Clinical and Experimental Obstetrics & Gynecology

*Clin. Exp. Obstet. Gynecol.* **2026**; 53(8): 48963
https://doi.org/10.31083/CEOG48963

*Original Research*

# SPARC Promotes the Proliferation and Migration of Endometriotic Stromal Cells via the PI3K/AKT and MAPK/ERK Signaling Pathways

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Academic Editor: Valerio Gaetano Vellone
Submitted: 9 December 2025 Revised: 10 April 2026 Accepted: 21 April 2026 Published: 19 August 2026

**Abstract**

**Background:** Endometriosis is a major contributor to infertility, and an effective curative treatment remains unavailable. *SPARC* has been identified as a differentially expressed gene in endometriosis between peritoneal and ovarian lesions. In this study, our aim was to comprehensively evaluate the effects of *SPARC* on the proliferation, migration, apoptosis, necrosis, and adhesion of endometriotic stromal cells (ESCs) *in vitro* and to explore the associated molecular signaling pathways. **Methods:** Using ESCs transfected with si-*SPARC* for gene silencing and those with *SPARC* overexpression; we evaluated cell proliferation through the Cell Counting Kit-8 (CCK-8) assay. Migratory capacity was examined using the wound-healing and Transwell assays. Cell apoptosis, necrosis, and adhesion capabilities were evaluated using the corresponding experimental methods. Immunohistochemistry was utilized to confirm *SPARC* expression in endometriotic lesions, and Western blot was conducted to assess protein expression. **Results:** The CCK-8 assay demonstrated that si-SPARC inhibited ESCs proliferation, whereas SPARC overexpression promoted it. Transwell and wound-healing assays showed that si-SPARC attenuated the migration ability of ESCs, whereas SPARC overexpression was associated with enhanced cell migration. Based on qualitative Hoechst/propidium iodide (PI) staining, SPARC did not show a significant effect on apoptosis or necrosis. Moreover, the study did not conclusively establish that SPARC regulates ESC adhesion to extracellular matrix (ECM) components, such as collagen I, fibronectin, poly-L-lysine, and laminin. Immunohistochemistry results further showed that SPARC expression was higher in ectopic endometrium compared with orthotopic endometrium. Finally, we observed that SPARC was involved in the regulation of the mitogen-activated protein kinases/extracellular regulated protein kinases (MAPK/ERK) and phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) signaling pathways. **Conclusions:** Our *in vitro* findings suggest that SPARC may promote ESC proliferation and migration, potentially via activation of the MAPK/ERK and PI3K/AKT signaling pathways. Qualitative Hoechst/PI staining showed no apparent effect of SPARC on apoptosis or necrosis, although subtle effects cannot be excluded. The effects of SPARC on ESCs' adhesion to the ECM were inconsistent, possibly reflecting differences between cell models.

**Keywords:** endometriosis; SPARC; endometriotic stromal cells; PI3K; cell signaling

## 1. Introduction

Endometriosis is a prevalent gynecological disorder characterized by the aberrant proliferation of endometrial tissue at extra-uterine locations, including the ovary, fallopian tube, bladder, and even the intestinal tract. This abnormal proliferation of endometrial tissue gives rise to a range of symptoms, such as menstrual irregularities, pelvic pain, and dyspareunia (pain during sexual intercourse). Endometriosis also significantly increases the risk of infertility [1]. The invasion and metastasis of endometriotic stromal cells (ESCs) underlie the pathological development of ectopic endometrium. On the other hand, ESC proliferation and death form the physiological basis for the cyclical expansion and shedding of the endometrium. Consequently, strategies aimed at halting the growth, invasion, and metastasis of ESCs represent a promising therapeutic approach for endometriosis [2].

The gene for Secreted Protein Acidic and Rich in Cysteine (*SPARC*) is located on human chromosome 5q33.1 and encodes a multifunctional glycoprotein that functions within the extracellular matrix (ECM) [3]. SPARC binds ECM components to regulate cell adhesion, proliferation, migration, and growth factor signaling, thereby playing a key role in tissue remodeling and damage [4]. Mature SPARC protein consists of three domains: the N-terminal (NT) domain has a low-affinity calcium-binding site, the F-spondin (FS) domain contains internal disulfide bonds and glycosylation sites, and the extracellular (EC) domain contains collagen-binding motifs and anti-proliferative peptides that specifically inhibit endothelial cell growth [5].

Recent evidence has demonstrated that SPARC is differentially expressed in the peritoneal and ovarian lesions of endometriosis [6]. However, it is not yet known whether SPARC can modulate the progression of endometriosis. In this study, we systematically evaluated the impact of SPARC on the proliferation, migration, apoptosis, and adhesion of ESCs in an *in vitro* environment. Additionally, we investigated the associated signaling pathways to gain a deeper understanding of the underlying molecular mechanisms.

Notably, SPARC is overexpressed in aggressive subclones of gynecological malignancies, where it consistently promotes cell proliferation, invasion, and metastasis. In ovarian cancer, its silencing suppresses these processes and induces apoptosis [4]. Similarly, high SPARC expression in cervical squamous cell carcinoma (CESC) drives tumor progression by enhancing proliferation, migration, and epithelial–mesenchymal transition (EMT) [7]. Furthermore, SPARC contributes to tumor-stroma crosstalk. For example, in endometrial cancer, it collaborates with fibronectin to activate fibroblasts, thereby accelerating cancer cell invasion [8]. Collectively, SPARC plays a multifaceted pro-tumorigenic role across gynecological cancers.

## 2. Materials and Methods

### *2.1 Cell Culture*

hEM15A cell line was obtained from American Type Culture Collection (ATCC). We tested for mycoplasma contamination and used the short tandem repeat (STR) test to verify cell origin. Cell morphology was confirmed by a pathologist before the experiments. Cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Biosharp, BL303A, Hefei, AnHui, China) mixed with 10% fetal bovine serum (FBS) (Biosharp, BL201A, Hefei, Anhui, China), 100 U/mL penicillin, and 100 U/mL streptomycin (Phygene, PH1513, Fuzhou, Fujian, China) in a humidified incubator at 37 °C with 5% $CO_2$.

Primary ESCs were a gift from Prof. Zongfeng Zhang, Gynecology Laboratory, Second Affiliated Hospital of Harbin Medical University. The primary cells were validated by flow cytometry analysis with CD10 antibody (detailed results are provided in the **Supplementary Materials File**). Flow cytometry data were analyzed using a hierarchical gating strategy. First, the cell population was initially identified on a forward scatter area (FSC-A) versus side scatter area (SSC-A) plot to exclude debris and non-cellular events. Next, single cells were selected by plotting FSC-A against FSC-W (or FSC-H) to exclude doublets and cell aggregates. Subsequently, live cells were gated based on the exclusion of a viability dye (e.g., DAPI-negative or PI-negative). Finally, the target cell populations were defined based on specific fluorescence markers, with thresholds established using unstained and fluorescence-minus-one (FMO) controls. Furthermore, all primary cells tested negative for mycoplasma contamination. All cultures (passages 3–5) were grown in Dulbecco's Modified Eagle Medium (DMEM) (Biosharp, BL301A, Hefei, Anhui, China) supplemented with 15% FBS (Biosharp, BL201A, Hefei, Anhui, China), 100 U/mL penicillin, and 100 U/mL streptomycin (Phygene, PH1513, Fuzhou, Fujian, China) in a cell incubator under standard conditions.

### *2.2 Transfection of Cells*

The SPARC overexpression (SPARC-OE) plasmid, featuring the complete coding sequence inserted into the pcDNA3.1+ vector, was synthesized by FengHuiShengWu (Changsha, Hunan, China). This construct, and the empty vector control, were subsequently purified from glycerol stocks using a mini-plasmid extraction kit (Solarbio, D1100, Beijing, China). The SPARC-OE plasmid was transfected into hEM15A cells and primary ESCs by Lipofectamine 8000 (Beyotime, C0533, Shanghai, China). An empty plasmid was transfected as an internal control.

Small interfering RNAs (siRNAs) were designed and produced by Sangon Biotech (Shanghai, China) (Table 1). Lipofectamine 8000 was used to transfect each of these siRNAs into the two cell lines independently, followed by incubation at 37 °C for 24 h. The knockdown potency of siRNA was assessed using quantitative reverse transcription polymerase chain reaction (qRT-PCR). In order to ensure their effectiveness, all siRNAs were transfected into the cells simultaneously in the subsequent *in vitro* cell experiments.

### *2.3 Wound Healing Assay*

The hEM15A siNC cells, hEM15A si-SPARC cells, hEM15A Vector cells, and hEM15A SPARC-OE cells ($5 \times 10^5$ cells/well) were grown to a density of approximately 90% in 6-well plates. The same procedure was performed for primary ESCs. A p1000 pipette tip was used to produce a single scratch in the middle of the plate. Photomicrographs were captured at 0 h and 24 h using an EVOS light microscope (ThermoFisher, Waltham, MA, USA). Cell migration fronts were subsequently quantified using ImageJ (v1.54h, LOCI, University of Wisconsin, Madison, WI, USA).

### *2.4 Migration Assay*

The hEM15A siNC cells, hEM15A si-SPARC cells, hEM15A Vector cells, or hEM15A SPARC-OE cells ($7 \times 10^4$ cells/well) were seeded into the upper chamber of a 24-well Transwell plate (Corning Inc., Corning, NY, USA) in serum-free medium. The same procedure was used for primary ESCs. The medium in the lower chamber was supplemented with 10% FBS. Following incubation for 24 h, non-migrated cells on the upper surface were removed with a cotton swab. Migrated cells were fixed with methanol, stained with 0.5% crystal violet (Phygene, PH1277, Fuzhou, Fujian, China), and imaged. An EVOS light microscope (ThermoFisher, MA) was used to take pic-

**Table 1. si-SPARC and control sequences.**

| si-RNA name | Sequence (5′ to 3′) | Base count (nt) with 3'-TT |
|---|---|---|
| hSparc-209-a | AAAUUCUCCUACUUCCACC | 21 |
| hSparc-209-s | GGUGGAAGUAGGAGAAUUU | 21 |
| hSparc-928-a | UUCUGCUUGAUGCCGAAGC | 21 |
| hSparc-928-s | GCUUCGGCAUCAAGCAGAA | 21 |
| hSparc-715-a | UAGUUCUUCUCGAAGUCCC | 21 |
| hSparc-715-s | GGGACUUCGAGAAGAACUA | 21 |
| Negative control | UUCUCCGAACGUGUCACGU | 21 |
| | ACGUGACACGUUCGGAGAA | 21 |
| Positive control (GAPDH) | GUAUGACAACAGCCUCAAG | 21 |
| | CUUGAGGCUGUUGUCAUAC | 21 |

GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

tures at 40× magnification after drying. Stained areas were quantified using ImageJ.

*2.5 Cell Counting Kit-8 (CCK-8) Assay*

The hEM15A siNC cells, hEM15A si-SPARC cells, hEM15A Vector cells, and hEM15A SPARC-OE cells ($1 \times 10^3$ cells/well) were seeded into 96-well plates with growth medium. The same procedure was performed for primary ESCs. CCK-8 (Beyotime, China) (10%) was added to each well on 24 h, 48 h, and 72 h. After 37 ℃-30 min incubation in cell incubator, the 450 nm absorbance was then measured.

*2.6 Cell Adhesion Assay*

A precoated 24-well plate was added by 300 µL of PBS containing collagen I (100 µg/mL), fibronectin (20 µg/mL), poly-L-lysine (100 µg/mL), or laminin (100 µg/mL) to each well and incubating the plate for 1 h. Subsequently, 300 µL of blocking buffer (0.5% bovine serum albumin [BSA] in medium) was added to each well. After 60 min, hEM15A siNC cells, hEM15A si-SPARC cells, hEM15A Vector cells, or hEM15A SPARC-OE cells ($8 \times 10^5$ cells/well) in serum-free medium were added for 90 min. Then, the culture medium was removed, and the plate was left with the remaining cells were fixed with 100 µL methanol for 10 min and subsequently stained with 0.5% crystal violet (Phygene, China) for 10 min. Finally, the plate was washed with PBS and dried at room temperature. The same procedure was performed for the primary ESCs. Photomicrographs were taken of each well, and the cells were counted.

*2.7 Apoptosis and Necrosis Assay*

A 6-well plate was used to grow hEM15A siNC cells, hEM15A si-SPARC cells, hEM15A Vector cells, and hEM15A SPARC-OE cells ($1 \times 10^3$ cells/well). Following 24 h incubation, 1 mL of cell staining buffer, 5 µL Hoechst 33342, and 5 µL propidium iodide (PI) (Beyotime, C1052, Shanghai, China) were added and mixed, and the plate was placed on ice for 30 min. The staining solution was then removed, and the cells were washed with PBS. The same procedure was performed for the primary ESCs. The cells were viewed under a fluorescent microscope after anti-fluorescent mounting solution was added. Hoechst 33342 stained the nuclei of all cells, while PI labeled cells with compromised membrane integrity. Apoptotic cells were identified by characteristic nuclear morphological changes, including condensation and fragmentation, as visualized under fluorescence microscopy. Representative images were selected to reflect the overall observations across experimental groups.

*2.8 qRT-PCR*

RNA was extracted from both treated hEM15A cells and primary ESCs using Trizol reagent (Life Sciences, Shanghai, China), as recommended by the manufacturer. The concentration of extracted RNA was measured using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA), then converted into cDNA using the HiScript II Q RT SuperMix kit (Vazyme, Nanjing, Jiangsu, China; R122-01). The program was set as follows: incubation at 37 °C for 2 min, 55 °C for 15 min, and 85 °C for 5 min. The resulting cDNA was stored at –20 °C for subsequent analysis.

The AceQ-quantitative polymerase chain reaction (qPCR) SYBR Green Master Mix (Vazyme, Q111-02) and a LightCycler 480 II real-time PCR system (Roche, Basel, Switzerland) were utilized for qPCR. The primer sequences (5′ to 3′) for SPARC were TGAGGTATCTGTGGGAGCTAAT (forward) and CCTTGCCGTGTTTGCAGTG (reverse). Pre-denaturation was performed at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 10 sec, and annealing and extension at 60 °C for 30 sec. The expression levels of target genes were normalized to that of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) using the $2^{-\Delta\Delta Ct}$ method.

*2.9 Immunohistochemistry*

A total of 32 cases of paraffin-embedded tissue were collected from patients who underwent surgery for endometriosis between 01/06/2023 and 01/06/2024 in the De-

partment of Obstetrics and Gynecology, Second Affiliated Hospital of Harbin Medical University. These comprised 20 cases of paired ovarian endometriosis and *in situ* endometrium, and 12 cases of abdominal wall endometriosis. The samples were analyzed on 05/02/2025. Prior to immunostaining, the slides containing tumor tissue sections were deparaffinized, rehydrated, and then immersed in a 3% $H_2O_2$/PBS solution for 15 min to deactivate endogenous peroxidase activity. Sections were then autoclaved in a buffered Tris-EDTA solution for two min at 121 °C to expose the antigen. After blocking with serum for 1 h, the sections were incubated overnight at 4 ℃ with anti-SPARC antibody (diluted 1:800, catalog number AF8043, Beyotime, China), followed by incubation with a secondary antibody for 1 h. Freshly prepared 3,3'-diaminobenzidine (DAB, Maxim Biotechnologies, DAB-0031, Fuzhou, Fujian, China) solution was then added to each slide. Finally, the slides were mounted with coverslips after undergoing hematoxylin counterstaining.

Each slide was categorized into four classes according to the intensity of cell staining: no positive staining (negative: 0 points), light yellow staining (weak positive: 1 point), brown/yellow staining (positive: 2 points), tan staining (strong positive: 3 points). The percentage of positive cells was also evaluated and categorized: ≤25% (1 point), 26%–50% (2 points), 51%–75% (3 points), and >75% (4 points). The final score for each sample was calculated by multiplying the two scores for intensity and percentage of cell staining.

### *2.10 Western Blot*

Cells were lysed using radioimmunoprecipitation assay (RIPA) solution (BioSharp, BL504, Hefei, Anhui, China). The extracted protein was mixed with 5× loading buffer (BioSharp, Hefei, Anhui, China), denatured by boiling, and then separated via SDS-PAGE (Epizyme, China). The protein was transferred onto a polyvinylidene fluoride (PVDF) membrane (0.45 µm), blocked with 5% nonfat dry milk for 1 h, and then incubated overnight at 4 °C with primary antibodies targeting PI3 Kinase p85α (Beyotime, China, AF7742; 1:1000), protein kinase B (AKT1/2/3) (Beyotime, China, AF1789; 1:1000), Phospho-AKT1 (Thr308) (Beyotime, China, AF5734; 1:1000), extracellular regulated protein kinases 1 (ERK1) (Beyotime, China, AF1315; 1:1000), Phospho-Erk1 (Thr202/Tyr204)/Erk2 (Thr185/Tyr187) (Beyotime, China, AF1891; 1:1000), and SPARC (Beyotime, China, AF8043; 1:1000). GAPDH (zs-bio, China, TA-08; 1:2000) served as the internal control. The next day, the membranes were washed three times with Tris-buffered saline with Tween 20 (TBST) (Solarbio, T1081, Beijing, China) and incubated with the following secondary antibodies for 1 h at room temperature: horseradish peroxidase-conjugated goat anti-rabbit/mouse IgG (H+L) (Beyotime, China, A0208/A0216; 1:1000). After adding ECL reagent (Beyotime, China), the membranes were visualized using a ChemiDoc imaging system (Bio-Rad, Hercules, CA, USA). Relative protein expression levels were quantified using ImageJ software based on the gray values of the protein bands.

### *2.11 Analysis of SPARC in the Turku Endometriosis Database*

The Turku Endometriosis Database [9] was used to compare SPARC gene-related information between patient and control groups using the link https://endometdb.utu.fi/gene_analysis/. After entering SPARC, the data was analyzed and a boxplot was chosen.

### *2.12 Acquisition of Microarray Data Information*

The Gene Expression Omnibus (GEO) database (https://ncbi.nlm.nih.gov/geo/) was used to select the GSE5108 dataset based on the GPL2895 platform. This includes 22 samples from endometriosis patients, comprising 11 pairs of ectopic and eutopic endometrium [10].

### *2.13 Identification of DEGs and GO/KEGG Enrichment Analysis*

GEO2R (https://ncbi.nlm.nih.gov/geo/geo2r/) was used to identify differentially expressed genes (DEGs) in the GEO dataset. Ectopic endometrial tissues were used as the experimental group, and eutopic endometrium as the control group. The GEO2R online analysis tool was used to analyze and screen out DEGs from the GSE5108 dataset. The screening criteria were $p < 0.05$ and $|\log FC| \geq 1.5$, with $\log FC \geq 1.5$ defined as up-regulated genes, and $\log FC \leq -1.5$ defined as down-regulated genes. $p < 0.05$ was used as the criterion for significance in both the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses.

### *2.14 Statistical Analysis*

Data were analyzed using Prism software 9.4.1 (GraphPad, San Diego, CA, USA). Two-tailed unpaired Student's *t*-test was employed for comparisons between two groups, and one-way analysis of variance (ANOVA) for comparisons across three or more groups. To ensure adequate statistical power, the sample size for each experiment was determined based on previous studies in the field and on our preliminary data. All quantitative data are presented as the mean ± standard deviation (SD) from at least three independent experiments. Differences were considered statistically significant at $*p < 0.05$, $** p < 0.01$, $*** p < 0.001$ and $**** p < 0.0001$.

## 3. Results

### *3.1 SPARC Expression Is Higher in Ectopic Than in Eutopic Endometrium in the Turku Endometriosis Database and the GEO Database*

Compared to controls, endometriosis patients showed higher levels of SPARC expression in their endometrium

and peritoneum. Additionally, analysis of the Turku Endometriosis Database revealed that the ovary and deep tissues expressed higher SPARC levels than the endometrium (Fig. 1A). According to this database, SPARC expression differs in the endometrium, peritoneum, deep tissue, and ovary, as well as in the various stages of endometriosis. The expression of SPARC in the endometrium of stage I–IV patients was higher than that of the control group. Moreover, SPARC expression in the peritoneum, deep tissues and ovary was different at different disease stages compared with the control group (Fig. 1B). SPARC was a differentially expressed gene in the endometriosis dataset GSE5108 from the GEO database, and showed significant upregulation (Fig. 1C). This finding led us to hypothesize that SPARC might play a functional role in the pathogenesis of endometriosis. KEGG enrichment analysis identified that SPARC expression was associated with PI3K/AKT and mitogen-activated protein kinases (MAPK) signaling pathways (Fig. 1D). This bioinformatic prediction directly guided our subsequent mechanistic investigation, which aimed to validate these specific pathways. GO biological process enrichment analysis focused mainly on cell migration, activation, adhesion, and immunity (Fig. 1E). Consequently, these GO terms framed the scope of our *in vitro* functional assays, which were designed to specifically test the effects of SPARC expression on cell proliferation, migration, and adhesion.

### *3.2 SPARC Regulates ESC Proliferation and Migration*

Following transfection with si-SPARC-209, si-SPARC-928, and si-SPARC-715 for 24 h, the two cell lines showed a significant decrease in SPARC RNA expression, as observed by qRT-PCR. Similarly, SPARC RNA levels were significantly increased after transfection with SPARC-OE (Fig. 2A). Western blot results revealed lower SPARC protein levels after interference for 24 h, and increased levels after SPARC overexpression for 24 h (Fig. 2B). Transwell assay showed that SPARC-OE increased the migration of hEM15A cells and primary ESCs under starvation conditions, whereas si-SPARC attenuated the migration of these cell lines (Fig. 2C). The wound healing experiment revealed that cells with increased SPARC expression exhibited improved scratch closure at 24 h, whereas cells in the si-SPARC group displayed poorer healing. The blue vertical line represents the fixed initial scratch boundary and the yellow line indicates the cell edge at 0h and 24h in Fig. 2D. The CCK-8 assay for cell proliferation in hEM15A cells and primary ESCs revealed that cell proliferation was greater in the SPARC overexpression group and lower in the si-SPARC group (Fig. 2E).

### *3.3 SPARC Did Not Regulate Apoptosis and Necrosis of ESCs and Did Not Affect Cell Adhesion to the Extracellular Matrix*

Observation of both cell lines by fluorescence microscopy showed that si-SPARC and SPARC-OE did not alter apoptosis (as revealed by Hoechst staining) or necrosis (as revealed by PI staining) (Fig. 3A). The effect of SPARC on cell adhesion to ECM proteins was evaluated in both hEM15A cells and primary ESCs. In primary ESCs, neither SPARC-OE nor si-SPARC significantly altered cell adhesion to Collagen I, fibronectin, poly-L-lysine, or laminin. In contrast, in hEM15A cells, both SPARC-OE and si-SPARC led to a marked reduction in adhesion specifically to Collagen I, whereas si-SPARC increased the adhesion of hEM15A cells to poly-L-lysine but decreased their adhesion to laminin (Fig. 3B). Taken together, these results suggest that SPARC does not substantially influence the adhesive capacity of endometrial stromal cells. The decreased adhesion to Collagen I observed exclusively in hEM15A cells is likely cell line–specific and does not reflect a general role of SPARC in regulating ECM adhesion in this context.

### *3.4 SPARC Is Associated With Activation of PI3K/AKT and MAPK/ERK in Endometriotic Stromal Cells*

Western blot assay confirmed that si-SPARC decreased the phosphorylation of ERK and AKT in ESCs, whereas overexpression of SPARC increased the phosphorylation of ERK and AKT (Fig. 4A). Immunohistochemical results showed a significantly higher staining intensity for SPARC in endometriotic tissues (including stroma and glands) of the ovary and abdominal wall compared with eutopic endometrium (including stroma and glands) (Fig. 4B).

## 4. Discussion

Endometriosis is one of the main causes of infertility, with complicated pathophysiologic mechanisms and unclear etiopathogenesis [2]. Despite its classification as a benign condition, endometriosis exhibits striking parallels to malignant neoplasms, characterized by aberrant cell proliferation, tissue invasion, and metastatic-like dissemination [11,12]. A widely accepted theory is that during menstruation, viable endometrial fragments undergo retrograde transport via the fallopian tubes into the peritoneal cavity [13,14]. Here, the cells implant and adhere to peritoneal surfaces, initiating a cascade of events that includes angiogenic remodeling, immune evasion, and progressive stromal infiltration, ultimately forming endometriotic lesions [15]. The aberrant proliferation and invasive capacity of ESCs underlie the pathological progression of ectopic endometrial lesions, while their regulated turnover maintains the endometrium's cyclical remodeling [16]. The targeting of ESC hyperactivity—including proliferation, invasion, and immune evasion—represents a promising therapeutic avenue, as demonstrated by a recent study that inhib-

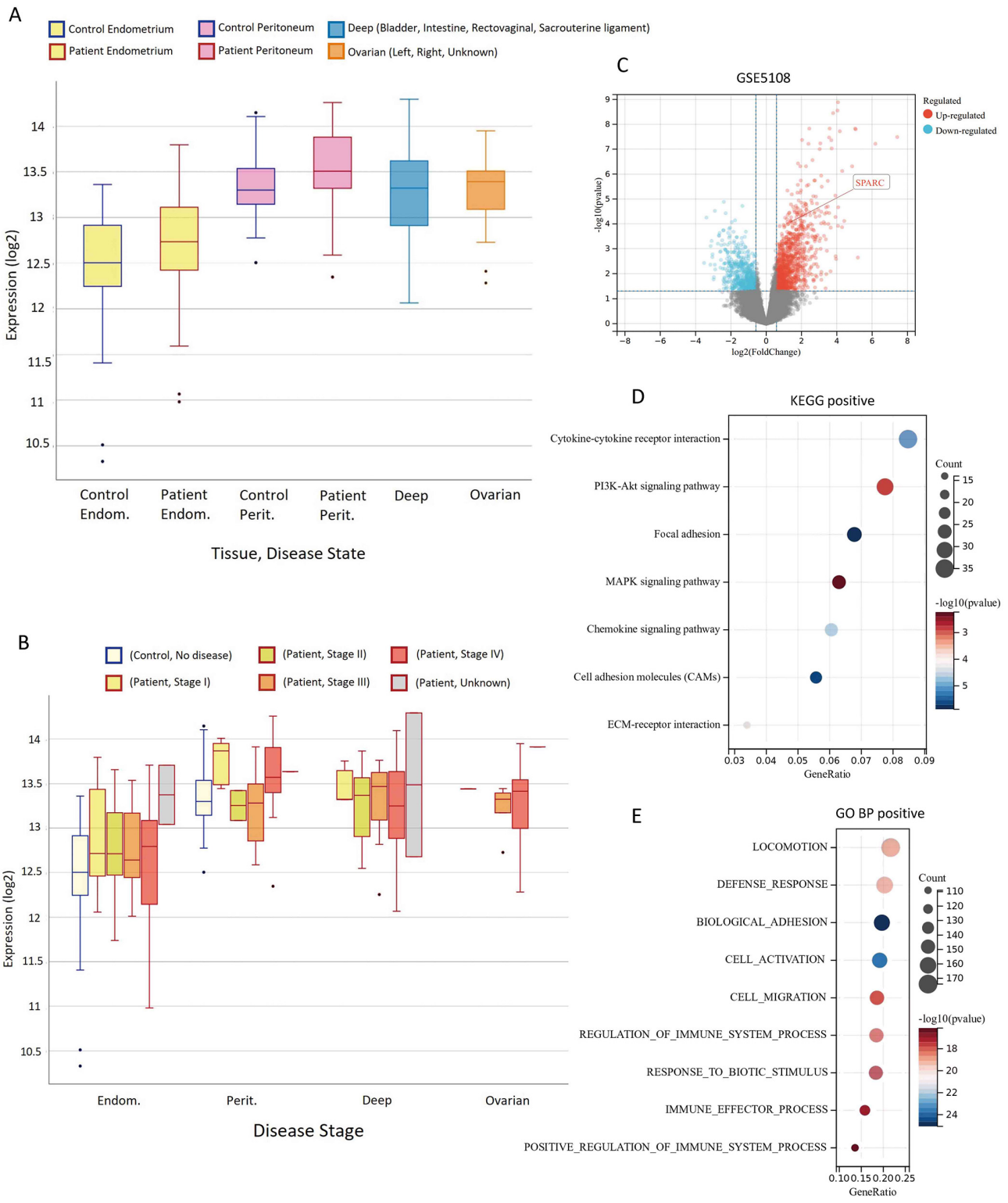


**Fig. 1. SPARC expression in endometriosis in the Turku Endometriosis Database and GEO database**. (A) SPARC expression in different endometriotic sites was shown by the Turku Endometriosis Database. (B) Turku Endometriosis Database revealed the SPARC expression in different stages of endometriosis. (C) SPARC was up-regulated in endometriosis in GSE5108. (D) The Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment bubble plot clearly showed the involvement of signaling pathways. (E) Bubble plot of Gene Ontology (GO) biological process analysis. SPARC, Secreted Protein Acidic and Rich in Cysteine; GEO, Gene Expression Omnibus; ECM, extracellular matrix.

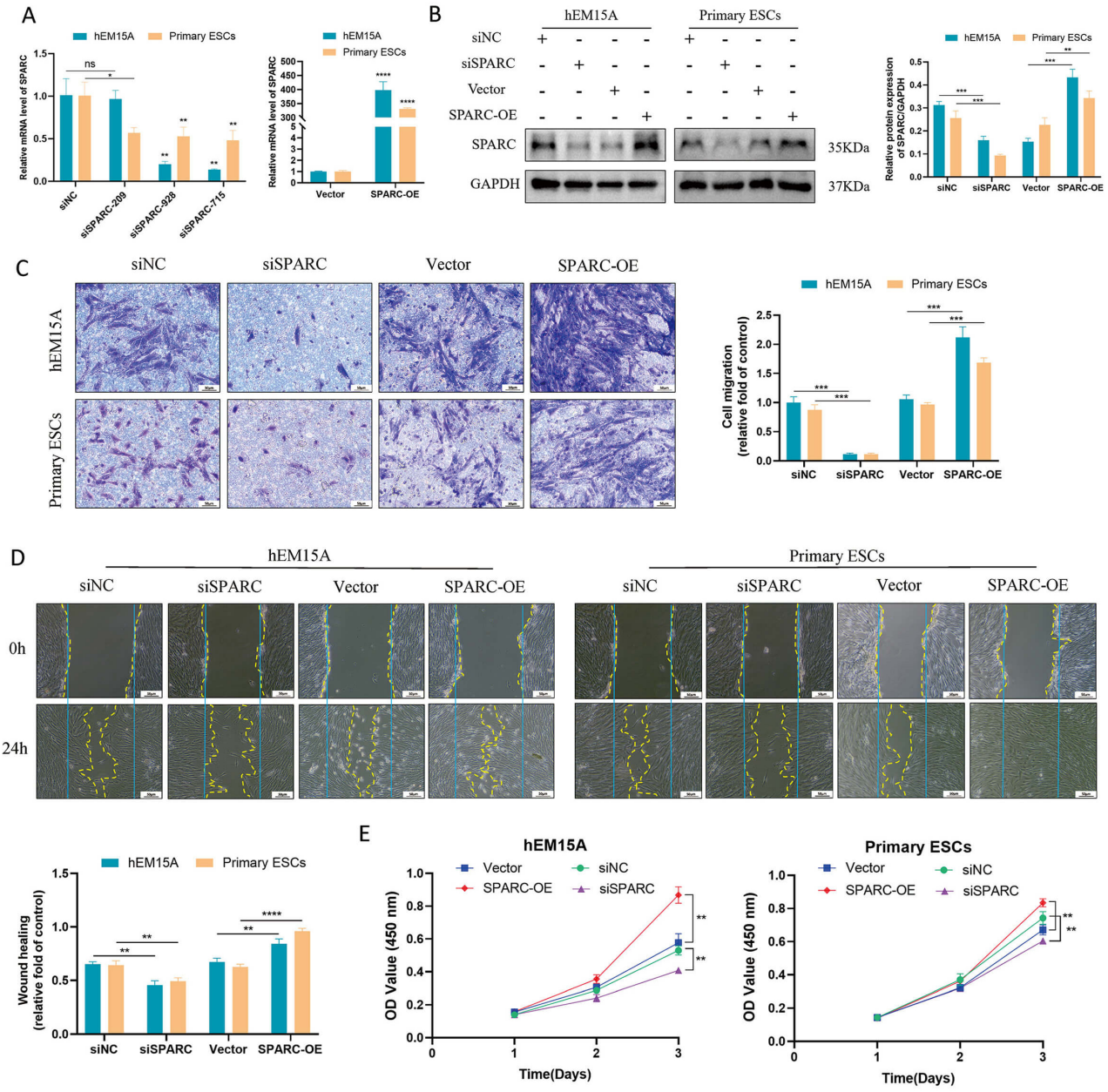


**Fig. 2. SPARC promotes endometriotic stromal cells proliferation and migration**. (A) The RNA levels of SPARC were altered by interference and overexpression in hEM15A and primary endometriotic stromal cells (ESCs) cell lines, according to Quantitative Real-time polymerase chain reaction. (B) Western blot results demonstrated that the protein level of SPARC was decreased and increased after interference and overexpression for 24 h, respectively. (C) Transwell assay showed that SPARC overexpression enhanced the migration of hEM15A cells and primary ESCs under starvation conditions, while the si-SPARC group attenuated the migration of the above two cell lines. Scale bar = 50 μm. (D) The results of the wound healing experiment showed that the scratch healing was better when the expression level of SPARC was increased at 24 h, while the healing of the si-SPARC group was worse at 24 h. The blue vertical line represents the fixed initial scratch boundary and the yellow line indicates the cell edge at 0 h and 24 h. Scale bar = 50 μm. (E) Cell Counting Kit-8 (CCK-8) time line plot of cell proliferation in both hEM15A cells and the primary ESCs: the cell proliferation ability was the strongest in the SPARC overexpression group and the weakest in the si-SPARC group. Data are representative of three independent experiments (n = 3). * indicates $p < 0.05$, ** indicates $p < 0.01$, *** indicates $p < 0.001$, **** indicates $p < 0.0001$, ns = not significant.

ited IDO1-mediated immune tolerance and NF-κB-driven inflammatory signaling [1]. Since the current therapeutic schedule only provides pain relief, the identification of reliable molecular targets and biomarkers is crucial [17,18,19]. SPARC was reported to be involved in regulating the proliferation and migration of gynecologic neoplasms, includ-

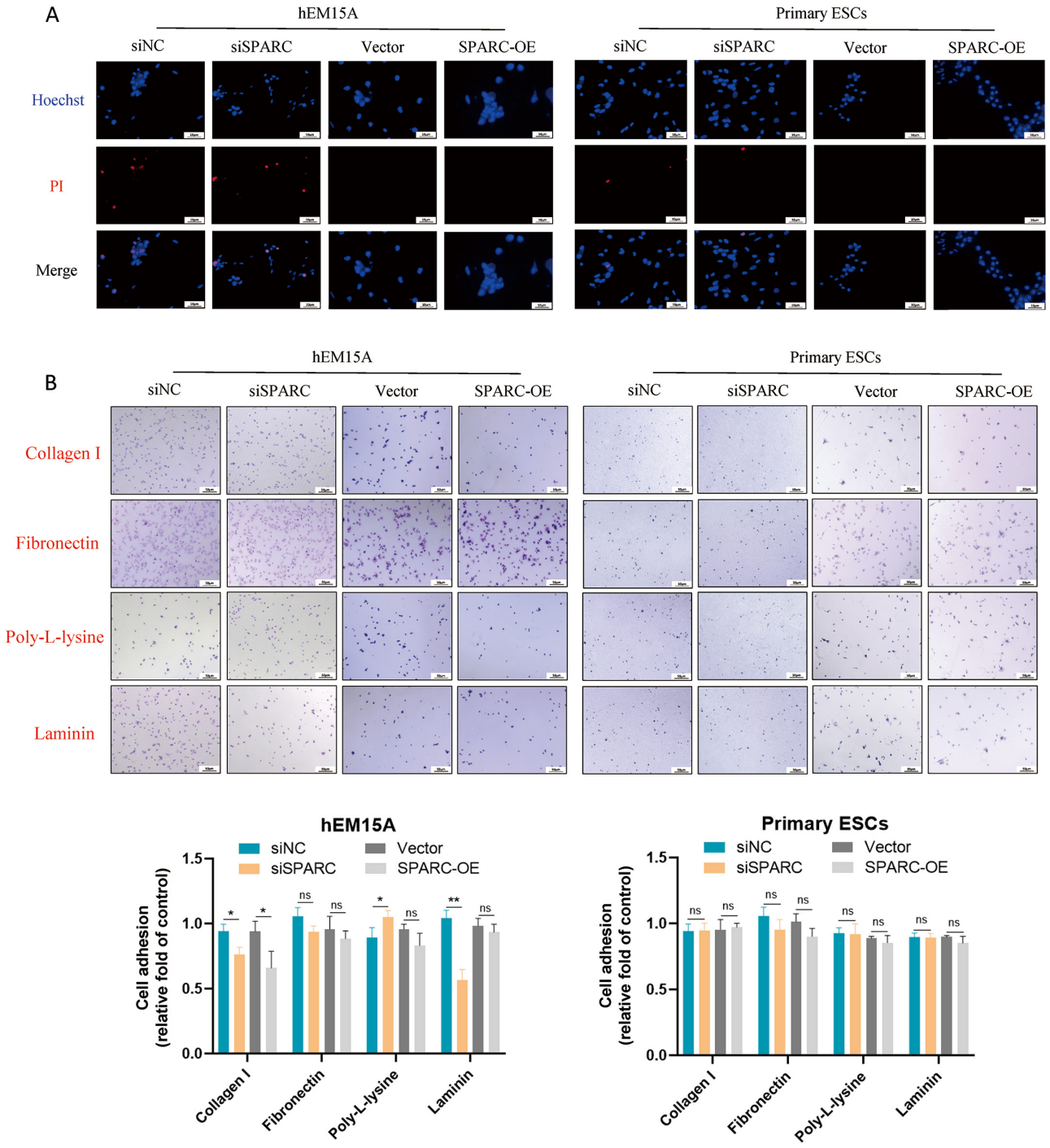


**Fig. 3. SPARC does not regulate apoptosis and necrosis of ectopic endometrial stromal cells and does not affect cell adhesion to the extracellular matrix**. (A) After staining the cells and observing them under a fluorescence microscope, the results indicated that si-SPARC and SPARC overexpression (SPARC-OE) did not alter apoptosis (stained with Hoechst) and necrosis (stained with PI) in both cell lines. Apoptotic cells were distinguished by Hoechst staining based on nuclear condensation and fragmentation. Scale bar = 10 μm. (B) Cell-to-matrix adherence test showed that: in the hEM15A cells, si-SPARC and SPARC-OE altered the ability of cells to adhere to different extracellular matrices. However, in the primary ESCs, altering SPARC expression did not significantly change cell adhesion ability. Scale bar = 50 μm. Data are representative of three independent experiments (n = 3). * indicates $p < 0.05$, ** indicates $p < 0.01$, ns = not significant.

ing endometrial carcinoma and ovarian cancer [4,20]. It was also shown to be a differentially expressed gene in endometriosis between peritoneal and ovarian lesions [6]. However, little is known about its functional significance

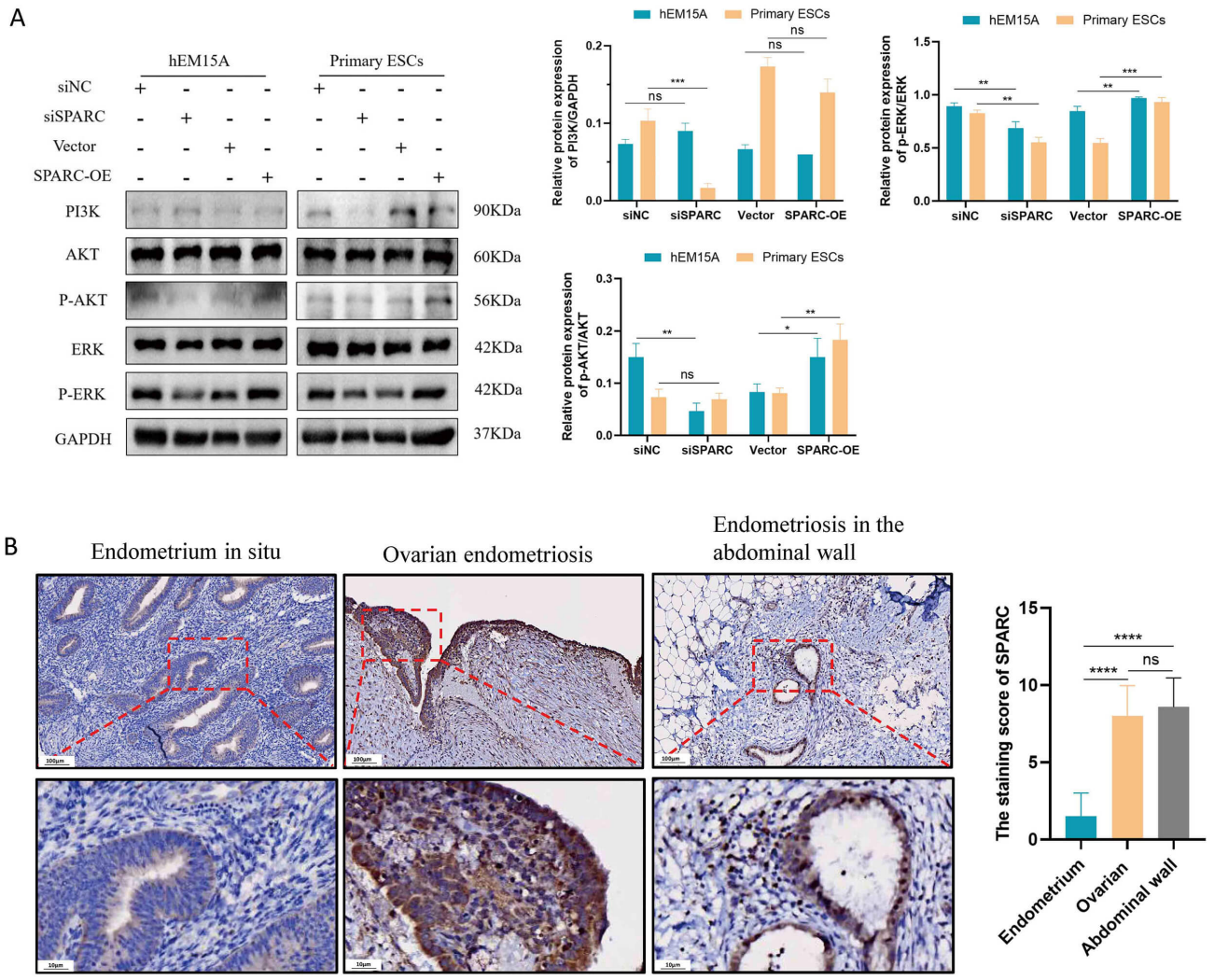


**Fig. 4. SPARC is associated with activation of PI3K/AKT and MAPK/ERK in endometriotic stromal cells**. (A) Western blot assay confirmed that si-SPARC attenuated the phosphorylation of ERK and AKT, and overexpression of SPARC enhanced the phosphorylation of ERK and AKT. (B) Immunohistochemical results showed that SPARC staining intensity was significantly higher in endometriotic tissues (including stroma and glands) of the ovary and abdominal wall than in eutopic endometrium (including stroma and glands). Scale bars represent 100 μm in the upper image, scale bars represent 10 μm in the enlarged views. * indicates $p < 0.05$, ** indicates $p < 0.01$, *** indicates $p < 0.001$, **** indicates $p < 0.0001$, ns = not significant. PI3K/AKT, phosphatidylinositol 3-kinase/protein kinase B; MAPK/ERK, mitogen-activated protein kinases/extracellular regulated protein kinases.

in endometriosis. Our study addresses this gap by elucidating the functional role of SPARC and its associated signaling pathways in ESCs. We found that dysregulation of SPARC contributes to the loss of cellular homeostasis in endometriosis.

The role of SPARC in regulating ESC adhesion to the ECM appears to be context-dependent, with our findings reflecting this complexity. As a canonical matricellular protein, SPARC is known to fine-tune cell-ECM interactions rather than simply promoting or inhibiting adhesion [21,22]. SPARC can bind to ECM components, modulate integrin clustering and signaling, and facilitate focal adhesion disassembly via its FS and EC domains to enable cell motility [23,24,25]. The discrepancies we observed between immortalized hEM15A cells and primary ESCs in the adhesion assays likely stem from fundamental differences in their ECM receptor repertoire and signaling networks. The immortalized line, with a potentially more homogenized signaling environment, may have a greater dependency on SPARC for modulating adhesion. Consistent with this, both SPARC overexpression and knockdown reduced hEM15A adhesion to Collagen I—a paradoxical result that can be explained by a biphasic, optimal-concentration-dependent model: an appropriate SPARC level is required for normal focal adhesion dynamics, and either excess or deficiency impairs adhesion. This context-dependent variability highlights an important limitation in the generalizability of our adhesion-related findings, as the

observed effects may not extend broadly across different experimental systems or physiological conditions.

Our data confirm that SPARC is highly expressed in clinical endometriotic tissues (ovarian and abdominal wall) and is associated with activation of the PI3K/AKT and MAPK/ERK pathways. This aligns with findings in other diseases. For instance, Deng et al. [26] reported that SPARC promotes cell proliferation, migration, invasion, and EMT in cholangiocarcinoma via PI3K/AKT activation. Thus, SPARC-mediated activation of core oncogenic pathways such as PI3K/AKT appears to be a conserved mechanism that drives invasive behavior, not only in cancers but also in the progression of endometriosis. From a translational perspective, these findings suggest that elevated SPARC expression could potentially serve as a biomarker; however, this remains hypothetical at this stage. Its detection might hypothetically indicate the presence of lesions or could potentially identify patients with a higher risk of multifocal or occult disease at other sites, but such applications would require rigorous validation in large, well-characterized patient cohorts. Furthermore, the assessment of SPARC levels in ectopic lesions could, in principle, inform prognosis and therapeutic strategy, although this possibility is speculative and awaits further investigation. This cross-disease commonality strengthens the therapeutic rationale for targeting SPARC and its downstream signaling. The potential utility of SPARC as a target is further highlighted by recent translational work. One example is the use of a targeted nano-delivery system (BSA@Mif NPs) designed to selectively target SPARC-overexpressing M2 macrophages in ectopic lesions. This strategy enhances drug accumulation in endometriotic tissue and modulates the local immune microenvironment, showing marked efficacy in a mouse model [27]. Nonetheless, the clinical applicability of targeting SPARC remains to be established, and any such strategies would require extensive preclinical and clinical evaluation.

*Limitations*

The negative apoptosis/necrosis findings in this study were limited by the sensitivity of the qualitative assay. Moreover, the results of the adhesion experiments likely reflect the complex role of SPARC as a matricellular regulator that primarily drives cell motility, with statistical power being a potential factor. The *in vitro* experiments were performed without formal power calculations and without correction for multiple comparisons. Further quantitative studies are required to clarify these issues.

The assessment of apoptosis and necrosis relied solely on qualitative fluorescence microscopy. Future studies employing quantitative methods such as flow cytometry with Annexin V/PI staining are required to definitively rule out any subtle effects of SPARC on cell death pathways. While our data show a significant correlation between SPARC expression levels and the phosphorylation status of AKT and ERK, the present study cannot fully distinguish between whether these changes represent direct, causative activation of these pathways, or whether they represent secondary, correlative adaptations. Additional experiments are needed to establish a direct causal link, such as co-immunoprecipitation to probe for physical interactions between SPARC-associated complexes and key components of the MAPK/ERK and PI3K/AKT molecular pathways, and kinase assays to directly assess the impact of SPARC on AKT/ERK phosphorylation *in vitro*. Until such direct evidence is obtained, our conclusions regarding pathway activation should be interpreted as indicative of a strong correlative relationship that is functionally significant in our model systems. Furthermore, the immunohistochemical analysis in this study was limited by the relatively small sample size and single-center design, which may affect the generalizability of the findings. Future prospective cohort studies with larger, multi-center samples are warranted to further validate the expression pattern and clinical significance of SPARC across different subtypes and stages of endometriosis.

## 5. Conclusions

Our *in vitro* results suggest that SPARC may promote ESC proliferation and migration, potentially via activation of the MAPK/ERK and PI3K/AKT pathways. Qualitative Hoechst/PI staining showed no obvious effect of SPARC on apoptosis or necrosis, though subtle effects cannot be excluded. The effect of SPARC on ESC adhesion to the ECM was inconsistent, possibly reflecting differences between cell models. These findings offer preliminary insights into endometriosis pathogenesis and raise the possibility of SPARC as a diagnostic or therapeutic target, which requires validation in larger cohorts and mechanistic studies.

## Availability of Data and Materials

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

## Author Contributions

XQL performed the literature search, *in vitro* experiments and drafted the manuscript. YH performed the statistical analysis. ZPW, SW and PL performed the IHC and revised the manuscript. SY and JFW designed the concept of the study and revised the whole manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

## Ethics Approval and Consent to Participate

This study has been approved by the Second Affiliated Hospital of Harbin Medical University, and the approval number is KY2024-277. The study was carried out in accordance with the guidelines of the Declaration of Helsinki. This was a retrospective study utilizing archived paraffin-embedded tissue blocks. The use and analysis of all the tissue samples were carried out strictly in accordance with the approved protocols after obtaining ethical approval. Written informed consent was obtained from all patients whose archived tissue blocks were used in this study.


## Acknowledgment

We thank all who helped us in writing the manuscript and Professor Zhang for his primary ESCs as a gift to support our project.

## Funding

This study was supported by the Research Project of China Primary Health Care Foundation (2023001).


## Conflicts of Interest

The authors declare no conflicts of interest.

## Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/CEOG48963.

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