

Quercetin modulates key biomarkers and inhibits cell proliferation in endometriosis

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Abstract. Endometriosis is a chronic disorder and the present study investigated the therapeutic potential of quercetin, derived from *Paeoniae Radix Alba* and *Hedysarum multijugum* Maxim (BSHQ), by identifying bioactive ingredients and key biomarkers with a focus on their molecular interactions in the context of endometriosis. The present study identified 32 bioactive ingredients from BSHQ, linked to 231 target genes. Gene Expression Omnibus datasets and Weighted Gene Co-expression Network Analysis were used to analyze differentially expressed genes and key co-expression modules. *In vitro* assays, including half-maximal inhibitory concentration, Cell Counting Kit-8 and 5-ethynyl-2'-deoxyuridine assays, assessed the impact of quercetin on cell proliferation in endometriosis cell lines. Molecular docking, cellular thermal shift assay (CETSA) and surface plasmon resonance (SPR) analyzed the interaction of quercetin with biomarkers. The present study identified 16 BSHQ-related genes. Single-cell RNA sequencing analysis showed distinct expression patterns of these genes in various cell types. Molecular docking confirmed strong binding between quercetin and these biomarkers. *In vitro* assays revealed quercetin markedly inhibited the proliferation of endometriosis cell lines, reducing cell proliferation and serpin family E member 1 (SERPINE1) expression. CETSA showed that quercetin can directly bind to SERPINE1 protein and enhance its thermal stability against temperature-induced degradation, a post-translational effect independent of expression regulation, and SPR analysis

further supported the direct interaction between quercetin and SERPINE1. Furthermore, SERPINE1 overexpression rescued the inhibitory effects of quercetin on proliferation, highlighting its critical role in mediating the effects of quercetin in endometriosis. The present study underscored SERPINE1 as a pivotal biomarker in endometriosis and revealed that quercetin effectively modulated key disease mechanisms, offering promising therapeutic potential. These findings provide a solid basis for future research aimed at exploring the role of quercetin in the treatment of endometriosis.

Introduction

Endometriosis is a chronic condition where endometrial-like tissue grows outside the uterus, leading to symptoms such as persistent pelvic pain, painful periods, pain during intercourse and infertility. The severity of the condition varies; some women experience only mild symptoms, whereas others face more severe issues such as ovarian cysts, adhesions and deep lesions (1). Pelvic pain, often worsened by menstruation and intercourse, is one of the primary challenges for affected individuals. Infertility is a notable concern, affecting ≤40% of women with endometriosis (2) and gastrointestinal problems are common, especially in more advanced stages of the disease (3). Diagnosing endometriosis often requires laparoscopy, as imaging methods are less effective, particularly in detecting deep lesions (4,5). Treatment typically involves hormonal therapies, including combined oral contraceptives, dienogest and gonadotropin-releasing hormone analogs, as well as surgical options, although symptoms often recur and long-term medication use can lead to side effects (6-8). Endometriosis can have a marked impact on quality of life, contributing to social and economic challenges (9).

Paeoniae Radix Alba, or Bai Shao, is a crucial herb in traditional Chinese medicine (TCM), known for its efficacy in regulating menstruation. Notably, it is commonly used to address gynecological concerns, such as dysmenorrhea, irregular periods and pelvic pain. *Paeoniae Radix Alba* possesses cramp-relieving properties and serves as a core herb in numerous TCM formulas targeting female reproductive health (10).

Hedysarum multijugum Maxim, known as Huang Qi, has been a cornerstone of TCM for centuries. It serves a notable

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role in supporting immune function, and is commonly used to treat conditions such as fatigue, spontaneous sweating and frequent colds. Additionally, it helps reduce edema, promotes wound healing and accelerates recovery after illness (11).

Hedysarum multijugum Maxim and *Paeoniae Radix Alba* both contain bioactive compounds that contribute to their therapeutic effects. The active ingredients of *Hedysarum multijugum* Maxim, such as saponins, polysaccharides and flavonoids, are known for their immune-boosting, anti-inflammatory and antioxidant properties (12). These qualities are particularly important in treating conditions such as endometriosis, where inflammation and tissue damage have an important role (13). When used together, *Hedysarum multijugum* Maxim and *Paeoniae Radix Alba* address both systemic and localized symptoms of conditions such as endometriosis, particularly pelvic pain and inflammation. Their combined effects have been supported by modern research, reinforcing their value in the treatment of gynecological conditions (14). The herbs both hold promise as complementary therapies for endometriosis, alongside conventional treatments.

In our previous study, we investigated hypoxia-related molecular mechanisms in endometriosis using integrated bioinformatics analyses and identified discoidin domain-containing receptor 2 as a potential diagnostic and therapeutic biomarker (15). However, this previous study focused primarily on hypoxia-associated gene signatures, extracellular matrix (ECM) remodeling and invasive behavior in endometriosis. By contrast, the present study aimed to investigate the pharmacological basis of *Paeoniae Radix Alba* and *Hedysarum multijugum* Maxim (BSHQ), with particular emphasis on BSHQ-derived active ingredients and the quercetin-serpin family E member 1 (SERPINE1) axis.

The present study aimed to identify BSHQ-related therapeutic targets and active compounds for endometriosis through network pharmacology, transcriptomics analysis, weighted gene co-expression network analysis (WGCNA), machine learning and reanalysis of published single-cell RNA sequencing (scRNA-seq) datasets. Following the identification of candidate biomarkers and active ingredients, *in vitro* experiments were performed to evaluate the biological effects of quercetin and its interaction with SERPINE1. The current study differs from our previous hypoxia-focused work by emphasizing the pharmacological mechanisms of BSHQ and experimentally validating the quercetin-SERPINE1 regulatory axis in endometriosis. The findings of the present study may provide novel therapeutic targets and strategies for the clinical treatment of endometriosis, offering a promising approach to improving patient outcomes.

Materials and methods

Screening of active ingredients and targets of BSHQ.

The active components of BSHQ were initially screened from the TCM Systems Pharmacology (TCMSP) database (<https://www.tcmsp-e.com/tcmsp.php>) using the criteria of oral bioavailability $\geq 20\%$ and drug-likeness ≥ 0.10 . Following this, the corresponding protein targets of the selected active components were identified within the TCMSP database. These protein targets were subsequently normalized using the UniProt database (<http://www.uniprot.org>) by selecting

the '*Homo sapiens*' taxonomy. Finally, Cytoscape 3.10.1 (<https://cytoscape.org/>) was employed to construct a network that illustrates the relationships between the active components and their target genes. Topological analysis of the network was performed using the NetworkAnalyst plugin (<https://www.networkanalyst.ca/>) embedded in Cytoscape to calculate and rank the degree of connectivity of each active ingredient.

Data acquisition. The datasets GSE7305, GSE11691 and GSE58178 were sourced from the Gene Expression Omnibus (GEO; <https://www.ncbi.nlm.nih.gov/geo/>) database and normalization analyses were subsequently performed (16–18). The GEO datasets used in the present study, GSE7305, GSE11691 and GSE58178, were also included in our previous study on hypoxia-related mechanisms in endometriosis (15); however, the present study was independently designed to investigate BSHQ-related pharmacological mechanisms and quercetin-associated therapeutic targets. All data preprocessing, gene screening, downstream analyses and figure generation were independently performed for the current study. The GSE7305 dataset includes 10 normal endometrial samples (paired eutopic endometrium from the same patients) and 10 endometriosis samples, and was analyzed using the GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array. The GSE11691 dataset consists of nine endometrial samples and nine matched ectopic endometriotic lesion samples, which are paired tissues collected from the same nine women with endometriosis. All samples were analyzed with the GPL96 [HG-U133A] Affymetrix Human Genome U133A Array. The GSE58178 dataset contains six normal endometrial samples (from healthy women without endometriosis) and six endometriosis samples, analyzed on the GPL6947 Illumina HumanHT-12 V3.0 expression beadchip. Endometriosis-related gene sets were retrieved from GeneCards (<https://www.genecards.org/>) using 'endometriosis' as the search key word. The GeneCards relevance score is an integrated metric automatically computed by the database based on multi-source evidence including disease-gene annotations, peer-reviewed literature, genome-wide association study data and mutation records, where a higher score indicates a stronger disease-gene association. Genes with a relevance score >1 were screened as candidate endometriosis-related genes, yielding a total of 1,359 genes. The GSE7846 dataset served as an independent validation cohort to verify the diagnostic performance of the identified hub genes. This dataset was also retrieved from the GEO database, containing 5 samples of primary cultured human endometrial endothelial cells (HEECs) isolated from the eutopic endometrium of disease-free premenopausal women and 5 HEEC samples from women with endometriosis, analyzed with the GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array. This dataset was initially reported in a previous study exploring the transcriptomic profile of endometrial endothelial cells in endometriosis (19).

Differentially expressed genes (DEGs) analysis. The DEGs between endometriosis samples and normal endometrial control samples analysis was conducted using the limma package (version 3.66.0; <https://bioconductor.org/packages/limma/>) in R software (version 4.3.1; <https://www.r-project.org/>), which utilizes a linear model to compute fold change (FC) values

and P-values. The Benjamini-Hochberg method was employed to control the false discovery rate. Genes with $\log_2\text{FCI} > 0.3$ and $P < 0.05$ were considered significantly differentially expressed. To visualize the expression profiles of the identified DEGs, heatmaps and volcano plots were generated using the pheatmap (version 1.0.13; <https://cran.r-project.org/package=pheatmap>) and ggplot2 (version 4.0.3; <https://ggplot2.tidyverse.org>) packages, respectively, in R software. Principal component analysis (PCA) was additionally performed to visualize the overall distribution of samples after normalization, and to assess the separation tendency between normal and endometriosis samples. Receiver operating characteristic (ROC) curve analysis was performed to assess the diagnostic performance of the key genes in differentiating ectopic from eutopic endometrial tissues. ROC curves were plotted using the pROC package (v1.18.0; <https://cran.r-project.org/package=pROC>) in R (v4.3.1) and the area under the curve (AUC) was calculated to evaluate the predictive accuracy of the model.

Gene ontology (GO) and kyoto encyclopedia of genes and genomes (KEGG) enrichment analyses. Chromosomal localization analysis of candidate genes was performed using the RIdiogram R package (version 0.2.2; <https://cran.r-project.org/web/packages/RIdiogram/index.html>) to visualize the chromosomal distribution of the genes. GO and KEGG enrichment analyses were conducted to identify the biological functions and pathways associated with the DEGs. Using the R package clusterProfiler (version 4.8.3; <https://bioconductor.org/packages/clusterProfiler/>), DEGs were analyzed for enrichment in biological processes (BP), cellular components (CC), molecular functions (MF) and pathways. Pearson correlation analysis was performed to calculate the expression correlation among candidate genes. Correlation plots were generated using the corrplot R package (version 0.92; <https://cran.r-project.org/web/packages/corrplot/index.html>), with color intensity indicating the strength of correlation. Chord plots were constructed using the circlize R package (version 0.4.16; <https://cran.r-project.org/web/packages/circlize/index.html>) to visualize the pairwise correlation relationships. The protein-protein interaction (PPI) network of the candidate genes was constructed using the STRING database (version 12.0; <https://string-db.org/>), with the organism limited to *Homo sapiens* and a minimum interaction score of 0.4 as the inclusion threshold. The network was then imported into Cytoscape 3.10.1, and the top 10 hub genes were identified via the maximal clique centrality (MCC) algorithm using the cytoHubba plugin (<https://apps.cytoscape.org/apps/cytohubba>).

WGCNA. WGCNA was performed to identify gene modules that are significantly associated with endometriosis. The normalized gene expression matrix derived from GEO datasets served as the input, with low-variance genes excluded to improve network stability and minimize noise. Sample clustering was conducted using the flashClust R package (version 1.01-2; <https://cran.r-project.org/web/packages/flashClust/index.html>). Using the WGCNA R package (version 1.72-1; <https://cran.r-project.org/package=WGCNA>), pairwise gene correlations were calculated and subsequently transformed into an adjacency matrix. A soft-thresholding

power of eight was selected according to the scale-free topology criterion, ensuring the construction of a robust and biologically relevant network. The topological overlap matrix (TOM) was then computed to assess gene connectivity and similarity within the network. Hierarchical clustering was employed to organize genes into distinct co-expression modules, with each module assigned a unique color for visualization purposes. Module eigengenes, which represent the first principal component of each module, were calculated to summarize the expression profiles of the modules.

scRNA-seq analysis. The single-cell transcriptomic dataset GSE213216 for endometriosis was retrieved from the GEO database (20). All downstream analyses were performed using the Seurat R package (version 4.3.0; <https://satijalab.org/seurat/>). First, quality control was performed to filter low-quality cells. After quality control, the filtered gene expression matrix was normalized using the LogNormalize method, and highly variable genes were identified via the FindVariableFeatures function. PCA was then conducted based on highly variable genes for linear dimensionality reduction. Subsequently, the uniform manifold approximation and projection (UMAP) method was applied for non-linear dimensionality reduction and 2D visualization of all cells, with the first 20 principal components selected as input. Cell clustering was performed using the FindNeighbors and FindClusters functions, and cell type annotation was conducted based on canonical marker genes. The expression patterns of target genes across cell types were visualized via feature plots, violin plots and heatmaps generated using the Seurat R package.

Machine learning methods. Machine learning techniques, specifically least absolute shrinkage and selection operator (LASSO) and support vector machine-recursive feature elimination (SVM-RFE), were employed to identify key genes from the DEGs. LASSO regression was conducted using the glmnet package in R (version 4.1-7; <https://cran.r-project.org/package=glmnet>), aiming to select the most relevant genes by shrinking regression coefficients and excluding non-informative features. Genes with non-zero coefficients were retained for further analysis. Following this, SVM-RFE was applied to rank the selected genes according to their significance in classification. This method, implemented via the e1071 package (version 1.7-12; <https://cran.r-project.org/package=e1071>), recursively removes the least influential features to enhance the performance of the model. Cross-validation was utilized to verify the stability and reliability of the feature selection process.

Nomogram construction and decision curve analysis (DCA). A nomogram was constructed based on the expression levels of the three identified hub genes (PTGER3, SERPINE1 and VCAM1) using the rms R package (version 6.7-1; <https://cran.r-project.org/web/packages/rms/index.html>). Each biomarker was assigned a weighted point according to its regression coefficient, and the total point was calculated to predict the risk of endometriosis. DCA was performed to evaluate the clinical utility of the nomogram using the rmda R package (version 1.6; <https://github.com/mbrown/rmda>), by

calculating the net benefit of the model across different risk thresholds (21).

Molecular docking. Small molecules were selected from the TCMSP database and their structures were prepared using AutoDockTools-1.5.7 (<https://ccsb.scripps.edu/mgltools/>). This preparation involves adding hydrogen atoms, calculating partial charges and exporting the structures as PDBQT-format files. Protein structures were obtained from the RCSB PDB database (version 2023; <https://www.rcsb.org>). These structures were processed with PyMOL (version 2.5.2; <https://pymol.org/>) and AutoDockTools-1.5.7, where water molecules were removed and hydrogen atoms were added. AlphaFold-predicted structures (version 4; <https://alphafold.ebi.ac.uk/>) underwent similar processing. Docking simulations were carried out using AutoDock Vina (version 1.2.5; <https://vina.scripps.edu/>), generating nine conformations for each ligand. The binding affinity was assessed based on the docking score, where values <-7 kcal/mol were considered indicative of strong binding. The conformations with the lowest binding free energy were selected for subsequent analysis. Non-covalent interactions were examined using Protein-Ligand Interaction Profiler (PLIP; version 2.1.7; <https://plip-tool.biotec.tu-dresden.de/>), and visualized with PyMOL. The docking models with a binding free energy of <-7 kcal/mol were chosen for further study.

Gene set enrichment analysis (GSEA). GSEA was performed to identify enriched biological pathways and MF terms associated with DEGs. The normalized gene expression matrix of the merged GEO datasets were ranked based on their association with the phenotype and used to generate a ranked gene list. Predefined gene sets from the Molecular Signatures Database (version 7.4; <https://www.gsea-msigdb.org/gsea/msigdb/index.jsp>) or custom gene sets were selected for analysis. GSEA was conducted using GSEA software (version 4.3.2; <https://www.gsea-msigdb.org/gsea/index.jsp>) and an enrichment score was calculated for each gene set to determine its significance. Statistical significance was assessed through permutation testing. Enrichment score plots and heatmaps were generated to visualize the results.

Cell lines and culture conditions. The VK2/E6E7 and 12Z endometriosis cell lines, obtained from Procell Life Science & Technology Co., Ltd., were cultured in high-glucose Dulbecco's Modified Eagle's Medium (cat. no. PM15021; Procell Life Science & Technology Co., Ltd.), supplemented with 10% fetal bovine serum (FBS; cat. no. 10099141C; Gibco; Thermo Fisher Scientific, Inc.). The cells were grown in a humidified incubator at 37°C with 5% CO₂ to ensure optimal growth conditions and maintain their viability for experimental purposes.

Cell transfection procedure. Transfection was performed on both 12Z and VK2/E6E7 endometriosis cell lines. Cells were seeded at a density of 2×10^5 cells/well in 6-well plates 24 h prior to transfection, reaching 70–80% confluence on the day of transfection. Prior to transfection, 1.5-ml micro-centrifuge tubes were UV-irradiated and Opti-MEM medium (cat. no. 31985070; Gibco; Thermo Fisher Scientific, Inc.) was pre-warmed to 37°C. Cells were washed twice with PBS

and the culture medium was then replaced with Opti-MEM. The transfection mixture was prepared by combining 125 μ l Opti-MEM, 2.5 μ g SERPINE1 overexpression plasmid and 4 μ l Lipo 8000 reagent (cat. no. C0533; Beyotime Biotechnology). The mixture was added dropwise to the wells and incubated at 37°C in a humidified 5% CO₂ incubator for 6 h. After incubation, the medium was replaced with fresh medium containing 10% FBS. For SERPINE1 overexpression experiments, the plasmid backbone used was pcDNA3.1(+), an ampicillin-resistant vector purchased from Beijing Tsingke Biotech Co., Ltd. Data S1 provides the complete coding sequence (consensus coding sequence region) of the human SERPINE1 gene (transcript variant NM_000602.5), which was used as the insert fragment for plasmid construction, the complete plasmid map is shown in Fig. S1. The negative control group was transfected with the corresponding empty pcDNA3.1 vector, which was obtained from the same supplier and also carried ampicillin resistance. For cell proliferation rescue assays, quercetin (cat. no. HY18085; MedChemExpress) treatment at the respective half-maximal inhibitory concentration (IC₅₀) concentration for each cell line was initiated 24 h after transfection. Cells were incubated at 37°C in a humidified 5% CO₂ incubator, and proliferation was measured at 0, 24, 48, 72 and 96 h after quercetin administration.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from cells treated with quercetin at the respective IC₅₀ concentration for each cell line for 24 and 48 h at 37°C in a 5% CO₂ humidified incubator (only data obtained at the 24 h timepoint are presented in the current study), with DMSO as the vehicle control or SERPINE1-overexpression transfected cells using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.), following the standard RNA extraction protocol, and cDNA was synthesized using the PrimeScript RT Reagent Kit (Takara Bio, Inc.) in accordance with the manufacturer's recommended protocol. qPCR was performed on the Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.) using 2X SYBR Green qPCR Master Mix (cat. no. G3320-15; Wuhan Servicebio Technology Co., Ltd.). Each 20- μ l reaction contained 4 μ l 2X SYBR Green Mix, 0.2 μ l SERPINE1 forward primer (25 μ M, 5'-CGCCTC TTCCACAAATCAGACG-3') and 0.2 μ l SERPINE1 reverse primer (10 μ M, 5'-GGCAGTTCCAGGATGTCGTAGT-3'), or 0.2 μ l GAPDH forward primer (25 μ M, 5'-ACCCACTCC TCCACCTTTGACG-3') and 0.2 μ l GAPDH reverse primer (25 μ M, 5'-TCTCTTCCTCTTGTGCTCTTG-3'), 0.5 μ l cDNA and nuclease-free water to adjust the final volume. The SERPINE1 target gene and GAPDH housekeeping gene were detected in independent reaction tubes, with only one pair of primers added per reaction. The cycling conditions were set as follows: Initial denaturation at 95°C for 10 min, followed by 40 cycles at 95°C for 15 sec and 60°C for 1 min. Relative expression levels of SERPINE1 were normalized to GAPDH using the $2^{-\Delta\Delta C_q}$ method (22). **Western blotting (WB).** For WB, cells were lysed in radioimmunoprecipitation assay buffer (cat. no. G2002; Wuhan Servicebio Technology Co., Ltd.) supplemented with 1% phenylmethylsulfonyl fluoride, and protein concentrations were measured by bicinchoninic acid assay (cat. no. G2026; Wuhan Servicebio Technology Co.,

Ltd.). Equal amounts of protein (30 μ g/lane) were separated by SDS-PAGE on 10% gels and transferred to polyvinylidene fluoride membranes. After blocking with 5% (w/v) non-fat skim milk (cat. no. D8340; Beijing Solarbio Science & Technology Co., Ltd.) for 1 h at room temperature, the membranes were incubated with anti-SERPINE1 (1:1,000; cat. no. ab28207; Abcam) and anti-GAPDH (1:5,000; cat. no. ab8245; Abcam) primary antibodies overnight at 4°C, followed by horseradish peroxidase-conjugated secondary antibodies (1:5,000; cat. nos. ab205718 and ab205719; Abcam) for 1 h at room temperature. Protein bands were detected using an enhanced chemiluminescence kit (cat. no. G2014; Wuhan Servicebio Technology Co., Ltd.) and semi-quantified by densitometric analysis using ImageJ software (version 1.53t; National Institutes of Health).

Cell counting kit-8 (CCK-8) assay. Cell proliferation was assessed using the CCK-8 assay kit (cat. no. G4103; Wuhan Servicebio Technology Co., Ltd.). Cells were seeded in 96-well plates and treated with quercetin at gradient concentrations (0, 1, 2, 4, 8, 16, 32 and 64 μ M) for 24, 48, 72 and 96 h at 37°C in a 5% CO₂ humidified incubator, with an equal volume of dimethyl sulfoxide (DMSO) as the vehicle control. At each time point, 10 μ l CCK-8 solution was added to each well and incubated for 2 h. The absorbance at 450 nm was measured using a microplate reader. The resulting optical density (OD) values were used to quantify cell proliferation and determine the effects of the treatments on cell proliferation.

IC₅₀ assay. The IC₅₀ of quercetin on endometriosis cell lines was determined using the CCK-8 cell viability assay. Cells in the logarithmic growth phase were digested, resuspended in complete medium and seeded into 96-well plates at a density of 5x10³ cells/well in 100 μ l complete medium. After 24 h of incubation for cell adhesion, quercetin was added at gradient concentrations (0, 1, 2, 4, 8, 16, 32 and 64 μ M), with six replicate wells for each concentration. A blank control with only culture medium was set simultaneously. After 24 h of drug treatment, the absorbance at 450 nm was measured to assess cell viability, and IC₅₀ values were calculated via dose-response curve fitting.

5-ethynyl-2'-deoxyuridine (EdU) assay. Cell proliferation was measured using the EdU assay kit (cat. no. G1601; Wuhan Servicebio Technology Co., Ltd.). Cells were divided into the DMSO solvent control group and quercetin treatment group: cells were treated with quercetin at the respective IC₅₀ concentration for each cell line, and incubated at 37°C in a 5% CO₂ humidified incubator for 24 h; the control group was treated with an equal volume of DMSO matching the solvent concentration of the quercetin treatment group. After treatment, cells were incubated with 10 μ M EdU for 2 h at 37°C, followed by fixation with 4% paraformaldehyde for 15 min at room temperature. Cells were then permeabilized using 0.5% Triton and stained according to the manufacturer's instructions, with Hoechst 33342 used for nuclear counterstaining. The EdU-positive cells were visualized and quantified under a fluorescence microscope. The percentage of EdU-positive cells was calculated to assess the proliferative activity of the treated cells.

Cellular thermal shift assay (CETSA). CETSA was performed to assess the stability of SERPINE1 in the presence of quercetin. Briefly, 12Z and VK2/E6E7 cells were treated with 20 μ M quercetin for 4 h at 37°C; an equal volume of DMSO was added to the DMSO control group. Following treatment, cells were harvested and resuspended in lysis buffer. The cell suspensions were subjected to gradient temperatures (37, 42, 47, 52, 57 and 62°C) for 3 min, followed by rapid cooling on ice. After three freeze-thaw cycles, cell lysates were collected and analyzed by WB to measure the levels of SERPINE1. The degree of SERPINE1 stabilization was determined by comparing the protein levels across different temperature treatments.

Surface plasmon resonance (SPR). SPR analysis was conducted to evaluate the interaction between quercetin and SERPINE1. Recombinant human SERPINE1 protein (cat. no. ab282405; Abcam) was immobilized on a CM5 sensor chip (cat. no. BR100530; Cytiva), and increasing concentrations of quercetin (0.3125, 0.625, 1.25, 2.5, 5 and 10 μ M) were injected over the surface at 25°C, with an association phase of 120 sec and a dissociation phase of 300 sec to allow binding. The changes in resonance units were monitored in real-time using a Biacore T200 system (Cytiva) to assess the binding affinity and kinetics. The dissociation constant (KD) was calculated from the binding curves to determine the strength of the interaction between quercetin and SERPINE1. Data analysis was performed using dedicated software (Biacore T200 Evaluation Software; version 3.2; Cytiva) to obtain precise binding parameters.

Statistical analysis. Statistical analysis was performed using GraphPad Prism 9.0 software (Dotmatics) and R software. Continuous data were first assessed for normality using the Shapiro-Wilk test. Normally distributed data are presented as the mean $\pm$ standard deviation, whereas non-normally distributed data are presented as median (interquartile range). For comparisons between two groups, unpaired Student's t-test was used for normally distributed data with equal variances, and unpaired Welch's t-test was applied when variances were unequal. P<0.05 was considered to indicate a statistically significant difference.

Results

Active ingredients and targets of BSHQ. In the present study, a total of 32 bioactive ingredients were identified from BSHQ. Among these, 12 ingredients were sourced from *Paeoniae Radix Alba*, while 22 originated from *Hedysarum multijugum* Maxim. Notably, kaempferol (MOL000422) and mairin (MOL000211) were found to be common to both herbs (Table SI). After eliminating duplicates, the 32 active ingredients were linked to 231 unique target genes. To explore the interactions between these active ingredients and their corresponding targets, the present study constructed a detailed interaction network using Cytoscape 3.10.1. This network visually represents the connections between the bioactive ingredients and their respective target genes. In the diagram, the orange rectangular nodes at the top denote the two constituent herbs of BSHQ, ovals represent the active ingredients and

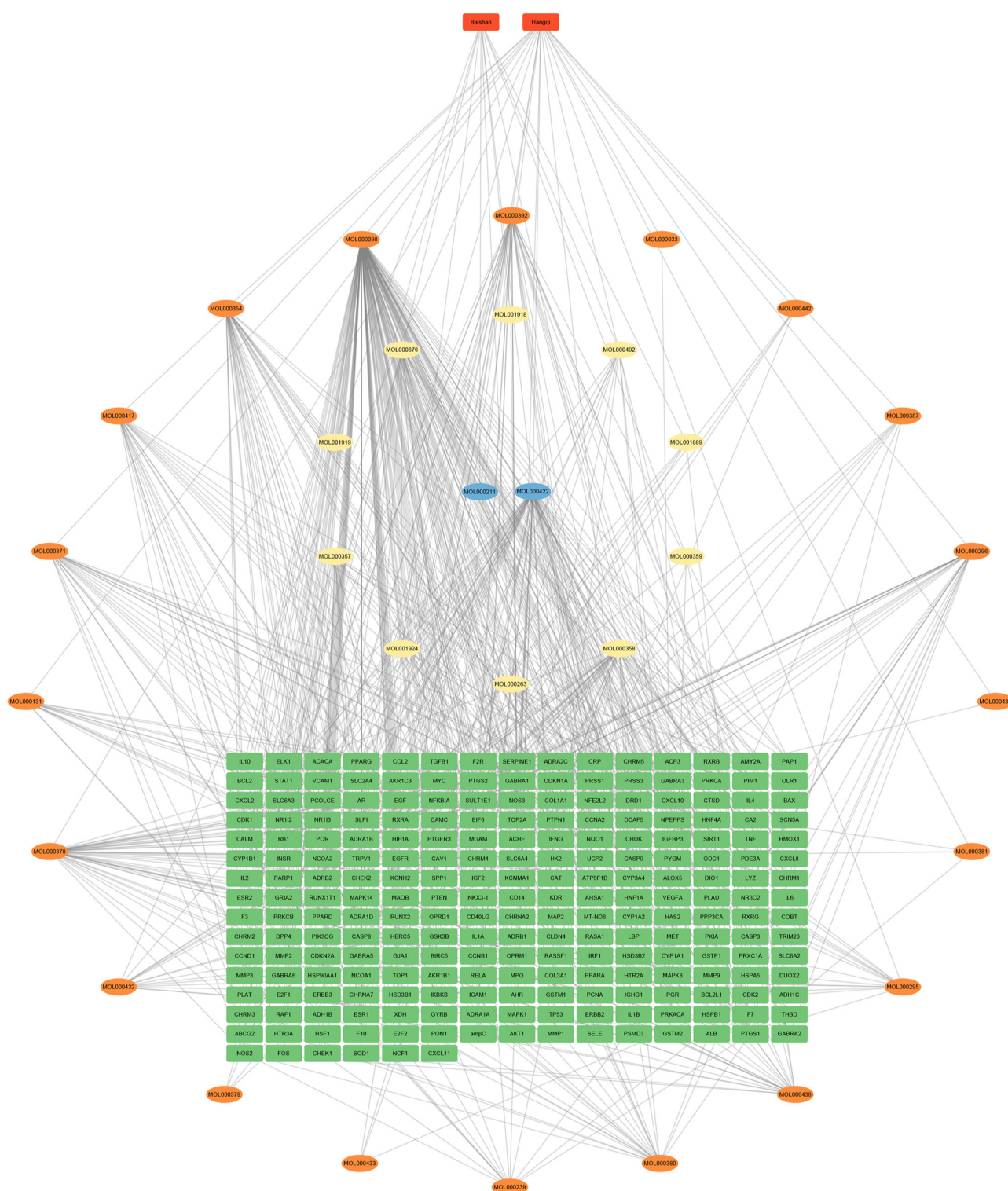


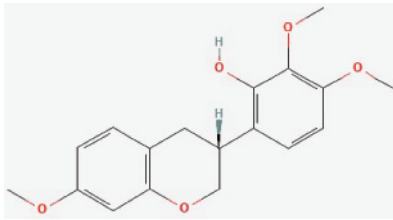
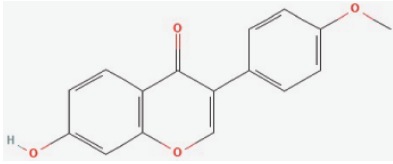
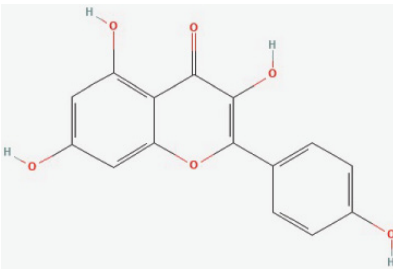
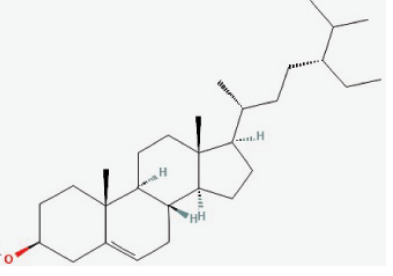
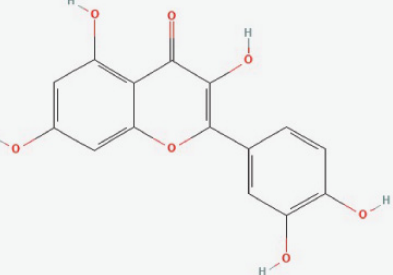
Figure 1. Network of the relationship between the active ingredients and the targets of *Paoniae Radix Alba* (Baishao) and *Hedysarum multijugum* Maxim (Huangqi).

the green rectangular nodes in the inner layer symbolize the target genes. This layout provides a clear and comprehensive overview of the interactions among these herbal ingredients and their targets (Fig. 1). Subsequent analysis within NetworkAnalyst (embedded in Cytoscape) was carried out to identify the top five active ingredients with the highest degree of connectivity in the network. The ingredients with the highest connectivity were identified as quercetin, kaempferol,

7-O-methylisomucronulatol, formononetin and β -sitosterol (Table I).

GEO dataset analysis. The GEO datasets GSE7305, GSE11691 and GSE58178 were retrieved from the GEO database and subsequently merged for further analysis. After normalization, boxplots of the processed data confirmed that the medians across all samples were comparable, indicating

Table I. Five core ingredients of *Paeoniae Radix Alba* and *Hedysarum multijugum* Maxim.

Molecule ID	Molecule name	Structure	OB, %	DL	Degree	Source
MOL000378	7-O-methylisomucronulatol		74.69	0.30	46	<i>Hedysarum multijugum</i> Maxim
MOL000392	Formononetin		69.67	0.21	39	<i>Hedysarum multijugum</i> Maxim
MOL000422	Kaempferol		41.88	0.24	64	<i>Hedysarum multijugum</i> Maxim
MOL000098	Quercetin		46.43	0.28	152	<i>Hedysarum multijugum</i> Maxim
MOL000358	β -sitosterol		36.91	0.75	39	<i>Paeoniae Radix Alba</i>

DL, drug-likeness; OB, oral bioavailability.

consistency in preprocessing steps (Fig. 2A-D). PCA was performed to visualize the global transcriptomic distribution of the normalized samples; a clear tendency toward separation was identified between the normal and endometriosis groups, supporting the presence of transcriptomic differences between the two tissue types (Fig. 2A-D). DEGs were identified using the criteria $|\log FCI| > 0.3$ and $P < 0.05$. The results of differential gene expression were visualized using a volcano plot (Fig. 2E).

In GSE7305, 2,977 genes were downregulated and 2,818 genes were upregulated. In GSE11691, there were 1,336 downregulated genes and 1,324 upregulated genes. In

GSE58178, 1,295 genes were downregulated and 1,384 genes were upregulated. In the merged datasets, 1,389 genes were downregulated and 1,445 genes were upregulated (Fig. 2E). To further illustrate these findings, a heatmap was generated to display the top 10 most upregulated and downregulated genes from the merged dataset (Fig. 2F).

WGCNA. The gene expression profiles from 25 endometriosis samples and 25 normal endometrial samples (merged from GSE7305, GSE11691 and GSE58178 datasets) were used to construct co-expression modules using the WGCNA algorithm. Sample clustering was performed using the flashClust

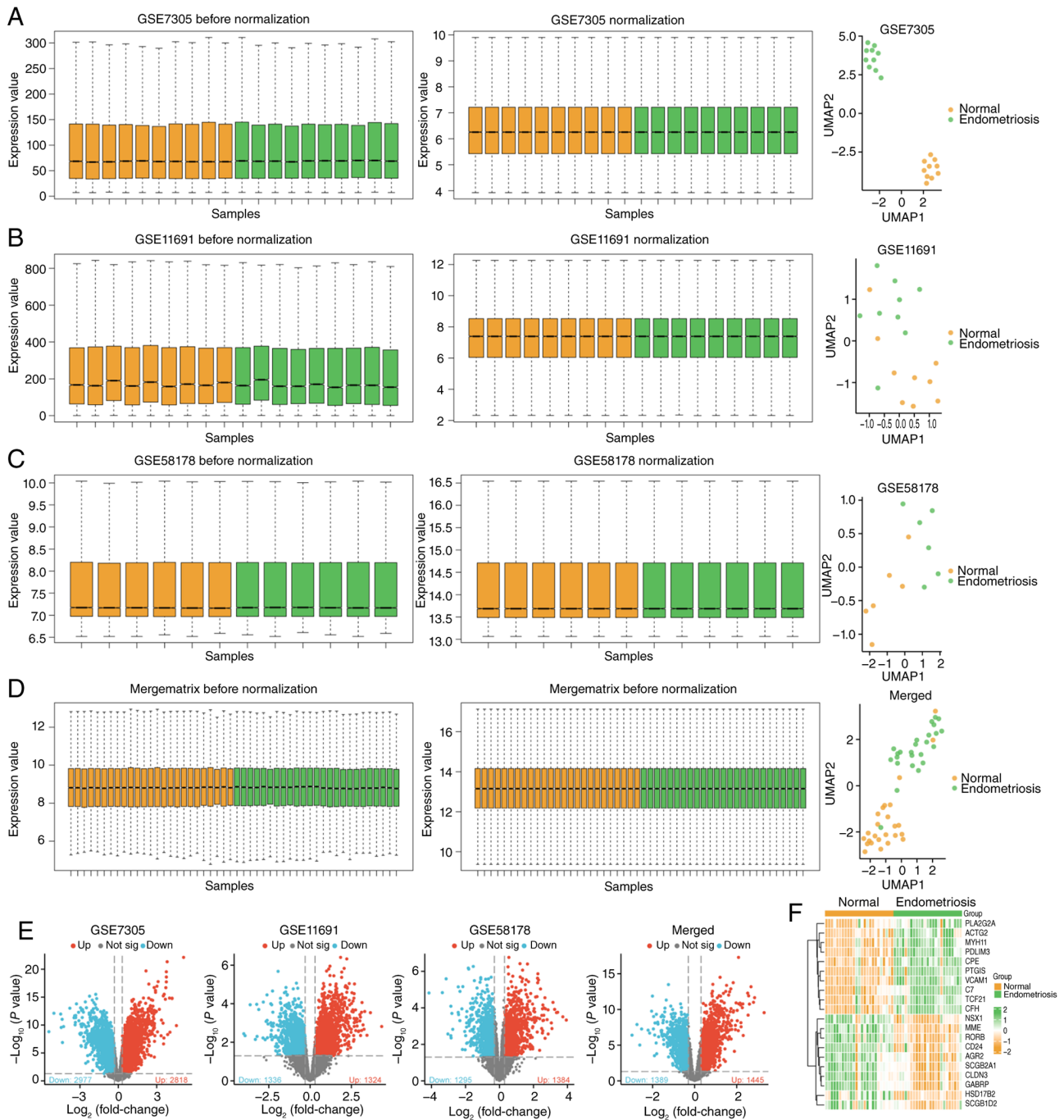


Figure 2. Gene Expression Omnibus dataset analysis. (A) GSE7305 dataset before normalization and after normalization, showing distributions of expression levels. PCA plot of GSE7305 with clear differentiation between normal and endometriosis groups. (B) GSE11691 dataset before normalization and after normalization, indicating expression distributions. PCA plot for GSE11691 with clear differentiation between normal and endometriosis groups. (C) GSE58178 dataset before normalization and after normalization, displaying expression patterns. PCA plots for GSE58178 with clear differentiation between normal and endometriosis groups. (D) Merged datasets before and after normalization. PCA plots with clear differentiation between normal and endometriosis groups. (E) Volcano plots for GSE7305, GSE11691, GSE58178 and the merged datasets. (F) Heatmap showing the top 10 most upregulated and downregulated genes from the merged dataset. PCA, principal component analysis.

package. When the soft-thresholding power was set to 8, the scale-free topology fit index reached 0.85, optimizing the average connectivity of the network (Fig. 3A). Hierarchical clustering was performed to organize genes into co-expression modules, and the gene dendrogram with module color assignments is shown in Fig. 3E. Applying the dynamic tree cut algorithm, four distinct gene co-expression modules were identified. The eigengene adjacency heatmap was generated

to visualize the correlation relationships among different modules (Fig. 3B). Subsequent analysis of the relationships between module eigengenes and clinical traits revealed that the turquoise module exhibited the strongest correlation with endometriosis, comprising 698 genes (Table SII; Fig. 3C). Furthermore, a strong positive correlation was observed between the module eigengenes and the genes within the turquoise module (Fig. 3D).

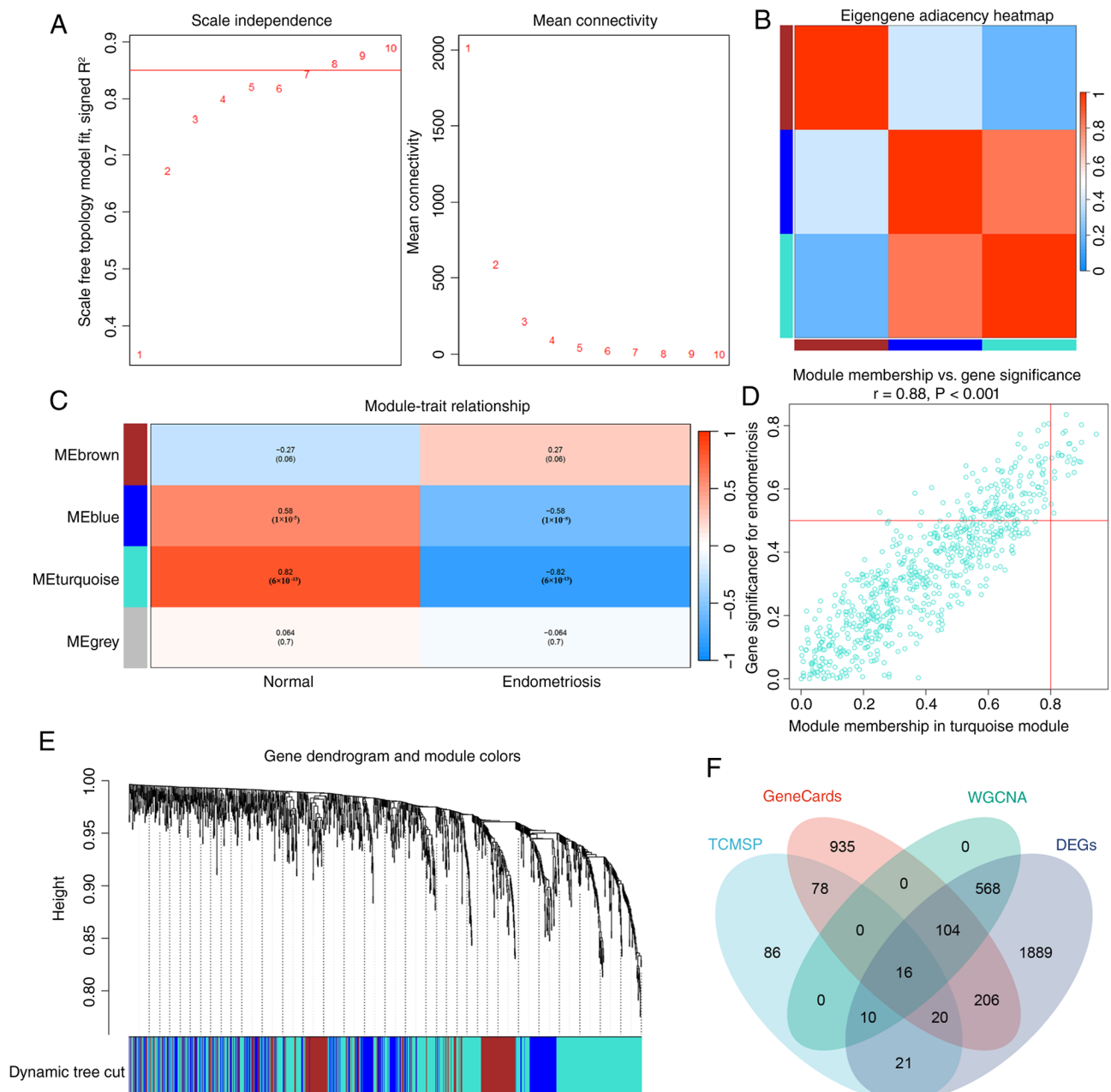


Figure 3. WGCNA. (A) Scale independence and mean connectivity analysis for the soft thresholding power in WGCNA, identifying the optimal power for network construction. (B) Eigengene adjacency heatmap illustrates the relationships between different modules in the WGCNA. The intensity of the color reflects the strength of correlation (from 0 to 1) between module eigengenes. (C) Module-trait relationships, with the correlation values between different modules (MEbrown, MEblue, METurquoise and MEGrey) and normal/endometriosis traits. The most significant module for endometriosis was METurquoise. (D) Scatter plot showing the relationship between module membership and gene significance for the turquoise module in endometriosis samples. A positive correlation was observed ($r=0.88$; $P<0.001$). (E) Gene dendrogram from hierarchical clustering of genes, colored by module membership. Modules were identified by dynamic tree cut, with color coding for different modules. (F) Venn diagram showing the overlap of genes from TCMSP, WGCNA, DEGs and GeneCards databases. A total of 16 common genes were identified as BSHQ-related genes. BSHQ, Paeoniae Radix Alba and *Hedysarum multijugum* Maxim; DEGs, differentially expressed genes; TCMSP, Traditional Chinese Medicine Systems Pharmacology; WGCNA, weighted gene co-expression network analysis.

Identification of BSHQ-related genes. A total of 1,359 endometriosis-related genes were retrieved from the GeneCards database (with a relevance score >1). The effective ingredients of BSHQ, as analyzed through TCMSP, along with endometriosis-related genes from GeneCards, the turquoise module genes from WGCNA and DEGs from the merged datasets, were intersected, resulting in the identification of 16 BSHQ-related genes (Fig. 3F). These 16 genes were as

follows: Cyclin dependent kinase inhibitor 1A (CDKN1A), claudin 4 (CLDN4), cytochrome P450 family 1 subfamily B member 1 (CYP1B1), estrogen receptor 1 (ESR1), Fos proto-oncogene (FOS), heme oxygenase 1 (HMOX1), heat shock protein family A member 5 (HSPA5), interleukin (IL)6, MET proto-oncogene (MET), matrix metalloproteinase 9 (MMP9), prostaglandin E receptor 3 (PTGER3), selectin E (SELE), SERPINE1, secretory leukocyte peptidase inhibitor

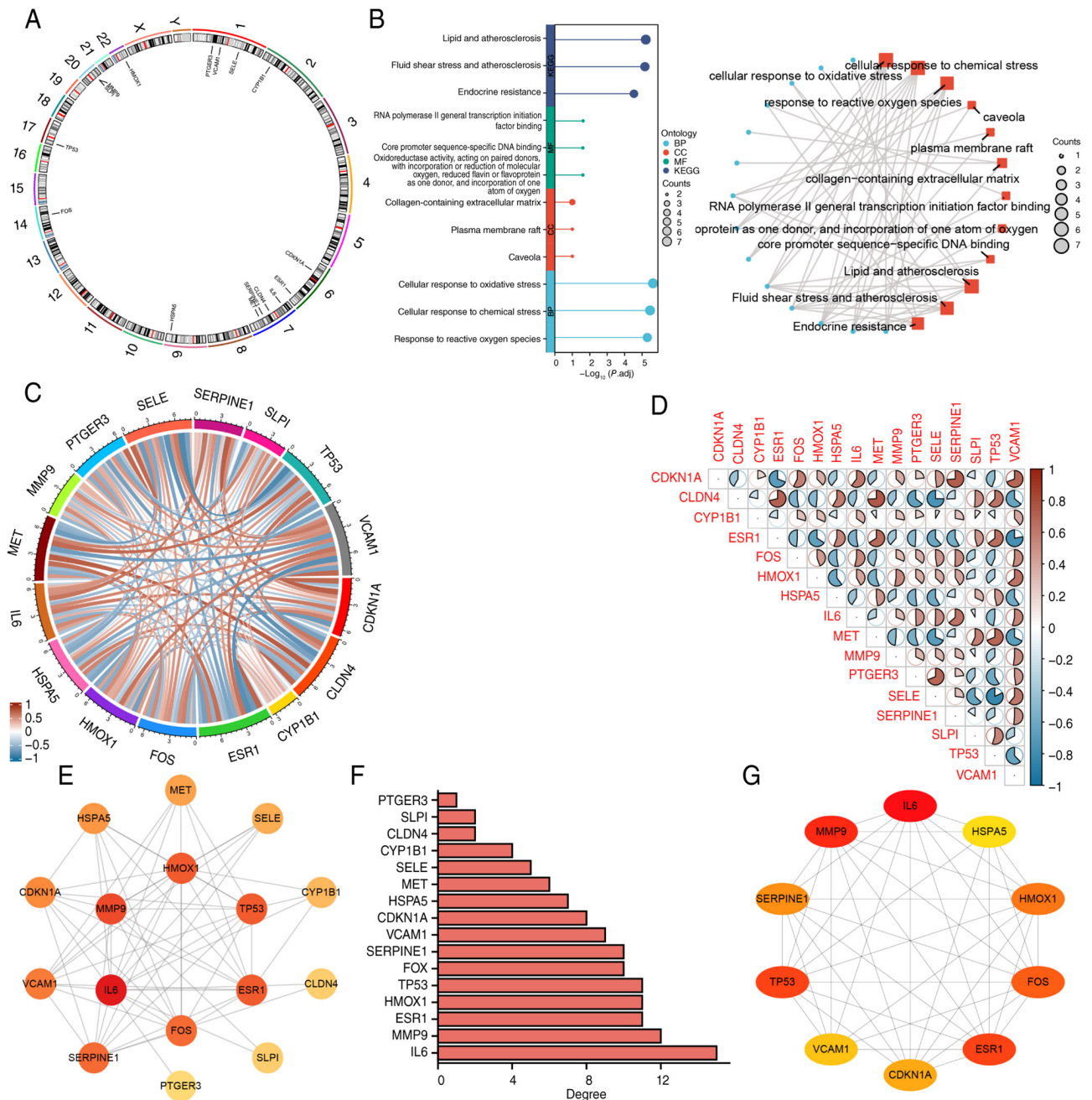


Figure 4. Network and enrichment analyses of BSHQ-related genes. (A) Circular plot showing the chromosomal locations of the BSHQ-related genes. Each chromosome is annotated with its chromosome number, and the significant BSHQ-related genes are marked with official gene symbol labels at their corresponding genomic positions. (B) GO and KEGG pathway enrichment analyses for the BSHQ-related genes. (C) Chord plots showing the relationships between the expression levels of the 16 BSHQ-related genes. The lines connecting genes indicate the strength and direction of their correlation, with red lines representing positive correlations and blue lines representing negative correlations. (D) Correlation plot illustrating the relationships between the expression levels of the 16 BSHQ-related genes, with the strength of the correlation indicated by the intensity of the color. (E) Protein-protein interaction network showing the interactions between the 16 key genes identified in the present study. Nodes represent proteins and edges between them indicate functional associations. The color intensity of the nodes reflects the relative importance of each gene, with darker nodes indicating higher importance. (F) Degree distribution plot showing the number of connections (degree) for each gene. (G) Network analysis of the top 10 BSHQ-related genes which were identified using the maximal clique centrality algorithm. BP, biological processes; BSHQ, *Paeoniae Radix Alba* and *Hedysarum multijugum* Maxim; CC, cellular components; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; MF, molecular functions.

(SLPI), tumor protein p53 (TP53) and vascular cell adhesion molecule 1 (VCAM1).

To explore the relationships between these 16 genes, the present study first performed chromosomal localization analysis (Fig. 4A). Following this, an enrichment analysis of the 16 genes was conducted. The top three most enriched items in each of the GO BP, CC and MF categories, and KEGG

pathways were identified. For GO BP terms, the top three enriched items were 'cellular response to oxidative stress', 'cellular response to chemical stress' and 'response to reactive oxygen species'. For GO CC terms, the top three were 'caveola', 'plasma membrane raft' and 'collagen-containing extracellular matrix'. For GO MF terms, the top three were 'RNA polymerase II general transcription initiation factor

binding', 'oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, reduced flavin or flavoprotein as one donor, and incorporation of one atom of oxygen' and 'core promoter sequence-specific DNA binding'. For KEGG pathways, the top three enriched items were 'lipid and atherosclerosis', 'fluid shear stress and atherosclerosis' and 'endocrine resistance' (Fig. 4B; Table SIII).

To further investigate the relationships between the expression levels of these 16 BSHQ-related genes, chord and correlation plots were constructed (Fig. 4C and D). In the correlation plot, the color intensities or color scale were used to indicate the strength of the correlation, with blue and red representing negative and positive correlations, respectively. Additionally, using the STRING database, a PPI network for the 16 BSHQ-related genes was constructed (Fig. 4E). The darker colors in the network indicate a higher influence on endometriosis. Fig. 4F displays the ranking of all 16 genes by degree. The top 10 hub genes (CDKN1A, ESR1, FOS, HMOX1, HSPA5, IL6, MMP9, SERPINE1, TP53 and VCAM) were identified using the MCC algorithm in the cytoHubba plug-in (Fig. 4G).

scRNA-seq analysis. The present study analyzed scRNA-seq data from endometriosis (GSE213216), applying rigorous raw data preprocessing. After normalizing the data and performing PCA to reduce dimensionality, the present study used the UMAP method to visualize all cells, which were subsequently grouped into 20 distinct clusters. Finally, the expression of the 16 BSHQ-related genes across different cell types was visualized. The analysis revealed distinct clusters of immune cell types based on their gene expression patterns. UMAP clustering analysis identified separate clusters corresponding to different cell types. Notably, clear separation was observed for B cells, dendritic cells, endothelial cells, fibroblasts, lymphocytes, mast cells, myeloid cells, natural killer (NK) cells and T cells, with clear delineation between these cell populations (Fig. 5A). These clusters were defined by their unique transcriptional profiles, as indicated by the different colors representing the various cell types.

Next, the present study examined the expression of the 16 BSHQ-related genes, CDKN1A, CLDN4, CYP1B1, ESR1, FOS, HMOX1, HSPA5, IL6, MET, MMP9, PTGER3, SELE, SERPINE1, SLPI, TP53 and VCAM1. The feature plots (Fig. 5B) and violin plots (Fig. 5C) illustrated the differential expression of these selected marker genes across the various immune cell types. Several of these genes, such as CDKN1A, FOS and HSPA5, were highly expressed in nearly all cell types, whereas others, including CLDN4, MET, MMP9 and SELE, exhibited low expression levels across almost all cell types. This highlights the diversity in gene expression profiles within the immune cell populations. Notably, SERPINE1 was highly expressed specifically in fibroblasts.

Finally, a heatmap of gene expression across all cell types was generated, revealing distinct expression patterns for each gene (Fig. 5D). The heatmap confirmed the tissue- and cell-type-specific expression of the selected genes, offering valuable insights into the regulatory mechanisms that govern immune cell functions.

Screening hub genes by machine learning. To identify key biomarkers for endometriosis, the present study applied a combination of LASSO regression and SVM-RFE algorithms for feature selection. The LASSO analysis was first conducted to identify the most predictive features, as illustrated in Fig. 6A and B, which shows the relationship between the fraction of deviance explained and the coefficients of each feature. As the model was regularized, the coefficients for each feature were progressively shrunk towards zero, with the optimal features emerging when the deviance explained by each feature plateaued. The SVM-RFE analysis, shown in Fig. 6C, was used to rank the features based on their importance. To refine the list of candidate biomarkers, the present study intersected the results from both the LASSO and SVM-RFE analyses. The Venn diagram (Fig. 6D) revealed three overlapping genes, which were selected as the most significant biomarkers for endometriosis: PTGER3, SERPINE1 and VCAM1.

Development and validation of a nomogram for endometriosis risk prediction. To further assess the diagnostic potential of the identified hub genes (PTGER3, SERPINE1 and VCAM1), the present study developed a nomogram incorporating these biomarkers to predict the risk of endometriosis. The nomogram (Fig. 6E) visually represents the weighted contributions of each gene to the overall risk score. The individual scores for each biomarker were calculated based on their expression levels, with PTGER3, SERPINE1 and VCAM1 each contributing specific points corresponding to their respective expression levels. The total score, which is the sum of the individual gene scores, was then used to compute the linear predictor. The nomogram revealed a linear predictor ranging from -6 to 7, with higher scores associated with an increased predicted risk of endometriosis. The total points, ranging from 0 to 220, can be used to stratify patients based on their likelihood of developing endometriosis. A risk score between 0.1 and 0.9 was observed across the merged GEO dataset cohort, making this a reliable tool for risk stratification in clinical practice.

Moreover, the clinical utility of the nomogram was validated through DCA (Fig. 6F), which evaluated the net benefit of applying the nomogram at different risk thresholds. The DCA curve demonstrated that using the nomogram to predict endometriosis risk provided a notable net benefit over traditional strategies (such as treating all or no patients), particularly at higher risk thresholds. This indicated that the nomogram, incorporating PTGER3, SERPINE1 and VCAM1, may represent a valuable tool for clinical decision-making and personalized risk assessment in endometriosis.

Diagnostic performance and validation of key biomarkers for endometriosis. To assess the diagnostic performance of the identified biomarkers, the present study generated ROC curves. In the merged datasets, PTGER3 achieved an AUC of 0.869, SERPINE1 had an AUC of 0.773 and VCAM1 showed an AUC of 0.939 (Fig. 6G), indicating that the three genes exhibited strong diagnostic potential for endometriosis. Boxplots showed that the expression levels of all three genes were significantly different between normal and endometriosis samples ($P < 0.05$).

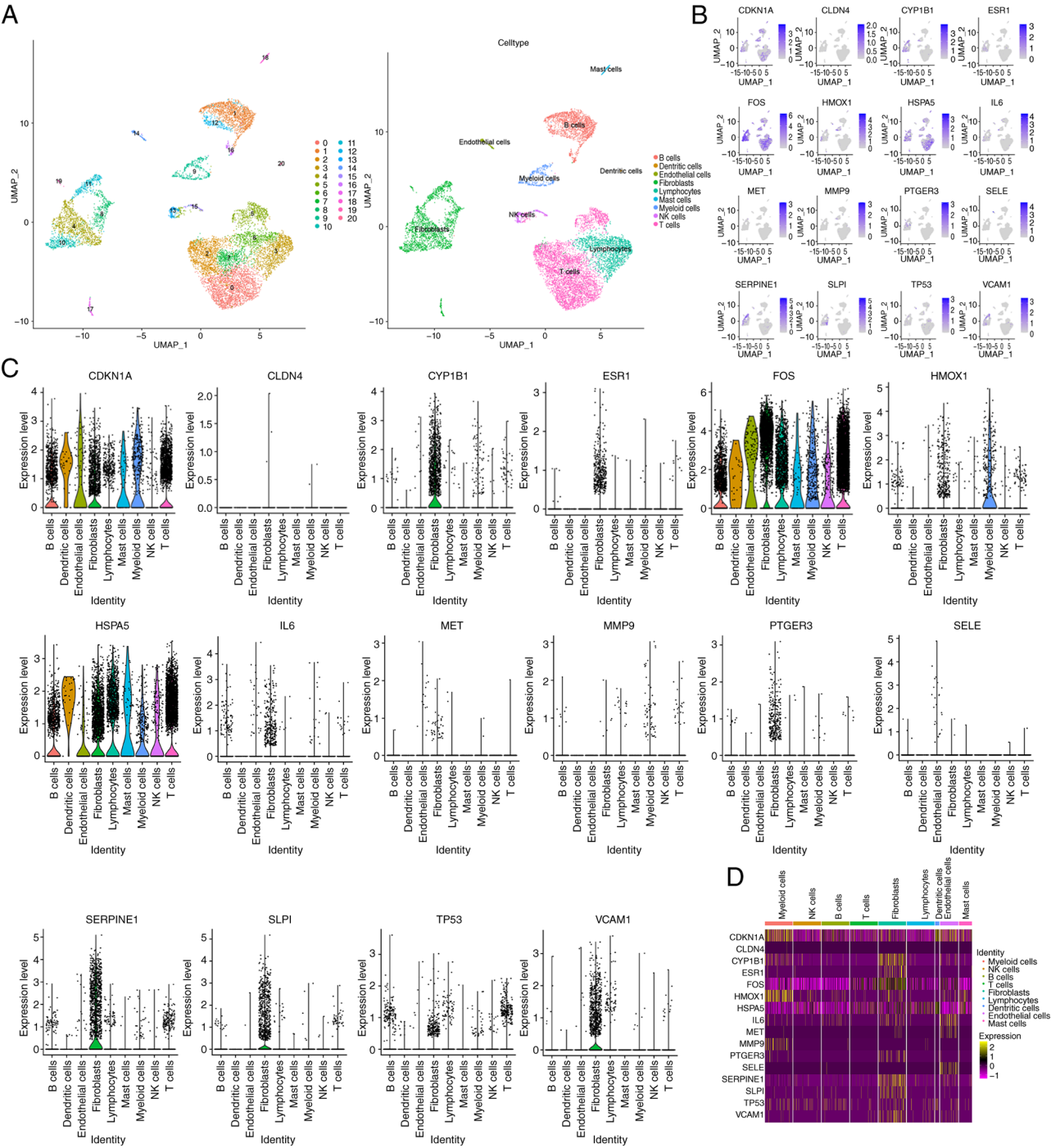


Figure 5. Single-cell RNA sequencing analysis of the BSHQ-related genes in endometriosis. (A) UMAP plots showing the clustering of different cell types in the endometriosis dataset. Each point represents a single cell and colors correspond to different cell types. (B) Expression of BSHQ-related genes visualized using feature plots. The color intensity represents the gene expression levels within each cell type. (C) Violin plots showing the expression levels of the 16 BSHQ-related genes across different cell types. Expression levels are distributed along the y-axis, with each cell type represented by a distinct color. (D) Heatmap showing the expression pattern of key genes across all cell types, highlighting the differential expression of genes in endometriosis-related cell populations. BSHQ, *Paenoniae Radix Alba* and *Hedysarum multijugum* Maxim; NK, natural killer; UMAP, uniform manifold approximation and projection.

Finally, the present study validated the performance of these biomarkers in an independent validation cohort (GSE7846). The ROC curve analysis in the validation set (Fig. 6H) revealed that SERPINE1 maintained high diagnostic value with an AUC of 0.920, whereas PTGER3 and VCAM1 exhibited reduced AUC values of 0.560 and 0.480, respectively. Expression analysis further confirmed the significant differential expression of SERPINE1, whereas PTGER3 and

VCAM1 did not show significant differences in the validation set. Therefore, the SERPINE1 gene was selected as the next research object.

Molecular docking. Based on the aforementioned network pharmacology analysis of BSHQ active ingredients and their target genes, quercetin was identified as a common BSHQ-derived bioactive ligand that interacts with the proteins

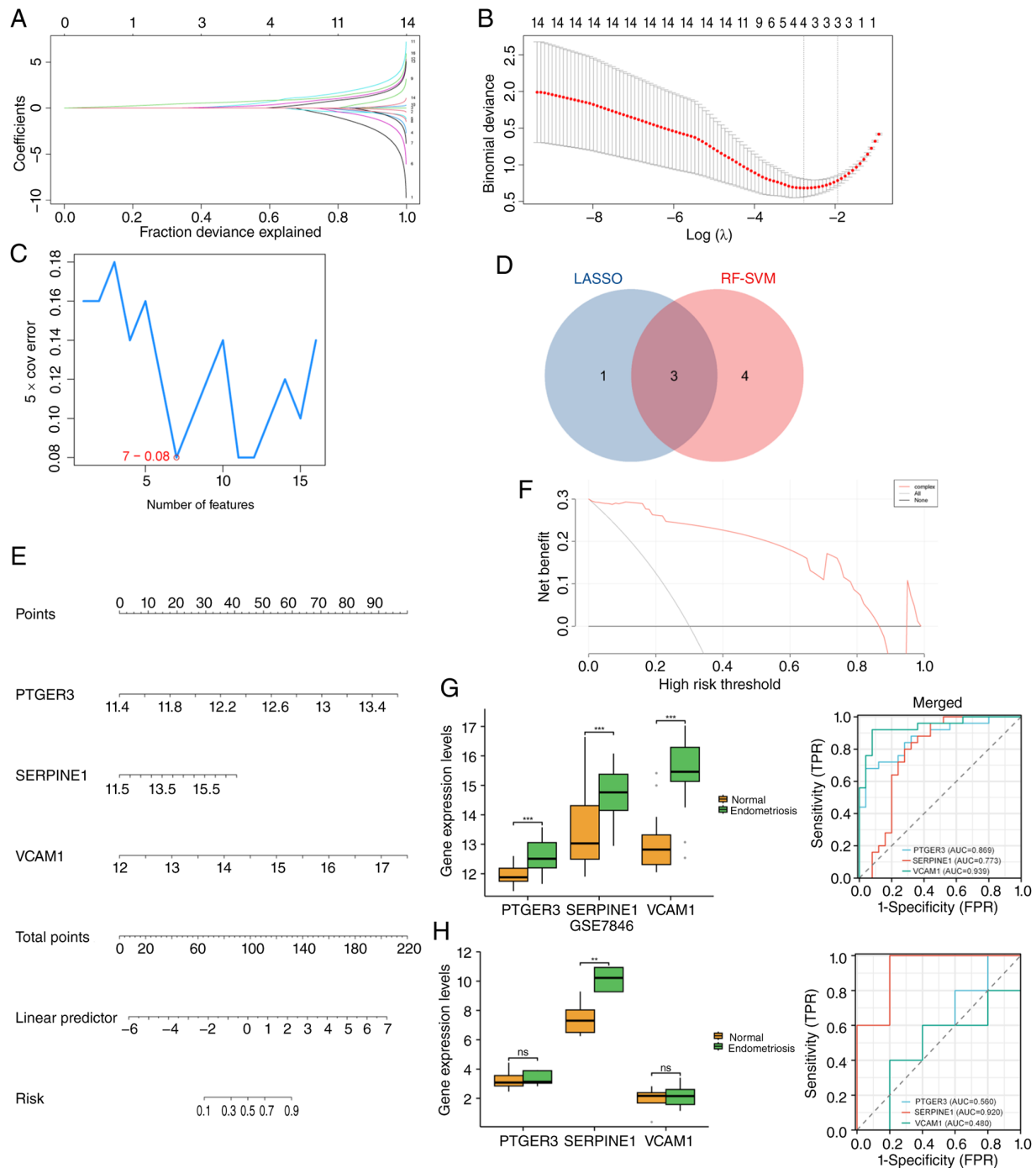
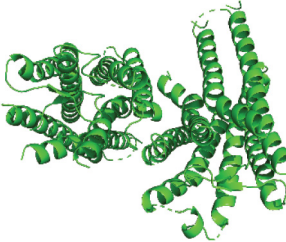
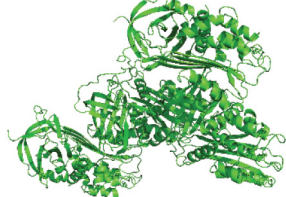
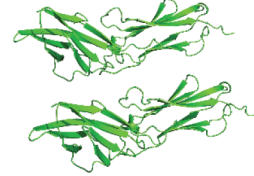


Figure 6. Machine learning analysis and identification of hub genes. (A) LASSO regression coefficient plot showing the selection of key genes based on their coefficients. The plot illustrates the shrinkage of coefficients as the regularization parameter increases. (B) LASSO cross-validation plot determining the optimal $\log(\lambda)$ value for the model. The red dot represents the selected λ with the minimal mean cross-validation error. (C) Cross-validation error plot for feature selection. (D) Venn diagram showing the overlap of genes selected by LASSO and SVM-RFE. Three hub genes were identified by both methods. (E) Nomogram for predicting risk based on the expression levels of the hub genes. The nomogram shows how to calculate the total score and the associated risk for each patient. (F) DCA for evaluating the clinical utility of the nomogram. The DCA plot assesses the net benefit of applying the nomogram at various risk thresholds, providing insights into its potential clinical application across different decision-making scenarios. (G) ROC curve analysis for the merged dataset, showing the predictive power of PTGER3, SERPINE1 and VCAM1 with AUC values. The boxplots display the distribution of expression of these three genes in normal and endometriosis samples. (H) ROC curve analysis for the GSE746 dataset used as a validation set, showing the predictive performance of PTGER3, SERPINE1 and VCAM1. The boxplots display the expression of these genes in both normal and endometriosis samples. ${}^{**}P < 0.01$, ${}^{***}P < 0.001$. AUC, area under the curve; DCA, decision curve analysis; FPR, false positive rate; LASSO, least absolute shrinkage and selection operator; PTGER3, prostaglandin E receptor 3; ROC, receiver operating characteristic; SERPINE1, serpin family E member 1; SVM-RFE, support vector machine-recursive feature elimination; TPR, true positive rate; VCAM1, vascular cell adhesion molecule 1.

encoded by PTGER3, SERPINE1 and VCAM1. The molecular structure file of quercetin was retrieved from the TCMSP database and subjected to docking preparation. Molecular docking

of the three proteins with the core active ingredient quercetin was conducted using AutoDock Vina, and the most optimal binding poses were selected. The resulting protein-ligand

Table II. Molecular docking information of quercetin and targets.

Protein	PDB ID	Structure	Affinity, kcal/mol
PTGER3	6AK3		-9.2
			-8.6
			-8.6
			-7.6
			-7.3
			-7.1
SERPINE1	1B3K		-9.1
			-9.0
			-8.9
			-8.7
			-8.3
			-8.2
VCAM1	1VCA		-7.3
			-7.2
			-6.8
			-6.7
			-6.3
			-6.3

Six affinity values are presented for each docking pair, representing the top six optimal binding conformations output by AutoDock Vina. Affinity (binding energy, kcal/mol) reflects the binding strength between quercetin and the target protein: Values >-4 kcal/mol indicate extremely weak or no binding; values between -7 kcal/mol and -4 kcal/mol indicate moderate binding; values <-7 kcal/mol indicate strong and stable binding. PTGER3, prostaglandin E receptor 3; SERPINE1, serpin family E member 1; VCAM1, vascular cell adhesion molecule 1.

docking structures showed that the binding free energies of all the optimal poses were <-7 kcal/mol (Table II). Molecular interaction analysis of the docking poses was performed using PLIP and visualization was carried out with PyMOL.

In the binding pocket of PTGER3, the amino acids Q339, Q103, T107 and T106 form hydrophobic interactions with quercetin. Additionally, amino acids F140 and T136 form hydrogen bonds with quercetin (Fig. 7A). In the binding pocket of SERPINE1, the amino acids V32, V284, E283, T214, Y220 and R30 participate in hydrophobic interactions with quercetin, whereas N31, E283, D285 and G218 form hydrogen bonds with quercetin (Fig. 7B). In the binding pocket of VCAM1, the amino acids P120 and Y119 interact hydrophobically with quercetin and R123, D122, A144 and I88 form hydrogen bonds with quercetin (Fig. 7C).

GSEA. Subsequently, based on the median expression level of the SERPINE1 gene, the present study classified the endometriosis samples from the merged dataset into two groups: The SERPINE1 low-expression group and the SERPINE1 high-expression group. DEGs (llogFC| >0.3 and P<0.05) between the two groups were identified, followed by GSEA to further investigate the biological pathways associated with these genes. GSEA was performed to identify the significantly enriched biological pathways within the dataset. The analysis revealed several prominent pathways with varying levels of enrichment, as indicated by their normalized

enrichment scores and corresponding statistical parameters. The enriched biological pathways included: ‘Focal adhesion PI3K-AKT-mTOR signaling pathway’, ‘Signaling by receptor tyrosine kinases’, ‘Signaling by interleukins’, ‘Nuclear receptors meta-pathway’, ‘Pathways in cancer’ and ‘Adipogenesis’ (Fig. 7D).

Effect of quercetin on proliferation in endometriosis cells. To evaluate the effects of quercetin on the proliferative capacity of the endometriosis cell lines VK2/E6E7 and 12Z, a IC₅₀ assay was performed.

The dose-response curve was constructed by plotting the relationship between the drug concentration and the percentage of cell proliferation. Through curve fitting, the IC₅₀ values for each cell line were calculated. The results demonstrated a dose-dependent decrease in cell proliferation as the drug concentration increased. For the 12Z cell line, the calculated IC₅₀ value was found to be 41.49 μM, whereas for the VK2/E6E7 cell line, the IC₅₀ value was 9.518 μM (Fig. 8A).

To further investigate the effects of quercetin on the proliferative capacity of the endometriosis cell lines VK2/E6E7 and 12Z, the present study employed both CCK-8 and EdU assays. The CCK-8 assay results revealed a significant reduction in cell proliferation following quercetin treatment, as evidenced by the substantial decrease in OD450 values for both cell lines. Specifically, for the 12Z cell line, the cell proliferation rate was significantly diminished at various time points, with a notable

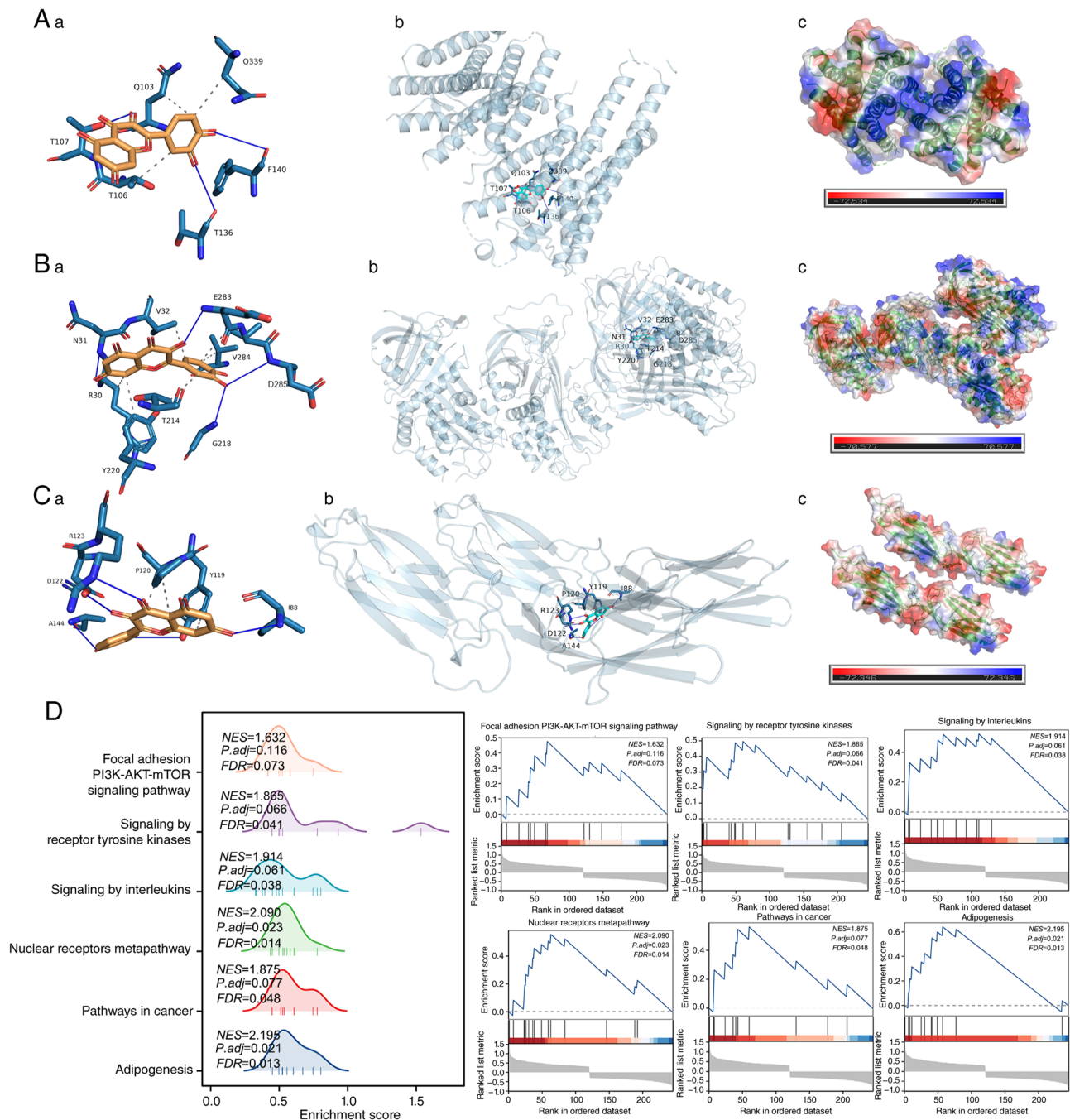


Figure 7. Molecular docking and GSEA. (Aa) 2D interaction diagram showing the binding interactions between PTGER3 and quercetin. (Ab) 3D structure of the PTGER3-quercetin complex, highlighting the binding pocket. (Ac) Electrostatic surface potential map of the PTGER3-quercetin complex, showing the distribution of charge (red for negative and blue for positive). (Ba) 2D interaction diagram showing the binding interactions between SERPINE1 and quercetin. (Bb) 3D structure of the SERPINE1-quercetin complex, highlighting the binding pocket. (Bc) Electrostatic surface potential map of the SERPINE1-quercetin complex, showing the distribution of charge (red for negative and blue for positive). (Cc) 2D interaction diagram showing the binding interactions between VCAM1 and quercetin. (Cb) 3D structure of the VCAM1-quercetin complex, highlighting the binding pocket. (Cc) Electrostatic surface potential map of the VCAM1-quercetin complex, showing the distribution of charge (red for negative and blue for positive). (D) GSEA showing the NES for various pathways. GSEA, Gene Set Enrichment Analysis; NES, normalized enrichment scores; PTGER3, prostaglandin E receptor 3; SERPINE1, serpin family E member 1; VCAM1, vascular cell adhesion molecule 1.

reduction observed at 24 h ($t=3.553$; $P<0.01$), 48 h ($t=34.59$, $P<0.001$), 72 h ($t=42.95$, $P<0.001$) and 96 h ($t=45.88$, $P<0.001$). Similarly, the VK2/E6E7 cell line demonstrated a pronounced decrease in cell proliferation across the same time intervals, with statistical significance observed at 24 h ($t=44.60$; $P<0.001$), 48 h ($t=58.21$; $P<0.001$), 72 h ($t=126.2$; $P<0.001$) and 96 h ($t=24.73$; $P<0.001$) (Fig. 8B). These findings indicated that

quercetin exerts a potent inhibitory effect on the proliferation of both VK2/E6E7 and 12Z cells, with the effect becoming more pronounced over time.

To further evaluate the effects of quercetin on cell proliferation, the present study conducted an EdU fluorescence staining assay using both the 12Z and VK2/E6E7 cell lines. The results demonstrated a significant reduction in the

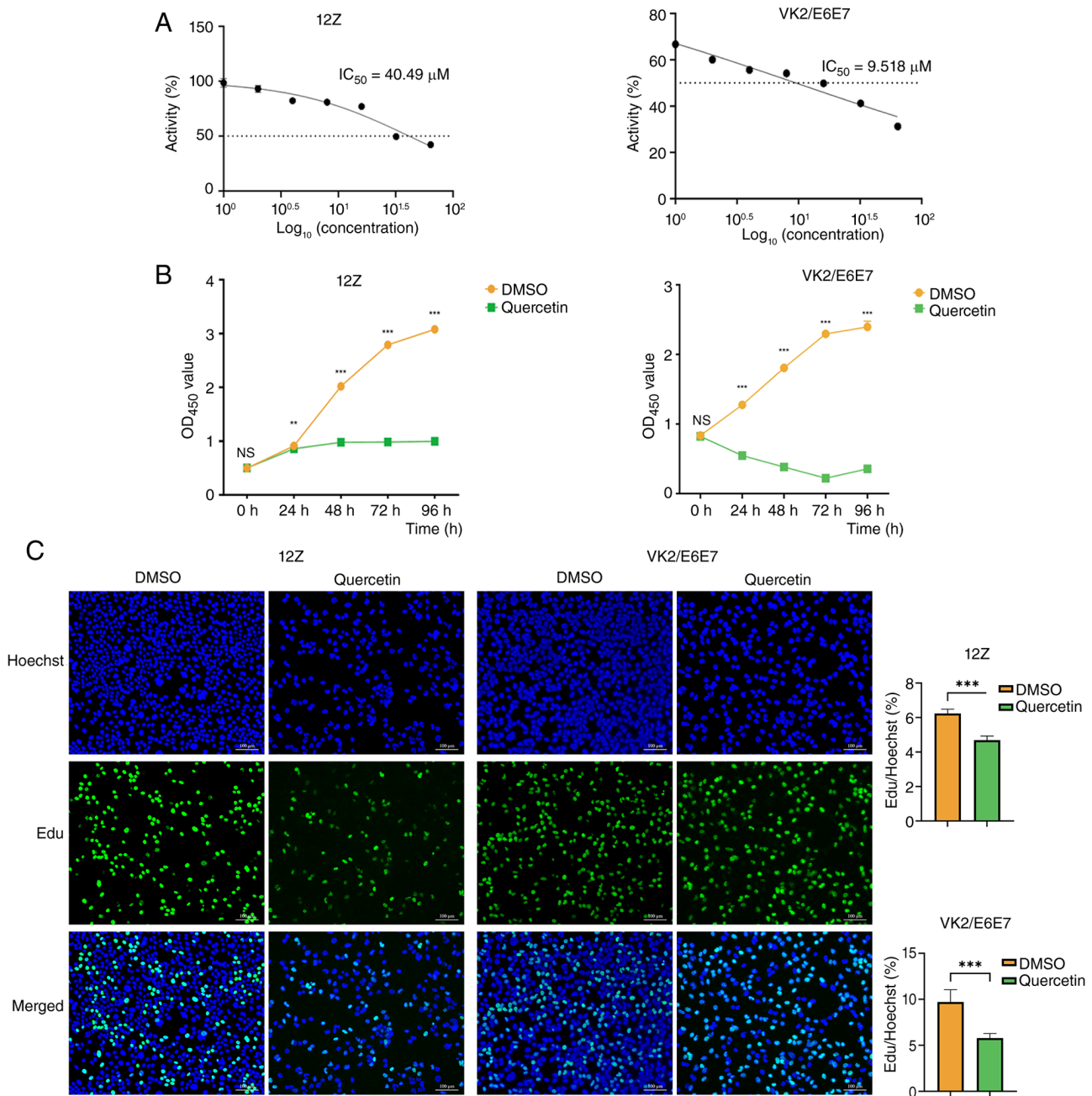


Figure 8. Effect of quercetin on the proliferation of endometriosis cells. (A) IC₅₀ values for quercetin in 12Z and VK2/E6E7 cells were determined, with the dose-response curves showing a dose-dependent decrease in cell viability. The IC₅₀ value for 12Z was 41.49 μM, while for VK2/E6E7 it was 9.518 μM. (B) Cell Counting Kit-8 assay results demonstrated a significant reduction in cell proliferation following quercetin treatment in both cell lines over 96 h. (C) EdU fluorescence staining revealed a significant decrease in the percentage of EdU-positive cells following quercetin treatment, with a marked reduction in both 12Z and VK2/E6E7 cells compared with the DMSO control group, all fluorescence micrographs in this panel were acquired at x10 magnification. ^{NS}P>0.05, ^{**}P<0.01, ^{***}P<0.001. DMSO, dimethyl sulfoxide; EdU, 5-ethynyl-2'-deoxyuridine; IC₅₀, half-maximal inhibitory concentration; OD, optical density.

EdU-positive cell rate after quercetin treatment, indicating a marked inhibition of cell proliferation (Fig. 8C). In the 12Z cell line, the EdU-positive cell rate was 6.234±0.249% in the DMSO control group, whereas it decreased to 4.692±0.241% in the quercetin-treated group, with a significant difference between the two groups (t=20.40; P<0.001). Similarly, for the VK2/E6E7 cell line, the EdU-positive cell rate in the DMSO control group was 9.708±1.337% and after treatment with quercetin, it decreased to 5.791±0.504%, with statistical significance observed (t=13.15; P<0.001). These findings confirmed that quercetin significantly inhibits the proliferative

capacity of both 12Z and VK2/E6E7 cells, as evidenced by the reduced EdU incorporation and the decreased number of proliferating cells.

Effect of quercetin on SERPINE1 expression in endometriosis cells. To investigate the effect of quercetin on SERPINE1 expression in 12Z and VK2/E6E7 cells, both RT-qPCR and WB were conducted at 24 and 48 h after treatment. The results of the RT-qPCR experiments demonstrated a significant reduction in SERPINE1 mRNA expression levels following quercetin treatment in both cell lines (Fig. 9A). In the 12Z

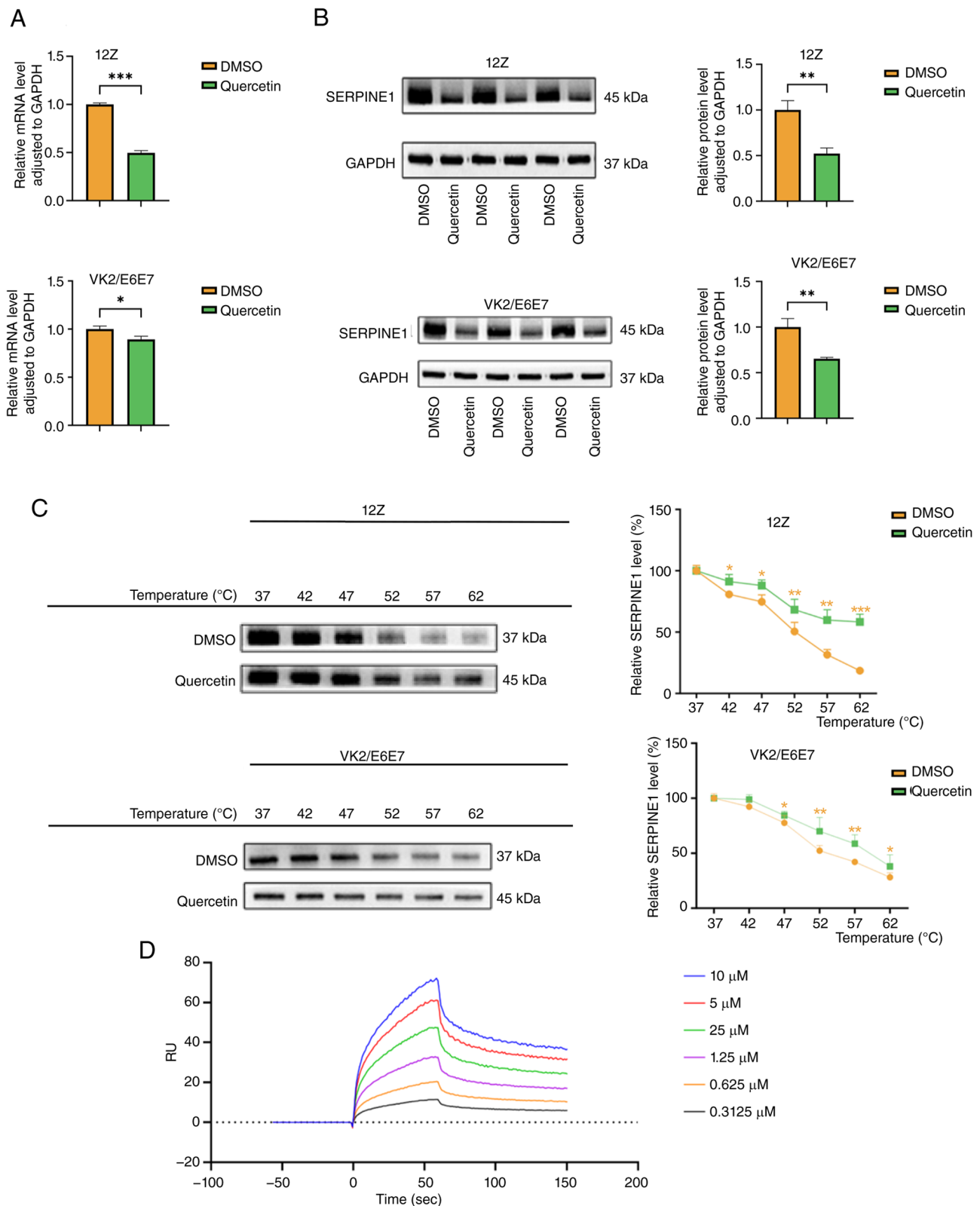


Figure 9. Effect of quercetin on SERPINE1 expression and stability in endometriosis cells. (A) Reverse transcription-quantitative PCR analysis showed a significant reduction in SERPINE1 mRNA expression in both 12Z and VK2/E6E7 cell lines following quercetin treatment, with marked differences compared with the DMSO control group. (B) Western blotting confirmed a substantial decrease in SERPINE1 protein expression in both cell lines after quercetin treatment. (C) Cellular thermal shift assay results indicated that quercetin effectively stabilized SERPINE1, preventing its degradation under heat stress in both cell lines. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. DMSO. (D) Surface plasmon resonance analysis revealed a strong binding affinity between quercetin and SERPINE1, with concentrations ranging from 0.3125 to 10 μ M. DMSO, dimethyl sulfoxide; RU, response unit; SERPINE1, serpin family E member 1.

cell line, SERPINE1 mRNA expression decreased from 1.000 ± 0.015 in the DMSO control group to 0.497 ± 0.023 in

the quercetin-treated group, with a highly significant difference ($t = 31.35$; $P < 0.001$). Similarly, in the VK2/E6E7 cell

line, the mRNA expression of SERPINE1 decreased from 1.000 ± 0.031 in the DMSO control group to 0.894 ± 0.033 in the quercetin-treated group, with a statistically significant reduction ($t=4.089$; $P=0.01$).

WB further confirmed these findings at the protein level (Fig. 9B). In the 12Z cell line, quercetin treatment led to a substantial reduction in SERPINE1 protein expression, which decreased from 1.000 ± 0.102 in the DMSO control group to 0.522 ± 0.061 in the quercetin-treated group, with a significant difference ($t=6.955$; $P=0.002$). Similarly, in VK2/E6E7 cells, SERPINE1 protein expression decreased from 1.000 ± 0.093 in the DMSO control group to 0.653 ± 0.015 in the quercetin-treated group, with a statistically significant reduction ($t=6.401$; $P<0.01$). These results collectively suggested that quercetin treatment may significantly inhibit both the mRNA and protein levels of SERPINE1 in 12Z and VK2/E6E7 cell lines, indicating its potential role in modulating the expression of key genes involved in the pathogenesis of endometriosis.

Evaluation of the interaction of quercetin with SERPINE1. To evaluate the effect of quercetin on the stability of cellular targets, the present study conducted a CETSA to assess the interaction between quercetin and SERPINE1 (Fig. 9C). The results showed that SERPINE1 protein levels decreased progressively with increasing temperature. However, this temperature-induced degradation was markedly mitigated in the quercetin-treated groups, suggesting that quercetin may effectively stabilize SERPINE1 by protecting it from temperature-dependent degradation.

Based on the findings from both molecular docking and CETSA, the present study preliminarily confirmed that quercetin can bind to SERPINE1. To further validate this interaction, the present study performed SPR experiments. The SPR sensorgram curves in Fig. 9D recorded real-time response units across serially diluted quercetin concentrations (0.3125 – $10 \mu\text{M}$). Fitting these response curves yielded a KD of $2.03 \times 10^{-6} \text{ M}$ for the interaction between quercetin and SERPINE1, indicating a strong affinity between the two (Fig. 9D). Consistent with this binding effect, quercetin treatment suppressed SERPINE1 mRNA and protein levels, thus reducing its expression. These results collectively supported the conclusion that quercetin interacts with SERPINE1, potentially influencing its stability and function in endometriosis cells.

SERPINE1 overexpression rescues cell proliferation inhibition induced by quercetin. To assess the overexpression efficiency of SERPINE1 in 12Z and VK2/E6E7 cells, the present study transfected both cell lines with a SERPINE1 overexpression plasmid, and performed RT-qPCR and WB at 24 and 48 h post-transfection, respectively. The RT-qPCR results demonstrated a significant increase in SERPINE1 mRNA expression in the overexpression groups for both cell lines (Fig. 10A). In the 12Z cell line, the SERPINE1 mRNA expression increased from 1.003 ± 0.095 in the DMSO control group to $2.8658.739 \pm 779.729$ in the SERPINE1 overexpression group, with a highly significant difference ($t=63.66$; $P<0.001$). Similarly, in the VK2/E6E7 cell line, SERPINE1 mRNA expression increased from 1.000 ± 0.019 in the DMSO control

group to $9,115.191 \pm 1479.679$ in the overexpression group, also showing a statistically significant increase ($t=10.67$; $P<0.001$).

WB further confirmed the successful overexpression of SERPINE1 at the protein level (Fig. 10B). In the 12Z cell line, SERPINE1 protein expression increased from 1.000 ± 0.139 in the DMSO control group to 2.399 ± 0.489 in the SERPINE1 overexpression group, with a significant difference ($t=4.769$; $P=0.009$). Similarly, in VK2/E6E7 cells, the SERPINE1 protein expression increased from 1.000 ± 0.119 in the DMSO control group to 1.880 ± 0.118 in the overexpression group, with a highly significant difference ($t=9.126$; $P<0.001$). These results indicated that transfection with the SERPINE1 overexpression plasmid effectively enhanced both mRNA and protein levels of SERPINE1 in 12Z and VK2/E6E7 cell lines, confirming the successful overexpression of SERPINE1 in these models.

To evaluate the effect of SERPINE1 overexpression on cell proliferation in the presence of quercetin, the present study transfected 12Z and VK2/E6E7 cells with a SERPINE1 overexpression plasmid and treated them with quercetin. Cell proliferation was assessed using the CCK-8 assay at multiple time points: 0, 24, 48, 72 and 96 h. The results from the CCK-8 assay indicated a significant increase in the OD450 values in the SERPINE1 overexpression + quercetin treatment groups compared with in the quercetin-only treatment groups, suggesting enhanced cell proliferation (Fig. 10C). In the 12Z cell line, the OD450 values at 24 h ($t=63.28$; $P<0.001$), 48 h ($t=26.05$; $P<0.001$), 72 h ($t=6.011$; $P<0.001$) and 96 h ($t=13.03$; $P<0.001$) were all significantly higher in the SERPINE1 overexpression group treated with quercetin. Similarly, in the VK2/E6E7 cell line, significant increases in cell proliferation were observed at all time points, with OD450 values at 24 h ($t=65.96$; $P<0.001$), 48 h ($t=70.81$; $P<0.001$), 72 h ($t=37.70$; $P<0.001$) and 96 h ($t=32.22$; $P<0.001$) showing a marked elevation in the SERPINE1 overexpression + quercetin treatment group compared with the quercetin-only treatment.

To further confirm these findings, EdU fluorescence staining was performed to assess cell proliferation. The results revealed a significant increase in the percentage of EdU-positive cells in the SERPINE1 overexpression + quercetin treatment groups compared with the quercetin-only treatment groups (Fig. 10D). In the 12Z cell line, the percentage of EdU-positive cells was $10.315 \pm 1.458\%$ in the quercetin-only treatment group and increased to $13.952 \pm 1.882\%$ in the SERPINE1 overexpression + quercetin treatment group, with a significant difference ($t=7.325$; $P<0.001$). Similarly, in the VK2/E6E7 cell line, the percentage of EdU-positive cells was $11.010 \pm 1.111\%$ in the quercetin-only group and increased to $13.002 \pm 1.466\%$ in the SERPINE1 overexpression + quercetin treatment group, also showing a significant increase ($t=5.080$; $P<0.001$). These findings collectively suggested that quercetin suppresses cell proliferation, while SERPINE1 overexpression reverses this inhibitory effect and restores cellular proliferative capacity in both 12Z and VK2/E6E7 endometriosis cells.

The results suggested that quercetin alone may suppress the proliferation of endometriosis cells and reduce endogenous SERPINE1 expression, whereas SERPINE1 overexpression counteracts this inhibitory effect. Specifically, SERPINE1 overexpression markedly increased cell proliferation in both 12Z and VK2/E6E7 cell lines in the presence of quercetin,

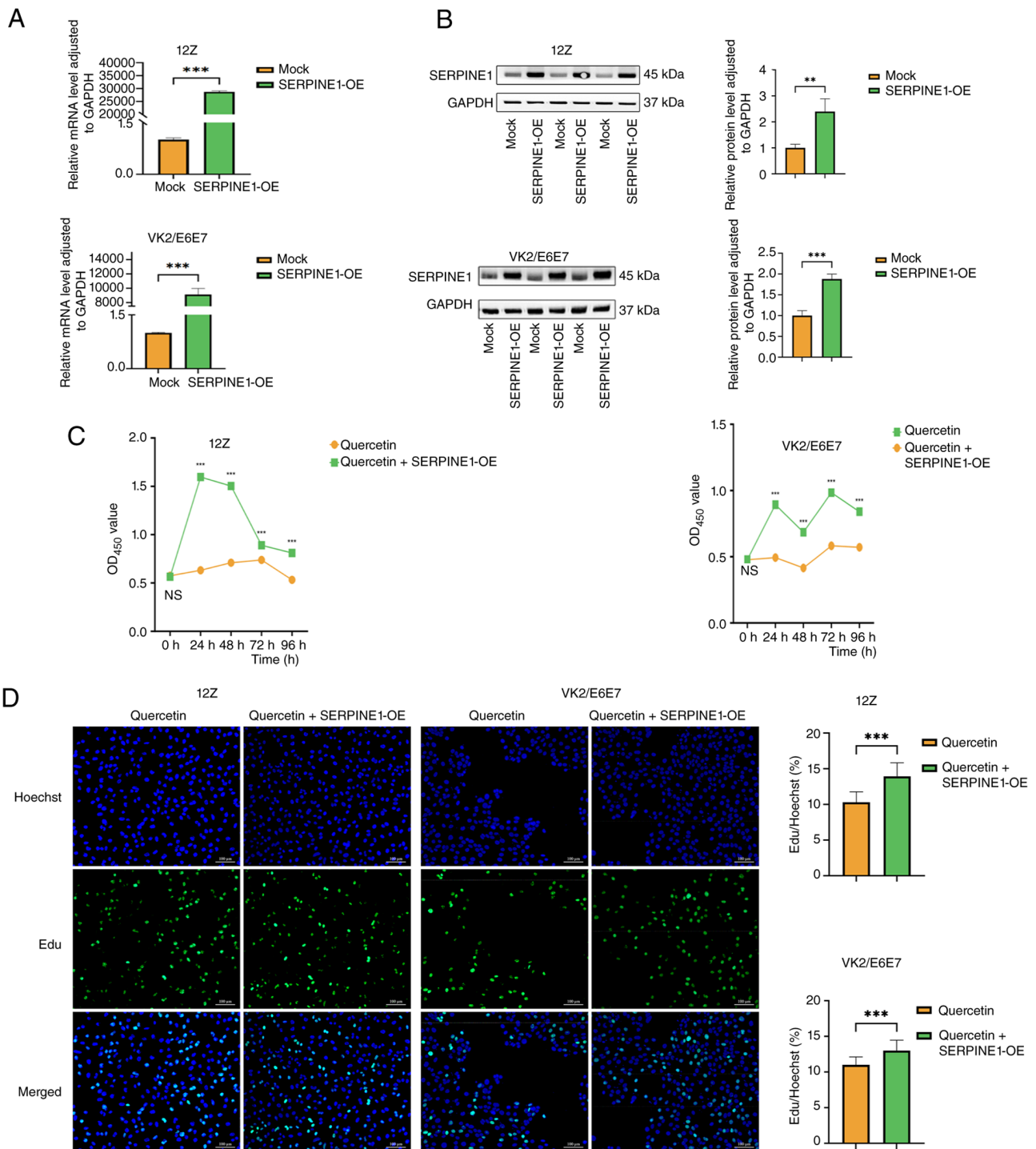


Figure 10. SERPINE1-oe reverses quercetin-induced cell proliferation inhibition. (A) Reverse transcription-quantitative PCR analysis showed a significant increase in SERPINE1 mRNA expression in both 12Z and VK2/E6E7 cell lines after transfection with the SERPINE1-oe plasmid. (B) Western blotting confirmed the successful oe of SERPINE1 at the protein level in both cell lines. (C) Cell Counting Kit-8 assay results indicated a significant increase in cell proliferation in the SERPINE1-oe + quercetin treatment groups compared with the quercetin-only treatment group, with higher OD₄₅₀ values at all time points. (D) EdU fluorescence staining demonstrated a marked increase in EdU-positive cells in the SERPINE1 oe + quercetin treatment groups. **P<0.01, ***P<0.001. EdU, 5-ethynyl-2'-deoxyuridine; OD, optical density; oe, overexpression; SERPINE1, serpin family E member 1.

as evidenced by the elevated OD₄₅₀ values and increased EdU-positive cell rates at all time points.

Discussion

The present study identified 32 bioactive ingredients from BSHQ, with 12 from *Paeoniae Radix Alba* and 22 from

Hedysarum multijugum Maxim. These ingredients demonstrated the notable pharmacological potential of these herbs in treating endometriosis. A total of 231 target genes were identified, highlighting their therapeutic value. The analysis revealed the interactions between bioactive ingredients and target genes, shedding light on the molecular mechanisms involved. Notably, quercetin, kaempferol and other compounds such as

7-O-methylisomucronulatol showed the highest connectivity, suggesting their critical role in modulating biological pathways related to endometriosis. The anti-inflammatory and antioxidant properties of quercetin may markedly contribute to the therapeutic effects of these herbs, modulating inflammatory responses, immune activity and tissue remodeling in endometriosis (23,24).

A comprehensive bioinformatics analysis was performed to identify 16 key genes associated with endometriosis, derived from herbal ingredients in BSHQ. The GO and KEGG pathway analyses provided crucial insights into the roles of these genes in endometriosis. These genes were involved in immune response, cell proliferation, inflammation and cell migration, all of which are fundamental to disease development. The GO analysis revealed that genes such as IL6, SERPINE1 and MMP9 were deeply involved in immune regulation and inflammation, pivotal factors in the progression of the disease (25-28). Meanwhile, genes such as CDKN1A, ESR1 and FOS may influence cell proliferation and migration, facilitating tissue invasion and growth in endometriosis. The KEGG analysis identified pathways related to oxidative stress, lipid metabolism and endocrine resistance. Genes such as HMOX1 and SERPINE1 serve key roles in oxidative stress responses, which contribute to the chronic inflammation seen in endometriosis (29). Additionally, VCAM1 is involved in immune cell adhesion and persistent inflammation, whereas ESR1 and PTGER3 are implicated in endocrine resistance, suggesting that targeting these pathways may improve the effectiveness of hormonal therapies for endometriosis (30,31).

For an improved understanding of the interactions of the 16 BSHQ-related genes in endometriosis, the present study constructed a PPI network and performed correlation analysis. The PPI network, built using the STRING database, identified key hub genes, with CDKN1A, ESR1, FOS, HMOX1, HSPA5, IL6, MMP9, SERPINE1, TP53 and VCAM1 emerging as the top 10 hub genes. These genes, characterized by high connectivity, may have critical roles in regulating key cellular processes, such as cell cycle progression, inflammation and tissue remodeling, and could serve as key regulators in the development of endometriosis.

The application of scRNA-seq has markedly enhanced the understanding of cellular diversity in endometriosis. The present study analyzed scRNA-seq data from endometriotic tissue and identified 20 distinct cellular clusters using UMAP clustering, providing clear insights into various immune cell types, including B cells, dendritic cells, fibroblasts, myeloid cells, NK cells and T cells. This highlights the immune complexity and heterogeneity within endometriotic tissue. The present study further examined the expression patterns of 16 BSHQ-related genes, which are involved in processes such as cell cycle regulation, inflammation and immune responses. Genes such as CDKN1A, FOS and HSPA5 were widely expressed across immune cells, indicating their involvement in proliferation, stress response and apoptosis. By contrast, genes such as CLDN4, MET, MMP9 and SELE exhibited more selective roles. Notably, SERPINE1 was highly expressed in fibroblasts, suggesting its critical role in tissue remodeling and the chronic inflammatory environment of endometriosis. Fibroblasts influence ECM formation and immune responses, potentially driving disease progression in endometriotic lesions (32). These findings underscored the immune

landscape of endometriosis and suggested that genes such as SERPINE1 could serve as potential biomarkers for diagnosis or monitoring. Targeting the pathways regulated by these genes may offer new therapeutic strategies for modulating immune responses and tissue remodeling in endometriosis.

In the present study, PTGER3, SERPINE1 and VCAM1 were identified as key biomarkers for endometriosis using LASSO regression and SVM-RFE algorithms. The ROC curve analysis revealed that PTGER3 and SERPINE1 possess strong diagnostic potential, effectively distinguishing between endometriosis and normal tissue. These genes are integral to the pathogenesis of the disease, underscoring their value as both diagnostic and therapeutic targets. Incorporating these biomarkers into a nomogram for risk prediction enhanced their clinical utility. This nomogram assigned weighted scores to each biomarker based on their expression levels, markedly improving the personalized diagnosis of endometriosis. By providing a quantitative risk score, the model allows for more accurate patient stratification, facilitating early intervention and tailored treatment approaches.

As a common bioactive flavonoid extracted from BSHQ, quercetin exerts multifaceted regulatory effects on pathological signaling pathways linked to endometriosis. Consistent with previous research, it exerts prominent anti-inflammatory activity: BSHQ has previously been shown to suppress macrophage activation, lower reactive oxygen species (ROS) levels and reduce the secretion of pro-inflammatory cytokines, including TNF- α and IL-1 β , thereby modulating the inflammatory microenvironment of endometriotic lesions (33,34). Prior reports have also confirmed that quercetin restrains endometriotic cell proliferation by inhibiting ERK1/2, p38 MAPK and AKT cascades to trigger cell cycle arrest and apoptosis (35). It also suppresses MMP2 and MMP9, enzymes critical for cell invasion and migration, further hindering disease progression (36). Moreover, quercetin serves a vital role in oxidative stress regulation by scavenging ROS and promoting the expression of antioxidant enzymes such as HMOX1 (24). Its ability to modulate hormonal resistance, particularly through the regulation of estrogen signaling, offers therapeutic advantages, especially when used in conjunction with hormonal therapies (37). Furthermore, quercetin interacts with key biomarkers, including SERPINE1, ESR1 and MMP9, potentially influencing the progression of endometriosis (38).

The present study identified SERPINE1 as a key target gene of quercetin and a central hub gene in BSHQ-related pathways in endometriosis. Located at 7q22.1, SERPINE1 encodes plasminogen activator inhibitor-1, which regulates fibrinolysis by inhibiting tissue-type and urokinase-type plasminogen activators. This activity is essential for ECM remodeling, cell migration and tissue repair. Elevated SERPINE1 expression has been linked to cancer progression, particularly in gastric cancer, where it regulates epithelial-mesenchymal transition (39). Additionally, SERPINE1 has a pivotal role in wound healing, immune responses and fibrosis, establishing it as a critical factor in chronic inflammatory diseases (40,41).

SERPINE1 serves a critical role in the inflammatory microenvironment of endometriotic lesions. Reduced SERPINE1 expression in endometrial stromal cells enhances the production of inflammatory cytokines through Toll-like receptor signaling, contributing to the inflammation observed in endometriosis (26). This inflammation accelerates disease progression by altering

cytokine production. As a protease inhibitor, SERPINE1 regulates ECM remodeling, which is essential for tissue invasion and lesion establishment. In endometriotic fibroblasts, SERPINE1 is abnormally upregulated, and this dysregulated upregulation leads to persistent ECM accumulation and lesion expansion (42). While SERPINE1 loss in stromal cells induces inflammation, its overexpression in fibroblasts promotes ECM deposition and lesion growth. Elevated SERPINE1 levels have been linked to endometrial cancer risk, and this cell-type-specific abnormal expression pattern makes it a promising biomarker for endometriosis (43). Additionally, SERPINE1 affects immune cell recruitment by altering cytokine secretion and ECM remodeling, facilitating immune cell infiltration and tissue invasion (44). Targeting SERPINE1 could offer therapeutic benefits by modulating inflammation and ECM remodeling, reducing lesion progression and alleviating symptoms (45). Its effect on hormonal regulation may also contribute to infertility in endometriosis (46). Environmental and oxidative stress factors further modulate SERPINE1 expression, exacerbating the chronic inflammatory state in endometriosis (47).

The present study highlighted the ability of quercetin to inhibit proliferation in endometriosis cells. CCK-8 and EdU assays both demonstrated significant reductions in cell proliferation following quercetin treatment. These findings suggested that quercetin suppresses cell proliferation in a dose- and time-dependent manner. The EdU assay further confirmed a decrease in DNA synthesis, indicating that quercetin effectively inhibits cell cycle progression, positioning it as a promising anti-proliferative agent for treating endometriosis.

The molecular analyses in the present study provided valuable insights into the interactions between quercetin and key biomarkers associated with endometriosis, specifically PTGER3, SERPINE1 and VCAM1. These findings underscored the potential of quercetin to modulate inflammation and tissue remodeling, both of which are critical processes in the disease. The analysis revealed that quercetin binds effectively to PTGER3, SERPINE1 and VCAM1, demonstrating strong binding affinities. These interactions suggest that quercetin may influence the prostaglandin E2 pathway via PTGER3, regulate inflammation and tissue remodeling through SERPINE1, and reduce immune cell infiltration by modulating VCAM1. Additionally, quercetin stabilizes SERPINE1 under stress conditions, protecting it from degradation at the protein level; simultaneously, quercetin suppresses abnormally high SERPINE1 transcription in endometriotic fibroblasts to restrict excessive ECM deposition and lesion growth. These findings suggest that quercetin can effectively modulate the inflammatory microenvironment, which is a key characteristic of endometriosis, positioning it as a promising therapeutic agent for the condition. By interacting with SERPINE1, quercetin further demonstrates its potential in treating endometriosis.

In the present study, the effects of quercetin on the proliferation of endometriosis cells were comprehensively assessed, revealing key insights into its potential therapeutic effects. The effect of quercetin on cell proliferation was evident across various cell lines, with both the IC₅₀ and proliferation assays demonstrating that quercetin markedly inhibited the proliferation of the 12Z and VK2/E6E7 endometriosis cell lines. This anti-proliferative effect was observed to be dose- and time-dependent, with greater reductions in cell proliferation

and DNA synthesis as the treatment duration extended. These findings align with previous studies on the anticancer properties of quercetin, highlighting its role in modulating cell cycle progression and survival pathways, which are crucial for the growth and persistence of endometriotic lesions (35,48). Furthermore, the ability of quercetin to inhibit DNA synthesis, as evidenced by the EdU assay, suggested that it could act as an effective agent to target the proliferative aspect of endometriosis.

An additional critical aspect explored in the present study was the influence of quercetin on the expression of SERPINE1, a protein involved in inflammation, ECM remodeling and cell migration, which are key contributors to endometriosis pathology. The results demonstrated that quercetin effectively downregulated both the mRNA and protein levels of SERPINE1 in 12Z and VK2/E6E7 cell lines. This finding is significant as SERPINE1 has been implicated in the regulation of chronic inflammation and tissue remodeling in endometriosis. The reduction in SERPINE1 expression following quercetin treatment suggested that quercetin may modulate the inflammatory microenvironment that characterizes endometriotic lesions. By decreasing SERPINE1 levels, quercetin could potentially alleviate some of the inflammation and ECM remodeling that exacerbate the disease. Moreover, this downregulation of SERPINE1 may further underscore the potential of quercetin to modulate key pathways involved in disease progression.

To further investigate the molecular interaction between quercetin and SERPINE1, the present study employed advanced techniques such as CETSA and SPR. These assays provided strong evidence that quercetin binds directly to SERPINE1, stabilizing the protein and preventing its degradation under stress conditions. The results from CETSA indicated that quercetin effectively stabilizes SERPINE1, protecting it from temperature-induced degradation. Normally, an increase in temperature leads to the denaturation of proteins, but in the presence of quercetin, the degradation of SERPINE1 was markedly reduced. This suggests that quercetin may bind directly to SERPINE1, preventing its destabilization under stressful conditions. The stabilizing effect observed in the CETSA experiment provides strong evidence that quercetin can interact with SERPINE1 in a manner that potentially influences its stability, which may be critical for maintaining proper cellular function in endometriotic lesions.

To further substantiate these findings, SPR analysis was performed, revealing a strong binding affinity between quercetin and SERPINE1. The KD value obtained from the SPR analysis indicated that quercetin interacts with SERPINE1 with high specificity and affinity, suggesting a potentially robust and biologically relevant interaction. This strong binding affinity highlights the potential of quercetin to influence the function of SERPINE1 within endometriotic tissue. The results from both CETSA and SPR experiments collectively support the hypothesis that quercetin interacts with SERPINE1; it may stabilize SERPINE1 protein to maintain basal stability at post-translational levels, while inhibiting its aberrantly high expression to block pathological activity; rescue experiments confirmed SERPINE1 overexpression reversed the therapeutic effects of quercetin on the disease process.

The interaction between quercetin and SERPINE1 is of particular importance in the context of endometriosis, as

SERPINE1 regulates ECM remodeling, which is central to the formation and growth of endometriotic lesions. The ability of quercetin to stabilize SERPINE1 may help modulate these processes by fine-tuning the uPA/tPA-plasmin-MMP proteolytic cascade that governs ECM turnover, potentially preventing excessive tissue invasion and fibrosis (49,50). Additionally, SERPINE1 is involved in immune cell recruitment and inflammatory response, both of which are key drivers of endometriosis pathology. By stabilizing SERPINE1, quercetin may help regulate these processes by preserving the homeostatic immunomodulatory function of SERPINE1 and restraining Toll-like receptor-mediated overproduction of inflammatory cytokines, contributing to a reduction in the chronic inflammation and tissue remodeling that exacerbate the condition (26,51).

The present study has several limitations that should be acknowledged. First, the bioinformatics analyses were based on publicly available datasets, and although multiple datasets were integrated and an independent validation cohort was used, potential heterogeneity among platforms and sample sources may still have influenced the results. Second, the functional experiments were performed primarily *in vitro* using 12Z and VK2/E6E7 cell lines, which cannot fully recapitulate the complex immune, hormonal and microenvironmental features of endometriosis *in vivo*. Third, although SERPINE1 was identified and validated as a key target of quercetin, the potential contributions of other candidate genes and signaling pathways identified in the present study were not thoroughly investigated. In addition, the direct therapeutic efficacy, pharmacokinetics and long-term safety of quercetin were not evaluated in animal models or clinical settings. Therefore, further studies using primary cells, animal models and clinical samples with standardized collection protocols and strict inclusion/exclusion criteria are required to validate the present findings and to further clarify the therapeutic potential and molecular mechanisms of quercetin in endometriosis.

In conclusion, the present study provided valuable insights into the therapeutic potential of quercetin in treating endometriosis, demonstrating its ability to inhibit cell proliferation and regulate key biomarkers such as SERPINE1. These results provide a solid basis for future studies, emphasizing the importance of clinical trials to evaluate the long-term effectiveness and safety of quercetin in treating endometriosis.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

XG, DR, XL and YX were responsible for designing the study. XG, DR, XC, RW, XL and YX were responsible for writing

the manuscript, extracting and analyzing data, interpreting the results and experiments. XG, DR XC confirm the authenticity of all the raw data. PZ, LW, MW, JZ, PL and DZ contributed to experiments and reviewing the article. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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