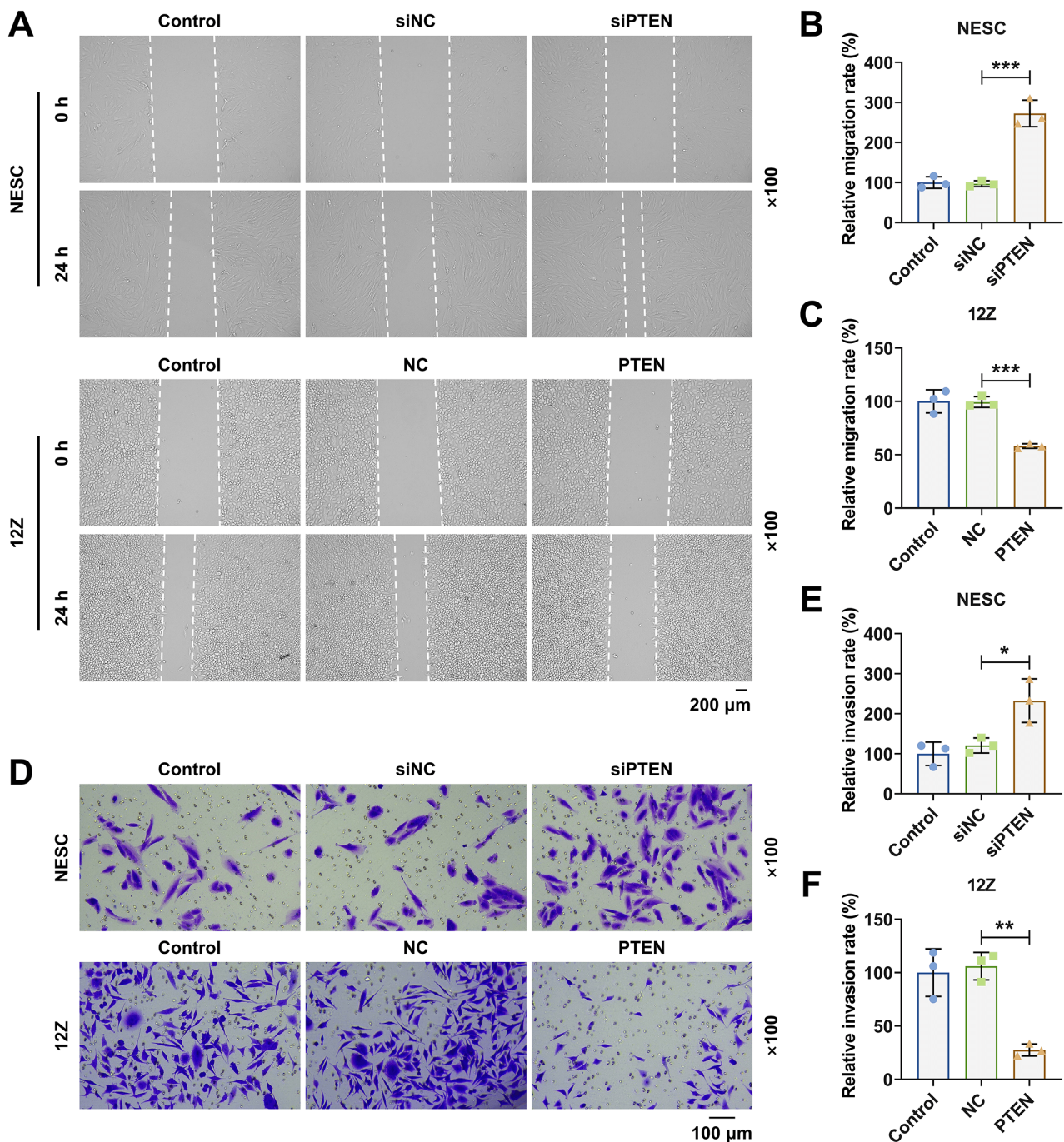


Fig. 5. PTEN modulates migration and invasion of endometrial stromal cells. (A–C) Scratch wound healing assay images of NESC and 12Z at 0 h and 24 h (magnification: $\times 100$, scale bar: 200 μ m). (D–F) Transwell invasion assay images of NESC and 12Z cells (magnification: $\times 100$, scale bar: 100 μ m). Data are presented as mean $\pm$ SD ($n = 3$ independent experiments). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Statistical graphs were created using GraphPad Prism (version 8.0, GraphPad Software, San Diego, CA, USA).

PTEN Regulates Migration and Invasion of Endometrial Cells

In cell migration and invasion assays, PTEN knock-down in NESC cells accelerated cell migration (as reflected by enhanced scratch wound healing) and increased invasion capacity (Fig. 5A,B,D,E, $p < 0.05$). Conversely, PTEN overexpression in 12Z cells slowed down cell migration,

impeded scratch wound healing, and reduced invasion capacity (Fig. 5A,C,D,F, $p < 0.01$). These data confirm that PTEN exerts an inhibitory effect on migration and invasion in both endometrial stromal cells and ectopic endometrial epithelial cells.

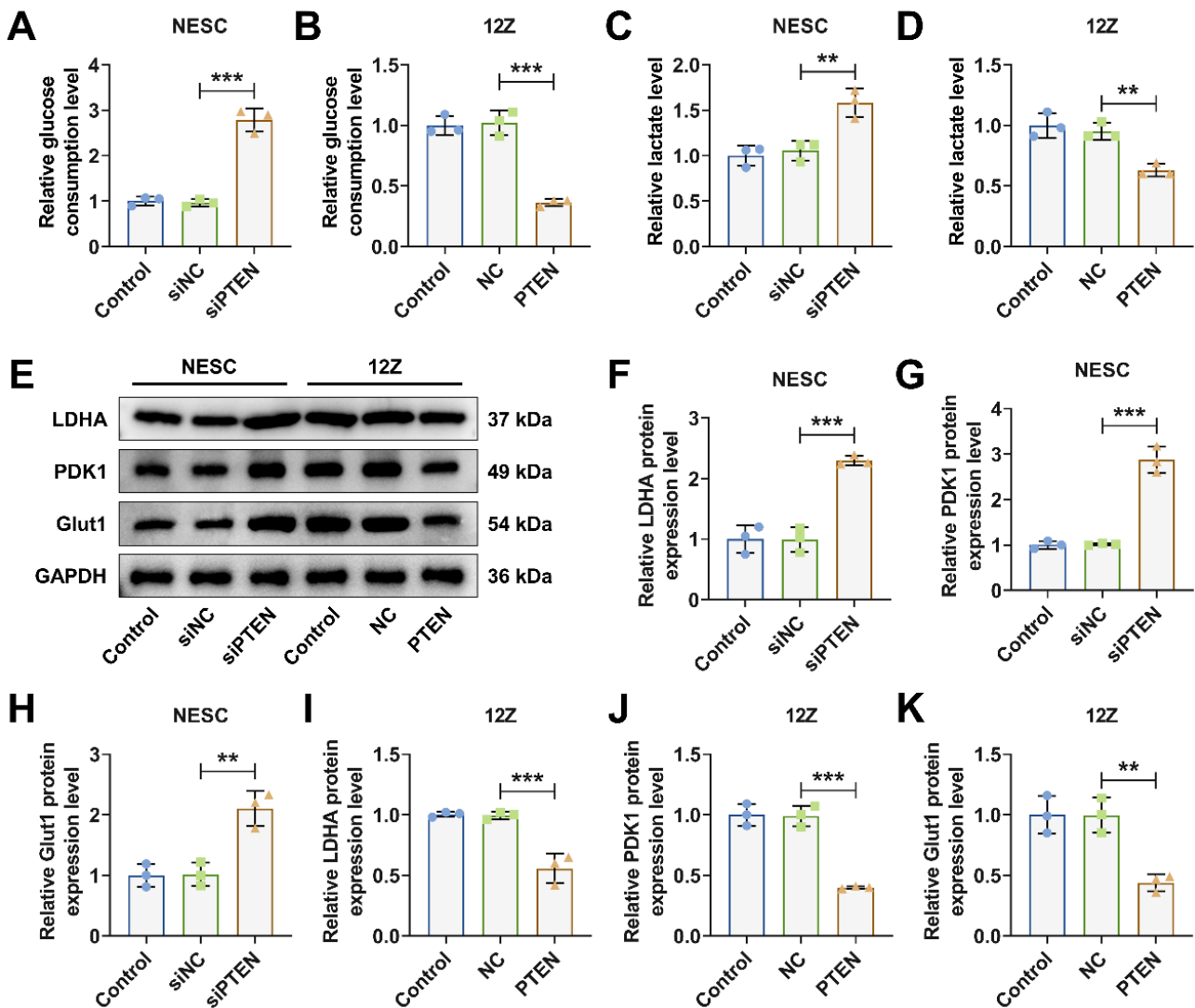


Fig. 6. PTEN regulates glucose metabolism and glycolysis-related protein expression in NESC and 12Z cells. (A,B) Quantitative analysis of relative glucose consumption in NESC and 12Z cells. (C,D) Quantitative analysis of relative lactate production in NESC and 12Z cells. (E–K) Western blot analysis of glycolysis-related proteins (LDHA, PDK1, Glut1) in NESC and 12Z cells; GAPDH served as the internal reference. Data are presented as mean $\pm$ SD ($n = 3$ independent experiments). ** $p < 0.01$, *** $p < 0.001$. Statistical graphs were created using GraphPad Prism (version 8.0, GraphPad Software, San Diego, CA, USA).

PTEN Regulates Glucose Metabolism and Glycolysis-Related Protein Expression in Endometrial Cells

In glucose metabolism assays, PTEN knockdown in NESC cells significantly increased both glucose consumption and lactate production levels; in contrast, PTEN overexpression in 12Z cells led to the opposite trends in these two indices (Fig. 6A–D, $p < 0.01$). Further detection of key glycolysis pathway proteins showed that PTEN knockdown in NESC cells upregulated the expression of LDHA, PDK1, and Glut1 proteins, while PTEN overexpression in 12Z cells downregulated their expression (Fig. 6E–K, $p < 0.01$).

PTEN Inhibitor SF1670 Suppresses PTEN Expression and Promotes Cell Functions

To mitigate cell-type-specific bias, SF1670, a PTEN inhibitor, was additionally applied to both cell lines in parallel. Western blot analysis showed that PTEN protein expression was downregulated in both NESC and 12Z cells (Fig. 7A–C, $p < 0.01$). Results of cell function assays indicated that SF1670 intervention enhanced the viability and proliferation capacity of both cell types (Fig. 7D–H, $p < 0.05$). In addition, after treatment with the PTEN inhibitor SF1670, the migration capacity of both NESC and 12Z cells was significantly enhanced (Fig. 8A–C, $p < 0.05$). Meanwhile, results of the Transwell invasion assay showed that SF1670 intervention also increased the invasion capacity

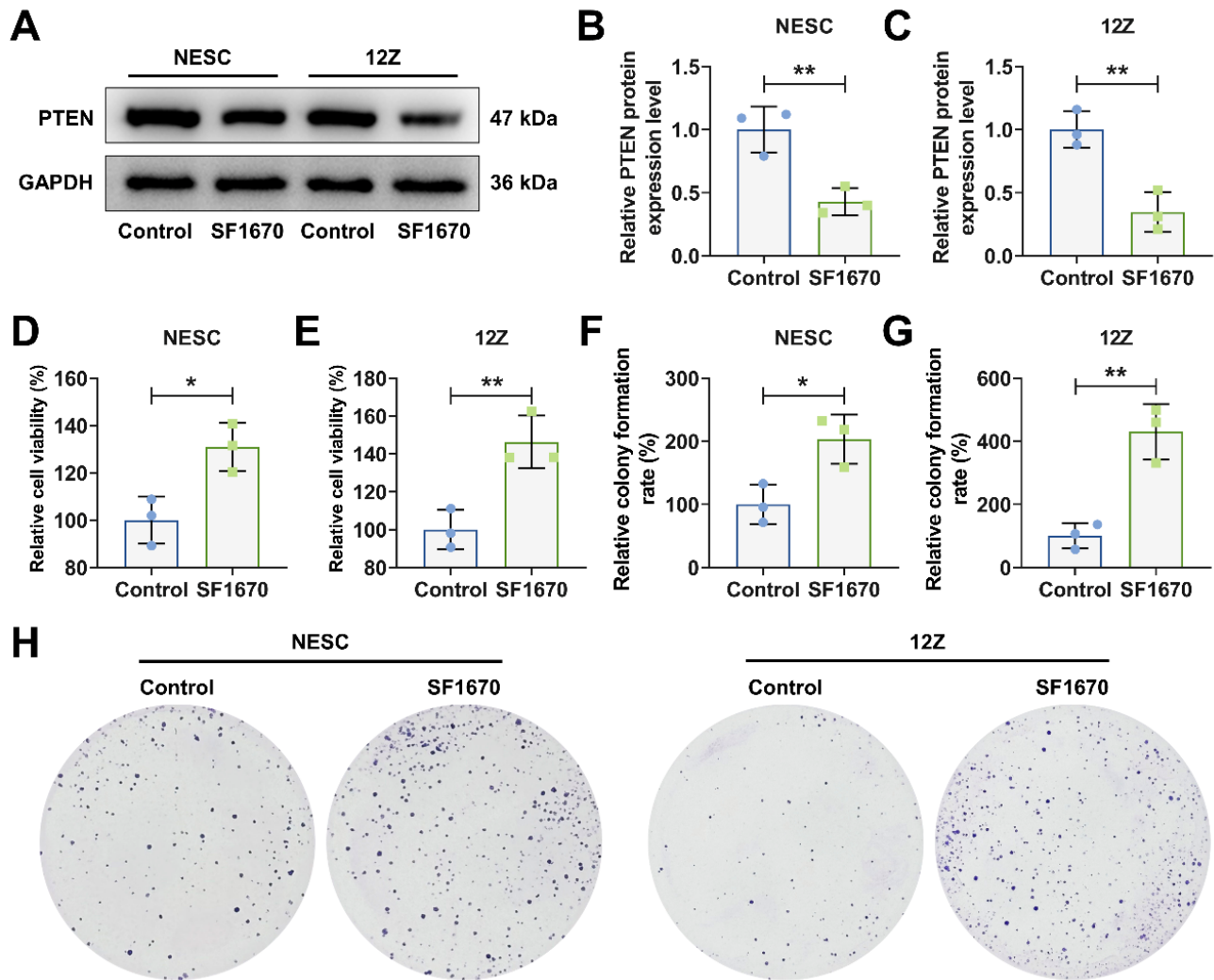


Fig. 7. PTEN inhibitor SF1670 suppresses PTEN expression and promotes cell viability and proliferation. (A–C) Western blot analysis of PTEN protein expression in NESC and 12Z cells treated with SF1670; GAPDH served as the internal reference. (D,E) Relative cell viability of each group detected by CCK-8 assay. (F–H) Quantification of cell proliferation in each group detected by colony formation assay. Data are presented as mean $\pm$ SD ($n = 3$ independent experiments). * $p < 0.05$, ** $p < 0.01$. Statistical graphs were created using GraphPad Prism (version 8.0, GraphPad Software, San Diego, CA, USA).

of both cell types (Fig. 8D–F, $p < 0.001$). In summary, the suppression of PTEN by SF1670 drives malignant biological behaviors in both stromal and ectopic epithelial cells of endometriosis.

PTEN Inhibitor SF1670 Promotes Glucose Metabolism and Glycolysis-Related Protein Expression in Endometrial Cells

After treatment with the PTEN inhibitor SF1670, both glucose consumption and lactate production levels increased in NESC and 12Z cells (Fig. 9A–D, $p < 0.05$). Meanwhile, the expression of key glycolysis pathway proteins (LDHA, PDK1, Glut1) was also upregulated (Fig. 9E–K, $p < 0.05$). These results align with the previously observed patterns of PTEN expression regulation, further indicating that PTEN suppresses glycolysis in both endometrial

stromal and ectopic epithelial cells, while PTEN inhibition enhances glycolytic activity in these cells.

PTEN Inhibitor SF1670 Promotes Ectopic Lesion Growth in a Mouse Model of Endometriosis

In a mouse model of EMs, intervention with the PTEN inhibitor SF1670 did not significantly alter mouse body weight (Fig. 10A), but notably increased the weight and volume of ectopic lesions (Fig. 10B–D, $p < 0.001$). Histopathological staining revealed that ectopic lesions in the SF1670-treated group exhibited denser glandular structures and increased stromal cellularity compared to the control group (Fig. 10E). Immunohistochemistry results showed that PTEN expression levels (positive signal: yellow-brown) in lesion tissues were significantly reduced in the SF1670-treated group (Fig. 10F,G, $p < 0.01$). Mean-

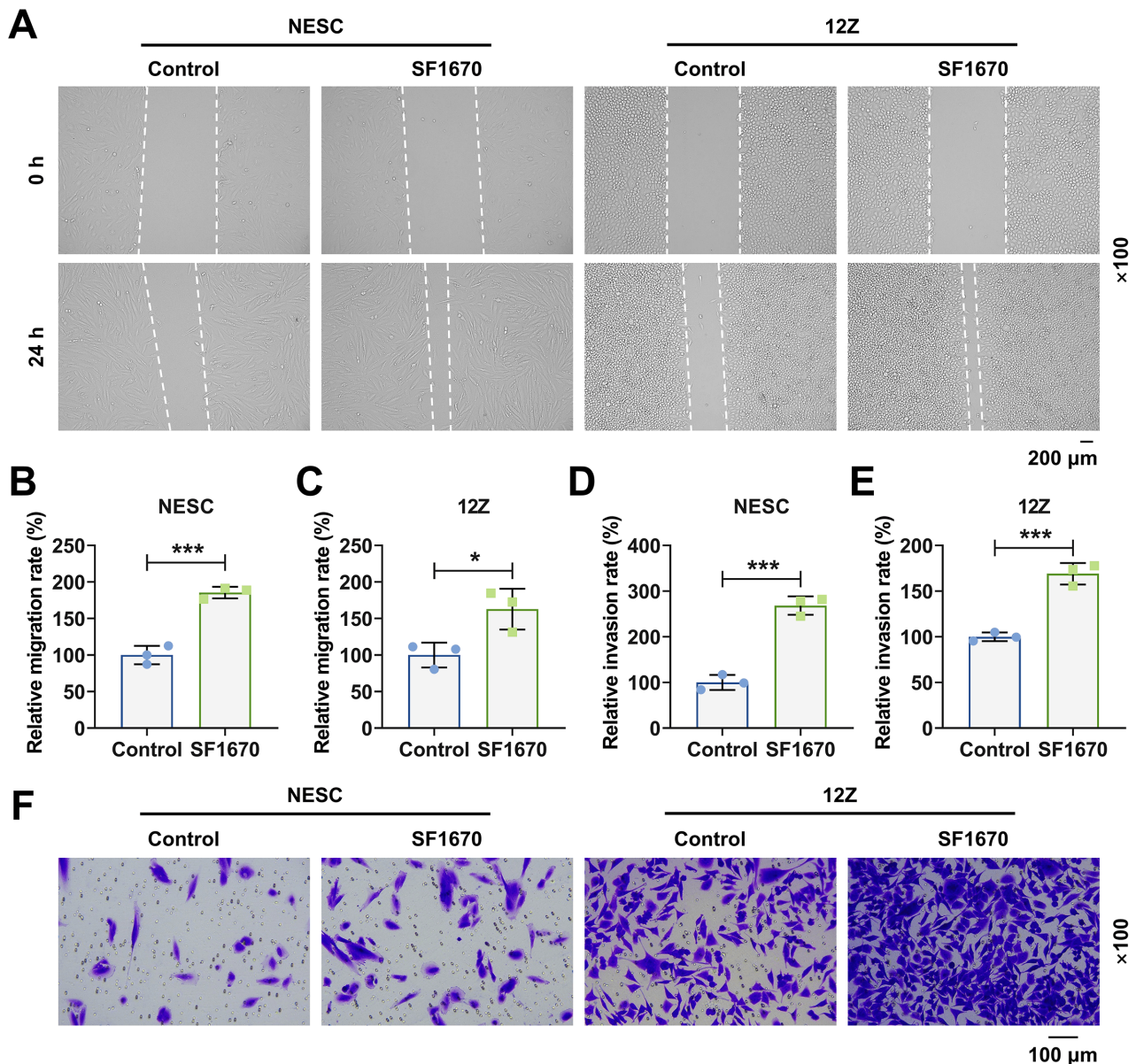


Fig. 8. PTEN inhibitor SF1670 promotes cell migration and invasion of NESC and 12Z cells. (A–C) Scratch wound healing assay images of NESC and 12Z at 0 h and 24 h (magnification: ×100, scale bar: 200 μm). (D–F) Transwell invasion assay images of NESC and 12Z cells (magnification: ×100, scale bar: 100 μm). Data are presented as mean ± SD (n = 3 independent experiments). * $p < 0.05$, *** $p < 0.001$. Statistical graphs were created using GraphPad Prism (version 8.0, GraphPad Software, San Diego, CA, USA).

while, glucose metabolism detection in peritoneal fluid demonstrated increased glucose consumption and lactate production in the SF1670 group (Fig. 10H,I, $p < 0.05$). Protein detection in lesion tissues also showed upregulated expression of glycolysis-related proteins LDHA, PDK1, and Glut1 in the SF1670 group (Fig. 10J,K, $p < 0.05$). These *in vivo* findings support the idea that suppression of PTEN function stimulates the growth and progression of endometriotic lesions, accompanied by enhanced glycolytic activity, which is consistent with the results observed in cellular models.

Discussion

The pathological process of EMs is closely associated with abnormal cell proliferation, invasion, and metabolic reprogramming. Although the role of aberrant glycolytic activation in EMs progression has been initially confirmed, and PTEN has received widespread attention as a key regulator of endometrial homeostasis [21,22], the synergistic regulatory mechanism between them in EMs remains unclear. Through multi-level experimental validation, this study systematically reveals that PTEN expression correlates with EMs progression and is inversely associated with

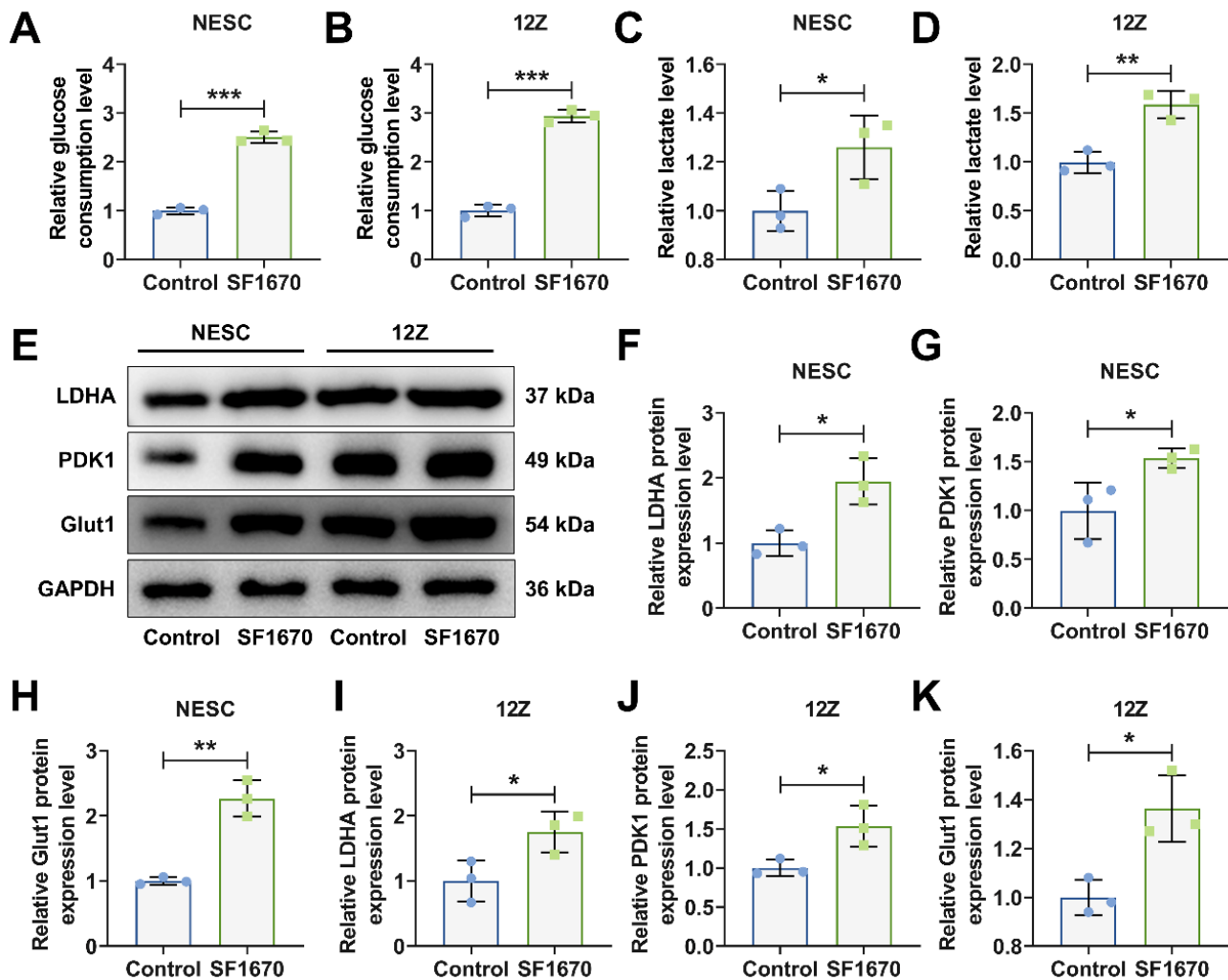


Fig. 9. PTEN inhibitor SF1670 promotes glucose metabolism and glycolysis-related protein expression in NESC and 12Z cells. (A,B) Quantitative analysis of relative glucose consumption in NESC and 12Z cells. (C,D) Quantitative analysis of relative lactate production in NESC and 12Z cells. (E–K) Western blot analysis of glycolysis-related proteins (LDHA, PDK1, Glut1) in NESC and 12Z cells; GAPDH served as the internal reference. Data are presented as mean $\pm$ SD ($n = 3$ independent experiments). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Statistical graphs were created using GraphPad Prism (version 8.0, GraphPad Software, San Diego, CA, USA).

glycolytic markers, suggesting that altered PTEN expression may contribute to metabolic reprogramming in EMs. It is important to note that the present study establishes a correlative association between PTEN expression and glycolytic activity, rather than a definitive causal mechanism. Future studies employing glycolysis-specific inhibitors in combination with PTEN manipulation are required to determine whether the observed phenotypic effects are mediated by or merely accompanied by changes in glycolytic metabolism.

This study revealed that PTEN is significantly down-regulated in moderate-to-severe EMs tissues and negatively correlated with key glycolytic proteins PDK1 and Glut1. Inhibition of aerobic glycolytic enzymes such as LDHA has been shown to alleviate EMs [23]. PTEN inactivation is common in human prostate cancer [24,25]. Additionally,

PTEN inactivation promotes the proliferation and migration of endometriotic cells [26]. Unlike previous studies, this research, by combining bioinformatics analysis with clinical sample validation, clarified the direct interaction among PTEN, EMs, and glycolysis-related genes. This study not only confirmed the PTEN deficiency in EMs but also revealed that the association between PTEN deficiency and aberrant glycolytic activation is not coincidental. LDHA-mediated lactate accumulation can reshape the lesion microenvironment and enhance cell invasiveness [27]. PDK1 blocks the tricarboxylic acid cycle, forcing cells to rely on glycolysis for energy [28], and Glut1 upregulation meets the energy demands of abnormal proliferation [29]. The synergistic activation of these three proteins is a crucial driver of EMs progression, and PTEN's inhibitory effect in this pathway fills the research gap regarding the association

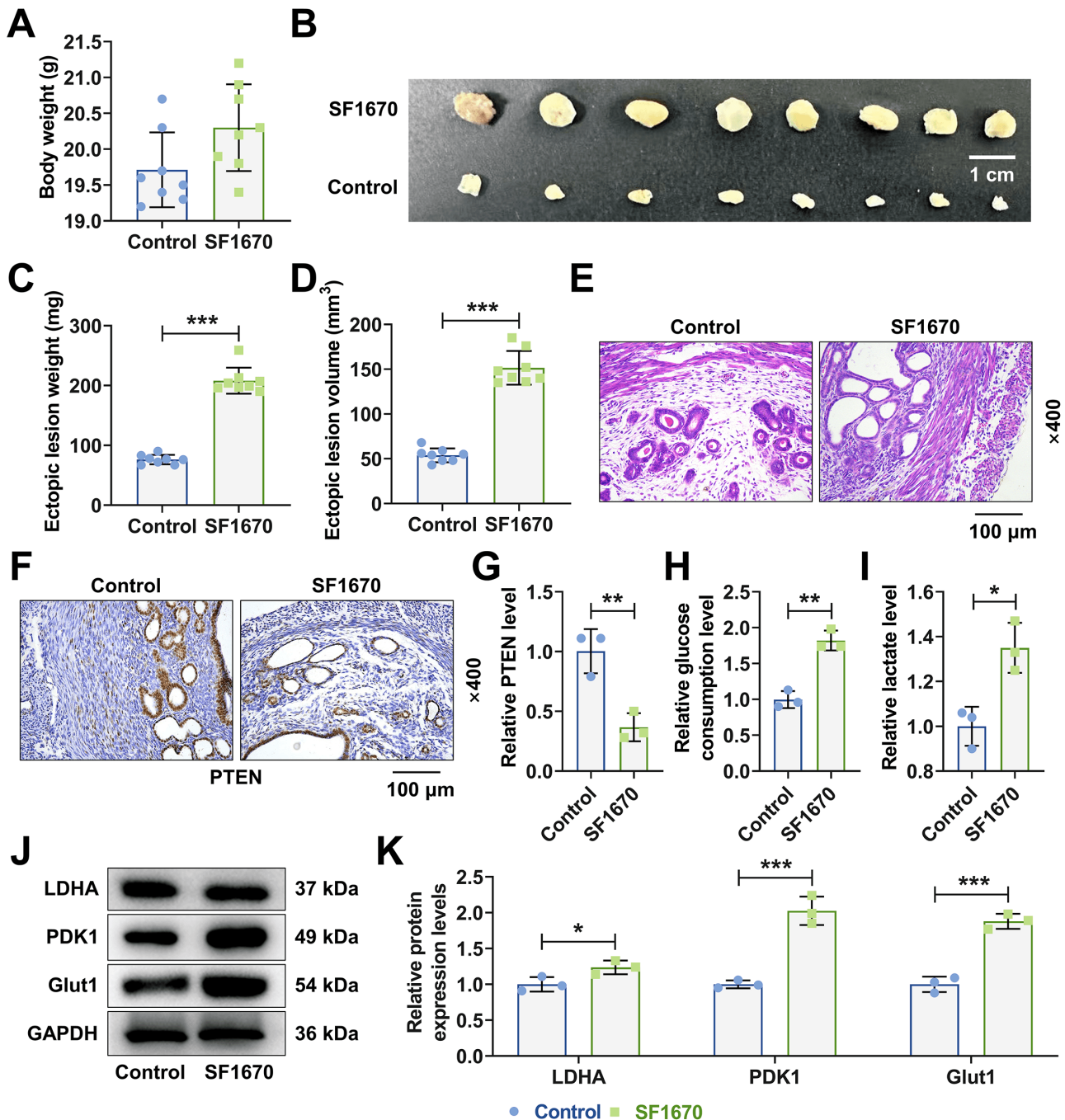


Fig. 10. PTEN inhibitor SF1670 promotes ectopic lesion growth in a mouse model of endometriosis. (A) Quantitative analysis of mouse body weight in the Control and SF1670 groups. (B) Representative images of ectopic lesions in the Control and SF1670 groups (scale bar: 1 cm). (C,D) Quantitative analysis of the weight and volume of ectopic lesions. (E) Hematoxylin and eosin (H&E) staining of ectopic lesion tissues (scale bar: 100 μm; magnification: ×400). (F,G) Immunohistochemical staining of PTEN in ectopic lesion tissues (scale bar: 100 μm; magnification: ×400). (H,I) Quantitative analysis of relative glucose consumption and lactate production in peritoneal fluid. (J,K) Western blot analysis of glycolysis-related proteins (LDHA, PDK1, Glut1) in ectopic lesion tissues; GAPDH served as the internal reference. Each group contained 8 mice. Data are presented as mean ± SD (n = 3 independent experiments) (A,C,D: n = 8 mice; G-I/K: n = 3 mice). **p* < 0.05, ***p* < 0.01, ****p* < 0.001. Statistical graphs were created using GraphPad Prism (version 8.0, GraphPad Software, San Diego, CA, USA).

between tumor suppressor genes and glycolysis within the metabolic regulatory network of EMs.

In cell experiments, PTEN knockdown or treatment with the inhibitor SF1670 significantly enhanced glycolytic

activity and malignant phenotypes of cells, while overexpression reversed this process. SF1670, a PTEN inhibitor, can suppress cell apoptosis and inflammation via the PTEN/AKT pathway [30]. In line with our finding that PTEN inactivation activates glycolysis, recent research has verified that PTEN loss induces M2 macrophage polarization by enhancing glycolytic activity in endometrial stromal cells [31]. *In vivo* experiments further validated the *in vitro* results: SF1670 intervention promoted the growth of ectopic lesions in EMs mice, accompanied by increased glucose consumption, lactate production in peritoneal fluid, and expression of key glycolytic proteins in lesions. EMs is closely linked to glycolysis; for instance, the carboxyl terminus of Hsc70-interacting protein (CHIP)/high mobility group protein 1 (HMGB1) pathway has been shown to regulate glycolysis in ovarian EMs [32]. These conclusions complement our study, indicating that different regulatory factors can affect EMs progression by targeting distinct links of the glycolytic pathway, while PTEN, as an upstream regulator, exerts a more global regulatory effect. Through a complete evidence chain of “bioinformatics analysis - cell experiments - animal models”, this study supports an associative link between PTEN and glycolytic activity in EMs progression.

Despite the important findings of this study, there are still limitations. NESC are normal endometrial stromal cells, whereas 12Z are endometriotic epithelial cells; their origins (normal vs. endometriotic endometrium), cellular lineages (stromal vs. epithelial), and culture conditions differ substantially. The use of two distinct cell lines introduces potential variability, and current data cannot fully exclude cell-type-specific effects. Moreover, bidirectional validation of PTEN knockdown and overexpression was not performed within the same cellular context: PTEN knockdown was conducted only in NESC, while PTEN overexpression was performed only in 12Z. While our results show consistent PTEN-mediated regulation of glycolysis in both cell types, the subtle differences in phenotypic magnitude may reflect intrinsic biological disparities between stromal and epithelial cells. Therefore, the observed baseline difference in PTEN expression between NESC and 12Z cannot be directly attributed to the disease state alone, as intrinsic biological disparities between stromal and epithelial cells, as well as differences in their normal versus endometriotic origins, may contribute to this variation. Thus, our conclusions should be cautiously interpreted as: PTEN inhibits glycolysis and malignant phenotypes in both stromal and epithelial cells of EMs, rather than exerting a universal effect across all endometrial cell subtypes. Further validation in primary human endometriotic cells and lineage-specific genetic models will help elucidate cell-type-dependent mechanisms. Additional limitations include the relatively small clinical sample size and the incomplete exploration of downstream signaling pathways (e.g., PI3K/AKT).

Conclusion

This study demonstrates that PTEN is significantly downregulated in endometriosis and its expression is inversely correlated with the levels of glycolytic markers LDHA, PDK1, and Glut1. Loss of PTEN is associated with enhanced proliferation, migration, invasion, and glycolytic activity in endometrial cells, whereas PTEN overexpression suppresses these malignant phenotypes. *In vivo*, PTEN inhibition accelerates ectopic lesion growth. These findings reveal an associative link between PTEN and glycolytic activity in endometriosis pathogenesis, supporting the PTEN-glycolysis interplay as a promising candidate pathway for further mechanistic investigation.

Availability of Data and Materials

The datasets analyzed or generated during the study are available from the corresponding author on reasonable request.

Author Contributions

JYZ: Conceptualization, Methodology, Validation, Writing - original draft, Writing - review & editing. AMS: Conceptualization, Methodology, Validation, Supervision, Writing - original draft, Writing - review & editing. SG and SSS: Data curation, Formal analysis, Investigation, Writing - review & editing. CJD: Data curation, Formal analysis, Investigation, Methodology, Supervision, Writing - review & editing. MLP: Methodology, Supervision, Writing - review & editing. All authors gave final approval of the version to be published. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

This study was conducted in accordance with the Declaration of Helsinki and approved by the Medical Ethics Committee of Sanmen People's Hospital (Ethics Approval Number: 2025094). Written informed consent was obtained from all patients prior to sample collection. Animal experiments were approved by the Laboratory Animal Welfare Ethics Committee of Zhejiang Laboratory Animal Center (Approval Number: ZJCLA-IACUC-20120024) and performed in accordance with applicable national laws and regulations, the Guidelines for the Ethical Review of Laboratory Animal Welfare, and the ARRIVE Guidelines 2.0.

Acknowledgment

Not applicable.

Funding

This study was funded by the Zhejiang Province Medical and Health Science and Technology Plan Project [No.2025KY479].

Conflict of Interest

The authors declare no conflict of interest.

References

- [1] Bonavina G, Taylor HS. Endometriosis-associated infertility: From pathophysiology to tailored treatment. *Frontiers in Endocrinology*. 2022; 13: 1020827. <https://doi.org/10.3389/fendo.2022.1020827>
- [2] Mariadas H, Chen JH, Chen KH. The Molecular and Cellular Mechanisms of Endometriosis: From Basic Pathophysiology to Clinical Implications. *International Journal of Molecular Sciences*. 2025; 26: 2458. <https://doi.org/10.3390/ijms26062458>
- [3] De Corte P, Klinghardt M, von Stockum S, Heinemann K. Time to Diagnose Endometriosis: Current Status, Challenges and Regional Characteristics-A Systematic Literature Review. *BJOG: an International Journal of Obstetrics and Gynaecology*. 2025; 132: 118–130. <https://doi.org/10.1111/1471-0528.17973> Erratum in: *BJOG: an International Journal of Obstetrics and Gynaecology*. 2025; 132: 1018. <https://doi.org/10.1111/1471-0528.18149>
- [4] Gou Y, Wang H, Wang T, Wang H, Wang B, Jiao N, et al. Ectopic endometriotic stromal cells-derived lactate induces M2 macrophage polarization via Mettl3/Trib1/ERK/STAT3 signalling pathway in endometriosis. *Immunology*. 2023; 168: 389–402. <https://doi.org/10.1111/imm.13574>
- [5] Kim BS, Kim B, Yoon S, Park W, Bae SJ, Joo J, et al. Warburg-like Metabolic Reprogramming in Endometriosis: From Molecular Mechanisms to Therapeutic Approaches. *Pharmaceuticals (Basel, Switzerland)*. 2025; 18: 813. <https://doi.org/10.3390/ph18060813>
- [6] Guo C, Na X, Guo Z, Jiao J, Yang M, Liang J, et al. Metabolic reprogramming in endometriosis: mechanisms and therapeutic prospects. *Journal of Advanced Research*. 2026. <https://doi.org/10.1016/j.jare.2026.04.069> (online ahead of print)
- [7] Lu C, Qiao P, Fu R, Wang Y, Lu J, Ling X, et al. Phosphorylation of PFKFB4 by PIM2 promotes anaerobic glycolysis and cell proliferation in endometriosis. *Cell Death & Disease*. 2022; 13: 790. <https://doi.org/10.1038/s41419-022-05241-6>
- [8] Wang Y, Xiu J, Yang T, Ren C, Yu Z. HSF1 promotes endometriosis development and glycolysis by up-regulating PFKFB3 expression. *Reproductive Biology and Endocrinology: RB&E*. 2021; 19: 86. <https://doi.org/10.1186/s12958-021-00770-9>
- [9] Zhang B, Liu M, Mai F, Li X, Wang W, Huang Q, et al. Interruption of KLF5 acetylation promotes PTEN-deficient prostate cancer progression by reprogramming cancer-associated fibroblasts. *The Journal of Clinical Investigation*. 2024; 134: e175949. <https://doi.org/10.1172/JCI175949>
- [10] Zhang C, Ma HM, Wu S, Shen JM, Zhang N, Xu YL, et al. Secreted PTEN binds PLXDC2 on macrophages to drive anti-tumor immunity and tumor suppression. *Developmental Cell*. 2024; 59: 3072–3088.e8. <https://doi.org/10.1016/j.devcel.2024.08.003>
- [11] Lin K, Zhou E, Shi T, Zhang S, Zhang J, Zheng Z, et al. m6A eraser FTO impairs gemcitabine resistance in pancreatic cancer through influencing NEDD4 mRNA stability by regulating the PTEN/PI3K/AKT pathway. *Journal of Experimental & Clinical Cancer Research: CR*. 2023; 42: 217. <https://doi.org/10.1186/s13046-023-02792-0>
- [12] Lv Z, Ren Y, Li Y, Niu F, Li Z, Li M, et al. RNA-binding protein GIGYF2 orchestrates hepatic insulin resistance through STAU1/PTEN-mediated disruption of the PI3K/AKT signaling cascade. *Molecular Medicine (Cambridge, Mass.)*. 2024; 30: 124. <https://doi.org/10.1186/s10020-024-00889-6>
- [13] Sun R, He SL, Liu HY, Wang XW, Li SH. Tumor Suppressor LINC02487 Inhibits the Progression of Cervical Cancer in Vitro by Regulating the PTEN/Akt/mTOR Pathway. *Discovery Medicine*. 2024; 36: 1732–1742. <https://doi.org/10.24976/Discovery.Med.202436187.159>
- [14] Tucker DR, Lee AF, Orr NL, Alotaibi FT, Noga HL, Williams C, et al. Somatic PTEN and ARID1A loss and endometriosis disease burden: a longitudinal study. *Human Reproduction (Oxford, England)*. 2025; 40: 296–309. <https://doi.org/10.1093/hmrp/deae269>
- [15] Tandon P, Gallo CA, Khatri S, Barger JF, Yepiskoposyan H, Plas DR. Requirement for ribosomal protein S6 kinase 1 to mediate glycolysis and apoptosis resistance induced by Pten deficiency. *Proceedings of the National Academy of Sciences of the United States of America*. 2011; 108: 2361–2365. <https://doi.org/10.1073/pnas.1013629108>
- [16] Lin F, Yin HB, Li XY, Zhu GM, He WY, Gou X. Bladder cancer cell-secreted exosomal miR-21 activates the PI3K/AKT pathway in macrophages to promote cancer progression. *International Journal of Oncology*. 2020; 56: 151–164. <https://doi.org/10.3892/ijo.2019.4933>
- [17] Liu L, Liu L, Yue C, Du S, Liu J, Yu Z. PYK2 promotes cell proliferation and epithelial-mesenchymal transition in endometriosis by phosphorylating Snail1. *Molecular Medicine (Cambridge, Mass.)*. 2025; 31: 155. <https://doi.org/10.1186/s10020-025-01218-1>
- [18] Li Y, Prasad A, Jia Y, Roy SG, Loison F, Mondal S, et al. Pretreatment with phosphatase and tensin homolog deleted on chromosome 10 (PTEN) inhibitor SF1670 augments the efficacy of granulocyte transfusion in a clinically relevant mouse model. *Blood*. 2011; 117: 6702–6713. <https://doi.org/10.1182/blood-2010-09-309864>
- [19] Huang F, He K. Rhizoma Zedoariae Inhibits G-CSF-Induced MDSCs-like Differentiation of THP-1 Cells. *Journal of Experimental and Clinical Application of Chinese Medicine*. 2024; 5: 12–22. <https://doi.org/10.62767/jecacm503.1126>
- [20] Li J, Huo S, Huang C, Cheng W, Liang S, Chen W, et al. Additive effects of curcumin and glucosamine on knee function, cartilage protection, and subchondral bone preservation in a post-traumatic osteoarthritis rat model. *Guidelines and Standards of Chinese Medicine*. 2025; 3: 69–80. <https://doi.org/10.1097/gscm.0000000000000053>
- [21] Wen X, Zhang J, Xu Z, Li M, Dong X, Du Y, et al. Highly expressed lncRNA H19 in endometriosis promotes aerobic glycolysis and histone lactylation. *Reproduction (Cambridge, England)*. 2024; 168: e240018. <https://doi.org/10.1530/REP-24-0018>
- [22] Zhou A, Cai Q, Hong Y, Lv Y. Down-Regulation of Casein Kinase 1α Contributes to Endometriosis through Phosphatase and Tensin Homolog/Autophagy-Related 7-Mediated Autophagy. *The American Journal of Pathology*. 2021; 191: 2195–2202. <https://doi.org/10.1016/j.ajpath.2021.08.015>
- [23] Cho MK, Jin L, Han JH, Jin JS, Cheon SY, Shin S, et al. Water-Extracted *Prunella vulgaris* Alleviates Endometriosis by Reducing Aerobic Glycolysis. *Frontiers in Pharmacology*. 2022; 13: 872810. <https://doi.org/10.3389/fphar.2022.872810>
- [24] Chaudagar K, Hieromnimon HM, Khurana R, Labadie B, Hirz T, Mei S, et al. Reversal of Lactate and PD-1-mediated Macrophage Immunosuppression Controls Growth

- of PTEN/p53-deficient Prostate Cancer. *Clinical Cancer Research: an Official Journal of the American Association for Cancer Research*. 2023; 29: 1952–1968. <https://doi.org/10.1158/1078-0432.CCR-22-3350>
- [25] Chaudagar K, Hieromnimon HM, Kelley A, Labadie B, Shafran J, Rameshbabu S, et al. Suppression of Tumor Cell Lactate-generating Signaling Pathways Eradicates Murine PTEN/p53-deficient Aggressive-variant Prostate Cancer via Macrophage Phagocytosis. *Clinical Cancer Research: an Official Journal of the American Association for Cancer Research*. 2023; 29: 4930–4940. <https://doi.org/10.1158/1078-0432.CCR-23-1441>
- [26] Yang Y, Ban D, Zhang C, Shen L. Downregulation of circ_0000673 Promotes Cell Proliferation and Migration in Endometriosis via the Mir-616-3p/PTEN Axis. *International Journal of Medical Sciences*. 2021; 18: 3506–3515. <https://doi.org/10.7150/ijms.63564>
- [27] Yu Y, Deng H, Wang W, Xiao S, Zheng R, Lv L, et al. LRP-PRC promotes glycolysis by stabilising LDHA mRNA and its knockdown plus glutamine inhibitor induces synthetic lethality via m⁶A modification in triple-negative breast cancer. *Clinical and Translational Medicine*. 2024; 14: e1583. <https://doi.org/10.1002/ctm2.1583>
- [28] Yang Q, Wu F, Zhang Y, Wang R. FOXM1 regulates glycolysis in nasopharyngeal carcinoma cells through PDK1. *Journal of Cellular and Molecular Medicine*. 2022; 26: 3783–3796. <https://doi.org/10.1111/jcmm.17413>
- [29] Shi Y, Kotchetkov IS, Dobrin A, Hanina SA, Rajasekhar VK, Healey JH, et al. GLUT1 overexpression enhances CAR T cell metabolic fitness and anti-tumor efficacy. *Molecular Therapy: the Journal of the American Society of Gene Therapy*. 2024; 32: 2393–2405. <https://doi.org/10.1016/j.ymthe.2024.05.006>
- [30] Fu HY, Shen L, Gao XS, Cui DX, Cui ZY. SF1670 inhibits apoptosis and inflammation via the PTEN/Akt pathway and thus protects intervertebral disc degeneration. *European Review for Medical and Pharmacological Sciences*. 2020; 24: 8694–8702. https://doi.org/10.26355/eurrev_202009_22806
- [31] Dai F, Li J, Liu Y. Phosphatase and tensin homolog deficiency induces M2 macrophage polarization by promoting glycolytic activity in endometrial stromal cells. *Biology of Reproduction*. 2025; 112: 640–650. <https://doi.org/10.1093/biolre/iaof016>
- [32] Sun Y, Wang Q, Wang M, Sun F, Qiao P, Jiang A, et al. CHIP induces ubiquitination and degradation of HMGB1 to regulate glycolysis in ovarian endometriosis. *Cellular and Molecular Life Sciences: CMLS*. 2022; 80: 13. <https://doi.org/10.1007/s00018-022-04637-z>