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Cdk5 regulates glutamine metabolism in colorectal cancer via the EZH2-GLS1 axis

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Abstract

Colorectal cancer (CRC) is a globally prevalent malignancy that poses a substantial threat to human health. Despite advancements in prevention, diagnosis, and treatment, CRC remains a formidable clinical challenge due to the incomplete elucidation of its pathological mechanisms. Glutamine, an abundant amino acid, exerts pivotal roles in energy production, redox homeostasis, macromolecular biosynthesis, and signal transduction within cancer cells. Elucidating the role of glutamine in CRC pathogenesis is therefore of profound significance. In this study, we investigated the regulatory role of Cyclin-dependent kinase 5 (Cdk5) in glutamine metabolism in CRC, employing both human CRC cell models and murine models. Our findings demonstrated that Cdk5 knockdown accelerated glutamine uptake while suppressing the proliferation of CRC cells. Further exploration of the underlying molecular mechanisms revealed that Cdk5 physically interacts with EZH2. Besides, Cdk5 phosphorylates EZH2 at specific sites, and then the PRC2 complex (centered around EZH2) catalyzes the production of H3K27me3, an inhibitory marker, to regulate the expression of genes involved in glutamine metabolism. At the same time, we also found that modulation of the Cdk5-EZH2 axis alters the epigenetic landscape of genes associated with glutamine transporters and tricarboxylic acid cycle (TCA) enzymes, resulting in reduced mitochondrial activity, impaired glutamine utilization in the TCA cycle, and decreased ATP production—collectively impacting the global glutamine metabolic processes in CRC cells. In in vivo experiments utilizing a murine CRC model, we established five experimental groups. Results showed that Dinaciclib treatment suppressed tumor growth in the CRC model, with this inhibitory effect being further potentiated upon combination with glutamine deprivation. These findings not only uncover the intricate interplay between Cdk5, EZH2, and glutamine metabolism in CRC but also offer novel insights into the pathogenic mechanisms of CRC and identify potential therapeutic targets.

Keywords Glutamine, Colorectal cancer, Cyclin-dependent kinase 5, Mitochondria, Tricarboxylic acid cycle, Glutaminase

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Introduction

Colorectal cancer (CRC) is one of the most common cancers worldwide, with its incidence and mortality rates on the rise. The pathogenesis of CRC is complex and multifactorial, involving genetic mutations, dietary habits, and environmental factors [1]. Despite significant progress in understanding CRC's molecular mechanisms, many aspects remain unclear, leading to challenges in prevention, early detection, and treatment.

Amino acid metabolic reprogramming is a hallmark of cancer cells, with glutamine being the most abundant amino acid in plasma. Glutamine plays a pivotal role in cancer cell proliferation, survival, and metastasis. Previous studies have shown that glutamine promotes cancer cell growth by driving the TCA cycle, supporting DNA replication, and lipid synthesis [2, 3]. Additionally, targeted glutamine metabolism has emerged as a promising therapeutic strategy for cancer treatment [4–6].

Cyclin-dependent kinase 5 (Cdk5), originally discovered in neuronal cells, is known for its role in neuronal development, differentiation, and axon growth [7–9]. Recent studies have implicated Cdk5 in cancer development, with its overexpression associated with tumor proliferation, migration, angiogenesis, chemotherapy resistance, and anti-tumor immunity [10]. Our previous studies have also shown that Cdk5 is overexpressed in CRC tissues and promotes CRC cell proliferation and migration [11].

Given the critical role of glutamine in cancer cell metabolism and the involvement of Cdk5 in cancer development, we hypothesized that Cdk5 might regulate glutamine metabolism in CRC cells. In this study, we aimed to investigate the role of Cdk5 in glutamine metabolism in CRC and its effects on CRC cell growth and metastasis.

Materials and methods

Cell culture

Human CRC cell lines SW480 and DLD-1 were obtained from ATCC and cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco,USA)supplemented with 10% fetal bovine serum (FBS)（BIOEXPLORER,USA）, 100 U/mL penicillin, and 100 mg/mL streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2.

Animal studies

Male C57BL/6 mice (18-22 g, aged 6–8 weeks) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. The mice were housed at the Wenzhou Medical University experimental animal center under specific pathogen-free conditions (12-h light/dark cycle, 21 ± 2 °C, humidity 50%±10%). All animal experiments complied with the guidelines of Wenzhou Medical University and were approved by the Animal Experimental Ethics Committee (wydw2022-0216).

To induce CRC, mice were injected with azoxymethane (AOM; 10 mg/kg, i.p.)(Sigma,USA) and given 2.5% dextran sodium sulfate (DSS)(Aladdin, CHN) in drinking water for 7 days, followed by 2 weeks of regular water. This cycle was repeated three times. Mice were randomly divided into five groups: control($n = 5$), AOM/DSS（$n = 5$）, AOM/DSS+glutamine($n = 5$), AOM/DSS+Dinaciclib($n = 5$), and AOM/DSS+glutamine+Dinaciclib($n = 5$). Glutamine (0.5 g/kg/day) was administered via gavage, and Dinaciclib (40 mg/kg, i.p.) was given twice weekly for 7 weeks, the diet of mice does not contain glutamine. After 12 weeks, the mice were euthanized, weighed, and examined. Tumor-bearing colorectal tissues were fixed in 4% paraformaldehyde（Solarbio, CHN）, and remaining colorectal tissues and cecal contents were stored at −80 °C.

Cell viability

Cell viability was assessed using the MTT assay. Cells were seeded in 96-well plates and treated with different concentrations of glutamine for 24, 48, and 72 hours. MTT (Beyotime,CHN)solution (5 mg/mL) was added, and cells were incubated for 4 hours. Formazan crystals were dissolved in DMSO(Solarbio, CHN), and absorbance was measured at 570 nm using a microplate reader.

Colony-formation assay

Cells were seeded in 6-well plates and cultured for 14 days. Colonies were fixed with methanol, stained with crystal violet, and counted manually.

Wound healing

Cells were scratched with a pipette tip and cultured in medium with or without glutamine. Wound closure was monitored at 0, 24, and 48 hours, and images were captured using a microscope.

Invasion assays

Cells were seeded in the upper chamber of a Boyden chamber coated with Matrigel. Medium containing 20% FBS was added to the lower chamber. After 48 hours, cells that invaded through the Matrigel were stained with crystal violet and counted.

Cell cycle analysis

Cells were treated with glutamine for 48 hours, fixed with 70% ethanol, and stained with propidium iodide (PI). Cell cycle distribution was analyzed using flow cytometry.

Reactive oxygen species (ros) levels

Cells treated with 0 or 2 mmol/L glutamine for 48 h were digested, centrifuged, and suspended in DCFH-DA

（Beyotime,CHN）staining solution (10 μmol/L) at 37 °C for 20 min. Cell suspensions were mixed every 3–5 minutes, washed three times with serum-free medium, and ROS levels were measured using flow cytometry.

Mitochondrial membrane potentials assay

Mitochondrial depolarization was measured using JC-1 (Beyotime, CHN) dye. NC and Cdk5 knockdown cells treated with or without glutamine for 48 h were collected, stained with JC-1 solution for 20 min at 37 °C, washed, and analyzed by flow cytometry.

Atp assay

Cells were treated with glutamine for 48 hours, lysed, and ATP levels were measured using an ATP assay kit (Beyotime, CHN). Data were normalized against protein concentrations and analyzed using GraphPad Prism 6.

Transfection with siRNA

Cells were transfected with specific siRNAs targeting Cdk5, EZH2, or control siRNA using Lipofectamine 3000. Specific siRNA sequences were:

CDK5 siRNA – 1 sense: 5'-GUCGAUGACCAGUUGAAGA(dT)(dT)-3',

antisense:

5'-UCUUCAACUGGUCAUCGAC(dT)(dT)-3';

CDK5 siRNA – 2 sense: 5'-UCUUCAACUGGUCAUCGAC(dT)(dT)-3',

antisense:

5'-ACACUUCAGAAGGUUCUGC(dT)(dT)-3'.

EZH2 siRNA – 1 sense: 5'-GCUCAAGAGGUUCAGACGA(dT)(dT)-3',

antisense:

5'-UCGUCUGAACCUCUUGAGC(dT)(dT)-3';

EZH2 siRNA – 2 sense: 5'-CAGAAACAGCUCUAGACAA(dT)(dT)-3',

antisense:

5'-UUGUCUAGAGCUGUUUCUG(dT)(dT)-3'.

The control siRNA sequences were as follows:

Sense:UUCUCCGAACGUGUCACGUTT;

antisense:

ACGUGACACGUUCGGAGAATT.

Transfection, infection, and stable expression of Cdk5-specific shRNA

Cdk5-specific shRNAs (#1, TRCN0000021465; #2, TRCN0000199652) were purchased from Sigma-Aldrich. VSV-G-pseudotyped lentiviruses were produced in 293T cells by co-transfecting these shRNAs or controls with Lipofectamine 2000, using packaging plasmids. Supernatants were collected, filtered, and used to infect DLD-1 and SW480 cells. Transduced cells were selected with puromycin (1 mg/mL) for 3–4 weeks.

Western blot

Proteins were extracted from cells or tissues, separated by SDS-PAGE, transferred to PVDF membranes, and probed with specific antibodies. Bands were visualized using an enhanced chemiluminescence detection system.

16S rDNA sequencing

Fecal samples from mice were collected, and DNA was extracted using the OMEGA Soil DNA kit. The V3–V4 regions of bacterial 16S rDNA were amplified and sequenced on the Illumina NovaSeq platform. Bacterial gene annotation and subsequent taxonomic analysis were carried out by Shanghai Personalbio Technology Co., Ltd.

2.15 enzyme-linked immunosorbent assay (elisa) for cytokine detection

Blood samples from mice were collected, and plasma was separated. Inflammatory cytokines (IL-6, IL-1β, TNF-α) were measured using ELISA kits.

Hematoxylin-eosin (he) staining and alcian blue-nuclear-fast red staining

He staining

Colon tissues were fixed in 4% paraformaldehyde（Solarbio, CHN）, embedded in paraffin, and sectioned. Sections were stained with hematoxylin and eosin (H&E) or Alcian blue-nuclear-fast red for histological analysis. Histomorphological changes were observed under a microscope, and the tissue was evaluated according to established criteria [12]. Densitometric analysis of the Alcian blue-positive area in the colon was carried out under a microscope.

Cells were fixed, permeabilized, and stained with specific antibodies followed by secondary antibodies conjugated to fluorescent dyes. Images were captured using a confocal microscope.

Quantitative reverse transcription polymerase chain reaction (RT-qPCR)

RNA was extracted from tissues, and cDNA was synthesized using a reverse transcription kit. Inflammatory genes were amplified using specific primers, and expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method. The specific paired primers synthesized by Beijing Qingke Biotechnology Co., Ltd. (Qingke, CHN) were as follows: tumor necrosis factor-α (TNF-α): 5′-TGAGATCCATGCCGTTGGC-3′ (antisense), 5′-CACGTCGTAGCAAACCACC-3′ (sense); interleukin-6 (IL-6): 5′-AAGTGCATCATCGTTCATACA-3′ (antisense), 5′-AGGATACCACTCCCAACAGACC-3′ (sense); interleukin-1β (IL-1β): 5′-TGCTGCGGGATTTGAAGCTG-3′ (antisense), 5′-AATGCCACCTTTTGACAGTGAT-3′ (sense); β-actin: 5′-CGCTCGTTGCCAATAGTG-3′ (antisense), 5′-GCTGTGCTATGTTGCTCTAG-3′ (sense); Ribosomal Protein L13a (RPL13a): 5'-TTGAGGACCTCTGTGTATTTGTCAA-3'

(antisense), 5'-CCTGGAGGAGAAGAGGAAAGAGA-3' (sense); Glutaminase1 （GLS1）: 5'-GCTGTGCTGTAG TCGTATCC-3'(antisense),
5'-GGAGAAGTTCCACCATTGCC-3'(sense).

CUT& RUN-qPCR

Firstly, the ConA Beads Pro magnetic beads were processed, and then cells were collected in a quantity of 5×10^5 cells per sample. After centrifugation and resuspension of the cells, they were incubated with ConA Beads Pro at 4 °C. After incubation, primary antibody and corresponding IgG control were added, and the cells were left to stand overnight at 4 °C. On the second day, the sample was incubated with pA/G-MNase at 4 °C for 1 hour, and then the product was fragmented by adding $CaCl_2$ and digested on ice for 1 hour. Subsequently, the fragmentation was terminated and the product was released for DNA extraction and qPCR quantitative detection. Primers for ChIP-qPCR was amplified with specific primers, and expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method. The specific paired primers synthesized by Beijing Qingke Biotechnology Co., Ltd. (Beijing, China) were as follows: GLS1: 5′-CCGGACAAGCT TCATCCCT-3′(antisense),
5′-CCCTCCCATGGCTTAAGTCG-3′(sense).

Targeted metabolomics analysis

Select five normal human colorectal cancer SW480 cells and five SW480 CDK5 gene knockout cells, then thaw the cell samples at 4 °C, mix with acetonitrile, vortex, and centrifuge at 4 °C. Dry the upper organic layer with nitrogen, treat with benzyl alcohol and trimethylchlorosilane, derivatize, and then mix with tetramethylammonium fluoride and acetonitrile. After centrifugation, the supernatant was analyzed using CalQuant-S HPLC/MS at a column temperature of 40 °C; Flow rate 0.4 mL/min; The injection volume is 5 μ L, and the mobile phase composition is A: 0.1% formic acid water, B: 0.1% formic acid acetonitrile. Finally, the data is normalized to cell counts.

Statistical analysis

Results are expressed as mean ± SD. Statistical significance was determined by Student's t-test or one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test using SPSS 24.0 and GraphPad Prism 8.02. Statistical significance was set at $p < 0.05$.

Results

Glutamine promotes the proliferation and clonal formation of colorectal cancer cells

To investigate the effect of glutamine on the growth of colorectal cancer cells, SW480 and DLD-1 cells were treated with 0, 0.2, 0.6, 2, and 4 mmol/L of glutamine. MTT and clonal formation assays were employed to assess the impact of glutamine on cell proliferation and colony formation at 24, 48, and 72 h. As shown in Fig. 1, colorectal cancer cells treated with various concentrations of glutamine exhibited a significant increase in both proliferation capacity and the number of clones compared to those treated without glutamine. This indicates that glutamine promotes the proliferation of colorectal cancer cells. Additionally, MTT and clonal formation experiments demonstrated that the growth of both colorectal cancer cell lines was optimal at a 2 mmol/L glutamine concentration. Therefore, this concentration was selected for subsequent treatments.

Cdk5 knockdown inhibits the proliferative ability of glutamine to colorectal cells

Cdk5, a serine/threonine kinase, was hypothesized to influence glutamine dependency in colorectal cancer cells. To test this, we generated SW480 and DLD-1 stable cell lines with Cdk5 knockdown using Cdk5 shRNA. Both normal colorectal cancer cells and Cdk5 knockdown cells were treated with 2 mmol/L glutamine. MTT assay results indicated that the proliferative effect of glutamine was significantly reduced in SW480 and DLD-1 cells with Cdk5 knockdown compared to their respective control cells (Fig. 2A). Similarly, clonal formation assays revealed that Cdk5 knockdown significantly diminished glutamine's ability to promote clonogenesis in both cell lines (Fig. 2B).

Cdk5 knockdown inhibits glutamine's ability to promote cell cycle progression

To explore the impact of Cdk5 knockdown on cell cycle progression, flow cytometry analysis was performed on SW480, DLD-1, and Cdk5 knockdown cells treated with either 0 or 2 mmol/L glutamine. As shown in Fig. 3, in the control cell group treated with 2 mmol/L glutamine, there was a significant decrease in the G0/G1 phase and an increase in the S + G2/M phases, indicating that glutamine promotes cell cycle progression. However, in Cdk5 knockdown cells, the ability of glutamine to enhance cell cycle progression was inhibited. Specifically, the proportion of cells in the S + G2/M phases was lower compared to the control group, indicating that Cdk5 knockdown impairs glutamine's ability to promote cell cycle progression.

Cdk5 knockdown inhibits glutamine's ability to promote colorectal cancer cell migration

Cell migration and invasion are critical factors in tumor metastasis. To assess the effect of glutamine on the migration and invasion of colorectal cancer cells (DLD-1 and SW480), we performed scratch and transwell assays (Fig. 4A–B). The results indicated that scratch healing was significantly impaired and the number of cells

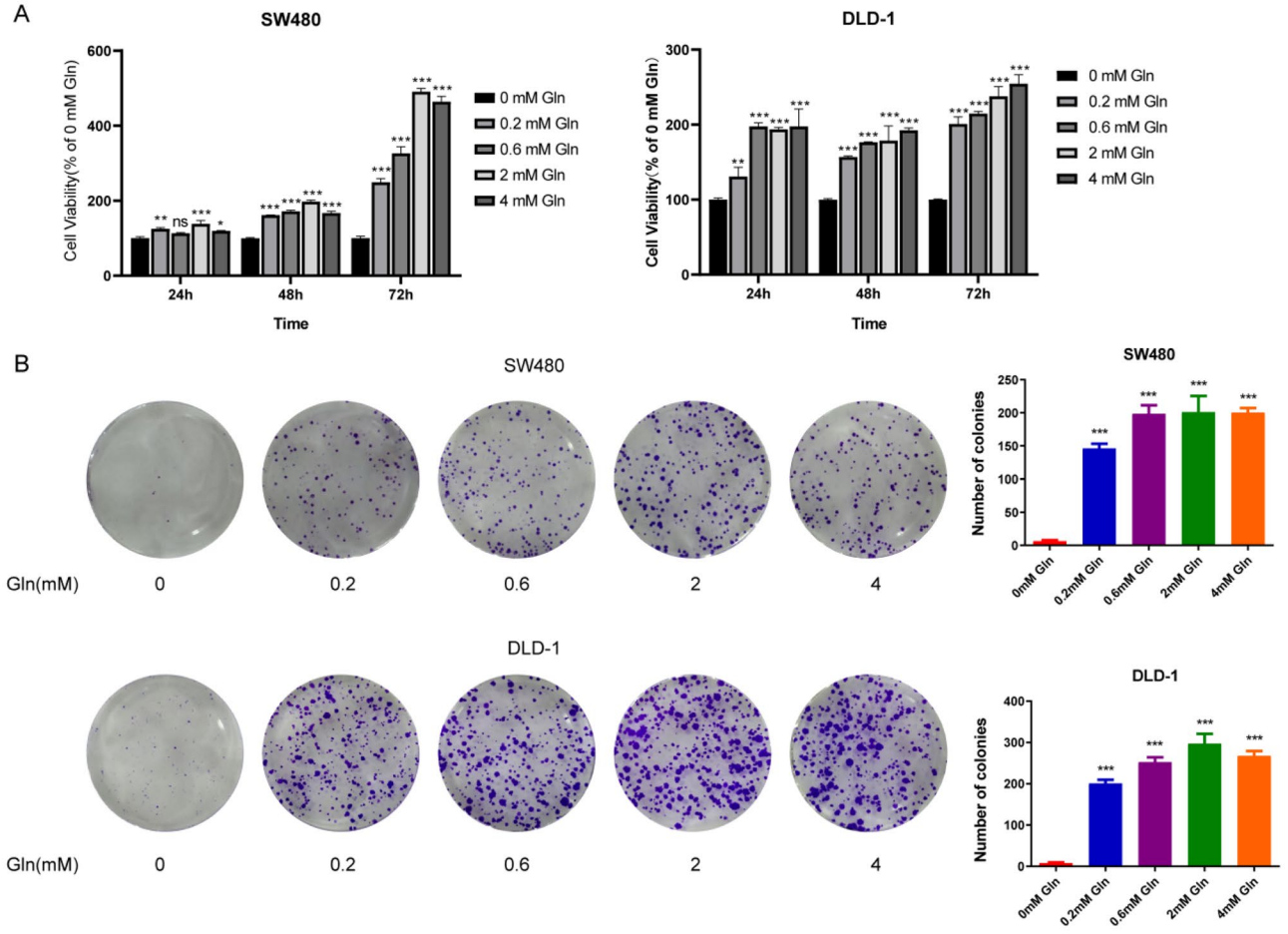

Fig. 1 Glutamine promotes the proliferation and clone formation ability of colorectal cancer cells. (**a**) MTT results of SW480, and DLD-1 cells treated with glutamine. (**b**) results of colony formation experiment of DLD-1 and SW480 treated with different concentrations of glutamine. Data from at least three independent experiments are expressed as mean ± sd. ns: $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

migrating into the subventricular chamber was notably reduced in the absence of glutamine. Additionally, Cdk5 knockdown cells showed a marked decrease in migration ability under the same glutamine treatment, with statistically significant differences observed. Furthermore, the migration-promoting effect of 2 mmol/L glutamine on SW480 and DLD-1 Cdk5 knockdown cells was significantly reduced compared to normal cells, suggesting that Cdk5 knockdown significantly inhibits glutamine's ability to promote colorectal cancer cell migration.

Cdk5 knockdown inhibits glutamine activation of IL-6/STAT3 and Wnt/β-catenin pathways

Given the role of Cdk5 and glutamine in colorectal cancer cell proliferation, and evidence that glutamine can modulate IL-6/STAT3 and Wnt/β-Catenin signaling pathways [13, 14], we conducted Western blot analysis to assess the expression of pathway-related proteins including β-catenin, STAT3, GLS1, IL-6, Cyclin D1, and C-MYC. As shown in Fig. 5A–B, there were no significant differences in the expression levels of IL-6/STAT3 and Wnt/β-Catenin signaling pathway proteins between NC cells and Cdk5 knockdown cells in the absence of glutamine. However, glutamine treatment promoted the expression of these signaling components, activating IL-6/STAT3 and Wnt/β-Catenin pathways. The levels of β-Catenin, Cyclin D1, P-STAT3, GLS1, IL-6, and C-MYC were significantly lower in Cdk5 knockdown cells compared to NC cells. Additionally, confocal microscopy showed that glutamine treatment enhanced the nuclear translocation of β-Catenin, while Cdk5 knockdown inhibited this process (Fig. 5C). These findings suggest that glutamine promotes colorectal cancer cell proliferation by activating IL-6/STAT3 and Wnt/β-Catenin signaling pathways through Cdk5.

Glutamine aggravated colorectal cancer tumor formation in C57BL/6J mice, and Cdk5 inhibitor dinaciclib inhibited the effect of glutamine

To verify the role of glutamine in colorectal cancer development, we established a mouse model of primary colorectal cancer using AOM+DSS [15]. Mice

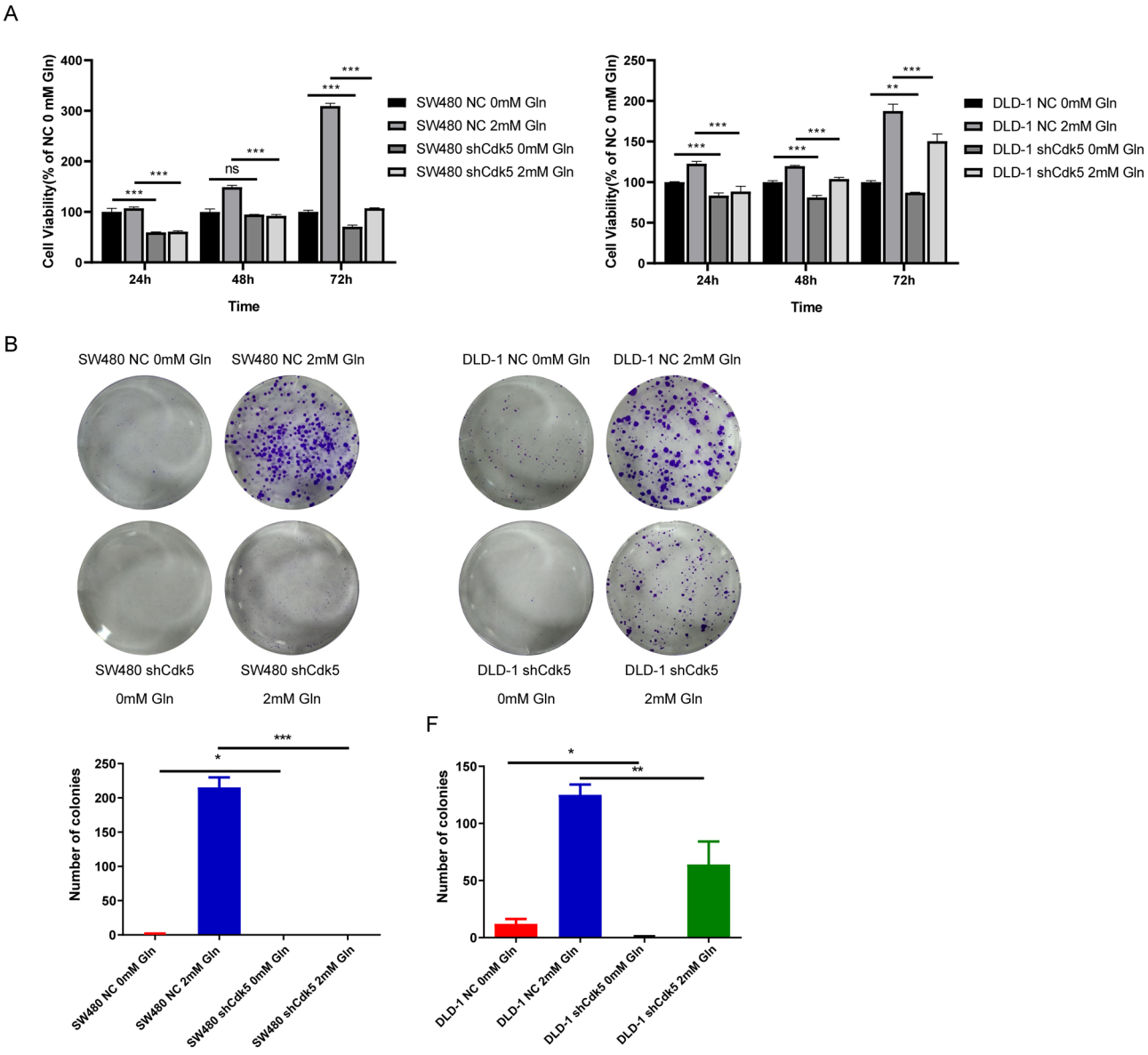

Fig. 2 Cdk5 knockdown inhibited glutamine's ability to promote cell proliferation and clone formation. (**a**) MTT results of SW480, DLD-1, SW480 shCdk5 and DLD-1 shCdk5 cells treated with different concentrations of glutamine. (**b**) clonal formation of SW480, DLD-1, SW480 shCdk5 and DLD-1 shCdk5 cells treated with different concentrations of glutamine. Data from at least three independent experiments are expressed as mean ± sd. ns: $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

were administered glutamine and the Cdk5 inhibitor Dinaciclib orally. As shown in Fig. 6A, DSS treatment resulted in significant weight loss in the model group, which was exacerbated by glutamine. Figure 6C illustrates rectal bleeding in each group, with the AOM/DSS group showing bleeding and the AOM/DSS+Glutamine group exhibiting more severe symptoms. The AOM/DSS+Glutamine+Dinaciclib group showed improved rectal bleeding, indicating that Dinaciclib positively influences the physiological state of the cancer mice.

Further evaluation of colorectal tumor formation involved dissection and observation of complete colorectal tissues. Figure 6B shows that the colorectal length was similar across all groups without significant statistical differences. Tumors primarily formed in the rectal segment (Fig. 6D). Mice in the glutamine intervention group had a higher number of and larger tumors compared to the AOM/DSS group, while Dinaciclib treatment significantly reduced tumor formation, consistent with the observed physiological changes. These results suggest that glutamine accelerates colorectal cancer tumor formation in C57BL/6J mice, while Dinaciclib mitigates this effect.

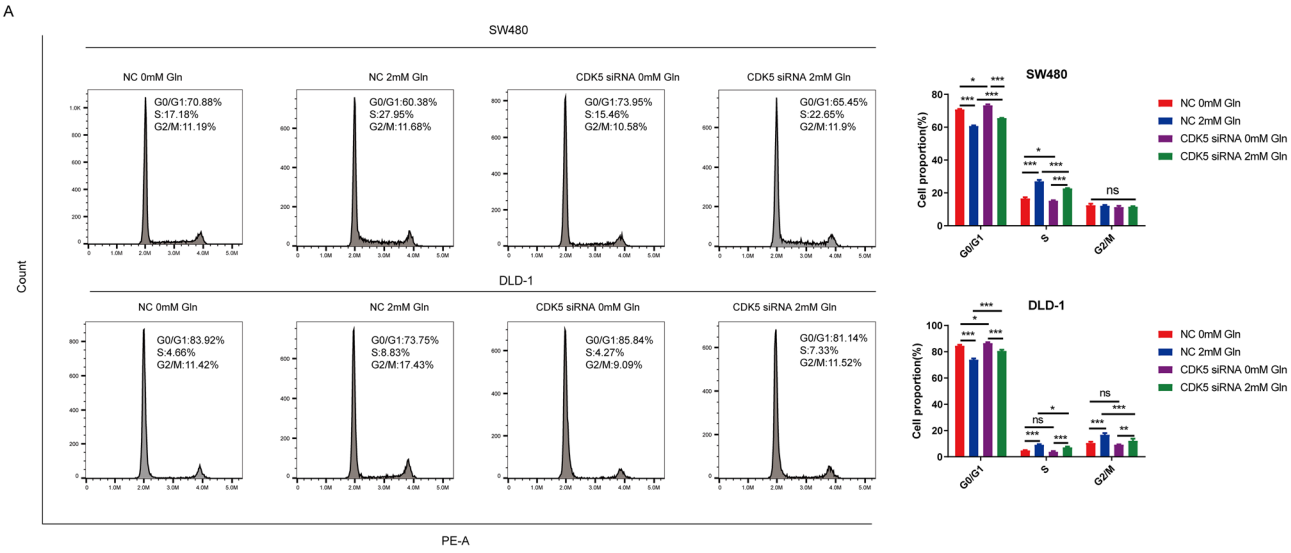

Fig. 3 Cdk5 knockdown inhibits glutamine's ability to promote cell cycle progression. (**a**) cell cycle results of SW480, DLD-1, SW480 knockdown, and DLD-1 knockdown cells treated with different concentrations of glutamine. Data from at least three independent experiments are expressed as mean ± sd. ns: $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Glutamine can up-regulate the expression of intestinal proliferative protein to accelerate the cancer process, and dinaciclib can inhibit the over-activation of proliferative pathways

To investigate the molecular mechanisms behind tumor formation differences among various mouse groups, we extracted proteins from tumor-containing intestinal tissues and assessed the expression levels of related proteins using Western blotting. As shown in Fig. 7A, the expression levels of cell proliferation and cycle-related proteins—P-STAT3, β-Catenin, Cdk5, Cyclin D1, and C-MYC—were elevated in the AOM/DSS group. Notably, these protein levels were further increased in the glutamine-treated group compared to the AOM/DSS group. Dinaciclib intervention significantly alleviated this effect. These results suggest that glutamine affects proliferation-related proteins through Cdk5, thereby influencing tumor formation, which aligns with our cell experiment findings.

Glutamine can increase the level of inflammation in the tumor and its peripheral tissues of colorectal cancer mice, and dinaciclib can inhibit the proinflammatory effect of glutamine

Chronic intestinal inflammation substantially raises the risk of colorectal cancer, with a progressive transition from enteritis to colorectal cancer. To assess inflammatory factor expression in different mouse groups, we used Western blotting and extracted RNA from tumor-containing intestinal tissues for cDNA synthesis and real-time fluorescence quantitative PCR. As shown in Fig. 8A–B, protein and mRNA levels of inflammatory factors IL-1β, TNF-α, and IL-6 were significantly higher in AOM/DSS group mice. Glutamine treatment further increased these inflammatory factors, while Dinaciclib+Glutamine group showed reduced levels, indicating Dinaciclib's ability to mitigate glutamine-induced inflammation. Additional ELISA results (Fig. 8C) confirmed that serum levels of IL-6, IL-1β, and TNF-α were elevated in the AOM/DSS group compared to the control group. Glutamine treatment further increased serum inflammatory factors. Thus, glutamine exacerbates inflammation in colorectal cancer mice, and Dinaciclib can counteract this effect.

Glutamine aggravated intestinal barrier destruction in mice with colorectal cancer, while Dinaciclib helped restore intestinal epithelial morphology

To evaluate the impact of glutamine and Cdk5 on intestinal tissue morphology and inflammation, we performed HE staining and Alcian blue staining on intestinal tissues. As depicted in Fig. 9A, the colonic mucosal structure in the Control group was intact with a uniform crypt structure. In contrast, AOM/DSS group mice displayed significant intestinal damage, including abnormal crypts, damaged epithelial cells, and inflammatory cell infiltration. Glutamine treatment worsened intestinal damage, while Dinaciclib reduced dysplasia and structural destruction. Alcian blue staining (Fig. 9B) showed that Dinaciclib mitigated glutamine-induced damage to the mucous layer. Western blot analysis revealed decreased expression of MUC2 and tight junction proteins (ZO-1 and Occludin) in the AOM/DSS group, further exacerbated by glutamine treatment, but attenuated by Dinaciclib (Fig. 9C). These findings indicate that glutamine worsens intestinal damage during colorectal cancer

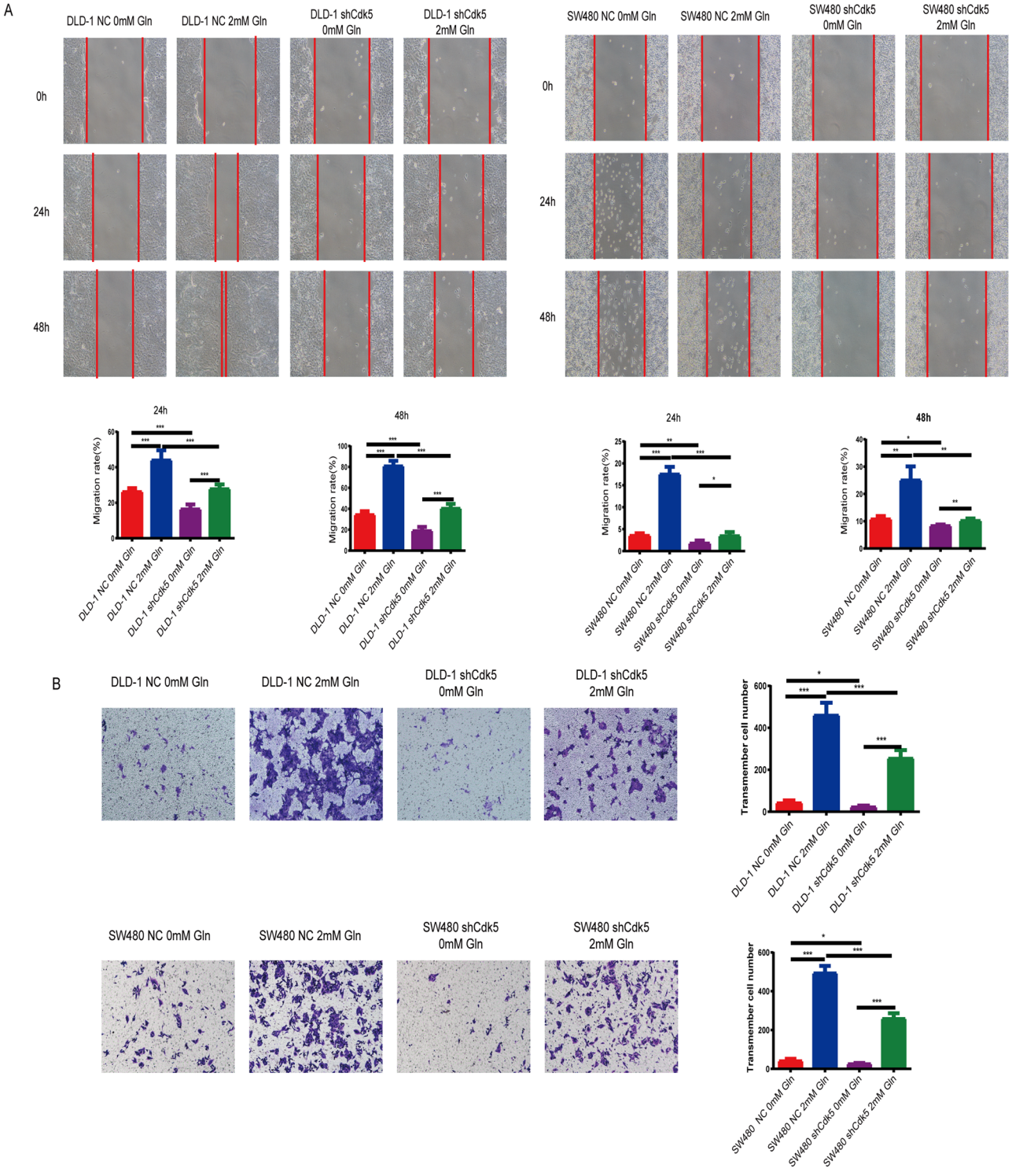

Fig. 4 Cdk5 knockdown inhibited the ability of glutamine to promote cell migration and invasion. (**a**) results of wound healing experiment of SW480, DLD-1, SW480 knockdown and DLD-1 knockdown cells treated with different concentrations of glutamine. (**b**) Transwell assay results of SW480, DLD-1, SW480 knockdown and DLD-1 knockdown cells treated with different concentrations of glutamine. Data from at least three independent experiments are expressed as mean ± sd. ns: $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

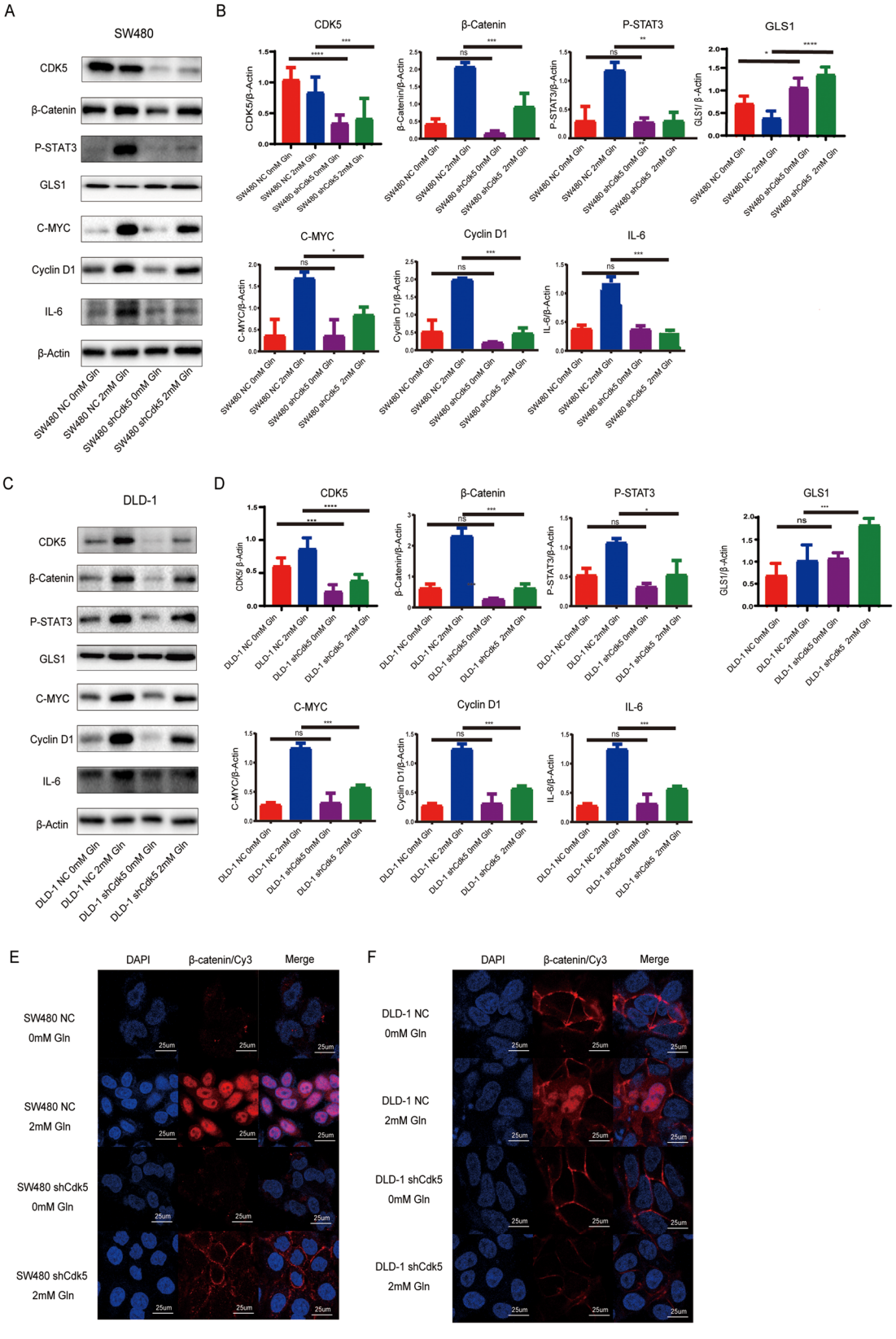

Fig. 5 (See legend on next page.)

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Fig. 5 Cdk5 knockdown inhibits glutamine activation of IL-6/P-STAT3 and Wnt/β-catenin signaling pathways. (**a**) expression of IL-6/P-STAT3 and Wnt/β-catenin signaling pathway proteins in SW480 and SW480 knockdown cells treated with different concentrations of glutamine. (**b**) expression of IL-6/P-STAT3 and Wnt/β-catenin signaling pathway proteins in DLD-1 and DLD-1 knockdown cells treated with different concentrations of glutamine. Data from at least three independent experiments are expressed as mean ± sd. (**c**) glutamine influences cellular localization of β-catenin via Cdk5. Cy: cyanine; DAPI:4′,6-diamidino-2-phenylindole. ns: $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

development, while Dinaciclib helps restore epithelial integrity.

Glutamine gavage leads to the enrichment of harmful flora during the development of colorectal cancer, while dinaciclib helps to maintain normal flora structure

The gut contains a vast array of microorganisms, primarily bacteria, which interact with the host and play a crucial role in colorectal cancer development [16]. To evaluate glutamine's impact on intestinal flora composition, we performed 16S rDNA sequencing on stool samples from each mouse group. Alpha-diversity analysis using Chao1, Shannon, and Simpson indices (Fig. 10A) showed no significant differences between groups. However, β-diversity analysis using the Jaccard distance algorithm and principal coordinate analysis (PCoA) (Fig. 10B) revealed distinct segregation in gut microbiota distribution among groups. At the phylum level (Fig. 10C), Firmicutes, which include beneficial bacteria, were less abundant in the AOM/DSS+Glutamine group compared to other groups. At the genus level (Fig. 10D–F), beneficial bacteria *Lactobacillus* and *Odoribacter* were significantly reduced in both AOM/DSS and AOM/DSS+Glutamine groups, while harmful bacteria *Turicibacter* and *Desulfovibrio* were more abundant. Dinaciclib helped restore bacterial community balance by counteracting glutamine's effects. These results suggest that glutamine alters specific taxa at both phylum and genus levels by up-regulating pro-inflammatory bacteria and down-regulating beneficial ones, an effect attenuated by Dinaciclib.

Cdk5 knockdown up-regulates the expression of glutamine transporter (ASCT2) and glutaminase (GLS1)

Alanine-serine-cysteine transporter 2 (ASCT2) is a key glutamine transporter in the ASC family, regulating intracellular glutamine levels [17]. Glutaminase 1 (GLS1) is the rate-limiting enzyme in glutamine catabolism, converting glutamine to glutamic acid, which is then converted to α-ketoglutaric acid by glutamate dehydrogenase (GLUD1) and participates in the tricarboxylic acid cycle [2, 18]. Preliminary results showed that Cdk5 knockdown significantly inhibited glutamine-induced proliferation in colorectal cancer cells. To explore Cdk5's mechanism, we assessed protein levels of ASCT2, GLS1, and GLUD1. Results (Fig. 11A) indicated increased expression of ASCT2 and GLS1 in Cdk5 knockdown cells, with no change in GLUD1. GLS1 activity was also higher in Cdk5 knockdown cells compared to NC cells (Fig. 11B). These findings suggest that Cdk5 influences GLS1 activity and glutamine's impact on colorectal cancer development, warranting further exploration of how Cdk5 regulates GLS1 protein expression.

Cdk5 knockdown indirectly regulates the expression of glutaminase GLS1 via EZH2

Previous research shows that Cdk1 and Cdk2 can regulate and activate EZH2, with their knockdown inhibiting EZH2 expression [19, 20]. EZH2 deletion up-regulates GLS1 expression [21]. We examined the relationship between Cdk5, EZH2, and GLS1 using siRNA transfection and Western blot analysis. Results (Fig. 12A–B) showed decreased EZH2 protein levels in Cdk5 knockdown cells. Knockdown of EZH2 with siRNA increased GLS1 protein levels, consistent with previous findings. Immunoprecipitation experiments (Fig. 12C–D). Meanwhile, we detected the expression level of H3K27me3 in EZH2 knockdown cells, and the results (Fig. 12E–F) showed a decrease in H3K27me3 level. Knocking out EZH2 with siRNA increased the level of GLS1 protein, which is consistent with previous findings. The immunoprecipitation experiment (Fig. 12G–H) showed that Cdk5 interacts with EZH2 and that EZH2 interacts with both Cdk5 and GLS1, suggesting Cdk5 regulates GLS1 expression through EZH2.

To further explore the regulatory mechanism of EZH2 on GLS1, we detected the expression levels of GLS1 and EZH2 in nuclear and cytoplasmic proteins of NC and EZH2 KD cells respectively. Results showed that both nuclear and cytoplasmic GLS1 levels increased (Fig. 13A–B). Next, we examined GLS1 mRNA expression, which revealed a marked upregulation in EZH2 KD cells—with the protein level increase consistent with the mRNA expression trend (Fig. 13C). This preliminarily indicated that EZH2 regulates GLS1 at the transcriptional level. To confirm the epigenetic basis of this regulation, we employed CUT&RUN qPCR to detect H3K27me3 enrichment at the GLS1 promoter. EZH2 KD cells exhibited reduced H3K27me3 enrichment at the GLS1 promoter, whereas NC cells maintained relatively high enrichment (Fig. 13D). Collectively, these experiments demonstrate that EZH2 regulates GLS1 via

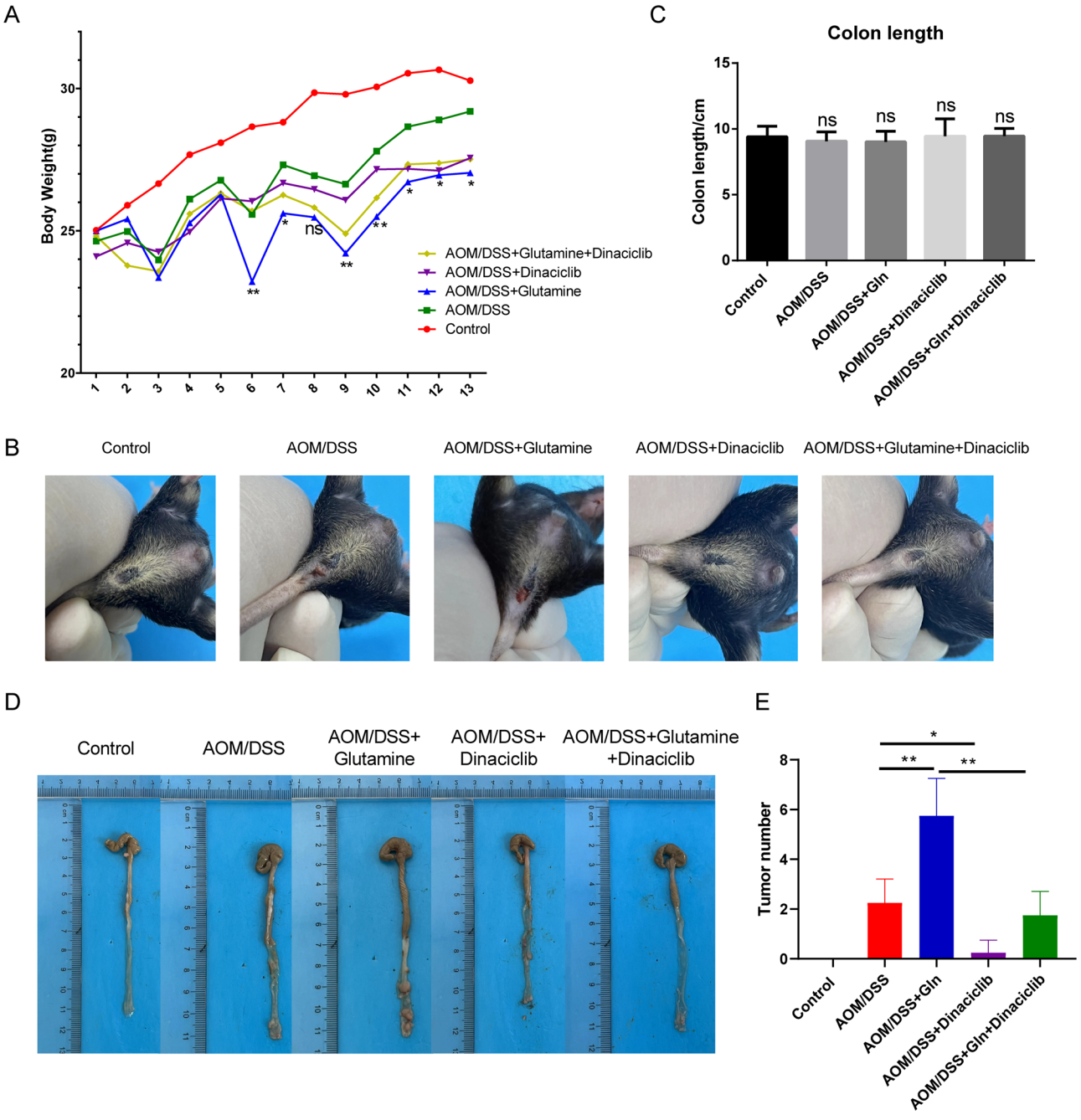

Fig. 6 Glutamine can aggravate colorectal cancer induced by AOM/DSS, and dinaciclib can inhibit the effect of glutamine. (**a**) body weight analysis of five groups of mice. (**b**) perianal appearance of five groups of mice. (**c**) colon length statistics of five groups of mice. (**d**) colorectal images of five groups of mice. (**e**) number of colorectal tumors in five groups of mice. Data from at least three independent experiments are expressed as mean ± sd. ns: $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

transcriptional repression: EZH2 inhibits GLS1 transcription through PRC2-catalyzed H3K27me3 deposition at the GLS1 promoter, thereby leading to increased GLS1 mRNA and protein levels upon EZH2 knockdown. Additionally, the concurrent upregulation of GLS1 in both nucleus and cytoplasm suggests a potential post-translational regulatory mechanism, indicating that these two regulatory modes may coexist.

Cdk5 knockdown affects the utilization of glutamine in colorectal cancer cells by inhibiting mitochondrial function

Mitochondria are crucial for ATP production, with glutamine entering the tricarboxylic acid (TCA) cycle to generate NADH and FADH2, which drive ATP synthesis [22]. Despite increased ASCT2 and GLS1 protein expression in Cdk5 knockdown cells, ATP production was reduced (Fig. 14A). Live cell staining with Mito-Tracker

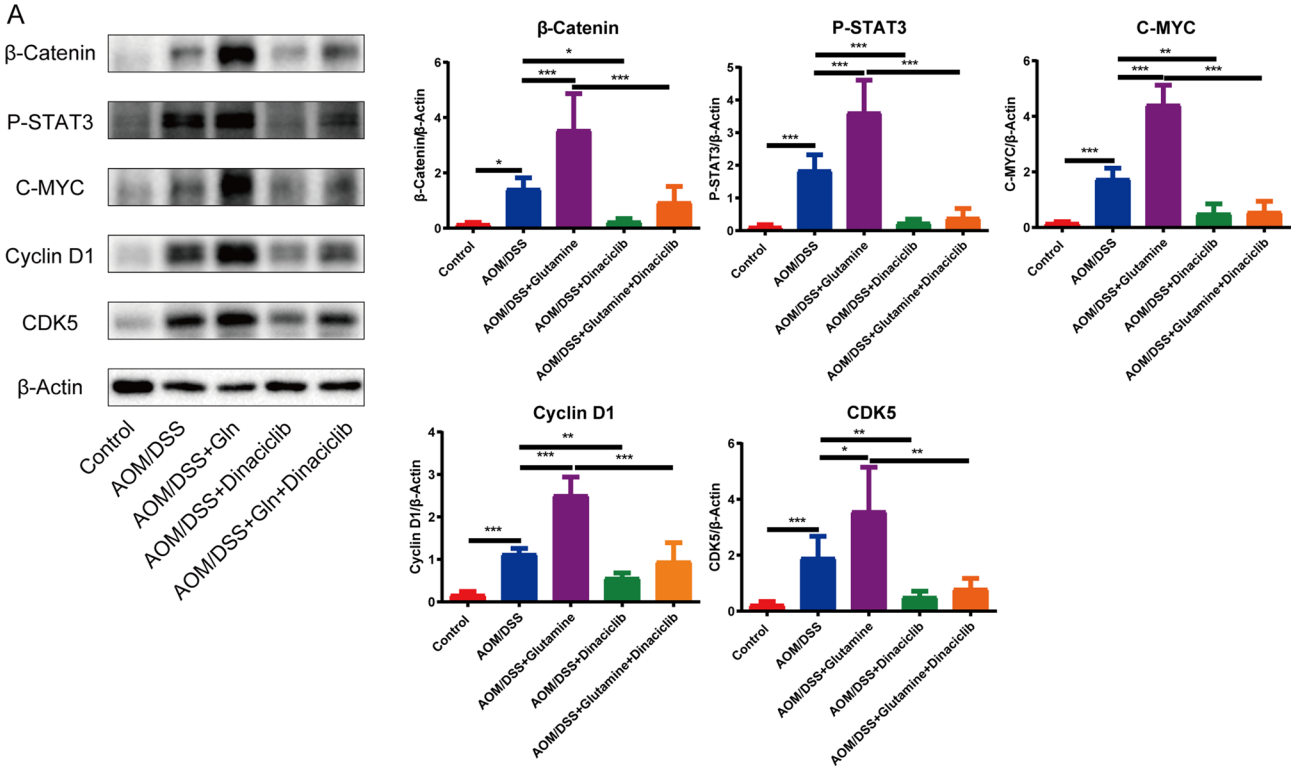

Fig. 7 Glutamine upregulated the expression level of value-added protein in the intestinal tissues of colorectal cancer mice, while Dinaciclib inhibited the effect of glutamine. (**A**) Western Blot results and statistical analysis of the expression level of value-added protein in mouse intestinal tissue. Data from at least three independent experiments are expressed as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

Red CMXRos and JC-1 probe showed decreased mitochondrial function and membrane potential (Fig. 14B–C). Elevated mitochondrial reactive oxygen species (ROS) levels were observed in Cdk5 knockdown cells, potentially compromising cellular structure and function (Fig. 14D). These findings suggest that Cdk5 knockdown impairs mitochondrial activity and decreases ATP production.

Cdk5 knockdown inhibited the protein expression of some tricarboxylic acid cycle metabolism enzymes

The TCA cycle is essential for energy production and biosynthetic intermediates [23]. Although the TCA cycle does not directly consume molecular oxygen or produce large amounts of ATP, the generated electrons are transferred to the electron transport chain for substantial ATP production [24]. Changes in the TCA cycle impact tumorigenesis and inflammation. We assessed protein expression levels of key TCA cycle enzymes—citrate synthase (CS), isocitrate dehydrogenase (IDH1, IDH2, IDH3), and α-ketoglutarate dehydrogenase complexes (OGDH, DLDH, DLST) (Fig. 15A). Results showed decreased CS expression and increased IDH2 expression in Cdk5 knockdown cells, with no change in DLDH and DLST. Thus, Cdk5 knockdown inhibits some TCA cycle enzyme expressions and the TCA cycle itself.

The metabolite content of the tricarboxylic acid cycle decreased in Cdk5 knockdown cells, while glutamate increase

We used HPLC-MS to analyze intracellular amino acids and TCA cycle metabolites. The results showed significant metabolic differences between SW480 NC and Cdk5 knockdown cells (Fig. 16A). Targeted amino acid detection (Fig. 16B–D) revealed increased glutamate content in Cdk5 knockdown cells ($p < 0.01$, Fig. 16C), consistent with increased ASCT2 and GLS1 protein levels. TCA cycle metabolites (citric acid, cis-aconitic acid, isocitric acid, fumaric acid, malic acid) were significantly reduced in Cdk5 knockdown cells, indicating that Cdk5 knockdown enhances glutaminolysis while inhibiting the TCA cycle and ATP production.

Discussion

Cancer cells are characterized by metabolic reprogramming, such as the Warburg effect and heightened dependence on glutamine [2, 25]. While glucose has traditionally been the focal point of tumor metabolism

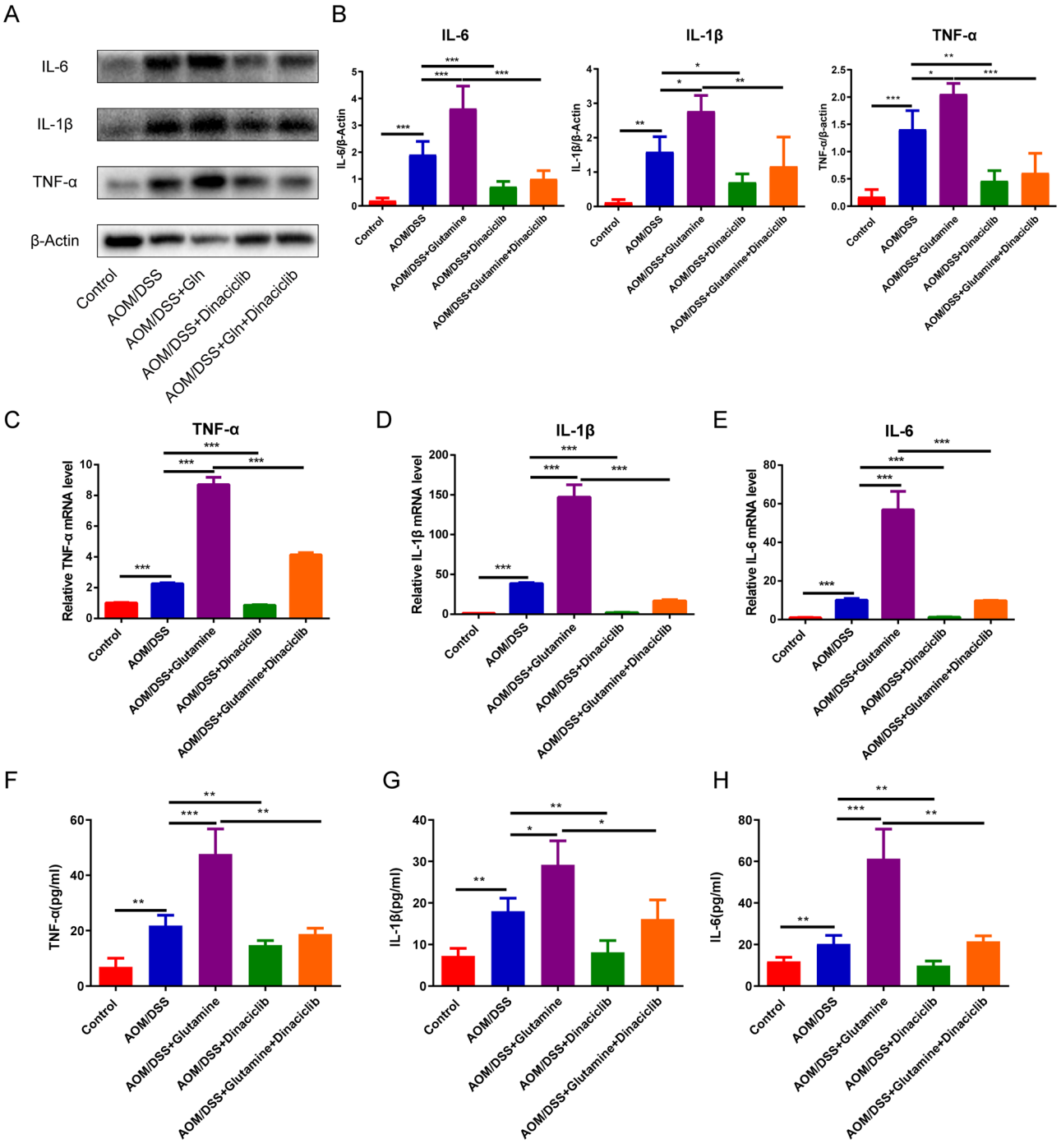

Fig. 8 Glutamine increased the expression of inflammatory factors in colorectal cancer mice, while dinaciclib inhibited the proinflammatory effect of glutamine. (**a**) expression levels of inflammatory cytokines IL-6, IL-1β and TNF-α in colon tissue of mice in each group. mRNA relative expression levels of inflammatory factors IL-6, IL-1β and TNF-α in colon tissue of mice in each group. (**c**) the contents of inflammatory factors IL-6, IL-1β and TNF-α in serum of mice in each group. Data from at least three independent experiments are expressed as mean ± sd. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

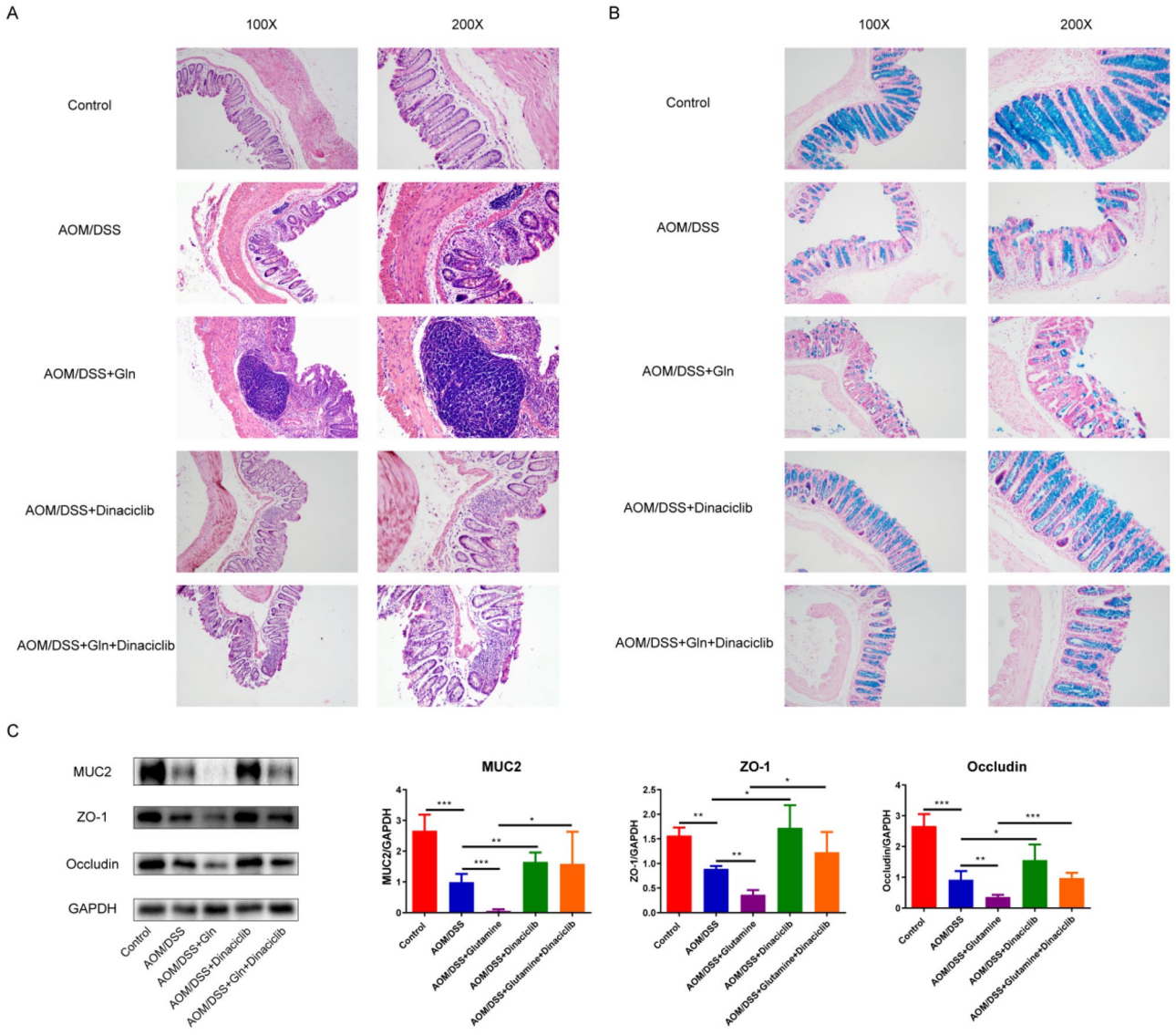

Fig. 9 Glutamine aggravated intestinal barrier damage in mice with colorectal cancer, and Dinaciclib helped restore intestinal epithelium. (**a**) he staining results of mice colon tissue. (**b**) results of Alcian blue staining of mouse colon tissue. (**c**) Representative images of colonic ZO-1, Occludin and claudin-1 in the colon from Western blot analysis. Data from at least three independent experiments are expressed as mean ± sd. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

research, the role of amino acid metabolism, particularly glutamine catabolism, has gained attention over the past decade [26]. Glutamine is vital for various cellular processes, including nitrogen metabolism, the tricarboxylic acid (TCA) cycle, glutathione production, and nucleotide and lipid synthesis [26]. Studies have shown that many cancer cells have an oncogene-dependent addiction to glutamine, making it a critical nutrient for tumor growth [27, 28]. Our functional tests with colorectal cancer cell lines DLD-1 and SW480 confirmed that glutamine promotes cell proliferation and clonogenicity (Fig. 1).

Research indicates that inhibition of Cdk4/6 in melanoma cells leads to increased intracellular glutamate levels and enhanced glutamine pathway activity [29], suggesting a role for the Cdk family in glutamine metabolism. As a member of the Cdk family, Cdk5 has been shown to regulate metabolic reprogramming, including glucose metabolism [30]. We explored whether Cdk5 also impacts glutamine metabolism and its effects on cancer cells. Our results revealed that Cdk5 knockdown inhibited glutamine-induced proliferation and cell cycle progression (Figs. 2, 3). Western blot analysis indicated that

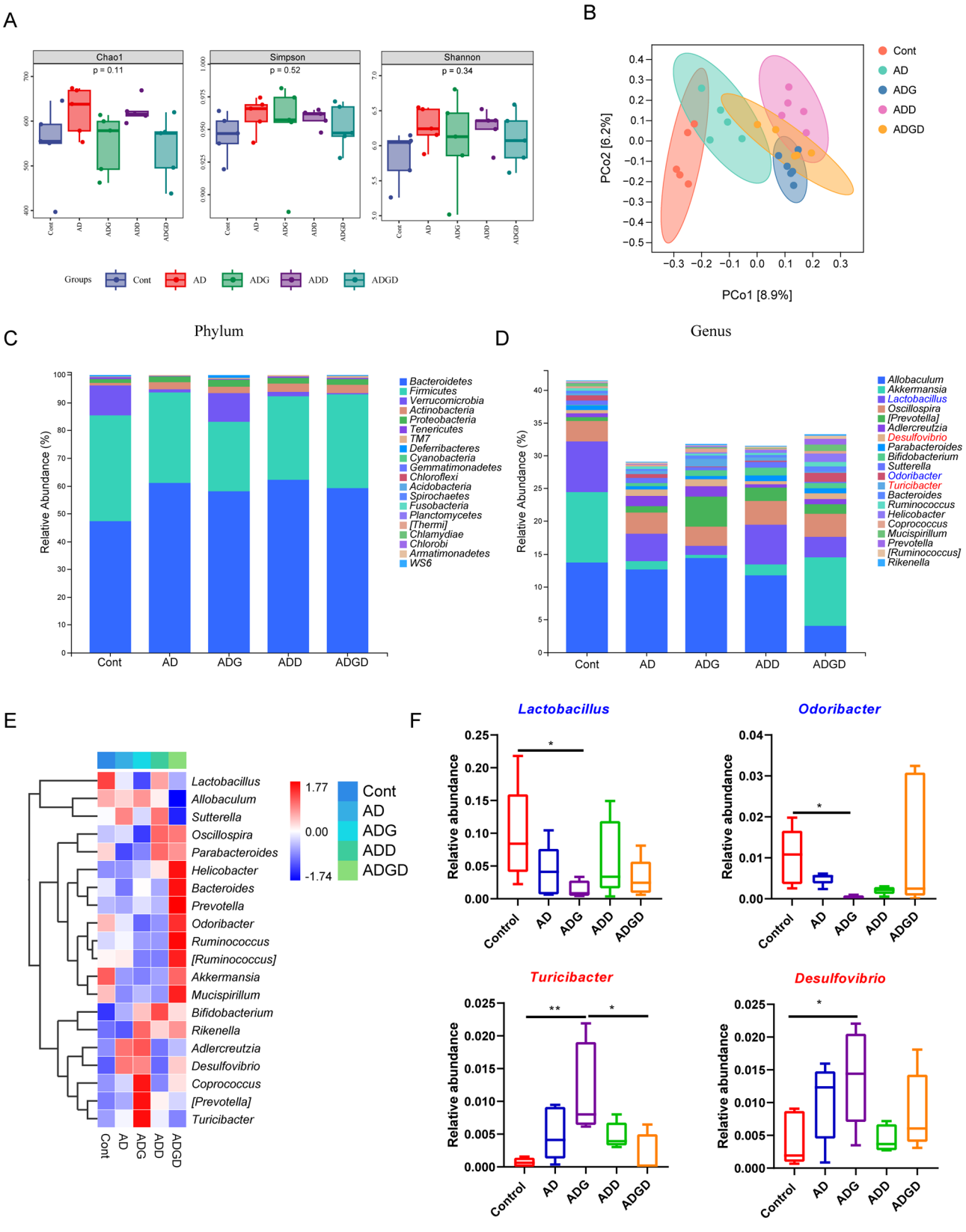

Fig. 10 (See legend on next page.)

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Fig. 10 Glutamine treatment aggravated intestinal flora dysregulation in mice with colorectal cancer. (**A**) Chao1($p=0.432$), Shannon ($p=0.185$) and Simpson ($p=0.368$) index analysis of alpha diversity of the gut microbiota ($n=5$ for each group). (**B**) PCoA distribution plot of the jazzard algorithm for beta diversity ($n=5$ for each group). (**C-D**) relative abundance of intestinal flora at the phylum level and genus level in different groups ($n=5$ for each group). (**E**) heat map of species composition at the genus level ($n=5$ for each group). (**F**) the relative content of *Lactobacillus*, *Odoribacter*, *Turicibacter*, *Desulfovibrio* in each group of mice. Data from at least three independent experiments are expressed as mean ± sd. Cont: control group; ad: AOM/DSS group. adg: AOM/DSS+Glutamine group; add: AOM/DSS+Dinaciclib group; ADGD: AOM/DSS+Glutamine+Dinaciclib group. * $p<0.05$, ** $p<0.01$

Cdk5 regulates glutamine activation of IL-6/STAT3 and Wnt/β-catenin pathways, with Cdk5 knockdown significantly reducing activation of these pathways (Fig. 5).

Glutamine is transported into mitochondria by SLC1A5 (ASCT2) and then converted to glutamate by glutaminase 1 (GLS1), with subsequent conversion to α-ketoglutarate (α-KG) by glutamate dehydrogenase 1 (GLUD1) or aminotransferases. α-KG enters the TCA cycle, generating ATP for cellular functions [31]. Our Western blot analysis showed that Cdk5 knockdown upregulated ASCT2 and GLS1, indicating enhanced glutamine uptake and metabolism (Fig. 11). GLS1 is a key enzyme in glutamine metabolism, and its increased expression in Cdk5 knockdown cells prompted us to investigate the regulatory mechanisms. EZH2, a component of the PRC2 complex, regulates GLS1 expression by catalyzing H3K27 methylation and inhibiting gene transcription [32]. Our findings showed reduced EZH2 expression in Cdk5 knockdown cells and increased GLS1 expression after EZH2 knockdown, suggesting that Cdk5 regulates GLS1 through EZH2. Immunoprecipitation experiments confirmed that EZH2 interacts with both Cdk5 and GLS1 (Fig. 12), supporting the idea that Cdk5 indirectly regulates GLS1 via EZH2.

To clarify how EZH2 regulates GLS1, we conducted experiments. First, comparing GLS1/EZH2 protein in nuclear/cytoplasmic fractions of EZH2 knockdown (EZH2 KD) and control (NC) cells showed EZH2 silencing significantly increased GLS1 in both compartments-suggesting EZH2 regulates GLS1 at both subcellular levels, with potential coordination between post-translational and transcriptional mechanisms. Next, GLS1 mRNA was significantly upregulated in EZH2 KD cells, mirroring the protein trend—confirming EZH2 primarily regulates GLS1 transcriptionally, not just translation or protein stability. CUT&RUN-qPCR showed reduced H3K27me3 at the GLS1 promoter in EZH2 KD cells (vs. high enrichment in NC cells), directly confirming EZH2 represses GLS1 transcription via H3K27me3 deposition (Fig. 13). Collectively, EZH2 primarily represses GLS1 transcription via H3K27me3-mediated silencing, with potential post-translational regulation in both compartments. This multi-layered control underscores the complexity of EZH2's regulation of metabolic enzymes like GLS1, relevant to EZH2-driven metabolic reprogramming in colorectal cancer.

To explore Cdk5's impact on mitochondrial glutamine utilization, we measured intracellular ATP levels and found reduced ATP production in Cdk5 knockdown cells (Fig. 14). Studies have shown that Cdk5 overexpression activates the TCA cycle, with high expression of TCA cycle-related genes in cancer cells [33]. Our results showed decreased mitochondrial fluorescence intensity, membrane potential, and reduced expression of citrate synthase (CS) with increased isocitrate dehydrogenase (IDH2) in Cdk5 knockdown cells (Fig. 15). HPLC-GS analysis revealed reduced TCA cycle metabolites and increased glutamate in Cdk5 knockdown cells (Fig. 16), indicating impaired TCA cycle activity and ATP production despite enhanced glutamine decomposition.

Normal epithelial cells and immune cells metabolize glutamine, but cancer cells have a higher glutamine uptake rate [34–37]. Our mouse model of colorectal cancer using AOM+DSS with glutamine 0.5 g/kg/day gavage and Dinaciclib treatment showed that glutamine accelerated tumor formation, while Dinaciclib inhibited this effect (Fig. 6) [38]. Western blot analysis of colon tissues revealed increased P-STAT3 and β-catenin expression in the AOM/DSS+Glutamine group, which was reduced by Dinaciclib (Fig. 5). This indicates that Cdk5 plays a crucial role in glutamine-promoted colorectal cancer progression.

The development of enteritis to bowel cancer is a continuous and dynamic process [39]. The continuous stimulation of inflammatory factors can cause damage to normal intestinal epithelial cells, and the long-term existence of intestinal inflammatory environment will greatly increase the risk of colorectal cancer [40]. The modeling method of AOM+DSS adopted in this study is a classical method to establish colitis-related colorectal cancer

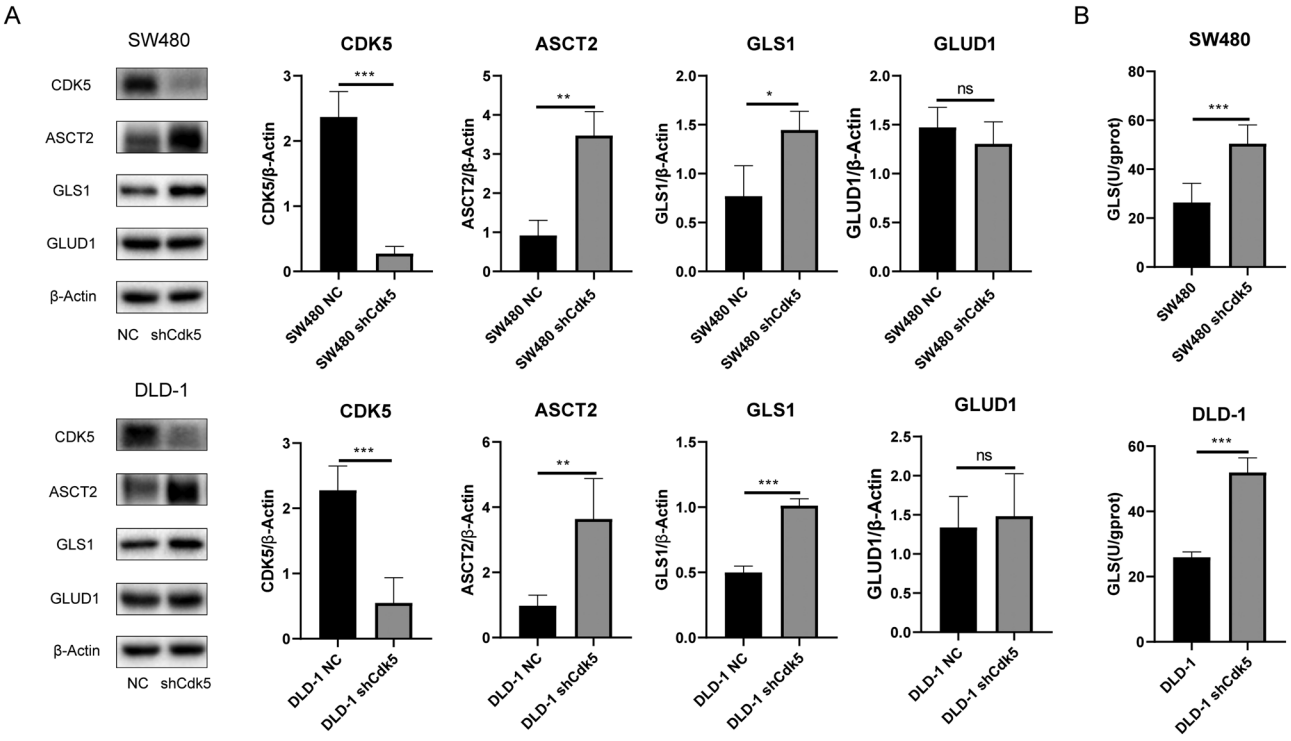

Fig. 11 Cdk5 knockdown upregulates the expression of ASCT2 and GLS1 proteins. (**A**) effect of Cdk5 knockdown on expression levels of ASCT2, GLS1 and GLUD1 proteins. (**B**) effect of Cdk5 knockdown on GLS1 enzyme activity. Data from at least three independent experiments are expressed as mean ± sd. ns: $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

model. DSS is sodium dextran sulfate, and the mouse colitis model induced by DSS has been widely used due to many similarities with human ulcerative colitis [41]. The results of this experiment showed that when glutamine was used in the modeling process, the weight loss of mice was more significant, and the blood of mice perianal stool was more serious. In addition, the study also found that glutamine increased the protein expression level and mRNA expression of inflammatory factors (IL-1β, TNF-α, IL-6) in colon tissue during modeling, while Dinaciclib inhibited the pro-inflammatory effect of glutamine. Serum levels of inflammatory factors in mice were measured by ELISA, and the results also showed increased levels of inflammatory factors (Fig. 8). Therefore, we believe that glutamine may promote the development of colorectal cancer by aggravating intestinal inflammation during the cancer process. However, some literature has found that glutamine also has a potential mechanism to promote inflammation. One of the mechanisms is that glutamine produces ammonia during metabolism, which may lead to intracellular acid-base imbalance and indirectly activate inflammatory pathways [42]. In addition, under certain pathological conditions, glutamine may exacerbate immune-mediated inflammatory responses by enhancing T lymphocyte differentiation and macrophage activity. For example, some autoimmune models have shown that glutamine supplementation is associated with excessive activation of Th17 cells [43]. Glutamine has a dual regulatory effect on inflammation in animals, which can alleviate intestinal inflammation and may also promote inflammatory responses under specific conditions. Its mechanism is closely related to metabolic pathways, immune regulation, and microenvironment.

The transition from enteritis to colorectal cancer involves continuous inflammation and damage to intestinal epithelial cells [39, 40]. Our AOM+DSS model showed that glutamine worsened weight loss, stool bleeding, and increased expression of inflammatory factors IL-1β, TNF-α, and IL-6 (Fig. 8). Dinaciclib alleviated these effects. Additionally, H&E and Alcian blue staining revealed that glutamine exacerbated intestinal

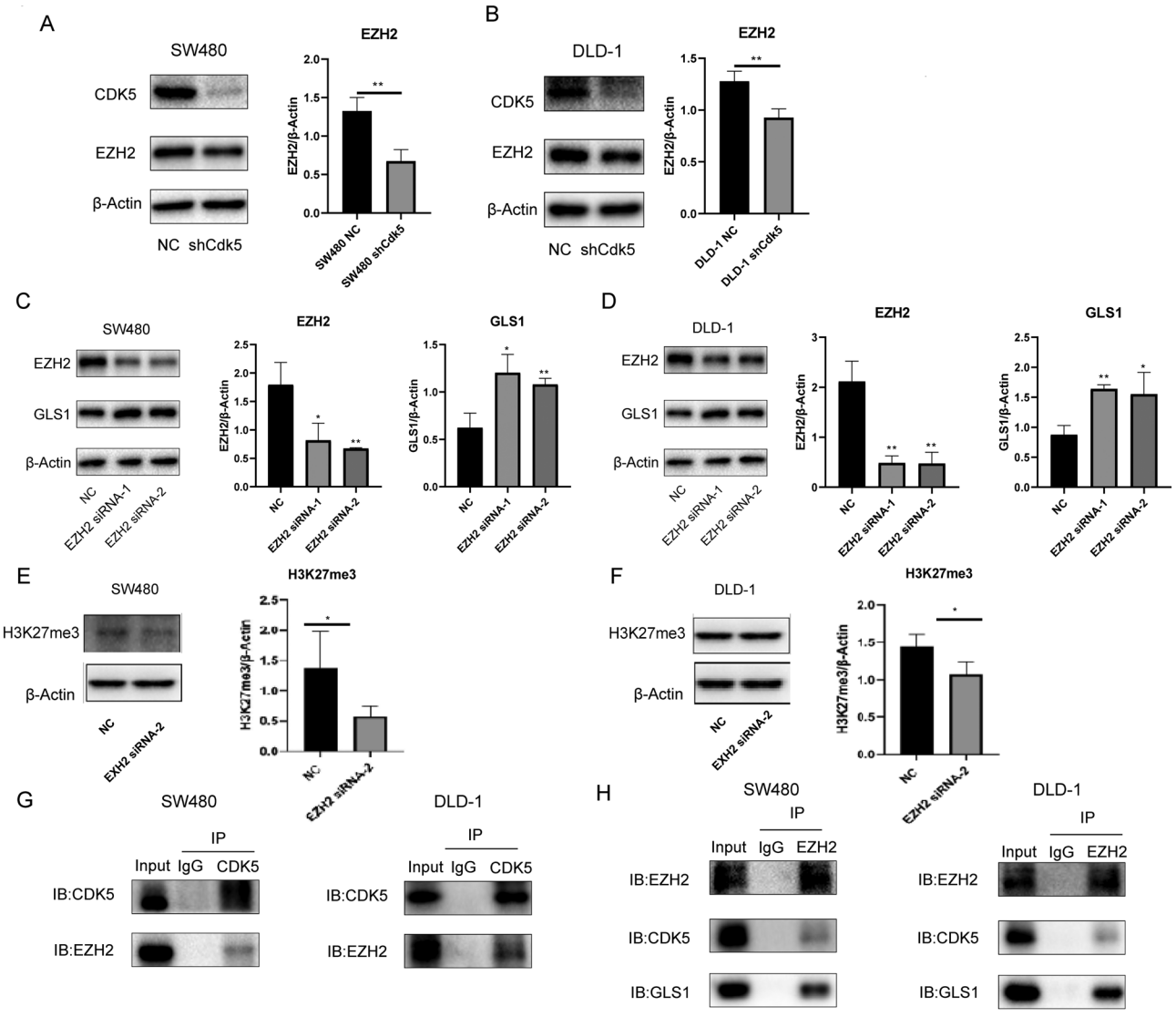

Fig. 12 Cdk5 indirectly regulates GLS1 protein expression through EZH2. (**A**) expression of EZH2 protein in Cdk5 knockdown cells. (**B**) effect of EZH2 small interfering rna on GLS1 protein expression after transfection. (**C-D**) the interaction between Cdk5, EZH2 and GLS1 proteins was detected by immunoprecipitation. (**E-F**) Expression of H3K27me3 protein in Cdk5 knockdown cells. Data from at least three independent experiments are expressed as mean ± sd. * $p < 0.05$, ** $p < 0.01$

damage, while Dinaciclib helped restore epithelial integrity (Fig. 9). 16S rDNA sequencing showed that glutamine altered intestinal microbiota, increasing harmful bacteria like *Desulfovibrio* and *Turicibacter* while decreasing beneficial bacteria such as *Lactobacillus* and *Odoribacter* (Fig. 10). This may be due to the interaction between glutamine metabolism and the host urea cycle. Glutamine is an important substrate in the host urea cycle, which can regulate nitrogen metabolism balance through the urease activity of gut microbiota. In colorectal cancer, dysbiosis of the gut microbiota can lead to abnormal activation of the urea cycle, promote differentiation of macrophages into immunosuppressive subtypes, and disrupt intestinal immune homeostasis [44]. The overactivation of the urea cycle is associated with the proliferation of pathogenic bacteria

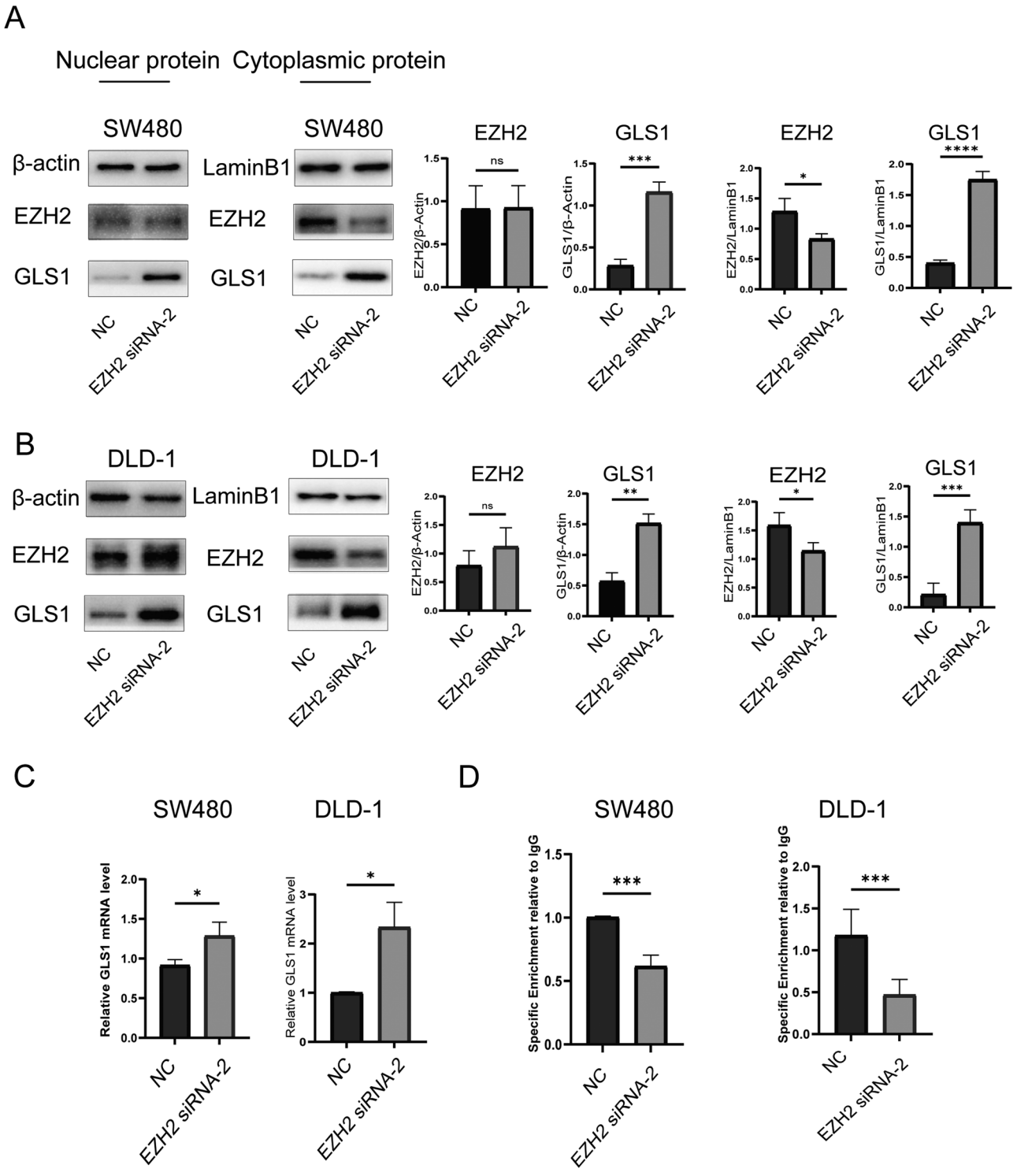

Fig. 13 EZH2 regulates the expression of GLS1 through H3K27me3. (**A**) expression levels of GLS1 and EZH2 proteins in nuclear fractions of EZH2 kd cells. (**B**) expression levels of GLS1 and EZH2 proteins in cytoplasmic fractions of EZH2 kd cells. (**C**) mRNA expression level of GLS1 in EZH2 kd cells. (**D**) enrichment of H3K27me3 in the GLS1 promoter region in EZH2 kd cells. Data from at least three independent experiments are expressed as mean ± sd. * $p < 0.05$, ** $p < 0.01$

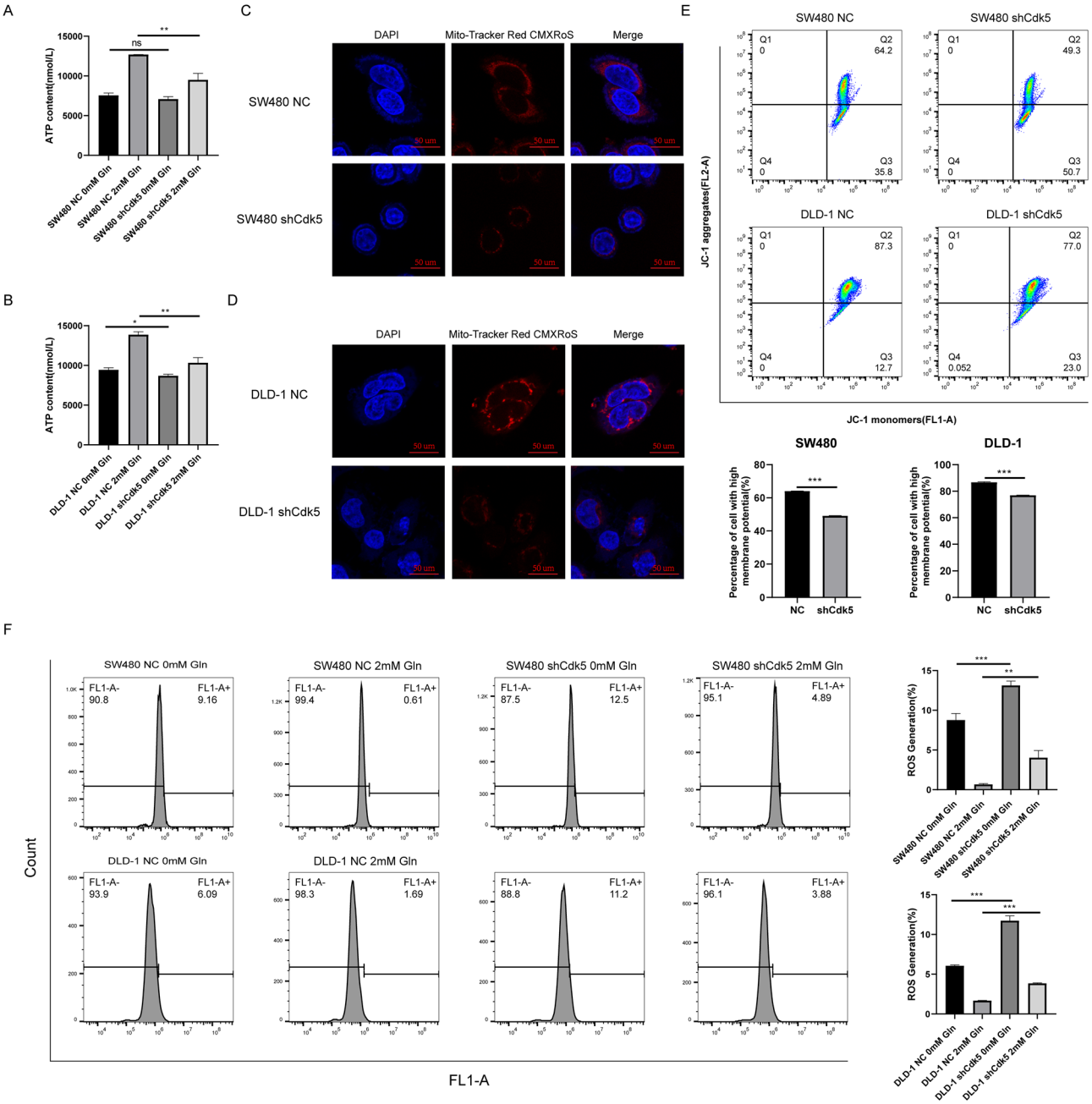

Fig. 14 Cdk5 knockdown suppresses mitochondrial function. (**A**) effects of glutamine and Cdk5 knockdown on intracellular atp levels. (**B**) mito-tracker red CMXRos probe staining results of mitochondria (scale bar 25 μm). (**C**) flow results and statistical diagram of mitochondrial membrane potential. (**D**) detection of reactive oxygen species. Data from at least three independent experiments are expressed as mean ± sd. ns: $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

in the gut microbiota, such as Clostridium nucleatum and Clostridium difficile, which further exacerbates inflammation and DNA damage [45]. In addition, the gut microbiota converts primary bile acids into secondary bile acids (such as deoxycholic acid) through 7α-dehydroxylation, which can promote tumorigenesis by activating pathways such as NF-κB. Glutamine may affect this process by regulating the activity of bacterial bile acid metabolism enzymes [46]. These changes suggest that glutamine disrupts intestinal barrier function and promotes colorectal cancer by affecting gut microbiota.

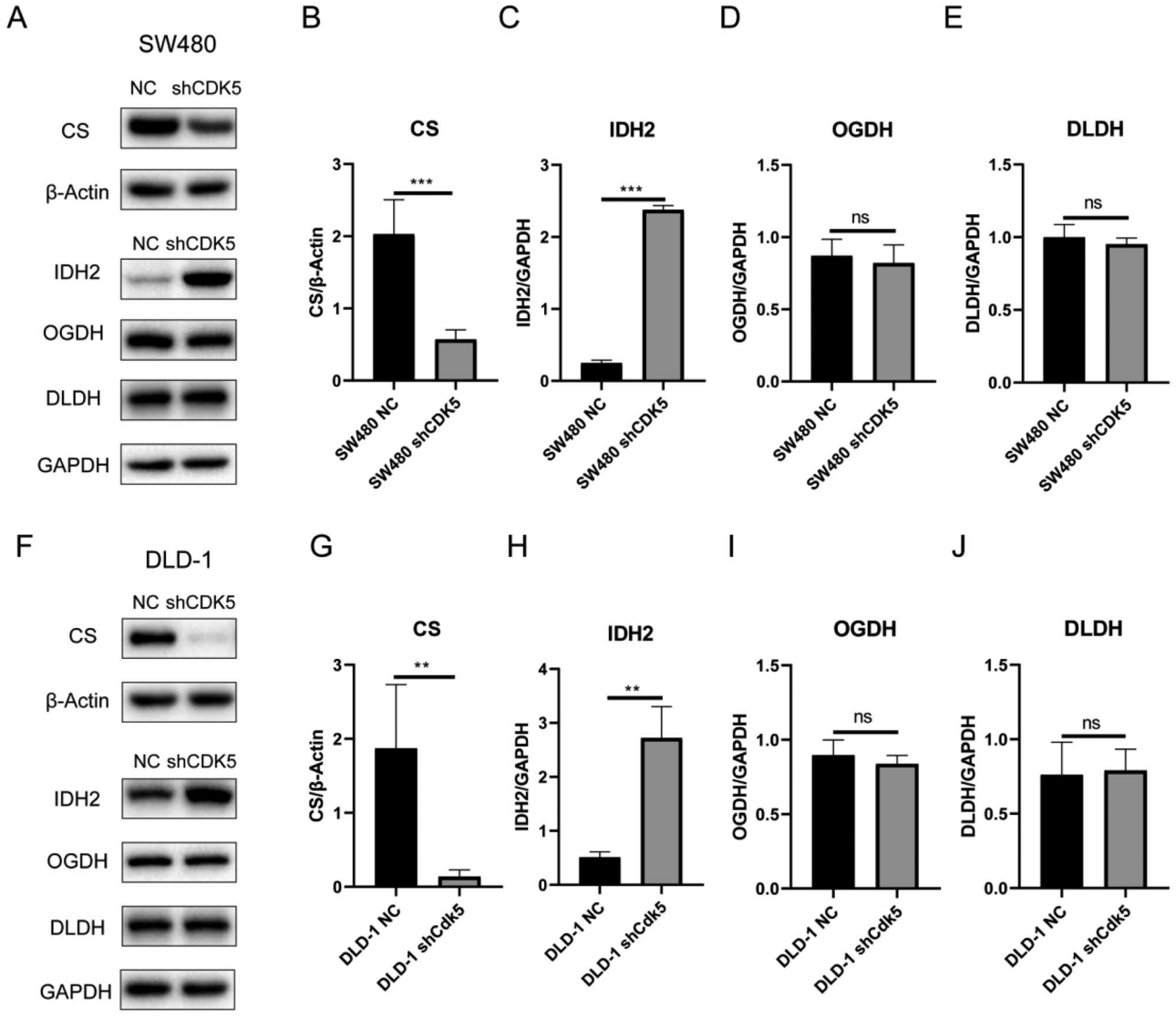

Fig. 15 Cdk5 knockdown inhibited the expression of some tricarboxylate circulating proteins. (**a**) protein expression of citrate synthetase (cs), isocitrate dehydrogenase 2 (IDH2) and α-ketoglutarate dehydrogenase complex (OGDH, DLDH). ata from at least three independent experiments are expressed as mean ± sd. ns: $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Our in vitro experiments demonstrate that glutamine activates the IL-6/STAT3 and Wnt/β-catenin signaling pathways through Cdk5, promoting colorectal cancer cell proliferation. Metabolite analysis reveals that Cdk5 regulates glutamine utilization in mitochondria, affecting ATP production. In vivo studies with glutamine gavage and Dinaciclib therapy show that glutamine accelerates colorectal cancer development by promoting intestinal inflammation and disrupting gut microbiota, while Cdk5 inhibition alleviates tumor formation (Fig. 17). These findings provide new insights into potential clinical strategies for treating colorectal cancer.

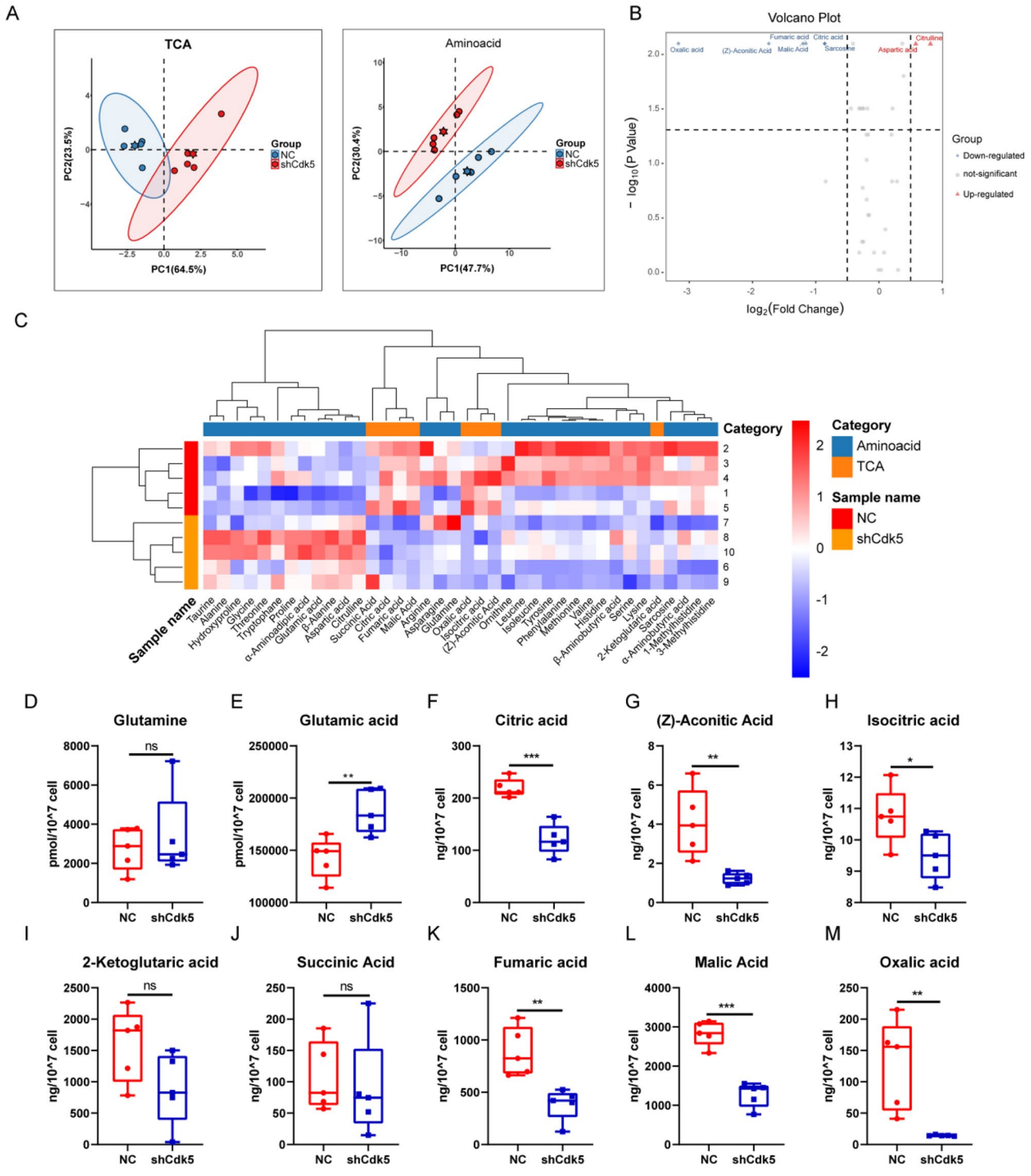

Fig. 16 Cdk5 knockdown inhibits the tricarboxylic acid cycle. (**a**) pcoa analysis ($n = 5$ for each group). (**b**) volcano plot ($n = 5$ for each group). (**c**) heat map ($n = 5$ for each group). (**d**) content of glutamine, glutamic acid, citric acid, cisaconite acid, isocitric acid, fumaric acid, malic acid and oxalic acid. ata from at least three independent experiments are expressed as mean ± sd. ns: $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

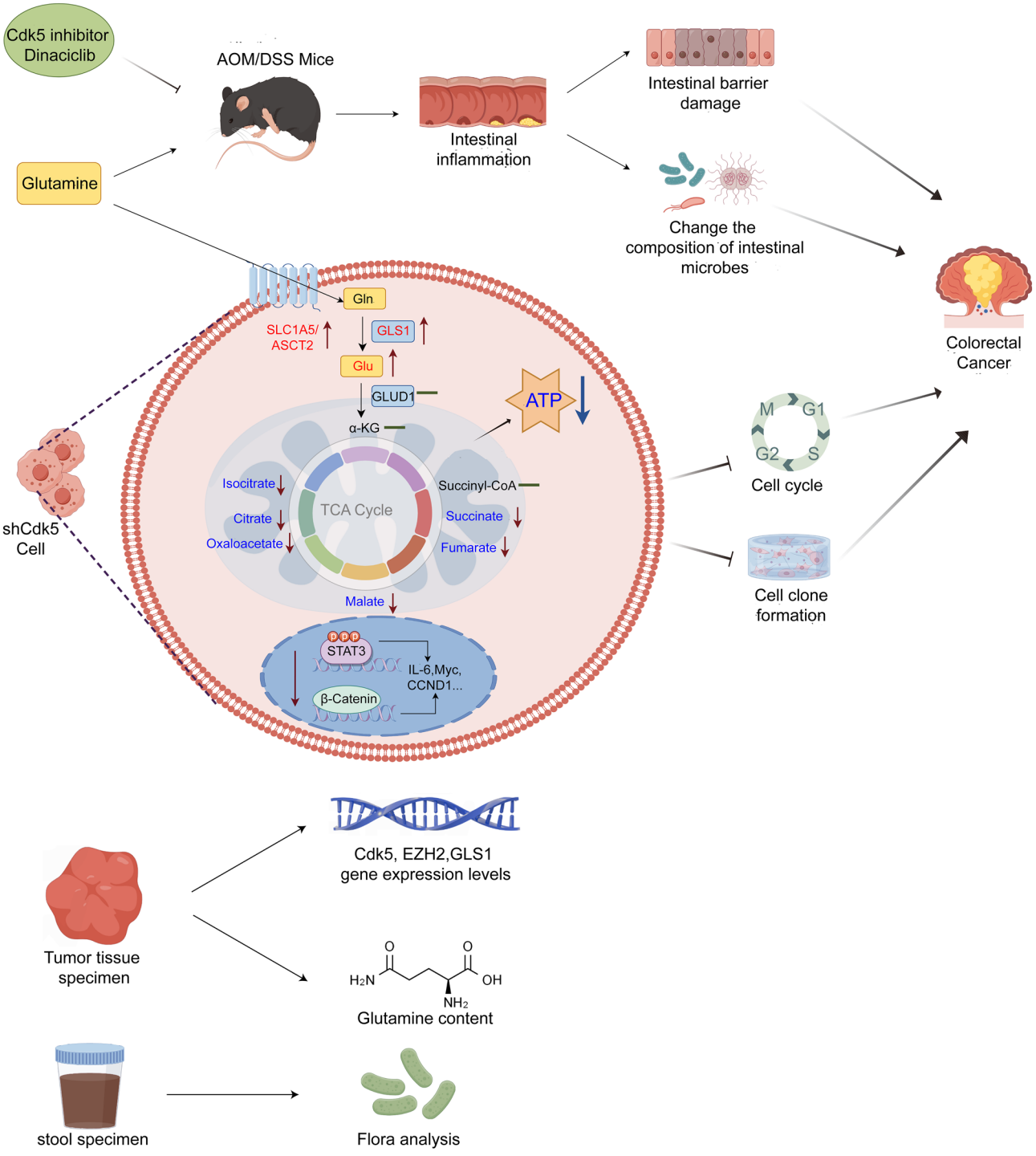

Fig. 17 Mechanism diagram of regulation of colorectal cancer progression by glutamine via cyclin-dependent kinase 5. In vitro and in vivo, glutamine can activate the IL-6/STAT3 and Wnt/β - catenin signaling pathways through Cdk5, regulating the proliferation of colorectal cancer cells; in addition, Cdk5 can further regulate the utilization of glutamine in mitochondria, providing energy for the proliferation of cancer cells

Abbreviations

CRC	Colorectal cancer
Cdk5	Cyclin-dependent kinase 5
Gln	Glutamine
Rb	Retinoblastoma
MTT	3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide
DMSO	Dimethyl sulfoxide
ELISA	Enzyme-linked immunosorbent assay
PCoA	Principal coordinate analysis
ASCT2	Glutamine transporter
GLS1	Glutaminase
GLUD1	Glutamate dehydrogenase
TCA	Tricarboxylic acid
CS	Citrate synthase
IDH2	Isocitrate dehydrogenase
EZH2	Enhancer of Zeste Homolog 2

Author contributions

QLW: Data curation, investigation, conceptualization, methodology, original draft preparation. XTZ: Data curation, investigation, conceptualization, methodology. XXW, XJL, JYCh, YZ, YL, XH: Investigation, data curation. JHL, YLL: Funding acquisition, supervision, project administration. XL: Funding acquisition, conceptualization, methodology, supervision, writing-reviewing and editing, project administration.

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Data availability

The datasets from this study can be accessed in online repositories. Specifically, they are available on the National Center for Biotechnology Information (NCBI) BioProject (https://www.ncbi.nlm.nih.gov/bioproject, Accession No. [PRJNA1138068](https://http://www.ncbi.nlm.nih.gov/bioproject/PRJNA1138068)), and on the National Center for Biotechnology Information (NCBI) SRA (https://www.ncbi.nlm.nih.gov/sra, Accession No. [PRJNA1138068] (https://http://www.ncbi.nlm.nih.gov/bioproject/PRJNA1138068)). Additionally, the datasets can be found on the National Genomics Data Center (NGDC) China National Center for Bioinformation (CNCB)/Beijing Institute of Genomics (BIG), Chinese Academy of Sciences (CAS) Open Archive for Miscellaneous Data (OMIX) (https://ngdc.cncb.ac.cn/omix/, Accession Nos. PRJCA027378).

Declarations

Ethics statement

Tables or Figures from Another Resource: N/A. Approval of the research protocolN/A. Informed consent: N/A. Registry and registration no. of the study/trial: N/A. Animal studies: All experiments involving live mice were approved by the Experimental Animal Ethics Committee of Wenzhou Medical University (Approval No. wydw2022-0216).

Competing interests

The authors declare no competing interests.

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