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TRIM11 regulated by m6A modification promotes the progression of cervical cancer by PHLPP1 ubiquitination

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Cervical cancer (CC) is a common cancer in women and a serious threat to women's lives. TRIM11 has been confirmed as a carcinogen in multiple cancers. Here, we will excavate the detailed mechanism of TRIM11 in CC. CC cell lines and nude mice were experimental subjects in this study. The abundance of genes and proteins was detected using qRT-PCR, western blot, and IHC. Cell proliferation, migration, and invasion were determined by CCK-8 assay, wound healing assay, and Transwell, respectively. The interactions among METTL14, TRIM11, and PHLPP1 were confirmed using RIP and co-IP, respectively. The stability of TRIM11 mRNA was examined by qRT-PCR with actinomycin D treatment. The m6A level of TRIM11 was detected by MeRIP assay. Results showed that TRIM11 levels were elevated in CC cells. TRIM11 depletion attenuated the proliferation, migration, and invasion of Hela and SiHa cells. Additionally, TRIM11 was modified with m6A, which was mediated by METTL14, and the stability of TRIM11 mRNA was enhanced by IGF2BP1 depending on the level of m6A modification. TRIM11 ubiquitinated PHLPP1 and led to reduced PHLPP1 expression at the protein level. PHLPP1 could further result in the dephosphorylation of AKT and inhibit AKT signaling. PHLPP1 knockdown neutralized TRIM11 silencing-mediated repression of malignant phenotypes of CC cells. TRIM11 mediated by the METTL14-IGF2BP1 axis promotes the AKT pathway to accelerate CC progression by mediating the ubiquitination of PHLPP, which might provide novel therapeutic targets for CC treatment.

Key words: m6A modification; IGF2BP1; PHLPP1; TRIM11; ubiquitination; CC

Cervical cancer (CC) is a common malignancy in women and a serious threat to women's lives. In 2020, approximately 604,000 cases of CC were diagnosed and approximately 342,000 cases died from it throughout the world [1]. Surgical resection, chemoradiotherapy, and molecular targeted therapy are the effective approaches in the state of early stage of CC [2, 3]. However, advanced CC is still a major challenge due to the invasive and lymphatic metastasis [4]. Molecularly targeted drugs such as VEGF inhibitors and PD-1 inhibitors have made incredible achievements in the field of cancer treatment [5, 6], but still do not meet clinical needs. Therefore, it is essential to explore new molecular targets for CC therapy.

RNA N6-methyladenosine (m6A) modification is widely found in around 25% of mRNAs and plays a crucial role in biological processes under physiological and pathological conditions through regulating pre-mRNA splicing, mRNA stability etc. [7]. m6A modification requires the involvement of three regulatory proteins, including methylated transferase (writers), demethylase (erasers), and methylated reading protein (readers) [8]. METTL14 as a writer and IGF2BP1 as a reader can mediate the m6A modification of mRNA to enhance the stability of RNA, thereby participating in the development of CC [9, 10]. As previously described, METTL14 expression was higher in CC and was associated with adverse prognosis [11]. Currently, the downstream targets of METTL14 in CC are not well understood and warrant further investigation.

Tripartite motif-containing 11 (TRIM11), a member of the TRIM family is an E3 ubiquitin ligase [12]. TRIM11 functioned as an oncogene in multiple cancers including CC and hepatocellular carcinoma [13, 14]. Liu et al. pointed

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out that the level of TRIM11 was elevated in CC patients and CC cells and exogenous TRIM11 knockdown inhibited the growth of CC [14], indicating that TRIM11 serves as a promoter in CC. Zhang et al. reported that TRIM11 is regulated by m6A modification in nasopharyngeal carcinoma [15]. However, whether m6A modification regulates the expression of TRIM11 in CC remains unknown. In addition, pleckstrin homology domain leucine-rich repeat protein phosphatases (PHLPPs) act as de-phosphorylases to participate in the activity of signaling pathways such as the PI3K/AKT pathway [16]. Previous studies proposed that PHLPP can dephosphorylate AKT and inhibit the activation of AKT, thereby exerting tumor inhibition [16, 17]. In hepatocellular carcinogenesis and chordoma, TRIM11 could mediate PHLPP1 ubiquitination to promote tumor progression [18]. However, it has not been reported whether TRIM11 activates the AKT pathway by mediating PHLPP1 ubiquitination in CC, which needs to be investigated in depth.

Through prediction using the RMBase V2.0 database, we found that TRIM11 has a potential binding relationship with IGF2BP1. Therefore, we believed that TRIM11 mRNA could be stabilized by IGF2BP1 depending on m6A modification. Based on these backgrounds, we reasonably postulate that TRIM11 regulated by the METTL14-IGF2BP1 axis mediates the ubiquitination and degradation of PHLPP1 to promote the progression of CC, which provides a new approach to suppress the progression of CC.

Material and methods

Cell culture. Normal endocervical epithelial cell line (End1/E6E7) and CC cell lines (Hela, SiHa, C33A, ME-180, MS751) were bought from ATCC (USA). All cells were maintained in DMEM medium (Thermo Fisher Scientific, USA) with 10% FBS (Thermo Fisher Scientific) and 1% antibiotics (Beyotime, China) under the condition of 5% CO_2 and 37°C.

Cell transfection. The small interfering RNA targeting TRIM11 (si-TRIM11) or METTL14 (si-METTL14) or IGF2BP1 (si-IGF2BP1) or PHLPP1 (si-PHLPP1), the short hairpin RNA targeting TRIM11 (sh-TRIM11) and their negative control groups (si-NC, sh-NC) were obtained from GenePharma (China). TRIM11 mutations (ΔR, ΔBB, ΔCC, ΔPS) were synthesized by GenePharma (China). Hela and SiHa cells were seeded into 6-well plates and incubated overnight. Then, cells were transfected with the above sequences and plasmids for 48 h using Lipofectamine™ 3000 (Invitrogen, USA) referring to the instruction. After 48 h, the transfected cells were used for subsequent experiments.

qRT-PCR. Total RNA was acquired from End1/E6E7 or CC cell lines without/with indicated transfection using TRIzol reagent (Beyotime). After cDNA synthesis was performed using Script Reverse Transcription Reagent Kit (TaKaRa, China), SYBR Premix Ex Taq II Kit (TaKaRa) was used for the qPCR process. Primer sequences were designed using the website of https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome and detailed sequences were as follows: TRIM11 (F): GAGAACGTGAACAGGAAGGAG; TRIM11 (R): CCATCGGTGGCACTGTAGAA; PHLPP1 (F): GTTCTGCCACTAATTGGTGGA; PHLPP1 (R): GCTGGGATGCAACCTTGGA; GAPDH (F): AGCCCAAGATGCCCTTCAGT; GAPDH (R): CCGTGTTCCTACCCCCAATG. All data were calculated by using the $2^{-\Delta\Delta Ct}$ formula. GAPDH served as a reference gene.

Western blot. The total protein was extracted from End1/E6E7 or CC cell lines without/with indicated transfection using RIPA buffer (Beyotime, China). The proteins of the transfected cells were extracted at 48 h after transfection. The protein concentration was quantified via the BCA protein kit (Beyotime, China). SDS-PAGE was employed to separate the proteins, and then the separated proteins were transferred onto the PVDF membrane. 5% BSA was used to block the PVDF membrane for 1 h. Then, the membrane was incubated with primary antibodies against METTL14 (ab300104, 1:1000, Abcam, UK), TRIM11 (ab111694, 1:3000, Abcam), PHLPP1 (22789-1-AP, 1:2000, Proteintech, China), AKT (10176-2-AP, 1:4000, Proteintech), p-AKT (28731-1-AP, 1:3000, Proteintech), HA tag (ab236632, 1:1000, Abcam), ubiquitin (ab134953, 1:5000, Abcam), and GAPDH (ab128915, 1:20000, Abcam) overnight at 4°C. Subsequently, the HRP-conjugated secondary antibody (AS1107, 1:5000, ASPEN, China), which was diluted with TBST buffer containing 5% BSA, was applied to incubate PVDF membrane for 1 h. Afterwards, membranes were immersed and reacted with an ECL chemiluminescent reagent (Beyotime, China). Using the Odyssey Clx Imaging System (Licor Biosciences, USA) captured the images of protein bands and using ImageJ software quantitatively analyzed the protein bands.

Cell count kit-8 (CCK-8) assay. Hela and SiHa cells without/with the indicated transfection were seeded onto 96-well plates and cultured for 24 h. CCK-8 solution (10 µl, Beyotime, China) was added to each well for 2 h. A spectrophotometer (Bio-Rad, Hercules, CA, USA) was employed to obtain the absorbance at 450 nm.

Scratch test. 1×10^4 cells/well Hela and SiHa cells with the indicated transfection were seeded onto 6-well plates. When the cells reached 80–90% confluence, a sterile 200 µl pipet tip was performed to scratch a line in plates. The cells were then cultured for 24 h and the widths of the scratch were recorded at 0 and 24 h.

Transwell assay. Hela and SiHa cells with the indicated transfection were seeded on the upper chamber filled with Matrigel (Becton, USA) containing FBS-free DMEM. The lower chamber was filled with DMEM supplemented with 10% FBS. After 24 h, the invasive cells on the bottom of the upper chamber were fixed with 95% alcohol and stained with 1% crystal violet. Finally, an inverted microscope (OLYMPUS, Japan) was performed to observe the stained cells.

m6A immunoprecipitation (MeRIP). MeRIP was performed to evaluate the m6A-modified level of TRIM11. Firstly, total RNA was isolated from Hela and SiHa cells using TRIzol regent. Then, anti-m6A (ab284130, Abcam) or anti-IgG (ab172730, Abcam) conjugated with A/G magnetic beads was used to incubate the RNA samples. Subsequently, the precipitated RNA was eluted and purified. m6A-modified TRIM11 mRNA was evaluated by qRT-PCR.

RNA immunoprecipitation (RIP) assay. To validate the interaction between METTL14/IGF2BP1 and TRIM11, RIP assay was performed using an RIP assay kit (Millipore, Billerica, MA, USA). Shortly, Hela and SiHa cells were washed with PBS and then were lysed with RIP Lysis Buffer. Afterward, the lysate was centrifuged to obtain the supernatants. Magnetic beads conjugated with anti-METTL14, anti-IGF2BP1, or IgG antibodies were added to the supernatants to immune-precipitate mRNA. IgG was used as a control. TRIM11 mRNA enrichment was determined using qRT-PCR.

RNA stability. As for the detection of RNA stability of TRIM11, firstly, actinomycin D (2 μg/ml) was used to prevent the process of gene transcription in HeLa and SiHa cells, which were transfected with si-NC, si-METTL14, si-IGF1BP2 after 48 h. Next, using RT-qPCR evaluated TRIM11 mRNA level after 0, 2, and 4 h.

Co-immunoprecipitation (Co-IP) assay. HeLa and SiHa cells were extracted with Co-IP buffer. The lysates were incubated overnight with the antibodies including IgG or TRIM11 (ab111694, Abcam) or PHLPP1(22789-1-AP, Thermo Fisher Scientific) antibody conjugated to Protein A/G beads (Santa Cruz Biotechnology, USA). After washing the beads, the immune-precipitates such as PHLPP1, ubiquitin, and TRIM11 were detected by western blot.

Tumor xenografts in nude mice. Ten male BALB/c nude mice (age 5–6 weeks, weight 18–20 g) were purchased from Hunan SJA Laboratory Animal Co., Ltd, (China). All mice obtained water and food *ad libitum* under a 12 h light/12 h dark condition. After 7 d, mice were divided into two groups including sh-NC, sh-TRIM11. Then, nude mice received subcutaneously injection with Hela cells (5.0×10^6 cells/mouse) which were infected with lentivirus carrying sh-NC or sh-TRIM11 (GenePharma, China), respectively. Tumor volumes in nude mice were measured and recorded every 5 days. The tumor volume was calculated according to the formula of $V = \text{length}*\text{width}^2/2$. After 25 d, mice were euthanized and the tumors in mice were harvested for subsequent experiments. The tumor weight of tumors was detected. Our study involving animal experiments was approved by the ethics committee of Hunan Provincial Maternal and Child Health Care Hospital.

Immunohistochemistry (IHC). After fixation with 4% paraformaldehyde solution and embeddedness with paraffin, 5 μm sections of tumor tissues were prepared. After repairing the antigen, the sections were blocked with 1% BSA. The sections were incubated with antibodies against Ki-67 (ab15580, Abcam), TRIM11 (ab111694, Abcam), or PHLPP1 (22789-1-AP, Proteintech,) overnight at 4 and then incubated with HRP-labelled antibody. The sections were counterstained with diaminobenzidine (DAB). Finally, the images were acquired using with an optical microscope (Japan).

Statistical analysis. Data were represented as mean ± standard deviation (SD). GraphPad Prism 9.0 t-test was used for comparison between two groups, while the analysis of variance (ANOVA) was used for pairwise comparison among multiple groups. All experiments were repeated at least three times independently. $p<0.05$ was considered as a significant difference.

Results

TRIM11 promoted the proliferation, migration, and invasion of CC cells. Here, the influences of TRIM11 on the proliferation, migration, and invasion of CC cells were probed. We observed TRIM11 expression was higher in CC cells (Hela, SiHa, C33A, ME-180, and MS751) than that in normal endocervical epithelial cells (End1/E6E7) (Figures 1A, 1B). As the expression of TRIM11 is higher in Hela and SiHa cells than in others, these two cell lines were used in subsequent experiments. We transfected si-TRIM11 into Hela and SiHa cells. As evidenced by qRT-PCR and western blot, TRIM11 was successfully knocked down in CC cells (Figures 1C, 1D). Biological functional analysis showed that TRIM11 knockdown evidently suppressed CC cell proliferation, migration, and invasion (Figures 1E–1G). The data suggested that TRIM11 silencing impaired the malignant properties of CC cells.

METTL14 resulted in the increase of TRIM11 mRNA m6A level. Here, we detected the m6A level of TRIM11 in CC by MeRIP assay. The results indicated that the m6A level of TRIM11 mRNA in CC cells was higher than that in End1/E6E7 cells (Figure 2A). Using the RMBase v2.0 database, TRIM11 mRNA was predicted to have a potential binding relationship with METTL14 (Supplementary Figure S1A). RIP further validated the interaction between METTL14 and TRIM11 mRNA (Figure 2B). In addition, METTL14 knockdown reduced TRIM11 expression and declined the m6A level of TRIM11 in HeLa and SiHa cells (Figures 2C–2E). Taken together, METTL14 promoted TRIM11 levels through m6A modification in CC cells.

IGF2BP1 promoted TRIM11 mRNA stability dependent on m6A modification. IGF2BP1 is an m6A reader, which plays a crucial role in the m6A modification of genes. The RMBase v2.0 database predicted the binding relationship between TRIM11 mRNA and IGF2BP1 (Supplementary Figure S1B). Moreover, we observed that anti-IGF2BP1 enriched TRIM11 mRNA in CC cells, validating the relationship between them (Figure 3A). Afterward, IGF2BP1 expression was silenced in CC cells using si-IGF2BP1 transfection (Figure 3C). IGF2BP1 silencing led to a reduction of TRIM11

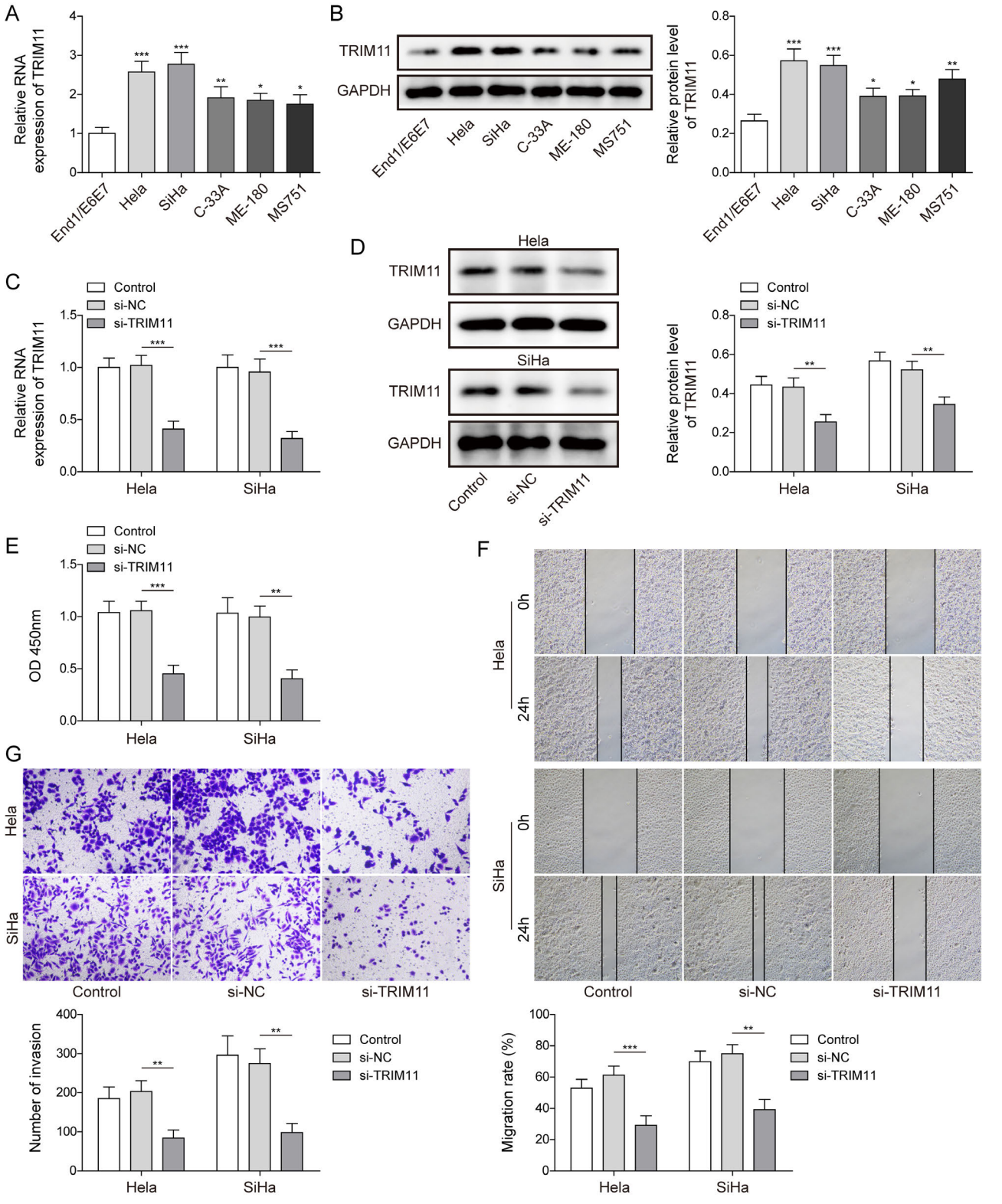

Figure 1. TRIM11 promoted the proliferation, migration, and invasion of CC cells. A, B) TRIM11 expression in CC cells (Hela, SiHa, C33A, ME-180, and MS751) and End1/E6E7 cells was measured using qRT-PCR and western blot. Hela and SiHa cells were transfected with si-TRIM11 and si-NC. C, D) TRIM11 was detected by qRT-PCR and western blot. E) Cell proliferation was tested by CCK-8 assay. F) Cell migration was estimated using scratch test. G) Cell invasion was investigated using Transwell. *p<0.05, **p<0.01, ***p<0.001

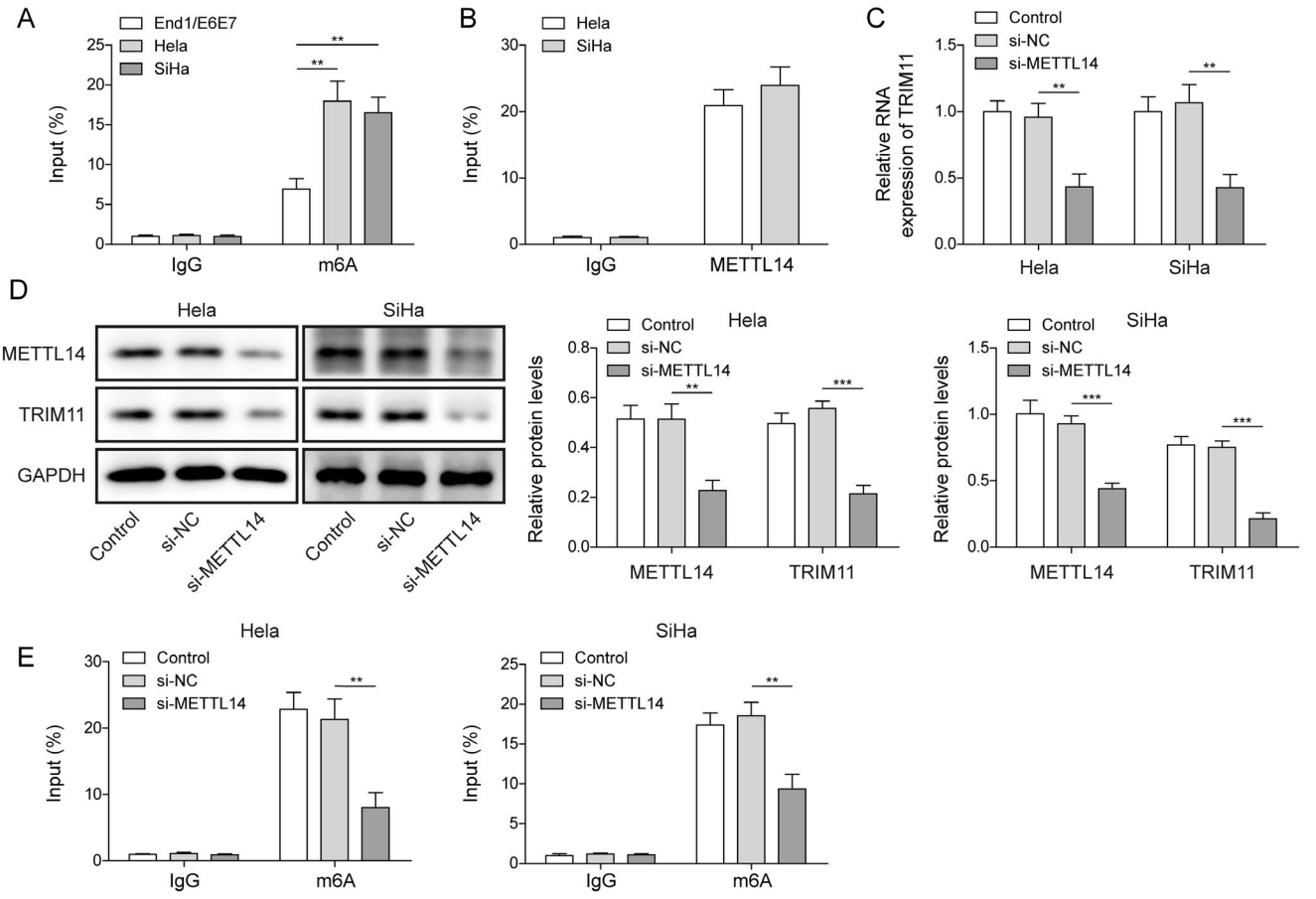

Figure 2. METTL14 resulted in the increase of TRIM11 mRNA m6A level. A) The m6A level of TRIM11 in Hela and SiHa cells and End1/E6E7 cells was detected by MeRIP assay. B) The interaction between METTL14 and TRIM11 mRNA was validated by RIP. Hela and SiHa cells were transfected with si-METTL14 and si-NC. C) TRIM11 mRNA levels were measured by qRT-PCR. D) The protein levels of TRIM11 and METTL14 were detected via western blot. E) The m6A level of TRIM11 was examined using MeRIP assay. *p<0.05, **p<0.01, ***p<0.001

expression (Figures 3B, 3C). To investigate whether the stability of TRIM11 mRNA is responsible for METTL14 or IGF2BP1, si-METTL14, or si-IGF2BP1 was transfected into HeLa and SiHa cells. Actinomycin D (5 μg/ml), a transcriptional inhibitor [19, 20], was applied to treat CC cells for 0, 2, and 4 h, respectively. The results indicated both si-METTL14 and si-IGF2BP1 appeared to attenuate the stability of TRIM11 mRNA (Figure 3D). In summary, IGF2BP1 enhanced the stability of TRIM11 mRNA in CC cells in an m6A modification-dependent manner.

TRIM11 activated the AKT signal by enhancing the ubiquitination of PHLPP1. Studies suggested that TRIM11 could ubiquitinate and degrade PHLPP1 to activate the AKT pathway in hepatocellular carcinogenesis and chordomas [18, 21]. Therefore, we explored whether TRIM11 could degrade PHLPP1 through ubiquitination and regulate the progression in CC. Firstly, the Co-IP assay revealed that PHLPP1 was successfully precipitated by anti-TRIM11 (Figure 4A), suggesting that PHLPP1 and TRIM11 interacted with each other. When TRIM11 was knocked down by si-TRIM11 transfection, PHLPP1 mRNA expression was unaffected (Figure 4B). However, protein expression of PHLPP1 was dramatically elevated by downregulating TRIM11 expression (Figure 4C). Knockdown of TRIM11 obviously reduced the phosphorylation level of AKT (Figure 4C), implying that TRIM11 promoted the activation of the AKT pathway. In addition, the ubiquitination of PHLPP1 was evaluated in CC cells transfected si-NC and si-TRIM11. The results revealed that TRIM11 silencing inhibited the ubiquitination of PHLPP1 prominently (Figure 4D). MG132, a proteasome inhibitor, which was used to inhibit protein ubiquitination degradation [22, 23], elevated PHLPP1 expression (Figure 4E). To further validate the domain of TRIM11 interacting with PHLPP1, TRIM11 deletion mutants lacking each of the individual domains within RING, B-Box, or coiled-coil domain (ΔR, ΔBB, ΔCC), or the entire PS region (ΔPS) were constructed (Figure 4F), which was designed according to a previous

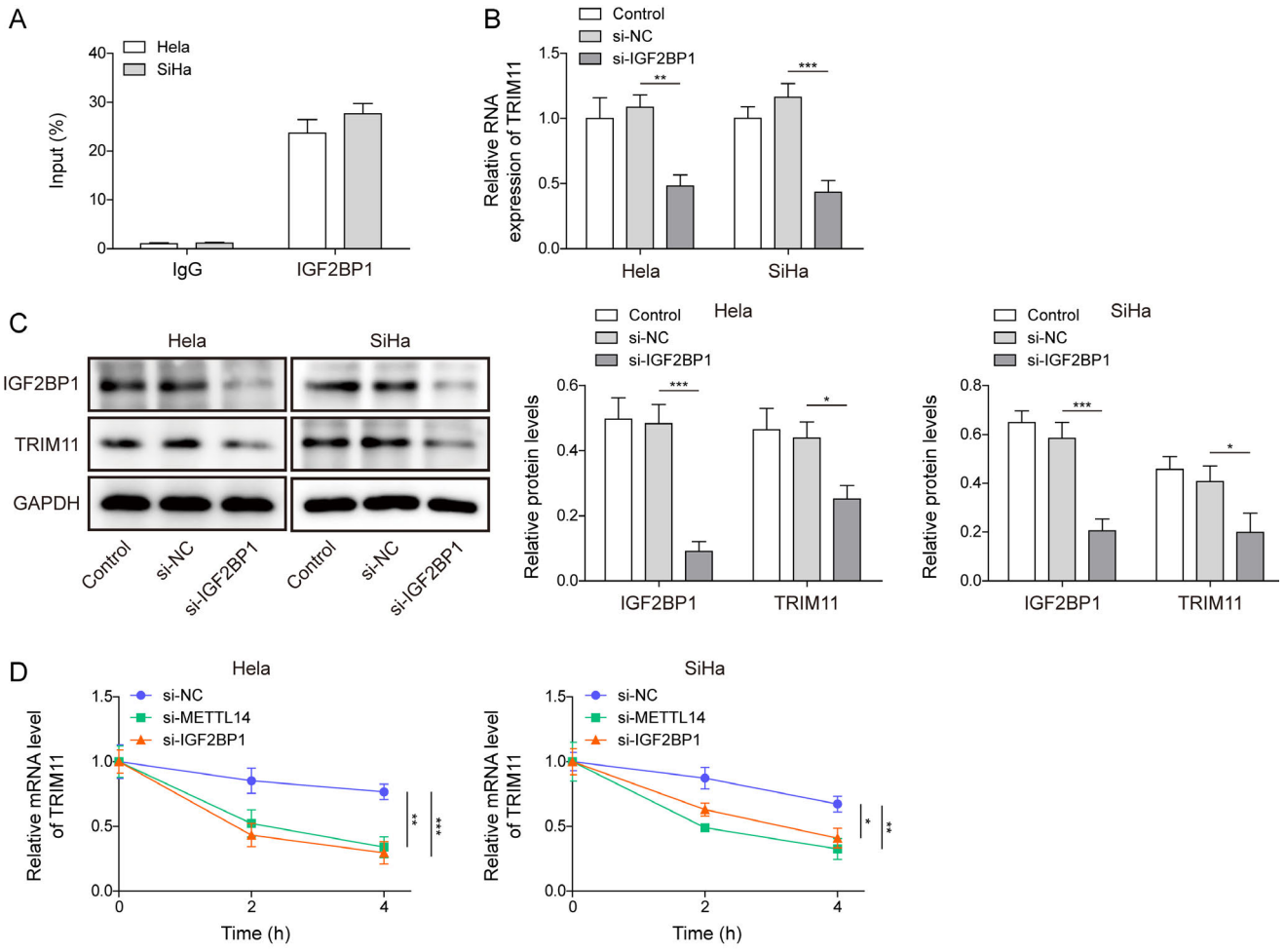

Figure 3. IGF2BP1 promoted TRIM11 mRNA stability dependent on m6A modification. A) The interaction between IGF2BP1 and TRIM11 mRNA was validated by RIP. Hela and SiHa cells were subjected to si-IGF2BP1 and si-NC transfection. B) TRIM11 mRNA levels were detected by qRT-PCR. C) The protein levels of TRIM11 and IGF2BP1 were detected via western blot. Hela and SiHa cells transfected with si-IGF2BP1, si-METTL14, and si-NC. D) The stability of TRIM11 was examined by qRT-PCR after actinomycin D (5 μg/ml) for 0, 2, 4 h. *p<0.05, **p<0.01, ***p<0.001

study [24]. TRIM11-ΔBB and -ΔPS retained the ability to interact with endogenous PHLPP1, which was determined using Co-IP assay (Figure 4G). However, TRIM11-ΔCC partially, and TRIM11-ΔR completely, lost the ability to interact with PHLPP1 (Figure 4G). The results of Fig 4F-G revealed that the RING region was the major domain of TRIM11 interacting with PHLPP1. These consequences showed that TRIM11 promoted PHLPP1 ubiquitination and AKT activation.

TRIM11 accelerated the proliferation, migration, and invasion of CC cells by ubiquitinating PHLPP1 and activating the AKT signaling. In this part, we investigated the effects of TRIM11 and PHLPP1 on CC cell proliferation, migration, and invasion. TRIM11-silenced CC cells were subjected to si-NC or si-PHLPP1 transfection. TRIM11 knockdown suppressed the protein levels of TRIM11 and p-AKT, while PHLPP1 levels were increased. However, PHLPP1 silencing restored the effects of TRIM11 knockdown on PHLPP1 and p-AKT levels and had no influence on TRIM11 levels (Figure 5A). In terms of malignant characteristics of CC cells, TRIM11 knockdown memorably attenuated the proliferation, migration, and invasion capabilities of CC cells, which were compromised by PHLPP1 silencing (Figures 5B–5D). Overall, TRIM11 knockdown restrained CC cell growth and metastasis via enhancing PHLPP1 expression.

TRIM11 silencing restrained tumor growth *in vivo* by elevating PHLPP1 expression. Furthermore, we investigated the effects of TRIM11 on tumor growth *in vivo* experiments. Nude mice were subcutaneously injected with Hela cells which were infected with lentivirus carrying sh-NC or sh-TRIM11. After 25 d, tumor weight and tumor volume growth curve were recorded. We found that TRIM11 silencing suppressed tumor weight and growth volume

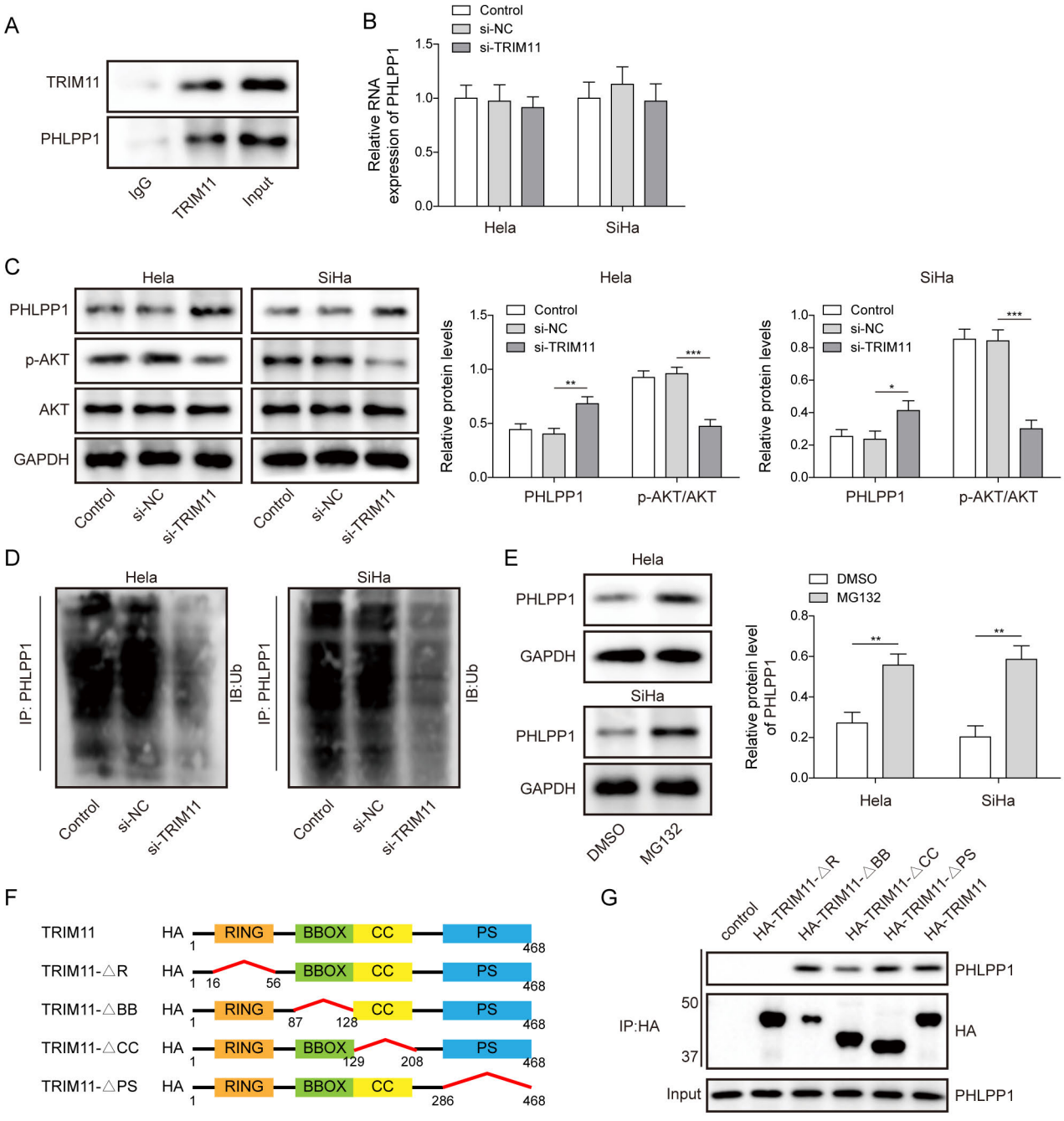

Figure 4. TRIM11 activated the AKT signal by enhancing the ubiquitination of PHLPP1. A) The interaction between PHLPP1 and TRIM11 was validated by Co-IP. Hela and SiHa cells were transfected with si-TRIM11 and si-NC. B) PHLPP1 mRNA levels were detected by qRT-PCR. C) Protein levels of PHLPP1, p-AKT, and AKT were measured using western blot. D) Ubiquitination of PHLPP1 was examined by Co-IP. E) The protein level of PHLPP1 in Hela and SiHa cells treated with DMSO or MG132 was detected using western blot. F) Schematic presentation of TRIM11 and its mutations. G) Interaction of TRIM11 proteins with endogenous PHLPP1 in Hela cells was analyzed using Co-IP assay. *p<0.05, **p<0.01, ***p<0.001

(Figures 6A–6C). Additionally, TRIM11 silencing resulted in decreased TRIM11 and Ki-67 expression and increased PHLPP1 expression in tumor tissues (Figure 6D). Our results revealed that TRIM11 silencing suppressed tumor formation and growth of CC cells in nude mice.

Discussion

To date, the incidence and death rate of CC are gradually decreasing due to human papillomavirus (HPV) vaccination and early screening [25, 26]. However, due to its insidious

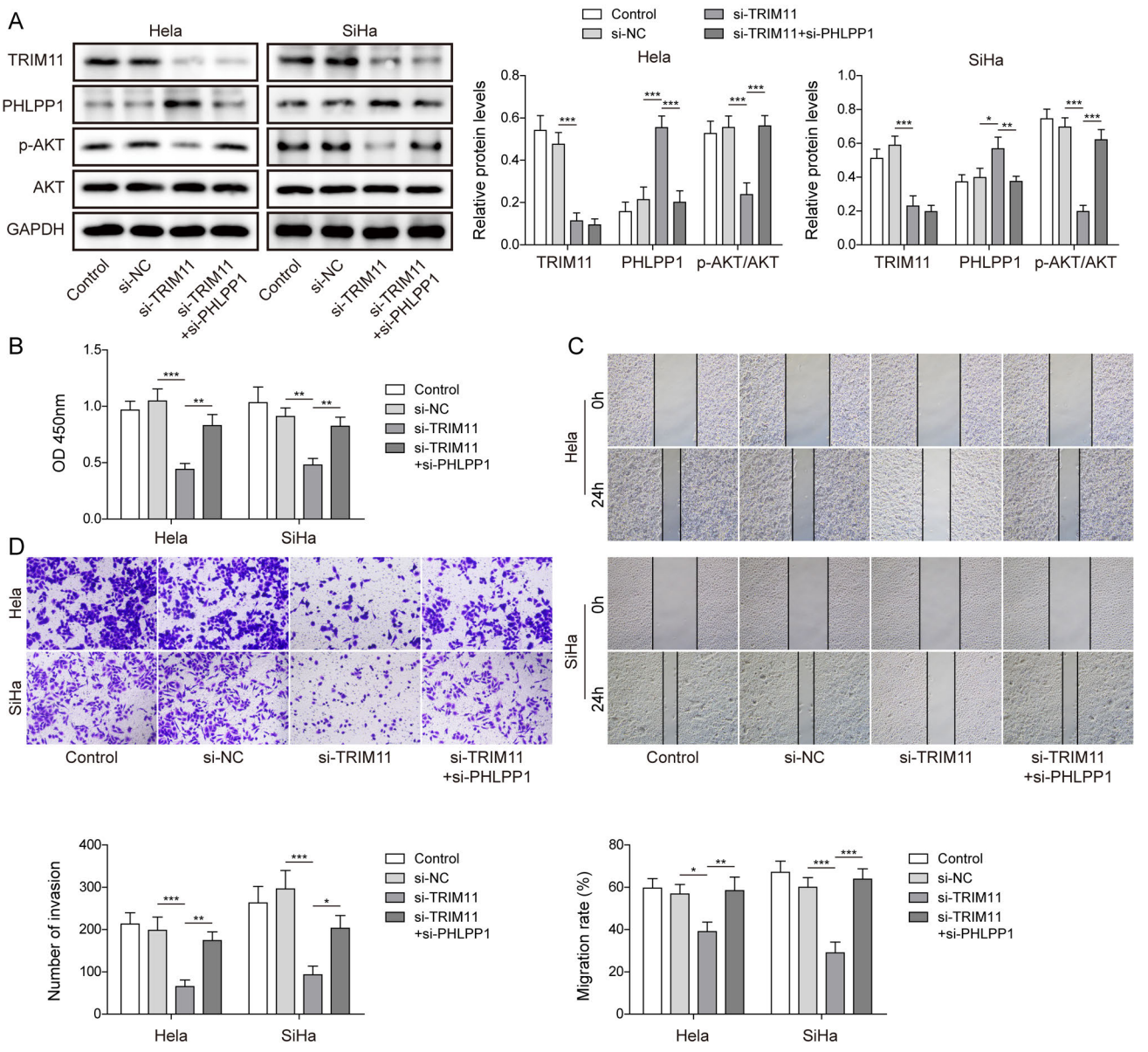

Figure 5. TRIM11 accelerated the proliferation, migration, and invasion of CC cells by ubiquitinating PHLPP1 and activating the AKT signal. Hela and SiHa cells were transfected with si-TRIM11 with/without si-PHLPP1. A) Protein levels of TRIM11, PHLPP1, p-AKT, and AKT were measured using western blot. B) Cell proliferation was tested by CCK-8 assay. C) Cell migration was estimated using scratch test. D) Cell invasion was investigated using Transwell. *p<0.05, **p<0.01, ***p<0.001

symptoms, there are still a significant number of CC patients in the advanced stage, resulting in a low survival rate [27]. Therefore, the approaches to inhibit the development and improve the prognosis of CC need to be investigated. In this study, METTL14 mediated TRIM11 expression in an m6A manner, and IGF2BP1 stabilized TRIM11 mRNA dependent on m6A modification in CC. In addition, TRIM11 mediated the ubiquitination degradation of PHLPP1 to activate the AKT signaling, thereby facilitating CC cell proliferation, migration, and invasion.

TRIM11 has been implicated as an oncogenic molecule in various cancers. In gastric cancer, TRIM11 was abnormally highly expressed and promoted cell growth and metastasis by activating the β-catenin signaling [28]. Similarly, TRIM11 was found to be upregulated in CC tissues and cells and TRIM11 knockdown suppressed the progression of CC through inhibiting cell growth and metastasis [14]. Our findings revealed that TRIM11 expression was enhanced in CC cells and its knockdown impaired the proliferative, migratory, and invasive capabilities of CC cells, which was

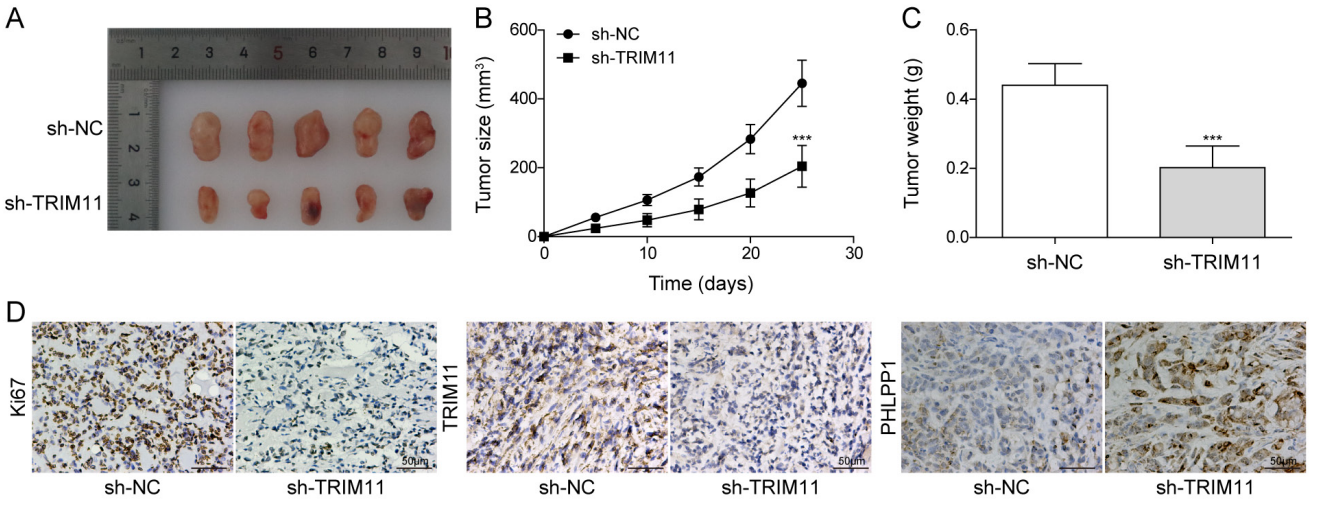

Figure 6. TRIM11 silencing restrained tumor growth *in vivo* by elevating PHLPP1 expression. Nude mice were treated with subcutaneous injection with sh-NC or sh-TRIM11-transfected Hela cells. A) The tumor images. B) The growth curve of tumor volume. C) The weight of tumors. D) The expressions of TRIM11, Ki-67, and PHLPP1 in tumor tissues were detected using IHC. *p<0.05, **p<0.01, ***p<0.001

consistent with previous studies. METTL14 plays a prominent role in m6A modification, which is widespread in eukaryotes and mediates multi-biological processes [29]. Geng et al. found that elevated METTL14 was observed in CC cell lines and METTL14 silencing evidently inhibited cell growth and metastasis in CC [11]. As previously described, Meningioma 1 (MN1) could be subjected to m6A modified by METTL14 to enhance MN1 expression, thereby promoting osteosarcoma progression [30]. In this work, we demonstrated that the level of m6A modification of TRIM11 mRNA was elevated in CC cells. More importantly, we for the first time demonstrated that METTL14 interacted with TRIM11 mRNA to mediate the m6A modification of TRIM11 and to elevate TRIM11. It has been shown that m6A readers including IGF2BPs could affect the stability of gene mRNA [31]. IGF2BP1 elevated HDAC4 mRNA and increased its RNA stability by recognizing METTL3-mediated m6A modification in sepsis-induced myocardial injury [32]. Here, we provided the first evidence that TRIM11 was mediated by METTL14-mediated m6A modification, and the stability of TRIM11 was enhanced by IGF2BP1 dependent on m6A modification, which contributed to CC cell proliferation, migration, and invasion. To sum up, the upregulation of TRIM11 expression in CC was at least partially mediated by the METTL14-IGF2BP1 axis.

TRIM11 is an E3 ubiquitin ligase, which reduces the protein levels of genes through ubiquitination [33]. A prior study revealed that TRIM11 destabilized Axin1 protein with ubiquitin modification to facilitate gastric cancer progression [33]. In hepatocellular carcinogenesis, TRIM11 ubiquitinated PHLPP1 to degrade and reduce the expression of PHLPP1 in HCC cells, thereby promoting HCC progression by activating the AKT signaling pathway [21]. PHLPP1 is involved in physiological and pathological processes by mediating the dephosphorylation of proteins, which affects the activity of multiple signaling pathways [34, 35]. For example, elevated degradation of PHLPP1 by ubiquitination could enhance p-AKT levels to activate the PI3K/AKT signaling pathway and enhance proliferation and metastasis in colorectal cancer [36]. In the current study, we confirmed that TRIM11 attenuated PHLPP1 expression at the protein level through ubiquitination in CC. Besides, we validated that the domain of TRIM11 interacting with PHLPP1 was mainly the RING region. Furthermore, PHLPP1 further inhibited the activation of AKT via dephosphorylation in CC. The functional experiments showed that TRIM11 activated AKT signaling to accelerate CC cell proliferation, migration, and invasion via ubiquitination of PHLPP1.

In conclusion, we found for the first time that METTL14 mediated m6A modification of TRIM11 and IGF2BP1 enhanced the stability of TRIM11 mRNA, further increasing the ubiquitination degradation of PHLPP1 and activating the AKT signaling, which accelerated CC cell proliferation, migration, and invasion. Our findings might provide new targets for CC treatment. Admittedly, our study has some limitations, namely the lack of clinical samples' validation, which will be explored in the future. In addition, the other targeted genes of TRIM11 regulated by the METTL3-IGF2BP1 axis deserve to be explored by interfering with METTL14, TRIM11, and IGF2BP1 and performing transcriptome sequencing in CC.

Supplementary information is available in the online version of the paper.

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TRIM11 regulated by m6A modification promotes the progression of cervical cancer by PHLPP1 ubiquitination

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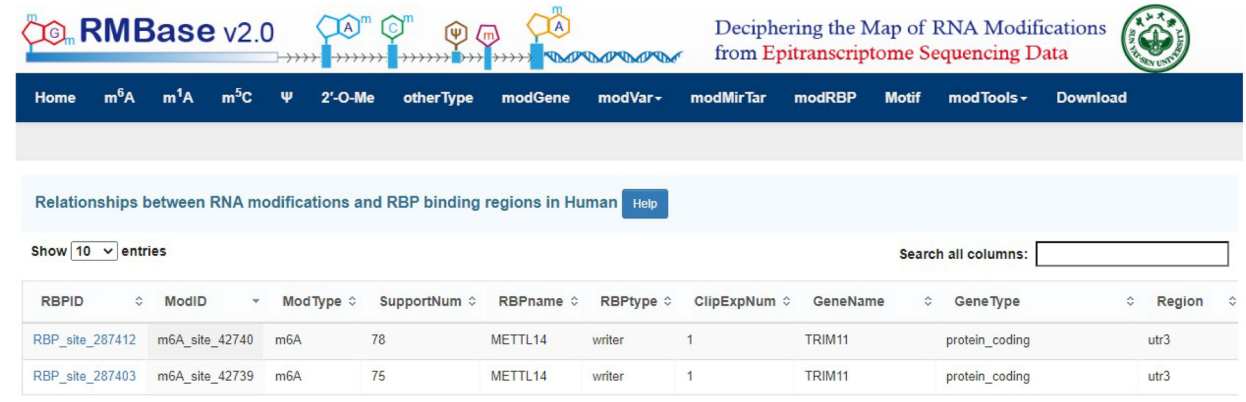
Supplementary Information

A



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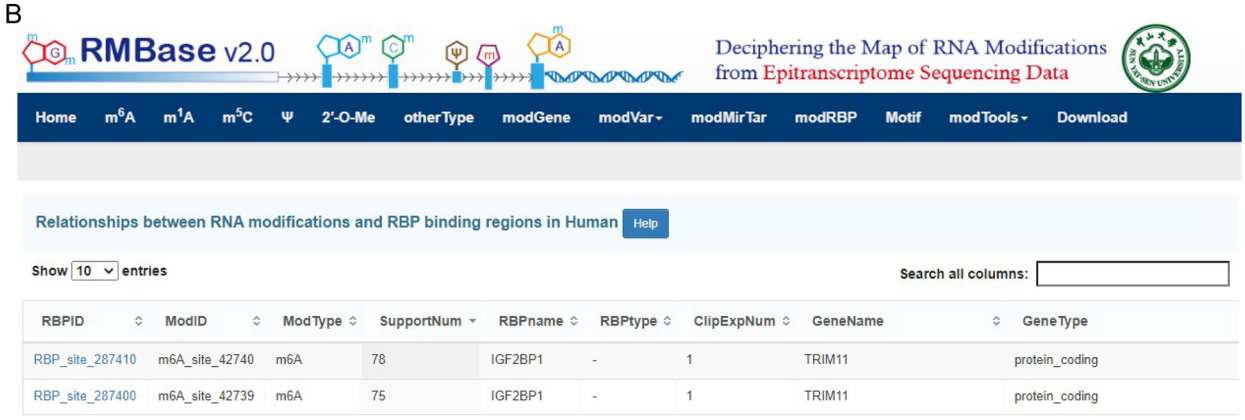

Supplementary Figure S1. RMBase v2.0 database predicted the binding relationship between TRIM11 mRNA and IGF2BP1.