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PHGDH Acts as an Oncogenic Accelerator by Activating the c-MYC/CDK2 Axis to Drive Cell Cycle Progression in Endometrial Cancer

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Abstract

Endometrial cancer (EC) is one of the most prevalent gynecological tumors with rising incidence and mortality rates[1]. Phosphoglycerate dehydrogenase (PHGDH), a key enzyme in serine biosynthesis, is closely related to the progression of various cancers, yet its role in EC pathogenesis remains poorly understood. Immunohistochemistry was used to detect the PHGDH expression and its correlation with clinical indicators in EC and normal endometrial tissues and to analyze its correlation with clinicopathological parameters. We explored the role of PHGDH in the progression of EC through in vitro and in vivo assays. Analysis of public databases and clinical specimens revealed that PHGDH expression is significantly elevated in EC tissues compared to normal endometrium, and high PHGDH levels correlate with poor patient prognosis. In vitro studies demonstrated that PHGDH promotes the proliferation of EC cells. *In vivo* assays showed that the PHGDH inhibitor NCT-503 effectively suppresses tumor growth, exhibiting selective efficacy across different cell lines. Transcriptome sequencing indicated that PHGDH influences multiple biological processes, including cell cycle. Mechanistically, PHGDH was found to regulate the cell cycle by modulating the PHGDH/c-MYC/CyclinA2/CDK2 axis. Functionally, PHGDH drives cancer progression through alterations in cell cycle regulation, whereas its pharmacological inhibition can counteract these effects. PHGDH exerts a tumor-promoting effect in endometrial cancer and represents a promising therapeutic target for inhibiting EC progression.

Introduction

Endometrial cancer (EC) ranks among the three most prevalent gynecological tumors affecting women's health, with both its incidence and disease-related mortality showing a persistent annual increase[2, 3]. While EC predominantly affects postmenopausal women, a rising incidence has been observed in younger populations in recent years. In several developed countries, endometrial cancer has now surpassed cervical cancer as the most common gynecological malignancy[1].

The development of endometrial cancer linked to metabolic disorders such as obesity, hypertension, diabetes[4–6]. Aberrant cellular metabolism, including disruptions in glucose, lipid, and amino acid pathways, plays an increasingly important role in EC progression. Serine, as a nonessential amino acid, along with glucose and glutamate, constitutes one of the three major metabolites of cancer cells. It contributes to the synthesis of nucleic acids, lipids, amino acids and cofactors, and has been confirmed to be vital not only for cell proliferation but also for multiple biosynthetic and signaling pathways. Serine is also essential for many cancer types, and enzymes involved in serine metabolism related are strongly related to tumor progression. For instance, serine / threonine protein kinase 4 (STK4) regulates EC cell proliferation and migration *in vitro*[7], while serine racemase (SRR) modulates the tumor immune microenvironment, affects EC metabolism, and hinders the key oncogenic process in EC[8].

Phosphoglycerate dehydrogenase (PHGDH), also known as 3-PGDH, catalyzes the first-rate limiting step in the serine biosynthesis pathway. Using NAD+/NADH as a cofactor, it converts 3-phosphoglycerate to 3-phosphohydroxypyruvate, thereby contributing to purine and serine synthesis[9], PHGDH is highly

expressed in various cancers, including breast cancer[10] ,lung cancer[11], glioma[12], cervical cancer[13], pancreatic cancer[14], colorectal cancer[15] and other cancers, and its overexpression is often associated with poor prognosis. Although a prognostic model for endometrial cancer has identified PHGDH as one of five metabolism-related genes[16]. its specific expression patterns and mechanisms in EC remain unclear. In this study, we aimed to elucidate the role of PHGDH in EC progression and its underlying mechanism through *in vivo* and *in vitro* experiments. Our findings suggest that PHGDH serve as a new prognostic biomarker and a potential therapeutic target in endometrial cancer.

Material and Methods

Bioinformatics Analysis

To evaluate the clinical relevance of PHGDH expression in uterine corpus endometrial carcinoma (UCEC), we analyzed PHGDH expression levels in pan-cancer datasets from The Cancer Genome Atlas (TCGA) via TIMER2.0 (comp-genomics. org). PHGDH expression specifically in UCEC were analyzed though GEPIA (cancer.pku.cn). Using UALCAN (uab.edu), we investigated the association between PHGDH expression and clinico-pathological features (tissue type, pathological stage, and grade). Finally, we assessed the prognostic impact of PHGDH expression in EC patients using TIMER2.0, TISDB (hku.hk), and UALCAN

Human-Tissue Samples

All clinical specimens were collected following approval by the Ethical Committee of the Affiliated Hospital of Qingdao University. A total of 110 tissue samples were obtained from patients with pathologically confirmed EC who underwent surgery at the Affiliated Hospital of Qingdao University from 2017 to 2022. All specimens were handled according to legal and ethical standards. No patients had previously received prior antitumor therapy. Clinical staging was performed according to the 2023 FIGO staging guidelines for endometrial cancer[17].

Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue sections were deparaffinized, rehydrated, and subjected to antigen retrieval. After blocking, sections were incubated with primary antibodies. Protein expression was visualized using brown pigmentation from the chromogen 3,3'-diaminobenzidine (DAB) hydrochloride. The slides were then counterstained, rinsed, dehydrated, cleared, and observed under an ECLIPSE E100 optical microscope (Nikon Corporation, Tokyo, Japan). The primary antibodies used were rabbit anti-PHGDH polyclonal antibody (Proteintech;14719-1-AP, 1:1000 dilution)[18, 19]. Images were captured using the ImageJ software to measure integrated optical density.

Cell Culture

Human EC cell lines AN3Ca, Ishikawa, HEC-1-A and RL95-2 were obtained from Medical Research Center of Affiliated Hospital of Qingdao university. T-hESCs were gathered from Qilu hospital of Shandong university. After detection, all cell lines were not infected with mycoplasma. Ishikawa and HEC-1-A cells were grown in DMEM medium (Pricella, PM150210), AN3Ca in RPMI-1640(Pricella, PM150110), RL95-2 and T-hESCs cells in iDMEM/F12 (Pricella, PM150312). All media were supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. All cells were cultured at 37℃ in a humidified atmosphere with 5% CO_2.

Transfection and Validation

Cells were transfected with siRNA using lipofectamine 8000 reagent (Beyotime, China) according to h the manufacturer's procedure within 24 h after seeded in the 6-well culture plates. RNA and total protein were extracted 48 or 72 h post-transfection for further analysis. To obtain stable PHGDH-overexpressing cell lines, AN3Ca and Ishikawa cells were transduced with recombinant lentivirus (Genechem) for 48 hours, followed by selection with 2 μg/mL puromycin for 72 hours. Blank lentivirus was used as a vector control. The efficiency of transfection was validated by western blot and quantitative real-time PCR (qPCR).

Cell Counting Kit-8 (CCK-8) assay

A total of 3×10^3 transfected cells were seeded into a 96-well plate and cultured for 24–72 hours. Subsequently, cells were incubated in medium with 10% CCK-8 reagent (Target Mol, USA, Cat# C0037) for 1 h at 37°C. The absorbance of the enzyme marker was measured at 450nm using a microplate reader.

Colony Formation Assays

A total of 7.5×10^2 AN3Ca or Ishikawa cells were seeded into 6-well plates. After 14 days, colonies were fixed with 4% paraformaldehyde for 30 min and stained with 0.1% (w/v) crystal violet (Beyotime, China). The number of colonies was counted using ImageJ software. Experiments were performed in triplicate.

5-Ethynyl-2'-deoxyuridine (EdU) Assay

A total of 3×10^3 AN3Ca or 1×10^3 Ishikawa cells were seeded in 96-well plates and incubated for 48 hours. Cells were then treated with 20μmoL EdU for 2 hours, fixed with 4% paraformaldehyde and stained with Hoechst33342 for nuclear visualization. Images were acquired using an inverted fluorescence microscope (Zeiss, Oberkochen, Germany), with at least five fields captured per assay[20].

Flow Cytometry (FCM)

For apoptosis analysis, transfected cells (1×10^6 per well) were seeded in 6-well plates and incubated for 48 hours. After collecting and washing cells twice with cold PBS, cells were stained with 5μl Annexin V-Alexa Fluor 647 and 10μl propidium iodide (PI) (40304ES60, Yeasen, China) for 15 min at room temperature. For cell cycle analysis, AN3Ca and Ishikawa cells treated with siRNA NC or siRNA PHGDH were seeded in six-well plates at 1×10^6 cells per well. Cells were harvested when they reached 70%–80% confluence. After being washed twice with cold PBS, cells were fixed overnight at 4ºC with cold 70% (v/v) ethanol. The following day, cells were incubated with PI and RNase. All experimental operations and steps were carried out following the instructions of the Cell Cycle Assay Kit (Elabscience, China). And then evaluated and analyzed with a CytExpert software (Beckman coulter, USA) for cell cycle analysis.

Protein Extraction and Western Blot Analysis

Cells were washed with cold PBS and lysed using RIPA buffer (Beyotime, China) containing PMSF (Solarbio, China). For western blot, protein concentrations were separated by 8% SDS—PAGE and transferred to PVDF Membranes. After blocking with 5% non-fat milk, membranes were incubated with primary antibodies at 4℃ overnight. On the following day, membranes were incubated with secondary antibodies (Beyotime, China, 1:5000). Protein bands were stained using Super ECL Detection Reagent (36208ES60, Yeasen, China). Band densities were evaluated using ImageJ. Primary antibodies: PHGDH (Proteintech, China, 1:200), GAPDH (Proteintech, China, 1:5000). Primary antibodies included:PHGDH (14719-1-AP, Proteintech, China), CyclinA2(27242-1-AP, Proteintech, China, 1:1000), GAPDH (ab8245, Abcam, USA), CDK2(10122-1-AP, Proteintech, China, 1:1000).

RNA Isolation and Reverse Transcription and Quantitative Real-Time PCR

Total RNA was extracted with Trizol reagent (Sigma, USA) and reversed transcription into cDNA using a cDNA synthesis kit (Yeasen, China). For mRNA expression analysis, qRT-PCR was performed using the SYBR Green Pro Taq HS Premix (1184ES03, YEASEN, China). Primers used were PHGDH (Forward: CTGCGGAAAGTGCTCATCAGT; Reverse: TGGCAGAGCGAACAATAAGGC). GAPDH served as the internal control. Relative gene expression levels were calculated using the 2^(-ΔΔCt) method.

Transcriptome Sequencing

AN3Ca and Ishikawa cells with PHGDH knockdown (24 hours post-transfection) were subjected to transcriptome sequencing by Chi Biotech. RNA quality was extracted using the Agilent 2100 bioanalyzer. Sequencing libraries were prepared and sequenced on an Illumina platform. Data analysis included quality control, read alignment, gene expression quantification, differential expression analysis, and functional enrichment.

Animal Experiment

Six-week-old female BALB/c nude mice were supplied by the Beijing Huafukang Biotechnology Co., Ltd. All animals were provided with sterilized food and water and then randomly divided to two groups (n = 3 per group). Approximately 5×10^5 Ishikawa and HEC-1-A cells were injected into right and left flank, respectively. The tumor volume [(length × width²)/2] and body weight were documented every 3 days. The treatment group received daily intraperitoneal injections of NCT-503 (40 mg/kg), while the control group received an equal volume of solvent. At the endpoint, tumors were excised, measured, and photographed.

Statistical Analysis

Data were presented as mean ± SEM as indicated. Comparisons between two groups were performed using the Student's t-test, while multiple group comparisons were analyzed by one-way ANOVA tests. The chi-square test was used to investigate the significance of the associations between patient variables such as age, tumor size, N classifications, and survival status. All statistical analyses were conducted using SPSS22.0 and GraphPad Prism 8.0). two-sided p-value < 0.05 was considered statistically significant.

Results

PHGDH overexpression is associated with malignant progression and poor prognosis in endometrial cancer

To investigate the relationship between PHGDH expression and clinical features. we first analyzed its expression across pan-cancer tissues using the TIMER2.0 database (Supplementary Fig. 1). PHGDH expression was significantly higher in EC tissues than in normal endometrial tissues ($P < 0.001$) (Fig. 1A). High PHGDH expression was associated with worse prognosis in EC patients (Fig. 1B), positively correlated with higher pathological grade (Fig. 1C), and significantly linked to advanced tumor stage (Fig. 1D). Among various pathological types, PHGDH expression was notably increased in the more aggressive serous carcinoma compared with endometrioid carcinoma (Fig. 1E).

Validation of the Correlation between PHGDH Expression and Clinicopathological Features in Endometrial Cancer Using a Clinical Cohort

We further validated these findings in an EC cohort from the Affiliated Hospital of Qingdao University. PHGDH expression was significantly higher in EC tissues compared with both normal and adjacent tissues (Fig. 2A-C). Clinical correlation analysis revealed that high expression of PHGDH was significantly associated with the larger tumor size (Fig. 2D) advanced stage (Fig. 2E), higher pathological grade (Fig. 2F), and deeper myometrial invasion (supplementary Table 1), consistent with the database analysis.

PHGDH Promotes the Proliferation of Endometrial Cancer Cells

Clinical analysis showed that PHGDH was significantly correlated with tumor size of EC, suggesting that PHGDH may be involved in tumor proliferation. After verifying basal PHGDH expression across EC cell lines, we selected AN3Ca and Ishikawa cells, which stably express high PHGDH levels, for functional studies (Fig. 3A, B). Knockdown of PHGDH using small interfering RNA was confirmed at both mRNA and protein levels (Supplementary Fig. 2). The CCK-8 assay confirmed that PHGDH inhibition significantly reduced cell viability in Ishikawa and AN3Ca cells compared with controls (Fig. 3C). Colony formation assay showed that PHGDH significantly promoted the colony forming ability of EC cells (Fig. 3D). EdU assays indicated that PHGDH silencing significantly suppressed DNA replication associated with proliferation (Fig. 4A, B). Collectively, these findings highlighted the important role of PHGDH in promoting the proliferation ability of EC cells, supporting its oncogenic role in EC.

In vivo Validation of the Proliferation-Promoting Role of PHGDH

To extend our *in vitro* findings, we established a subcutaneous xenograft mouse model using the low-PHGDH cell line HEC-1-A and the high-PHGDH cell line Ishikawa, which were inoculated into opposite flanks of the same mice (Fig. 5A, B). Mice were treated with the PHGDH inhibitor NCT-503. The inhibitor NCT-503 did not significantly affect tumor growth in HEC-1-A xenografts (Fig. 5B, C) but notably reduced tumor size in Ishikawa-derived tumors (Fig. 5B, D), indicating that high PHGDH expression promotes EC cell proliferation *in vivo* (Fig. 5E).

PHGDH Promotes Cell Cycle Progression and Proliferation in Endometrial Cancer by Modulating the Nuclear Localization of c-MYC

To further explore the mechanism underlying PHGDH-driven EC progression, we performed transcriptome sequencing on AN3Ca cells with PHGDH knockdown. Enrichment analysis revealed that differentially expressed genes were primarily involved in biological processes such as amino acid biosynthesis, steroid biosynthesis, inositol phosphate metabolism, regulation of DNA metabolic process, carbon metabolism, DNA replication, G1/S change of cell cycle, FOXO signaling pathway and AMPK signaling pathway (Fig. 6A). Among these, cell cycle alterations was one of the most prominent. Subsequently, we verified the effect of PHGDH on the cell cycle in cells. Cell cycle analysis indicated that PHGDH knockdown inhibited the G0/G1 to S phase transition, thereby suppressing proliferation (Fig. 6B, C). CyclinA2 played an important role in both S phase and G2/M, and CDK2 played an important role in the middle and late G1 and S phase[21]. We found that PHGDH silencing markedly downregulated key cell cycle regulators Cyclin A2 and CDK2 at the protein level (Fig. 6D).

From the transcriptomic sequencing data, we observed a significant correlation between PHGDH expression and cell proliferation-related genes, including the transcription factor c-MYC (Supplementary Fig. 3). Given that c-MYC is a well-established positive regulator of cell proliferation and a classical

transcriptional activator of CDK2 and Cyclin A2, we initially hypothesized that PHGDH promotes cell cycle progression by upregulating CDK2 and Cyclin A2 via c-MYC[22]. However, subsequent Western blot analysis revealed that total c-MYC protein levels remained largely unchanged following PHGDH knockdown (Fig. 7A). Our prior IHC analysis of tumor tissues showed strong nuclear PHGDH positivity in some patients with advanced, poorly differentiated EC (Supplementary Fig. 4). This observation, coupled with existing evidence suggesting that nuclear-localized PHGDH may function synergistically with c-MYC in a manner independent of its enzymatic activity[23], led us to speculate that PHGDH might influence the transcriptional activity of c-MYC by affecting its subcellular distribution. To test this, we performed cytoplasmic and nuclear fractionation assays. The results demonstrated that while PHGDH silencing did not alter total c-MYC protein abundance, it significantly increased c-MYC levels in the nucleus and correspondingly decreased them in the cytoplasm (Fig. 7B, C). These findings suggest that PHGDH may modulate MYC nuclear localization to upregulate CDK2 and Cyclin A2 expression, thereby enhancing cell proliferation. Finally, flow cytometry demonstrated that the PHGDH inhibitor NCT-503 significantly inhibited S-phase progression in PHGDH-high Ishikawa cell line (Fig. 7D, E), suggesting that endometrial cancers with high PHGDH expression are more likely to benefit from NCT-503 treatment.

Discussion

Endometrial cancer incidence and mortality rates continue to rise annually[2, 3]. While most patients are diagnosed at an early stage with a favorable prognosis, effective treatment options for advanced disease remain limited. In recent years, PHGDH, a key metabolic enzyme, has been found to play a significant role in the development and progression of various malignant tumors. Its expression level is closely associated with tumor type, clinical stage, and patient prognosis[9, 14, 15]. Studies have shown that PHGDH is highly expressed in multiple cancers, including breast cancer, lung cancer, colorectal cancer, and EC, and correlates significantly with larger tumor size, higher pathological grade, advanced stage, and poor prognosis[9, 14, 15, 24, 25], Our study in EC similarly revealed that PHGDH expression is significantly higher in tumor tissues compared to normal tissues and is closely associated with tumor aggressiveness, depth of myometrial invasion, and progression-free survival. These findings suggest that PHGDH may serve not only as a potential prognostic biomarker for various solid tumors but also as a useful indicator for assessing disease progression and therapeutic response.

Mechanistically, PHGDH contributes to tumorigenesis and progression through multiple pathways. Traditionally, PHGDH has been recognized as the rate-limiting enzyme in the serine biosynthesis pathway, supporting rapid cancer cell proliferation by regulating intracellular serine metabolism and influencing nucleotide and lipid synthesis[16, 26]. However, growing evidence indicates that its oncogenic role extends far beyond its metabolic function. Our study in EC discovered that PHGDH affects the nuclear localization of c-MYC, a critical oncogenic transcription factor known to upregulate the transcription of cyclins and influence biological processes such as chromatin remodeling and histone acetylation[22, 27–30]. CDK2, whose activity is essential for initiating DNA replication by activating the MCM helicase, forms core regulatory complexes with cyclins to drive the cell cycle[31]. As a classical

regulator of both CDK2 and cyclins, c-MYC is central to this process[32, 33]. We found that PHGDH promotes the nuclear translocation of c-MYC, which subsequently upregulates the expression of cyclin A2 and CDK2. This establishes the "PHGDH/c-MYC/Cyclin A2/CDK2" axis, significantly promoting the G1/S phase transition and driving tumor cell proliferation. This axis has been partially validated in other cancers such as breast and liver cancers, suggesting that the role of PHGDH in regulating the cell cycle may be broadly applicable[23]. Furthermore, PHGDH is involved in regulating other biological processes such as autophagy and antioxidant stress, and can influence gene expression through epigenetic modifications in certain tumors, thereby expanding its functional repertoire in cancer progression[34–37]. Notably, the subcellular localization of PHGDH may be closely tied to its function. Our study observed marked nuclear enrichment of PHGDH in some cases of advanced, poorly differentiated EC, where it co-localized with c-MYC. A similar phenomenon has been reported in liver cancer, where nuclear PHGDH can form a transcription co-activation complex with c-MYC, exerting pro-tumorigenic effects independently of its enzymatic activity[23]. This differential subcellular localization offers a new perspective for understanding the functional heterogeneity of PHGDH in tumors and implies that its non-metabolic functions may play a significant role in cancer progression.

Given the pivotal role of PHGDH in cancer, therapeutic strategies targeting this molecule have gained considerable research interest. Several PHGDH inhibitors, such as the small molecule compounds NCT-503 and NCT-502, have entered preclinical research stages and demonstrated potent anti-tumor activity in models of PHGDH-high cancers[38, 39]. Our research showed that NCT-503 significantly inhibits the proliferation of PHGDH-high EC cells and effectively suppresses tumor growth in animal models, while exhibiting limited effects on PHGDH-low cells. This provides experimental support for a personalized treatment approach based on PHGDH expression levels. Moreover, PHGDH inhibitors have shown synergistic anti-tumor effects when combined with conventional chemotherapeutic agents (e.g., cisplatin, gemcitabine) or targeted drugs in various tumor models[13, 26, 40–42]. They may also help reverse resistance to certain therapies, such as sorafenib, erlotinib, and bortezomib[43–45], thereby enhancing their clinical translation potential.

Despite the promising outlook, PHGDH-targeted therapy faces several challenges. First, PHGDH performs vital physiological functions in normal tissues, particularly in the nervous system. Systematic evaluation is required to determine whether inhibiting its activity may lead to side effects such as neurotoxicity. Second, tumor cells might compensate for the metabolic deficit caused by PHGDH inhibition through metabolic reprogramming or activation of alternative signaling pathways, potentially leading to drug resistance. Therefore, future research needs to further elucidate the mechanistic subtypes of PHGDH function in specific cancers and develop more precise inhibitor delivery systems or combination regimens to improve therapeutic specificity and reduce adverse effects.

In summary, PHGDH is highly expressed in endometrial cancer tissues and correlates with poor prognosis. PHGDH promotes endometrial cancer proliferation both *in vitro* and *in vivo*. PHGDH potentially induced nuclear localization of MYC, which upregulates the cell cycle proteins CDK2 and

Cyclin A2 to drive tumor growth. PHGDH inhibitors effectively suppress the growth of PHGDH-high endometrial cancers. These findings highlight PHGDH as a novel prognostic marker for endometrial cancer and suggest that targeting PHGDH represents a promising therapeutic strategy in endometrial cancer.

Abbreviations

EC, Endometrial cancer; PHGDH, 3-phosphoglycerate dehydrogenase; STK4, Serine / threonine protein kinase 4; SRR, serine racemase; UCEC, uterine corpus endometrial carcinoma;

Declarations

Disclosure:

Consent to Participate and Consent to Publish

Consent to publish has been obtained from all participants, and the relevant author declaration has been submitted as a separate file in the Supplementary Information.

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Conflict of Interest

The authors declare no potential conflicts of interest.

Ethics Statement

Approval of the research protocol by an Institutional Reviewer Board: Ethics approval was obtained from the Institutional Review Board of The Affiliated Hospital of Qingdao University.

Informed Consent: All samples were collected with the informed consent of the patients and approved by the Ethics Committee of the Affiliated Hospital of Qingdao University (QYFY WZLL 27380).

All procedures involving human participants were performed in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments. This study was approved by the Ethics Committee of the Affiliated Hospital of Qingdao University (approval number: QYFY WZLL 27380).

Registry and the Registration No. of the study/trial: N/A.

Animal Studies: All animal experiments were approved by the Animal Ethics Committee of Qingdao University(20231201BALB/c-nu3520240111254). All animal procedures were approved by the Institutional Animal Care and Use Committee. At the end of the experiment, the nude mice were euthanized by cervical dislocation in accordance with the AVMA Guidelines on Euthanasia (2020 Edition). The procedure was performed by trained personnel, and tumor tissues were immediately harvested for subsequent analysis.

Author Contributions: Yu Chen: writing – original draft, methodology, investigation, conceptualization, formal analysis, visualization. **Ying Ning**: project administration, methodology, software, date curation, writing – review and editing. **Yufei Yang**: investigation. **Zeyu Lin**: investigation. **Xinyan Gao**: investigation, validation. **Yan Kong**: investigation, validation. **Ke Lei**: project administration, methodology, writing – review and editing. **Zhumei Cui**: funding acquisition, supervision, resources, writing – review and editing.

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Figures

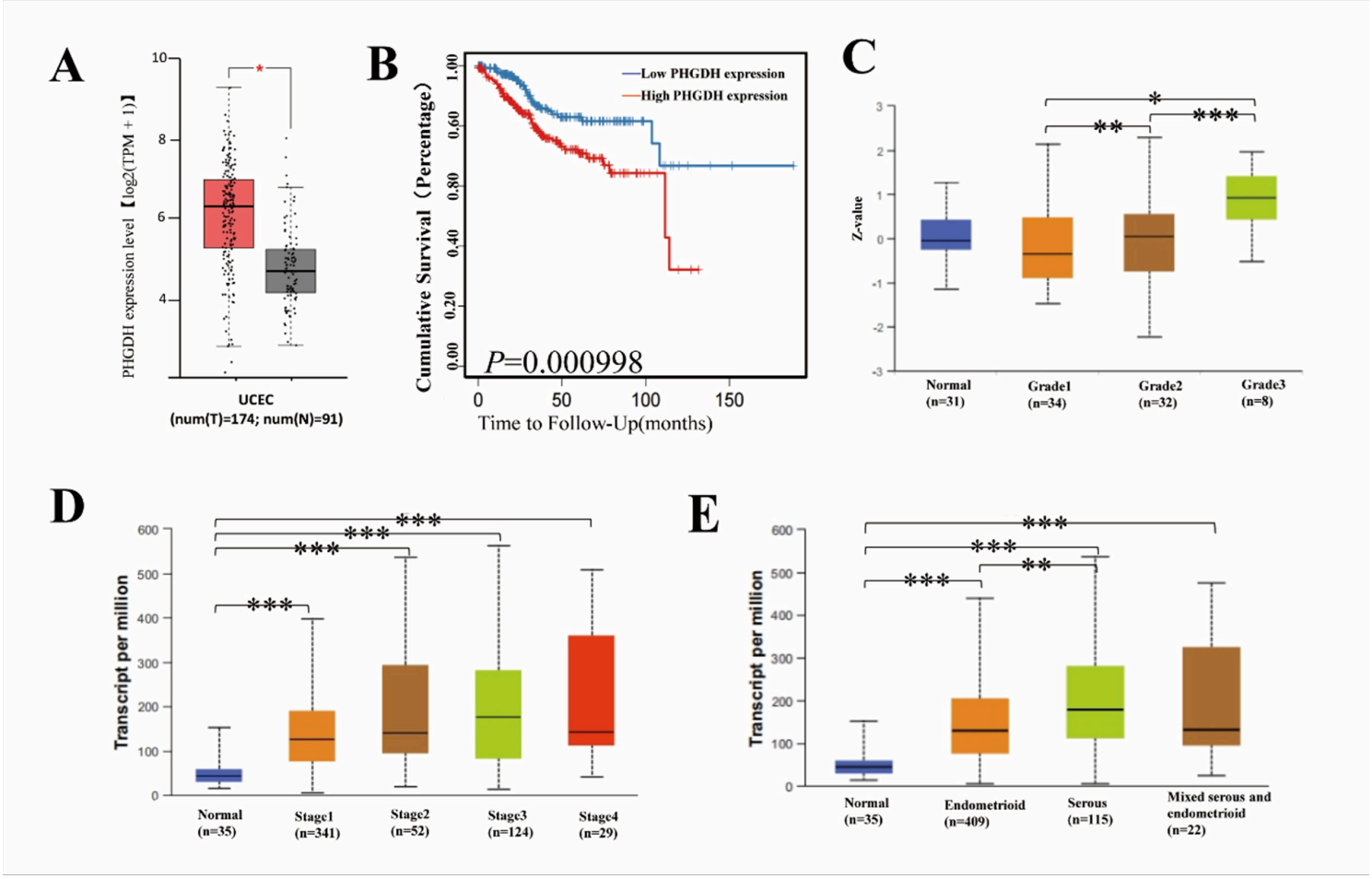


Figure 1

High PHGDH expression is correlated with clinicopathologic features in patients with endometrial carcinoma by bioinformatics analysis. (A) PHGDH expression levels in tumor samples and normal samples in UCEC. (B) Survival analysis based on the protein expression of PHGDH from UALCAN database on UCEC patients. (C) Protein expression of PHGDH in UCEC based on individual cancer grades (D) Expression of PHGDH in UCEC based on individual cancer stages. (E) Expression of PHGDH in UCEC based on histological subtypes. (*: $P<0.05$；**：$P<0.01$；***：$P<0.001$； ****：$P<0.0001$). Results were present as the mean±SEM. UCEC: uterine corpus endometrial carcinoma.

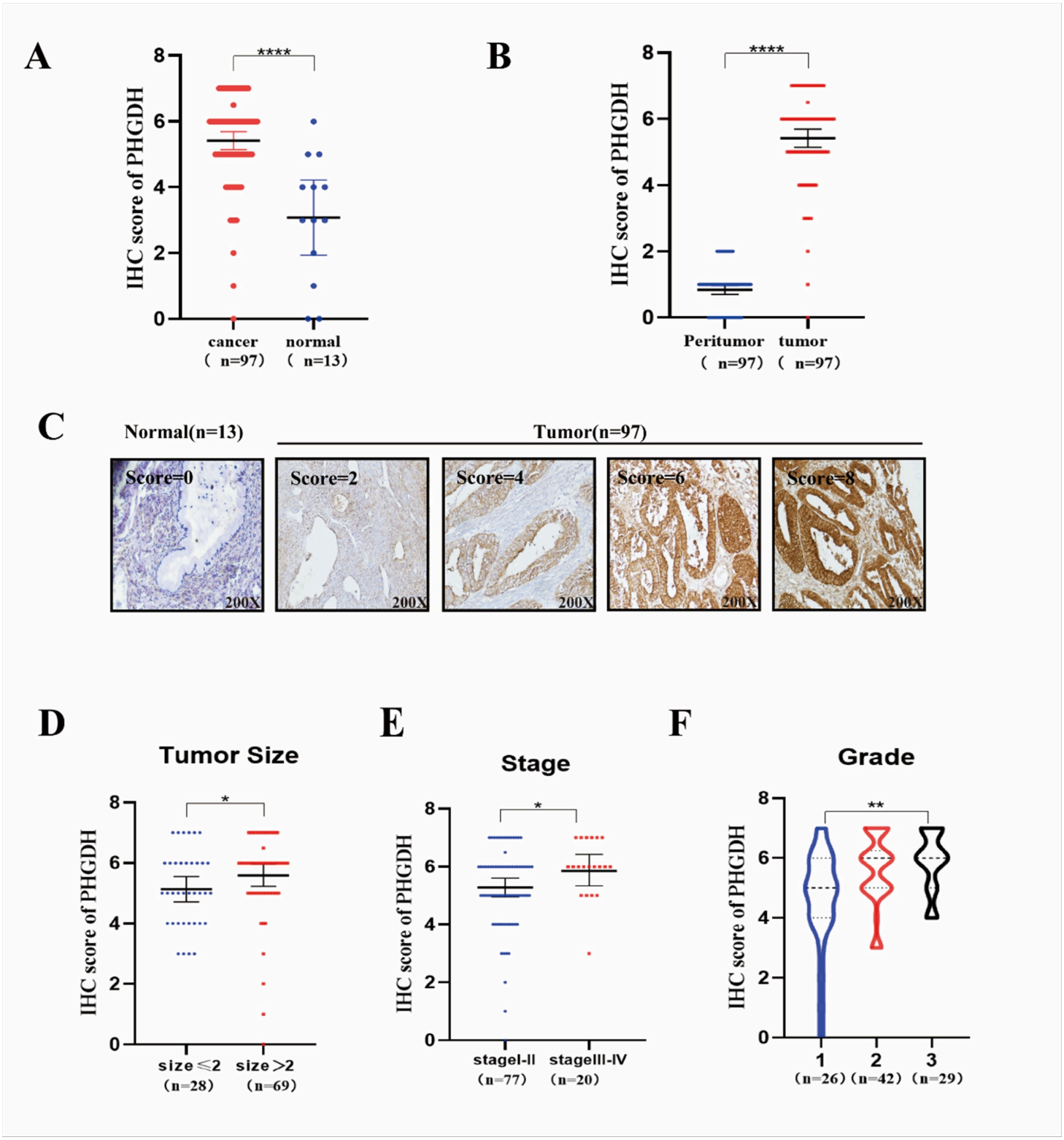


Figure 2

The significance of PHGDH in EC by clinical samples. (A) The expression of PHGDH in paired tumor and normal tissues from our hospital by IHC and the statistical analysis of the percentage of positive cells. (B) The expression of PHGDH in paired tumor and para-tumoral tissues from our hospital by IHC and the statistical analysis of the percentage of positive cells. (C)IHC assays to show the expression levels of PHGDH in UCEC tissue samples and matched normal samples. (D-F) 100% Stacked Bar assessing

PHGDH expression in patients with endometrial carcinoma according to different clinical characteristics; D: tumor size. E: Clinical Stage; F: Histologic grade. (*; $P<0.05$ **; $P<0.01$ ***; $P<0.001$ ****; $P<0.0001$). Results were present as the mean±SEM. IHC: immunohistochemistry.1: Grade1; 2: Grade2; 3: Grade3.

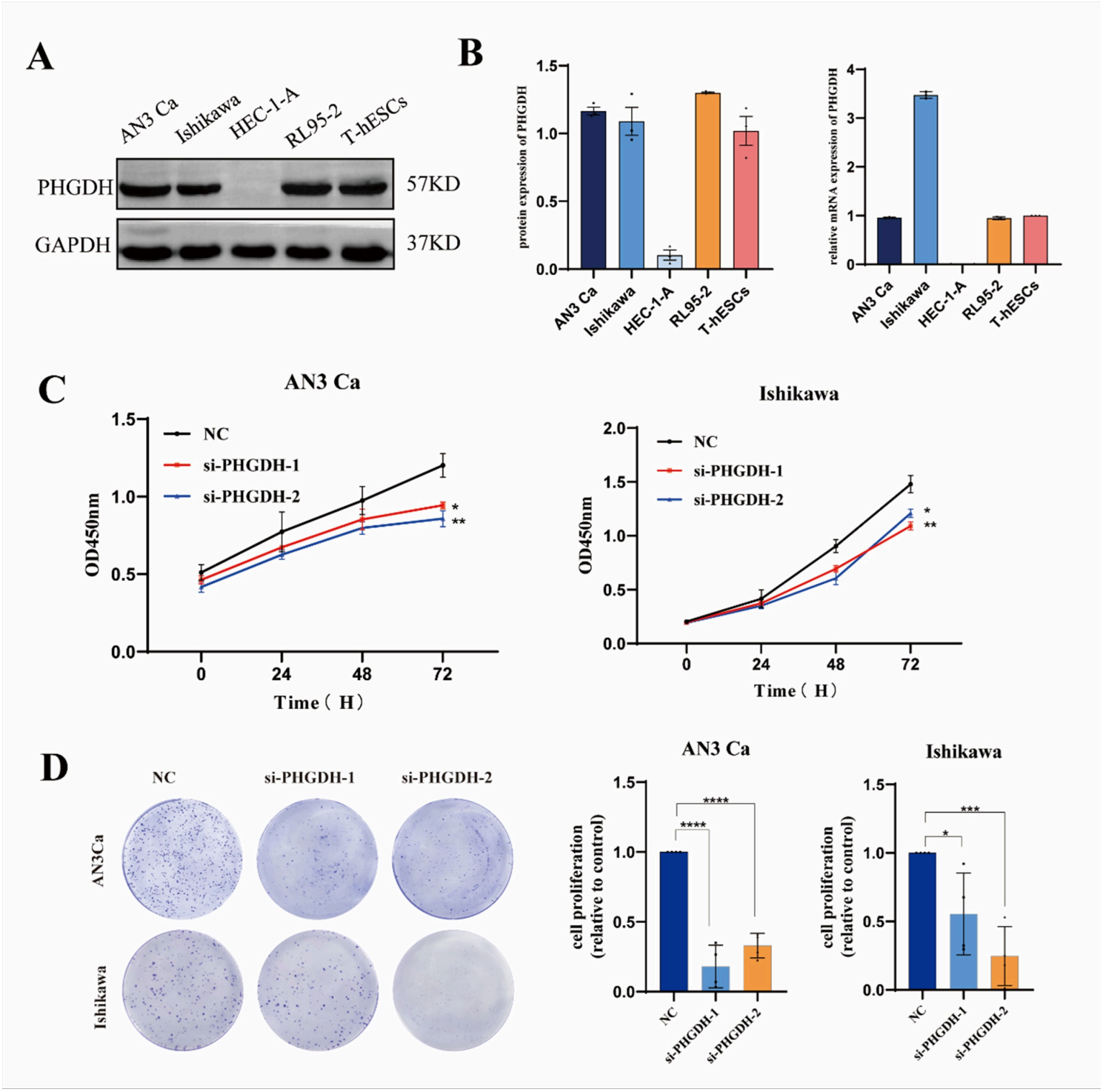


Figure 3

PHGDH knockdown inhibited cell proliferation in vitro. (A) The expression of PHGDH in endometrial carcinoma cell lines by Western blot. (B) The protein and relative RNA expression of PHGDH in endometrial carcinoma cell lines. (C) CCK-8 assay, (D) colony formation and (E,F) EdU Assay

demonstrated that knockdown of PHGDH in AN3Ca and Ishikawa cells induced a remarkable reduction in cell proliferation (*; $P<0.05$ ** $P<0.01$ *** $P<0.001$ **** $P<0.0001$). Results were present as the mean±SEM.

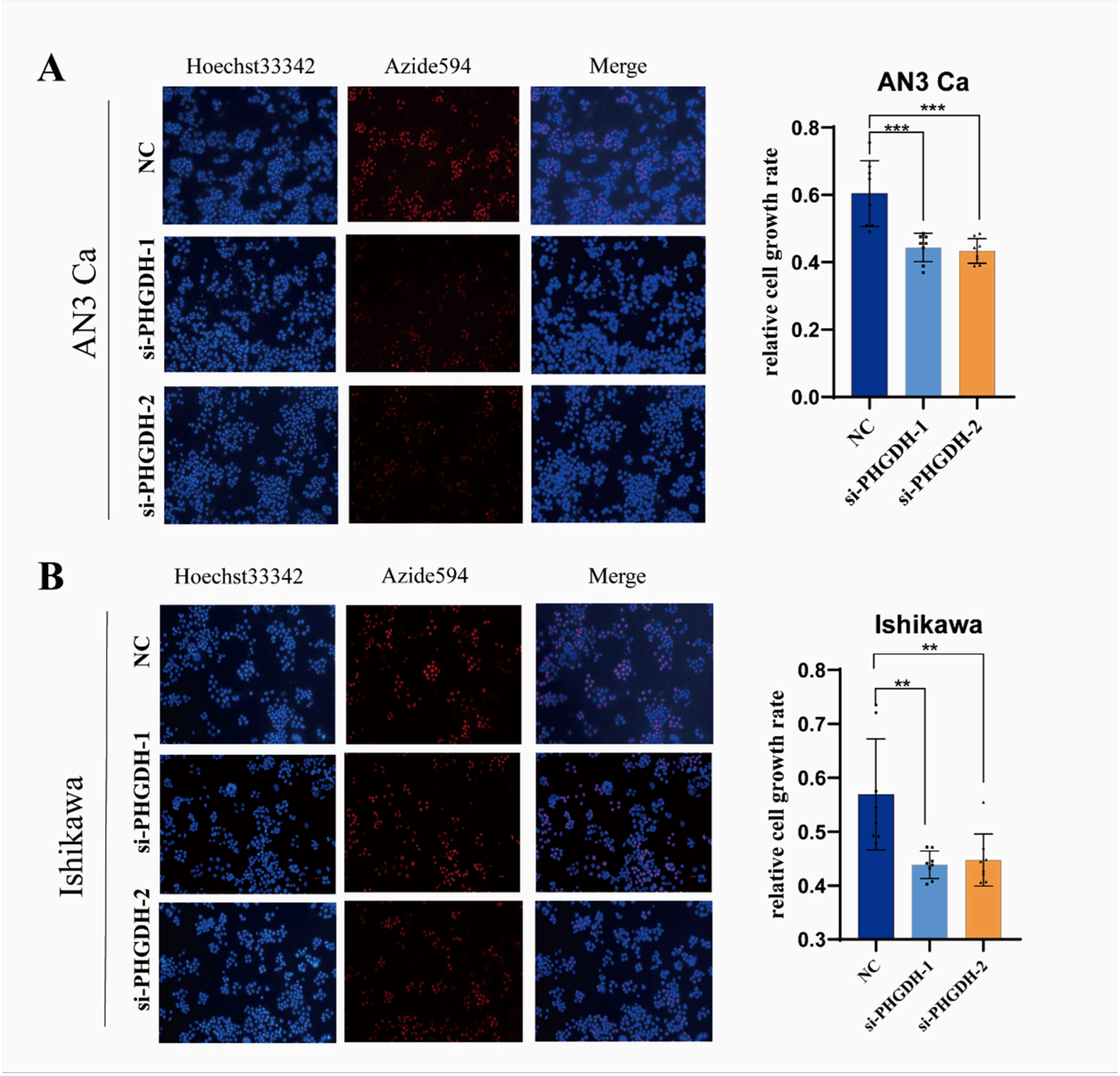


Figure 4

EdU Assay. (A) EdU Assay demonstrated that knockdown of PHGDH in AN3Ca induced a remarkable reduction in cell proliferation. (B) EdU Assay demonstrated that knockdown of PHGDH in Ishikawa cells induced a remarkable reduction in cell proliferation. (*P 0.05 ** P 0.01 *** P 0.001). Results were present as the mean±SEM.

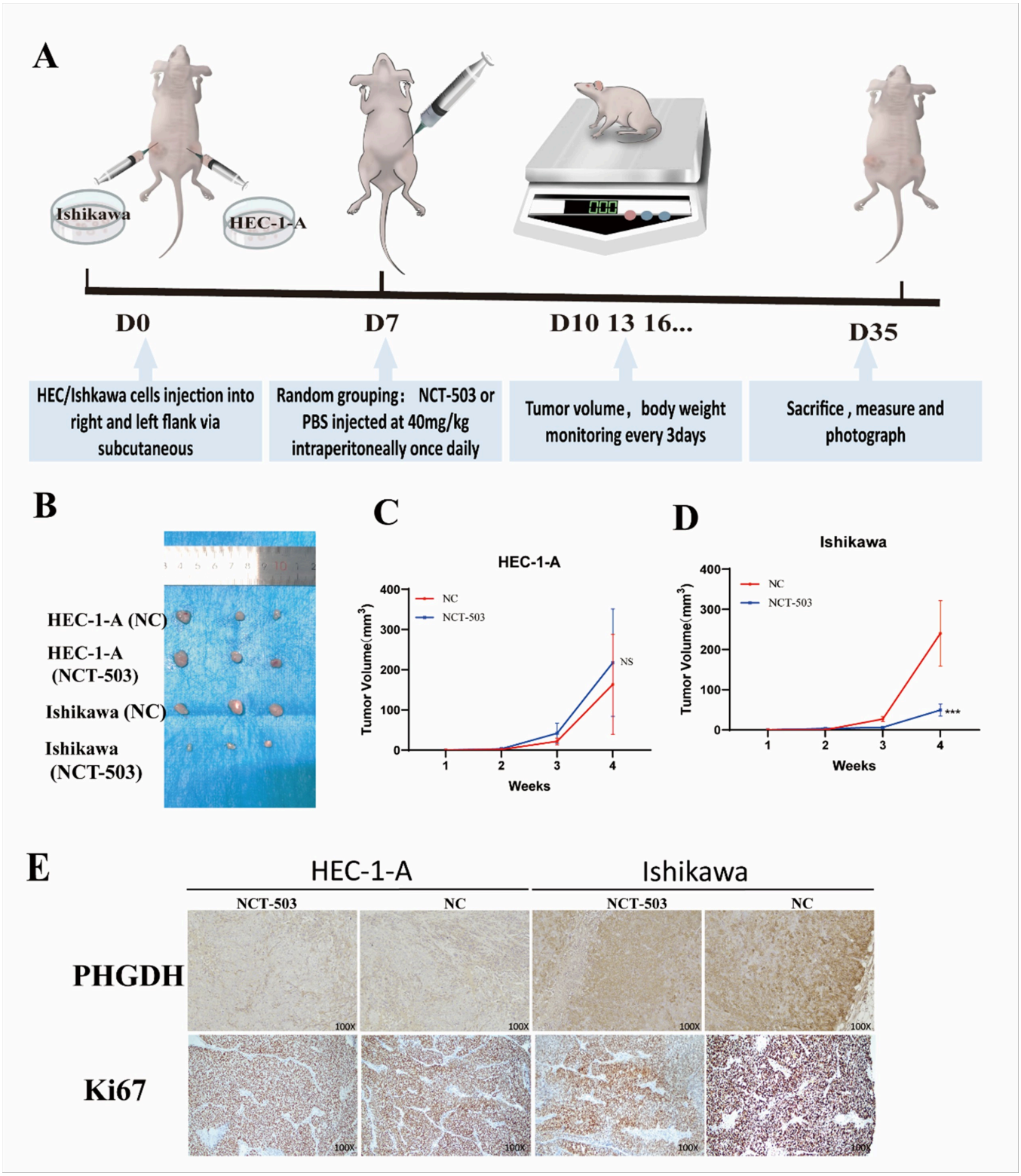


Figure 5

Experimental results of xenograft mouse model. (A) Figure of subcutaneous tumor inoculation site in xenograft mouse model and mouse experimental flow chart; (B) The size of subcutaneous tumors in each group; (C) Line graph of tumor volume growth in HEC-1-A group; (D) Line graph of tumor volume growth in Ishikawa group (E) immunohistochemistry (IHC) assays to show the expression levels of

PHGDH and Ki67 in the tumor tissue sections of xenograft mouse model. (NS: $P > 0.05$ * * *: $P < 0.001$). Results were present as the mean±SEM.. NS, not significant.

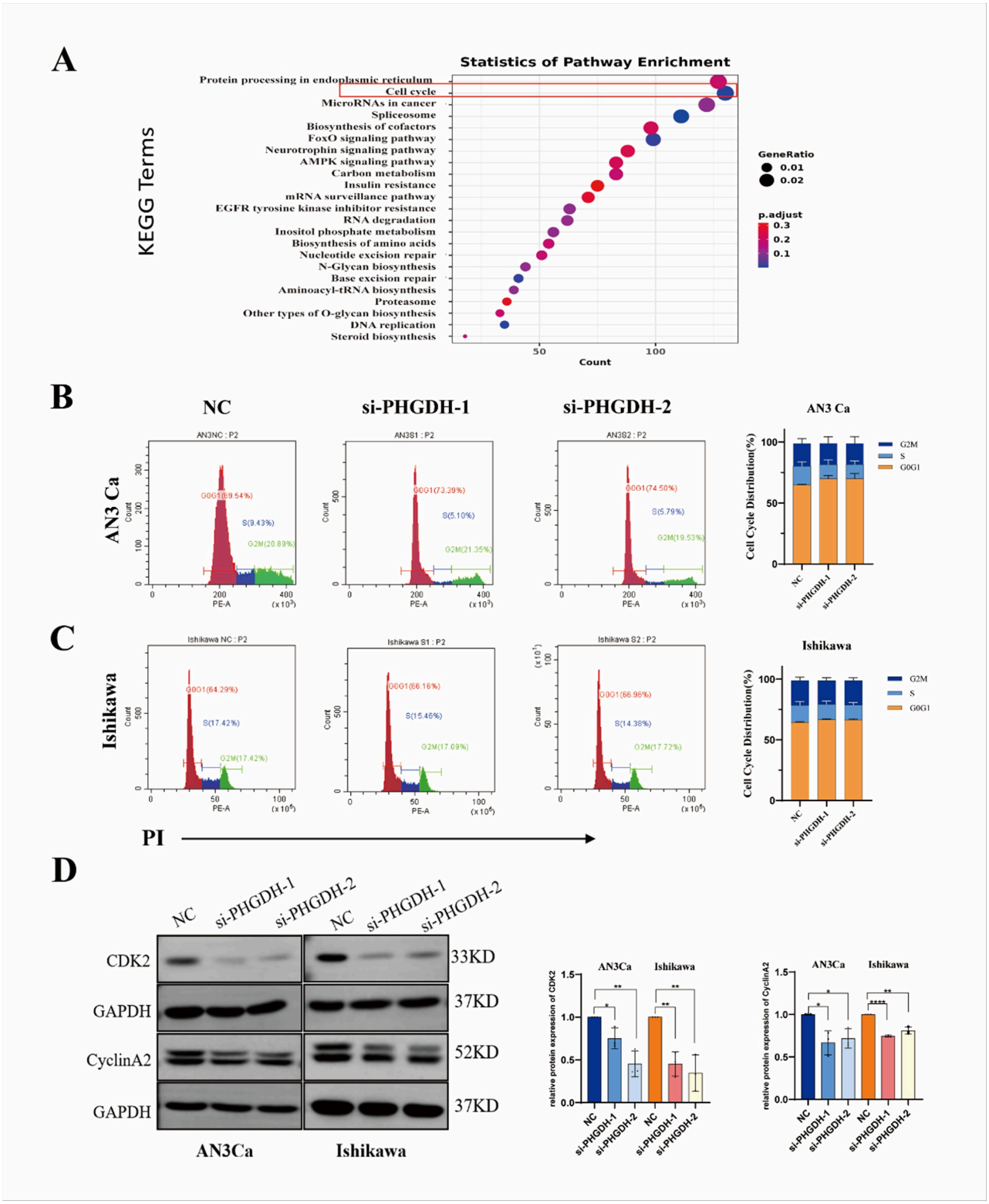


Figure 6

PHGDH expression affects cell cycle to promote EC progression. (A) Differential gene KEGG analysis; (B) Flow cytometry revealed that knocking down of PHGDH in AN3Ca cells mainly affects the G0/G1 phase

of the cell cycle. (C) Flow cytometry revealed that knocking down of PHGDH in Ishikawa cells mainly affects the G0/G1 phase of the cell cycle. (D) After PHGDH protein knockdown treatment, the expression changes of cell cycle marker proteins CDK2 and CyclinA2; NS*P*0.05**P*<0.05***P*<0.01****P*<0.001 *****P*<0.0001. Results were present as the mean±SEM.

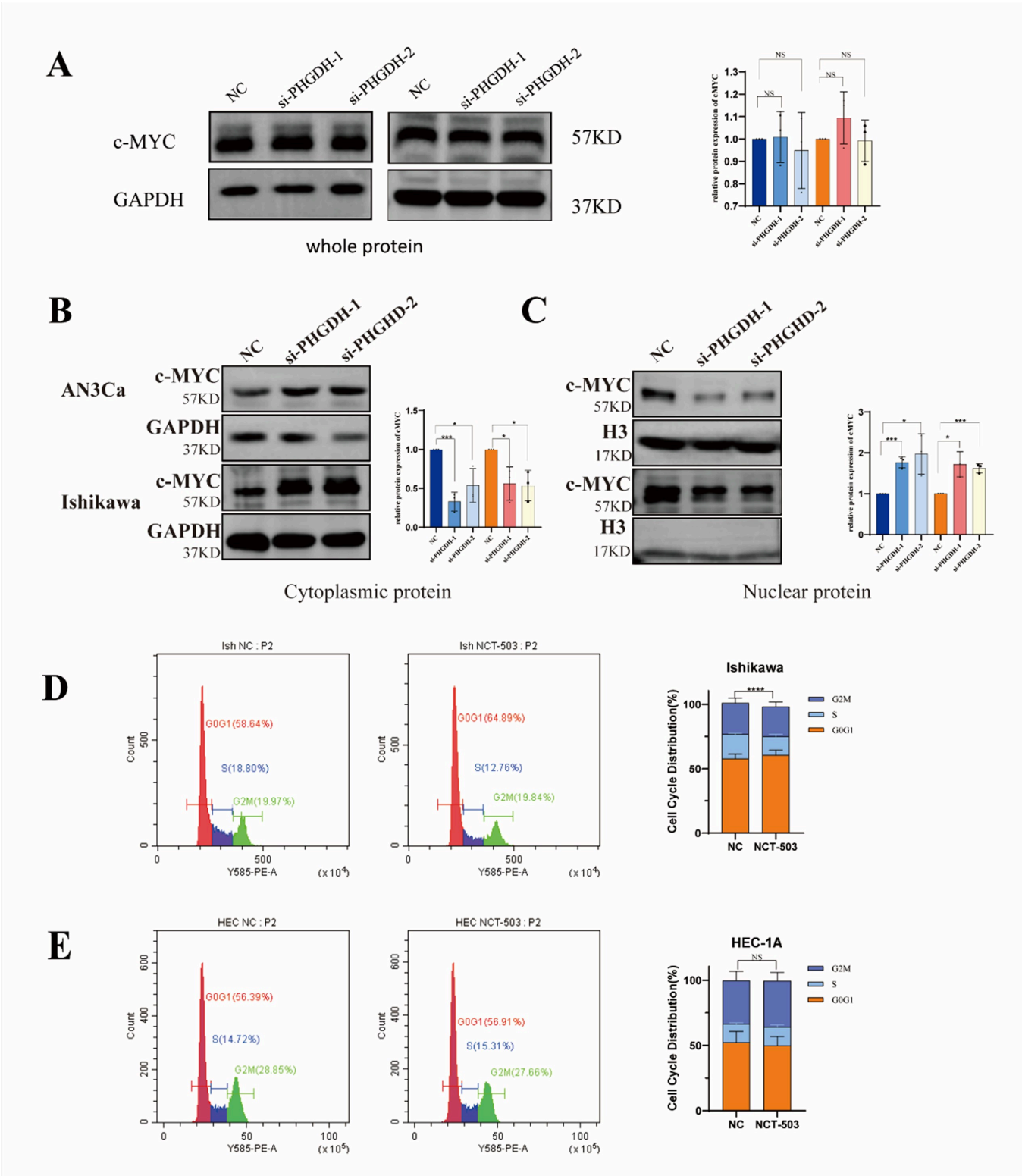


Figure 7

PHGDH expression affects DNA replication and cell cycle to promote EC progression. (A) The whole protein expression of c-MYC that knockdown of PHGDH in AN3Ca and Ishikawa cells by western blot. (B) The cytoplasmic protein expression of c-MYC that knockdown of PHGDH in AN3Ca and Ishikawa cells by western blot; (C) The nuclear protein expression of c-MYC that knockdown of PHGDH in AN3Ca and Ishikawa cells by western blot; (DE) Ishikawa and HEC-1A cells were treated with NCT-503 for cell cycle detection; The experimental results are representative pictures on the left and statistical graphs on the right. All experiments were repeated three times （NS：*P*＞0.05；*：*P*<0.05；**：*P*<0.01；***：*P*<0.001； ****：*P*<0.0001）. Results were present as the mean±SEM. NS: not significant.