



Tripartite motif 52 (TRIM52) promotes proliferation, migration, and regulation of colon cancer cells associated with the NF- κ B signaling pathway

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Background: With the advancement of early detection and treatment, the incidence of colon cancer (CC) has declined steadily worldwide; however, the mortality remains unacceptably high. *Tripartite motif 52 (TRIM52)* is a member of the family of highly conserved RBCC (a RING-finger, two B-boxes, and a predicted alpha-helical Coiled-Coil domain were linked to the N-terminal region in sequence) proteins with more than 70 isoforms, which plays an important role in tumorigenesis through different signaling pathways. How it regulates the development of CC remains unknown.

Methods: Western blot was used to reveal that TRIM52 protein expression is up-regulated in CC cells. The Analysis of The Cancer Genome Atlas (TCGA) database was used to find the different expressions of TRIM52 between colon cancer tissues and normal colonic epithelial tissues. Cell proliferation assays, migration and invasion assays, and apoptosis were used to verify the changes in cell function after knockdown or overexpression of TRIM52 in CC cells. After that, the key proteins of the nuclear factor (NF)- κ B signaling pathway were validated by western blot to explore the role of TRIM52 in the NF- κ B signaling pathway. Finally, in order to explore the potential sites of TRIM52, LPS and PDTC were employed to activate and block the NF- κ B signaling pathway, and the key proteins of the NF- κ B signaling pathway were validated by western blot.

Results: TCGA database revealed that TRIM52 expression was elevated in CC tissues and correlated with prognosis. It was verified that TRIM52 promoted the proliferation, migration, and invasion of CC cells, and inhibited cell apoptosis. Most of the tripartite motif proteins (TRIMs) have ubiquitin ligase activity related to their highly conserved RING structure. Detection of the key proteins of the NF- κ B signaling pathway in CC cells revealed that TRIM52 activated the NF- κ B signaling pathway.

Conclusions: We confirmed that TRIM52 promotes proliferation, migration, and invasion while inhibiting apoptosis of CC cells. The regulatory effect of TRIM52 on CC cells is related to the activation of the NF- κ B signaling pathway. As TRIM52 acted as an upstream stimulator, stimulating the transfer of P65 into the nucleus to activate the NF- κ B signaling pathway, it may provide a potential target for prognosis prediction and treatment of CC.

Keywords: Colon cancer (CC); *tripartite motif 52 (TRIM52)*; NF- κ B signaling pathway; cell proliferation

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Introduction

Recently, the incidence of malignant tumors has been increasing. The latest report from the National Cancer Center states that colorectal cancer (CRC) ranked second in cancer incidence and fifth in cancer mortality in China in 2019 (1). Relevant data from the United States show that the incidence of CRC in men has decreased at a rate of 3% per year over the past 5 years, with no significant change in the incidence rate in women (2). As a cancer with high morbidity and mortality, the mechanism underlying the development of CRC remains unclear and requires further exploration.

The tripartite motif protein (TRIM) family has been found to widely exist in humans (with more than 70 species having been identified) and some multicellular animals. The TRIM protein family is a group of proteins with highly conserved RING, B-box, double helix, and C-terminus (RBCC) structures in order from N-terminal to C-terminal (3). In addition, TRIM proteins are divided into 11 subgroups according to the C-terminal sequence (4,5), among which the PRY/SPRY sequence, also known as the B30.2 sequence, is the most common sequence (5).

A significant portion of the TRIM protein family comprises ubiquitin ligase (E3) activity (6,7), which plays a considerable role in mediating post-translational modifications of the protein by binding to ubiquitin-binding enzymes (E2). E3 activity is closely related to its RING structure. Ubiquitination is a post-translational modification of proteins that is very important in tumorigenesis. Previous studies on the TRIM protein family have shown that TRIM proteins play diverse roles in tumorigenesis. Most of them play a role in promoting tumor progression and metastasis, while others play a tumor-suppressive role (8-10). Certain TRIM family members have been shown to be potential molecular biomarkers for cancer diagnosis and prognosis, and potential therapeutic targets (11).

In mammals, partial gene sequences of Tripartite motif 52 (*TRIM52*) are duplicated from partial genes of *TRIM41*, mainly in the RING and B-box structural domains, and have been present in primate mammals for a long time, implying that *TRIM52* plays an essential role in certain human biological processes. It has been found that *TRIM52* is differentially expressed in different tumor cells. It has been reported that *TRIM52* promotes tumorigenesis in ovarian cancer by activating the nuclear factor (NF)- κ B signaling pathway. Knocking-down of *TRIM52* in ovarian cancer cells suppresses cell invasion, cell migration and cell proliferation, while inducing apoptosis (12).

Overexpression of *TRIM52* promoted the biological activity of hepatocellular carcinoma. Downregulation of *TRIM52* significantly upregulated p21 and PPM1A expression but suppressed the expression of MMP2, which induced dephosphorylation of Smad2/3 in hepatocellular carcinoma cells. In addition, down-regulation of *TRIM52* inhibited the ubiquitination of PPM1A. Upregulation of PPM1A in hepatocellular carcinoma cells significantly suppressed the *TRIM52*-mediated cell biological activity. It can be seen that *TRIM52* interacted with PPM1A (13). Downregulation of *TRIM52* has been shown to inhibit kidney cancer progression (14). Non-coding (lnc)RNA *TRIM52-AS1* knockdown inhibited the proliferation and metastasis of hepatocellular carcinoma cells. It has been shown to promote the growth and metastasis of hepatocellular carcinoma via miR-218-5p/ROBO1 (15). It was reported that *TRIM52* upregulation promotes proliferation and invasiveness through activation of the Wnt/ β -catenin pathway in lung cancer cells (16). The role that *TRIM52* plays in colon cancer (CC) cells remains unknown. CRC is one of the most common cancers around the world as mentioned above. It has been reported that the possible mechanisms by which NF- κ B can contribute to colon carcinogenesis include the activator of antiapoptotic gene expression, enhanced cell survival, and proliferation, regulation of angiogenesis, and promotion of metastasis of cancer cells (17). This study aimed to determine the different expression levels of *TRIM52* in normal colon epithelial cells and CC cells, explore its effect on CC cells, and identify the underlying mechanisms associated with the NF- κ B signaling pathway. We present the following article in accordance with the MDAR reporting checklist (available at <https://jgo.amegroups.com/article/view/10.21037/jgo-22-317/rc>).

Methods

Cell lines

We obtained the following cell lines: SW1116 (TCHu174, human colon cancer cells), SW480 (TCHu172, human colon cancer cells), SW620 (TCHu101, human colon cancer cells), LOVO (TCHu82, human colon cancer cells), and HT29 (TCHu103, human colon cancer cells) from the National Collection of Authenticated Cell Culture, Chinese Academy of Sciences (Shanghai, China); HCT116 (human colon cancer cells) and NCM460 (human normal mucosal epithelial cells) from the American-Type Culture Collection (Manassas, VA, USA). Cells were maintained in Dulbecco's modified Eagle medium (DMEM) with 10% fetal bovine serum (FBS)

at 37 °C with 5% CO₂. Cells were used in the following assays when they were in the logarithmic growth phase.

Western blot

Protein expressions of *TRIM52* and NF-κB signaling pathway associated proteins were tested by western blot (WB). Cell protein was collected from CC cells by radioimmunoprecipitation assay (RIPA) buffer [1 mM with phenylmethylsulfonyl fluoride (PMSF)]. A bicinchoninic acid (BCA) protein assay kit (Thermo Fisher Scientific, Waltham, MA, USA) was used to determine protein concentrations. Lysate samples were separated by electrophoresis on a 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel at 120v and subsequently transferred to polyvinylidene fluoride (PVDF) membranes. The membranes were incubated with primary antibodies for at least 12 hours at 4 °C. After the primary antibody was incubated, horseradish peroxidase (HRP)-conjugated secondary antibody was incubated with the membranes for 2 hours in a room temperature environment. Signals were detected using chemiluminescent substrate (ECL; Bio-Rad, Richmond, CA, USA). The band intensity was measured by ImageJ software (National Institutes of Health, Bethesda, MD, USA). Primary antibodies NF-κB P65 (ab32536), phospho-NF-κB P65 (ab76302), IKKβ (ab32135), IKKα (ab32041), IKBα (ab32518), phospho-IBKα (ab133462), and β-actin (ab8226) were purchased from Abcam (Cambridge, MA, USA). Antibodies against *TRIM52* (sc-398954) were purchased from Santa Cruz Biotechnology (Dallas, TX, USA). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH; G9545) served as an internal control and was obtained from Sigma Aldrich (St. Louis, MO, USA). Phosphor-IKKα/β (Ser180/181) (abs130641) was obtained from Absin Bioscience Inc. (Shanghai, China).

Cell migration and invasion assays

Transwell experiments were employed for the cell migration and invasion assays. The upper chamber of the Transwell (Corning, Corning, NY, USA) was coated with Matrigel (Becton, Dickinson, and Co. Biosciences, Franklin Lakes, NJ, USA) at 37 °C in a 5% CO₂ incubator for 30 minutes for the cell invasion assay. Indicated cells were trypsinized, resuspended in DMEM without serum, and placed in the upper chamber (5×10⁴ cells/well). After that, DMEM with 10% FBS was added to the lower chamber. After 24 hours,

a cotton swab was used to remove the cells in the upper chamber. Cells migrating into the lower chamber were washed by the phosphate-buffered saline (PBS), fixed in the 4% paraformaldehyde, and stained by the 0.5% crystal violet. Eventually, the cells were counted under a microscope in 4 random fields. The conditions for the cell migration assay were the same as the cell invasion assay, except for the Matrigel.

Flow cytometry for cell apoptosis

After the transfection for 48 hours, cells were harvested and washed with PBS. Then, the cells were stained with both Annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI) in sequence at 4 °C for 30 minutes in a light-proof environment. Flow cytometry data were collected on the FACScan instrument (Becton Dickinson and Co., Mountain View, CA, USA).

Cell proliferation assay

Cell proliferation assay was detected using a Cell Counting Kit-8 (CCK-8; Dojindo Laboratories, Kumamoto, Japan) according to the manufacturer's instructions. Each assay was performed 6 times.

NF-κB activation assays

Lipopolysaccharide (LPS; an activator of NF-κB P65), and pyrrolidine dithiocarbamate (PDTC; an inhibitor of NF-κB P65) were employed. The LPS (Sigma Aldrich, USA) was co-incubated with the knocked-down *TRIM52* HCT116. The PDTC (MedChemExpress, Princeton, NJ, USA) was added to the overexpressed *TRIM52* SW620. Protein expressions of nuclear P65 and plasmin P65 were detected to verify the activation of the NF-κB signaling pathway.

Statistical analysis

Data were acquired from at least 3 independent experiments. Statistics were performed in GraphPad Prism7 (GraphPad Software Inc., San Diego, CA, USA). Statistical significance was considered when P<0.05.

Knock down and overexpression of *TRIM52* in CC cells

The small interfering (si)RNA (RiboBio, Guangzhou,

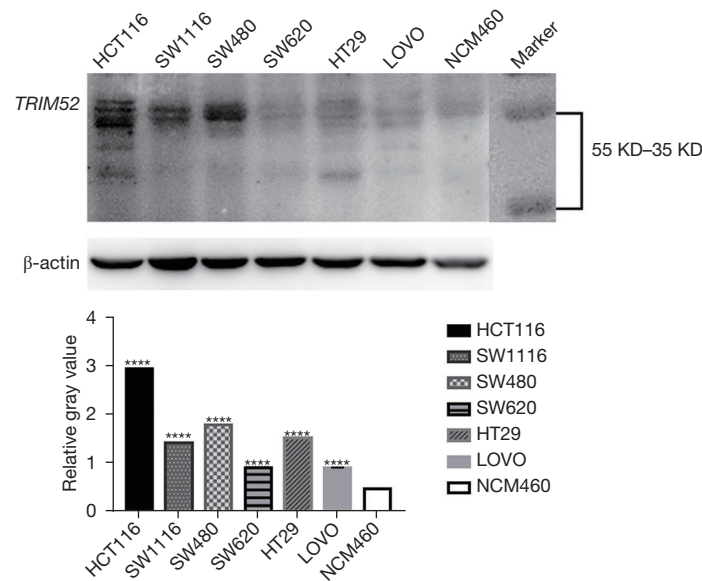


Figure 1 Differential expression of *TRIM52* in different colon cancer cell lines. ****, $P < 0.0001$.

China) (position 756–774, AGAAATACTGGAAGCATAC, Si-*TRIM52* 002; position 975–993, GGCCATGTGCTTT AACAC, Si-*TRIM52* 003) was added to the HCT116 in the logarithmic growth phase according to the manufacturer's instructions for 24 hours. The knocking down effect was verified by WB. The coding sequence of *TRIM52* was amplified by reverse transcription polymerase chain reaction (RT-PCR) with the following primers: (forward 5'-CGGAATTCATGGCTGGTTATGCCACTA CTC-3' and reverse 5'-CGGGATCCTTACTGATTATA GGCCTTGCTG-3'), and constructed into pCDH-CMV-MCS-EF1-CopGFP-T2A-puro (Syngenta Biotechnology Inc., Durham, NC, USA). Lentiviral empty vector was used as a control group. The *TRIM52* overexpression lentivirus and control (Vector) lentiviruses were transfected by Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Results

TRIM52 protein expression is up-regulated in CC cells

The CC cells HCT116, SW620, HT29, SW1116, LOVO, and SW480, and the normal colon epithelial cells, NCM460, were cultured, and the expression of *TRIM52* was detected after extracting total cell protein

by WB. Among CC cells, the expression in HCT116 was the highest, in order of SW480, HT29, SW1116, and LOVO. The expression of *TRIM52* was lowest in SW620, which was about 1.6 times more than that in normal colon epithelial cells NCM460 (Figure 1).

TRIM52 is upregulated in tumor tissues analyzed by TCGA database

The University of Alabama Cancer Database (UALCAN; <http://ualcan.path.uab.edu/index.html>) was used to find relevant data on *TRIM52* expression in CC, and data analysis was performed to speculate on the possible role of *TRIM52* in CC. By summarizing TCGA and UALCAN data, it was found that *TRIM52* was highly expressed in tumor tissues compared with normal tissues (Figure 2A), and *TRIM52* was significantly highly expressed in stage IV tumor tissues, which was statistically different compared with other stages (Figure 2B). This indicated that *TRIM52* might be a marker of poorer prognosis. It was also found that the expression of *TRIM52* correlated with the survival of tumor patients, and by comparing the survival curves, there was a statistical difference between the high expression *TRIM52* group and the low expression group, indicating that *TRIM52* may be related to the development of CC and is an indicator of poorer prognosis (Figure 2C).

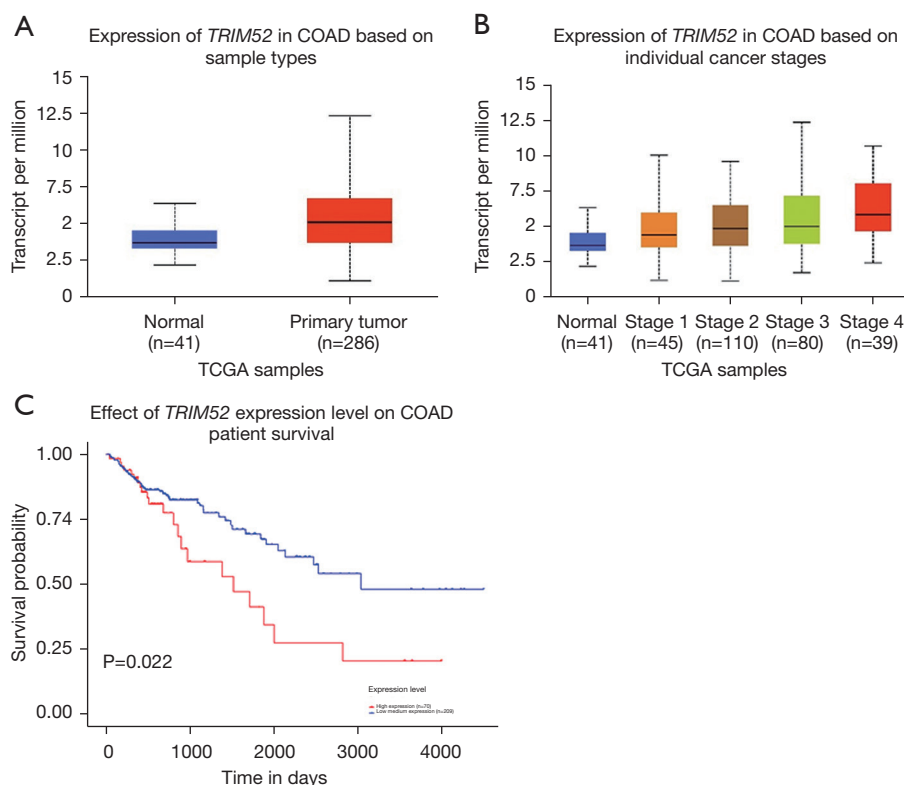


Figure 2 *TRIM52* is upregulated in tumor tissues analyzed by TCGA database. (A) Expression of *TRIM52* in 286 colon cancer tissues and 41 normal tissues were analyzed by TCGA database, *TRIM52* is upregulated in tumor tissues ($P < 0.05$). (B) Expression of *TRIM52* in normal colon epithelial tissues and each stage of colon cancer was analyzed by TCGA database data. (C) Survival analysis of patients with high and low expression of *TRIM52* in tumor tissues by TCGA database. TCGA, The Cancer Genome Atlas.

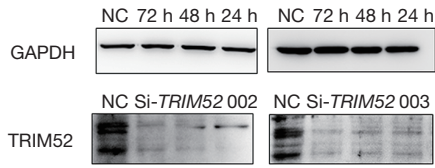
Knockdown of *TRIM52* suppresses cell proliferation, migration, and regulation in CC cells

The knockdown effect of siRNA on *TRIM52* in HCT116 started to show at 24 hours after transfection and lasted at least until 72 hours (Figure 3A). After knocking down the expression of *TRIM52* in HCT116 cells, the proliferation (Figure 3B), invasion (Figure 3C), and migration (Figure 3D) were significantly decreased compared with the untreated HCT116 cell group and the NC control groups. These results showed that the percentage of cells in the S phase decreased from 24.89% to 19.03% after the knockdown of *TRIM52* compared to the NC control group, with a statistically significant decrease in the percentage of cells in the S phase (Figure 3E). It was suggested that cellular DNA replication was reduced. Knocking down the expression of *TRIM52* in HCT116 cells promoted cell apoptosis and the percentage of late apoptotic cells increased by about 30% (Figure 3F).

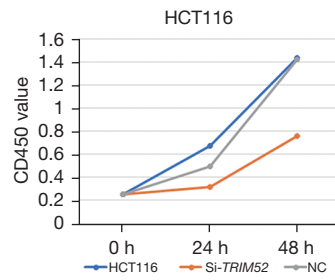
Overexpression of *TRIM52* suppresses CRC cell proliferation, migration, and regulation in CC cells

The *TRIM52*/pCDH-CMV-MCS-EF1-CopGFP-T2A-puro plasmid was more effective for overexpression of *TRIM52* in SW620, and its overexpression effect started to show at 24 hours after transfection (Figure 4A). The proliferation (Figure 4B), invasion (Figure 4C), and migration (Figure 4D) ability of SW620 were significantly enhanced after overexpression of *TRIM52* by plasmid compared to untreated SW620. The percentage of S-phase cells increased from 30.01% to 40.31% after overexpression of *TRIM52* in SW620, which was a statistically significant, suggesting an increase in cellular DNA replication (Figure 4E). Apoptosis was significantly inhibited, and the percentage of early and late apoptosis was reduced by about 20%, which was statistically different from the percentage of late apoptosis after the overexpression of *TRIM52* in SW620 (Figure 4F).

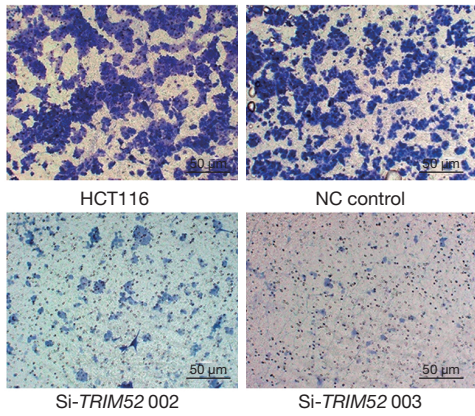
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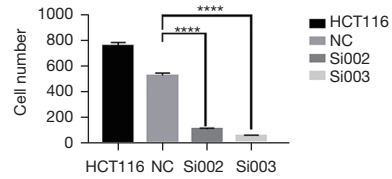
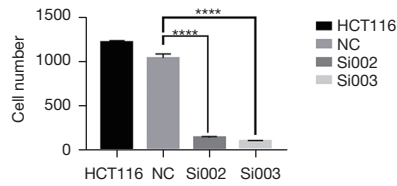
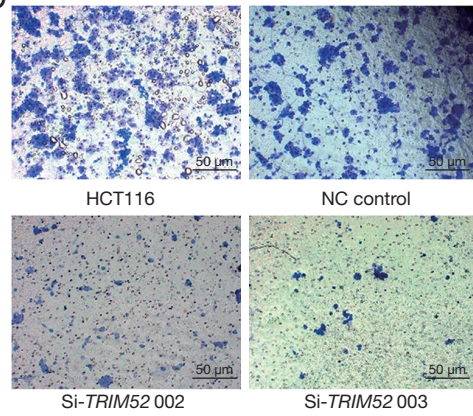
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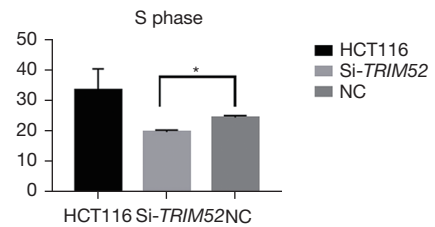
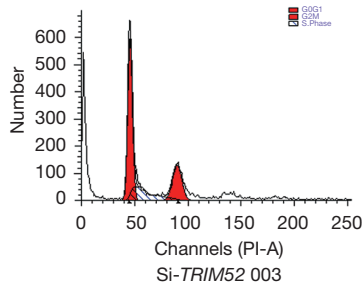
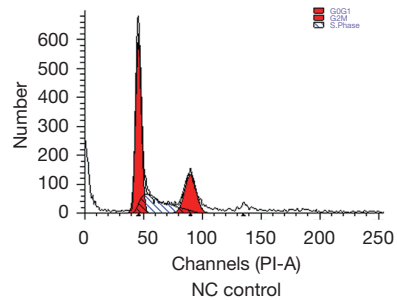
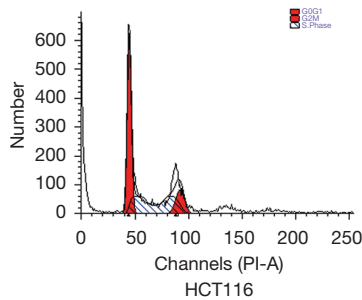
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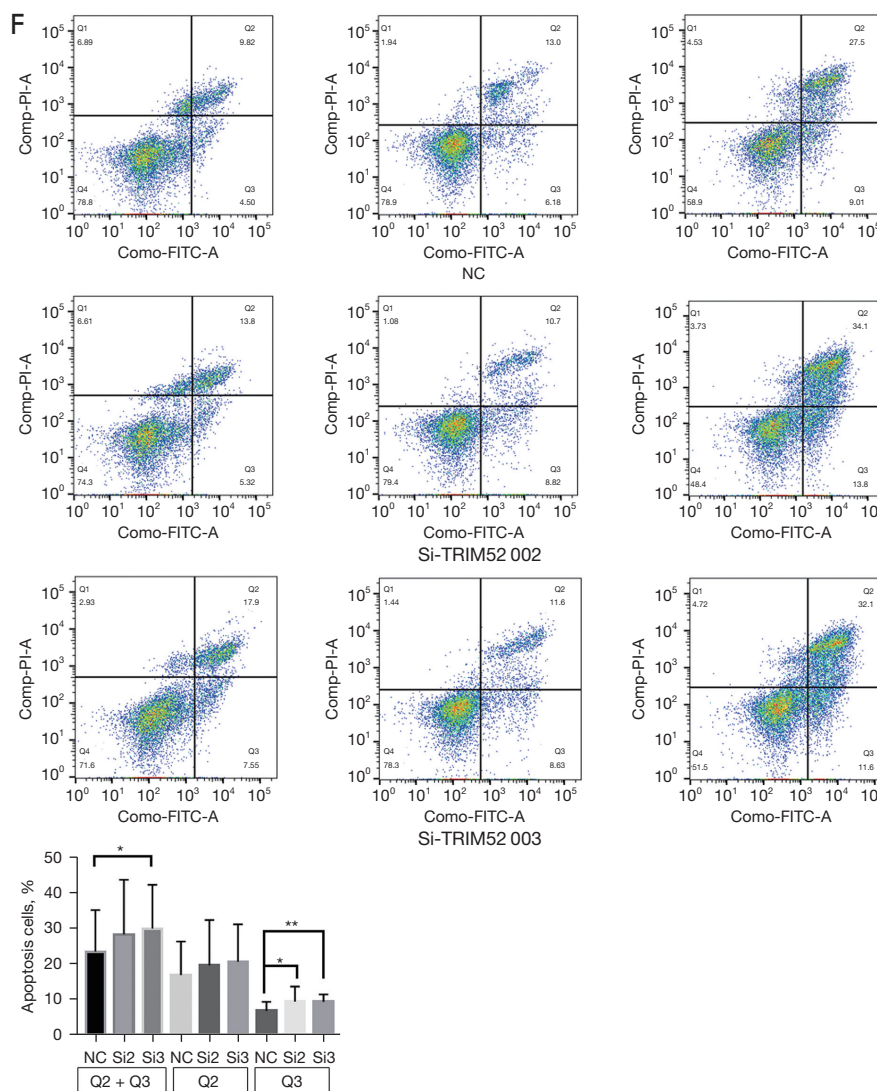
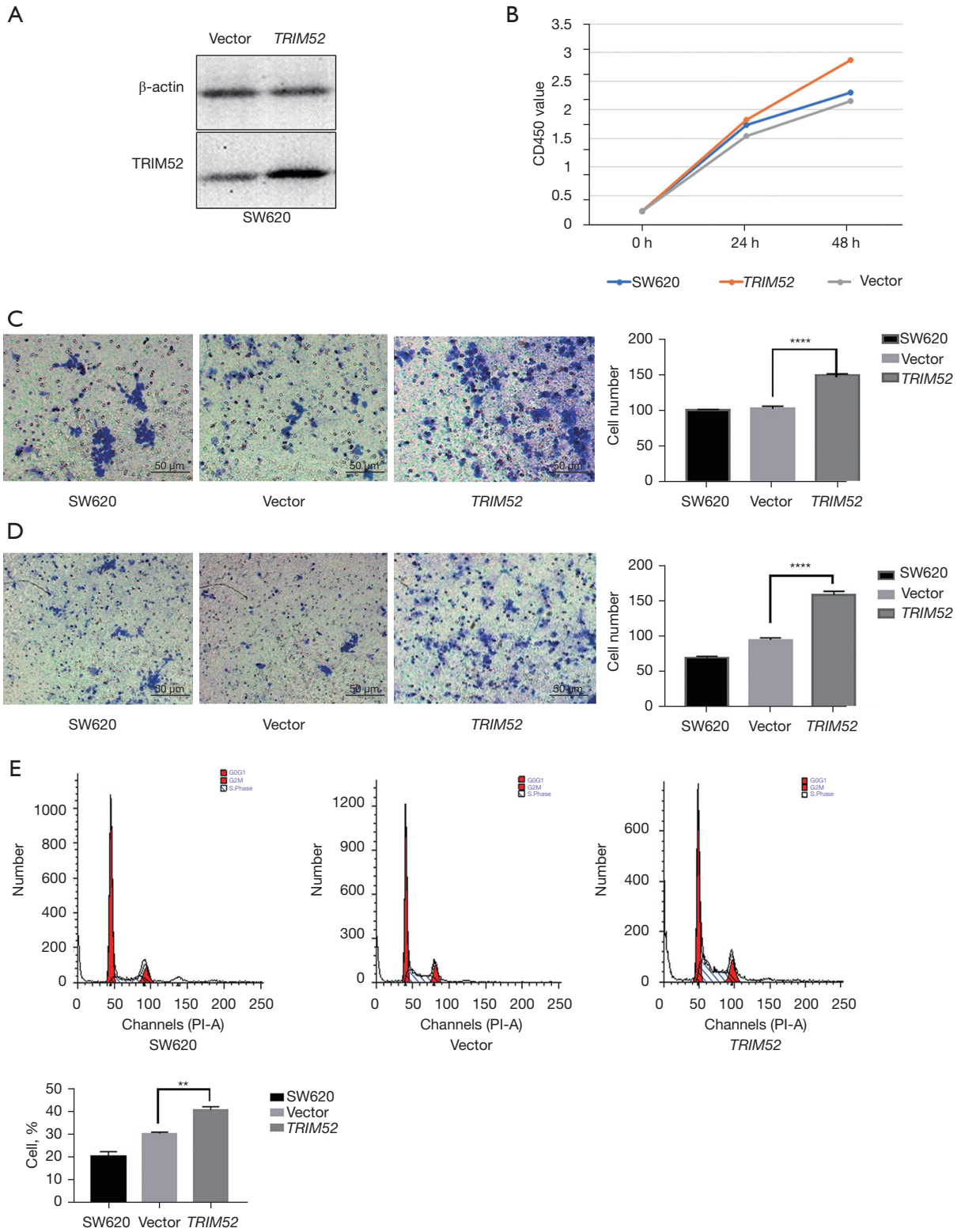


Figure 3 Knockdown of *TRIM52* suppresses cell proliferation, migration, and regulation in colon cancer cells. NC: HCT116 cells transfected with negative control. si-*TRIM52* 002: cell line transfected with *TRIM52* gene off SiRNA002. si-*TRIM52* 003: cell line transfected with *TRIM52* gene off SiRNA003. *, $P < 0.1$; **, $P < 0.01$; ****, $P < 0.0001$. (A) The knockdown effect of Si-*TRIM52* on *TRIM52* in HCT116 started to show at 24 h after transfection, lasting at least 72 h. (B) HCT116 cells were spread 2,000 cells per well in 96-well plates, 6 replicate wells were transfected with siRNA at a concentration of 100 nM, and the absorbance at 450 nm was measured by CCK-8 cell proliferation assay kit, 6 values were averaged, and the curve was plotted against the OD value. (C) HCT116 cell line was transfected with *TRIM52* gene off SiRNA and NC control for 24 hours, after digesting 50,000 cells were added to each Transwell upper chamber, 500 μ L of medium containing 10% FBS was added to the lower chamber, incubated overnight in the incubator, fixed in methanol, stained with 0.1% crystalline violet, and the cells were observed under the microscope and photographed. (D) Diluted Matrigel was added to the bottom of the upper chamber of each Transwell and completely solidified. 50,000 cells were added to the upper chamber and 500 μ L of medium containing 10% FBS was added to the lower chamber, incubated overnight in the incubator, fixed in methanol, stained with 0.1% crystalline violet, and photographed under the microscope. (E) HCT116 cells were transfected by Si-*TRIM52* 003, fixed overnight in 75% cold ethanol, and flow cytometric detection of fluorescence intensity after PI staining. Statistical analysis of the data was performed. (F) HCT116 cells were transfected by Si-*TRIM52* for 24 h. Fluorescence intensity was measured by flow cytometry after collecting supernatant and double staining of all cells with PI/Annexin V-FITC in the pressed wall. Statistical analysis of data was performed. CCK-8, Cell Counting Kit-8; siRNA, small interfering RNA; OD, optical density; FBS, fetal bovine serum; PI, propidium iodide.



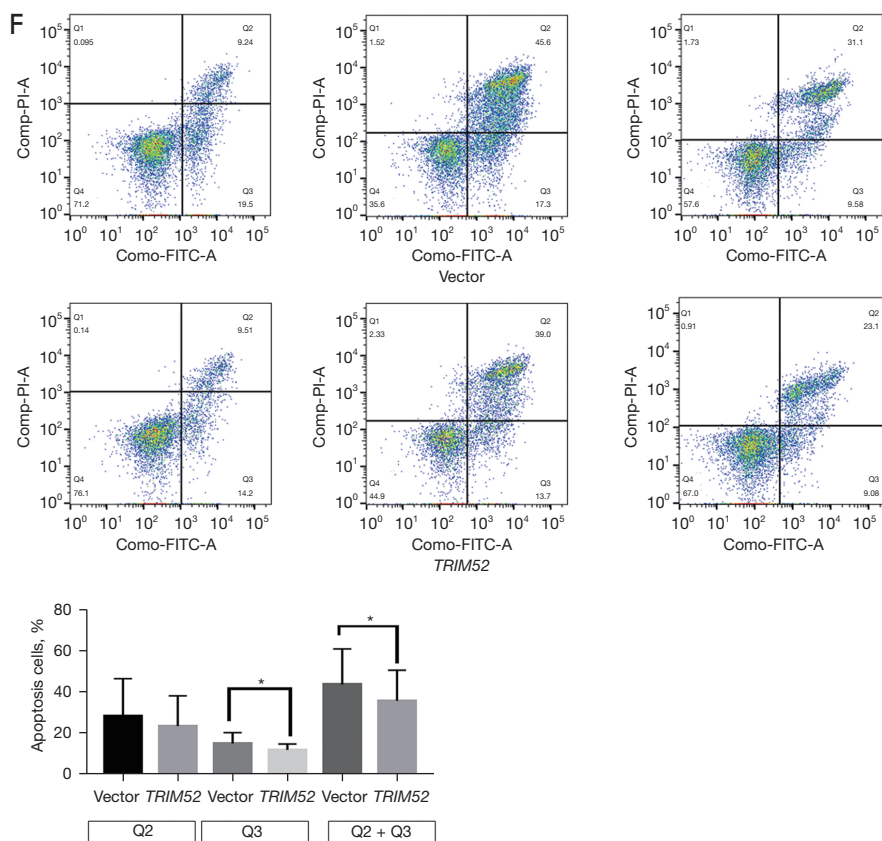


Figure 4 Overexpression of *TRIM52* in SW620 suppresses cell proliferation, migration, and invasion. SW620: untreated SW620 cells. Vector: SW620 cells transfected with pCDH-CMV-MCS-EF1-CopGFP-T2A-puro empty vector as control. *TRIM52*: cell line transfected with *TRIM52*/pCDH-CMV-MCS-EF1-CopGFP-T2A-puro. *, $P < 0.1$; **, $P < 0.01$; ****, $P < 0.0001$. (A) SW620 cells were inoculated in 12-well plates, and after the density reached about 60–70%. SW620 cells were transfected by *TRIM52*/pCDH-CMV-MCS-EF1-CopGFP-T2A-puro and pCDH-CMV-MCS-EF1-CopGFP-T2A-puro control empty vector, and total cell protein was extracted at 48 and 72 h after transfection, and WB assay was performed. *TRIM52*/pCDH-CMV-MCS-EF1-CopGFP-T2A-puro plasmid was more effective for overexpression of *TRIM52* in SW620, and its overexpression effect started to show at 24 h after transfection. (B) SW620 cells were spread 20,000 cells per well in 96-well plates with 6 replicate wells, transfected with *TRIM52*/pCDH-CMV-MCS-EF1-CopGFP-T2A-puro, and the medium was changed after 8 h, and the absorbance at 450 nm was measured at different time points by CCK-8 cell proliferation assay kit, and the 6 values were averaged to plot the curve against the OD value. (C) SW620 cell line was transfected with *TRIM52*/pCDH-CMV-MCS-EF1-CopGFP-T2A-puro and pCDH-CMV-MCS-EF1-CopGFP-T2A-puro empty vector as a control for 24 h. After digestion and counting, cells were resuspended with serum-free culture medium, and each Transwell was added to the upper chamber of 50,000 cells, 500 μ L of medium containing 10% FBS was added to the lower chamber, incubated overnight in the incubator, fixed in methanol, stained with 0.1% crystalline violet, and observed under the microscope for photographs. (D) Diluted Matrigel was added to the bottom of the upper chamber of each Transwell and completely clotted. SW620 cell line was transfected with *TRIM52*/pCDH-CMV-MCS-EF1-CopGFP-T2A-puro and pCDH-CMV-MCS-EF1-CopGFP-T2A-puro empty vector as a control for 24 h. 50,000 cells were added to each Transwell upper chamber and 500 μ L of medium containing 10% FBS was added to the lower chamber, incubated overnight in the incubator, fixed in methanol, stained with 0.1% crystalline violet, and observed under the microscope for photographs. (E) SW620 cells were transfected with *TRIM52*/pCDH-CMV-MCS-EF1-CopGFP-T2A-puro, fixed overnight in 75% cold ethanol, and flow cytometric detection of fluorescence intensity after PI staining. Statistical analysis of data was performed. (F) SW620 cells were transfected with *TRIM52*/pCDH-CMV-MCS-EF1-CopGFP-T2A-puro and control for 24 h, and the fluorescence intensity was detected by flow cytometry after collecting the supernatant and double staining of all cells with PI/Annexin V-FITC in the pressed wall. Statistical analysis of data was performed. CCK-8, Cell Counting Kit-8; WB, Western blot; OD, optical density; FBS, fetal bovine serum; PI, propidium iodide.

TRIM52 is present in CC cells as a factor that promotes NF- κ B activation

By knocking down the expression of *TRIM52* in HCT116, the expression of p-IKK β was decreased, and while the expression of p-IKB was slightly decreased, the expression of P-P65 was decreased significantly both in SiR-*TRIM52* 002 and SiR-*TRIM52* 003 group (the gray value of P-P65: NC = $9.01 \times 10^5 \pm 41.28$, SiR-*TRIM52* 002 = $6.45 \times 10^5 \pm 88.49$, SiR-*TRIM52* 003 = $3.27 \times 10^5 \pm 126.7$, n=3, P<0.0001). On the contrary, overexpression of *TRIM52* protein in SW620 resulted in the slightly elevated expression of IKK β , the increased expression of p-IKK β , the increased expression of p-IKB, and the relatively increased expression of P-P65 (the gray value of P-P65: Vector = $8.86 \times 10^4 \pm 8.65$, *TRIM52* = $3.17 \times 10^5 \pm 95.35$, n=3, P<0.0001) (Figure 5A). Transport of P65 from cytoplasm to nucleus is a characteristic change of the NF- κ B signaling pathway activation. It is generally believed that phosphorylated P65, the active form of P65, is present in the nucleus. The results illustrate that knocking down the expression of *TRIM52* in HCT116 decreased the expression of p-P65 and the NF- κ B signaling pathway was inhibited, while the overexpression of *TRIM52* promoted the NF- κ B signal pathway activation (Figure 5A). The proteins in the cytoplasm and nucleus were further extracted and examined by WB. It was found that knockdown of *TRIM52* expression in HCT116 increased the expression of P65 in the cytoplasm and decreased the expression of P65 in the nucleus (the gray value of nucleus-P65: NC = $2.11 \times 10^5 \pm 89.9$, SiR-*TRIM52* 002 = $9.97 \times 10^4 \pm 68.04$, SiR-*TRIM52* 003 = $1.66 \times 10^5 \pm 314.2$, n=3, P<0.0001). This indicated that the transfer of P65 from the cytoplasm to the nucleus was blocked, and the activation of the NF- κ B signaling pathway was inhibited, while overexpression of *TRIM52* in SW620 resulted in the opposite (the gray value of nucleus-P65: Vector = $2.40 \times 10^5 \pm 89.26$, *TRIM52* = $2.74 \times 10^5 \pm 402.6$, n=3, P<0.0001) (Figure 5B). Therefore, the presence of *TRIM52* worked as a promoter of the NF- κ B signal pathway in CC tumor cells.

TRIM52 is closely related to the NF- κ B signaling pathway and acts as an agonist in the NF- κ B signaling pathway, with its site of action in the upstream pathway of NF- κ B complex activation

It has been reported that LPS binding to Toll-like receptor 4 (TLR4) causes activation of NF- κ B (18). LPS was known as an activator of the NF- κ B signaling pathway

by promoting the phosphorylation of IKB. After LPS treatment with HCT116, p-IKB was found to be reduced and p-IKB ubiquitination hydrolysis was stimulated, which promoted activation of the NF- κ B signaling pathway. The knockdown of *TRIM52* expression in the HCT116 cell line still significantly reduced the expression of p-P65 (the gray value of P-P65: NC = $2.89 \times 10^5 \pm 95.4$, SiR-*TRIM52* = $1.69 \times 10^5 \pm 51.49$, n=3, P<0.0001) and slightly reduced the expression of p-IKB (the gray value of p-IKB: LPS+NC = $4.40 \times 10^5 \pm 87.55$, LPS+SiR-*TRIM52* = $2.79 \times 10^5 \pm 89.27$, n=3, P<0.0001), indicating that *TRIM52* still had an inhibitory effect on the NF- κ B signaling pathway after LPS activation, and the site where *TRIM52* inhibited the NF- κ B signaling pathway acted upstream of the phosphorylation of IKB. In contrast, after inhibition of NF- κ B complex activity by PDTC, overexpression of *TRIM52* in the SW620 cell line had little effect on the rise of p-P65 expression, and p-P65 was significantly reduced and almost none was expressed (Figure 6A,6B).

Discussion

The *TRIM52* protein belongs to the C-V isoform of the TRIM protein family, with 1 RING structure at a time from the N-terminal to the C-terminal, a B-Box2 structural domain, and lacking a distinct double helix structural domain and C-terminus. It has been shown as a promoter in ovarian cancer (12), hepatocellular carcinoma (13,15), and renal cancer (14), while its role in the development of CC had not been clarified

Our study demonstrated that *TRIM52* is significantly highly expressed in CC cell lines. Cell proliferation and cell cycle showed a statistically significant decrease of approximately 20.83% in the proportion of S-phase cells in HCT116 cells following knockdown of *TRIM52*. In contrast, when *TRIM52* was overexpressed in SW620, its G2M phase cells increased by about 29.03%. This result fully illustrated that *TRIM52* plays a role in the cell cycle to promote cellular DNA replication, and most cells start DNA replication and enter the cell division process after DNA formation of 4-ploidy. The cell cycle results showed that there was a slight increase in the ratio of cells in the G2M phase (the second peak), with no significant statistical difference, which may be related to factors such as the inactivation of key proteins associated with cell division.

In addition, *TRIM52* promoted the proliferation of CC cells and accelerated the cell cycle process, which may be related to the promotion of DNA replication in CC cells.

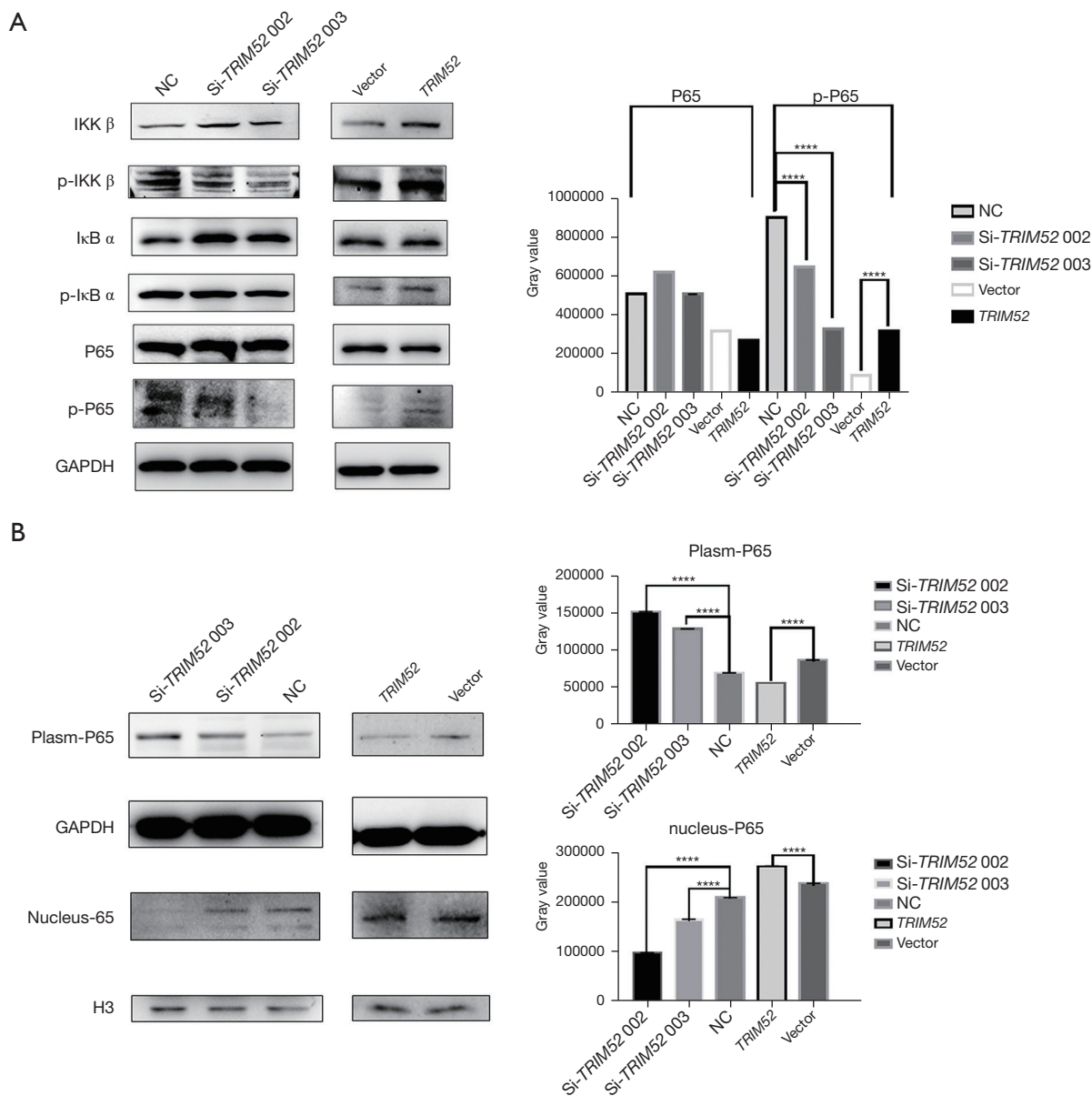


Figure 5 *TRIM52* is present as a factor that promotes NF-κB activation. NC: HCT116 cells transfected with negative control. siR-*TRIM52* 002: cell line transfected with *TRIM52* gene off SiRNA 002. siR-*TRIM52* 003: cell line transfected with *TRIM52* gene off SiRNA 003. Vector: SW620 cells transfected with pCDH-CMV-MCS-EF1-*TRIM52*: Cell line transfected with *TRIM52*/pCDH-CMV-MCS-EF1-CopGFP-T2A-puro as control. ****, P<0.0001. (A) HCT116 cells were transfected with SiR-*TRIM52* 002, SiR-*TRIM52* 003, NC control, and SW620 after transfection with empty vector and *TRIM52* overexpression plasmid. Total cellular proteins were extracted to verify NF-κB-related signaling pathway proteins by WB. (B) After HCT116 cells were transfected with SiR-*TRIM52* 002, SiR-*TRIM52* 003, NC control, SW620 transfected with empty vector and *TRIM52* overexpression plasmid, proteins were extracted from cytoplasm and nucleus, respectively, and changes in NF-κB P65 were verified by WB. WB, Western blot.

In terms of cell migration and invasion, knock-down of *TRIM52* expression in the HCT116 cells significantly reduced cell migration and invasion, while overexpression of

TRIM52 protein in SW620 enhanced its migration ability. The above results suggested that *TRIM52* plays a role in promoting cell migration and invasion in HCT116 and

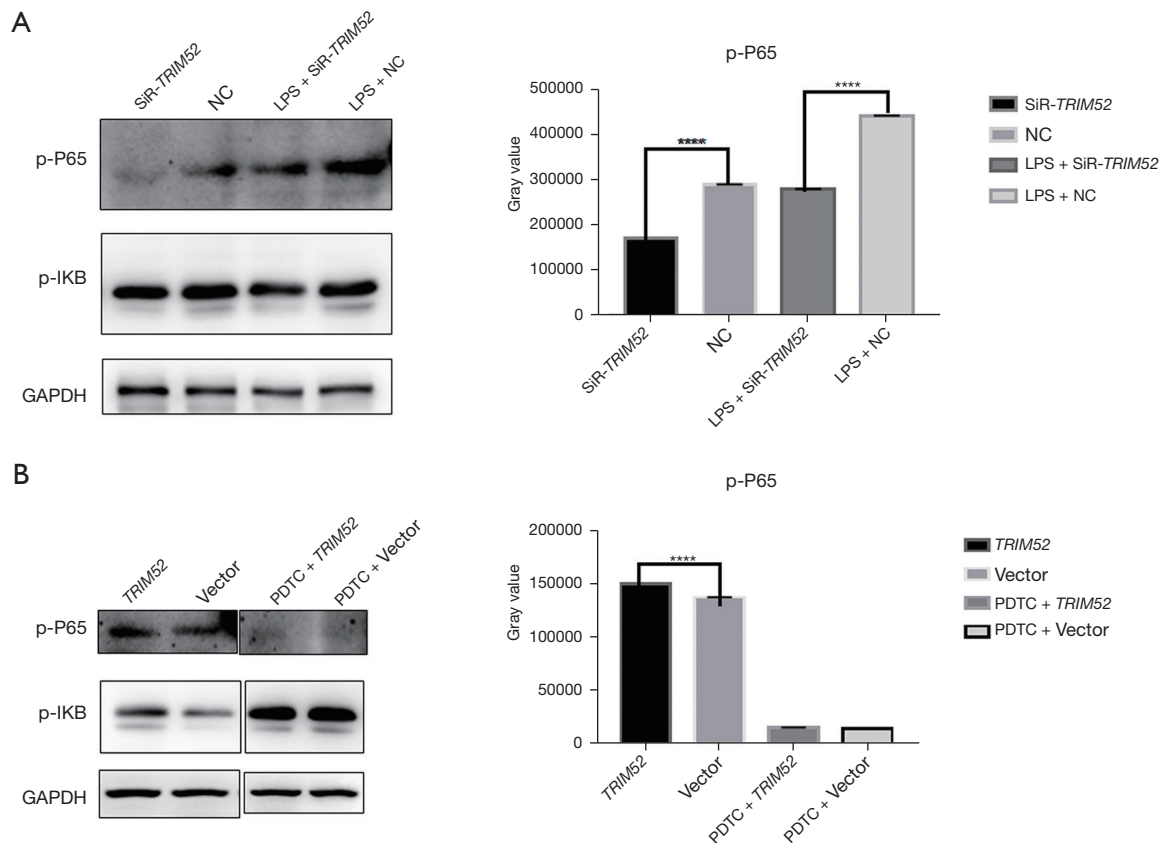


Figure 6 *TRIM52* acts as an agonist in the NF- κ B signaling pathway, with its site of action in the upstream pathway of NF- κ B complex activation. NC: HCT116 cells transfected with negative control. siR-*TRIM52* 002: cell line transfected with *TRIM52* gene off SiRNA 002. siR-*TRIM52* 003: cell line transfected with *TRIM52* gene off SiRNA 003. vector: SW620 cells transfected with pCDH-CMV-MCS-EF1-*TRIM52*: Cell line transfected with *TRIM52*/pCDH-CMV-MCS-EF1-CopGFP-T2A-puro as control. ****, $P < 0.0001$. (A) LPS was cotreated with SiR-*TRIM52* or NC control in HCT116 cells, and total cell protein was extracted after 48 h for WB assay to verify the intracellular expression changes of p-P65 and p-IKB. (B) PDTC was co-treated with *TRIM52* overexpression plasmid or empty vector control in SW620 cells, and total cell protein was extracted for WB assay after 48 h to verify the changes of p-P65 and p-IKB expression in the cells. PDTC, pyrrolidine dithiocarbamate; LPS, lipopolysaccharide; WB, Western blot.

SW620 CC cells, which is closely related to the metastasis of tumors.

The results of apoptosis showed that expression of *TRIM52* significantly changed (about 20–30%) in the proportion of apoptotic cells in CC cell lines HCT116 and SW620 in the middle and late stages, and exerted an inhibitory role on the cells. It was also confirmed that the presence of *TRIM52* served as a promoting factor of tumor development in CC cell lines HCT116 and SW620. It was shown that *TRIM52* promoted cell proliferation by facilitating the DNA replication process in CC cells and promoting cell progression from pre-division to the division phase. Apoptosis experiments demonstrated that *TRIM52*

acts as an inhibitor of apoptosis in CC cells.

It is widely accepted that activation of the NF- κ B signaling pathway promotes tumor cell proliferation, metastasis, and inhibits apoptosis of tumor cells (19–22). Phosphorylation and ubiquitination of IKB are important events in the activation of the NF- κ B signaling pathway. TRIM family is closely associated with the activation of the NF- κ B signaling pathway due to its conserved RBCC structure, especially the RING structure, which gives it E3 ubiquitin ligase activity (23–25). The regulation of the TRIMs for the NF- κ B signaling pathway is diverse, and some members of the triple structural domain protein family are involved in the activation of the NF- κ B signaling

pathway, for example, TRIM1 is involved in the tumor necrosis factor (TNF) α /LPS-induced activation of the NF- κ B signaling pathway (26), *TRIM52* acts as a positive regulator to regulate the NF- κ B signaling pathway (27), and TRIM22 also has an activating effect on the NF- κ B signaling pathway (28). It has been reported that TRIM22/activates NF- κ B signaling in glioblastoma by accelerating the degradation of I κ B α (29); TRIM47 activates NF- κ B signaling via PKC- ϵ /PKD3 stabilization and contributes to endocrine therapy resistance in breast cancer (30), and TRIM10 activates the NF- κ B signaling pathway in osteosarcoma (31). In contrast, some members of the triple structural domain protein family down-regulate the NF- κ B signaling pathway in some tumors, namely, TRIM39 inhibits the NF- κ B signaling pathway by stabilizing actin expression (32), and TRIM45 inhibits tumor cell proliferation by suppressing the NF- κ B signaling pathway (33). The highly conserved RBCC structure of the TRIM protein family, an E3 ubiquitin ligase, characterizes the relationship between *TRIM52* and the NF- κ B signaling pathway in tumor cells. After knocking down the TRIM52 protein expression in HCT116 tumor cells, p-IKK β expression was reduced and p-IKB expression was slightly decreased. Also, p-P65 and intracellular P65 were found to be reduced. The result demonstrated the inhibition of the NF- κ B signaling pathway from qualitative and localization perspectives. In contrast, the expression of p-IKK β and p-IKB increased in the SW620 cells overexpressing *TRIM52*. The expression of P-P65 and intranuclear P65 increased, which indicated that the NF- κ B signaling pathway was activated after overexpression of TRIM52 protein in tumor cells, and it was inferred that *TRIM52* was closely related to the activation of the NF- κ B signaling pathway. Further experiments revealed that the inhibitory effect of knocking down *TRIM52* on the NF- κ B signaling pathway in tumor cells persisted after activation of the NF- κ B signaling pathway by LPS. As an upstream activator of NF- κ B, LPS was seen to be an upstream promoter of *TRIM52* for the NF- κ B signaling pathway. Meanwhile, after PDTC inhibited the NF- κ B complex activity, the activation effect of *TRIM52* for the NF- κ B signaling pathway in the SW620 cell line was not obvious. It can also be inferred that *TRIM52*, as an upstream stimulator of the NF- κ B signaling pathway, lost its stimulating effect via blockade from the downstream PDTC. It was suggested that *TRIM52* is closely related to the NF- κ B signaling pathway in CC tumor cells and acts as an upstream stimulator of the NF- κ B signaling pathway to stimulate the transfer of P65 into the nucleus to activate the

NF- κ B signaling pathway.

In vivo experiments can be employed in the future to further explore the role of *TRIM52* in colon carcinogenesis. In addition, the specific binding sites of *TRIM52* require verification by further experiments.

Conclusions

Our study demonstrated that *TRIM52* was significantly overexpressed, promoting proliferation, migration, and invasion while inhibiting apoptosis in CC cells. Our study also revealed that the regulation effect of *TRIM52* on CC cells is closely related to the NF- κ B signaling pathway, and *TRIM52* serves as an upstream factor stimulating the transfer of P65 into the nucleus to activate the NF- κ B signaling pathway, which provides a potential target for prognosis prediction or treatment of CC.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at <https://jgo.amegroups.com/article/view/10.21037/jgo-22-317/rc>

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <https://jgo.amegroups.com/article/view/10.21037/jgo-22-317/coif>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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