Article

Discovery Medicine 2026; 38(208): 1478–1491
https://doi.org/10.24976/Discov.Med.202638208.115

The Driving Effect of CSNK1E/DVL1 on the Malignant Development of Colorectal Cancer

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Submitted: 18 September 2025 Revised: 12 March 2026 Accepted: 20 March 2026 Published: 20 May 2026

Background: Casein kinase I isoform epsilon (CSNK1E), a gene intricately linked to the circadian clock, has been found to be highly expressed in colorectal cancer (CRC). Elevated expression of CSNK1E correlates with shorter disease-free survival in patients, suggesting a significant, albeit not yet fully understood, role in CRC pathogenesis. The specific molecular mechanisms by which CSNK1E contributes to tumor development and progression remain largely unclear. Given its involvement in key signaling pathways, this study aims to elucidate the functional role of CSNK1E and its underlying mechanisms in CRC, with particular emphasis on its relationship with the Wnt/β-catenin pathway, a cornerstone of CRC oncogenesis.

Methods: CRC cells underwent the intervention on the expression of CSNK1E and dishevelled segment polarity protein 1 (DVL1), a protein interacting with CSNK1E in Wnt/β-catenin signaling. The efficiency of gene modulation was evaluated by qRT-PCR. The interaction between these two genes was validated using Co-immunoprecipitation assays. Phenotypic experiments were subsequently conducted in CRC cells, and Western blot analysis was conducted to assess the status of Wnt/DVL1/β-catenin signaling.

Results: Knockdown of CSNK1E inhibited proliferation ($p < 0.01$), hindered cell cycle transition from the G1 phase to the S phase ($p < 0.05$), while inducing apoptosis of CRC cells ($p < 0.001$). Moreover, CSNK1E was found to bind to DVL1; CSNK1E knockdown significantly decreased the expression of DVL1 and β-catenin in CRC cells ($p < 0.05$), whereas CSNK1E overexpression exerted an opposite effect ($p < 0.05$). DVL1 upregulation counteracted the inhibitory effects of CSNK1E knockdown, while DVL1 downregulation offset the promotive effects of CSNK1E overexpression on CRC ($p < 0.05$).

Conclusion: This study unveils that CSNK1E is a pivotal oncoprotein in CRC. It promotes tumor progression by interacting with and stabilizing DVL1, thereby facilitating the activation of the canonical Wnt/β-catenin signaling pathway. These results highlight the CSNK1E/DVL1 axis as a potential novel therapeutic target for CRC treatment.

Keywords: colorectal cancer; casein kinase I isoform epsilon; dishevelled segment polarity protein 1; cell cycle; Wnt/β-catenin signaling

Introduction

Colorectal cancer (CRC) represents the third most prevalent cancer and the second leading cause of cancer-related mortality [1]. It arises from conventional adenomas, which progress to carcinomas, followed by the development of metastasis, indicative of a poor prognosis [2]. Although the early detection and removal of precancerous adenomas, enabled by extensive screening, have substantially reduced CRC incidence and the related mortality [2], approximately 20% of patients present synchronous metastases at diagnosis [3], and up to 25%–50% early-stage CRC cases develop metastasis [1]. Given that the benefits of chemotherapy and targeted therapy for treating metastatic disease would reach a plateau [3], novel strategies need to be established to satisfy the unmet need of CRC treatment.

Circadian rhythms are biological systems that display a 24-hour oscillation and are required for the implementation of the correct physiology and behaviour in the appropriate time window each day [4]. Disruption of the circadian rhythm correlates with cancer development, low therapy efficacy, and poor prognosis [5], which is evidenced by the fact that patients with metastatic CRC who maintain normal circadian rhythms exhibit a 5-fold higher survival rate than those with severely disturbed circadian rhythms [6]. In addition, both the anti-tumor effects and toxicity of therapy vary depending on the timing of administration within the circadian rhythm [7]. Circadian rhythm is generated and maintained by circadian clock genes (CCGs), whose alterations profoundly impact tumorigenesis by modulating cell proliferation, cell cycle transition, and apoptosis [8,9], with cell cycle transition being the hotspot of the research investigating the role of CCGs in cancer [10]. Casein kinase I isoform epsilon (CSNK1E), a member of the casein kinase I (CKI) protein family and also known as CK1ϵ or CK1epsilon, is a CCG that is highly expressed in CRC samples and closely linked to shorter disease-free survival (DFS) in CRC patients [11]. Moreover, in CRC, CSNK1E induces inactivation of glycogen synthase

kinase3β (GSK3β), thereby promoting the activation of β-catenin [12], a canonical mechanism driving CRC cell cycle transition [13]. The above findings suggest a regulatory role of CSNK1E in CRC development and progression by impacting cancer cell cycle transition through regulating β-catenin activation.

β-catenin serves as a central effector of the Wnt/β-catenin signaling pathway, a complex network of protein interactions that are critically involved in embryogenesis and tumorigenesis [14]. Aberrant activation of the Wnt/β-catenin signaling pathway in cancer is associated with increased incidence, promoted malignant progression, poor prognoses, and elevated mortality, owing to its regulation of cell survival, differentiation, and proliferation, and cell cycle [15]. This pathway is identified as a key driver of CRC occurrence and progression [16]. Notably, the activity of CSNK1E, a key kinase component within the pathway, is often regulated through protein-protein interactions. For instance, phosphorylation of Frizzled 6 (FZD6) by CSNK1E requires the scaffolding protein dishevelled (DVL) [17]. DVL proteins act as critical molecular hubs, transmitting Wnt signal from its upstream receptors to downstream effectors [18]. They are recruited and phosphorylated/activated upon Wnt signaling activation, and then disrupt the formation of the Axin/GSK3β/anaphase-promoting complex (APC), a complex mediating the destruction of β-catenin at the cell membrane, thereby leading to the accumulation of cytoplasmic β-catenin to trigger Wnt signaling cascades [19,20]. In addition, nuclear translocation of DVL proteins positively regulates Wnt/β-catenin signaling [21]. Bioinformatics analysis using STRING reveals that one of the DVL homologs, dishevelled segment polarity protein 1 (DVL1), is co-expressed with CSNK1E. Based on the above theoretical bases, we hypothesized that CSNK1E might activate CRC tumorigenesis by activating Wnt/β-catenin signaling through the modulation of DVL1 expression.

In this study, through gain- and loss-of-function experiments, we investigated the role of CSNK1E in CRC tumorigenesis with a focus on its regulation of Wnt/β-catenin signaling, aiming to provide novel insights into the pathogenesis of CRC and potential avenues for clinical therapeutic strategies.

Materials and Methods

Cell Culture

The human CRC cell lines (HCT116 and SW620 cells) were purchased from Procell (CL-0096 and CL-0225B, Wuhan, China), and were cultured in DMEM (11965092, ThermoFisher, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; HY-P2352, MedChemExpress, Monmouth Junction, NJ, USA) and 1% penicillin-streptomycin (15140148, ThermoFisher, USA). Cell culture was performed at 37 °C in a moistened 5% CO_2 incubator. All cell lines were validated by short tandem repeat (STR) profiling and confirmed to be mycoplasma-free.

Cell Transfection

Prior to transfection, the coding sequence (CDS) of *CSNK1E* and *DVL1* was subcloned into pcDNA3.1 vectors (VT1001, YouBio, Changsha, China) for constructing overexpressing vectors, with an empty vector as a negative control (NC, 5′-CTAGAGAACCCACTGCTTAC-3′). The CDS region of *CSNK1E* and *DVL1* can be found in **Supplementary Materials**. Short hairpin RNA targeting *CSNK1E* (sh*CSNK1E*; TR320314) and *DVL1* (sh*DVL1*; TR304872), along with the shNC (TR20003), were all procured from OriGene (Rockville, MD, USA). These constructs were transfected individually (except DVL1 overexpression plasmid and sh*DVL1*) or in combination (sh*CSNK1E* plus pCMV6-Entry vector, sh*CSNK1E* plus *DVL1*, *CSNK1E* plus shNC, *CSNK1E* plus sh*DVL1*, shNC plus pCMV6-Entry vector) into HCT116 and SW620 cells using Lipofectamine 3000 transfection reagent (L3000015, ThermoFisher, USA). Briefly, 1×10^4 cells were inoculated in each well of 96-well plates, and cultured until 90% confluence. The above plasmids were combined with Lipofectamine 3000 transfection reagent, Opti-MEM medium and P3000 reagent and incubated at 37 °C for 15 min to form gene-lipid complexes. Cells were then treated with these complexes and cultured at 37 °C for 48 h. Transfection efficiency was assessed using quantitative reverse transcription polymerase chain reaction (qRT-PCR). The sequences of shCSNK1E and shDVL1 were as follows:

sh*CSNK1E* (forward): 5′-CATCTGGCTCTGAGTTATAAA-3′;

sh*CSNK1E* (reverse): 5′-TTTATAACTCAGAGCCAGATG-3′;

sh*DVL1* (forward): 5′-TCTGGAGTAGGGATCTAATTT-3′;

sh*DVL1* (reverse): 5′-AAATTAGATCCCTACTCCAGA-3′.

shNC (forward): 5′-TTCTCCGAACGTGTCACGT-3′;

shNC (reverse): 5′-ACGTGACACGTTCGGAGAA-3′.

Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted from HCT116 and SW620 cells with/without transfection using Trizol reagent (15596026, ThermoFisher, USA). The first-strand cDNA was synthesized from 1 µg of total RNA employing a reverse transcription kit (K1622, Yaanda Biotechnology, Beijing, China). Sequentially, qPCR was carried out with a system (LightCycler 96, Roche, Indianapolis, IN, USA) using Eastep qPCR Master Mix (LS2062, Promega, Madison, WI, USA) under the typical cycling conditions below: 95 °C for 10 min, followed by 40 cycles of 95 °C

Table 1. Primer sequences of related genes.

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)
CSNK1E (human)	CGTGTGGGGAACAAGTACCG	GATGTTGGCACCCAGGTAGAT
DVL1 (human)	GAGGGTGCTCACTCGGATG	GTGCCTGTCTCGTTGTCCA
GAPDH (human)	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG

CSNK1E, casein kinase I isoform epsilon; *DVL1*, dishevelled segment polarity protein 1; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase.

for 15 seconds (s) and 60 °C for 1 min. The relative mRNA expression was quantified using the $2^{-\Delta\Delta Ct}$ method [22], with glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) used as a normalizer. Primer sequences are listed in Table 1.

5-Ethynyl-2′-Deoxyuridine (EdU) Assay

Cell proliferation was evaluated using the BeyoClick EdU-647 proliferation kit (red fluorescence, C0081S, Beyotime, Shanghai, China). Briefly, after transfection, HCT116 and SW620 cells were placed in 6-well plates, cultured at 37 °C overnight. The EdU working solution (220 µL) was added to 2 mL of the culture media containing the above cells. After 2 h of incubation, cells were harvested and fixed with 4% paraformaldehyde (P0099, Beyotime, China) at room temperature for 15 min, followed by permeabilization with 0.3% Triton X-100 (X100, Sigma-Aldrich, St. Louis, MO, USA) at room temperature for 10 min. Later, 0.5 mL Click reaction mixture (containing Click Reaction Buffer, $CuSO_4$, Azide-647, and Click Additive Solution) was supplemented to each well and incubated in the dark for 30 min. Cell nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) staining solution (blue fluorescence, C1005, Beyotime, China). Finally, EdU-positive cells were observed under a confocal microscope (Axio Imager 2, Carl Zeiss, Oberkochen, Germany) at ×200 magnification.

Colony Formation Assay

HCT116 and SW620 cells, with/without transfection, were transferred (1×10^3/well) into 6-well culture plates and cultivated in complete media for 14 days. When colonies were visible, they were carefully washed twice, fixed with 4% paraformaldehyde, and stained with 0.1% crystal violet (R40052, Thermo Scientific, USA). The number of stained colonies was counted under an optical microscope (IX73, Olympus, Tokyo, Japan).

Flow Cytometry

Cell apoptosis was checked using Annexin V-FITC/propidium iodide (PI) Apoptosis Detection Kit (E-CK-A211, Elabscience, Wuhan, China). HCT116 and SW620 cells (1×10^5), with or without transfection, were centrifuged at 300 ×g for 5 min, washed with phosphate-buffered saline (PBS; P2272, Sigma-Aldrich, USA), and resuspended in 500 µL Annexin V Binding Buffer. Then, 5 µL Annexin V-FITC and 5 µL PI reagent were added to the cells, followed by 15-min incubation in the dark. Apoptotic cells were analyzed by a flow cytometer (Cytoflex, Beckman Coulter, Brea, CA, USA), with CellQuest software (version 5.1, BD Biosciences, San Jose, CA, USA) utilized for quantitative assessment.

For the cell cycle assay, transfected/non-transfected HCT116 and SW620 cells (1×10^6 cells/mL) were washed once with PBS and resuspended in 1 mL of ice-cold PBS. 300 µL of the cell suspension was fixed in 5 mL of 80% ice-cold EtOH at –20 °C. 24 h later, the cells were rehydrated with PBS for 3 h. Cells were pelleted by centrifugation and resuspended in 300 µL of PBS supplemented with 60 µg/mL PI and 50 µg/mL RNase A (R4875, Sigma-Aldrich, USA), then cultivated in the dark for 30 min. Ultimately, cell cycle profiles were analyzed using a flow cytometer combined with the CellQuest software.

Western Blot Analysis

Total protein was extracted from non-transfected/transfected HCT116 and SW620 cells using RIPA lysis buffer (20-188, Sigma-Aldrich, USA) containing 1% protease inhibitor cocktail (P8465, Sigma-Aldrich, USA), and then its concentration was measured using the BCA kit (A53227, ThermoFisher, USA). The protein extraction (25 µg) was separated by SDS-PAGE gel (1615100, BIO-RAD, Hercules, CA, USA), and then transferred onto polyvinylidene fluoride membranes (1620256, BIO-RAD, USA). The membranes were blocked with 5% skimmed milk at room temperature for 1 h and washed with PBS. Then, they were incubated with primary antibodies against DVL1 (A10536, 75 kDa, 1:500, abclonal, Wuhan, China), β-catenin (ab32572, 86 kDa, 1:5000, Abcam, UK), CSNK1E (ab302638, 47 kDa, 1:1000, Abcam, UK) and GAPDH (ab8245, 37 kDa, 1:500, Abcam, UK) overnight incubation at 4 °C. Thereafter, the membranes were washed with PBS thrice and further probed with the secondary antibodies of goat anti-rabbit (ab97051, Abcam, UK) and goat anti-mouse (ab205719, Abcam, UK) at room temperature for 1 h. Blot signals were visualized with SignalFire ECL reagent (6883, Cell Signaling Technology, Danvers, MA, USA) on a luminescence imager (LAS4000, Fujifilm, Tokyo, Japan). Image-Pro Plus (6.0 version, Media Cybernetics, Silver Spring, MD, USA) was then utilized for grayscale analysis.

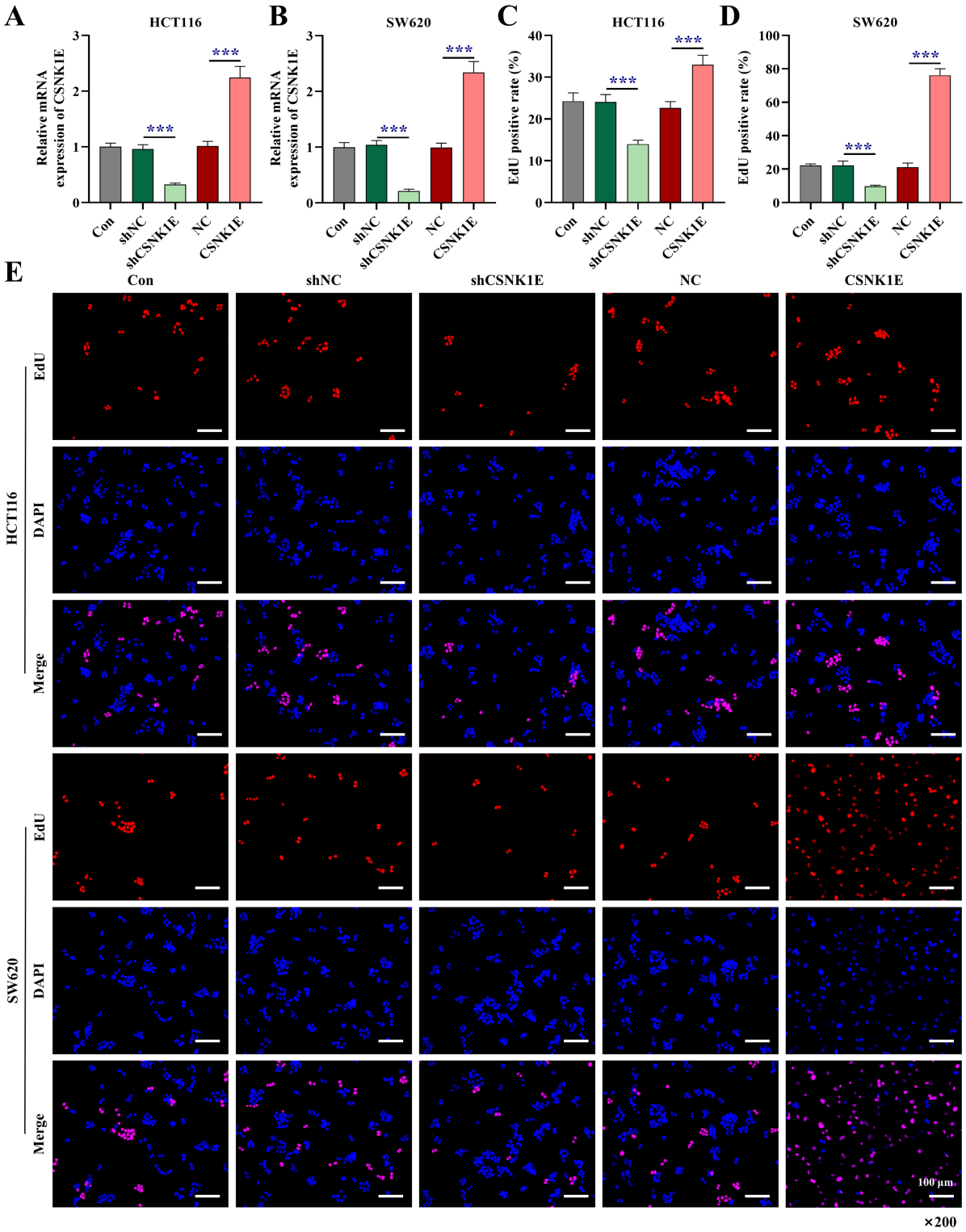

Fig. 1. CSNK1E positively modulated the proliferation of CRC cells. (A,B) The expression of CSNK1E in CRC cells was assessed using qRT-PCR. GAPDH was used as the normalizer. (C–E) The proliferation of CRC cells was evaluated using the EdU assay. Red fluorescence: EdU-positive cells. Blue fluorescence: cell nuclei. Resolution: 100 μm, ×200. ***$p < 0.001$. CRC, colorectal cancer; CSNK1E, casein kinase I isoform epsilon; EdU, 5-ethynyl-2′-deoxyuridine; DAPI, 4′,6-diamidino-2-phenylindole; qRT-PCR, quantitative reverse transcription polymerase chain reaction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Con, blank control; NC, negative control; shCSNK1E, short hairpin RNA against CSNK1E; shNC, short hairpin RNA against NC.

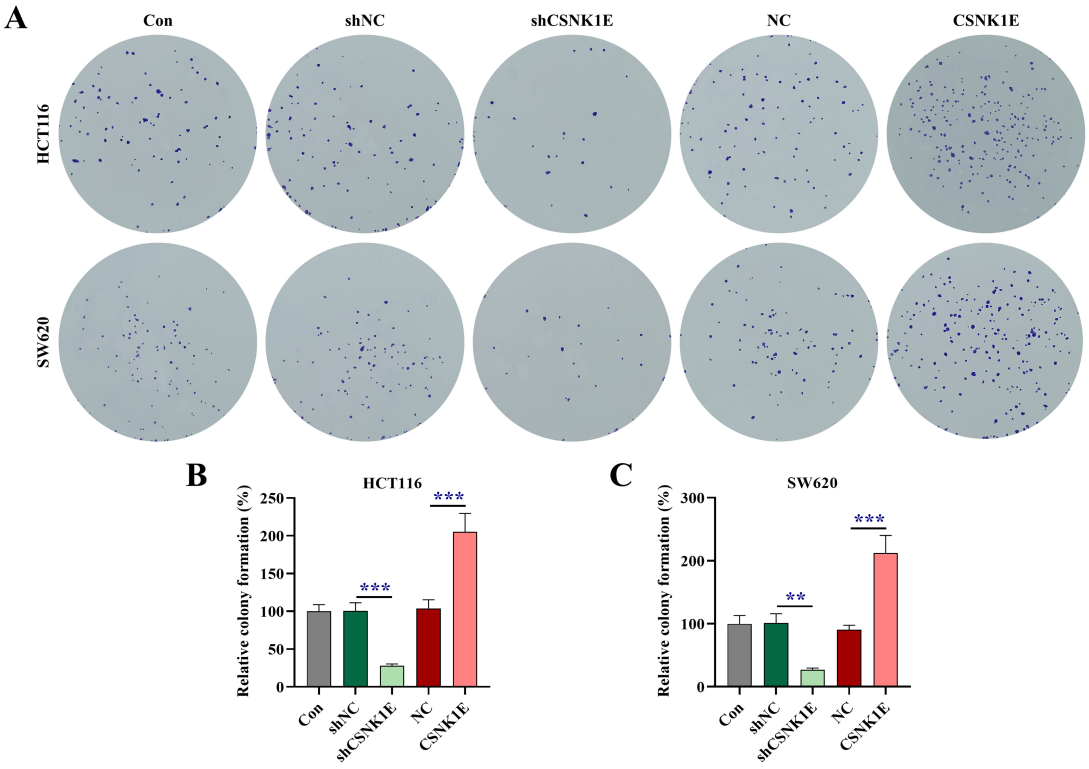

Fig. 2. CSNK1E promoted the proliferation of CRC cells. (A–C) The proliferation of CRC cells was evaluated using the colony formation assay. $^{**}p < 0.01$, $^{***}p < 0.001$. CRC, colorectal cancer; CSNK1E, casein kinase I isoform epsilon; Con, blank control; NC, negative control; shCSNK1E, short hairpin RNA against CSNK1E; shNC, short hairpin RNA against NC.

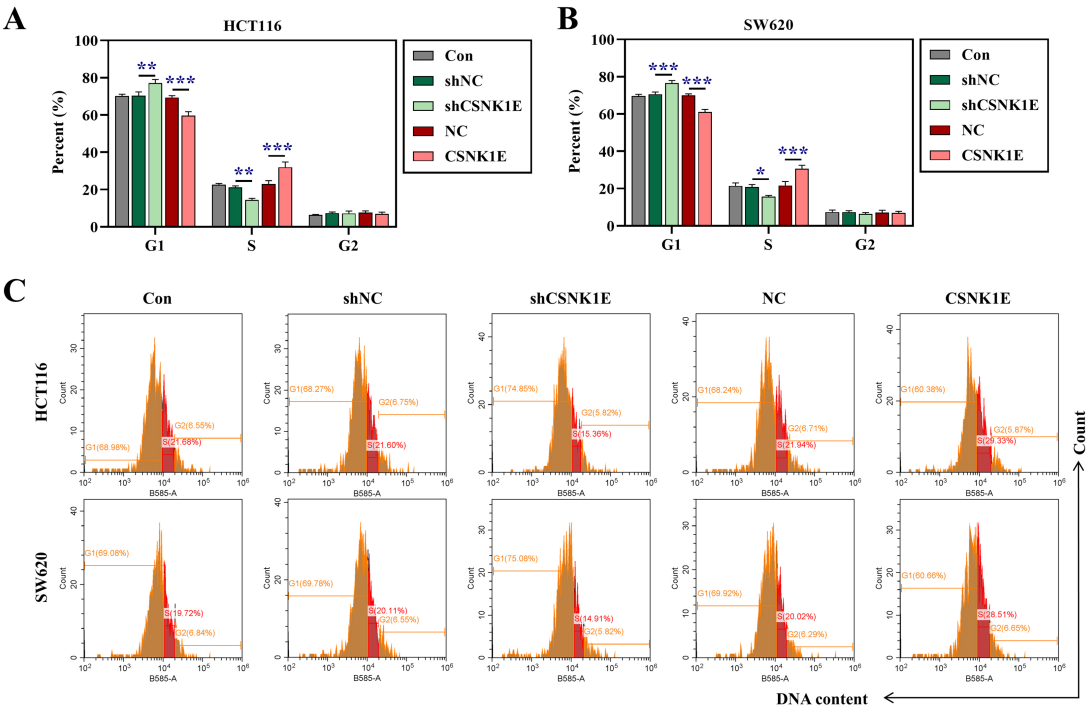

Fig. 3. CSNK1E positively impacted cell cycle transition of CRC cells. (A–C) The cell cycle transition of CRC cells was detected using flow cytometry. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. CRC, colorectal cancer; CSNK1E, casein kinase I isoform epsilon; Con, blank control; NC, negative control; shCSNK1E, short hairpin RNA against CSNK1E; shNC, short hairpin RNA against NC.

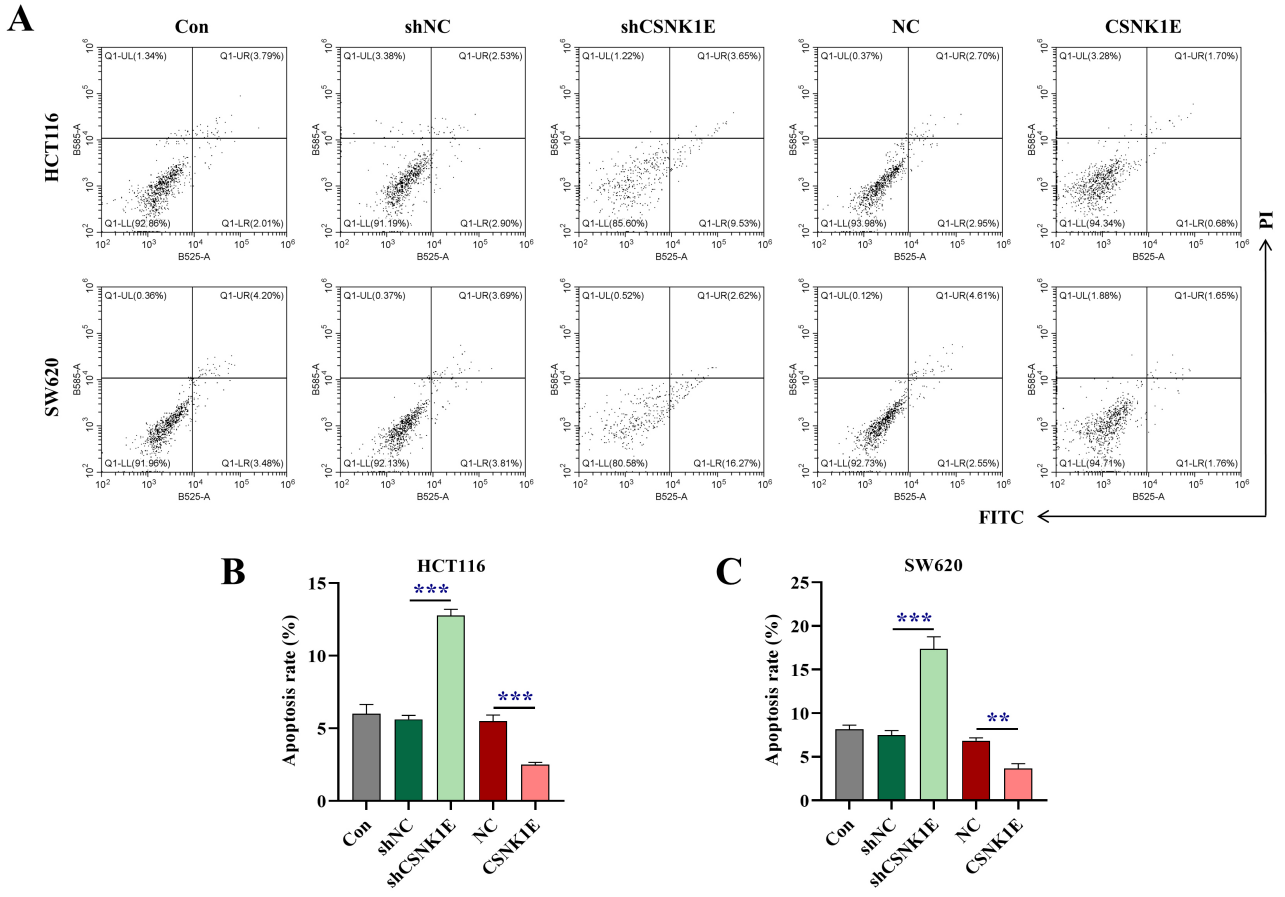

Fig. 4. CSNK1E negatively affected the apoptosis of CRC cells. (A–C) The apoptosis of CRC cells was detected using flow cytometry. $^{**}p < 0.01$, $^{***}p < 0.001$. CRC, colorectal cancer; CSNK1E, casein kinase I isoform epsilon; Con, blank control; NC, negative control; shCSNK1E, short hairpin RNA against CSNK1E; shNC, short hairpin RNA against NC.

Co-Immunoprecipitation (Co-IP) Assay

The binding relationship between CSNK1E and DVL1 was confirmed using Pierce Co-IP kits (26149, ThermoFisher, USA). In brief, lysates from HCT116 and SW620 cells (1×10^6) were prepared using IP Lysis Buffer and then precleared with Agarose Resin. The lysates were centrifuged at 2000 $\times$g for 20 min at 4 °C to obtain the supernatant. Then, the supernatants were incubated at 4 °C overnight with agarose resin coupled to antibodies against CSNK1E (ab270997, Abcam, Cambridge, UK) or DVL1 (sc-166303, Santa Cruz, Dallas, TX, USA), or with normal rabbit immunoglobulin G (ab171870, Abcam, UK) as a negative control to generate immunocomplexes. After being eluted using elution buffer (21009, ThermoFisher, USA), the immunocomplexes were subjected to Western blot analysis for examination of the enrichment of DVL1 or CSNK1E.

Statistical Analysis

All statistical analyses were performed with Graphpad Prism (version 8.0, GraphPad Software Inc., San Diego, CA, USA). The results from triplicate experiments were presented as mean $\pm$ standard deviation, with one-way analysis of variance (ANOVA) employed for the comparison among multiple groups. A $p < 0.05$ was regarded as statistically significant.

Results

CSNK1E Positively Modulated the Proliferation of CRC Cells

In this study, two human CRC cell lines (HCT116 and SW620 cells) were used to investigate the potential function of CSNK1E during the development of CRC. CSNK1E expression in HCT116 and SW620 cells (hereinafter referred to as CRC cells) was modulated through transfection. qRT-PCR confirmed that shCSNK1E effectively knocked down CSNK1E, whereas the CSNK1E overexpression plasmid successfully increased its expression (Fig. 1A,B, $p < 0.001$). Subsequently, the proliferation assay was carried out utilizing the EdU kit and showed that CSNK1E overexpression increased the number of EdU-positive CRC cells, while CSNK1E knockdown exerted the opposite effects, indicating a pro-proliferative role of CSNK1E overexpression (Fig. 1C–E, $p < 0.001$). Colony

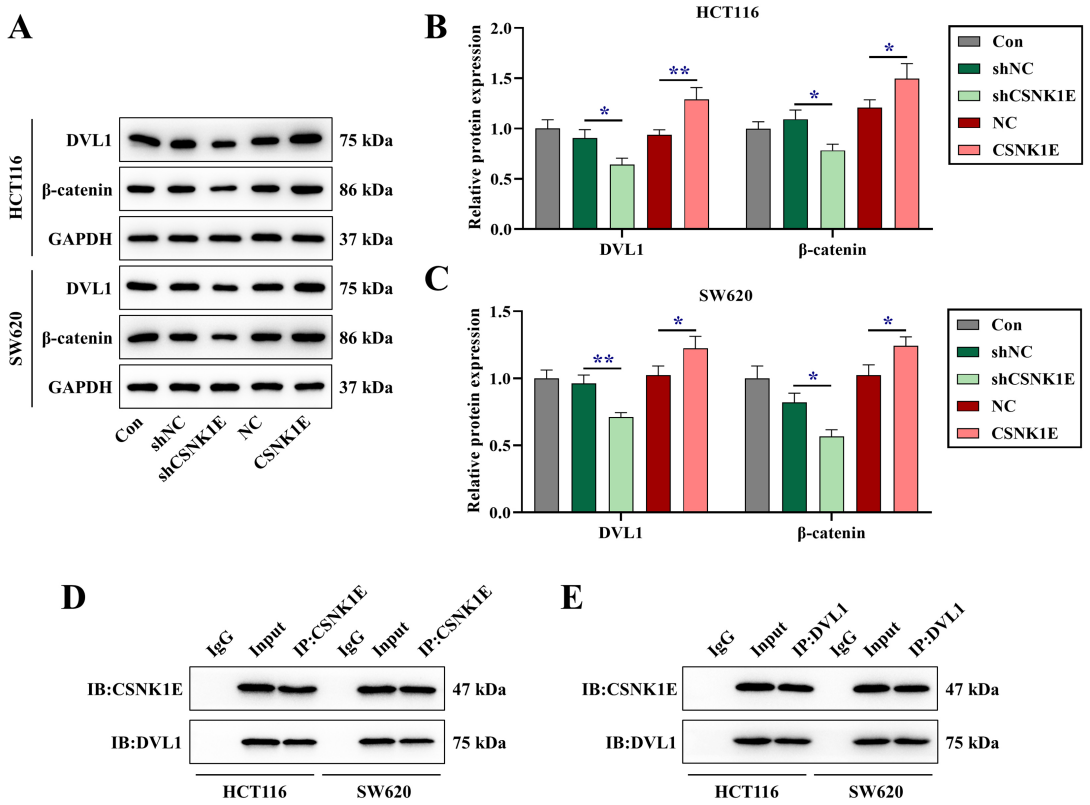

Fig. 5. CSNK1E interacted with DVL1 and its expression was positively correlated with DVL1 expression and Wnt signaling activation in CRC cells. (A–C) The protein levels of DVL1 and β-catenin in CRC cells were assessed using Western blot analysis. GAPDH was used as the normalizer. (D,E) The interaction between CSNK1E and DVL1 was validated through Co-immunoprecipitation assay in HCT116 and SW620 cells. *$p < 0.05$, **$p < 0.01$. CRC, colorectal cancer; CSNK1E, casein kinase I isoform epsilon; DVL1, dishevelled segment polarity protein 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Con, blank control; NC, negative control; shCSNK1E, short hairpin RNA against CSNK1E; shNC, short hairpin RNA against NC; IgG, immunoglobulin G; IB, immunoblotting; IP, immunoprecipitation.

formation assay was also conducted for assessing cell proliferation, and suggested that shCSNK1E-transfected CRC cells formed fewer colonies, whereas CRC cells overexpressing CSNK1E formed a greater number of colonies (Fig. 2A–C, $p < 0.01$).

CSNK1E Positively Impacted Cell Cycle Transition, but Negatively Affected the Apoptosis of CRC Cells

Flow cytometry revealed that CSNK1E knockdown induced CRC cell cycle arrest at the G1 phase and reduced the distribution of the cells at the S phase; in contrast, CSNK1E overexpression reduced cell distribution at the G1 phase and induced cell cycle arrest at the S phase in CRC cells (Fig. 3A–C, $p < 0.05$). Additionally, CSNK1E knockdown was demonstrated to promote apoptosis in CRC cells, while overexpression of CSNK1E inhibited apoptosis of CRC cells (Fig. 4A–C, $p < 0.01$).

CSNK1E Interacted With DVL1 and Its Expression Was Positively Correlated With DVL1 Expression and Wnt Signaling Activation in CRC Cells

Remarkably, the analysis using STRING identified DVL1 as a protein co-expressed with CSNK1E. Western blot analysis showed that CSNK1E knockdown or overexpression in CRC cells resulted in downregulation or upregulation of DVL1, accompanied by diminished or elevated β-catenin expression (Fig. 5A–C, $p < 0.05$). Co-IP assays further denoted that DVL1 protein was enriched by anti-CSNK1E antibody, and CSNK1E was precipitated by anti-DVL1 antibody (Fig. 5D,E), indicating that CSNK1E and DVL1 are present in the same protein complex in CRC cells. Although these results suggest a close association, they do not definitively exclude the possibility that the interaction is mediated by other adapter proteins.

CSNK1E Elevated DVL1 Expression to Promote CRC Cell Proliferation

To further explore the role of DVL1 in mediating the effects of CSNK1E on CRC development, DVL1 ex-

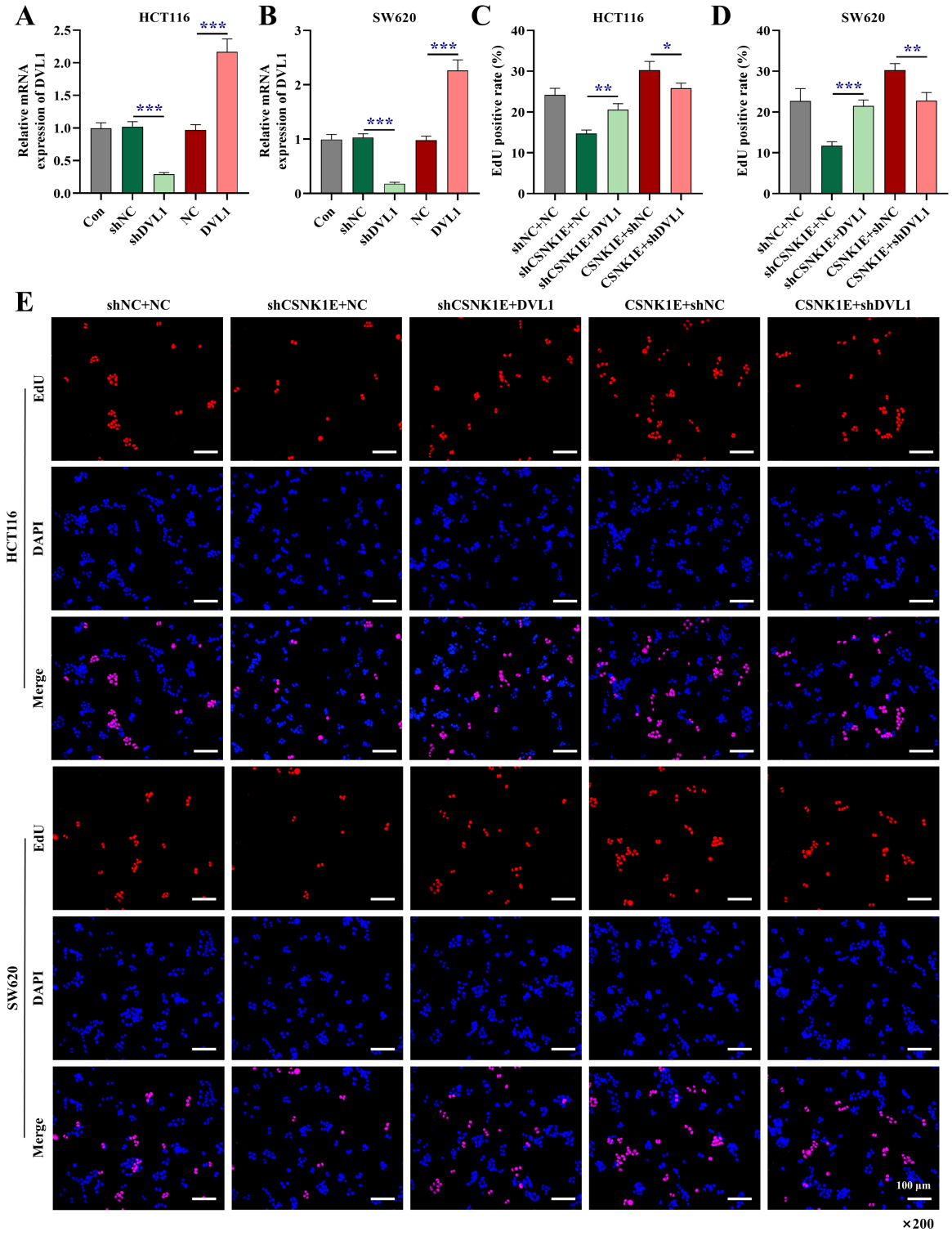

Fig. 6. CSNK1E positively modulated the proliferation of CRC cells by elevating DVL1. (A,B) The expression of DVL1 in CRC cells was assessed using qRT-PCR. GAPDH was used as the normalizer. (C–E) The proliferation of CRC cells was evaluated using the EdU assay. Red fluorescence: EdU-positive cells. Blue fluorescence: cell nuclei. Resolution: 100 μm, ×200. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. CRC, colorectal cancer; CSNK1E, casein kinase I isoform epsilon; DVL1, dishevelled segment polarity protein 1; EdU, 5-ethynyl-2′-deoxyuridine; DAPI, 4′,6-diamidino-2-phenylindole; qRT-PCR, quantitative reverse transcription polymerase chain reaction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Con, blank control; NC, negative control; shCSNK1E/shDVL1, short hairpin RNA against CSNK1E or DVL1; shNC, short hairpin RNA against NC.

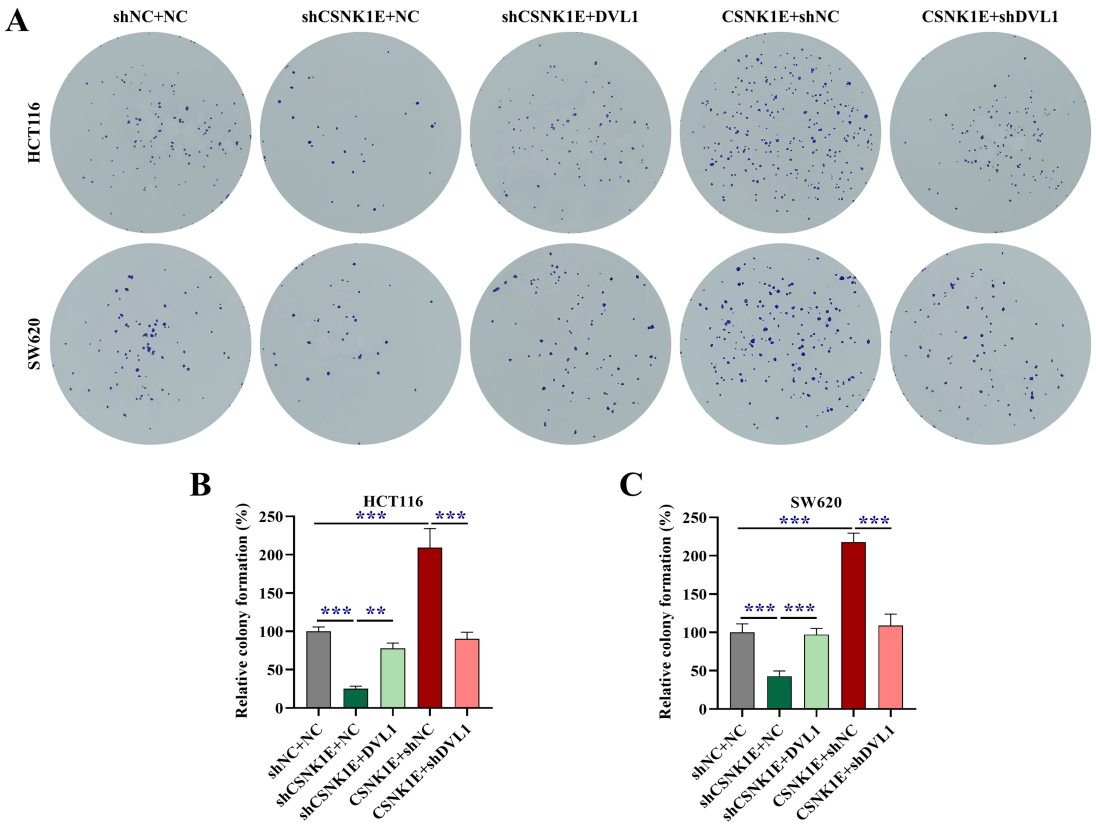

Fig. 7. CSNK1E promoted the proliferation of CRC cells by elevating DVL1. (A–C) The proliferation of CRC cells was evaluated using the colony formation assay. $^{**}p < 0.01$, $^{***}p < 0.001$. CRC, colorectal cancer; CSNK1E, casein kinase I isoform epsilon; DVL1, dishevelled segment polarity protein 1; Con, blank control; NC, negative control; shCSNK1E/shDVL1, short hairpin RNA against CSNK1E or DVL1; shNC, short hairpin RNA against NC.

pression in CRC cells was modulated via transfection with shDVL1 or DVL1 overexpression plasmid, and the transfection efficiency was verified to be high, as evidenced by the markedly downregulated or upregulated DVL1 expression (Fig. 6A,B, $p < 0.001$). Furthermore, EdU assay demonstrated that DVL1 upregulation mitigated the reduction in EdU-positive CRC cells caused by CSNK1E knockdown, while DVL1 downregulation counteracted the increase in the EdU-positive cells induced by CSNK1E overexpression (Fig. 6C–E, $p < 0.05$). Colony formation assays showed that DVL1 upregulation attenuated the inhibitory effect of CSNK1E knockdown, whereas DVL1 downregulation diminished the promotive effect of CSNK1E overexpression on CRC cell colony formation (Fig. 7A–C, $p < 0.01$).

CSNK1E Upregulated DVL1 Level to Drive CRC Cell Cycle Transition and Inhibit Apoptosis

DVL1 upregulation alleviated CSNK1E knockdown-induced CRC cell cycle arrest at the G1 phase to increase the distribution of CSNK1E-underexpressing CRC cells at the S phase (Fig. 8A–C, $p < 0.05$). On the contrary, DVL1 downregulation negated the transition of the G1 phase to the S phase driven by CSNK1E overexpression (Fig. 8A–C, $p < 0.05$). Meanwhile, the promotive effect of CSNK1E knockdown on CRC cell apoptosis was offset by DVL1 upregulation, while DVL1 downregulation counteracted CSNK1E overexpression-induced inhibition on CRC cell apoptosis (Fig. 9A–C, $p < 0.001$).

CSNK1E Prompted DVL1 Expression to Activate Wnt Signaling in CRC Cells

Transfection-mediated DVL1 upregulation or downregulation neutralized the inhibitory effect of CSNK1E knockdown or the promoting effect of CSNK1E overexpression on the expression of DVL1 in CRC cells (Fig. 10A–C, $p < 0.05$). Notably, the inhibition by CSNK1E knockdown on the expression of β-catenin in CRC cells was reversed by DVL1 upregulation, whereas DVL1 downregulation negated the promotion by CSNK1E overexpression on the β-catenin expression (Fig. 10A–C, $p < 0.05$).

Discussion

CRC is considered curable when detected at early stages [23]. However, early-stage tumors are prone to metastasize, posing major challenges for comprehensive

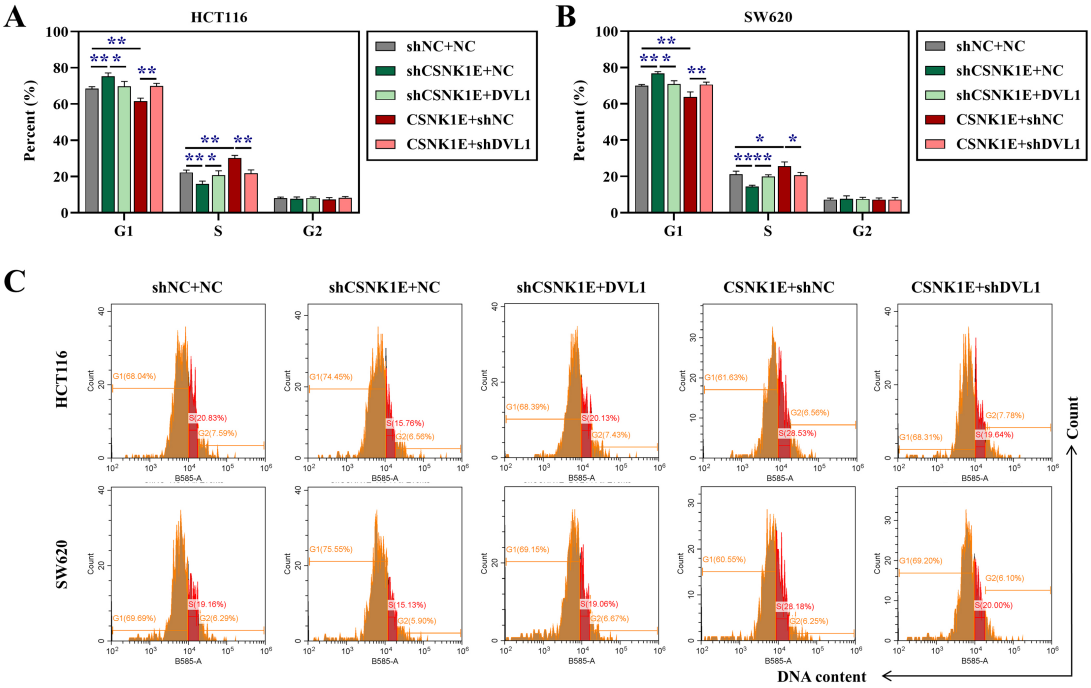

Fig. 8. CSNK1E upregulated DVL1 level to drive CRC cell cycle transition. (A–C) The cell cycle transition of CRC cells was detected using flow cytometry. $*p < 0.05$, $**p < 0.01$. CRC, colorectal cancer; CSNK1E, casein kinase I isoform epsilon; DVL1, dishevelled protein homolog 1; NC, negative control; shCSNK1/shEDVL1, short hairpin RNA against CSNK1E or DVL1; shNC, short hairpin RNA against NC.

CRC treatment, in which targeted therapies play a central role [24]. Inducing cell cycle arrest is a common mechanism underlying targeted therapies and is particularly effective in CRC [12]. Therefore, identification of novel targeted therapies that hinder cell cycle transition might contribute to a conspicuous improvement in the outcomes of CRC patients.

Twenty-four-hour circadian rhythms enable most organisms to physiologically produce regular oscillations, thus synchronizing them with the environmental changes [25]. Malfunction of CCGs, which orchestrate the rhythm, accelerates the development and progression of various cancers by modulating cell processes, including cell cycle transition [26]. In CRC, multiple CCGs are dysregulated, and among them, CSNK1E expression exhibits a higher level and serves as a major prognostic marker predicting a shorter DFS time in CRC patients [11]. Also, CSNK1E has been recorded as a synthetic lethal partner to a tumor suppressive protein, TP53, which is frequently mutated in CRC, and deletion of CSNK1E results in the death of TP53-mutant CRC cells [27]. These findings hint that CSNK1E may be oncogenic in CRC. CCGs have been proven to modulate cell cycle transition, as well as proliferation and apoptosis, both of which closely relate to cell cycle transition during the tumor process [9]. CRC cells are featured with uncontrolled proliferation [25]. CSNK1E knockdown causes the decline of the cell proliferation rate [28]. Depletion of CSNK1E reduces the viability of glioblastoma cells [29]. Consistently, in our study, the proliferation of CRC cells was positively proportional to CSNK1E expression. The mechanism underlying uncontrolled cell proliferation is the dysregulation of the cell cycle; rapid cell division, which is indicative of high proliferation, often results from alterations in cell cycle machinery, such as facilitated transition from the G1 phase to the S phase of DNA synthesis [30]. This transition is pivotal to eukaryotic cell proliferation, and its deregulation is a key contributor to carcinogenesis [31]. Knocking down CSNK1E has been shown to induce fibrosarcoma-cell-selective cytotoxicity by generating cell cycle arrest at the G2/M phase [28]. However, our results depicted that the entry from the G1 to S phase in CRC cells was driven by CSNK1E overexpression, while CSNK1E deficiency resulted in cell cycle arrest at the G1 phase. This discrepancy highlights a context-dependent role of CSNK1E in cell cycle regulation, suggesting that its function may be wired into distinct checkpoint networks in different cancer types. Since an altered cell cycle may arrest cells with irreparable DNA damage at a certain phase or prevent cells with damaged DNA from repairing and directly drive them into the subsequent phase, thereby activating apoptotic pathways [32], our results suggest that CSNK1E may negatively regulate apoptosis in CRC cells. CSNK1E deficiency induces apoptosis of glioblastoma cells [29] and fibrosarcoma cells [28]. Building on these foundational roles in proliferation and survival, our study further elucidates a key mechanism: CSNK1E drives CRC progression

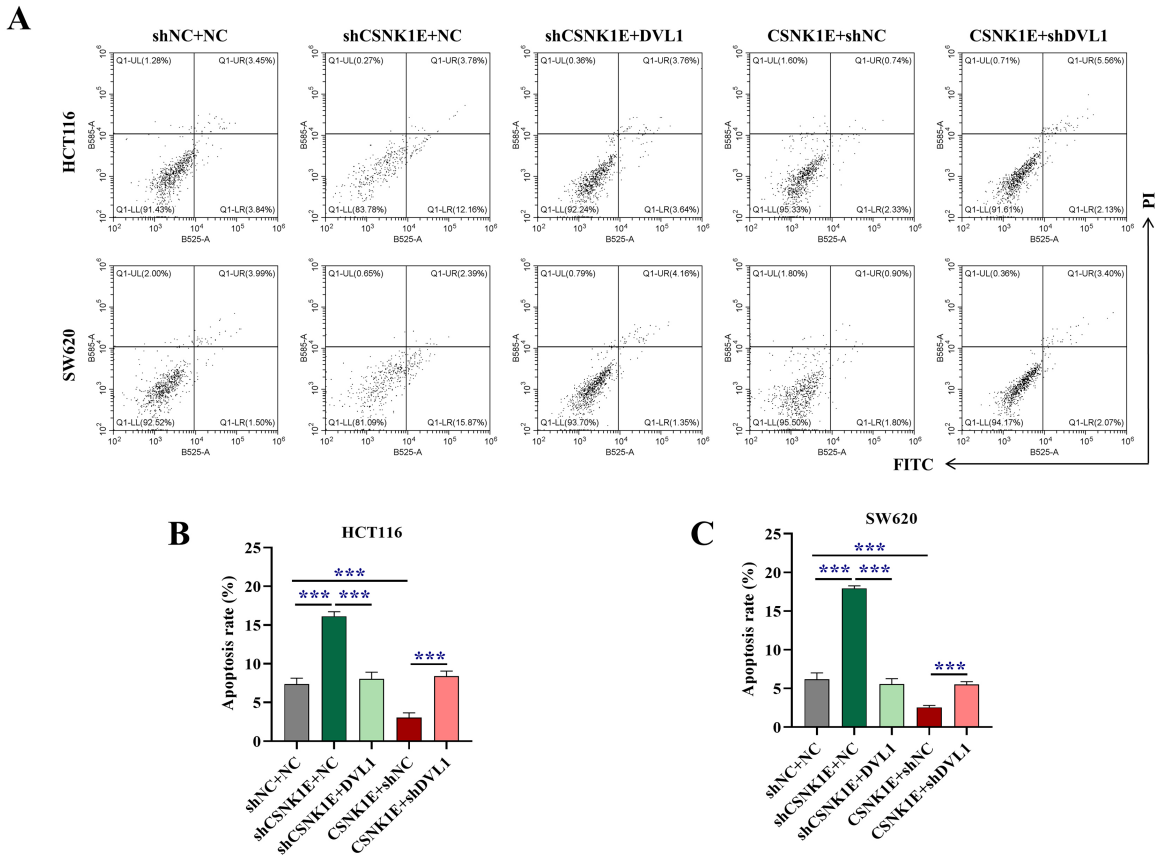

Fig. 9. CSNK1E upregulated DVL1 level to inhibit CRC cell apoptosis. (A–C) The apoptosis of CRC cells was detected using flow cytometry. ***$p < 0.001$. CRC, colorectal cancer; CSNK1E, casein kinase I isoform epsilon; DVL1, dishevelled protein homolog 1; NC, negative control; shCSNK1/shEDVL1, short hairpin RNA against CSNK1E or DVL1; shNC, short hairpin RNA against NC.

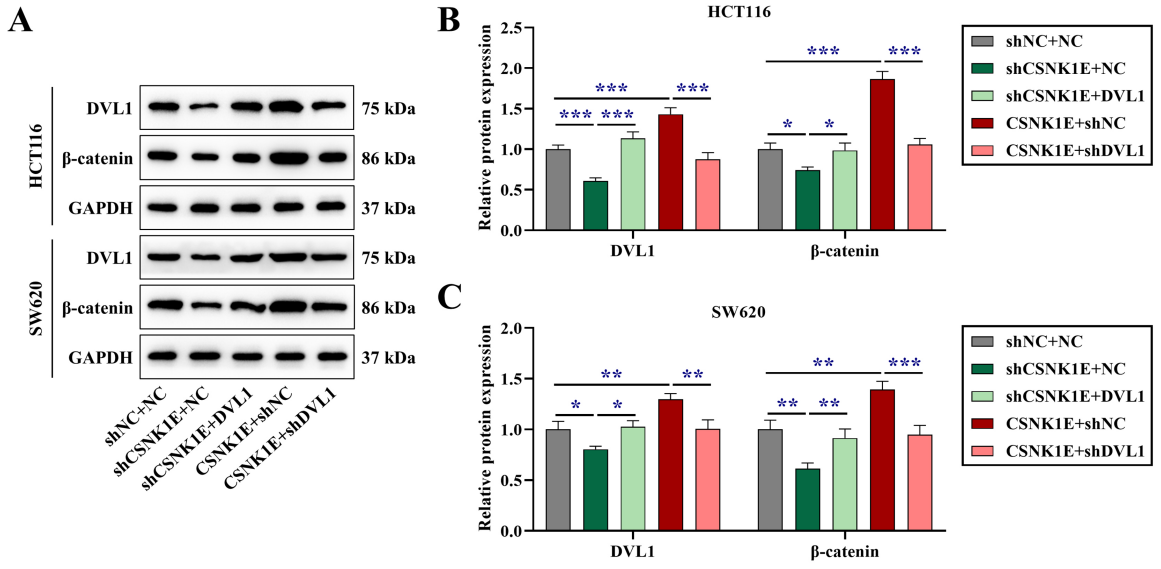

Fig. 10. CSNK1E prompted DVL1 expression to activate Wnt signaling in CRC cells. (A–C) The protein levels of DVL1 and β-catenin in CRC cells were assessed by Western blot analysis. GAPDH was used as the normalizer. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. CRC, colorectal cancer; CSNK1E, casein kinase I isoform epsilon; DVL1, dishevelled segment polarity protein 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; NC, negative control; shCSNK1/shEDVL1, short hairpin RNA against CSNK1E or DVL1; shNC, short hairpin RNA against NC.

by directly interacting with and stabilizing DVL1, a core component of the oncogenic Wnt/β-catenin pathway. This positions the CSNK1E/DVL1 axis as a novel functional module linking circadian dysregulation to a cornerstone signaling pathway in CRC. The above results from our study reveal that CSNK1E contributes to CRC development by promoting CRC cell cycle transition to enhance CRC cell proliferation and suppress CRC cell apoptosis, suggesting that CSNK1E can be a promising therapeutic target for improving the clinical outcomes in CRC patients.

CSNK1E is a key protein of the Wnt/β-catenin pathway, and its effect on tumorigenesis, including cell cycle transition, is mediated by regulating this pathway [29]. The Wnt/β-catenin pathway is initiated by the binding of extracellular Wnt ligands to their membrane receptor, after which DVL is activated and multimerized with Axin to inactivate the destruction complex that mediates the proteasome-induced degradation of β-catenin to stabilize β-catenin; the stabilized β-catenin further translocates into the nucleus, where it associated with T-cell factor (TCF)/lymphoid enhancing factor (LEF) transcription factors to facilitate the expression levels of genes involved in cell proliferation, survival, differentiation, and migration [19,33]. CSNK1E is one of the core components of the β-catenin destruction complex and, in the absence of Wnt, cooperates with GSK3β to phosphorylate β-catenin at serine 45 and threonine 41, thereby promoting its degradation [34]. Of note, DVL has been established as a dual function adaptor that can inhibit this signaling in addition to inducing its activation; for example, the phosphorylation of DVLs by CSNK1E triggers a negative feedback loop by interaction with an E3 ubiquitin ligase, Huwe1/EEL-1, which inhibits DVL multimerization, thereby blocking Wnt/β-catenin signaling [35]. Also, DVL recruits zinc ring finger protein 3 (ZNRF3)/ring finger protein 43 (RNF43) for the degradation of Wnt receptors and thus negatively controls the Wnt pathway activity [36]. Our study discovered that CSNK1E overexpression positively regulated the expression of DVL1 and β-catenin in CRC cells, whereas DVL1 downregulation/upregulation generated a trend inverse to that of CSNK1E overexpression/knockdown. Concretely, DVL1 downregulation/upregulation attenuated the effect of CSNK1E overexpression/knockdown on CRC development and β-catenin expression in CRC cells. This indicates that, contrary to its canonical role in the destruction complex or in mediating negative feedback, CSNK1E can function as a positive regulator upstream of DVL1 in CRC. These findings collectively suggest that the oncogenic role of CSNK1E in CRC is attributed to the DVL1-dependent activation of Wnt/β-catenin signaling. This newly identified CSNK1E/DVL1 axis thus represents a context-specific mechanism contributing to the sustained activation of a pivotal oncogenic pathway in colorectal cancer.

This study has certain limitations that warrant further investigation in future research. Firstly, although our Co-IP results demonstrate an interaction within the cellular context, this assay does not exclude the possibility that the interaction is mediated by other proteins. Therefore, future studies using direct binding assays are needed to confirm a physical interaction between CSNK1E and DVL1. Moreover, our findings are derived from cellular models. Validating the pathological relevance of the CSNK1E/DVL1 axis in clinical specimens and its oncogenic function *in vivo* using animal models is crucial for advancing its translational potential.

Conclusion

In conclusion, the present study unveils the oncogenic role of CSNK1E and the related mechanisms, while demonstrating that knockdown of CSNK1E decreases the expression of DVL1 to block Wnt/β-catenin signaling and hence inhibits CRC development by inducing CRC cell cycle arrest. This study implies that the induction of cell cycle arrest by targeting CSNK1E to block Wnt/β-catenin signaling can be a potential option for CRC treatment.

Availability of Data and Materials

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

Author Contributions

WL and JC designed the research study; JY and ZJ performed the research; BZ and DW collected and analyzed the data. ZJ has been involved in drafting the manuscript and all authors have been involved in revising it critically for important intellectual content. All authors gave final approval of the version to be published. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.24976/Discov.Med.202638208.115.

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