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Original Article

Livin promotes tumor progression through YAP activation in ovarian cancer

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Abstract: Ovarian cancer is a gynecological malignant tumor with a high morbidity. Livin is a novel member of the inhibitor of apoptosis protein family, which is expressed in various malignant tumors and is suggested to be a poor prognostic factor. However, the prognostic significance of Livin and the molecular mechanisms by which Livin promotes ovarian cancer progression are poorly understood. In this study, the upregulation of Livin was confirmed both in primary specimens from ovarian cancer patients and in ovarian cancer cell lines compared to normal controls *in vitro*. Overexpression of specific Livin transcripts promoted cell growth and migration *in vitro,* while knockdown of Livin expression suppressed these cellular processes. These effects of the Livin gene were also demonstrated in a xenograft mouse model. Mechanistic studies further revealed that Livin promotes the proliferation and invasion of ovarian cancer cells by activating the transcriptional coactivator YAP, a critical component of the Hippo signaling pathway. Furthermore, we revealed that inhibition of YAP by short-hairpin RNA prevents the growth and invasion of ovarian cancer cells *in vivo* and *in vitro*. Therefore, Livin may be a potential novel therapeutic target for the treatment of ovarian cancer.

Keywords: Ovarian cancer, Livin, Hippo/YAP signaling pathway, apoptosis

Introduction

Ovarian cancer is a common malignant tumor in women, and its incidence is increasing worldwide [1]. Ovarian cancer has unique characteristics of difficult diagnosis and early metastasis [2]. However, the molecular mechanisms underlying the metastasis of ovarian cancer have not been clarified. Therefore, finding the molecular mechanism of ovarian cancer development is of great significance for the treatment of ovarian cancer patients.

Livin is a member of the inhibitor of apoptosis protein (IAP) family encoded by the BIRC7 gene, which consists of a single baculoviral IAP repeat domain and a RING domain at the C-terminus. The protein inhibits apoptosis by inhibiting the proteolytic activation of caspases, such as caspase-3, -7 and -9, and it plays an important role in tumorigenesis and chemoresistance [3]. Overexpression of Livin has been observed in a variety of cancers, including lung, colon, and prostate cancers, but Livin is rarely detected in normal adult tissues. It is thought that Livin protein expression may be an early event in the occurrence of HCC [4]. Aberrant Livin expression has been demonstrated in human malignancy and tumor tissue cells in most tumors, and the expression of Livin correlated with *in vitro* drug resistance, advanced tumor stage, and poor outcome [5-9]. Knockdown of Livin has been shown to reduce tumor cell proliferative potential and/or induce sensitization toward proapoptotic stimuli in glioma cells [7], lung cancer cells [10] or neuroblastoma cells [11]. Together, these studies suggest that Livin may be essential for the progression of many tumors. However, the significance of Livin in ovarian cancer cells and the underlying molecular mechanisms have not been investigated.

The Hippo pathway was originally discovered as a key factor in the control of Drosophila organ size and was recently found to be important in human cancer [12-14]. Yes-associated protein 1 (YAP1), a 65-kDa proline-rich phosphoprotein and a transcription coactivator, is a major downstream target of the Hippo signaling pathway [15-17]. Regulation of the Hippo signaling path-

way is known to be mediated by phosphorylation and subcellular localization of YAP1. Activation of the Hippo signaling pathway induces phosphorylation of YAP1, which prevents translocation to the nucleus. When the Hippo signaling pathway is inactivated, dephosphorylated YAP1 translocates to the nucleus, interacts with transcription factors, and eventually causes the proliferation of cells of various organ systems [18, 19]. Recently, YAP1 has been suggested to be a potent oncogene, and it was found to be elevated in several types of cancers compared to normal controls [19-23]. In addition, YAP1 is implicated in the EMT program in diverse cancers, including liver, colon, prostate, ovarian, and breast cancers [24-26]. However, the expression of YAP1 and its correlation with Livin in ovarian cancer have rarely been reported.

In this study, combining *in vitro* and *in vivo* analyses, we investigated i) the difference in Livin expression in both primary specimens from ovarian cancer patients and ovarian cancer cell lines compared to normal controls; ii) the effects of overexpression and downregulation of Livin on ovarian cancer cell progression *in vitro* and *in vivo*; and iii) the relationship between Livin and the activation and nuclear translocation of transcriptional coactivator YAP, the key component of the Hippo signaling pathway and the functional role of this relationship. Our study indicates that the activation of YAP/TAZ by Livin drives the progression of ovarian cancer cells *in vitro* and *in vivo*. Therefore, Livin could be a potential novel therapeutic target for the treatment of ovarian cancer in the clinic.

Materials and methods

Patients and tissue specimens

A total of 44 ovarian cancer samples and 21 normal samples were collected from the Department of Gynecology, the First Affiliated Hospital of Harbin Medical University. The samples were quickly frozen in liquid N_2 after collection immediately and then stored at -80°C before use. The research ethics committee of the First Affiliated Hospital Harbin Medical University approved the research in accordance with the Declaration of Helsinki.

Cell culture and reagents

Immortalized epithelial cell lines (HOSE and FTE188) were obtained from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences, Shanghai Institute of Cell Biology (Shanghai, China). The ovarian cancer cell lines (A2780, HO-8910, HO-8910PM, CoC1, Caov-3, OV-1063, SKOV3 and OVCAR-3) were purchased from National Infrastructure of Cell Line Resource (Beijing, China). Immortalized ovarian epithelial cell lines and ovarian cancer cell lines were cultured as described previously.

Lentivirus vector construction and siRNA transfection

The lentivirus vectors for knockdown and overexpression of Livin were produced by GenePharma (Shanghai, China). The shRNAs against Livin (shLivin) and siRNAs against YAP/TAZ (si-YAP/TAZ) and negative control (NC) were synthesized by GenePharma (Shanghai, China). The siRNA sequence against Livin was 5'-CAGGCCATCAGGACAAGGT-3'. The siRNA sequences against YAP/TAZ were as follows: si-YAP#1: 5'-CCCAGTTAAATGTTCACCAAT-3'; siYAP#2: CAGGTGATACTATCAACCAAA-3'. siTAZ#1: 5'-CAGCCAAATCTCGTGATGAAT-3'; siTAZ#2: 5'-CCAGGAACAAACGTTGACTTA-3'. Ovarian cancer cells were transfected with shRNAs and siRNAs with Lipofectamine 3000 Reagent (Thermo, USA) according to the manufacturer's protocol.

Real-time PCR

All RNA of tissues and cells was isolated by TRIzol reagent (Invitrogen, USA), and 1 μg of total RNA was used to reverse-synthesize cDNA template following the Reverse Transcription kit instructions (Applied Biosystems Co., Ltd. USA) for the SYBR Green PCR (Takara, Japan) assay. The PCR primers and reaction conditions were produced as described previously, and the expression of Livin, CYR61, CTGF, and CCND1 was normalized to GAPDH. Each PCR experiment was performed at least 3 times independently, and the relative expression value was expressed by $2^{-\Delta\Delta}$Ct.

Western blotting (WB)

The ovarian cancer tissue and normal tissue were minced, homogenized and digested by using RIPA lysis buffer (with protease and phosphatase inhibitor, Thermo Scientific). The cells were scraped from the culture plate on ice and lysed with RIPA lysis buffer (with protease and phosphatase inhibitor, Thermo Scientific). The resulting suspensions were centrifuged at 13,000 g for 20 minutes at 4°C, and the super-

natant of protein was collected. Protein samples were prepared for PAGE. Proteins were then transferred to a PVDF membrane (Bio-Rad) for immunoblotting with relevant antibodies. The following antibodies were used in this study: Livin (ab11983), YAP (sc-271134), pSer127-YAP (PA5-17481), Lamin B (sc-374015), LAST1 (CST#3477), TAZ (ab84927), β-actin (ab115777), and GAPDH (CST#5174).

In vivo lung metastasis model

Six-week-old male BALB/c nude mice were used for the *in vivo* lung metastasis model. Ovarian cancer cells were injected into the tail vein of mice. The bioluminescence signal of lung metastasis from day 7 to day 28 was determined. After 4 weeks, all mice were sacrificed, and then immunohistochemical analysis and hematoxylin/eosin (HE) staining were performed.

Immunohistochemical (IHC) staining

The tumor tissues were embedded in paraffin, cut into 4 μm sections, and either HE stained or treated with corresponding antibodies for IHC evaluation. Slides were incubated with anti-Ki-67 for 2 h at 37°C. The second antibody was incubated for 1 h at room temperature (RT), and then diaminobenzidine (DAB) was used as chromogen. A terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) kit was used for apoptosis measurement according to the manufacturer's protocol. The brown dots within the nucleus were considered Ki-67- and TUNEL-positive cells.

In vivo tumor growth model

One hundred microliters of ovarian cancer cells transfected with shLivin or interference control vector were injected subcutaneously into each nude mouse at a density of 1×10^8 cells/ml in saline. We measured the volume of ovarian tumors every 5 days and then sacrificed the mice and isolated and weighed the tumors after 30 days.

Wound healing assay

The ovarian cancer cells were collected and implanted into a 6-well plate at a density of 5×10^5/mL. The cells were induced and cultivated to a confluence of nearly 90%. The ovarian cancer cells were scraped using a 200-μL pipette tip and cultured in FBS-free medium. Typical images were taken under the microscope 0 and 24 h after the scratches were formed.

Transwell assay

To evaluate the migration ability of ovarian cancer, a polycarbonate membrane filter coated with gelatin and 24-well perforated chambers (Corning) coated with Matrigel were used. The cells were suspended in medium without FBS, and the transformed cell density was 5×10^4/mL; 100 μL of the cell suspension was added to the insert, and 600 μL of medium containing 20% FBS (HyClone, FBS) was added to the lower chamber. After 24 h, the cells were stained with crystal violet (0.1%) for 15 minutes and then washed twice with PBS. ImageJ was used to observe the cells and count. The cells were observed with an optical microscope, and then different fields (6 fields per group) were randomly selected for counting.

Colony formation assay

Ovarian cancer cells were plated into 12-well plates (1×10^3/mL). The colonies were stained with 0.1% crystal violet (Sigma-Aldrich) after 2 weeks of culture and then washed with PBS twice. Visible violet colonies were counted and analyzed.

Cell counting Kit-8 (CCK-8) assay

The cell viability of ovarian cancer cells was evaluated by CCK-8 assay according to the manufacturer's protocol (MCE, China). Ovarian cancer cells were seeded in a 96-well plate at a density of 2×10^3 cells/mL and transfected with shRNA or plasmid 24 h after implantation. After culturing for 2 days, the medium in each well was replaced with 100 ml of culture medium containing 10 ml of CCK-8 solution. The absorbance at 450 nm was measured by a spectrometer (Thermo Fisher, Rockford, IL, USA).

Statistical analysis

Data are presented as the mean ± SEM. Statistical analyses were performed with GraphPad Prism software (version 8.0). Statistical significance was compared via unpaired Student's t test or one-way analysis of variance (ANOVA) followed by Bonferroni's multiple as

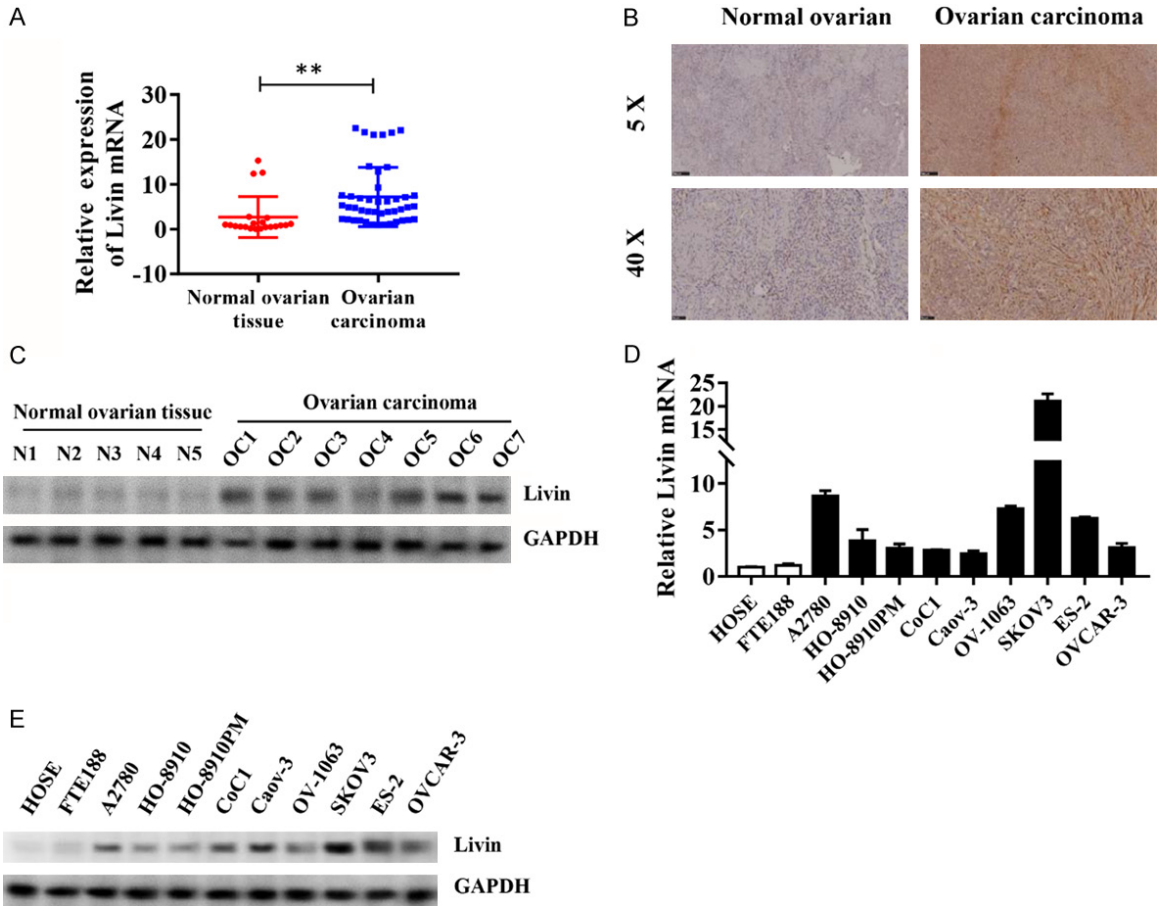


Figure 1. Livin is upregulated in human ovarian carcinoma. A. The mRNA level of Livin in ovarian tissue and normal ovarian tissue. B. IHC staining of Livin in ovarian carcinoma tissue and normal ovarian tissue from different clinical samples. C. WB analysis of Livin in ovarian carcinoma tissue and normal ovarian tissue from different clinical samples. D, E. The mRNA and protein levels of Livin in different ovarian cancer cell lines (HOSE 6-3, FTE188, A2780, HO-8910, HO-8910PM, CoC1, Caov-3, OV-1063, SKOV3 and OVCAR-3). **P < 0.01.

appropriate. Differences were considered statistically significant at the level of $P < 0.05$.

Results

Livin is upregulated in human ovarian carcinoma

We analyzed the expression of Livin in the patient samples collected during ovarian surgery. Compared with normal ovarian tissues, the mRNA level of Livin is higher in ovarian tissue (**Figure 1A**). Then, we measured the expression of Livin in ovarian carcinoma tissue and normal ovarian tissue from different clinical samples by IHC staining and WB and found that Livin was increased in all ovarian carcinoma samples (**Figure 1B** and **1C**). Next, we detected the expression of Livin in human ovarian surface epithelial (HOSE) cells and fallopian tube epithelial (FTE188) cells and different ovarian carcinoma cell lines (A2780, HO-8910, HO-8910PM, CoC1, Caov-3, OV-1063, SKOV3 and OVCAR-3) at the mRNA and protein levels. The results showed that Livin expression was consistently upregulated in ovarian carcinoma cell lines, especially in A2780 and SKOV3 cells (**Figure 1D** and **1E**). These findings indicate that Livin is increased in ovarian carcinoma.

Livin promotes the proliferation and migration of ovarian cancer cells

To explore the function of Livin in ovarian carcinoma, we transfected ovarian carcinoma cell lines with lentivirus carrying Livin or control vector. We measured cell viability at different times (0 h, 24 h, 48 h, 72 h and 96 h) after infection and found that compared with the vector control cells, A2780 and SKOV3 cells infected with

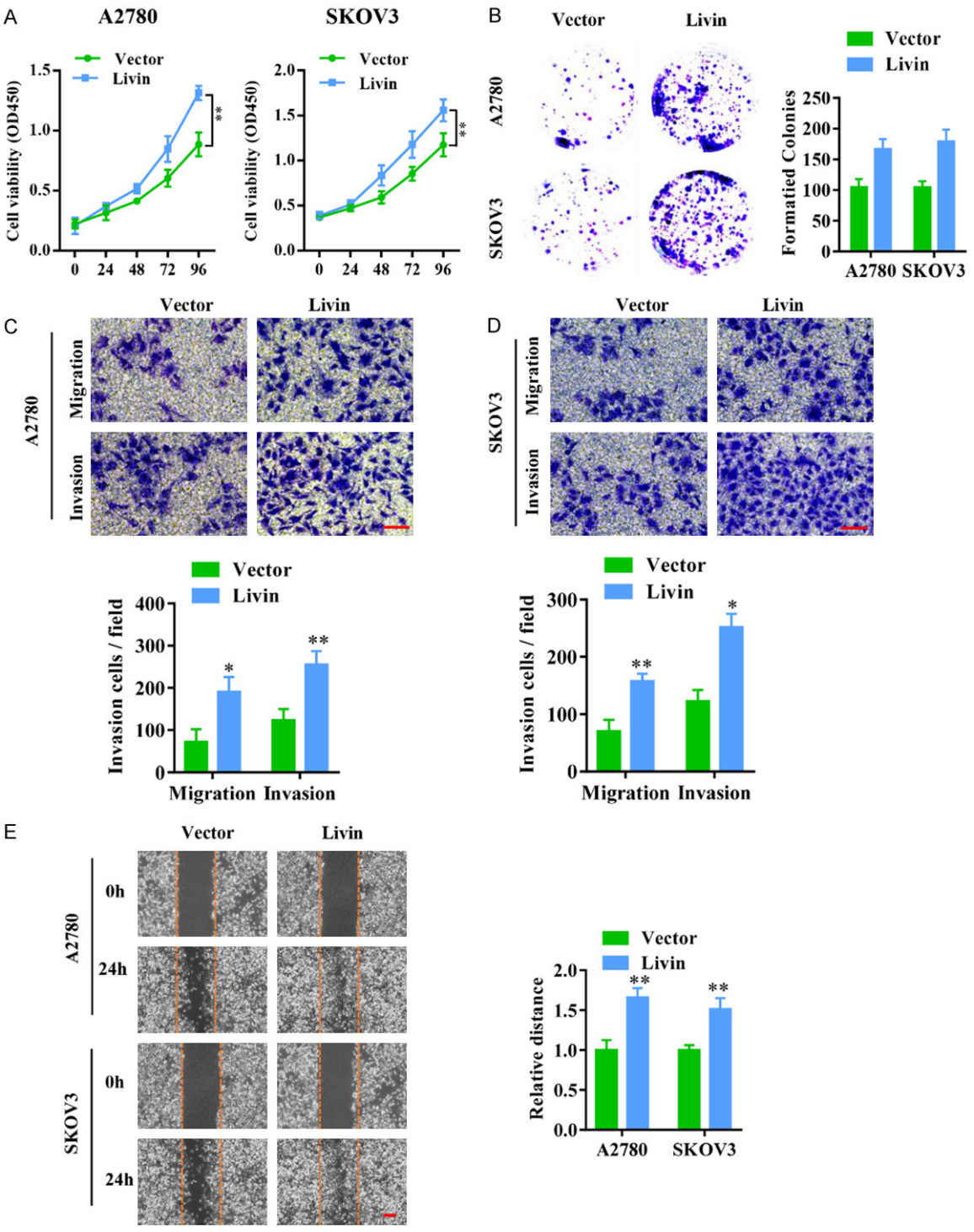


Figure 2. Livin overexpression promotes the proliferation and migration of ovarian cancer cells. A. The cell viability of A2780 and SKOV3 cells was evaluated by CCK-8. B. The colony formation of A2780 and SKOV3 cells transfected with vector and Livin. C, D. The invasive ability of A2780 and SKOV3 cells was measured by Transwell assay. E. The migration potential of A2780 and SKOV3 cells was evaluated by the wound healing test. Scale bars: 200 μm. *P < 0.05, **P < 0.01.

Livin showed increased cell viability (**Figure 2A**). Next, we performed colony formation assays of A2780 and SKOV3 cells infected with lentivirus carrying Livin or vector. Our data indicate that Livin overexpression significantly increased the colony formation of ovarian carci-

noma cells (**Figure 2B**). By measuring the migration and invasion ability of ovarian cancer by Transwell assay and wound healing assay, we found that the upregulation of Livin increased the cell migration and invasion abilities of ovarian cancers (**Figure 2C-E**).

To further explore the function of Livin in ovarian carcinoma progression, we infected A2780 and SKOV3 cells with lentivirus carrying specific short-hairpin RNA (shRNA) targeting Livin (shLivin-1 and shLivin-2), and the knockdown efficiency was verified by WB (**Figure 3A**). Colony formation assays, Transwell assays and wound healing assays were performed to examine the effects of downregulation of Livin on cell colony formation, migration and invasion of ovarian carcinoma cells. Our results suggest that Livin downregulation suppressed cell viability, colony formation, migration and invasion of ovarian carcinoma cells (**Figure 3A-E**). These results indicate that Livin plays important roles in the process of cell proliferation, migration, and invasion of ovarian carcinoma cells.

Livin promotes ovarian carcinoma growth and metastasis in vivo

We next used a xenograft mouse model to explore the effect of Livin inhibition on ovarian cancer cell growth. A2780 cells stably infected with lentivirus encoding shLivin or empty vector were subcutaneously injected into nude mice. After four weeks, we found that shLivin significantly reduced the tumor volume and tumor weight of shLivin mice compared with the control group mice (**Figure 4A** and **4B**). In addition, IHC analysis showed that the proportion of Ki-67-positive cells decreased, while the proportion of TUNEL-positive cells increased in all shLivin groups compared with the control group (**Figure 4C**). These results indicate that silencing Livin expression inhibits the growth of ovarian cancer cell-derived tumors in the mouse xenograft model. Next, we evaluated whether Livin influences the metastasis of ovarian carcinoma cells *in vivo*. A2780 cells infected with lentivirus carrying shLivin were injected into the tail vein of BALB/c nude mice. The luciferase signals were measured at day 28 after injection to monitor the xenograft growth of ovarian carcinoma in the lung. We found that the shLivin group showed lower luciferase signals than the control group at day 28 after injection (**Figure 4D**). HE staining of the lungs of the different groups also showed that shLivin inhibited the occupation of ovarian carcinoma cells in the lungs (**Figure 4D**). Altogether, we discovered that shLivin effectively inhibits the growth of A2780 cells and their metastasis to the lungs of nude mice compared to the control mice (**Figure 4D**).

Livin activates YAP/TAZ in human ovarian cancer cell lines

To investigate whether Livin promotes the progression of ovarian cancer cells by activating the YAP/TAZ signaling pathway, we measured the protein expression and translocation of YAP from the cytosol to the nucleus in A2780 and SKOV3 cells by immunofluorescence (IF) and WB analysis (**Figure 5A-D**). IF and WB analysis of biochemically fractionated nuclear and cytoplasmic extracts after Livin upregulation clearly showed the nuclear accumulation of YAP (**Figure 5A-D**). Accordingly, the expression of the YAP TEAD-regulated genes *CTGF*, *CYR61* and *CCND1* was found to be significantly increased when Livin was upregulated (**Figure 5E**), confirming the functional association between Livin and YAP activity.

Livin acts through LATS, Rho/ROCK, and actin cytoskeleton to activate YAP/TAZ

The key protein mediating the phosphorylation of YAP is LATS, which is one of the core components of the Hippo pathway [27-29]. LATS regulates target gene expression by phosphorylating, thereby restricting YAP and transcriptional coactivator with PDZ-binding motif (TAZ) in the cytoplasm [28, 29]. To investigate whether LATS1 is involved in YAP/TAZ regulation by Livin, we assessed the kinase activity of LATS1 immunopurified from control or Livin-transfected cells. As shown in **Figure 6A**, Livin overexpression markedly reduced LATS1 kinase activity, which was coincident with YAP/TAZ dephosphorylation. Pretreatment of cells with botulinum toxin C3, a specific inhibitor of Rho GTPases, blocked YAP/TAZ activation by Livin (**Figure 6B**). We also found that pretreatment with GSK429286 and Y27632, two inhibitors of Rho-associated protein kinase (ROCK), also strongly suppressed Livin-induced YAP/TAZ dephosphorylation (**Figure 6C**). The Rho/ROCK pathway potently modulates actin cytoskeleton dynamics, particularly stress fiber formation, in response to various GPCR agonists. We therefore examined whether cytoskeletal reorganization contributes to YAP/TAZ activation in res-

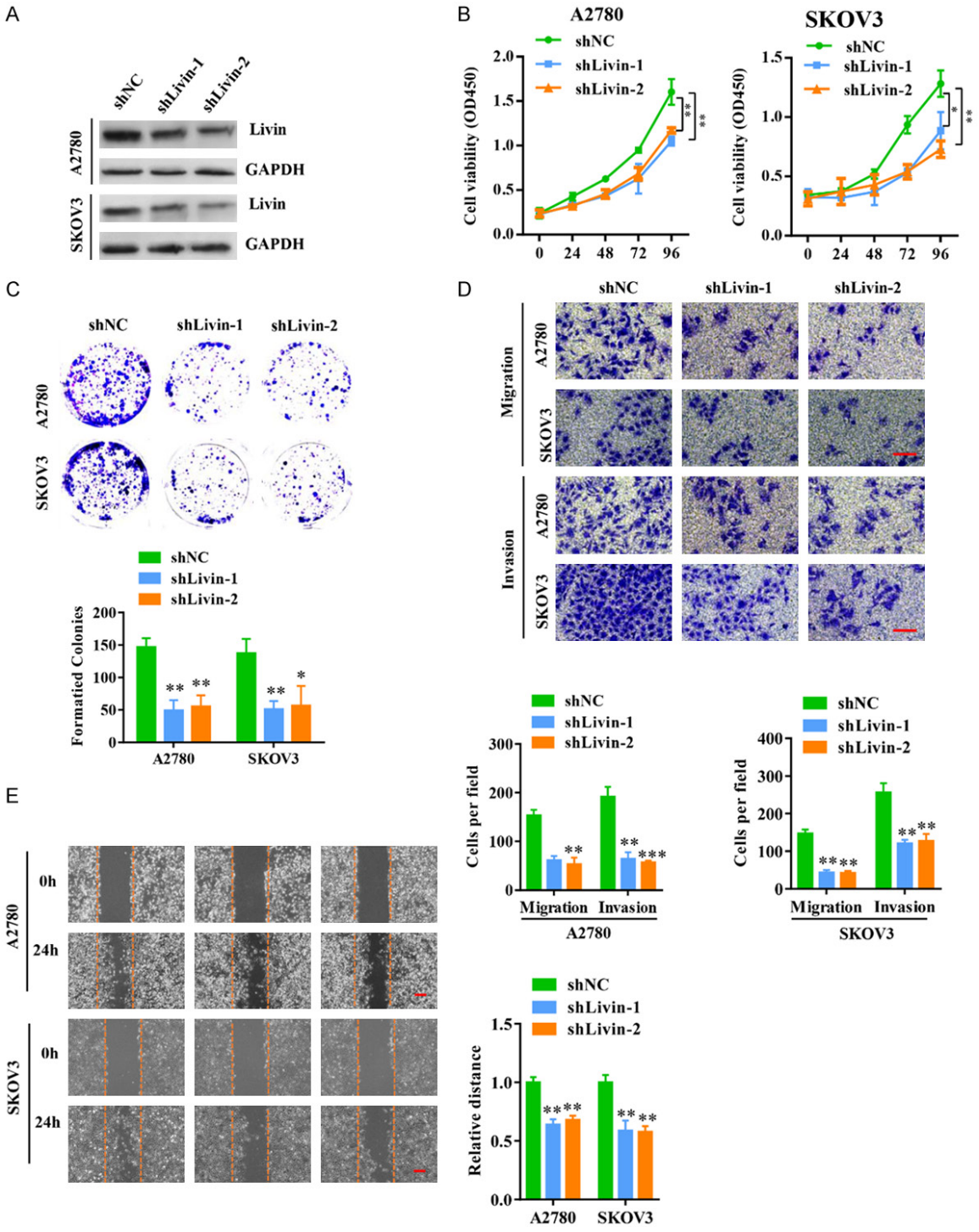


Figure 3. Knockdown of Livin impairs the proliferation and migration of ovarian cancer cells. A. The Livin knockdown efficiency measured by WB. B. The effect of Livin knockdown on A2780 and SKOV3 cell proliferation was detected by CCK-8 assay at the indicated times. C. The colony formation of A2780 and SKOV3 cells treated with shNC and shLivin. D. Migration ability and invasion ability were determined after knockdown of Livin in A2780 and SKOV3 cells using a migration assay and a Transwell assay. E. The migration potential of A2780 and SKOV3 cells treated with shNC and shLivin was evaluated by the wound healing test. Scale bars: 200 μm. *P < 0.05, **P < 0.01, ***P < 0.001.

ponse to Livin overexpression. Treatment with latrunculin B (Lat B), an F-actin destabilizing reagent, blocked Livin-induced YAP/TAZ dephosphorylation (**Figure 6C**). Taken together,

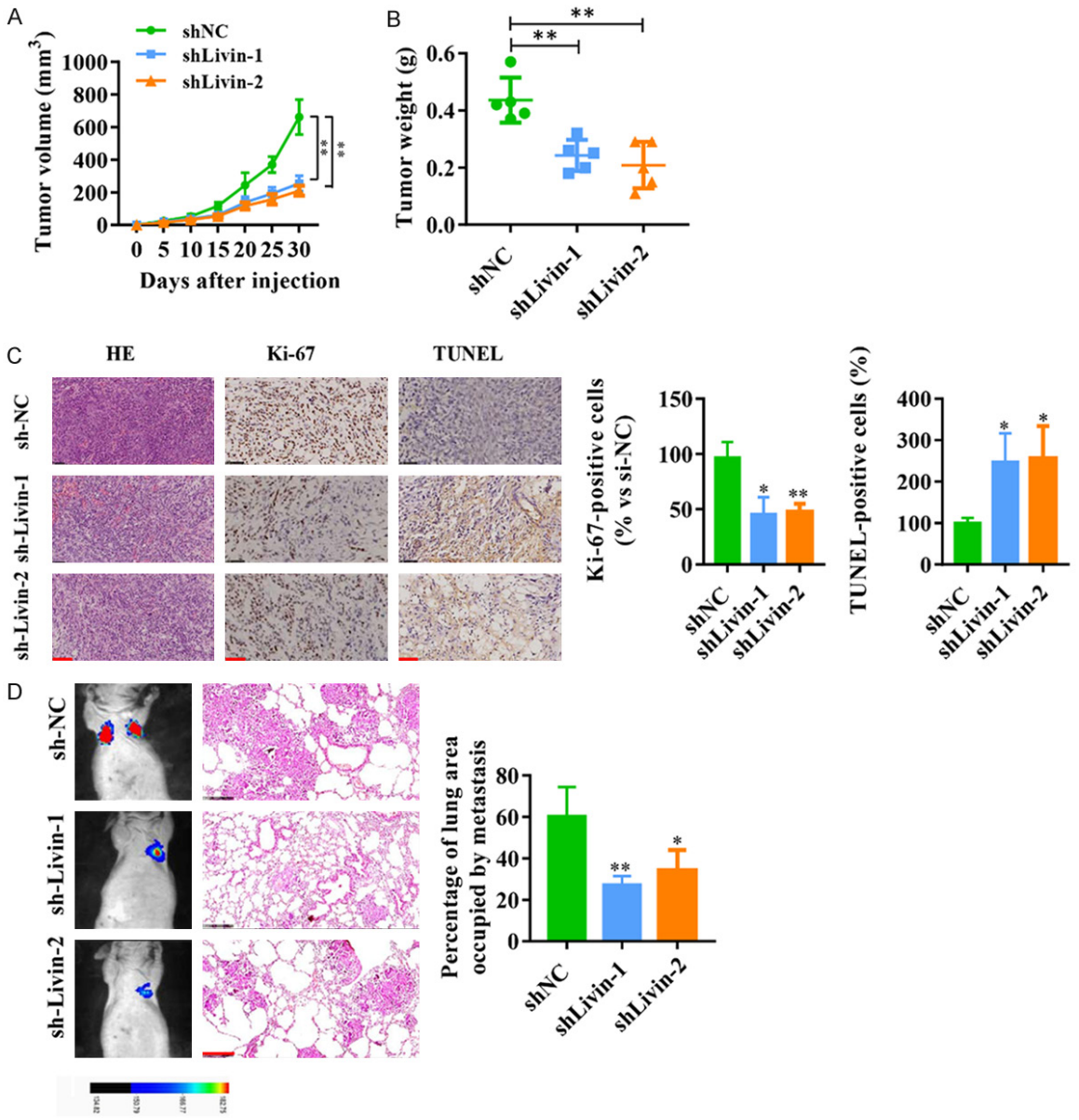


Figure 4. Livin knockdown inhibits the growth and metastasis of ovarian cancer cells in vivo. A. Quantitative analysis of the tumor volume at the indicated times. B. Quantitative analysis of the tumor weight. C. HE, Ki-67 and TUNEL immunohistological staining of tumors from shNC and shLivin cells. Scale bars: 100 μm. D. In vivo bioluminescent imaging of nude mice at day 28 after ovarian cancer cell implantation and HE staining for lung occupancy of shLivin- or shNC-expressing ovarian cancer cells. Scale bars: 250 μm. *P < 0.05, **P < 0.01.

the above data support a model in which Livin acts through Rho/ROCK and actin cytoskeleton to regulate the Hippo pathway, leading to YAP/TAZ activation.

Livin promotes cell migration and cell proliferation through YAP/TAZ

Because YAP/TAZ activities are significantly elevated upon Livin overexpression in ovarian cancer cells, we investigated the role of YAP/TAZ in Livin-regulated cellular functions by knocking down YAP/TAZ expression. The knockdown efficiency was confirmed by WB (**Figure 7A**). Livin promoted the proliferation of A2780 cells as determined by the colony formation assay, and this effect was largely abolished by YAP/TAZ knockdown (**Figure 7B**), suggesting an essential role of YAP/TAZ in mediating Livin-induced ovarian cancer cell proliferation. Our results showed that Livin overexpression enhanced cell proliferation, whereas knockdown

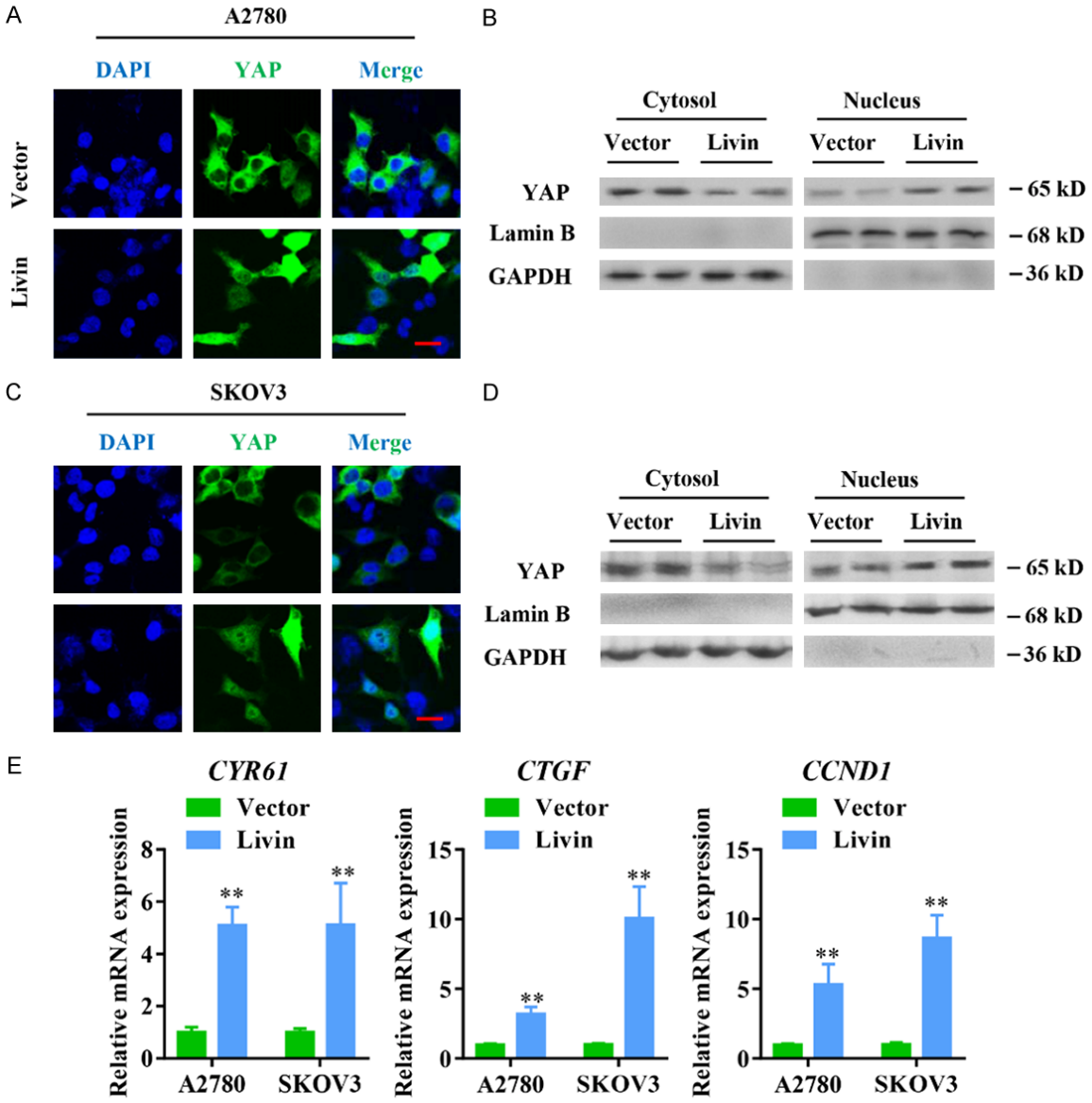


Figure 5. Livin activates YAP/TAZ in human ovarian cancer cell lines. A, C. Immunofluorescence staining of YAP nuclear localization in A2780 and SKOV3 cells after 24 h transfection with Livin or vector. B, D. WB analysis of YAP in the cytosol and nucleus in A2780 and SKOV3 cells after 24 h transfection with Livin or vector. E. qPCR analysis of the expression of CYR61, CTGF and CCND1 in A2780 and SKOV3 cells after 24 h of treatment. Scale bars: 25 μm. **P < 0.01.

of YAP/TAZ significantly blocked the promigratory and proinvasive effects of Livin (**Figure 7C**). These observations indicated that YAP/TAZ play a critical role in vin-induced cell growth.

Livin promotes tumorigenesis and metastasis of ovarian cancer through YAP/TAZ

To determine whether Livin promotes the tumorigenesis and metastasis of ovarian cancer through YAP/TAZ *in vivo*, tumor growth in response to treatment was investigated in a xenograft mouse model. A2780 cells stably transfected with Livin or empty vector were injected into nude mice treated with or without shYAP/TAZ. After four weeks, we found that Livin overexpression significantly increased tumor volume and tumor weight compared with those in the vector group, while these effects were abolished when YAP/TAZ was downregulated (**Figure 8A**). In addition, YAP/TAZ knockdown reversed the increased numbers of Ki-67-po-

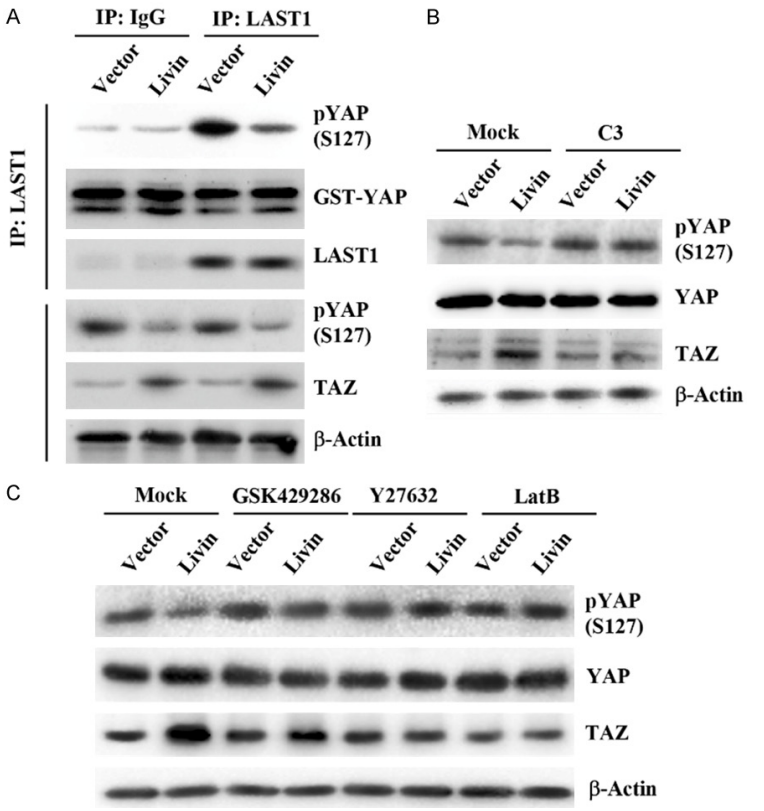


Figure 6. Livin acts through LATS, Rho/ROCK, and actin cytoskeleton to activate YAP/TAZ. A. Livin overexpression markedly reduced LATS1 kinase activity and dephosphorylated YAP/TAZ. B. Inactivation of Rho GTPase prevents YAP/TAZ dephosphorylation by Livin stimulation. C. Treatment with ROCK inhibitor GSK429286 (0.5 mmol/L) or Y27632 (1 mmol/L) and actin cytoskeleton inhibitor latrunculin B (Lat B; 1 mg/mL) abolished the YAP/TAZ activation induced by Livin.

sitive cells induced by Livin overexpression. In addition, TUNEL staining showed that YAP/TAZ knockdown partially abolished the apoptosis inhibition induced by Livin (**Figure 8B**). Next, we evaluated whether YAP/TAZ knockdown can inhibit the effects of Livin on ovarian carcinoma metastasis *in vivo*. SKOV3 cells infected with Livin overexpression lentivirus were injected into the tail vein of BALB/c nude mice with or without YAP/TAZ knockdown, and luciferase signals were measured at day 28 to observe the growth of ovarian cancer xenografts in the lung (**Figure 8C**). We found that Livin significantly increased tumor metastasis *in vivo*. Significantly, the tumor metastasis effects of Livin were partially reversed when YAP/TAZ was downregulated. Taken together, these data revealed that Livin promotes the tumorigenesis and metastasis of ovarian cancer *in vivo* in a YAP/TAZ-dependent manner.

Discussion

Ovarian cancer has the highest mortality rate of malignant tumors in women [30]. Until now, the molecular mechanisms underlying the metastasis of ovarian cancer have not been clarified. In this study, by combining *in vitro* and *in vivo* analyses, we demonstrated Livin as a tumor promoter in ovarian cancer. Livin is upregulated both in primary specimens from ovarian cancer patients and in ovarian cancer cell lines compared to controls, suggesting that Livin may function as a promoter of ovarian cancer and may have clinical value for ovarian cancer therapy. Furthermore, Livin overexpression promotes ovarian cancer cell growth and migration *in vitro,* while downregulation shows an inhibitive effect during these cellular processes. These effects of the Livin gene were also demonstrated in a xenograft mouse model. Further mechanistic studies revealed that Livin promotes the proliferation and invasion of ovarian cancer cells by activating the transcriptional coactivator YAP, a critical component of the Hippo signaling pathway. Furthermore, we revealed that knockdown of YAP prevented the growth and invasion of ovarian cancer cells. Our study revealed that Livin may be a potential therapeutic target for the treatment of ovarian cancer.

Livin is a member of the IAP family and has been shown to be expressed in multiple malignant tumors, such as superficial bladder cancer tumors [31, 32], neuroblastoma [11, 33], adrenocortical tumors [34], colorectal tumors [35, 36], melanoma and acute lymphoblastic leukemia [37]. Our study proved that Livin is significantly overexpressed in ovarian cancer patients and related cell lines compared to controls, and similar results were demonstrated by other authors. It has been reported that Livin plays an important role in the regulation of apoptosis with subsequent modulation of the

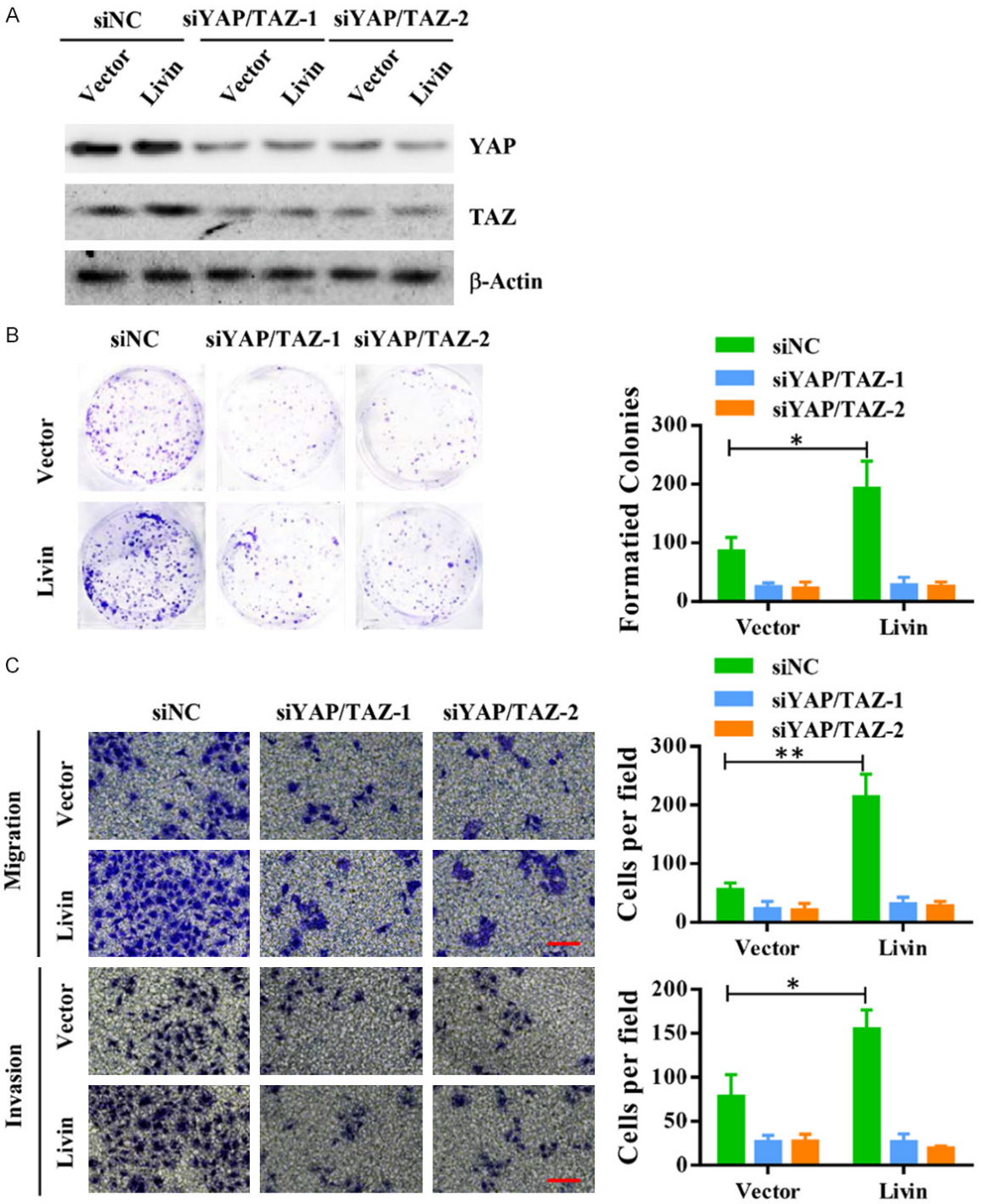
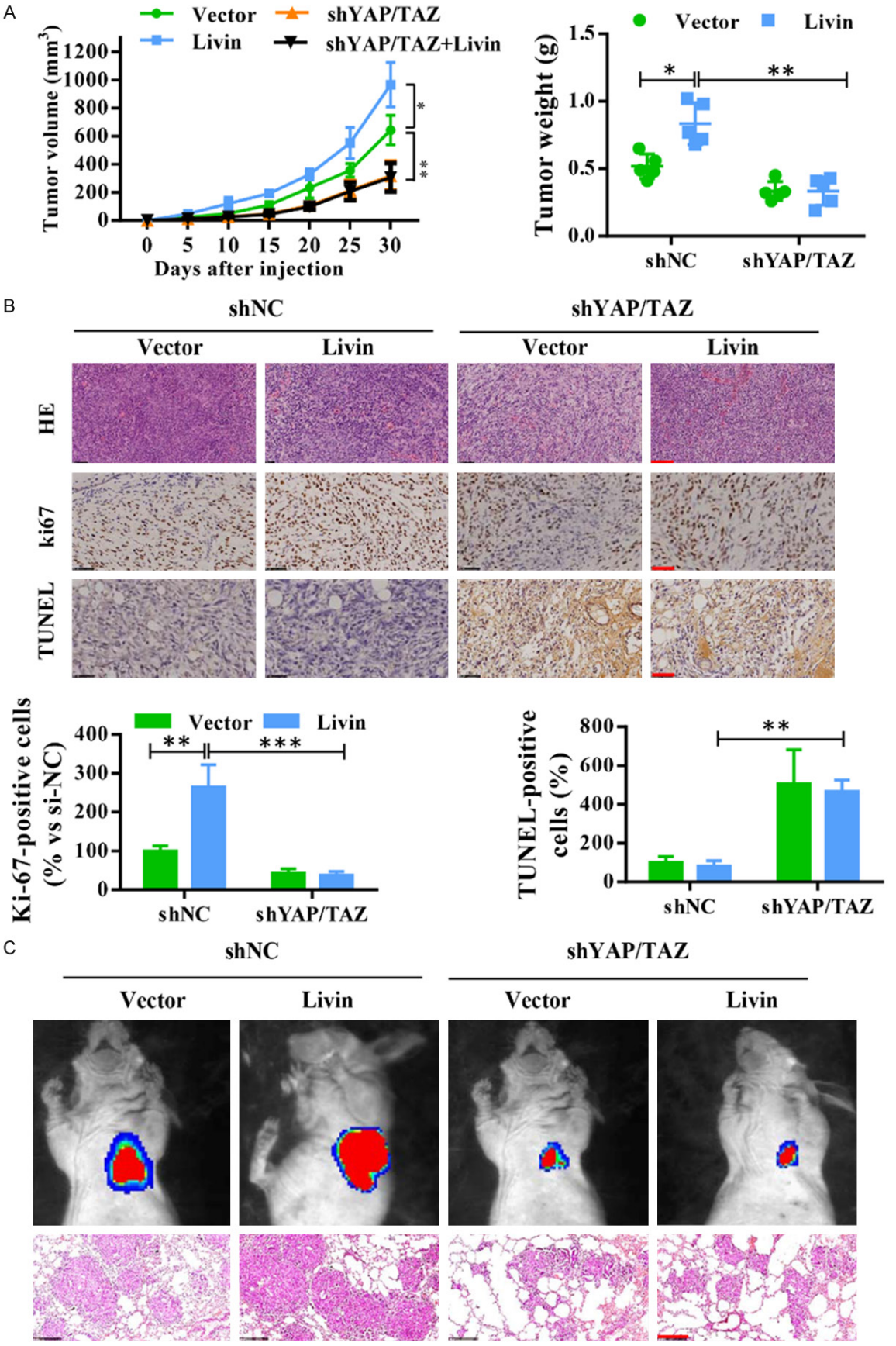


Figure 7. Livin promotes cell migration and cell proliferation through YAP/TAZ. A. The knockdown efficiency of YAP and TAZ was confirmed by WB. B. Livin overexpression promoted the proliferation of A2780 cells as determined by the colony formation assay. C. Knockdown of YAP/TAZ significantly blocked the promigration and proinvasion effect of Livin. Scale bars: 200 μm. *P < 0.05, **P < 0.01.

cell cycle and cell proliferation. The antiapoptotic activity of Livin is mainly mediated by the direct inhibition of apoptosis executers, such as caspase-3, -7 and -9 [34]. Using shRNA to downregulate the expression of Livin, we also demonstrated that Livin can inhibit apoptosis while promoting the proliferation of ovarian cancer cells *in vivo*.


A
Vector
Livin
shYAP/TAZ
shYAP/TAZ+Livin
Tumor volume (mm3)
1200
1000
800
600
400
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Days after injection
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**
Vector
Livin
Tumor weight (g)
1.5
1.0
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shNC
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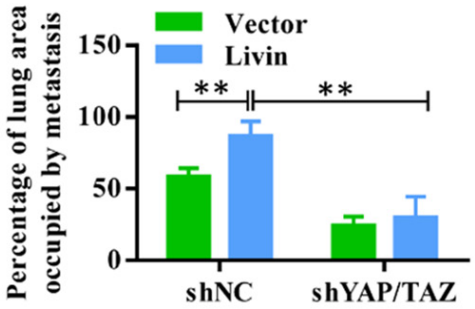

Figure 8. Livin promotes tumorigenesis and metastasis of ovarian cancer through YAP/TAZ. A. Quantitative analysis of the tumor volume at the indicated time and quantitative analysis of the tumor weight. B. HE, Ki-67 and TUNEL immunohistological staining of tumors from different groups. Scale bars: 100 μm. C. *In vivo* bioluminescent imaging of nude mice at day 28 after ovarian cancer cell implantation and HE staining for lung occupancy of different groups. Scale bars: 250 μm. **P < 0.01, ***P < 0.001.

The Hippo-YAP/TAZ pathway plays an important role in regulating ovarian development and carcinogenesis [38-40]. Importantly, the Hippo pathway acts as an important inhibitor of tissue growth and reduces organ size by controlling cell proliferation, apoptosis, and migration in response to a variety of extracellular and intracellular signals [41, 42]. Therefore, we speculate that the promotion of tumor progression by Livin in ovarian cancer is related to abnormal activation of the Hippo-YAP/TAZ pathway. In this study, we first identified the role of YAP/TAZ in Livin-mediated tumor promotion: Livin promotes ovarian cancer proliferation and invasion by activating YAP, while knockdown of YAP/TAZ prevents the progrowth and proinvasive effects of Livin in ovarian cancer cells.

The core components of the Hippo pathway consist of a series of kinases, including MST1/2, MAP4Ks, and LATS1/2; MST1/2 or MAP4Ks phosphorylate and activate LATS. The activation of LATS regulates target gene expression by inhibiting YAP and TAZ. Inhibition of LATS activity causes dephosphorylation of YAP/TAZ, which allows YAP/TAZ to translocate to the nucleus and promote the expression of target genes [15, 16, 28]. In this study, we found that LATS is involved in YAP/TAZ regulation by Livin, which can markedly reduce LATS1 kinase activity with dephosphorylation of YAP/TAZ. We further found that a specific inhibitor of Rho GTPases blocked YAP/TAZ activation by Livin, and pretreatment with GSK429286 and Y276-32, inhibitors of Rho-associated protein kinase (ROCK), strongly suppressed Livin-induced YAP/TAZ dephosphorylation. The Rho/ROCK pathway potently modulates actin cytoskeleton dynamics, particularly stress fiber formation [43]. We therefore examined whether cytoskeletal reorganization contributes to YAP/TAZ activation in response to Livin overexpression. Treatment with Lat B, an F-actin destabilizing reagent, blocked Livin-induced YAP/TAZ dephosphorylation. Our data support a model in which Livin acts through Rho/ROCK and the actin cytoskeleton to regulate the Hippo pathway, leading to YAP/TAZ activation during ovarian cancer cell progression.

In summary, our findings indicate that the activation of YAP/TAZ by Livin drives the invasion and proliferation of ovarian cancer cells *in vitro* and *in vivo*. We predict that Livin is a potential novel therapeutic target for the treatment of ovarian cancer that may be applied in the future.

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Disclosure of conflict of interest

None.

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