scientific reports

OPEN

# Dysregulated GLUT1 results in the pathogenesis of preeclampsia by impairing the function of trophoblast cells

Jingyuan Pei[1,3], Yangyou Liao[1,3], Xiaoxian Bai[1,3], Min Li[1], Jing Wang[1], Xiaotong Li[1], Hongshuo Zhang[2], Linlin Sui[1]✉ & Ying Kong[1]✉

**Preeclampsia (PE) is a common placental-origin complication of pregnancy and a major cause of morbidity and mortality among pregnant women and fetuses. However, its pathogenesis has not been elucidated. Effective strategies for prevention, screening, and treatment are still lacking. Studies have indicated that dysfunction of placental trophoblast cells, such as impaired syncytialization, proliferation, and epithelial-mesenchymal transition processes, plays a crucial role in the development of PE. Glucose transporter 1 (GLUT1) is a key protein regulating glucose transport in placental tissues. However, the effect of GLUT1 on the function of trophoblast cells in PE is not well understood. In this study, we found that GLUT1 expression is reduced in PE placental tissues. GLUT1 promotes the syncytialization process by increasing the glucose uptake ability of BeWo cells. Additionally, GLUT1 promotes the proliferation, migration, and invasion capabilities of HTR-8/SVneo cells by regulating MAPK and PI3K/AKT signaling pathways. Overall, these findings provide a new insight into understanding the biological functions of GLUT1, clarifying the pathogenesis of PE, and identifying diagnostic and therapeutic targets for PE.**

**Keywords** GLUT1, Preeclampsia, Trophoblast cells, Syncytialization, Migration and invasion, Proliferation

Preeclampsia (PE) is a common placental-origin complication of pregnancy, characterized by new-onset hypertension after 20 weeks of gestation, often accompanied by symptoms such as proteinuria or hepatic/renal dysfunction[1]. PE is a major cause of morbidity and mortality among pregnant women and fetuses, resulting in approximately 70,000 maternal and 500,000 fetal deaths globally each year[2]. However, the pathogenesis and pathophysiology of PE remain incompletely elucidated, and currently, there are no effective means for its prevention, screening, or treatment. Placental trophoblast cell (TC) dysfunction plays a pivotal role in the development of PE, and the premature delivery of the placenta is the only definitive treatment[3,4]. Placental cytotrophoblast cells (CTBs) differentiate along two pathways. In the villous pathway, CTBs fuse intercellularly to form multinucleated syncytiotrophoblast cells (STBs), a process known as TC syncytialization[5,6]. STBs are in direct contact with maternal blood and are responsible for the transport of nutrients, gases, and metabolic wastes between the mother and fetus[7,8]. Studies have shown that impaired TC syncytialization is a significant factor contributing to the onset of PE[9,10]. Therefore, identifying key regulators of TC syncytialization and analyzing their roles in the development of PE will facilitate the development of new biomarkers and therapeutic approaches in the future, which are crucial for maintaining maternal and fetal health.

In the extravillous pathway, CTBs proliferate and differentiate into highly invasive extravillous trophoblast cells (EVTs), which recognize, adhere to, and degrade the extracellular matrix and invade the maternal myometrium and endothelium, thereby remodeling the maternal vasculature and increasing the placental blood supply[11,12]. Research indicates that the transformation of proliferative CTBs into invasive EVTs involves epithelial-mesenchymal transition (EMT)[13]. Dysregulation of EMT in TCs can lead to insufficient migration and invasion of EVTs, resulting in shallow placental implantation and impaired remodeling of spiral arteries[14]. Insufficiently remodeled vessels at the maternal-fetal interface cannot accommodate the increased blood volume of late pregnancy, which induces PE[15]. Thus, it can be seen that impaired TC proliferation and dysregulation

[1]Department of Biochemistry and Molecular Biology, College of Basic Medical Sciences, Dalian Medical University, 9 West Section, Lvshun South Road, Dalian 116044, Liaoning Province, China. [2]Advanced Institute for Medical Sciences, Dalian Medical University, Dalian 116044, China. [3]These authors contributed equally: Jingyuan Pei, Yangyou Liao and Xiaoxian Bai. ✉email: linlin19810622@sina.com; yingkong@dmu.edu.cn

of EMT are the critical pathological processes of PE[16]. Consequently, investigating the molecular mechanisms underlying TC aberrant proliferation and EMT could provide valuable insights for early intervention in PE and offer reliable therapeutic targets for this disease.

Glucose is the most widespread and significant monosaccharide in nature. It serves as the primary energy source for organisms, generating ATP through pathways such as glycolysis to fuel cellular activities. Additionally, glucose is a precursor for the synthesis of other biomolecules and participates in intercellular recognition processes. The concentration of glucose within organisms is tightly regulated, which is essential for ensuring normal cellular functions and overall health[17]. Due to its highly hydrophilic nature, glucose requires transport proteins to traverse the hydrophobic biological membranes. The uptake and release of glucose in cellular or subcellular compartments are coordinated by glucose transporters (GLUTs)[18]. During embryonic development, placental glucose transport is primarily regulated by GLUT1[19], which is ubiquitously expressed in CTBs, STBs, EVTs, endothelial cells, and the villous stroma[20]. This suggests that GLUT1 may be essential for maintaining the biological functions of TCs. It has been shown that glucose uptake is increased during TC syncytialization[21]. GLUT1 expression is downregulated in PE[22]. However, how GLUT1 dynamically regulates glucose metabolism and signaling networks, thereby impacting TC function, remains poorly understood, and its impact and mechanisms in the development of PE require further investigation.

This study focuses on the pathological aspects of PE, using the placental TCs, a pivotal mediator in maternal-fetal exchange, as the entry point. Through clinical tissue samples, in vitro syncytialization models, and cellular function assays, we investigate the effects and molecular mechanisms of GLUT1 on TC function and the development of PE. Overall, this research provides a new perspective for understanding the biological functions of GLUT1, clarifying the pathogenesis of PE, and identifying diagnostic and therapeutic targets for PE.

## Materials and methods

### Clinical samples

Placental tissues from normal pregnant women and PE patients were collected from the First Affiliated Hospital of Dalian Medical University. All patients supplied written informed consent, and the study was approved by the Ethics Committee (PJ-KS-KY-2024-325) from the First Affiliated Hospital of Dalian Medical University. All experiments were performed in accordance with relevant guidelines and regulations. Pathologists performed pathological diagnoses on the samples using Hematoxylin and Eosin (HE) staining.

### Immunohistochemistry (IHC)

Paraffin-embedded placental tissue sections were deparaffinized with xylene and hydrated with graded concentrations of ethanol, then heated in citrate buffer for antigen retrieval. The tissues were blocked with peroxidase solution and goat serum, followed by overnight incubation at 4 °C with specific primary antibodies. Immunodetection was conducted using a biotin-streptavidin reaction system (ZSGB-Bio, China). Finally, the nuclei were stained with hematoxylin, the sections were dehydrated and sealed, and the images were taken under a microscope (Nexcope, China). The antibody information is shown in Table S1.

### Cell culture

The human choriocarcinoma cell line BeWo was purchased from Wuhan Pricella Bio Co. (China) and cultured in DMEM/F12 medium (Thermo Fisher Scientific, USA) containing 10% fetal bovine serum (FBS). The human extravillous trophoblast cell line HTR-8/SVneo, donated by the Chinese Academy of Sciences, was cultured in RPMI-1640 medium (Thermo Fisher Scientific, USA) containing 10% FBS.

### Cell transfection

The targeting GLUT1 overexpression plasmids (pLenti-CMV), siRNA, and corresponding negative controls were purchased from the Public Protein/Plasmid Library (China). Cells were cultured in 35 mm dishes to 70% confluence and transfected with 100 mM siRNA or 5 µg overexpression plasmid using 5 µL lipofectamine 2000 (Invitrogen, USA). The interference sequences are shown in Table S2. Inhibitors were employed individually during the transfection process as follows: 5µM LY294002 (Selleckchem, USA), 10µM SB203580 (Sigma-Aldrich, USA), 10µM PD98059 (Sigma-Aldrich, USA) to specifically assess their effects in isolation.

### Western blot

Total proteins in tissues and cells were extracted using lysis buffer containing protease and phosphatase inhibitors (KeyGen, China). Protein concentration was determined using a BCA Protein Assay Kit (Thermo Fisher Scientific, USA). Equal amounts of protein samples (30 µg) were separated by 10% SDS-PAGE and subsequently transferred onto nitrocellulose membranes (PALL, UK). The membranes were blocked with 5% skimmed milk at room temperature for at least 2 h, followed by overnight incubation at 4 °C with specific primary antibodies. After incubation with HRP-conjugated secondary antibodies at room temperature for 1 h, immunodetection was performed using an ECL detection reagent (Tanon, China) and visualized on a Tanon imaging system. β-actin was used as an internal control for normalization to determine protein expression levels. The antibody information is shown in Table S1.

### Quantitative real-time PCR (qPCR)

Total RNA in tissues and cells was extracted using TRIzol reagent (TaKaRa, Japan) according to the manufacturer's instructions. cDNA was synthesized using a reverse transcription kit (Vazyme, China). Then the experiments were conducted using the SYBR Premix Ex Taq II kit (TransGen, China) on a Real-Time PCR Detection System (Thermo Fisher Scientific, USA). Results were normalized to β-actin and analyzed using the $2^{-\Delta\Delta Ct}$ method. The primer sequences are shown in Table S3.

### Immunofluorescence (IF)
Cells were inoculated onto cell-crawling slices. Cells were fixed with 4% paraformaldehyde at room temperature for 20 min, then blocked with goat serum for 1 h. Cells were incubated with specific primary antibodies overnight at 4 °C, followed by 1 h of incubation with secondary antibodies at room temperature in the dark. Finally, cell nuclei were stained with DAPI for 5 min at room temperature, and images were captured using a fluorescence microscope (Nexcope, China). The antibody information is shown in Table S1.

### Glucose concentration measurement
The glucose content in tissues and cells was determined using a Glucose Assay Kit (Beyotime, China). For tissues, lysis buffer was added at a ratio of 10 μL/mg of tissue, homogenized after lysing at 4 °C for 30 min, followed by centrifugation to collect the supernatant. For cells, 100 μL of lysis buffer was added to each well of a 6-well plate, lysed at 4 °C for 30 min, followed by centrifugation to collect the supernatant. A mixture of 20 μL of sample and 170 μL of o-toluidine detection reagent was vortexed, heated at 95 °C for 8 min, then cooled to 4 °C. The absorbance at 630 nm was measured, and the glucose concentration was calculated based on the standard curve.

### Glucose uptake assay
After experimental treatment, cells were incubated with a complete medium containing 50 μM 2-NBDG (MCE, USA) for 1 h in a cell culture incubator. Subsequently, cells were observed and imaged under a fluorescence microscope.

### Cell proliferation assay
Cell proliferation ability was reflected by cell viability assays using the CCK-8 (APExBIO, USA) reagent. The experimentally treated cells were seeded in 96-well plates at a density of 5000 cells per well. At designated time points, serum-free medium containing 1% CCK-8 was added. After incubating for 4 h in a cell culture incubator, absorbance at 450 nm was measured.

### Colony formation assay
The experimentally treated cells were seeded in 6-well plates at a density of 3,000 cells per well and incubated in a cell culture incubator for about 2 weeks until visible cell colonies formed. After fixation with methanol for 20 min at room temperature, the cells were stained with 0.2% crystal violet for 30 min. Image J software was used to calculate the number of cell clones after taking photos.

### Cell migration and invasion assay
Cell migration and invasion capacities were evaluated using Transwell chambers (Corning, USA). For the invasion assay, Matrigel (Corning, USA) was evenly coated in the upper chamber and allowed to solidify. Subsequently, $6\times10^4$ cells suspended in 200 μL of serum-free medium were seeded in the upper chamber, and 500 μL of medium containing 10% FBS was added to the lower chamber. After incubating for 36 h, the Transwell chambers were fixed with methanol at room temperature for 30 min and stained with 0.2% crystal violet for 30 min. The cells on the upper surface were removed with a cotton swab, and invaded cells on the lower surface were observed and imaged under a microscope. The migration assay follows the same steps as the invasion assay, but without Matrigel coating, and the incubation duration is 24 h.

### Wound healing assay
Equal amounts of experimentally treated cells were inoculated in 6-well plates. Once adhered, a sterile 200 μL pipette tip was used to scratch a straight line across the confluent cell layer. After washing with PBS and adding serum-free medium, images of the scratch were taken at 0 h and 24 h under a microscope. The width of the scratch was analyzed using Image-Pro Plus 6.0 software, and the decrease in scratch width represented the migration distance.

### Statistical analysis
All experiments were performed at least three times independently. Data error bars are presented as mean $\pm$ standard deviation. Statistical analyses were conducted using GraphPad Prism 9.0 software, Unpaired two-tailed Student' s *t*-tests were used to analyze two unpaired samples. One-way ANOVA was used to analyze multiple unpaired samples. $P<0.05$ was considered statistically significant.

## Results
### Impairment of TC function in PE placental tissues
To delve deeper into the alteration in TC function during the pathogenesis of PE, we collected placental tissues from clinically normal pregnant women and PE patients. HE staining revealed normal vascular morphology in the villi of healthy placentas. However, in PE placentas, the TC basement membrane was thickened, interstitial fibrin deposition increased, syncytial knots were more frequent, and there was an increase in the number of vessels (Fig. 1A). IHC results indicated that the syncytialization marker β-hCG was primarily expressed in STBs, and its expression was significantly lower in PE placental tissue compared to the normal group (Fig. 1B). Additionally, the protein and mRNA levels of syncytialization markers, including Syncytin-1 (ERVW-1), Syncytin-2 (ERVFRD-1), GCM1, OVOL1, and β-hCG (CGB3), were reduced in PE placental tissues (Fig. 1C, D). Hence, it is evident that the process of TC syncytialization is impaired in PE placental tissues.

Concurrently, we examined the expression of proliferation- and EMT-related molecules in placental tissues. The results showed that, compared to the normal group, the expression of the pro-apoptotic protein BAX was elevated, whereas the expression of cell cycle proteins Cyclin B1, Cyclin D1, PCNA, and the anti-apoptotic

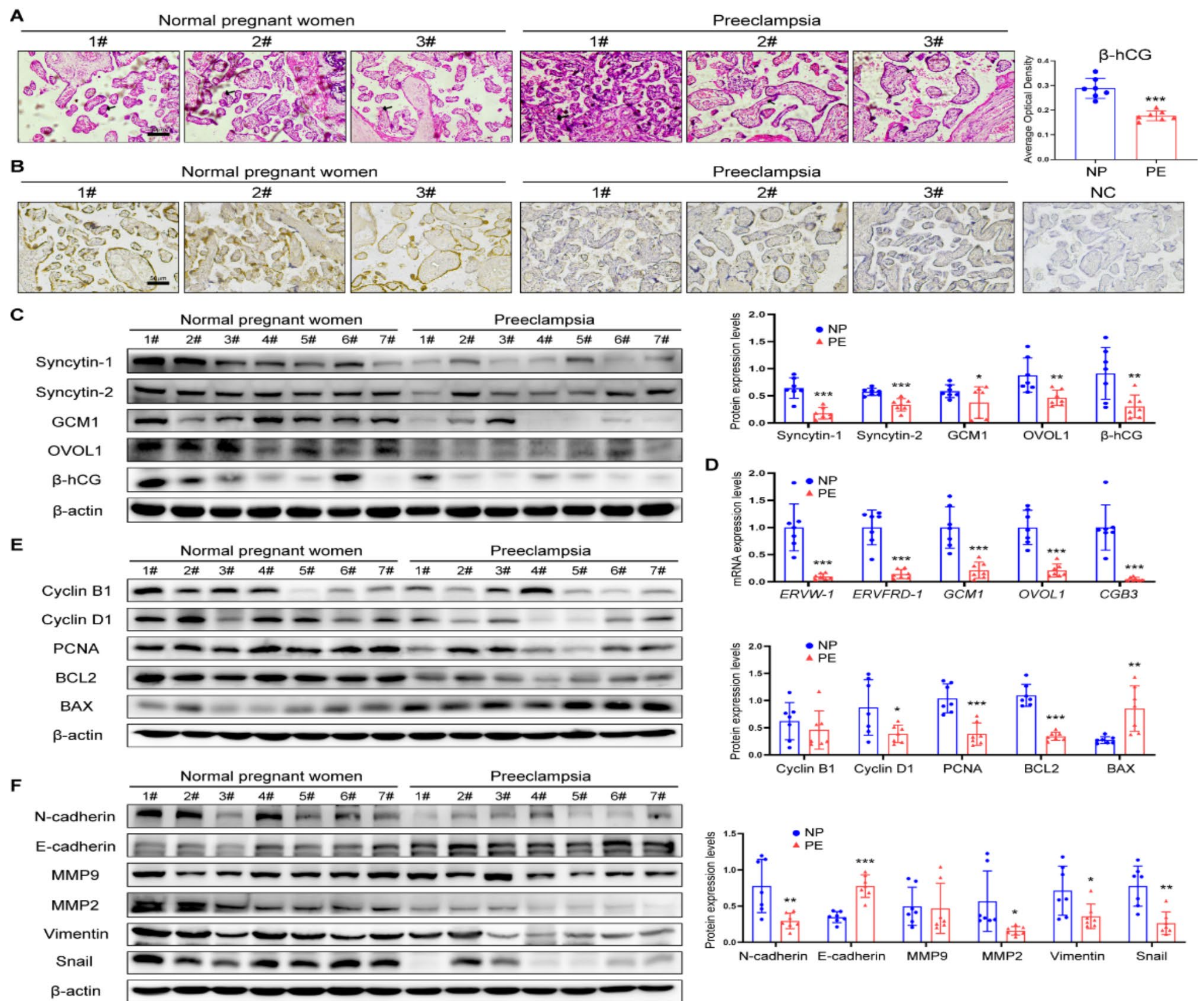


**Fig. 1**. Expression of syncytialization, proliferation, and EMT markers in placental tissues. (**A**) Representative HE staining of placental tissues; black arrows point to CTBs. (**B**) IHC detection of β-hCG expression; (**C**,**D**) western blot (**C**) and qPCR (**D**) detection of syncytialization marker expression; (**E**,**F**): western blot detection of proliferation (**E**) and EMT (**F**) marker expression. (Scale bar = 50 μm, *$P<0.05$, **$P<0.01$, ***$P<0.001$).

protein BCL2 was decreased in PE placental tissues (Fig. 1E). On the other hand, there were also differences in the expression of EMT-related molecules: E-cadherin expression was increased, while N-cadherin, MMP2, Vimentin, and Snail expressions were decreased in PE placental tissues (Fig. 1F). Overall, these findings suggest that the syncytialization, proliferation, migration, and invasion capabilities of TC are significantly compromised during the pathogenesis of PE.

## Reduced GLUT1 expression in PE placental tissues

To investigate the relationship between GLUT1 and the development of PE, we examined GLUT1 expression in the placental tissues. Through IHC, western blot, and qPCR assays, we found that GLUT1 expression was significantly reduced in the placental tissues of PE compared with normal pregnant women (Fig. 2A–C). This suggests that GLUT1 expression is closely associated with the onset and progression of PE.

## Increased GLUT1 expression during the syncytialization of BeWo cells

To explore the association between GLUT1 expression and TC syncytialization, we established an in vitro syncytialization model by inducing BeWo cell fusion with Forskolin (FSK)[23]. After treating BeWo cells with varying concentrations of FSK for different durations, it was observed that compared to the control group, the expression of GLUT1 and syncytialization markers, such as OVOL1, which is expressed in human placenta and was robustly induced following stimulation of trophoblast differentiation. Disruption of OVOL1 abrogated cytotrophoblast fusion and inhibited the expression of a broad set of genes required for trophoblast cell fusion and hormonogenesis. These proteins were increased with 20 and 50 μM FSK for 12 and 24 h, with the most

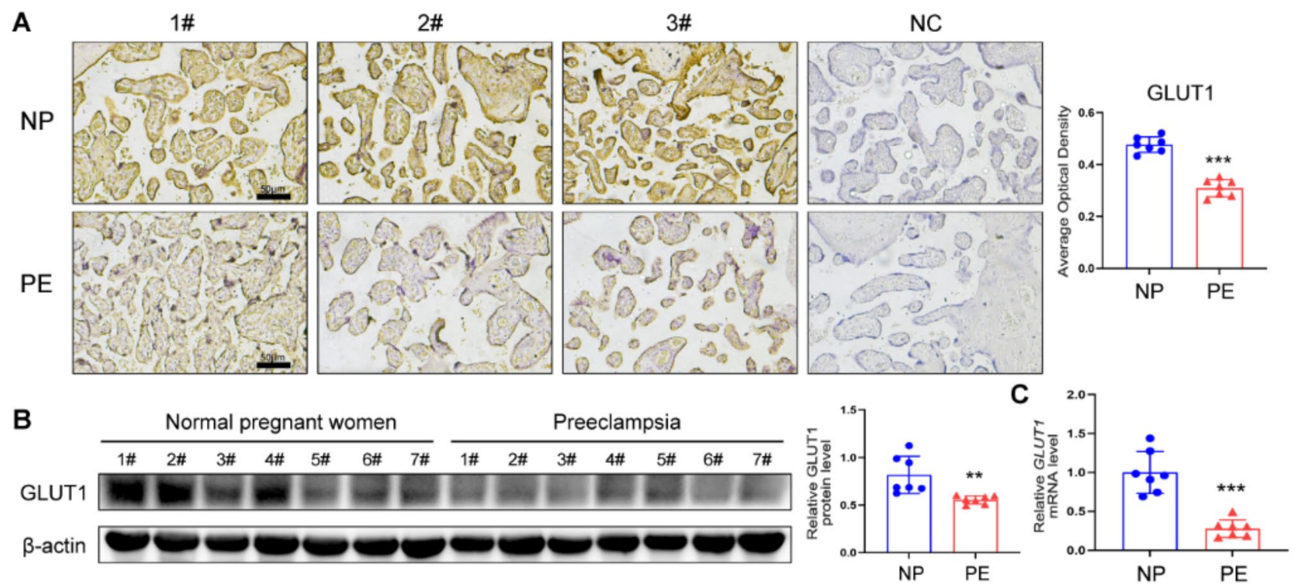


**Fig. 2.** Expression differences of GLUT1 in the placental tissues. (**A**–**C**): IHC (**A**), western blot (**B**), and qPCR (**C**) detection of GLUT1 expression. (Scale bar = 50 μm, $^{**}P < 0.01$, $^{***}P < 0.001$).

significant effect observed with 20 μM FSK for 24 h (Fig. 3A). However, when another trophoblast cell line, HTR-8/SVneo, was treated with the optimal concentration of 20 μM FSK for varying durations, no significant changes in syncytialization marker expression were noted (Fig. 3B). E-cadherin, a protein that is highly expressed on the cell membrane and participates in cell adhesion, is commonly used as a marker to detect TC fusion[24]. After treating BeWo with 20 μM FSK for various durations, it was found by staining for E-cadherin that induction for 12 h had significantly increased cell fusion, with the number of fused cells increasing as the treatment duration extended (Fig. 3C). This indicates that 20 μM FSK treatment for 24 h significantly induces syncytialization of BeWo cells, and this condition was subsequently used for induction. Meanwhile, these results suggest that GLUT1 may play an important role in the TC syncytialization process.

## GLUT1 promotes the syncytialization of BeWo cells

The above results suggest that GLUT1 may be closely associated with TC syncytialization. Subsequently, we regulated the expression of GLUT1 in BeWo cells to investigate its impact on the syncytialization process. The findings showed that downregulating GLUT1 resulted in decreased expression of syncytialization markers, whereas upregulating GLUT1 showed the opposite result (Fig. 4A, B). Through staining for E-cadherin, it was found that reducing GLUT1 led to decreased cell fusion, while increasing GLUT1 produced the opposite outcome (Fig. 4C). It can be seen that GLUT1 plays an indispensable role in TC syncytialization and can promote this process.

## GLUT1 promotes syncytialization by enhancing glucose uptake in BeWo cells

How does GLUT1 influence the TC syncytialization? We hypothesize that GLUT1 may regulate syncytialization by affecting glucose uptake capacity. Examination of clinical placental tissues using a glucose assay kit revealed that the content of glucose was significantly lower in PE placental tissues compared with the normal group (Fig. 5A). This implies that there is a correlation between glucose concentration and the pathogenesis of PE. Subsequently, we added D-Glucose to the syncytialization model and found that the expression of both GLUT1 and syncytialization markers increased with increasing concentration (Fig. 5B). This indicates that glucose can promote TC syncytialization. However, when the glucose treatment concentration reached 30 mM, the syncytialization process was inhibited. Further, regulating GLUT1 expression after the addition of D-Glucose, we found that downregulating GLUT1 reversed the expression changes of syncytialization markers induced by elevated glucose (Fig. 5C). Additionally, using the glucose assay kit and 2-NBDG reagent showed that adding D-Glucose increased intracellular glucose concentration (Fig. 5D) and cellular glucose uptake capacity (Fig. 5E), and downregulating GLUT1 showed opposite effects, whereas down-regulation of GLUT1 after adding D-Glucose reversed these changes. The above results suggest that GLUT1 can promote TC syncytialization by enhancing the glucose uptake capability of cells.

## GLUT1 promotes the proliferation capacity of HTR-8/SVneo cells

During the pathogenesis of PE, TCs not only suffer from impaired syncytialization but also experience obstacles in their proliferation and metastatic capabilities. Subsequently, we utilized HTR-8/SVneo cells to investigate the impact of GLUT1 expression changes on the proliferative ability of TCs. After confirming that GLUT1 expression was significantly altered (Fig. 6A, B), we observed that downregulating GLUT1 significantly inhibited cell viability and colony-forming ability by CCK-8 and colony formation assays, while upregulating GLUT1 showed

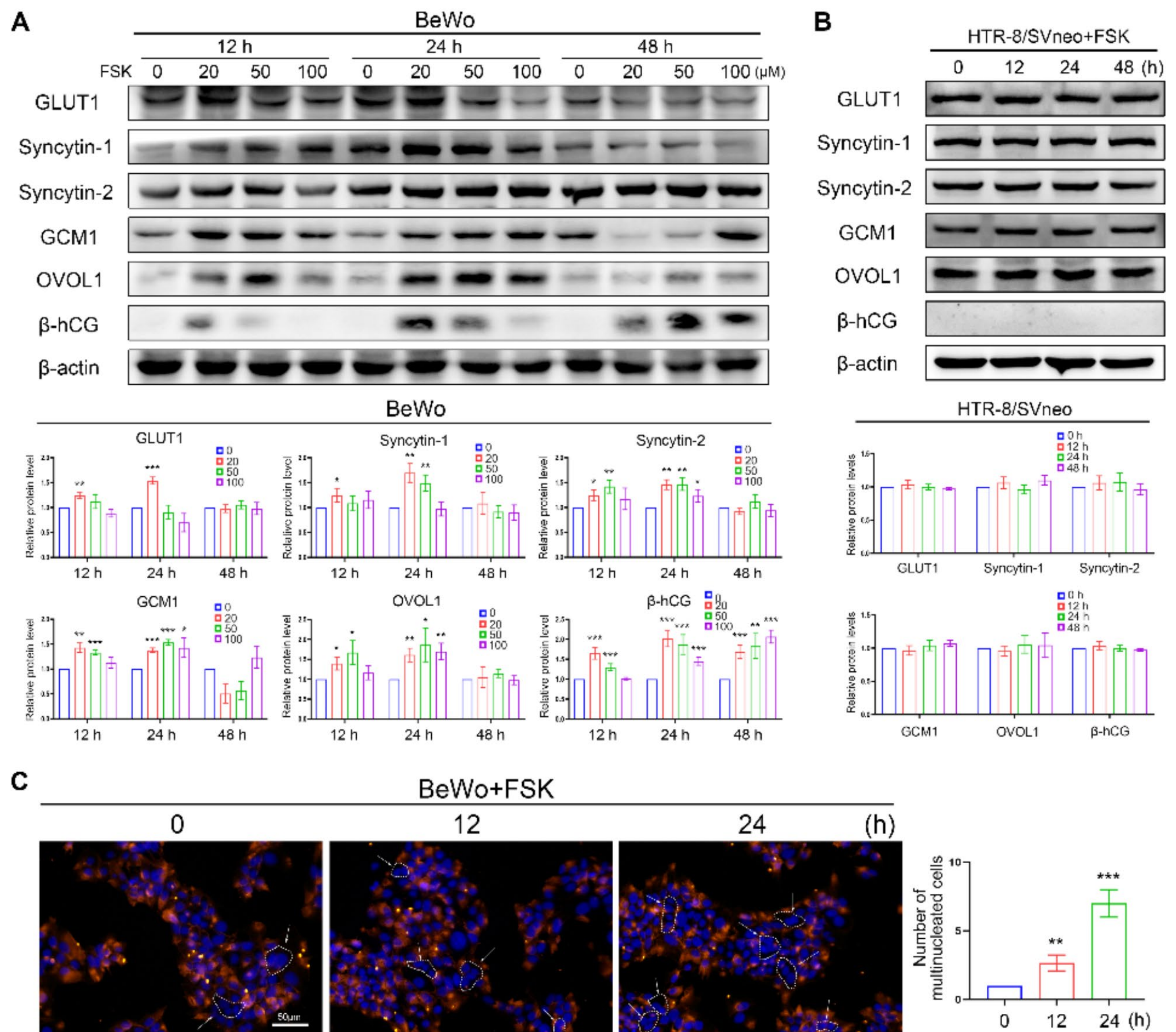


**Fig. 3**. Expression of GLUT1 during the syncytialization of BeWo cells. (**A**,**B**) Western blot detection of syncytialization marker expression treated with various concentrations of FSK in BeWo (**A**) and HTR-8/SVneo (**B**) cells; (**C**) IF detection of the fusion of BeWo cells treated with 20 μM FSK over time. (Scale bar = 50 μm, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

the opposite effect (Fig. 6C, D). It was also noted that downregulating GLUT1 led to decreased expression of proliferation-related proteins and increased expression of apoptosis-related proteins, and upregulating GLUT1 resulted in opposite outcomes (Fig. 6E). Overall, GLUT1 can promote the proliferation capacity of HTR-8/SVneo cells.

## GLUT1 promotes the migration and invasion capabilities of HTR-8/SVneo cells

The effects of changes in GLUT1 expression on the migration and invasion capabilities of TCs were also investigated in HTR-8/SVneo cells. Considering that knock-down or overexpressing GLUT1 impacts the proliferation rate of cells, we utilized serum-free media in the transwell and wound healing assays to minimize the influence of cell proliferation on migration capability. We observed that downregulating GLUT1 significantly inhibited cellular migration and invasion capabilities, with a twofold difference in change, while upregulating GLUT1 showed the opposite effect, despite potential limited contributions from changes in proliferation rates (Fig. 7A, B). Additionally, it was noted that downregulating GLUT1 altered the expression of EMT-related proteins, and upregulating GLUT1 reversed these changes (Fig. 7C). Overall, GLUT1 can promote the migration and invasion capabilities of HTR-8/SVneo cells.

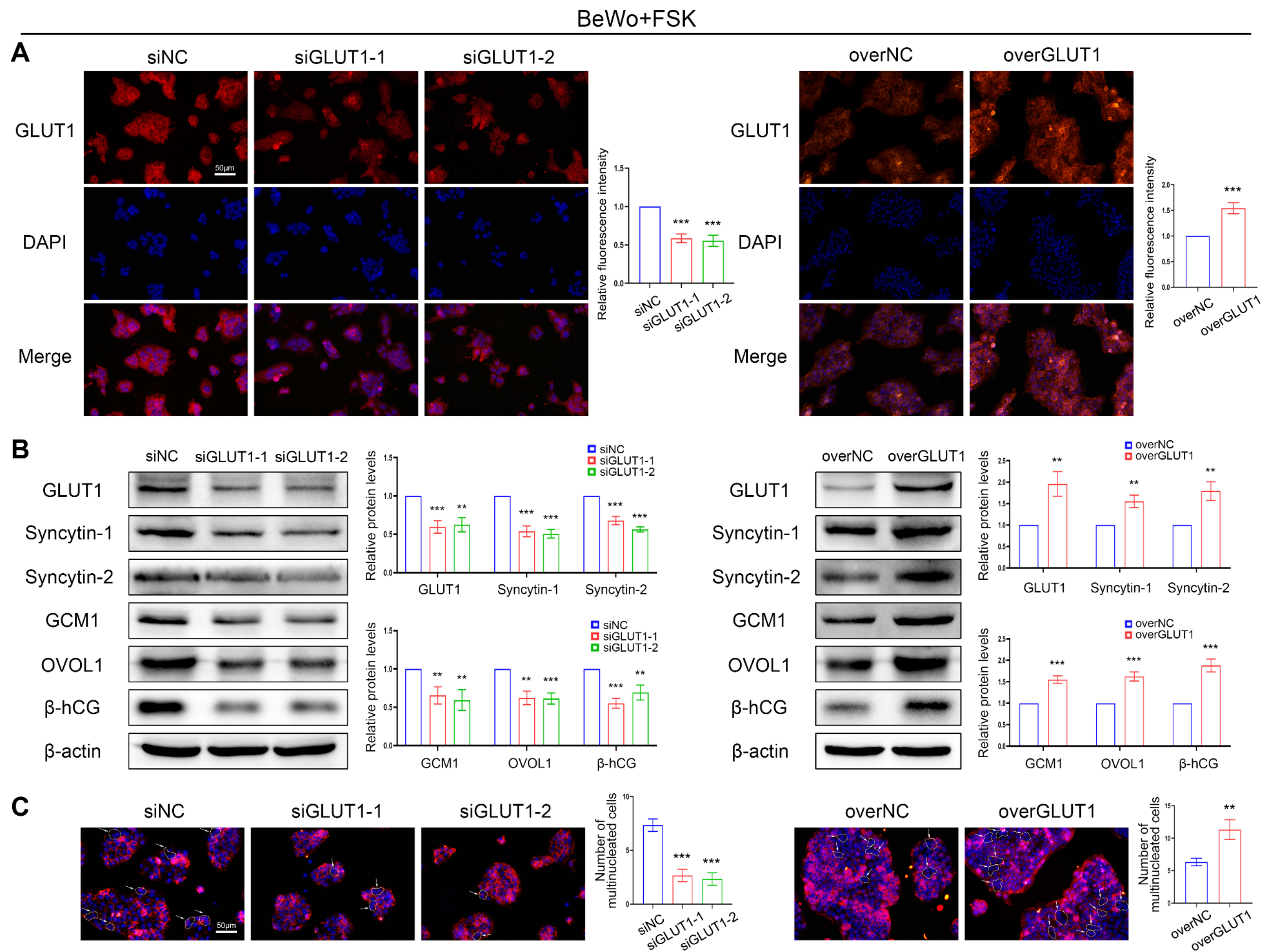


**Fig. 4.** Effect of GLUT1 during the syncytialization of BeWo cells. (**A**) IF detection of GLUT1; (**B**) western blot detection of syncytialization marker expression after regulated GLUT1 expression; (**C**) IF detection of cell fusion after regulated GLUT1 expression. (Scale bar = 50 μm, $^{**}P<0.01$, $^{***}P<0.001$).

## GLUT1 promotes the proliferation and metastatic capabilities of HTR-8/SVneo cells through the PI3K/AKT and MAPK signaling pathways

The above results indicate that GLUT1 promotes the proliferation, migration, and invasion capabilities of HTR-8/SVneo cells. Further, we explored the molecular mechanisms by which GLUT1 induced alterations in the biological functions of TCs. It was shown that the PI3K/AKT and MAPK signaling pathways are major regulators of EMT and can be involved in the pathogenesis of PE by affecting the differentiation and angiogenesis of EVTs[25]. Consequently, we assessed the expression of signaling pathway-related molecules after regulating GLUT1 expression. The results demonstrated that downregulating GLUT1 resulted in a decrease of the phosphorylation molecules p-AKT, p-ERK, and p-p38 without affecting the expression of AKT, ERK, p38, and JNK, while there was no significant change in p-JNK, and the opposite result occurred with upregulating GLUT1 (Fig. 8A). This indicates that GLUT1 can activate the PI3K/AKT and MAPK signaling pathways.

To further investigate whether the PI3K/AKT and MAPK signaling pathways are directly involved in the processes of GLUT1 affecting TC proliferation, migration, and invasion, we treated GLUT1 overexpressing HTR-8/SVneo cells with the PI3K inhibitor LY294002, the ERK inhibitor PD98059, and the p38 inhibitor SB203580. It was revealed that adding LY294002 and SB203580 significantly reversed the proliferation induced by GLUT1 overexpression, while PD98059 had no noticeable effect on it (Fig. 8B, C). Adding LY294002 and PD98059 significantly reversed the migration and invasion caused by GLUT1 overexpression, while SB203580 had no noticeable effect on it (Fig. 8D). Additionally, western blot results showed that by adding different signaling pathway inhibitors to GLUT1-overexpressing cells, only the phosphorylation of their respective target molecules was inhibited without affecting the expression of GLUT1 and other signaling pathway molecules (Fig. 8E). This suggests that the PI3K/AKT, ERK, and p38 signaling pathways operate independently in the process of GLUT1-affecting changes in TC function, with no cross-talk between them. Overall, these results indicate that the PI3K/AKT and p38 signaling pathways were involved in the proliferation of HTR-8/SVneo cells mediated by GLUT1, while the PI3K/AKT and ERK signaling pathways were involved in the metastasis of HTR-8/SVneo cells mediated by GLUT1.

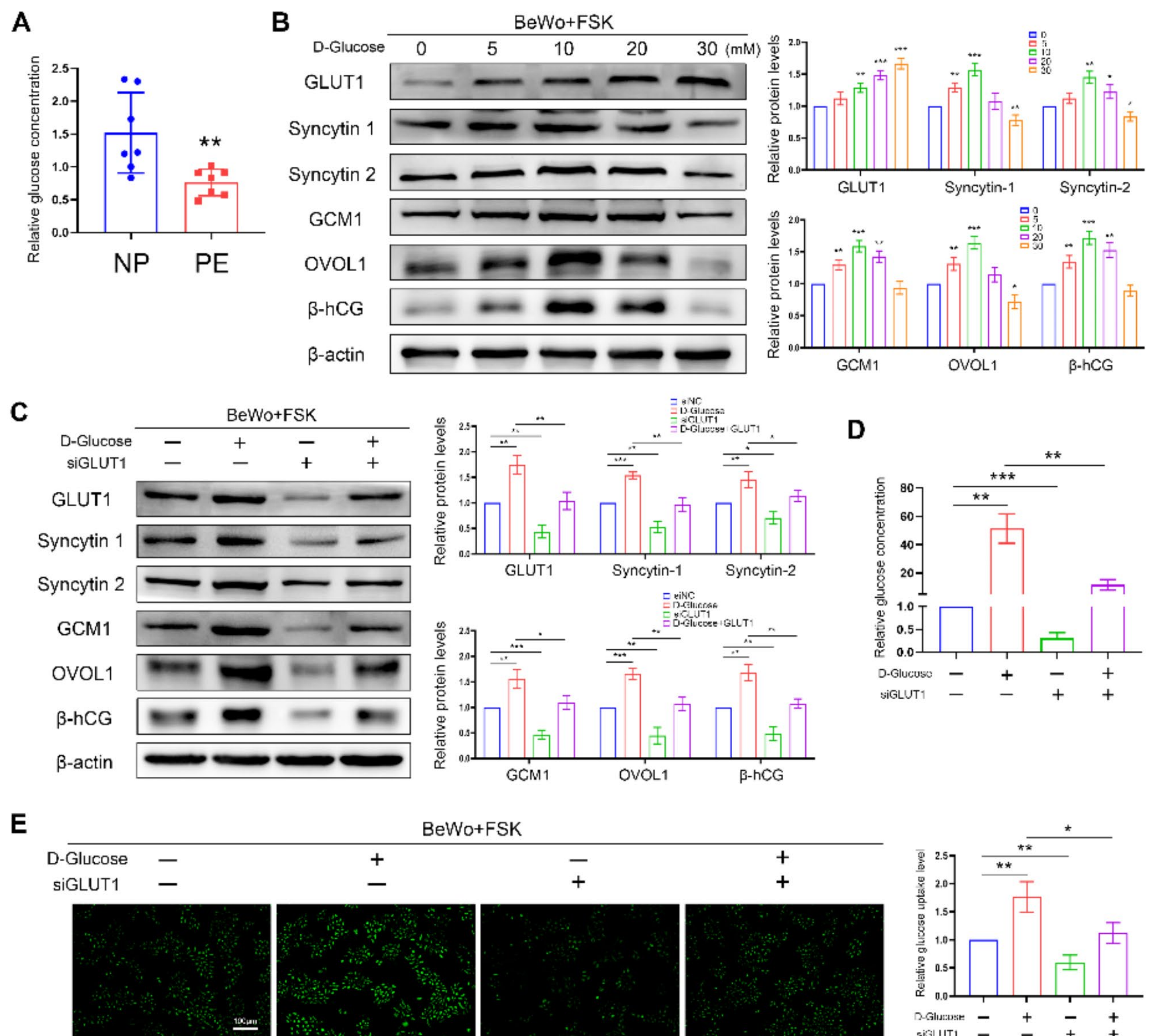


**Fig. 5**. GLUT1 affects the syncytialization of BeWo cells by regulating glucose uptake. (**A**) Glucose assay kit detection of glucose content in placental tissues; (**B**,**C**) western blot detection of syncytialization markers expression following treatment with various concentrations of D-Glucose (**B**) and D-Glucose with siGLUT1 (**C**); (**D**,**E**) glucose assay kit (**D**) and 2-NBDG kit (**E**) detection of intracellular glucose content (**D**) and glucose uptake capacity (**E**) after treatment with D-Glucose and siGLUT1. (Scale bar = 100 μm, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$).

## Discussion

PE is a common and severe complication of pregnancy, affecting approximately 5–7% of pregnancies and being a major cause of maternal and fetal morbidity and mortality[2]. However, the pathogenesis and pathophysiology of PE have not been fully elucidated. The dysfunction of placental TC plays a central role in its development, and early delivery of the placenta remains the only definitive treatment[3,4]. Increasing research has shown that the process of TC fusion and differentiation is dysregulated in the placentas of PE patients[26–28]. Compared to normal placentas, the expression of Syncytin-1, Syncytin-2, and their transcription factor GCM1 is reduced in the placentas of PE patients[29–31]. Consistent with this, we collected placental tissues from normal pregnant women and PE patients and revealed that β-hCG expression is decreased in PE placental tissues by IHC. Similarly, both protein and mRNA expression levels of syncytialization markers were decreased in the placental tissues of PE patients. In addition, MacLennan et al. found intermittent, multiple vacuoles, and thinning of STBs in the placentas of PE patients by electron microscopy[32]. Similar results were obtained in this study. After HE staining, we noted that while the villi in normal pregnant women's placentas were well-matured with orderly arranged STBs nuclei, the villi in PE patients showed poor maturation and disorganized STBs nuclei. On the other hand, some studies have shown that insufficient invasion and abnormal proliferation of EVTs are closely associated

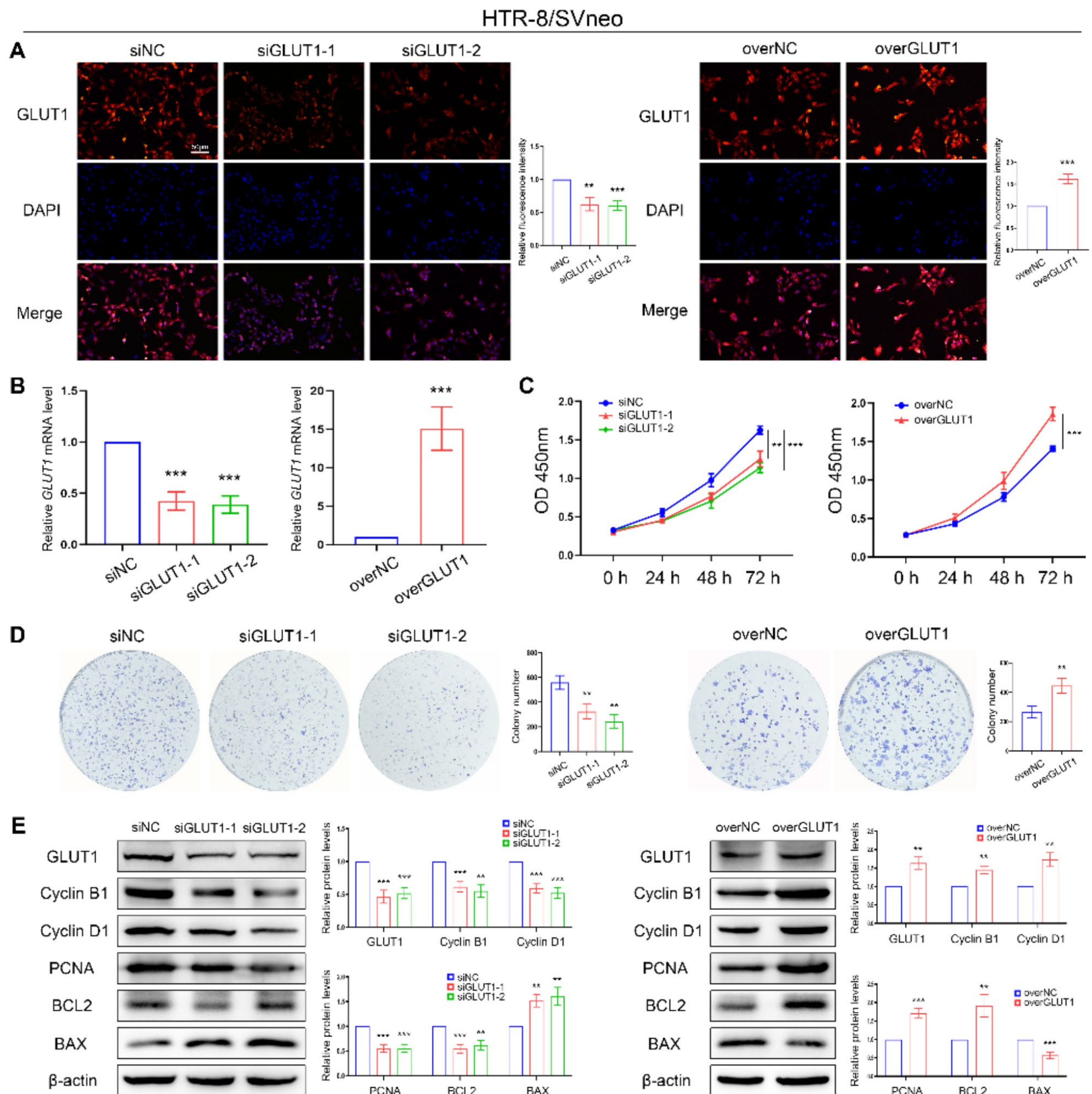


**Fig. 6**. Effect of GLUT1 on the proliferation capacity of HTR-8/SVneo cells. (**A**) IF detection of GLUT1; (**B**) qPCR detection of GLUT1 mRNA expression; (**C**,**D**) CCK8 (**C**) and colony formation assays (**D**) detection of the effect of GLUT1 expression changes on proliferation; (**E**) western blot analysis detection of the effect of GLUT1 expression changes on proliferation- and apoptosis-related protein expression. (Scale bar = 50 μm, $^{**}P < 0.01$, $^{***}P < 0.001$).

with the onset of PE. Xu et al. found that the endogenous peptide Apelin-36 could alleviate LPS-induced cellular inflammation and apoptosis and enhance the invasion and migration of TC by inhibiting the GRP78/ASK1/JNK signaling pathway[33]. Similarly, Jia et al. discovered that HOXB3 promotes the proliferation, invasion, and migration of TC by mediating the Notch/Wnt/β-catenin pathway, thus mitigating the progression of PE[34]. Our results confirmed that in PE placental tissues, the expression of the pro-apoptotic protein BAX was increased, while the expression of the anti-apoptotic protein BCL2 and the cell proliferation marker PCNA, as well as EMT markers, was decreased. The above evidence suggests that impairment of TC function in the placenta is a significant contributor to PE. Therefore, investigating the key regulatory factors affecting TC function will aid in understanding the physiological and pathological processes involved in placental formation and development, as well as the occurrence and progression of PE.

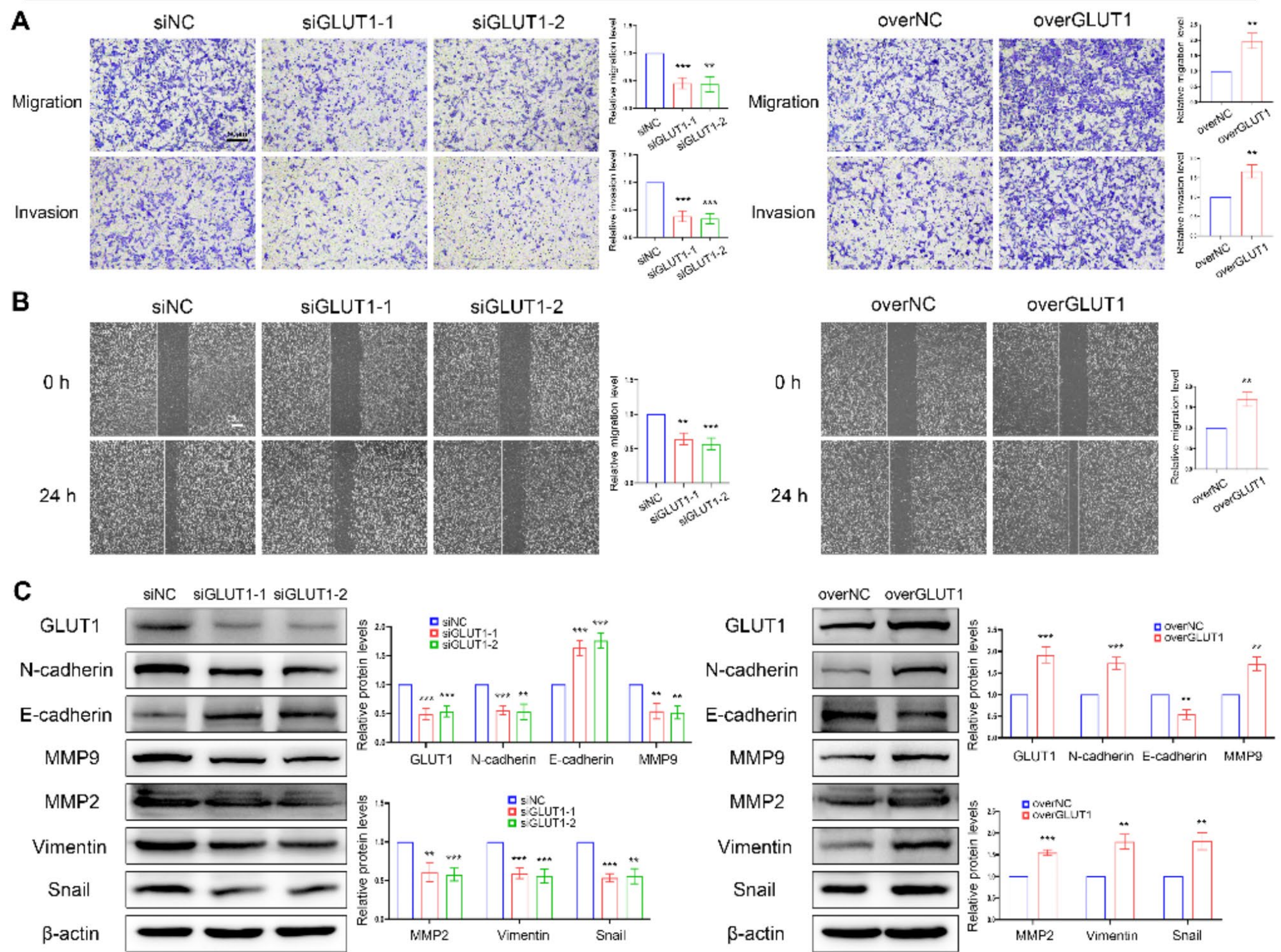


**Fig. 7**. Effects of GLUT1 on the migration and invasion capabilities of HTR-8/SVneo cells. (**A**,**B**): Transwell (**A**) and wound healing assays (**B**) detection of the effect of GLUT1 expression changes on migration and invasion; (**C**) western blot detection of the effect of GLUT1 expression changes on EMT marker expression. (Scale bar = 50 μm, $^{**}P < 0.01$, $^{***}P < 0.001$).

Sugar is the primary source of energy for the human body and an essential precursor for tissue formation, but it also has the role of promoting fat oxidation, conserving protein, and maintaining liver detoxification. Glucose is the most widely distributed and significant monosaccharide in nature. It serves as an energy source for living cells and as an intermediate in metabolism, essential for sustaining normal life activities[35]. However, it has been shown that high concentrations of glucose (HG) can induce the production of inflammatory factors and ROS, thereby causing damage to TC function[36]. Treatment of STBs with HG and insulin has been shown to inhibit the expression of syncytialization markers[37]. Given that HG treatment of TC can induce gestational diabetes-like symptoms, in this study, we opted to treat BeWo cells with lower concentrations of glucose. The results demonstrated that, within a certain concentration range, glucose promoted the expression of syncytialization markers. However, when the glucose treatment concentration reached 30 mM, the cellular syncytialization process was inhibited. These findings are consistent with the results of related research.

Glucose uptake is fundamental for cellular metabolism, growth, and proliferation, and it also serves as the primary substrate for fetal oxidative metabolism. Efficient glucose transport in the placenta is crucial for fetal development. Among the glucose transporter family, GLUT1 has been extensively studied and plays a vital role in placental glucose transport[38]. Our previous study showed that progesterone regulates glucose metabolism through GLUT1 to improve endometrial receptivity[39] and that GLUT1 activates the hexosamine biosynthesis pathway to play an important role during embryo implantation[40]. Abnormal expression of GLUT1 has been reported to cause pregnancy-related diseases. In PE patients, a significant decrease in GLUT1 expression in the decidua tissue has been observed, leading to aberrant glycolysis and destructive decidualization, ultimately resulting in PE[41]. Our results also demonstrate reduced GLUT1 expression in PE placental tissues. Ogura K et al. found that during induced syncytialization of BeWo cells with 8-bromo-cAMP, cellular glucose uptake was increased, and GLUT1 expression was upregulated[21]. The results of this study are consistent with the above conclusions. However, after observing that the changes in GLUT1 expression were synchronized with the degree

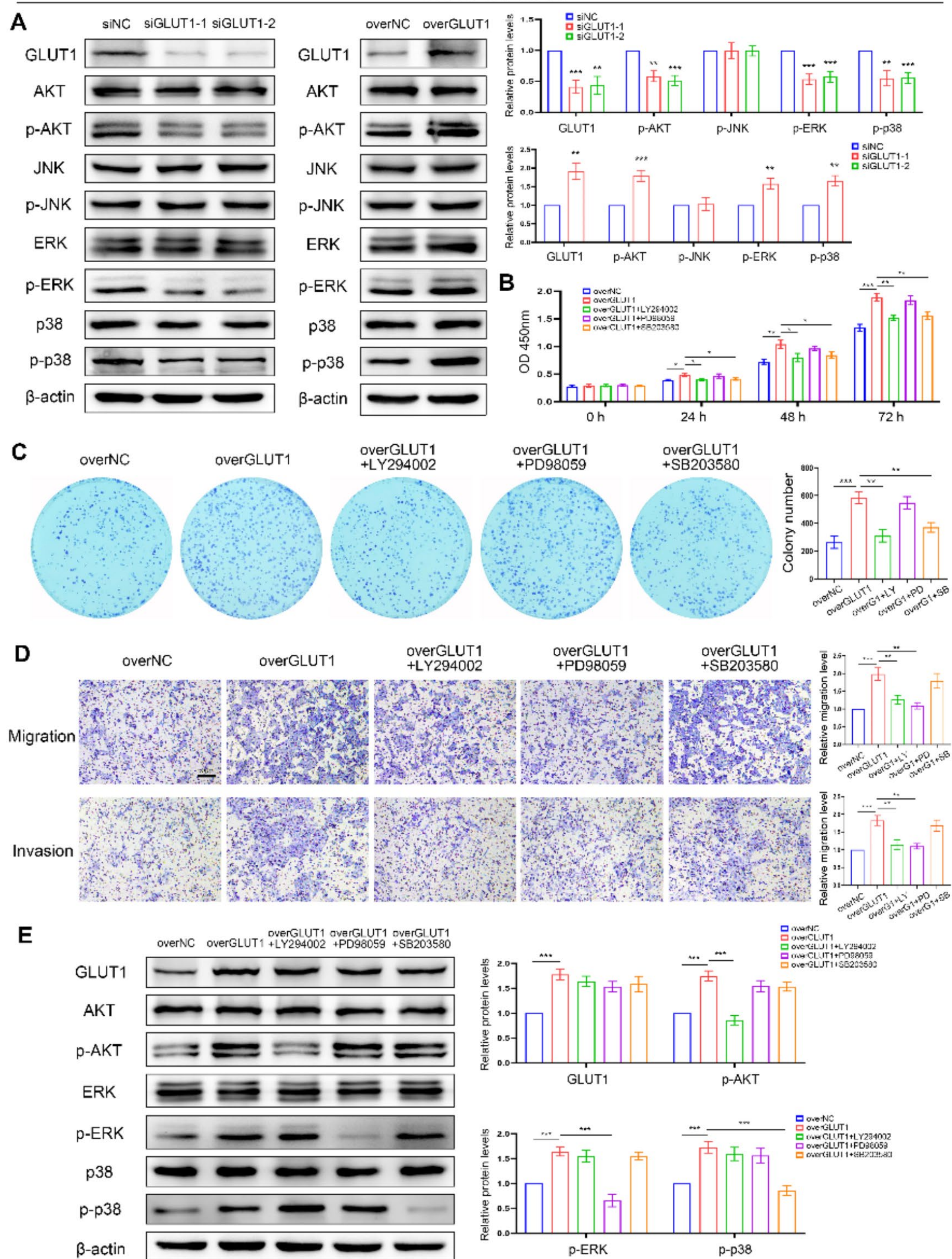


**Fig. 8**. Effects of PI3K/AKT and MAPK signaling pathways on GLUT1 induced proliferation and metastasis in HTR-8/SVneo cells. (**A**) western blot detection of PI3K/AKT and MAPK signaling pathways-related proteins expression after regulated GLUT1 expression; (**B**,**C**) CCK8 (**B**) and colony formation assays (**C**) detection of the effects of transfecting cells with an overexpression plasmid for GLUT1 and adding various signaling pathway inhibitors on proliferation; (**D**) Transwell detection of the effects on migration and invasion; (**E**) western blot detection of the effect on signaling pathways-related proteins expression after adding different signaling pathway inhibitors in overexpress GLUT1 cells. (Scale bar = 50 μm, *$P<0.05$, **$P<0.01$, ***$P<0.001$).

of syncytialization, we further modulated the expression level of GLUT1 and found that it could accelerate the process of TC syncytialization. Research has identified differences in GLUT1 content between the microvillous membrane and basal membrane of STBs[42]. The permeability of the basal membrane and the expression of GLUT1 on its surface are critical regulatory factors affecting transmembrane glucose transport[43,44]. This implies that the localization of GLUT1 in TC varies and its expression is regulated differently, although the exact mechanisms of this regulation remain to be elucidated.

GLUT1 plays a crucial role in the progression of various diseases. Down-regulation of GLUT1 by siRNA can significantly inhibit the invasion of MCF-7 cells and the expression of MMP2 and MMP9[45]. The lncRNA GAL can promote the migration and invasion abilities of colorectal cancer cells by stabilizing GLUT1[46]. GLUT1 is overexpressed in intrahepatic cholangiocarcinoma tissues, associated with shorter survival in patients, and its overexpression promotes proliferation, migration, and invasion of cancer cells[47]. Our results indicate that overexpression of GLUT1 enhances proliferation, migration, and invasion of HTR-8/SVneo cells, as well as the expression of proliferation and EMT markers. Therefore, how does GLUT1 participate in the regulation of cellular biological functions? In fact, GLUT1 not only acts as a glucose transporter but also as a regulatory factor in signal cascades. It has been shown that knockout of GLUT1 inhibits glucose uptake, proliferation, migration, and invasion of triple-negative breast cancer cells through the EGFR/MAPK pathway and the integrin β1/Src/FAK signaling pathway[48]. There is a reciprocal regulation between GLUT1 and the MEK/ERK cascade, and reduced GLUT1 expression can inhibit force-mediated upregulation of the receptor activator nuclear factor-κB ligand RANKL[49]. miR-340 regulates the PI3K/AKT pathway by targeting GLUT1, inhibiting proliferation and inducing apoptosis of bladder cancer cells[50]. In this study, we found that GLUT1 regulated the proliferation, migration, and invasion of HTR-8/SVneo cells by activating the PI3K/AKT and MAPK signaling pathways.

In summary, this study provides new insights into the pathogenesis of PE. We found that GLUT1 expression was reduced in PE placental tissues, and the processes of syncytialization, proliferation, and EMT in TC were impaired. GLUT1 enhances the glucose uptake capacity of BeWo cells, thereby promoting TC syncytialization. Additionally, GLUT1 facilitates the proliferation, migration, and invasion of HTR-8/SVneo cells by modulating the MAPK and PI3K/AKT signaling pathways. However, there are limitations in our study. The mechanisms by which glucose metabolism drives TC syncytialization require further investigation. Moreover, the specific regulatory mechanism between GLUT1 and these signaling pathways remains unclear. These provide the direction for our future work.

## Data availability

The data used and/or analysed during the current study available from the corresponding author on reasonable request.

Received: 24 May 2024; Accepted: 26 September 2024
Published online: 10 October 2024

## References

1. American College of Obstetricians and Gynecologists' Committee on Practice Bulletins—Obstetrics. Gestational hypertension and preeclampsia: ACOG Practice Bulletin, Number 222. *Obstet. Gynecol.* **135** (6), e237–e260 (2020).
2. Magee, L. A. et al. The 2021 International Society for the Study of Hypertension in Pregnancy classification, diagnosis & management recommendations for international practice. *Pregnancy Hypertens.* **27**, 148–169 (2022).
3. Farah, O. et al. Trophoblast lineage-specific differentiation and associated alterations in preeclampsia and fetal growth restriction. *Placenta*. **102**, 4–9 (2020).
4. Melchiorre, K., Giorgione, V. & Thilaganathan, B. The placenta and preeclampsia: villain or victim? *Am. J. Obstet. Gynecol.* **226** (2S), S954–S962 (2022).
5. Aplin, J. D. Developmental cell biology of human villous trophoblast: current research problems. *Int. J. Dev. Biol.* **54** (2–3), 323–329 (2010).
6. Bischof, P. & Irminger-Finger, I. The human cytotrophoblastic cell, a mononuclear chameleon. *Int. J. Biochem. Cell. Biol.* **37** (1), 1–16 (2005).
7. Lager, S. & Powell, T. L. Regulation of nutrient transport across the placenta. *J. Pregnancy*. **2012**, 179827 (2012).
8. Tetro, N. et al. The placental barrier: the Gate and the fate in drug distribution. *Pharm. Res.* **35** (4), 71 (2018).
9. Gauster, M. et al. Factors involved in regulating trophoblast fusion: potential role in the development of preeclampsia. *Placenta*. **30** (Suppl A), S49–54 (2009).
10. Redman, C. W. & Staff, A. C. Preeclampsia, biomarkers, syncytiotrophoblast stress, and placental capacity. *Am. J. Obstet. Gynecol.* **213** (4 Suppl), S9e1–S11 (2015).
11. He, N. et al. Human extravillous trophoblasts penetrate decidual veins and lymphatics before remodeling spiral arteries during early pregnancy. *PLoS One*. **12** (1), e0169849 (2017).
12. Abbas, Y. et al. Investigation of human trophoblast invasion in vitro. *Hum. Reprod. Update*. **26** (4), 501–513 (2020).
13. Davies, E., Pollheimer, J. & Yong, J. Epithelial-mesenchymal transition during extravillous trophoblast differentiation. *Cell. Adh Migr.* **10** (3), 310–321 (2016).
14. Illsley, N. P. et al. Trophoblast invasion: lessons from abnormally invasive placenta (placenta accreta). *Placenta*. **102**, 61–66 (2020).
15. Moser, G. et al. Extravillous trophoblasts invade more than uterine arteries: evidence for the invasion of uterine veins. *Histochem. Cell. Biol.* **147** (3), 353–366 (2017).
16. Burton, G. J. et al. Pre-eclampsia: pathophysiology and clinical implications. *BMJ*. **366**, l2381 (2019).
17. Han, H. S. et al. Regulation of glucose metabolism from a liver-centric perspective. *Exp. Mol. Med.* **48** (3), e218 (2016).
18. Thorens, B. & Mueckler, M. Glucose transporters in the 21st Century. *Am. J. Physiol. Endocrinol. Metab.* **298** (2), E141–E145 (2010).
19. Illsley, N. P. Glucose transporters in the human placenta. *Placenta*. **21** (1), 14–22 (2000).
20. Illsley, N. P. & Baumann, M. U. Human placental glucose transport in fetoplacental growth and metabolism. *Biochim. Biophys. Acta Mol. Basis Dis.* **1866** (2), 165359 (2020).
21. Ogura, K. et al. 8-bromo-cyclicAMP stimulates glucose transporter-1 expression in a human choriocarcinoma cell line. *J. Endocrinol.* **164** (2), 171–178 (2000).
22. Lüscher, B. P. et al. Placental glucose transporter (GLUT)-1 is down-regulated in preeclampsia. *Placenta*. **55**, 94–99 (2017).

23. Wice, B. et al. Modulators of cyclic AMP metabolism induce syncytiotrophoblast formation in vitro. *Exp. Cell. Res.* **186** (2), 306–316 (1990).
24. Butler, T. M. et al. Integrin-linked kinase can facilitate syncytialization and hormonal differentiation of the human trophoblast-derived BeWo cell line. *Reprod. Biol. Endocrinol.* **7**, 51 (2009).
25. Fitzgerald, J. S. et al. Signal transduction in trophoblast invasion. *Chem. Immunol. Allergy*. **88**, 181–199 (2005).
26. Racca, A. C. et al. A novel regulator of human villous trophoblast fusion: the Krüppel-like factor 6. *Mol. Hum. Reprod.* **21** (4), 347–358 (2015).
27. Wang, H. et al. A positive feedback self-regulatory loop between miR-210 and HIF-1α mediated by CPEB2 is involved in trophoblast syncytialization: implication of trophoblast malfunction in preeclampsia†. *Biol. Reprod.* **102** (3), 560–570 (2020).
28. Choi, S. et al. Leveraging optimized transcriptomic and personalized stem Cell technologies to Better Understand Syncytialization defects in Preeclampsia. *Front. Genet.* **13**, 872818 (2022).
29. Bainbridge, S. A. et al. Effects of reduced Gcm1 expression on trophoblast morphology, fetoplacental vascularity, and pregnancy outcomes in mice. *Hypertension*. **59** (3), 732–739 (2012).
30. Vargas, A. et al. Reduced expression of both syncytin 1 and syncytin 2 correlates with severity of preeclampsia. *Reprod. Sci.* **18** (11), 1085–1091 (2011).
31. Zhuang, X. W. et al. Decreased expression and altered methylation of syncytin-1 gene in human placentas associated with preeclampsia. *Curr. Pharm. Des.* **20** (11), 1796–1802 (2014).
32. MacLennan, A. H., Sharp, F. & Shaw-Dunn, J. The ultrastructure of human trophoblast in spontaneous and induced hypoxia using a system of organ culture. A comparison with uultrastructural changes in pre-eclampsia and placental insufficiency. *J. Obstet. Gynaecol. Br. Commonw.* **79** (2), 113–121 (1972).
33. Xu, R. et al. Apelin-36 alleviates LPS-induced trophoblast cell injury by inhibiting GRP78/ASK1/JNK signaling. *Tissue Cell.* **82**, 102057 (2023).
34. Jia, X. et al. HOXB3 promotes trophoblast cell proliferation, invasion, and migration to alleviate preeclampsia via mediating the Notch/Wnt/β-catenin pathway. *Eur. J. Pharmacol.* **960**, 176015 (2023).
35. Dienel, G. A. Brain glucose metabolism: integration of energetics with function. *Physiol. Rev.* **99** (1), 949–1045 (2019).
36. Negi, M. et al. Allopurinol inhibits excess glucose-induced trophoblast IL-1β and ROS production. *Reproduction*. **159** (1), 73–80 (2020).
37. Rogers, L. M. et al. Impact of metabolic stress on BeWo Syncytiotrophoblast function. *Chembiochem*. **24** (24), e202300410 (2023).
38. Sibiak, R. et al. Fetomaternal expression of glucose transporters (GLUTs)-Biochemical, Cellular and clinical aspects. *Nutrients*. **14** (10), 2025 (2022).
39. Zhang, H. et al. Progesterone regulates glucose metabolism through glucose transporter 1 to promote endometrial receptivity. *Front. Physiol.* **11**, 543148 (2020).
40. Zhang, H. et al. O-GlcNAc modification mediates aquaporin 3 to coordinate endometrial cell glycolysis and affects embryo implantation. *J. Adv. Res.* **37**, 119–131 (2021).
41. Yang, M. et al. Dysregulated GLUT1 may be involved in the pathogenesis of preeclampsia by impairing decidualization. *Mol. Cell. Endocrinol.* **540**, 111509 (2022).
42. Joshi, N. et al. Altered expression of nutrient transporters in syncytiotrophoblast membranes in preeclampsia placentae. *Placenta*. **139**, 181–189 (2023).
43. Barta, E. & Drugan, A. Glucose transport from mother to fetus–a theoretical study. *J. Theor. Biol.* **263** (3), 295–302 (2010).
44. Vardhana, P. A. & Illsley, N. P. Transepithelial glucose transport and metabolism in BeWo choriocarcinoma cells. *Placenta*. **23** (8–9), 653–660 (2002).
45. Sun, X. F. et al. High-concentration glucose enhances invasion in invasive ductal breast carcinoma by promoting Glut1/MMP2/MMP9 axis expression. *Oncol. Lett.* **13** (5), 2989–2995 (2017).
46. Li, B. et al. LncRNA GAL promotes colorectal cancer liver metastasis through stabilizing GLUT1. *Oncogene*. **41** (13), 1882–1894 (2022).
47. Tiemin, P. et al. Dysregulation of the miR-148a-GLUT1 axis promotes the progression and chemoresistance of human intrahepatic cholangiocarcinoma. *Oncogenesis*. **9** (2), 19 (2020).
48. Oh, S. et al. Glut1 promotes cell proliferation, migration and invasion by regulating epidermal growth factor receptor and integrin signaling in triple-negative breast cancer cells. *BMB Rep.* **50** (3), 132–137 (2017).
49. Wang, Y. et al. Transcriptional activation of glucose transporter 1 in orthodontic tooth movement-associated mechanical response. *Int. J. Oral Sci.* **10** (3), 27 (2018).
50. Xu, G. et al. Overexpression of miR-340 inhibits cell proliferation and induces apoptosis of human bladder cancer via targeting Glut-1. *BMC Urol.* **21** (1), 168 (2021).


## Author contributions

Y. K.and L.L. S. projected this study. J.Y. P. and Y.Y. L. prepared the manuscript. J.Y. P. and X.X. B. carried out cell experiments. M. L. and J. W. analyzed the data. X.T. L. conducted the clinical studies. H.S. Z. revised the manuscript. All authors reviewed, edited, and approved the manuscript.

## Funding

This study was supported by the National Natural Science Foundation of China (31971209), the Liaoning Revitalization Talents Program (XLYC2211013).

## Declarations

## Competing interests

The authors declare no competing interests.

## Ethics statement

The study was performed in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the First Affiliated Hospital of Dalian Medical University (approval number: PJ-KS-KY-2024-325). All research procedures were carried out in accordance with relevant guidelines and regulations of the Ethics Committee of the First Affiliated Hospital of Dalian Medical University. Informed consent was obtained from all participants and/or their legal guardians for this study.

## Additional information

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1038/s41598-024-74489-z.

**Correspondence** and requests for materials should be addressed to L.S. or Y.K.

**Reprints and permissions information** is available at www.nature.com/reprints.

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

© The Author(s) 2024