TRIM11 Posttranscriptionally Modulated by miR-5193 Facilitates Tumor Growth and Metastasis of Prostate Cancer

Yue Pan, MM[1], Hui Yu, BM[1], and Feng Lu, MM[1] ⓘD

Abstract

Objective: Tripartite motif-containing protein 11 (TRIM11), an E3 ubiquitin ligase, possesses a pro-tumor property. Still, the detailed functions of TRIM11 remain not well characterized in prostate cancer. **Methods:** PC-3 and DU145 prostate cancer cells were transfected with small interfering RNAs (siRNAs) or lentiviruses for TRIM11 deficiency or overexpression, and microRNA-5193 (miR-5193) mimics were utilized for overexpressing miR-5193. Proliferation, apoptosis, migration, and invasion were examined through CCK-8, colony formation, flow cytometry, wound healing, and transwell assays. MAP kinase-extracellular signal-regulated kinase (MEK1/2) and extracellular signal-regulated kinase (ERK)1/2 activities were detected via immunoblotting. Murine xenograft models were established. Interactions of TRIM11 with miR-5193 were demonstrated via dual luciferase reporter. **Results:** TRIM11 deficiency or miR-5193 overexpression exerted antiprostate cancer effects through suppression of proliferation, migration, and invasion as well as enhancement of apoptosis in PC-3 and DU145 cells. The mechanisms by which TRIM11 deficiency or miR-5193 overexpression involved the inactivation of MEK1/2 and ERK1/2. miR-5193 downregulated TRIM11 expressions in prostate cancer cells, and their interactions were confirmed. Further, up-regulated miR-5193 weakened the effects of TRIM11 overexpression on enhancing proliferation, migration, invasion, and activity of MEK1/2 and ERK1/2 as well as alleviating apoptosis of prostate cancer cells. In murine xenograft models, TRIM11 upregulation facilitated tumor growth, which was alleviated by miR-5193 overexpression. **Conclusion:** These findings described the oncogenic role of TRIM11 in prostate cancer biology, which was post-transcriptionally modulated by miR-5193.

Keywords

TRIM11, prostate cancer, miR-5193, proliferation, metastasis

Abbreviations

ADT, androgen deprivation therapy; TRIM11, tripartite motif-containing protein 11; AKT, protein kinase B; GLUT1, glucose transporter type 1; DUSP6, dual-specificity phosphatase 6; ERK, extracellular signal-regulated kinase; MEK, MAP kinase-ERK kinase; PI3K, phosphoinositide 3-kinase; UBE2N, ubiquitin-conjugating enzyme E2N; TAX1BP1, ubiquitin-binding protein Tax1 binding protein1; DMEM, Dulbecco's modified Eagle medium; miR-5193, microRNA-5193; siRNA, small interfering RNA; NC, negative control; RT-qPCR, quantitative real-time polymerase chain reaction; CCK8, cell counting kit-8; wt, wild-type; mut, mutant; OE-TRIM11, TRIM11-overexpressed; CRAF, cellular RAF gene.

Introduction

Prostate cancer remains the most frequently diagnosed cancer in males,[1] with an estimated 1,414,259 new cases (7.3%) and 375,304 new deaths (3.8%).[2] Patients with localized prostate cancer with low to moderate risk of relapse typically have favorable outcomes with a 99% 10-year overall survival rate if this disease can be detected and treated early.[3] Curative

[1] Department of Urology, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou City, Zhejiang Province, China

Corresponding Author:
Feng Lu, Department of Urology, The First Affiliated Hospital of Wenzhou Medical University, Ouhai District, Wenzhou, Zhejiang Province 325000, China.
Email: lufens@163.com

treatment of localized disease comprises active surveillance, radical prostatectomy as well as ablative radiotherapy.[4] Treatment for recurrent patients following prostatectomy contains salvage radiotherapy and/or androgen deprivation therapy (ADT) against local recurrence, or with ADT plus chemotherapy or new androgen signaling-targeted drugs against systemic recurrence.[5,6] Nevertheless, most cases ultimately experience resistance.[7] Hence, it is required for discovering novel treatments that are capable of targeting prostate cancer cells as well as reducing the influence on healthy tissues. In-depth comprehension of additional molecular alterations during prostate cancer progression is essential for the development of novel targets and therapeutics to prolong patients' survival.

Tripartite motif-containing protein 11 (TRIM11) is an E3 ubiquitin ligase, which belongs to the tripartite motif (TRIM) protein family.[8] The carcinogenic properties of TRIM11 have been characterized in previous studies. For instance, TRIM11 heightens proliferative capacity along with glycolytic process for breast carcinoma cells through modulating AKT/glucose transporter type 1 (GLUT1) signaling[9] or stabilizing estrogen receptor α.[10] Both in gastric cancer[11] and lung cancer,[12] TRIM11 strengthens tumor growth and metastases through activating β-catenin signaling or dual-specificity phosphatase 6 (DUSP6)-mediated extracellular signal-regulated kinase (ERK)1/2 signaling.[13] TRIM11 is capable of promoting proliferation, migration as well as chemoresistance in anaplastic thyroid cancer.[14] TRIM11 inhibition stimulates apoptosis and alleviates cervical cancer cellular progression by phosphoinositide 3-kinase/AKT signaling.[15] Recent research has unveiled that TRIM11 weakens ferritinophagy as well as gemcitabine sensitivity via ubiquitin-conjugating enzyme E2N (UBE2N)/ubiquitin-binding protein Tax1 binding protein1 (TAX1BP1) pathway in pancreatic ductal adenocarcinoma.[16] More importantly, a recent report conducted by Guo et al has indicated that knocking-down TRIM11 can enhance paclitaxel sensitivity to prostate cancer cells.[17] However, how TRIM11 affects the occurrence and development of prostate cancer is still unclear.

Bioinformatics analysis has indicated that TRIM11 could be post-transcriptionally modulated by microRNAs to modulate the progression of numerous cancers. For example, TRIM11 serves as a target of miR-24-3p to induce the proliferative potentials of colon cancer, breast cancer, and mantle cell lymphoma, respectively.[18-20] Our previous study has indicated that TRIM11 is overexpressed in prostate cancer and its expression can be downregulated by microRNA-5193 (miR-5193).[21] Interestingly, another study conducted by Song et al[22] also demonstrated that miR-5193 is an important regulator of TRIM11 to function as a tumor suppressor in the development of ovarian cancer. It was capable of weakening proliferative and migratory capacities of ovarian cancer cells through post-transcriptionally modulating TRIM11.[22] Still, the mechanisms of TRIM11 and miR-5193 in prostate cancer remain not well characterized. In our work, we hypothesize that TRIM11 may be also post-transcriptionally modulated by miR-5193, and therefore affects the progression of prostate cancer.

Materials and Methods

The reporting of this study conforms to ARRIVE 2.0 guidelines.[23]

Cell Culture

Human prostate cancer cell lines PC3 and DU145 (ATCC, USA) were maintained in Dulbecco's modified Eagle medium (DMEM) (Gibco, USA) plus 10% fetal bovine serum along with 1% penicillin–streptomycin in a humidified environment of 5% CO_2/95% air at 37 °C.

RNA Interference

PC3 or DU145 cell line was seeded into six-well plates (1 × 10^5 cells per well). When the confluence reached 75%, the cells were transfected with negative control of small interfering RNA (siRNA) (si-NC), si-TRIM11-395, si-TRIM11-725, si-TRIM11-1214, miR-5193 mimics or NC mimics utilizing Lipofectamine 2000 (Invitrogen). The sequences included: si-NC: 5′-UUCUCCGAACGUGUCACGUTT-3′, 5′-ACGU GACACGUUCGGAGAATT-3′; si-TRIM11-395: 5′-CCGAA GACCUCAAGGCGAATT-3′, 5′-UUCGCCUUGAGGUCUU CGGTT-3′; si-TRIM11-725, 5′-GGCUGCUGCAGGACAU CAATT-3′, 5′-UUGAUGUCCUGCAGCAGCCTT-3′; si-TRIM11-1214, 5′-GGAUCUUUCUGGACUACGATT-3′, 5′-UCGUAGUCCAGAAAGAUCCTT-3′.

Quantitative Real-Time Polymerase Chain Reaction

Total RNA extraction was implemented with Trizol (10606ES60; Yeasen), followed by synthesis of cDNA with cDNA Synthesis SuperMix (11123ES60; Yeasen). Quantification of the mRNA expressions was conducted with 12K quantitative real-time polymerase chain reaction (RT-qPCR) instrument (ABI) and qPCR SYBR Green Master Mix (11202ES08; Yeasen). Primer sequences included: TRIM11, 5′-GGACGCAATGCTGTTCCAAG-3′, CAAACTCGC CCAGCACATTC-3′; GAPDH, 5′-TCAAGAAGGTGGTGAA GCAGG-3′, 5′-TCAAAGGTGGAGGAGTGGGT-3′; miR-5193, 5′-AGTGCAGGGTCCGAGGTATT-3′, 5′-CGCGTCCTCCTCTA CCTCAT-3′; U6, 5′-GCTTCGGCAGCACATATACTAAAAT-3′, 5′-CGCTTCACGAATTTGCGTGTCAT-3′.

Cell Viability Assay

PC3 or DU145 cell line (5000/100 μl) was planted onto 96-well plates. At indicated times, 10 μl cell counting kit-8 (CCK8; CK04; Dojindo) was mixed with the medium. Following 1-h incubation, PC3 or DU145 cells were exposed to 10 μl stop buffer. Cell viability was evaluated via examining the absorbance at 450 nm.

Flow Cytometry

Flow cytometry was implemented via apoptosis Detection Kit (AD10; Dojindo). PC3 or DU145 cell line was washed

with PBS, along with 0.25% trypsin digestion for 3 min. Afterwards, they were centrifuged at 1000 rpm for 5 min, and the supernatant was discarded. $1 \times$ Annexin V binding reagent was utilized for preparing cellular suspension with 1×10^6 cells/ml. Incubation of 5 µl Annexin V-FITC conjugate with 100 µl cellular suspension was implemented for 10 min away from the light. Thereafter, incubation with 5 µl of PI was conducted for 10 min away from the light, followed by 400 µl of $1 \times$ Annexin V binding reagent. Within 1 h, apoptosis was examined through flow cytometer.

Colony Formation Assay

PC3 or DU145 cell line was seeded into six-well plates at 2000 cells per well. DMEM was exchanged other day. Colonies were counted following 2 weeks. After discarding the supernatant and washing, fixation was conducted with 4% paraformaldehyde (P0099; Beyotime) for 15 min. The fixative was discarded as well as 0.1% crystal violet (C0121; Beyotime) staining was achieved for 15 min. After washing with PBS, number of colonies was counted and photographed utilizing an IX71 inverted phase contrast microscope (Olympus).

Wound Healing Assay

With a marker, horizontal lines were drawn on the back of the 6-well plate evenly, about every 0.5–1 cm, across the holes. PC3 or DU145 cells were plated in six-well plates, adhered overnight, and the cell density was controlled to reach 100% on the second day. The next day, scratches were made on the back utilizing 200 µl pipette tips. Following the removal of the streaked cells, serum-free DMEM was added. At 0 h along with 24 h, photographs were taken with an IX71 inverted phase contrast microscope (Olympus).

Transwell Assay

Matrigel (354234; BD) was diluted 40-fold with pre-chilled medium. In total, 100 µL of Matrigel was added to the invasion chamber that was placed in a 24-well plate for 30 min. PC3 or DU145 cells were adjusted to 1×10^6 cells/ml. In total, 100 µL cellular suspensions were added onto the upper chamber as well as 600 µL serum-containing DMEM was added onto the lower chamber. The chamber was taken out after 24 h. The cells were fixed with 4% paraformaldehyde (P0099; Beyotime) for 15 min, along with 0.1% crystal violet (C0121; Beyotime) for 15 min. With cotton swabs, removal of unpenetrated cells within the filter surface was conducted. Photographs were acquired with an IX71 inverted phase contrast microscope (Olympus).

Immunoblotting

PC3 or DU145 cells were lysed by RIPA lysis buffer (BL504A; Biosharp) on ice for 15 min. Cell lysates were harvested and centrifuged at 12,000 rpm at 4 °C for 5 min. The supernatant was transferred to a new 1.5 ml EP tube. In total, 3 µl of BCA kit (BL521A; Biosharp) was applied for examining total protein content. $5 \times$ Loading Buffer was added, followed by heat at 100 °C for 10 min. 12% SDS polyacrylamide separating gel was prepared. The protein was transferred onto PVDF membrane, which was sealed with 5% milk/TBST for 1 h. Primary antibody including TRIM11 (cat.no. ab111694; Abcam; 1:1000), ERK1/2 (cat.no. 16443-1-AP; Cell signaling; 1:1000), p-ERK1/2 (cat.no. 28733-1-AP; Proteintech; 1:2000), MAP kinase-extracellular signal-regulated kinase (MEK)1/2 (cat.no. 8727T; Cell signaling; 1:1000), p-MEK1/2 (cat.no. 9154T; Cell signaling; 1:1000), GAPDH (cat.no. 60004-1-Ig; Proteintech; 1:10000) was diluted with 1% BSA/PBST, and the membrane was sealed by hybridization bag, and cooled at 4 °C overnight. The membrane was placed in horseradish peroxidase-labeled goat anti-rabbit or anti-mouse secondary antibody (cat.no. A21020 or A21010; Abbkine; 1:10000) diluted with 5% milk/PBST, along with incubation for 1 h. The protein bands were developed with ECL luminescent fluid and analyzed with fully automatic chemiluminescence imaging system (5200Multi; Tanon).

Lentivirus Construction and Transfection

Virus coating was performed using psPAX2 and pMD2.G plasmids, and pMD2.G, psPAX2 and pCDH-TRIM11 plasmids were mixed in a mass ratio of 1:3:4. 293T cells were cultured in 10 cm dishes, and transfection was carried out when the cells reached 70% confluence. In total, 20 µg of plasmids were used per dish, and 20 µl Lipofectamine 2000 (Invitrogen) was used for transfection. Serum-free DMEM with no double antibody was used for culture. After 6 h, it was replaced with DMEM without double antibody and serum to continue the culture. The cell culture medium was collected at 24 and 48 h after transfection, filtered with a 0.2 µm filter, and directly used to culture PC3 cells. PC3 cells were cultured in 6-well plates, 2 mL of virus-containing cell culture medium was added, along with 10 ug/mL polybrene. After 24 h, the cells were replaced with fresh DMEM without double antibody and serum to continue the culture. In total, 48 h after infection, 2 µg/mL puromycin was added for screening. After 2 weeks of culture, transfection effects were evaluated with RT-qPCR.

Dual Luciferase Reporter

The 3′UTR wild-type (TRIM11-wt) along with mutant (TRIM11-mut) sequences of TRIM11 gene were synthesized and incorporated into fluorescent reporter plasmids, respectively. Transfection of luciferase reporter plasmid along with miR-5193 mimics or NC mimics into 293 T cells was implemented via Lipofectamine 2000 reagent (Invitrogen). The fluorescence activity was detected 48 h later utilizing Dual-Luciferase Reporter Assay System (Promega).

Xenograft Tumor Model

Fifteen male nude mice weighting 20 to 24 g (4 weeks; Charles River Laboratories) were randomized to control group, TRIM11-overexpressed (OE-TRIM11) + NC mimics group

as well as OE-TRIM11 + miR-5193 mimics group (n = 5). All mice were raised in a specific pathogen-free environment for one week. A total of 0.2 mL cell suspensions (5×10^6) of normal PC3 cells or PC3 cells stably transfected with OE-TRIM11 were planted subcutaneously on the side of the back of nude mice.[24] When the tumors grew to the size of rice grains after 2 weeks, miR-5193 mimics or PBS (K813; Shanghai Gao Chuang) was injected with equal volumes, once every 3 days, for a total of 8 injections. The tumor volumes (v) were detected as well as recorded every 3 days in accordance with the formula: $v = \pi/6 \times$ short diameter$^2 \times$ long diameter. The sodium pentobarbital was mixed with sterile saline to make a 3% solution and injected intraperitoneally at a dose of 50 mg/kg (1-1.25 mg per mouse).[25] The mice were sacrificed by cervical dislocation and mortality was confirmed by cessation of heartbeat. The excised tumors were weighed at 4 weeks. The dissected tumors were stored in liquid nitrogen. We followed the Guide for the Care and Use of Laboratory Animals, eighth Edition.[26] During this experiment, we make efforts to minimize the number of animals utilized and to decrease their suffering. Animal experiments were approved by the animal ethics committee of our hospital (approval number: WYYY-AEC-2020-063; Signature date: 7 December 2020).

Statistical Analysis

In vitro experiments were performed in triplicate, and each experiment was repeated three times. In vivo experiments were performed using six mice per group. Statistical analysis was implemented with GraphPad Prism 8.0.1 (LaJolla, CA, USA). Measurement data are displayed as mean ± standard deviation. Student's *t*-tests or one-way analyses of variance were applied. Statistical significance was set at $P < .05$.

Results

TRIM11 Deficiency Induces Apoptosis as Well as Impairs Proliferative Capacity in Prostate Cancer Cells

The oncogenic role of TRIM11 in prostate carcinoma biology was probed in two prostate cancer cell lines, PC-3 along with DU145. TRIM11 knockout cell lines were produced through siRNA transfections. In Figure 1A, si-NC did not affect TRIM11 mRNA expressions than blank cells. Three TRIM11 siRNAs (si-TRIM11-395, si-TRIM11-725, or si-TRIM11-1214) significantly lowered TRIM11 mRNA expressions than si-NC. Si-TRIM11-395 presented the optimal knockout effect on TRIM11 mRNA expressions, which was applied for subsequent assays. CCK-8 was carried out for determining whether TRIM11 was required for prostate cancer progression. Cell viability of PC-3 and DU145 was significantly restrained at 24, 36, 48, and 72 h following TRIM11 expressions were knocked out (Figure 1B and C). Higher apoptosis rate was noted in TRIM11-depleted prostate cells (Figure 1D to F). Additionally,

TRIM11 depletion impaired clone formation ability of PC-3 or DU145 cell line (Figure 1G to I).

TRIM11 Loss Restrains the Motility and Invasiveness of Prostate Carcinoma

The migratory along with invasive capacities of tumors allow them to spread with the tissues and metastasize to distant organs.[27] For investigating the role of TRIM11 in prostate cancer metastases, prostate cancer cellular migration and invasion were estimated via wound healing or Transwell experiment. In Figure 2A to C, migratory capacities of PC-3 or DU145 cell line were significantly impaired via TRIM11 loss. Additionally, depleted TRIM11 in prostate cells displayed dramatic reduction in invasive capacities (Figure 2D to F).

TRIM11 Mediates MEK/ERK Pathway in Prostate Cancer

Targeting MEK/ERK pathway is a viable treatment strategy against prostate cancer.[13] Limited evidence indicates that TRIM11 mediates MEK/ERK pathway in diabetic nephropathy[28] and lung cancer.[13] Immunostaining results showed that TRIM11 depletion did not affect ERK1/2 or MEK1/2, but remarkably decreased phosphorylated ERK1/2 and MEK1/2 both in PC-3 along with DU145 cell lines (Figure 3A to F). This indicated that TRIM11 mediated MEK / ERK signaling activation in prostate cancer.

MiR-5193 Overexpression Represses Cell Growth, Migration and Invasion of Prostate Cancer Cells

Limited evidence indicates that TRIM11 is post-transcriptionally modulated by miR-5193.[22] Herein, we further probed the function of miR-5193 in prostate cancer biology. MiR-5193 expressions were dramatically increased in PC-3 or DU145 cell line with miR-5193 mimics than NC-mimics (Figure 4A and B). In Figure 4C and D, miR-5193-overexpressed PC-3 or DU145 cell line presented significantly lower viability. Moreover, apoptosis of PC-3 or DU145 cell line was significantly heightened through miR-5193 mimics (Figure 4E to G). As shown in Figure 4H to J, clone formation abilities of two prostate cancer cell lines were impaired by miR-5193 mimics. Additionally, miR-5193-overexpressed PC-3 or DU145 cell line presented attenuated migratory (Figure 4K to M) as well as invasive capacities (Figure 4N to P).

MiR-5193 post-Transcriptionally Modulates TRIM11 and Attenuates MEK/ERK Signaling Activation in Prostate Cancer Cells

Further analysis showed that TRIM11 expressions were significantly lower in miR-5193 mimics-transfected PC-3 or DU145 cell line (Figure 5A to D). Additionally, miR-5193 mimics did not alter ERK1/2 or MEK1/2 but decreased phosphorylated
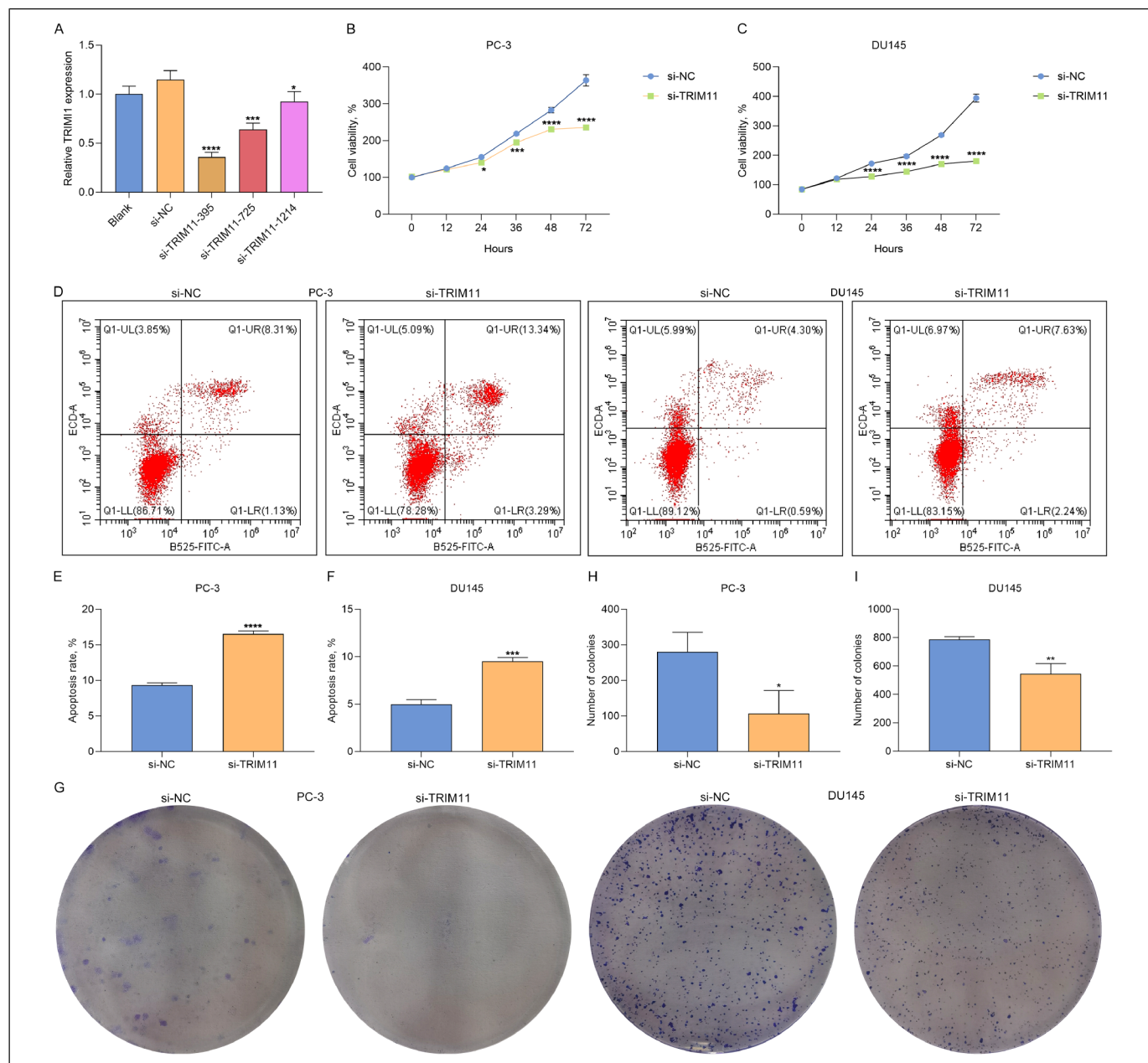
Figure 1. TRIM11 deficiency induces apoptosis and impairs proliferative capacity in prostate cancer cells. (A) Transfection effect of si-TRIM11-395, si-TRIM11-725 or si-TRIM11-1214 on TRIM11 mRNA expressions in PC-3 cells via RT-qPCR. (B, C) Cell viability of PC-3 or DU145 cell with si-NC or si-TRIM11 transfections. (D-F) Apoptosis of PC-3 or DU145 cell line with si-NC or si-TRIM11 transfections. (G-I) Number of colonies of PC-3 or DU145 cell line with si-NC or si-TRIM11 transfections. $*P < .05$; $**P < .01$; $***P < .001$; $****P < .0001$. Abbreviations: TRIM11, tripartite motif-containing protein 11; NC, negative control; RT-qPCR, quantitative real-time polymerase chain reaction.

ERK1/2 and MEK1/2 in PC-3 or DU145 cell line (Figure 5E to H), demonstrating that miR-5193 attenuated MEK/ERK signaling activation. Dual luciferase reporter showed that fluorescence activity was dramatically decreased under transfection with TRIM11-wt and miR-5193 mimics (Figure 5I). Nevertheless, no significant difference in fluorescence activity was investigated when co-transfection with TRIM11-mut and miR-5193 mimics. Thus, TRIM11 can be post-transcriptionally modulated by miR-5193.

Overexpression of miR-5193 Prohibits Prostate Cancer Cell Growth, Migration and Invasion Through TRIM11

We further probed the underlying mechanisms by which TRIM11 and miR-5193 participated in prostate cancer progression and metastases. We firstly overexpressed TRIM11 in PC3 cells (Figure 6A). Overexpressed TRIM11 facilitated viability of PC3 cells, weakened via miR-5193 mimics (Figure 6B). In Figure 6C and D, PC3 cellular apoptosis was alleviated via
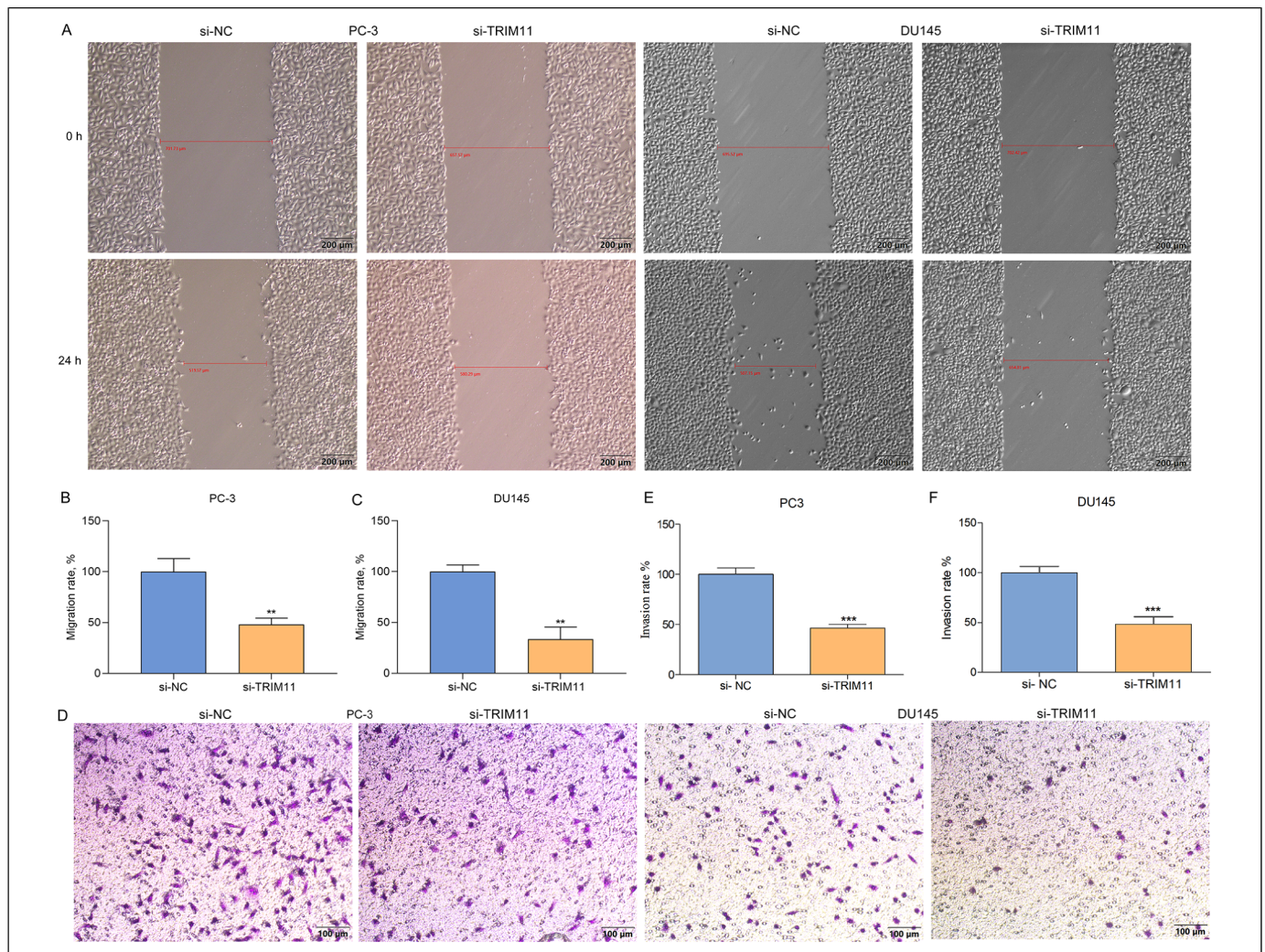
Figure 2. TRIM11 loss restrains the motility and invasiveness of prostate cancer cells. (A-C) Photographs and quantification for wound healing experiment of TRIM11-depleted PC-3 or DU145 cell line at 0 and 24 h. Scale bar: 200 μm. (D-F) Photographs and quantification for transwell invasion experiment of TRIM11-depleted PC-3 or DU145 cell line. Scale bar: 100 μm. **$P < .01$; ***$P < .001$.

TRIM11, which was reversed by miR-5193 mimics. In agreement, overexpressed TRIM11 heightened colony formation capacity of PC3 cells, weakened through miR-5193 mimics (Figure 6E and F). Moreover, PC3 cellular migration and invasion were heightened via overexpressed TRIM11, which were weakened by miR-5193 mimics (Figure 6G to J). Overall, miR-5193 facilitated prostate cancer cell growth and metastases through TRIM11.

MiR-5193 Attenuates MEK/ERK Signaling Activation Through TRIM11

In TRIM11-overexpressed PC-3 cells, miR-5193 mimics decreased TRIM11 expressions (Figure 7A and B). Overexpressed TRIM11 did not alter ERK1/2 or MEK1/2 but increased phosphorylated ERK1/2 and MEK1/2 in PC-3 cells (Figure 7C to F). Oppositely, miR-5193 mimics alleviated the increase in phosphorylated

ERK1/2 and MEK1/2 in TRIM11-overexpressed PC-3 cells. MiR-5193 attenuated MEK/ERK signaling activation through TRIM11 in prostate carcinoma cells.

MiR-5193 Alleviates Tumor Growth in Vivo Through TRIM11

Murine xenograft models were set up utilizing control-, TRIM11-overexpressed (OE-TRIM11) + NC mimics- or OE-TRIM11 + miR-5193 mimics-PC3 cells. After 21 days, the xenografts were collected. Xenografts formed by OE-TRIM11 + NC mimics-PC3 cells were bigger than control cells (Figure 8A and B). Oppositely, OE-TRIM11 + miR-5193 mimics group had a smaller tumor volume than OE-TRIM11 + NC mimics group. The tumor growth curve indicated that tumors in OE-TRIM11 + NC mimics group grew faster than the control group (Figure 8C). In comparison to OE-TRIM11 + NC mimics group, tumor growth was slower in OE-TRIM11 + miR-5193 mimics group. The xenografts were also weighed. In
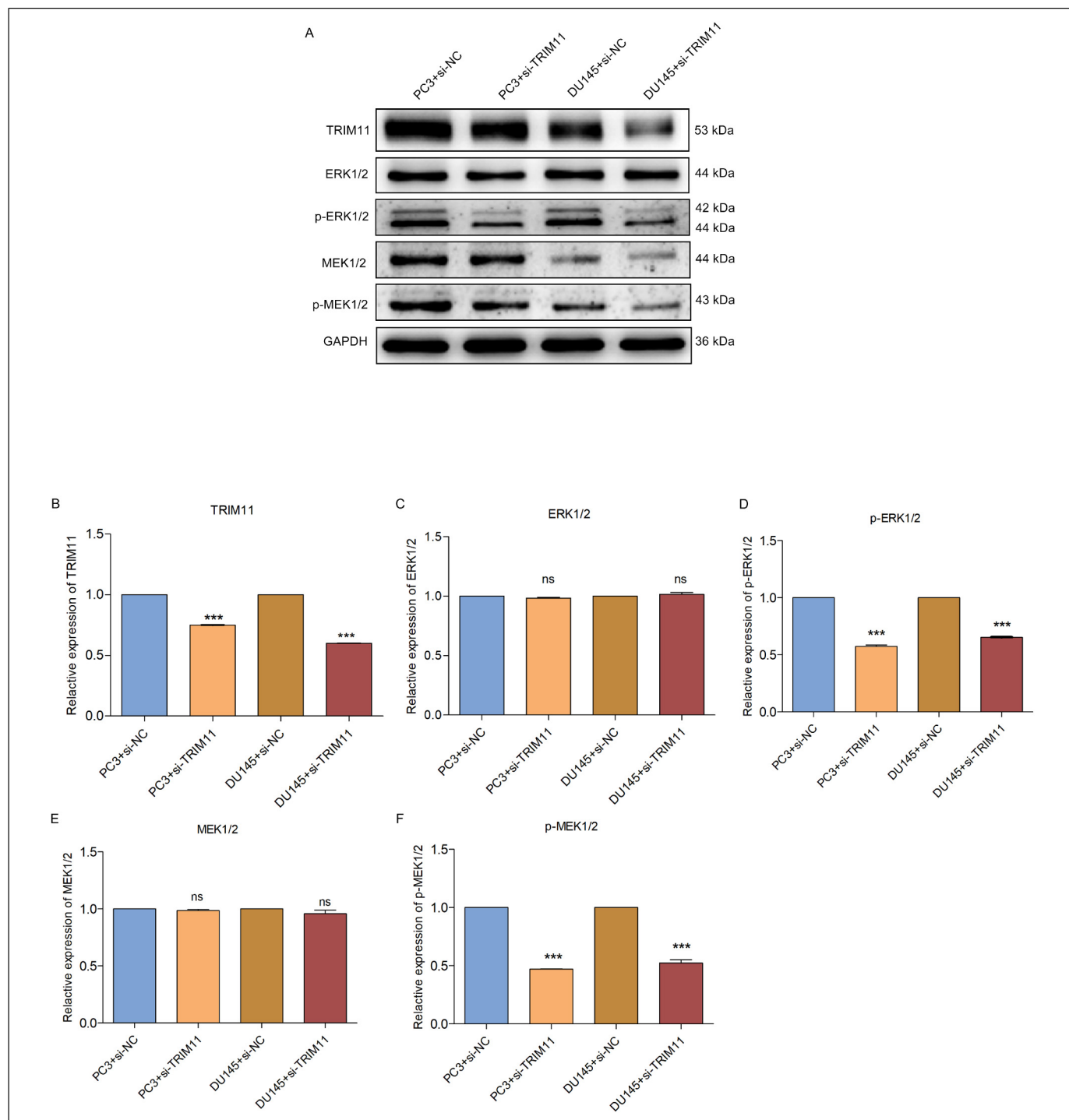
Figure 3. TRIM11 mediates MEK/ERK pathway in prostate cancer. (A-F) Immunoblotting of TRIM11, ERK1/2, p-ERK1/2, MEK1/2, and p-MEK1/2 in TRIM11-depleted PC-3 or DU145 cell line. Ns: no significance; ***$P < .001$. Abbreviations: ERK, extracellular signal-regulated kinase; MEK, MAP kinase-extracellular signal-regulated kinase; OE-TRIM11, tripartite motif-containing protein 11-overexpressed.

Figure 8D, xenografts formed by OE-TRIM11 + NC mimics-PC3 cells were heavier in comparison to control cells. Compared with OE-TRIM11 + NC mimics group, smaller tumor weigh was found in OE-TRIM11 + miR-5193 mimics group. Hence, miR-5193 enabled to alleviate tumor growth in vivo through TRIM11. Thereafter, we examined miR-5193 and

TRIM11 expressions in xenografts. Lower miR-5193 and higher TRIM11 expressions were found in OE-TRIM11 + NC mimics group than the control group (Figure 8E and F). Oppositely, compared with OE-TRIM11 + NC mimics group, miR-5193 expressions were up-regulated and TRIM11 expressions were down-regulated in OE-TRIM11 + miR-5193 mimics group.
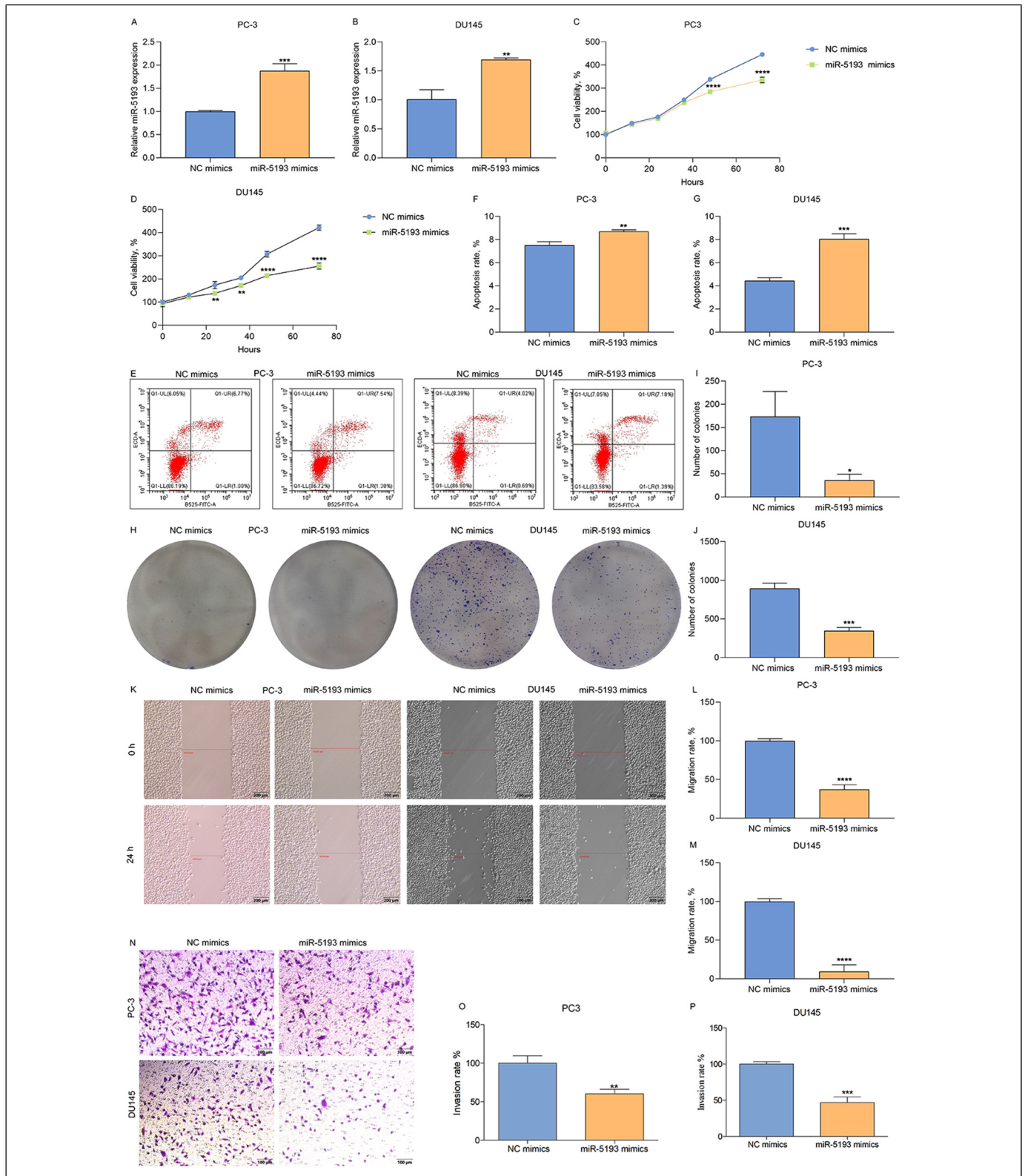
Figure 4. MiR-5193 overexpression represses cell growth, migration and invasion of prostate cancer cells. (A, B) MiR-5193 expressions in PC-3 or DU145 cell line with miR-5193 mimics. (C, D) Cell viability of PC-3 or DU145 cell line with miR-5193 mimics. (E-G) Apoptosis rate of PC-3 or DU145 cell line with miR-5193 mimics. (H-J) Number of colonies of PC-3 or DU145 cell line with miR-5193 mimics. (K-M) Wound healing for the migratory capacity of PC-3 or DU145 cell line with miR-5193 mimics. Scale bar: 200 μm. (N-P) Transwell invasion experiment for the invasive capacity of PC-3 or DU145 cell line with miR-5193 mimics. Scale bar: 100 μm. $*P < .05$; $**P < .01$; $***P < .001$; $****p < .0001$. Abbreviations: miR-5193, microRNA-5193; OE-TRIM11, tripartite motif-containing protein 11-overexpressed.
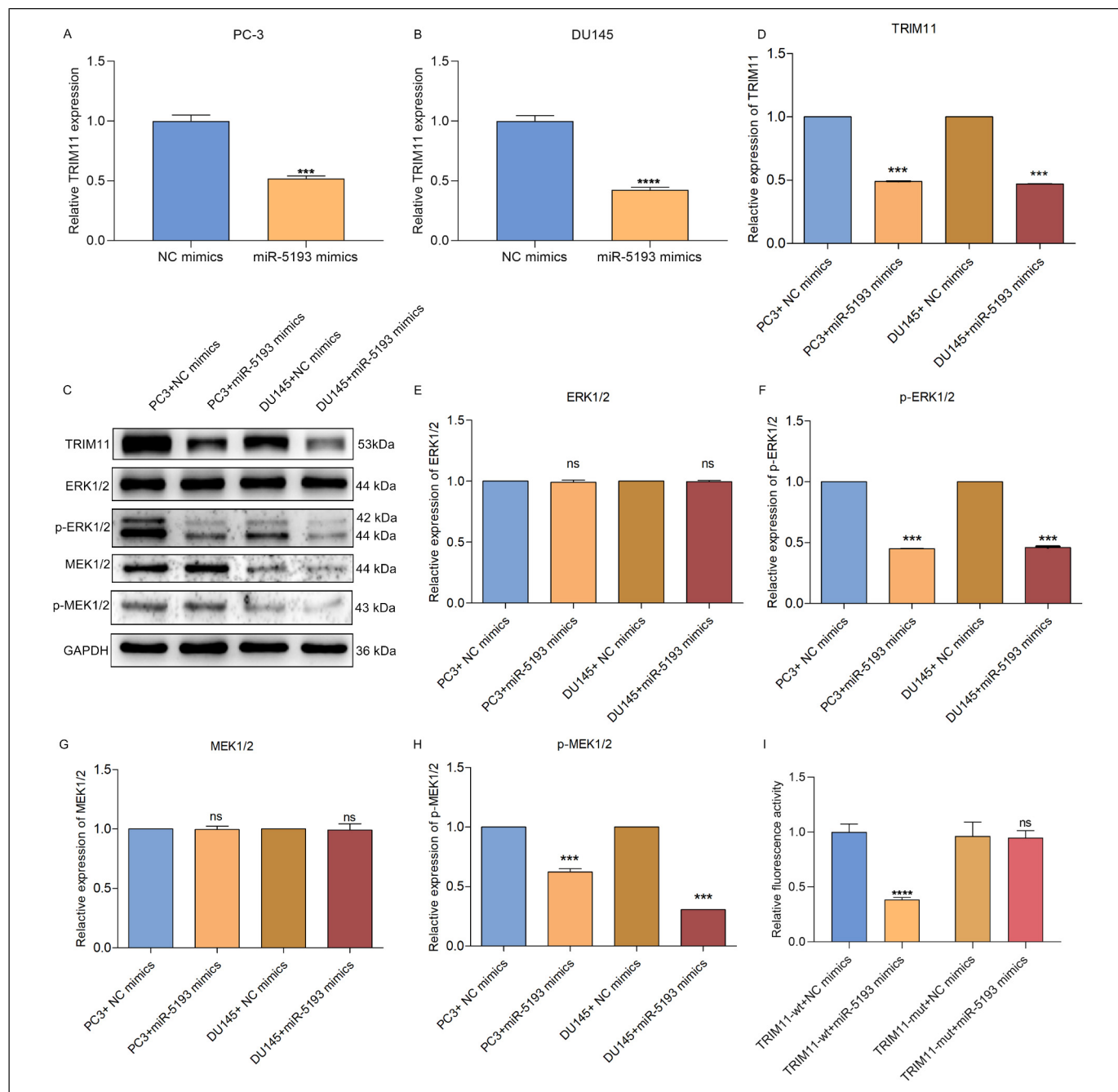
Figure 5. MiR-5193 post-transcriptionally modulates TRIM11 and attenuates MEK/ERK signaling activation in prostate cancer cells. (A, B) TRIM11 mRNA expressions of PC-3 or DU145 cells with miR-5193 mimics. (C-H) Immunoblotting of TRIM11, ERK1/2, p-ERK1/2, MEK1/2, and p-MEK1/2 in PC-3 or DU145 cell line with miR-5193 mimics. (I) Dual luciferase reporter between TRIM11-wt or TRIM11-mut and miR-5193. Ns: no significance; ***$p < .001$; ****$p < .0001$. Abbreviations: ERK, extracellular signal-regulated kinase; MEK: MAP kinase-ERK kinase; miR-5193, microRNA-5193; OE-TRIM11, tripartite motif-containing protein 11-overexpressed; wt, wild-type; mut, mutant.

Discussion

Prostate cancer ranks as the fifth leading cause of cancer-related death among males worldwide.[29] Recently, a significantly increasing incidence and mortality trend was observed in prostate cancer patients in China.[30] The recurrence rate of prostate cancer patients is still high (approximately 40%) although various therapeutic options can successfully cure the majority of patients.[31,32] Therefore, exploring the potential molecular targets for prostate cancer treatment is an urgent problem to be solved. This study mainly discussed the underlying functions of TRIM11 in the pathogenesis of prostate cancer, indicating that TRIM11 contributes to the malignancy of prostate cancer via post-transcriptionally modulated by miR-5193.
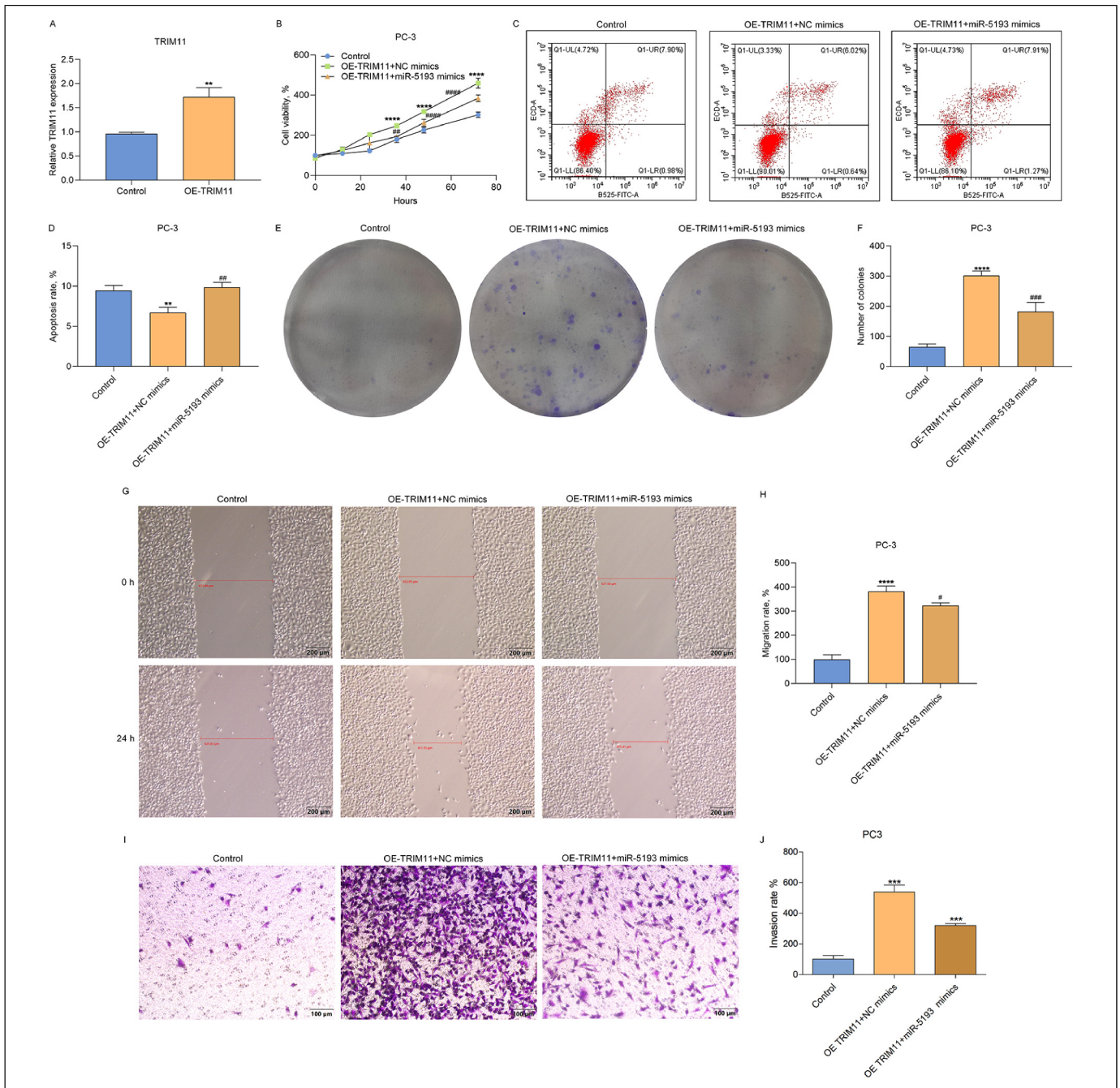
Figure 6. Overexpression of miR-5193 prohibits prostate cancer cell growth, migration and invasion through TRIM11. (A) TRIM11 mRNA expressions in PC-3 cells with TRIM11-overexpressed (OE-TRIM11). (B) Cellular viability, (C, D) apoptotic level, (E, F) number of colonies, (G, H) migratory capacity, (I, J) invasive capacity of control-, TRIM11-overexpressed (OE-TRIM11) + NC mimics or OE-TRIM11 + miR-5193 mimics-PC3 cells. Scale bar: 200 μm (Wound healing assay); Scale bar: 100 μm (Transwell assay). Compared with control, **$P < .01$; ****$P < .0001$. Compared with OE-TRIM11 + NC mimics, #$P < .05$; ##$P < .01$; ###$P < .001$; ####$P < .0001$. Abbreviations: miR-5193, microRNA-5193; OE-TRIM11, tripartite motif-containing protein 11-overexpressed; NC, negative control.

TRIM11 up-regulations have been reported in prostate cancer more than normal tissues, and correlate to poor outcomes,[21] but its specific function in tumorigenesis remains indistinct. SiRNA is a powerful gene silencing tool that enables selectively suppress specific genes, displaying great potential in gene therapy.[33,34] Public data demonstrated that

inhibition of TRIM11 dramatically prohibited the proliferative capacities of cells in breast, lung, and ovarian cancers.[9,12,35] Consistently, our findings also demonstrated that knockout TRIM11 enabled to alleviate PC3 and DU145 cell proliferation as well as heighten apoptosis.[21] These results implied that knocking-down TRIM11 was helpful for the suppressive
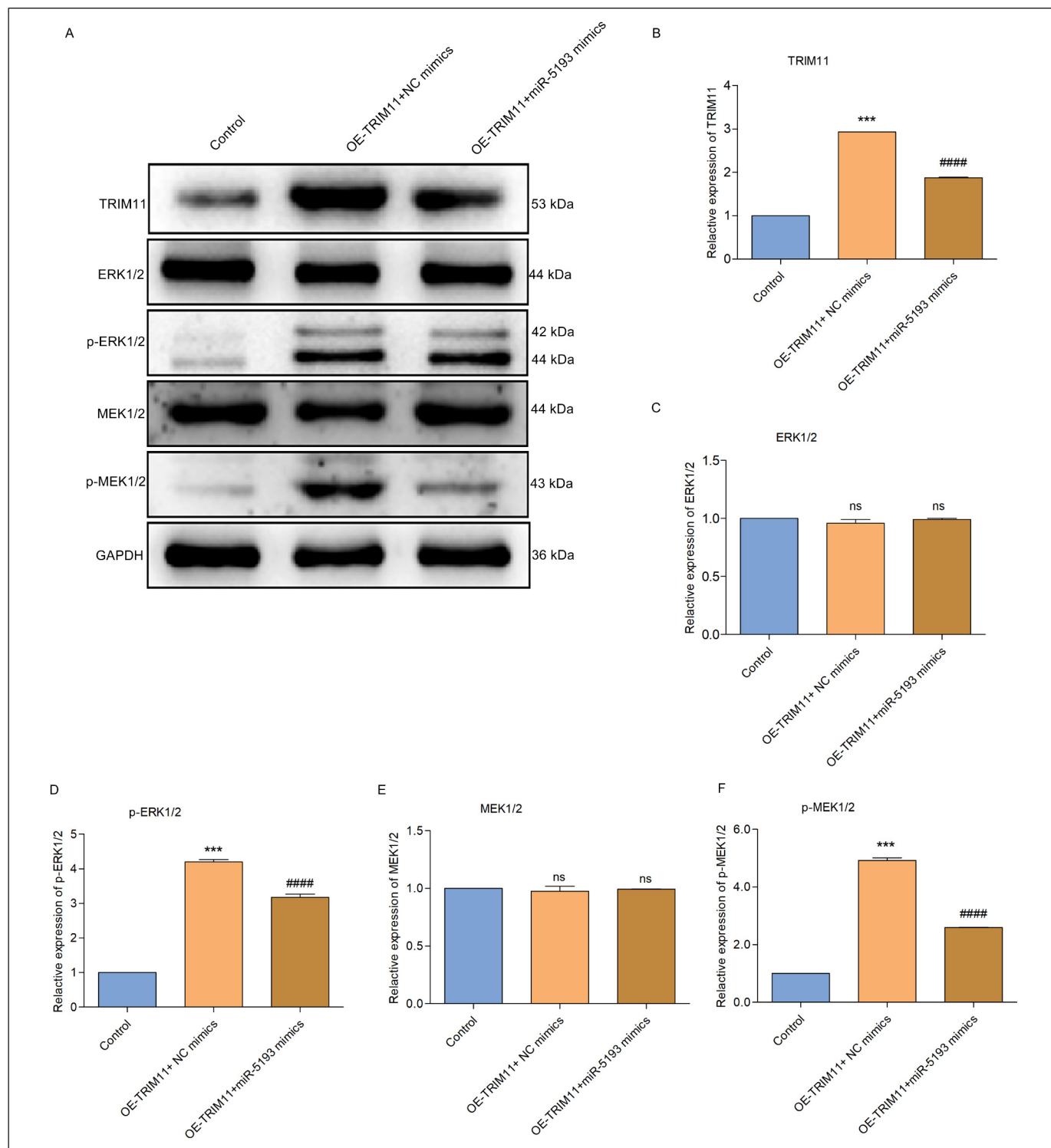
Figure 7. miR-5193 attenuates MEK/ERK signaling activation through TRIM11. (A-F) Immunoblotting of TRIM11, ERK1/2, p-ERK1/2, MEK1/2, and p-MEK1/2 in control-, TRIM11-overexpressed (OE-TRIM11) + NC mimics or OE-TRIM11 + miR-5193 mimics-PC3 cells. Compared with control, ***P < .001; compared with OE-TRIM11 + NC mimics, ####P < .0001; ns: no significance. Abbreviations: ERK, extracellular signal-regulated kinase; MEK, MAP kinase-ERK kinase; miR-5193, microRNA-5193; OE-TRIM11, tripartite motif-containing protein 11-overexpressed; NC, negative control.

effect on cell growth in prostate cancer. Standard therapy of nonmetastatic prostate cancer, to prevent metastasis, remains ADT.[36] Nevertheless, most patients will eventually develop castration-resistant prostate cancer, and thus proves challenging to treat, with a five-year survival of 30%.[37] Impeding prostate cancer metastases enables to ameliorate patients'
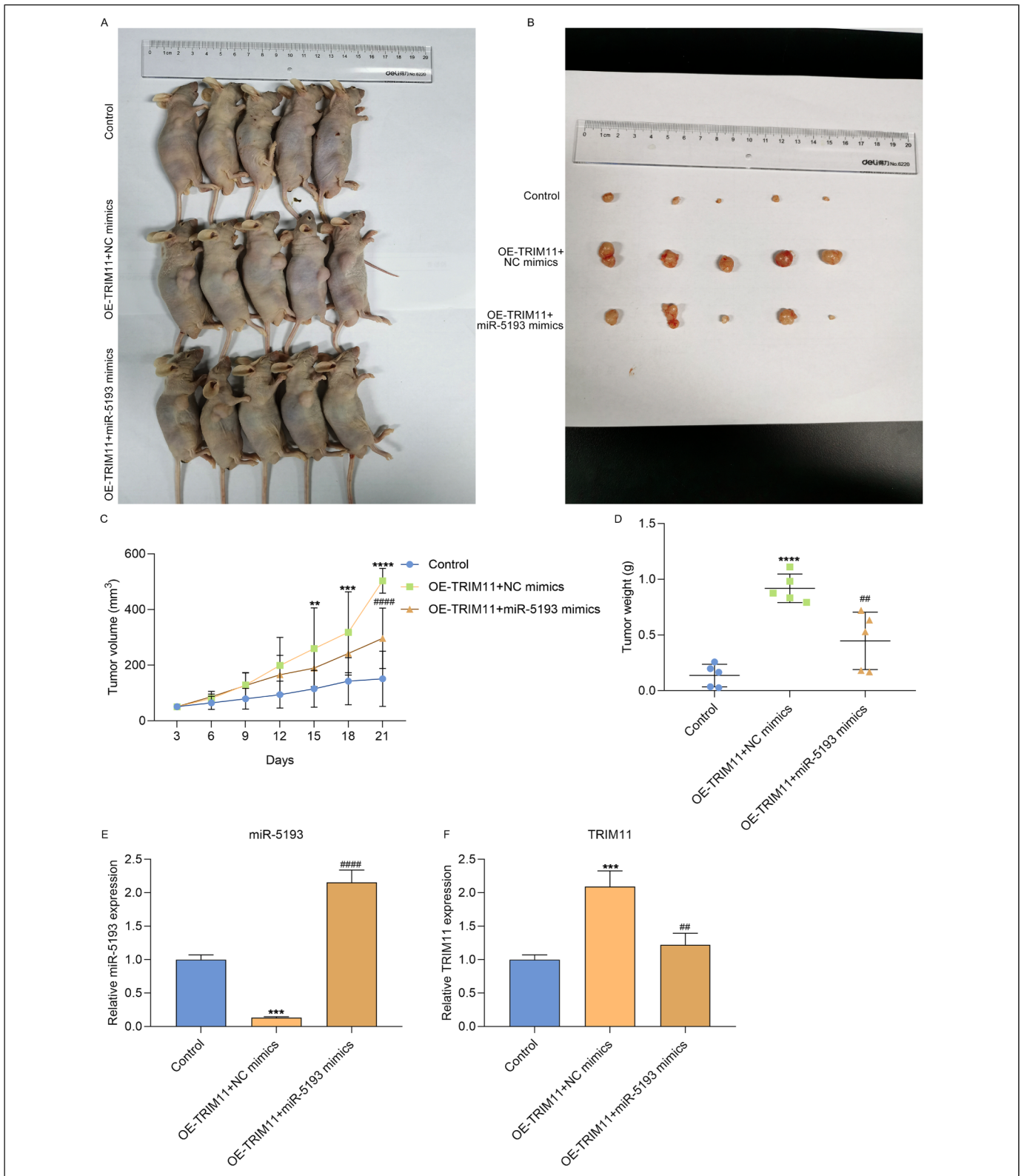
Figure 8. MiR-5193 alleviates tumor growth in vivo through TRIM11. (A, B) Photographs of nude mice and excised subcutaneous tumors through implanting control-, TRIM11-overexpressed (OE-TRIM11) + NC mimics- or OE-TRIM11 + miR-5193 mimics-PC3 cells. (C, D) Quantification of tumor volume and weight. (E, F) miR-5193 and TRIM11 expressions in excised subcutaneous tumors via RT-qPCR. Compared with control, $**P < .01$; $***P < .001$; $****P < .0001$. Compared with OE-TRIM11 + NC mimics, $^{##}P < .01$; $^{####}P < .0001$. Abbreviations: OE-TRIM11, tripartite motif-containing protein 11-overexpressed; miR-5193, microRNA-5193; NC, negative control; RT-qPCR: quantitative real-time polymerase chain reaction.

prognosis.[38,39] Due to cancer metastases as an extremely complex process triggered by diverse crucial events, comprehending the molecular mechanisms underlying these events is beneficial for clinical management.[40-42] Additionally, determining and targeting vital genes involved in prostate cancer metastases is essential to develop future therapeutic regimens to control metastatic spread.[3,43,44] Migration and invasion of tumor cells represent two crucial biological phenomena during metastases.[45,46] Herein, loss of TRIM11 decreased the migratory and invasive capacities of prostate cancer cells. In line with our experimental data, several researchers have determined that TRIM11 is correlated to migration and invasion in several human cancers. For instances, Lan et al found that TRIM11 triggered migration, invasion as well as EMT of gastric cancer via heightening β-catenin activity.[11] Tang et al[14] detected the pro-migration effect of TRIM11 in anaplastic thyroid cancer. Wang et al[12] also reported that TRIM11 enabled to heighten migration and invasion of lung cancer. Liu et al[15] demonstrated the inhibition effect of TRIM11 deficiency on migration and invasion of cervical cancer. On the basis of these findings, TRIM11 enabled to facilitate the migratory and invasive potentials of prostate cancer cells.

MEK/ERK signaling is a classical signaling pathway for cancer therapy. The cytoplasmic Ser/Thr kinases ERK1 and ERK2 were found to promote cell cycling[47]; and ERK1/2 activity was shown to be enhanced by yet other cytosolic kinases, MEK1/2, that phosphorylate the conserved Thr/Tyr in the activation loop of ERK1/2.[48] Further investigation of the kinase cascade revealed that cellular RAF gene (CRAF) is the upstream kinase that phosphorylates MEK1 at Ser222 and MEK2 at Ser218 that regulates the activity of MEK, and through which ERK,[49] thus rank-ordering the MAPK signaling from RAS, RAF, MEK, and finally to ERK.[50] In clinical practice, MEK inhibitors including trametinib and cobimetinib have been applied to the treatment of melanoma and non-small-cell lung cancer to limit the metastatic capacities.[51,52] In the progression of prostate cancer, phosphorylated ERK1/2 is higher in castration-resistant prostate cancer in comparison to primary patients.[53] Additionally, ERK1/2 phosphorylation is linked to biochemical failure following radical prostatectomy, which is independent of clinicopathologic factors. MEK/ERK pathway heightens androgen receptor element-induced gene transcriptions as well as DNA synthesis of prostate carcinoma.[54] MEK/ERK phosphorylation facilitates prostate tumorigenesis.[55] Hence, we speculated molecules targeting MEK / ERK pathway to limit its activity might be a viable therapeutic strategy against refractory metastatic prostate cancer. As expected, we found that TRIM11 knockdown reduced MEK / ERK signaling activity in prostate cancer. We believed that downregulation of TRIM11 could inactivate MEK / ERK signaling to attenuate prostate cancer progression.

The post-transcriptional regulation of TRIM11 by miRNAs has been uncovered in numerous human cancers such as colon cancer, breast cancer and mantle cell lymphoma.[18-20] Additionally, our previous study in 2019 has confirmed that TRIM11 was a direct target of miR-5193.[21] Meanwhile, Song et al[22] demonstrated that miR-5193 alleviated proliferative and migratory abilities of ovarian cancer cells through targeting TRIM11. Therefore, in the current study, we speculated TRIM11 may be post-transcriptionally regulated by miR-5193 to exert oncogene role in prostate cancer progression. Interestingly, we demonstrated that overexpression of miR-5193 reduced the levels of TRIM11, suggesting that TRIM11 was negatively modulated by miR-5193. These results further implied that miR-5193 could attenuate the influences of TRIM11 overexpression on prostate cancer progression, which was confirmed in our researches, namely upregulation of miR-5193 ameliorating the promoting effects of TRIM11 overexpression on cell growth, migration, and invasion in vitro and tumor growth in vivo. At the same time, miR-5193 upregulation also reversed the activation effects of TRIM11 overexpression on MEK/ERK signaling pathway. We, therefore, concluded that TRIM11 could be post-transcriptionally modulated by miR-5193 and then activate MEK/ERK signaling pathway to facilitate tumor growth and metastasis of prostate cancer.

Some limitations exist in the current study. First, only miR-5193 mimics were used in cell culture, and miR-5193 inhibitor should be added in future studies to make our results more rigorous. Second, in vivo experiments exploring the interactions among TRIM11, miR-5193 and MEK/ERK signaling may be needed. Third, there may be some homology differences between mouse and human. Further clinical experiments are indispensable to validate these findings. Fourth, the levels of up-stream regulatory factors of MEK/ERK signaling such as CRAF, RAS, and RAF should be also tested.

Conclusion

Collectively, TRIM11 was determined as a driver and therapeutic target of prostate cancer, post-transcriptionally modulated by miR-5193. Thus, our study provided preclinical evidence for TRIM11 as a promising therapeutic target for clinical application.

ORCID iD

Feng Lu ![ORCID] https://orcid.org/0000-0002-0732-8522

References

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71(3):209-249. doi:10.3322/caac.21660

2. Tukachinsky H, Madison RW, Chung JH, et al. Genomic analysis of circulating tumor DNA in 3,334 patients with advanced prostate cancer identifies targetable BRCA alterations and AR resistance mechanisms. *Clin Cancer Res*. 2021;27(11):3094-3105. doi:10.1158/1078-0432.Ccr-20-4805

3. Matsubara N, Chi KN, Özgüroğlu M, et al. Correlation of prostate-specific antigen kinetics with overall survival and radiological progression-free survival in metastatic castration-sensitive prostate cancer treated with Abiraterone acetate plus prednisone or placebos added to androgen deprivation therapy: post hoc analysis of phase 3 LATITUDE study. *Eur Urol*. 2020;77(4):494-500. doi:10.1016/j.eururo.2019.11.021

4. Morris WJ, Tyldesley S, Rodda S, et al. Androgen suppression combined with elective nodal and dose escalated radiation therapy (the ASCENDE-RT trial): an analysis of survival endpoints for a randomized trial comparing a low-dose-rate brachytherapy boost to a dose-escalated external beam boost for high- and intermediate-risk prostate cancer. *Int J Radiat Oncol Biol Phys*. 2017;98(2):275-285. doi:10.1016/j.ijrobp.2016.11.026

5. Rosenthal SA, Hu C, Sartor O, et al. Effect of chemotherapy with docetaxel with androgen suppression and radiotherapy for localized high-risk prostate cancer: the randomized phase III NRG oncology RTOG 0521 trial. *J Clin Oncol*. 2019;37(14):1159-1168. doi:10.1200/jco.18.02158

6. Vaishampayan UN, Heilbrun LK, Monk P 3rd, et al. Clinical efficacy of enzalutamide vs bicalutamide combined with androgen deprivation therapy in men with metastatic hormone-sensitive prostate cancer: a randomized clinical trial. *JAMA Netw Open*. 2021;4(1):e2034633. doi:10.1001/jamanetworkopen.2020.34633

7. Sun W, Deng Y, Zhao M, et al. Targeting therapy for prostate cancer by pharmaceutical and clinical pharmaceutical strategies. *J Control Release*. 2021;333:41-64. doi:10.1016/j.jconrel.2021.01.010

8. Chen L, Zhu G, Johns EM, Yang X. TRIM11 Activates the proteasome and promotes overall protein degradation by regulating USP14. *Nat Commun*. 2018;9(1):1223. doi:10.1038/s41467-018-03499-z

9. Song W, Wang Z, Gu X, et al. TRIM11 Promotes proliferation and glycolysis of breast cancer cells via targeting AKT/GLUT1 pathway. *Onco Targets Ther*. 2019;12:4975-4984. doi:10.2147/OTT.S207723

10. Tang J, Luo Y, Tian Z, et al. TRIM11 Promotes breast cancer cell proliferation by stabilizing estrogen receptor α. *Neoplasia*. 2020;22(9):343-351. doi:10.1016/j.neo.2020.06.003

11. Lan Q, Tan X, He P, Li W, Tian S, Dong W. TRIM11 Promotes proliferation, migration, invasion and EMT of gastric cancer by

activating β-catenin signaling. *Onco Targets Ther*. 2021;14:1429-1440. doi:10.2147/ott.S289922

12. Wang X, Shi W, Shi H, et al. TRIM11 Overexpression promotes proliferation, migration and invasion of lung cancer cells. *J Exp Clin Cancer Res*. 2016;35(1):100. doi:10.1186/s13046-016-0379-y

13. Wang XL, Lu SC, Sun C, et al. Tripartite motif protein 11 (TRIM11), an oncogene for human lung cancer via the DUSP6-mediated ERK1/2 signaling pathway. *Cancer Biol Ther*. 2021;22(4):324-332. doi:10.1080/15384047.2021.1902912

14. Tang J, Tian Z, Liao X, Wu G. SOX13/TRIM11/YAP Axis promotes the proliferation, migration and chemoresistance of anaplastic thyroid cancer. *Int J Biol Sci*. 2021;17(2):417-429. doi:10.7150/ijbs.54194

15. Liu W, Wu Z, Wang L, Wang Q, Sun X, Niu S. Knockdown of TRIM11 suppresses cell progression and apoptosis of cervical cancer cells via PI3 K/AKT pathway. *Am J Transl Res*. 2021;13(9):10328-10340.

16. Shang M, Weng L, Xu G, et al. TRIM11 Suppresses ferritinophagy and gemcitabine sensitivity through UBE2N/TAX1BP1 signaling in pancreatic ductal adenocarcinoma. *J Cell Physiol*. 2021;236(10):6868-6883. doi:10.1002/jcp.30346

17. Guo H, Deng C, Liang T, et al. Tripartite motif-containing protein 11 reverses paclitaxel resistance in prostate cancer drug-resistant cells by mediating family with sequence similarity 46B expression. *Crit Rev Eukaryot Gene Expr*. 2022;32(7):67-76. doi:10.1615/CritRevEukaryotGeneExpr.2022043323

18. Yin Y, Zhong J, Li SW, et al. TRIM11, A direct target of miR-24-3p, promotes cell proliferation and inhibits apoptosis in colon cancer. *Oncotarget*. 2016;7(52):86755-86765. doi:10.18632/oncotarget.13550

19. Zhang Y, Zhang J, Lin JI, et al. TRIM11 promotes proliferation and invasion of breast cancer cell line MCF7 by targeting miR-24-3p. *Basic Clin Med*. 2018;38(4):451-457.

20. Dong L, Zhang H, Zan T, Han J, Xue Q, Sun Y. LncRNA LUADT1 is upregulated in mantle cell lymphoma and modulates TRIM11 by sponging miR-24-3p to inhibit cell apoptosis. *Crit Rev Eukaryot Gene Expr*. 2021;31(5):33-40. doi:10.1615/CritRevEukaryotGeneExpr.2021038929

21. Pan Y, Zhang R, Chen H, Chen W, Wu K, Lv J. Expression of tripartite motif-containing proteactiin 11 (TRIM11) is associated with the progression of human prostate cancer and is downregulated by MicroRNA-5193. *Med Sci Monit*. 2019;25:98-106. doi:10.12659/MSM.911818

22. Song Z, Guo Q, Wang H, et al. miR-5193, regulated by FUT1, suppresses proliferation and migration of ovarian cancer cells by targeting TRIM11. *Pathol Res Pract*. 2020;216(11):153148. doi:10.1016/j.prp.2020.153148

23. Percie du Sert N, Hurst V, Ahluwalia A, et al. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. *Br J Pharmacol*. 2020;177(16):3617-3624. doi:10.1111/bph.15193

24. Jiang Z, Zhao J, Zou H, Cai K. CircRNA PTPRM promotes non-small cell lung cancer progression by modulating the miR-139-5p/SETD5 axis. *Technol Cancer Res Treat*. 2022;21:15330338221090090. doi:10.1177/15330338221090090

25. Liang D, Tian C, Zhang X. lncRNA MNX1-AS1 promotes prostate cancer progression through regulating miR-2113/MDM2 axis. *Mol Med Rep*. 2022;26(1). doi:10.3892/mmr.2022.12747

26. Guide for the Care and Use of Laboratory Animals. 8th ed. 2011. The National Academies Collection: reports funded by National Institutes of Health.

27. Zhu X, Chen L, Liu L, Niu X. EMT-mediated acquired EGFR-TKI resistance in NSCLC: Mechanisms and strategies. *Front Oncol*. 2019;9:1044. doi:10.3389/fonc.2019.01044

28. Chen W, Peng R, Sun Y, et al. The topological key lncRNA H2k2 from the ceRNA network promotes mesangial cell proliferation in diabetic nephropathy via the miR-449a/b/Trim11/Mek signaling pathway. *Faseb j*. 2019;33(10):11492-11506. doi:10.1096/fj. 201900522R

29. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin*. 2015;65(2):87-108. doi:10.3322/caac.21262

30. Xia C, Dong X, Li H, et al. Cancer statistics in China and United States, 2022: Profiles, trends, and determinants. *Chin Med J (Engl)*. 2022;135(5):584-590. doi:10.1097/CM9.0000000000002108

31. Roehl KA, Han M, Ramos CG, Antenor JA, Catalona WJ. Cancer progression and survival rates following anatomical radical retropubic prostatectomy in 3,478 consecutive patients: long-term results. *J Urol*. 2004;172(3):910-914. doi:10.1097/01.ju.0000134888.22332.bb

32. Boorjian SA, Eastham JA, Graefen M, et al. A critical analysis of the long-term impact of radical prostatectomy on cancer control and function outcomes. *Eur Urol*. 2012;61(4):664-675. doi:10. 1016/j.eururo.2011.11.053

33. Ren R, Du Y, Niu X, Zang R. ZFPM2-AS1 Transcriptionally mediated by STAT1 regulates thyroid cancer cell growth, migration and invasion via miR-515-5p/TUSC3. *J Cancer*. 2021;12(11):3393-3406. doi:10.7150/jca.51437

34. Wang X, Cheng G, Miao Y, et al. Piezo type mechanosensitive ion channel component 1 facilitates gastric cancer omentum metastasis. *J Cell Mol Med*. 2021;25(4):2238-2253. doi:10.1111/jcmm. 16217

35. Chen Y, Sun J, Ma J. Proliferation and invasion of ovarian cancer cells are suppressed by knockdown of TRIM11. *Oncol Lett*. 2017;14(2):2125-2130. doi:10.3892/ol.2017.6432

36. Armstrong AJ, Shore ND, Szmulewitz RZ, et al. Efficacy of enzalutamide plus androgen deprivation therapy in metastatic hormone-sensitive prostate cancer by pattern of metastatic spread: ARCHES post hoc analyses. *J Urol*. 2021;205(5):1361-1371. doi:10.1097/ju. 0000000000001568

37. Armstrong AJ, Szmulewitz RZ, Petrylak DP, et al. ARCHES: A randomized, phase III study of androgen deprivation therapy with enzalutamide or placebo in men with metastatic hormone-sensitive prostate cancer. *J Clin Oncol*. 2019;37(32):2974-2986. doi:10.1200/jco.19.00799

38. Murthy V, Maitre P, Kannan S, et al. Prostate-only versus whole-pelvic radiation therapy in high-risk and very high-risk prostate cancer (POP-RT): outcomes from phase III randomized controlled trial. *J Clin Oncol*. 2021;39(11):1234-1242. doi:10.1200/jco.20. 03282

39. Hofman MS, Lawrentschuk N, Francis RJ, et al. Prostate-specific membrane antigen PET-CT in patients with high-risk prostate cancer before curative-intent surgery or radiotherapy (proPSMA): a prospective, randomised, multicentre study. *Lancet*. 2020;395(10231):1208-1216. doi:10.1016/s0140-6736(20)30314-7

40. Fizazi K, Tran N, Fein L, et al. Abiraterone Acetate plus prednisone in patients with newly diagnosed high-risk metastatic castration-sensitive prostate cancer (LATITUDE): final overall survival analysis of a randomised, double-blind, phase 3 trial. *Lancet Oncol*. 2019;20(5):686-700. doi:10.1016/s1470-2045(19)30082-8

41. Kerkmeijer LGW, Groen VH, Pos FJ, et al. Focal boost to the intraprostatic tumor in external beam radiotherapy for patients with localized prostate cancer: results from the FLAME randomized phase III trial. *J Clin Oncol*. 2021;39(7):787-796. doi:10. 1200/jco.20.02873

42. Boevé LMS, Hulshof M, Vis AN, et al. Effect on survival of androgen deprivation therapy alone compared to androgen deprivation therapy combined with concurrent radiation therapy to the prostate in patients with primary bone metastatic prostate cancer in a prospective randomised clinical trial: Data from the HORRAD trial. *Eur Urol*. 2019;75(3):410-418. doi:10.1016/j.eururo.2018.09.008

43. Lestingi JFP, Guglielmetti GB, Trinh QD, et al. Extended versus limited pelvic lymph node dissection during radical prostatectomy for intermediate- and high-risk prostate cancer: early oncological outcomes from a randomized phase 3 trial. *Eur Urol*. 2021;79(5):595-604. doi:10.1016/j.eururo.2020.11.040

44. Saad F, Efstathiou E, Attard G, et al. Apalutamide plus Abiraterone acetate and prednisone versus placebo plus Abiraterone and prednisone in metastatic, castration-resistant prostate cancer (ACIS): a randomised, placebo-controlled, double-blind, multinational, phase 3 study. *Lancet Oncol*. 2021;22(11):1541-1559. doi:10.1016/s1470-2045(21)00402-2

45. Sun Y, Niu X, Wang G, Qiao X, Chen L, Zhong M. A novel lncRNA ENST00000512916 facilitates cell proliferation, migration and cell cycle progression in ameloblastoma. *Onco Targets Ther*. 2020;13:1519-1531. doi:10.2147/ott.S236158

46. Qiao X, Niu X, Shi J, et al. Wnt5a regulates ameloblastoma cell migration by modulating mitochondrial and cytoskeletal dynamics. *J Cancer*. 2020;11(18):5490-5502. doi:10.7150/jca.46547

47. Boulton TG, Nye SH, Robbins DJ, et al. ERKs: A family of protein-serine/threonine kinases that are activated and tyrosine phosphorylated in response to insulin and NGF. *Cell*. 1991;65(4):663-675. doi:10.1016/0092-8674(91)90098-j

48. Crews CM, Erikson RL. Purification of a murine protein-tyrosine/threonine kinase that phosphorylates and activates the Erk-1 gene product: Relationship to the fission yeast byr1 gene product. *Proc Natl Acad Sci U S A*. 1992;89(17):8205-8209. doi:10.1073/pnas.89.17.8205

49. Alessi DR, Saito Y, Campbell DG, et al. Identification of the sites in MAP kinase kinase-1 phosphorylated by p74raf-1. *EMBO J*. 1994;13(7):1610-1619. doi:10.1002/j.1460-2075.1994.tb06424.x

50. Kolch W, Heidecker G, Lloyd P, Rapp UR. Raf-1 protein kinase is required for growth of induced NIH/3T3 cells. *Nature*. 1991;349(6308):426-428. doi:10.1038/349426a0

51. Welsh SJ, Corrie PG. Management of BRAF and MEK inhibitor toxicities in patients with metastatic melanoma. *Ther Adv Med Oncol*. 2015;7(2):122-136. doi:10.1177/ 1758834014566428

52. Kim C, Giaccone G. MEK Inhibitors under development for treatment of non-small-cell lung cancer. *Expert Opin Investig Drugs*. 2018;27(1):17-30. doi:10.1080/13543784. 2018.1415324

53. Nickols NG, Nazarian R, Zhao SG, et al. MEK-ERK signaling is a therapeutic target in metastatic castration resistant prostate cancer. *Prostate Cancer Prostatic Dis*. 2019;22(4):531-538. doi:10.1038/s41391-019-0134-5

54. Carey AM, Pramanik R, Nicholson LJ, et al. Ras-MEK-ERK signaling cascade regulates androgen receptor element-inducible gene transcription and DNA synthesis in prostate cancer cells. *Int J Cancer*. 2007;121(3):520-527. doi:10.1002/ijc.22715

55. Liu W, Xie A, Tu C, Liu W. REX-1 represses RASSF1a and activates the MEK/ERK pathway to promote tumorigenesis in prostate cancer. *Mol Cancer Res*. 2021;19(10):1666-1675. doi:10.1158/1541-7786.Mcr-20-0974