

## Original Article

## Terrestrosin D promotes autophagy and apoptosis of breast cancer cells through PSMD1-dependent activation of P53 pathway

Li-Ling Jia <sup>a</sup>\*, Cheng-Jie Wu <sup>b</sup>, Pei-Wen Ye <sup>b</sup>, Qian Zhang <sup>b</sup>, Hua Liu <sup>a</sup>, Tu-Ping Li <sup>a</sup>, Xiao-Lei Hu <sup>b,\*</sup>

<sup>a</sup> Department of Anaesthesia, Second Hospital of Shanxi Medical University, Taiyuan, Shanxi, 030001, PR China

<sup>b</sup> Breast Center, Department of General Surgery, Southern Medical University Nanfang Hospital, Guangzhou, Guangdong, 510515, PR China

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## ABSTRACT

**Background and purpose:** Breast cancer, particularly triple-negative breast cancer (TNBC), poses a significant threat to women's health. In tumor cells, autophagy and apoptosis are double-edged swords, playing complex roles in cancer progression and treatment. This study aimed to investigate whether Terrestrosin D (TED) exerts antitumor effects on TNBC by modulating autophagy and apoptosis, and to elucidate the underlying molecular mechanisms.

**Methods:** The antiproliferative and pro-apoptotic effects of TED on TNBC cells were assessed using CCK-8, EdU assay, Live/Dead staining, and flow cytometry. Autophagy was monitored through immunofluorescence and confocal microscopy. RNA sequencing was performed to identify the pathways and molecular targets involved. The anti-TNBC effects of TED were further evaluated in vivo using tumor xenograft models. Western blotting was conducted to validate the relationship between PSMD1, P53, and TED-induced antitumor activity.

**Results:** TED exhibited significant antitumor effects both in vitro and in vivo. Cellular phenotypic analyses revealed that TED promoted autophagy and apoptosis. Transcriptomic analyses indicated that TED stabilizes P53 expression and activates the P53 signaling pathway by inhibiting the function of PSMD1.

**Conclusion:** TED exhibits potent antitumor effects on TNBC by promoting autophagy and apoptosis. It achieves this through PSMD1 inhibition, stabilizing P53 expression, and activating the P53 pathway. Notably, this study is the first to demonstrate that TED directly targets PSMD1, a key proteasomal regulator, thereby unveiling a novel mechanism for P53 stabilization in TNBC. These findings provide new insights into the therapeutic modulation of the PSMD1 - P53 axis by natural compounds and support the development of TED as a multi-functional agent for aggressive breast cancers.

## Introduction

Breast cancer, the most prevalent malignancy among women, accounts for approximately 32 % of all cancer cases (Sung et al., 2021). Triple-negative breast cancer (TNBC) represents a particularly aggressive subtype characterized by a lack of effective treatment options and clear prognostic markers (Bianchini et al., 2016). Due to its non-responsiveness to current targeted therapies, TNBC is associated with poor clinical outcomes (Garrido-Castro et al., 2019). Identifying

new therapeutic agents for TNBC is therefore of significant clinical importance.

Terrestrosin D (TED) is a major spirostanol saponin isolated from *Fructus Tribuli*, a traditional medicine with a history spanning thousands of years (Sun et al., 2022). Previous studies have demonstrated TED's therapeutic potential in alleviating inflammatory damage (Qiu et al., 2019; Guo et al., 2022), lung fibrosis (Yang et al., 2022), and its ability to inhibit tumor growth and angiogenesis in prostate cancer (Wei et al., 2014). However, its effects on TNBC remain unexplored. This

**Abbreviations:** 3-MA, 3-methyladenine; AMPK, adenosine 5'-monophosphate-activated protein kinase; Baf-A1, bafilomycin A1; BAX, BCL-2-associated X protein; BCL2, B-cell lymphoma-2; CETSA, cellular thermal shift assay; CHX, cycloheximide; CQ, chloroquine; DARTS, drug affinity responsive target stability; FER-1, ferrostatin-1; MDM2, murine double minute2; mTOR, mammalian target of rapamycin; PTX, paclitaxel; TED, terrestrosin D; TTM, tetrathiomolybdate; TNBC, triple-negative breast cancer; UPS, ubiquitin-proteasome system.

\* Corresponding author.

E-mail address: [xlhu@smu.edu.cn](mailto:xlhu@smu.edu.cn) (X.-L. Hu).

study aims to investigate the antitumor activity of TED in TNBC and elucidate its underlying mechanisms.

Apoptosis, a tightly regulated programmed cell death process, is critical for eliminating malignant or hyperplastic cells and controlling tumor progression (Elmore, 2007). Autophagy, a strictly regulated catabolic process, also plays a dual role in cancer, functioning as both a tumor suppressor and promoter depending on the context (Levy et al., 2017). Autophagy and apoptosis are intricately connected and often share upstream regulatory signals during cancer cell death (Mariño et al., 2014). While TED has been shown to induce both autophagy and apoptosis in various cancers, the precise mechanisms underlying these effects, particularly in TNBC, remain undefined.

The tumor suppressor P53 plays a pivotal role in regulating apoptosis and autophagy (Wang et al., 2016; Aubrey et al., 2018). Normally expressed at low levels, P53 levels rise in response to DNA damage, promoting apoptosis by upregulating pro-apoptotic factors such as BAX and downregulating anti-apoptotic factors like BCL2 (Wu and Deng, 2002; Zhang et al., 2021; Wang, 2023). P53 also regulates autophagy through both transcription-dependent and transcription-independent pathways. In the transcription-independent pathway, P53 activates AMPK, which in turn inhibits mTOR to induce autophagy (Thoreen and Sabatini, 2005; Ge et al., 2022). In the transcription-dependent pathway, P53 promotes autophagy by upregulating genes such as PTEN, TSC1, or DRAM, thereby negatively regulating mTOR activity (Lorin et al., 2010; Chen et al., 2020; Kato et al., 2021; Luo et al., 2021).

The ubiquitin–proteasome system (UPS) is a critical proteolytic mechanism that maintains protein homeostasis by degrading ubiquitinated proteins. The 26S proteasome complex, a key component of the UPS, consists of the 20S core and 19S regulatory particles. Among its subunits, the non-ATPase 1 (PSMD1) protein is a vital component of the 19S regulatory particle and is closely associated with cancer progression and aggressiveness. PSMD1 mediates the degradation of key proteins, including NOXA and P53, thereby regulating cancer cell proliferation and apoptosis.

This study demonstrates that TED exerts significant antitumor effects on TNBC both *in vitro* and *in vivo*. Mechanistically, TED inhibits PSMD1, stabilizing P53 expression and activating the P53 signaling pathway. These findings suggest that TED is a promising therapeutic agent for TNBC and provide valuable insights into the molecular mechanisms underlying its antitumor activity.

## Materials and methods

### Reagents

The Anti-Cancer Active Compound Library and Terrestroisin D (TED) were purchased from TargetMol (Boston, USA). Additional reagents, including Z-VAD-FMK (FMK), Ferrostatin-1 (FER-1), Baflomycin A1 (Baf-A1), Tetrathiomolybdate (TTM), 3-Methyladenine (3-MA), Chloroquine (CQ), Cycloheximide (CHX), and MG132, were obtained from Selleck Chemicals (Houston, USA). Antibodies used in this study include Cleaved PARP (9541T, 1:1000, CST), Cleaved Caspase-3 (25,128–1-AP, 1:1000, Proteintech), Bax (A19684, 1:1000, ABclonal), Bcl-2 (A19693, 1:1000, ABclonal), GAPDH (60,004–1-Ig, 1:10,000, Proteintech), Beclin-1 (11,306–1-AP, 1:1000, Proteintech), P62 (18,420–1-AP, 1:5000, Proteintech), LC3 (14,600–1-AP, 1:1000, Proteintech), P53 (10,442–1-AP, 1:5000, Proteintech), MDM2 (66,511–1-Ig, 1:1000, Proteintech), PSMD1 (A16420, 1:1000, ABclonal), HA (51,064–2-AP, 1:5000, Proteintech), SNAI1 (13,099–1-AP, 1:1000, Proteintech), N-cadherin (22,018–1-AP, 1:2000, Proteintech), Vimentin (A11952, 1:1000, ABclonal), E-cadherin (20,874–1-AP, 1:5000, Proteintech), and Claudin-1 (28,674–1-AP, 1:1000, Proteintech).

### Cell culture and treatment

Cell lines used in this study were obtained from the National

Collection of Authenticated Cell Cultures (Shanghai, China). These included the human mammary epithelial cell line MCF10A, breast cancer cell lines MCF7, T47D, BT474, SKBR3, MDA-MB-468, BT-549, and the human embryonic kidney epithelial cell line 293FT. MCF10A was cultured in cell-specific medium (Pricella Biotechnology, Wuhan, China), while other cell lines were maintained in DMEM supplemented with 10 % fetal bovine serum (FBS, ExCell Bio, Shanghai) and 1 % penicillin-streptomycin (Pricella Biotechnology, Wuhan, China). Cells were cultured at 37 °C in a humidified atmosphere of 5 % CO<sub>2</sub>, confirmed to be free of mycoplasma contamination, and passaged no >30 times post-thawing.

### Cell apoptosis assay

Apoptosis was assessed using the Annexin V-FITC/PI Apoptosis Detection Kit (Beyotime, Shanghai, China). Cells were incubated with the Annexin V-FITC/PI working solution for 15 min at room temperature in the dark. Analysis was performed using a FACScan flow cytometer (BD Biosciences, San Jose, CA, USA).

### EdU assay

The EdU-594 Kit (Beyotime, Shanghai, China) was used to assess cell proliferation. Cells were treated with drugs and incubated with the EdU working solution for 2 h, followed by fixation, permeabilization, and addition of the EdU reaction solution. Proliferating cells were visualized by Alexa Fluor 594 under a fluorescence microscope, and nuclei were counterstained with DAPI.

### Live/dead staining assay

The cytotoxic effects of TED were analyzed using the Calcein/PI Cell Viability/Cytotoxicity Assay Kit (Beyotime, Shanghai, China). Treated cells were incubated with calcein AM/PI staining solution for 30 min at 37 °C, protected from light. Live cells displayed green fluorescence (calcein AM), while dead cells exhibited red fluorescence (PI). Images were captured using a fluorescence microscope, and dead cell percentages were calculated.

### Cell viability assay

Cells were seeded in 96-well plates, treated with TED for specified durations, and incubated with CCK8 reagent (APExBIO, Houston, USA) for 2 h at 37 °C. Absorbance was measured at 450 nm using a microplate reader.

### GFP-LC3/mRFP-GFP-LC3 puncta assay

MDA-MB-231 and MDA-MB-468 cells were infected with GFP-LC3 or mRFP-GFP-LC3 lentivirus to establish stable cell lines. Following drug treatment, autophagosome formation was assessed using fluorescence microscopy. Double-positive (yellow) puncta indicated autophagosome formation, while red puncta marked autophagosome maturation.

### Confocal co-localization of LC3 and lysosomal markers

Cells were seeded on glass coverslips and treated with TED or DMSO for 24 h. After treatment, cells were fixed with 4 % paraformaldehyde for 20 min at room temperature, followed by permeabilization with 0.1 % Triton X-100 for 10 min. Cells were then blocked with 5 % bovine serum albumin (BSA) and incubated overnight at 4 °C with primary antibodies against LC3 and LAMP1. After washing, cells were incubated with Alexa Fluor 488-conjugated secondary antibody for LC3 (green) and Alexa Fluor 594-conjugated secondary antibody for LAMP1 (red) for 1 h at room temperature. Nuclei were counterstained with DAPI. Fluorescence images were acquired using a confocal laser scanning

microscope.

#### Drug affinity responsive target stability (DARTS)

Cell lysates treated with or without TED were incubated for 30 min at room temperature, followed by digestion with proteinase K. Digestion was halted with PMSF, and candidate protein expression was assessed via Western blotting.

#### Cellular thermal shift assay (CETSA)

Cell lysates were treated with TED or DMSO, incubated for 1 h, and divided into aliquots heated at 50–66 °C. Supernatants were collected and analyzed via Western blotting.

#### Co-IP assay

Cell lysates were incubated with antibodies overnight at 4 °C. Protein A/G beads were added, incubated for 3 h, and washed. The samples were analyzed via Western blotting.

#### Transwell assays

50,000 cells were seeded into the upper chamber of Transwell inserts with serum-free medium and TED. DMEM with 15 % FBS was added to the lower chamber. After 24 h, cells were fixed, stained with crystal violet, and analyzed microscopically.

#### Quantitative real-time PCR

Total RNA was extracted from MDA-MB-231 cells using TRIzol reagent following the manufacturer's instructions. cDNA was synthesized using a reverse transcription kit. Quantitative real-time PCR (qPCR) was performed using SYBR Green Master Mix on a StepOnePlus™ Real-Time PCR System (Applied Biosystems). GAPDH was used as an internal control. Relative gene expression levels were calculated using the 2<sup>-ΔΔCt</sup> method. The primers used in this study are listed below: TMEFF1 forward, 5'- TTGTTGGAAAGAAAGATGATGGA -3' and reverse, 5'- GATGCAGTAACCATTGAGGTTT -3'; PAK6 forward, 5'- GACTC-CATCCTGCTGACCCCTC -3' and reverse, 5'- CACCTCAGTGGCATA-CAAAGACC -3'; GAPDH forward, 5'-CATGAGAAGTATGACAACAGCCT-3' and reverse 5'-AGTCCTCCACGATAACAAAGT-3'; HSPA6 forward, 5'- CAAGGTGCCCGTATGCTAC -3' and reverse 5'- GCTCATTGAT-GATCCGCAACAC -3'; DRD5 forward, 5'- CTATTTCAGACCCCTCCGCT -3' and reverse 5'- CTGCCCTGTCTGTGCCAAT -3'; CASP8 forward, 5'- GAAGATAATCAACGACTATG -3' and reverse 5'- TTCAC-TATCCTGTTCTCT -3'; IGFBP3 forward, 5'- TGTGGCCATGACTGAG-GAAA -3' and reverse 5'- TGCCGACCTCTGGGTTT -3';

#### Western blotting

Proteins were extracted with RIPA lysis buffer containing protease inhibitors and quantified using a BCA kit. Samples were subjected to SDS-PAGE, transferred to PVDF membranes, blocked, and incubated with primary and secondary antibodies. Protein bands were detected using ECL reagents and a chemiluminescence system.

#### Tumor xenograft assay

Orthotopic TNBC models were established by injecting  $2 \times 10^6$  MDA-MB-231 cells into the mammary fat pads of BALB/c nude mice. After tumor formation, mice were treated with TED bi-daily for three weeks. Tumor tissues and major organs were collected for analysis. Tumor volume was calculated as  $V = (\text{length} \times \text{width}^2) / 2$ . All animal experiments were approved by the Institutional Animal Care and Use Committee of Nanfang hospital, under the ethical approval number IACUC-

LAC-20,250,307-006, and conducted in accordance with the ARRIVE guidelines and institutional regulations for the care and use of laboratory animals.

#### Statistical analysis

Data are presented as mean  $\pm$  SD. Statistical analysis was performed using GraphPad Prism 8.0. Statistical significance was denoted as \* ( $0.01 < p < 0.05$ ), \*\* ( $0.001 < p < 0.01$ ), and \*\*\* ( $p < 0.001$ ).

## Results

#### TED dose-dependently induced TNBC cell death

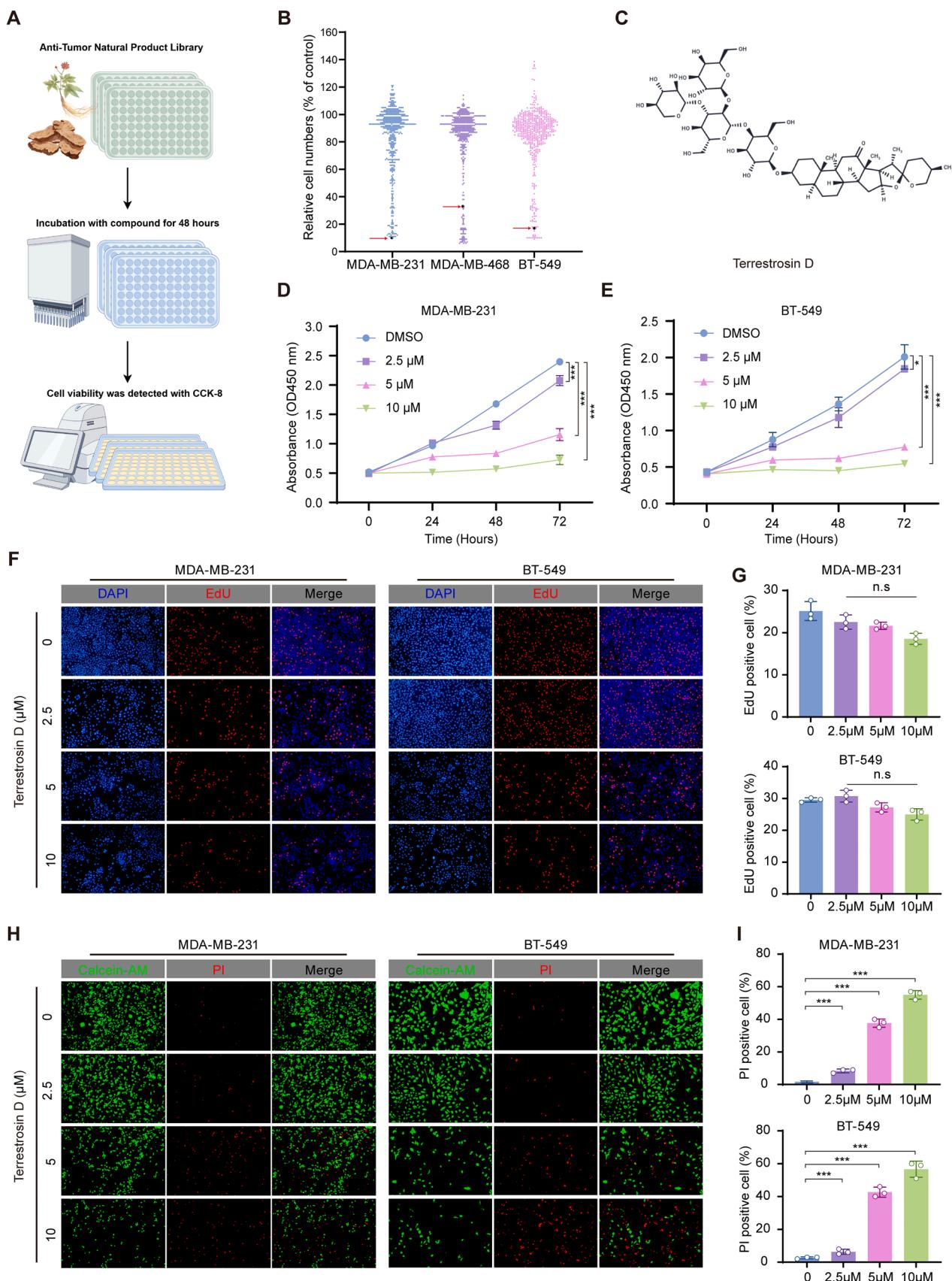
Based on a Natural Product Library consisting of 500 natural products derived from plants, animals, or microorganisms with known or potential antitumor activity, a method for screening compounds was applied to identify compounds with anticancer properties in TNBC (Fig. 1A). First, the inhibitory efficacy of 500 compounds against TNBC cell lines was assessed at a concentration of 10 μM. 46 compounds with inventory rates  $>50\%$  were selected (Fig. 1B and Supplementary Table 1). Subsequently, we conducted a comprehensive literature search of these 46 compounds and discovered that there is currently no existing literature regarding the anti-tumor efficacy of TED in breast cancer. Therefore, TED became our research focus (Fig. 1C). To guide the experimental design, we first determined the IC<sub>50</sub> values of TED in multiple cell lines. MDA-MB-231 and BT-549 cells exhibited the highest sensitivity (IC<sub>50</sub>  $\approx$  5 μM), while MCF-10A cells showed relatively low sensitivity (Supplementary Fig. 1 A-D). Therefore, a concentration gradient of 2.5–10 μM was employed in the CCK-8 assay and subsequent in vitro experiments to ensure coverage of the biologically relevant range. The CCK8 experiments demonstrated a dose-dependent reduction in cell viability following TED treatment (Fig. 1D, E). Subsequently, we conducted an EdU proliferation assay in conjunction with a live or dead staining assay in cell treated with TED. Interestingly, compared with the DMSO group, TED did not exert a significant inhibitory effect on cell proliferation (Fig. 1F, G), conversely, it exhibited a notable ability to induce cell death in TNBC cells (Fig. 1H, I). The findings imply that TED has the potential to impede the progression of breast cancer tumors through its facilitation of cell death.

#### TED promotes apoptosis and autophagy in TNBC

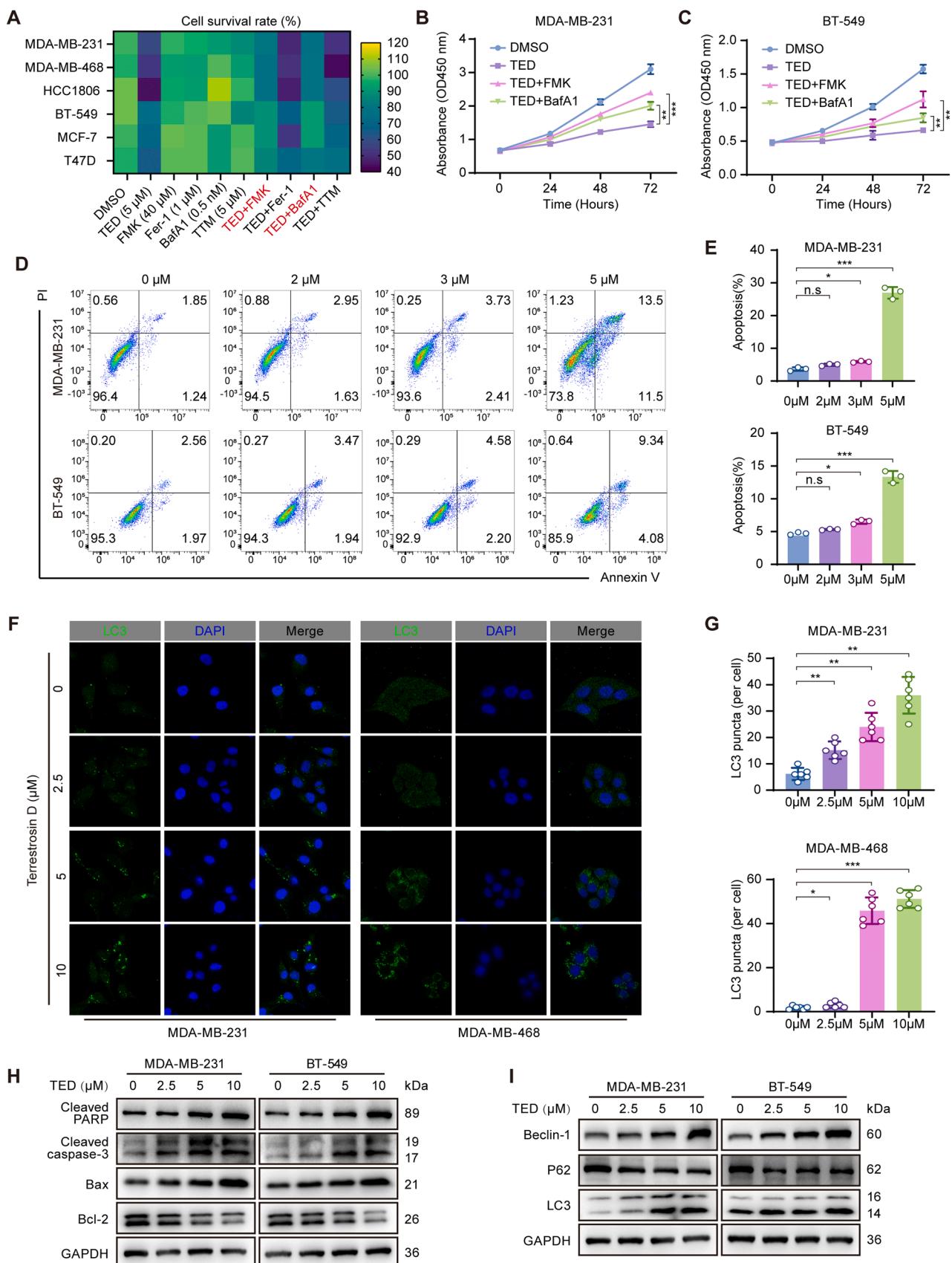
To revealing the mechanisms by which TED facilitates TNBC cell death, we used a panel of cell death inhibitors to rescue the cell viability under TED treatment including copper chelator (tetraethylomolybdate, TTM), iron chelator (ferrostatin-1, Fer-1), autophagy inhibitor (baflomycin A1, Baf-A1), and apoptosis inhibitor (Z-VAD-FMK, ZMK). Our results indicated that the autophagy inhibitor and apoptosis inhibitor partially restored TED induced cell death, while the copper and iron chelators had no significant effect (Fig. 2A, B, C).

To confirm the effect of TED on TNBC cell apoptosis, flow cytometry was employed to assess the apoptotic rate in TNBC cells, as illustrated in Fig. 2D and E. The results demonstrated that TED treatment significantly increased apoptosis in TNBC cells. Furthermore, results of the Western blot demonstrated that the TED treatment led to an increase in the expression of the pro-apoptotic protein Bax, cleaved PARP and cleaved caspase-3, while there was a reduction in the expression of the anti-apoptotic protein Bcl-2. (Fig. 2H).

A substantial body of evidence indicates that the activation of cellular autophagy is associated with the promotion of apoptosis. To observe the effect of TED on autophagy, we transfected the GFP-LC3 plasmid into TNBC cells to construct the GFP-LC3 single fluorescence autophagy indicator system. The results obtained from confocal indicated a notable increase in autophagosomes (green puncta) following TED treatment and it was concentration dependent (Fig. 2F, G).



**Fig. 1. Screening of natural compounds identifies Terrestrosin D (TED) as an anticancer agent for TNBC.** (A) Overview of the natural product library screening process. (B) Initial screening results showing compounds with inhibitory rates above 50 %. (C) Chemical structure of Terrestrosin D. (D, E) Dose-dependent inhibition of TNBC cell viability by TED as assessed by CCK8 assays. (F, G) EdU staining and quantification of proliferation following TED treatment. (H, I) PI staining for cell Live/Dead evaluation. Bars, SDs; \* 0.01 < p < 0.05, \*\* 0.001 < p < 0.01, and \*\*\* p < 0.001.



**Fig. 2. TED induces apoptosis and autophagy in TNBC cells.** (A-C) Inhibitor assays showing partial rescue of cell viability using apoptosis and autophagy inhibitors. (D, E) Flow cytometry analysis demonstrates increased apoptotic cell populations following TED treatment. (F, G) Confocal microscopy of GFP-LC3 puncta indicating autophagosome accumulation. (H, I) Western blot analysis of autophagy- and apoptosis-related markers in TED-treated cells. Bars, SDs; \* 0.01 <  $p$  < 0.05, \*\* 0.001 <  $p$  < 0.01, and \*\*\*  $p$  < 0.001.

Consistently, the above results were confirmed by the increased LC3 and Beclin1 expression and decreased P62 expression in the TED-treated TNBC cells (Fig. 2I).

To determine that the accumulation of autophagosomes as well as the increase in LC3 was due to TED promoting autophagic flow rather than the blockage of the autolysosomal degradation pathway. A dual fluorescence-labeled indicator system for LC3 was constructed (Fig. 3A). The early autophagy inhibitor 3-MA (3-Methyladenine) (blocking the activation of early autophagy) and the late autophagy inhibitor CQ (Chloroquine) (inhibiting the fusion of autophagosome and lysosome) were added to TED at the same time. The accumulation of early autophagosomes (yellow puncta, GFP+ and RFP+) as well as late autophagosomes (red puncta, GFP– and RFP+) was observed by fluorescence microscopy (Fig. 3B). The results showed that the initiation of autophagy could be greatly inhibited by 1 mM 3-MA, and autophagy activated by TED was inhibited. Compared with CQ concentration of 1 μM, 10 μM could completely block the late process of autophagy, and the number of autophagosomes was further increased when TED and CQ were added at the same time. Moreover, TED had little effect on the number of late autophagosomes (Fig. 3C, D, E, F). WB results also showed the same trend (Fig. 3G, H). These results suggest that the effect of TED on autophagy is through promoting the initiation of early autophagic flow rather than inhibiting late lysosomal fusion. Overall, the aforementioned results indicate that TED can exert concentration-dependent promotion of autophagy and, consequently, apoptosis in TNBC cells.

To better complement the findings from the mRFP-GFP-LC3 system, we performed a confocal co-localization analysis of LC3 and the lysosomal marker LAMP1. The results showed a significant increase in LC3–LAMP1 co-localization following TED treatment, indicating enhanced autophagosome–lysosome fusion. This finding supports the conclusion that TED facilitates the progression of autophagy to the degradation stage, further confirming its role in activating a complete autophagic response (Supplementary Fig. 2).

To investigate whether TED-induced apoptosis is mediated through the activation of autophagy, we used the autophagy inhibitor 3-methyladenine (3-MA) to block autophagy and evaluated the apoptosis rate via flow cytometry. In both MDA-MB-231 and BT-549 cells, we observed that TED-induced apoptosis was markedly attenuated upon 3-MA treatment (Fig. 3I). These results suggest that autophagy plays a pivotal role in mediating TED-induced apoptosis in TNBC cells, indicating that apoptosis is at least partially dependent on autophagic activation.

#### TED activates the P53 pathway

To elucidate the potential mechanisms by which TED promotes apoptosis. We performed transcriptome sequencing of MDA-MB-231 cells treated with TED or DMSO for 48 h, with three biological replicates ( $n = 3$ ) per group. To validate the reliability of the RNA-seq data, We selected six representative differentially expressed genes (TMEFF1, PAK6, HSPA6, DRD5, CASP8, and IGFBP3) for qPCR validation. The mRNA expression levels determined by qPCR were in strong agreement with the transcriptomic data, further supporting the robustness of the RNA-seq analysis (Supplementary Fig. 3). The differential genes of the two groups were subjected to KEGG analysis, and found the involvement of the p53 signaling pathway, a classical pathway known to be associated with cellular autophagy and apoptosis (Fig. 4A). P53 protein is a key protein in the p53 pathway and has been reported as a tumor suppressor protein. It can regulate the expression of a variety of genes, including apoptosis-related genes such as BAX, BCL2, CASPASE, and up-regulate upstream regulators such as PTEN, AMPK, mTOR to promote autophagy through a transcription-dependent pathway. We added TED to TNBC cells and by WB we found that TED could increase P53 expression in cells in a concentration-dependent manner (Fig. 4B). Therefore, we speculated that TED might inhibit the protein degradation

of p53. We then added CHX to inhibit protein synthesis in TNBC cells and observed the degradation of P53 at different time points (0, 1, 2, 3, 4, 5 h). Compared to the DMSO control group, the TED group was found to have a slower rate of p53 protein degradation (Fig. 4C).

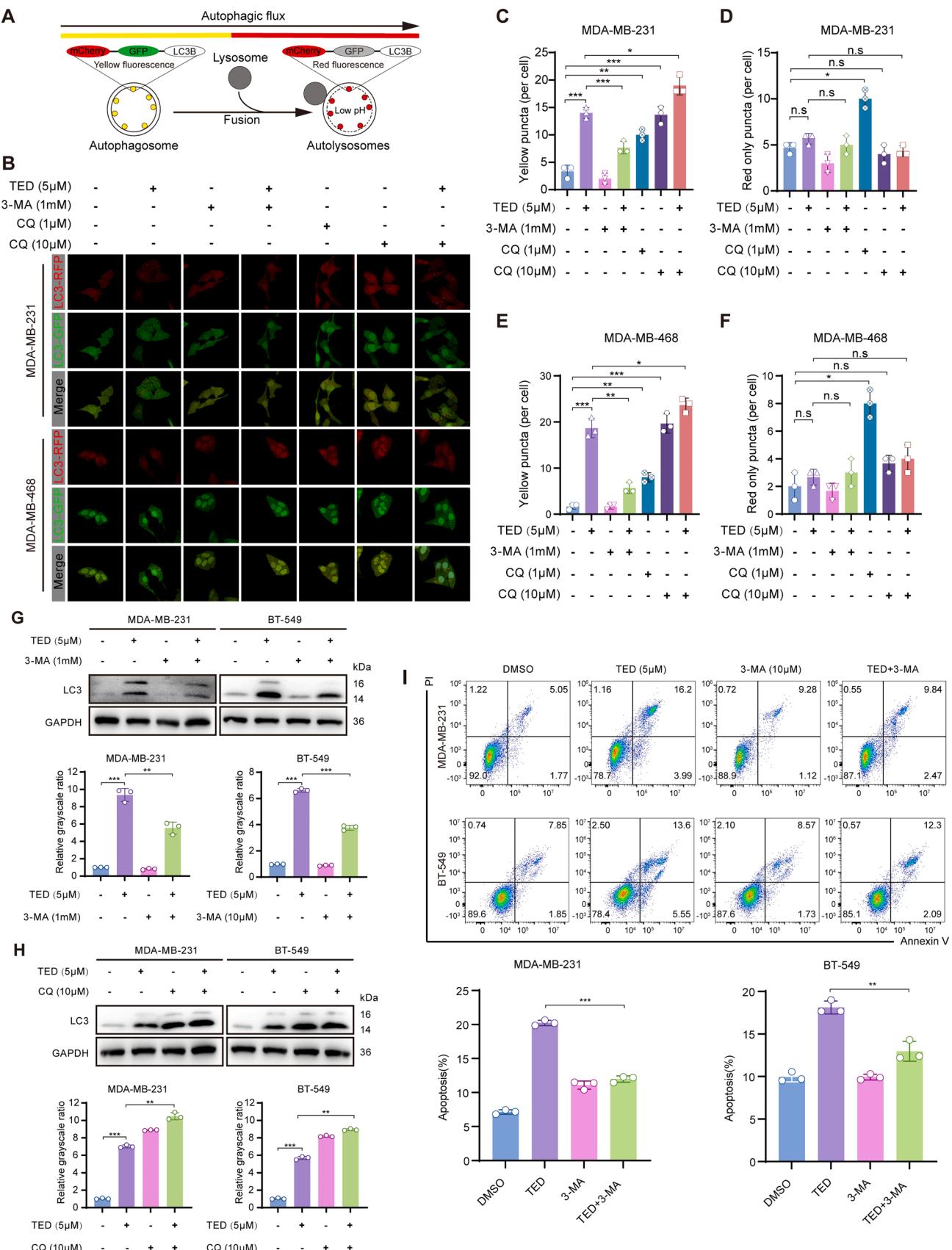
Considering that MDM2-dependent ubiquitination degradation is the major mode of P53 degradation, we observed by CoIP assay that the binding of p53 to MDM2 was significantly reduced after the addition of TED, which may account for the increased P53 expression (Fig. 4D). We then wondered whether TED could be a direct target for P53 or MDM2, thus protecting p53 from degradation by direct binding. We therefore used the CETSA assay, which is a direct method of measuring the degree of drug binding to the target. However, P53 and MDM2 are not directly targeted by TED (Fig. 4E, F).

#### TED directly targets PSMD1 and relies on PSMD1 to inhibit tumor progression

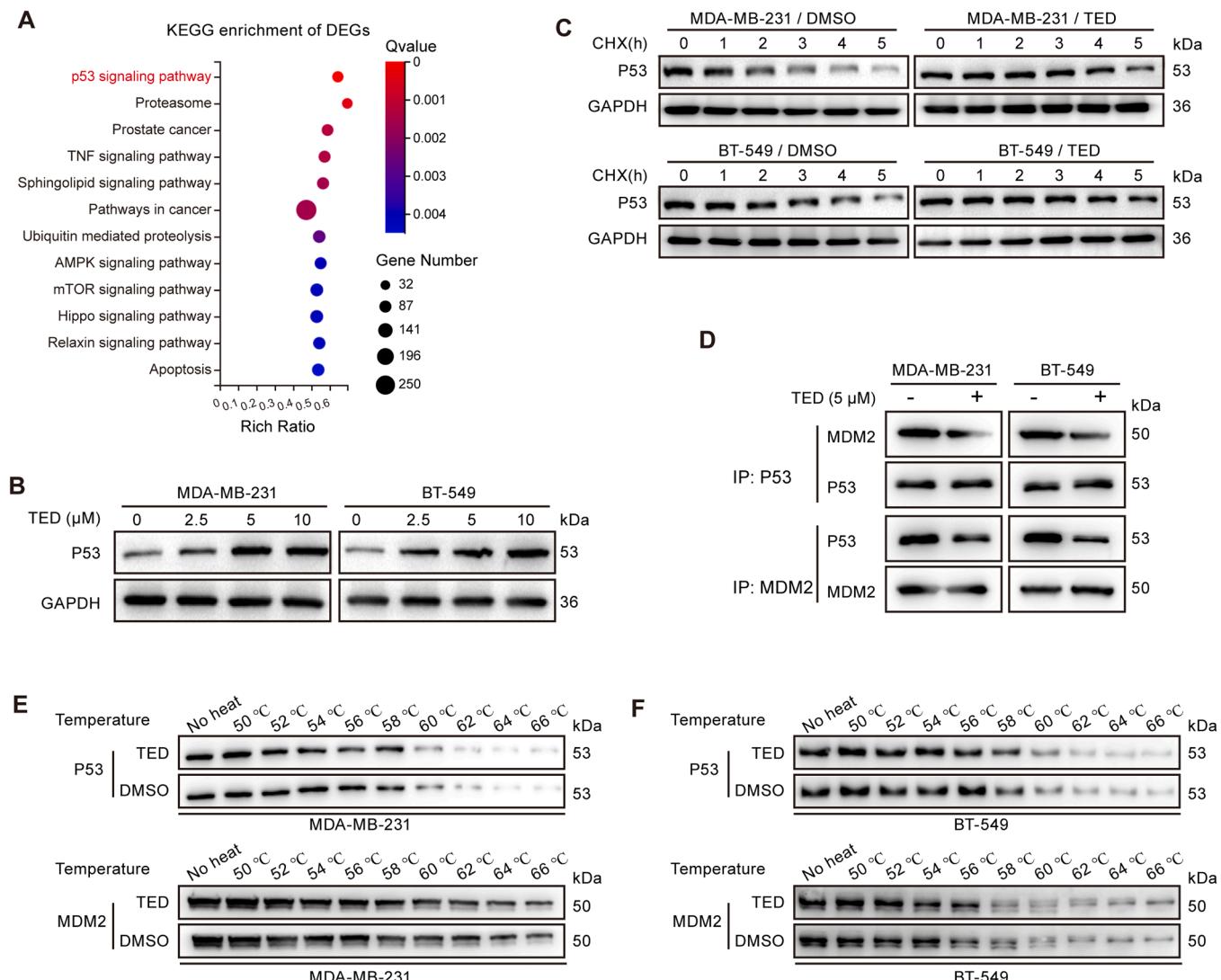
In order to find out how TED inhibits P53 degradation, we used the transcriptome data after treatment and the CLUE database to predict the direct targets of TED (Fig. 5A, B, C). Specifically, if the expression pattern of differential genes after treatment is very similar to the differential gene expression pattern of a gene that is knocked down or overexpressed, then, We believe that this gene may be the target of the drug. In our results, PSMD1, PSMA1 and CTSB had higher similarity, while PSMD1 had the highest score and was significantly higher than the other genes (Fig. 5C). Therefore, the PSMD1 gene became the focus of our consideration. We hypothesized that TED might inhibit PSMD1 expression. So whether TED regulates its expression by directly binding to PSMD1. CETSA assay confirmed our conjecture. The results showed that the PSMD1 protein became more stable after the addition of TED, and the amount of degraded protein decreased significantly with the increase of temperature (Fig. 5D). Another experimental method for drug target identification, the DARTS assay, can also be used for drug target screening and validation, based on the principle that the binding of a small molecule drug to a target protein leads to a decrease in the sensitivity of the target protein to proteases. As shown in the Fig. 5E, our results indicate that TED can protect PSMD1 from degradation in the presence of different protease concentrations.

We hypothesised that TED exerts its anti-tumour activity through PSMD1. Therefore, we knocked down PSMD1 in tumour cells to observe whether the anticancer activity of TED was affected. CCK8 results showed that the effect of TED in inhibiting cell viability was attenuated after knocking down PSMD1 (Fig. 5F). WB results showed that TED's regulatory effect on apoptosis-related genes BAX, BCL2 as well as autophagy-related genes LC3 was decreased (Fig. 5G).

In addition, we investigated the expression of PSMD1 in breast cancer and its oncogenic effects in vitro and in vivo. In breast cancer, the expression of PSMD1 was significantly higher than that in normal tissues (Fig. 6A), and the high expression of PSMD led to a worse prognosis for patients (Fig. 6B). WB validation of breast cancer clinical samples collected at hospital also showed that PSMD1 was highly expressed in breast cancer (Fig. 6C). In cells, PSMD1 expression was also higher in the breast cancer cell line than in the normal cell line MCF10A (Fig. 6D). We subsequently constructed stable transient cell lines knocking down as well as overexpressing PSMD1 in triple-negative breast cancer cell lines (Fig. 6E, Supplementary Fig. 4). The results of the CCK8 experiments showed that knock down PSMD1 inhibited cell viability in vitro, and conversely, overexpression of PSMD1 resulted in an increase in cell viability (Fig. 6F, G, H, I). For in vivo experiments, we constructed a subcutaneous transplantation tumour model in nude mice. The results showed that knockdown of PSMD1 resulted in reduced tumour weight and volume compared with the control group (Fig. 6K, L), and the opposite was true for overexpression (Fig. 6M, N). The results of the in vivo and in vitro experiments suggest that PSMD1 may play a role as a pro-carcinogenic gene in breast cancer, and knockdown of PSMD1 can inhibit the progression of breast cancer.



**Fig. 3. TED promotes autophagic flux in TNBC cells.** (A) Schematic of dual-fluorescence LC3 system. (B-F) Confocal microscopy of autophagosomes and auto-lysosomes after treatment with TED and inhibitors (3-MA and CQ). (G, H) Western blot analysis confirms TED-induced autophagic flux. (I) Apoptosis analysis by flow cytometry in TNBC cells treated with TED  $\pm$  3-MA, showing that 3-MA reduces TED-induced apoptosis. Bars, SDs; \*  $0.01 < p < 0.05$ , \*\*  $0.001 < p < 0.01$ , and \*\*\*  $p < 0.001$ .



**Fig. 4. TED activates the P53 signaling pathway.** (A) KEGG analysis of transcriptomic data identifies P53 pathway involvement. (B) Western blot showing concentration-dependent increase in P53 expression after TED treatment. (C) Cycloheximide assays indicate reduced P53 degradation in TED-treated cells. (D) Co-IP assays reveal decreased P53-MDM2 binding following TED treatment. (E, F) CETSA assays confirmed that TED did not directly bind to P53 and MDM2.

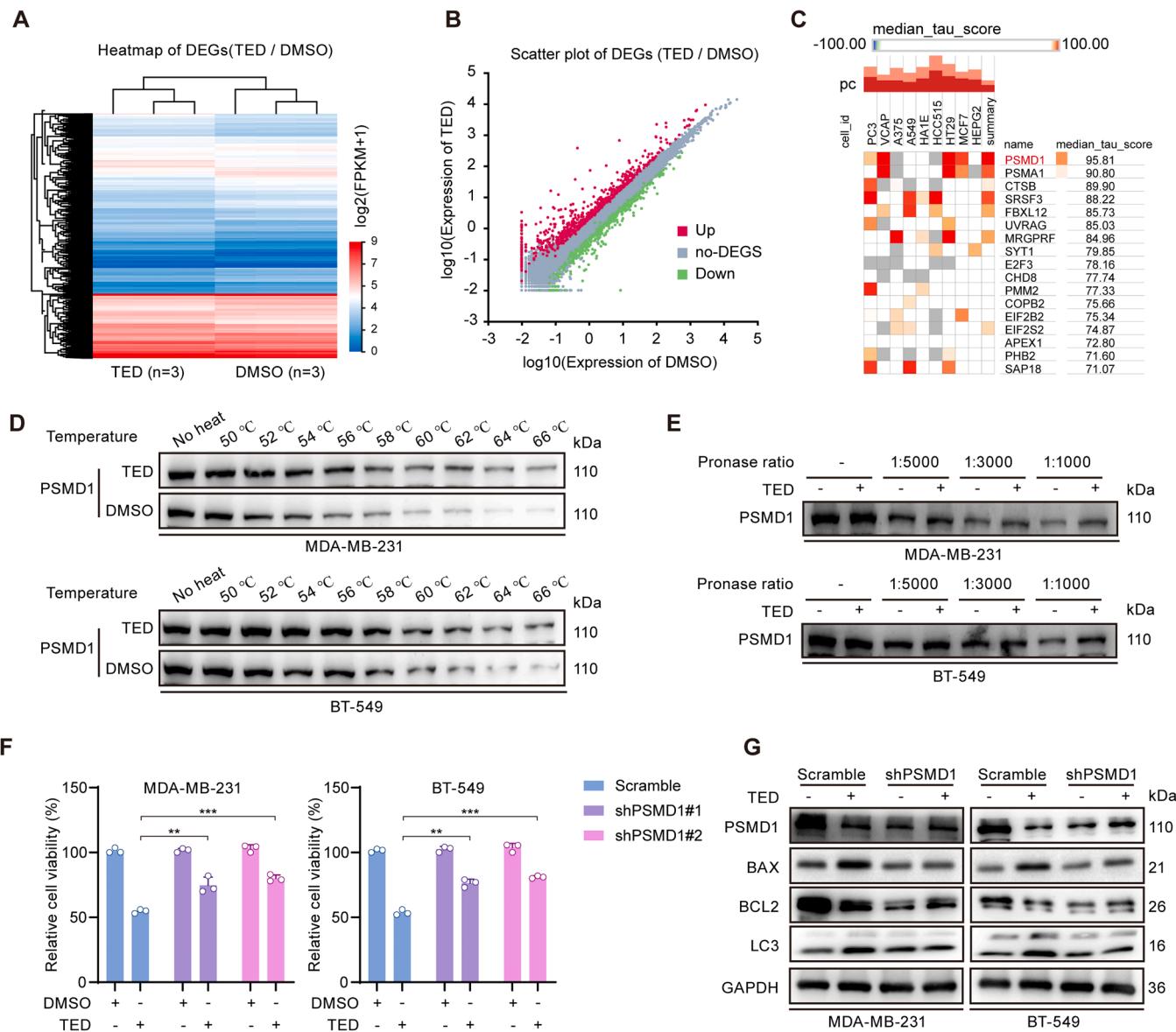
#### PSMD1 promotes ubiquitination degradation of P53

Our previous findings suggest that TED can activate the P53 pathway by indirectly inhibiting ubiquitinated degradation of P53. As a direct target of TED, PSMD1 may be a key protein mediating P53 protein. The PSMD1 protein is a 26 s protease subunit, increased proteasome activity causes altered degradation of some proteins. Therefore, we wanted to verify whether PSMD1 expression causes degradation of P53. First, we found increased expression of P53 protein in PSMD1 knockdown cell lines and decreased P53 expression after overexpression of PSMD1 (Fig. 7A, B). Subsequently, protein degradation experiments involving CHX showed that the rate of P53 degradation was significantly accelerated after overexpression of PSMD1 (Fig. 7C). In general, proteins are degraded in two ways, one is the autophagic lysosomal degradation pathway and the other is the ubiquitin proteasomal degradation pathway. To determine the mode of P53 degradation in breast cancer cells, we introduced the proteasome inhibitor MG132 and the lysosomal inhibitor CQ in stable cell lines overexpressing PSMD1, respectively. It was found that the P53 degradation process mediated by PSMD1 was reverted by the addition of MG132, i.e., inhibition of the ubiquitin proteasome degradation pathway. Whereas CQ was unable to reply PSMD1-mediated P53 degradation (Fig. 7D). Here, we found that

PSMD1 was inhibiting P53 expression by promoting the ubiquitin proteasome degradation pathway of P53 and thereby inhibiting P53 expression. Subsequently, we performed an Ubiquitination assay to detect the ubiquitination level of P53 in cell lines overexpressing as well as knocking down PSMD1 by simultaneous overexpression of HA-UB followed by the addition of MG132 to inhibit proteasomal degradation of the protein. In both MDA-MB-231 and BT-549 cells, P53 ubiquitination was increased after PSMD1 overexpression and decreased after PSMD1 knockdown (Fig. 7E, F). The above results suggest that PSMD1 can promote the ubiquitination degradation of p53. And TED may have caused autophagy and apoptosis in TNBC cells by inhibiting the expression of PSMD1, which in turn led to the increased expression of P53, thus activating the P53 pathway.

#### TED inhibited TNBC cell growth in vivo

To investigate the inhibitory effect of TED on breast cancer in vivo, we established an orthotopic injection model of breast cancer. Mice were randomly divided into three groups: vehicle control, TED low dose (5 mg/kg), and TED high dose (10 mg/kg). TED was administered intraperitoneally every 2 days for a total of 20 days, while the control group received saline injections. At the endpoint, tumors and major



**Fig. 5.** TED targets PSMD1 to stabilize P53. (A-C) Transcriptomic and CLUE database analysis identify PSMD1 as a potential TED target. (D, E) CETSA and DARTS assays confirm TED-PSMD1 binding. (F, G) Knockdown of PSMD1 reduces TED's effects on cell viability and autophagy/apoptosis-related markers. Bars, SDs; \* 0.01 <  $p$  < 0.05, \*\* 0.001 <  $p$  < 0.01, and \*\*\*  $p$  < 0.001.

organs (lung, liver, kidney) were harvested, and serum was collected (Fig. 8A). We compared the size of the tumor and found that TED could significantly inhibit the growth of the tumor without affecting the weight of the nude mice (Fig. 8B, C, D, E). The two indicators of ALT and AST in the serum of the nude mice were also in the normal range (Fig. 8F, G), indicating that TED did not cause damage to the liver function of the nude mice, which was also proved by HE staining of the liver. In addition, HE staining of lung and kidney was consistent with normal (Fig. 8H). These results indicate that TED can inhibit the growth of breast cancer cells *in vivo* without obvious toxic side effects. We extracted proteins from the collected tumors and examined the expression of PSMD1, P53, BAX, BCL2, and LC3, and the results were consistent with the cellular experiments (Fig. 8I).

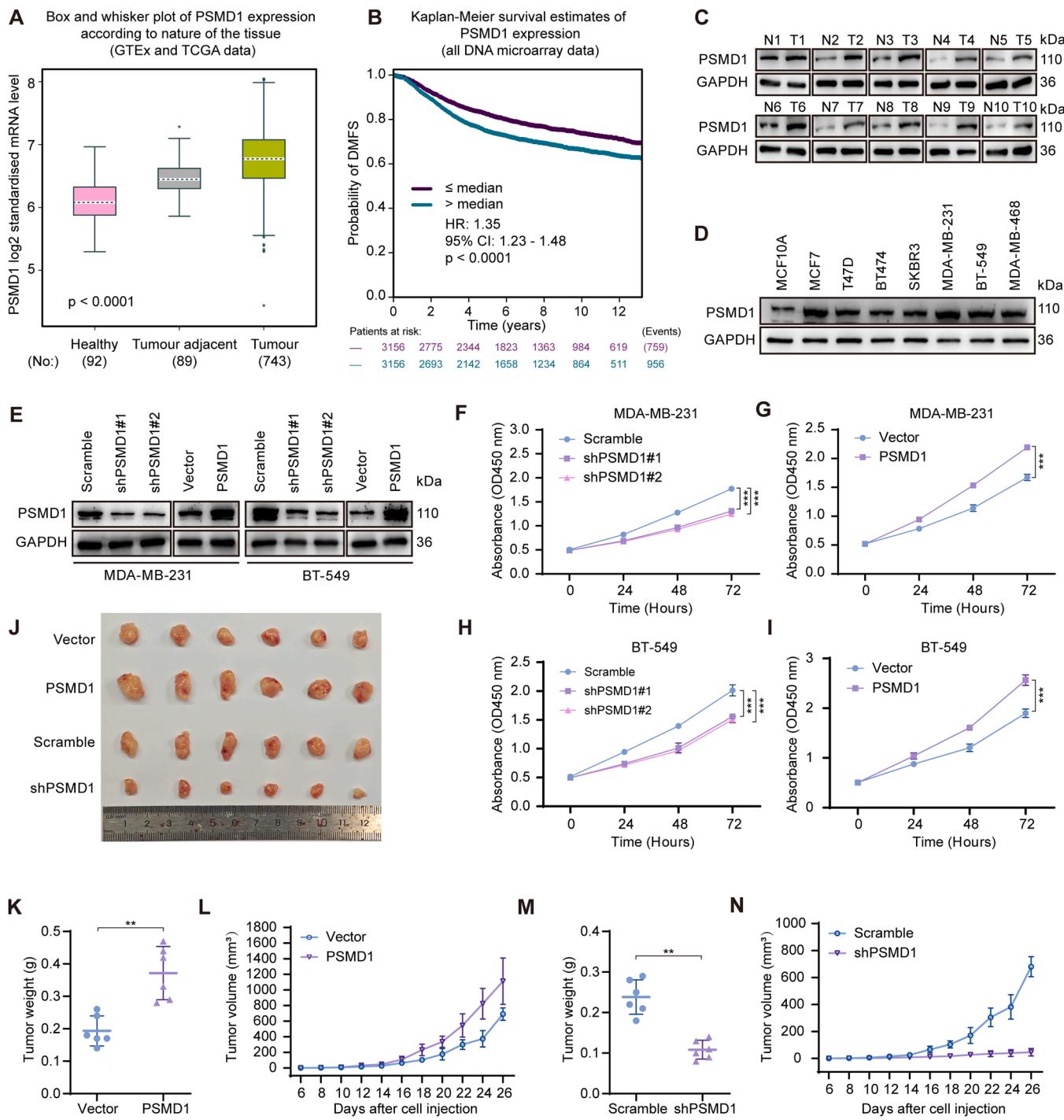
#### TED inhibits the metastasis of breast cancer cells and enhances the efficacy of chemotherapy

Tumor metastasis and poor chemotherapy effect of breast cancer patients are urgent clinical problems to be solved. Here, we wanted to

investigate whether TED can improve breast cancer in these two aspects. Transwell assay showed that the migration and invasion ability of MDA-MB-231 and BT-549 cells were significantly inhibited with the increase of TED concentration. At the protein level, the epithelial cell surface markers E-cadherin and Claudin-1 were up-regulated, while the mesenchymal markers N-cadherin, SNAIL and Vimentin were down-regulated after TED treatment, which was consistent with the results of cell experiments. We evaluated the impact of combining low doses of TED and PTX using the CCK8 assay. As illustrated in the figure, the combination of TED and PTX demonstrated a significantly greater effect compared to the control group, and exhibited superior cytotoxicity compared to PTX administered alone. Collectively, our results show that TED can inhibit tumor cell metastasis and improve the sensitivity of chemotherapy drugs, suggesting that TED may have great potential in clinical treatment.

#### Discussion

Autophagy and apoptosis are tightly interconnected processes

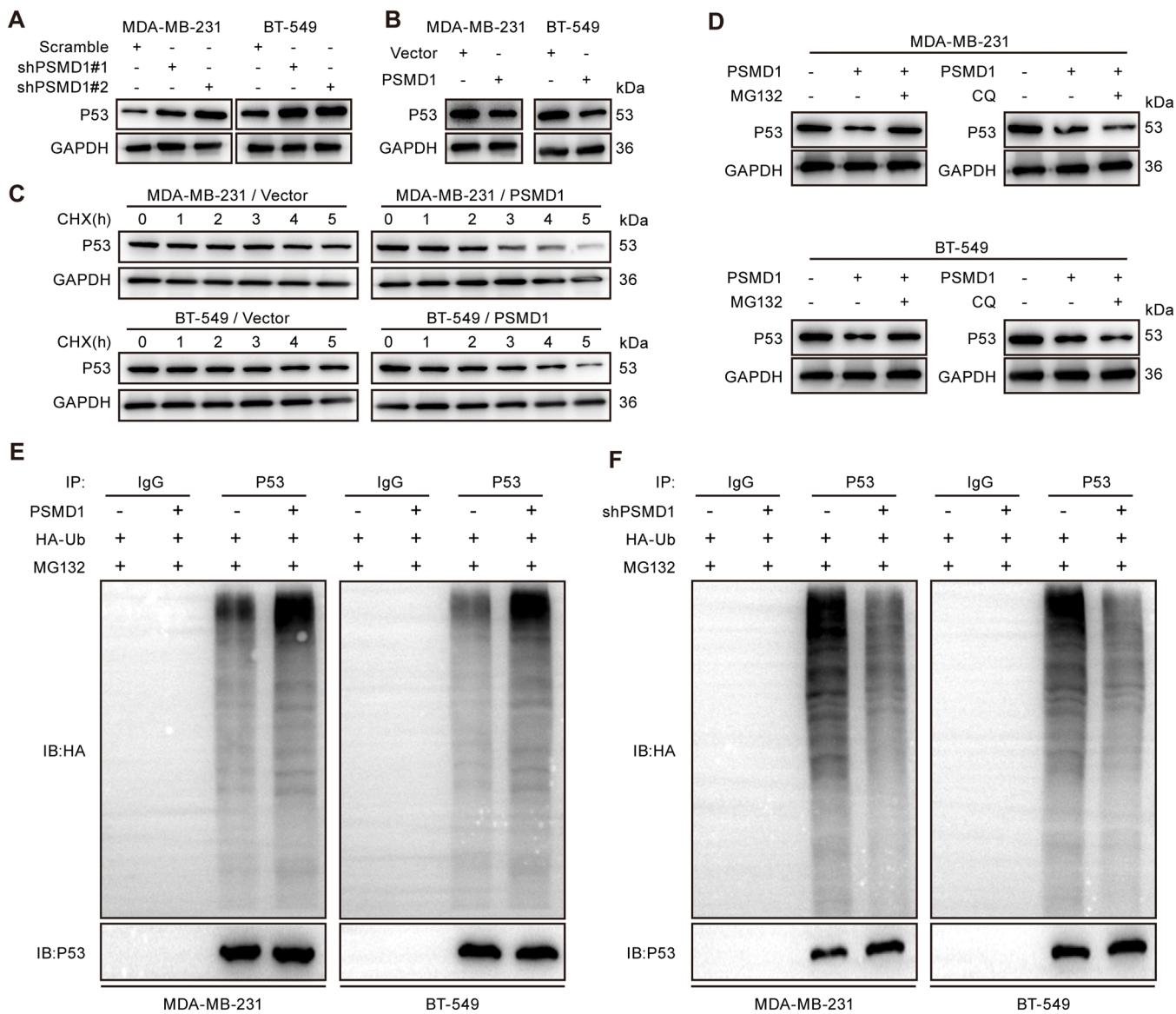


**Fig. 6. PSMD1 as a pro-oncogenic factor in TNBC.** (A, B) PSMD1 expression is significantly elevated in breast cancer tissues and correlates with poor prognosis. (C) PSMD1 was highly expressed in tumor tissues. (D) PSMD1 expression is higher in TNBC cell lines than in normal cells. (E-I) Knockdown of PSMD1 reduces cell viability and tumor progression in vitro. (J-N) In vivo experiments confirm reduced tumor weight and volume following PSMD1 knockdown. Bars, SDs; \* 0.01 < p < 0.05, \*\* 0.001 < p < 0.01, and \*\*\* p < 0.001.

critical to maintaining cellular homeostasis. While both can act as tumor suppressors, they are often dysregulated in cancer, contributing to tumor progression and therapeutic resistance. TED's dual ability to enhance autophagic flux and induce apoptosis in TNBC cells is consistent with prior studies that highlight the synergistic interplay between these pathways in promoting cell death in cancer (Mariño et al., 2014). For instance, TED increased autophagosome formation and expression of autophagy-related proteins LC3 and Beclin-1 while reducing P62 levels, indicating activation of autophagic flux rather than inhibition of

lysosomal degradation. Simultaneously, TED upregulated pro-apoptotic proteins Bax and cleaved caspase-3 while downregulating the anti-apoptotic protein Bcl-2. This dual activation may provide a more robust therapeutic strategy against TNBC, a cancer type notoriously resistant to conventional therapies (Lehmann et al., 2011).

The pivotal role of P53 as a tumor suppressor is well-established. It regulates critical processes such as DNA repair, cell cycle arrest, apoptosis, and autophagy. In TNBC, mutations or dysregulation of P53 often lead to aggressive tumor growth and poor prognosis (Aubrey et al.,



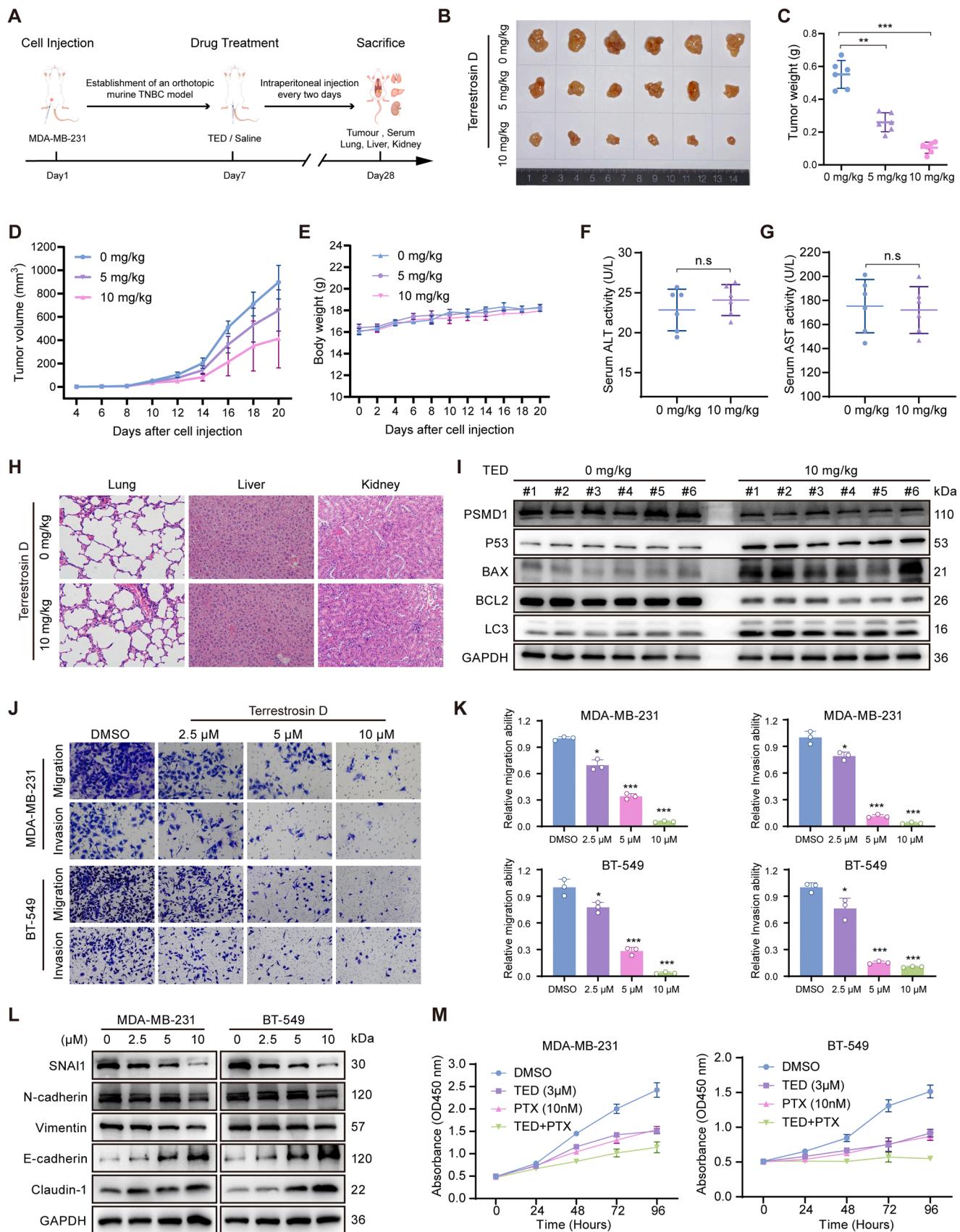
**Fig. 7. PSMD1 promotes ubiquitin-proteasomal degradation of P53.** (A, B) P53 expression increases after PSMD1 knockdown and decreases after PSMD1 overexpression. (C) Cycloheximide assays reveal accelerated P53 degradation with PSMD1 overexpression. (D) MG132 proteasome inhibitor reverses P53 degradation mediated by PSMD1. (E, F) Ubiquitination assays confirm enhanced P53 ubiquitination with PSMD1 overexpression and reduced ubiquitination with PSMD1 knockdown.

2018). P53 has been widely recognized as a crucial regulator of autophagy in addition to its classical roles in apoptosis and cell cycle arrest. The impact of P53 on autophagy is context-dependent and can vary based on its subcellular localization. Nuclear P53 can transcriptionally activate autophagy-related genes such as DRAM1, SESN1/2, and AMPK, thereby promoting autophagic flux (Crighton et al., 2006; Budanov and Karin, 2008). Conversely, cytoplasmic P53 has been shown to inhibit autophagy through mTOR activation under certain conditions (Tasdemir et al., 2008). In our study, TED-mediated stabilization of P53 may contribute to enhanced autophagic activity by favoring nuclear localization and transcriptional activation of autophagy regulators. These findings suggest that TED may activate a coordinated tumor-suppressive program involving both apoptosis and autophagy through the P53 pathway, reinforcing its potential as a multi-functional therapeutic agent in TNBC.

Our results show that TED stabilizes P53 expression by inhibiting its degradation via the ubiquitin-proteasome pathway. Specifically, TED directly binds to PSMD1, a key component of the 19S regulatory particle

in the proteasome complex, thereby preventing P53 ubiquitination and subsequent degradation. This novel mechanism highlights PSMD1 as a critical mediator of TNBC progression and a promising therapeutic target. Furthermore, the downregulation of PSMD1 not only inhibited TNBC cell viability but also enhanced P53 stability, reinforcing its role in tumor suppression.

In addition to its role in regulating P53 stability, recent studies have implicated PSMD1 in other cancer-related signaling pathways, including NF- $\kappa$ B and Wnt/ $\beta$ -catenin. PSMD1, as a key component of the 26S proteasome regulatory subunit, contributes to the degradation of I $\kappa$ B $\alpha$ , thereby facilitating NF- $\kappa$ B activation and promoting cancer cell survival and inflammation-driven tumor progression. Moreover, PSMD1 has been shown to influence Wnt signaling by modulating  $\beta$ -catenin degradation, linking proteasomal regulation to cancer stemness and metastatic potential. These findings suggest that PSMD1 may serve as a broader oncogenic regulator beyond its interaction with P53, and its inhibition may affect multiple tumor-promoting pathways. Further studies are warranted to explore these alternative mechanisms in the



**Fig. 8. TED inhibits TNBC growth in vivo without significant toxicity.** (A-E) TED treatment reduces tumor size and weight in an orthotopic TNBC mouse model (Each group included six mice ( $n = 6$ )). (F-H) Liver and kidney functions remain unaffected, as confirmed by ALT/AST levels and histological analysis. (I) Western blot analysis of tumor tissue shows consistent expression patterns of PSMD1, P53, and related markers. (J-L) Effects of TED on migration, invasion, and EMT markers. (M) enhances the efficacy of chemotherapy. Bars, SDs; \*  $0.01 < p < 0.05$ , \*\*  $0.001 < p < 0.01$ , and \*\*\*  $p < 0.001$ .

context of TNBC.

Notably, PSMD1 has been implicated in various malignancies as a pro-oncogenic factor (Adler et al., 2023; Park et al., 2023). Its high expression in breast cancer tissues correlates with poor prognosis, as observed in our study and supported by previous reports. By targeting PSMD1, TED effectively disrupts proteasome function, restoring P53 activity and suppressing tumor progression. This aligns with earlier findings where proteasome inhibitors demonstrated efficacy in stabilizing P53 and inducing apoptosis in cancer cells (Cao et al., 2020).

In addition to its cytotoxic effects, TED significantly inhibited TNBC metastasis by modulating epithelial-to-mesenchymal transition (EMT). EMT markers such as E-cadherin and Claudin-1 were upregulated, while N-cadherin, Vimentin, and SNAIL were downregulated following TED treatment. These findings suggest that TED not only suppresses primary tumor growth but also reduces metastatic potential, addressing one of the most critical challenges in TNBC management (Lamouille et al., 2014).

Moreover, the combination of TED with paclitaxel (PTX) showed a synergistic effect in reducing cell viability, suggesting its potential to enhance chemotherapy efficacy. Given that TNBC patients often experience poor responses to chemotherapy due to intrinsic resistance, TED could serve as a chemosensitizer, improving treatment outcomes and reducing the required doses of conventional drugs, thereby minimizing side effects (Yin et al., 2020).

Importantly, our study is the first to report TED as a direct inhibitor of PSMD1, linking proteasomal regulation to P53-mediated autophagy and apoptosis in TNBC (Fig. 9). Previous studies have largely focused on global proteasome inhibition, whereas our findings pinpoint PSMD1 as a specific and actionable node. This advances the understanding of selective proteasome modulation and opens a new avenue for targeted

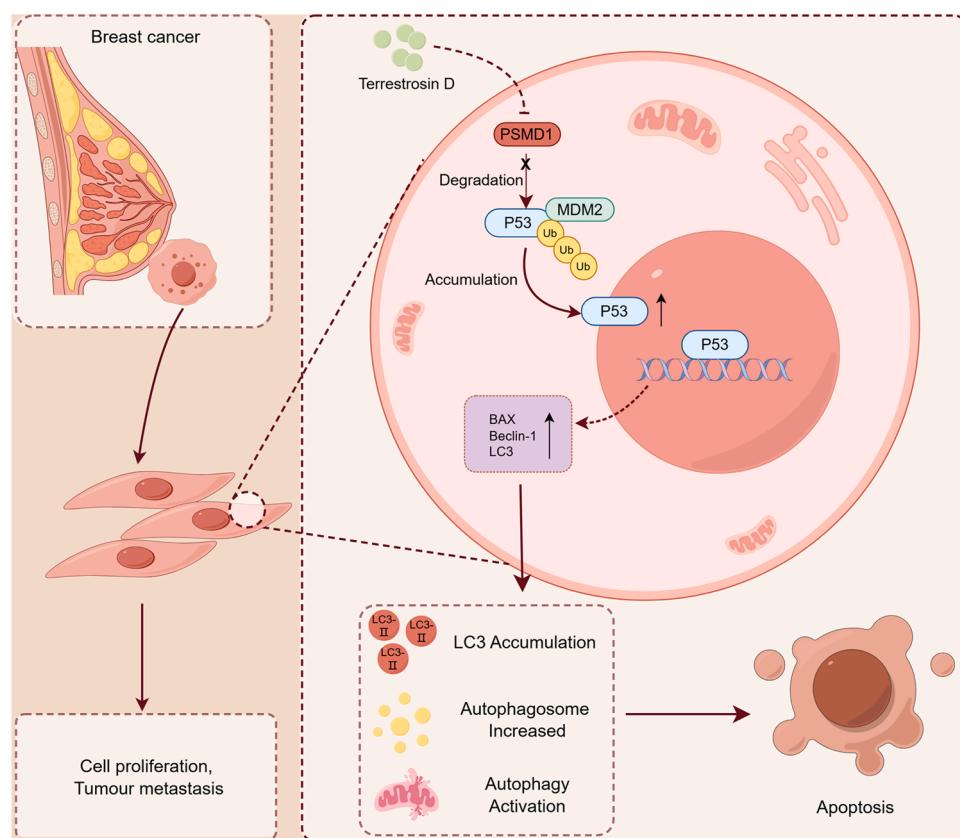
cancer therapy using natural compounds.

While this study highlights TED's therapeutic potential, further research is warranted to investigate its pharmacokinetics, long-term toxicity, and efficacy in clinical settings. Although our *in vivo* data showed no obvious signs of hepatorenal toxicity at therapeutic doses, a recent study has reported potential hepatorenal adverse effects of TED at higher concentrations or prolonged exposure (Sun et al., 2022). Therefore, systematic toxicological evaluation under various dosing conditions will be essential to ensure its safety profile. Moreover, elucidating the broader implications of PSMD1 inhibition in other cancer types and identifying potential off-target effects of TED will be critical for its successful clinical translation.

In conclusion, TED represents a promising candidate for TNBC therapy by targeting PSMD1 to stabilize P53, promote autophagy, and induce apoptosis. Its ability to inhibit metastasis and enhance chemotherapy sensitivity underscores its potential as a multifaceted therapeutic agent. These findings contribute to the growing body of evidence supporting the role of natural compounds in cancer treatment and provide a foundation for future clinical investigations.

#### CRediT authorship contribution statement

**Li-Ling Jia:** Writing – review & editing, Writing – original draft, Data curation, Conceptualization, Funding acquisition. **Cheng-Jie Wu:** Methodology, Investigation. **Pei-Wen Ye:** Investigation. **Qian Zhang:** Investigation. **Hua Liu:** Software. **Tu-Ping Li:** Software. **Xiao-Lei Hu:** Supervision, Funding acquisition, Formal analysis, Conceptualization.



**Fig. 9.** Proposed molecular mechanism by which Terrestrosin D (TED) exerts antitumor effects in triple-negative breast cancer (TNBC). TED directly inhibits PSMD1, a subunit of the 26S proteasome complex, thereby preventing the ubiquitin-mediated degradation of P53. Stabilized P53 activates its downstream signaling pathways, promoting both apoptosis and autophagy. TED also suppresses epithelial–mesenchymal transition (EMT), contributing to reduced tumor progression and metastasis.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.phymed.2025.156883](https://doi.org/10.1016/j.phymed.2025.156883).

## References

- Adler, J., Oren, R., Shaul, Y., 2023. Depleting the 19S proteasome regulatory PSMD1 subunit as a cancer therapy strategy. *Cancer Med.* 12, 10781–10790.
- Aubrey, B.J., Kelly, G.L., Janic, A., Herold, M.J., Strasser, A., 2018. How does p53 induce apoptosis and how does this relate to p53-mediated tumour suppression. *Cell Death Differ.* 25, 104–113.
- Bianchini, G., Balko, J.M., Mayer, I.A., Sanders, M.E., Gianni, L., 2016. Triple-negative breast cancer: challenges and opportunities of a heterogeneous disease. *Nat. Rev. Clin. Oncol.* 13, 674–690.
- Budanov, A.V., Karin, M., 2008. p53 Target genes sestrin1 and sestrin2 connect genotoxic stress and mTOR signaling. *Cell* 134, 451–460.
- Cao, Y., Zhu, H., He, R., Kong, L., Shao, J., Zhuang, R., Xi, J., Zhang, J., 2020. Proteasome, a promising therapeutic target for multiple diseases beyond cancer. *Drug Des. Devel. Ther.* 14, 4327–4342.
- Chen, L., Liu, S., Tao, Y., 2020. Regulating tumor suppressor genes: post-translational modifications. *Signal. Transduct. Target. Ther.* 5, 90.
- Crighton, D., Wilkinson, S., O'Prey, J., Syed, N., Smith, P., Harrison, P.R., Gasco, M., Garrone, O., Crook, T., Ryan, K.M., 2006. DRAM, a p53-induced modulator of autophagy, is critical for apoptosis. *Cell* 126, 121–134.
- Elmore, S., 2007. Apoptosis: a review of programmed cell death. *Toxicol. Pathol.* 35, 495–516.
- Garrido-Castro, A.C., Lin, N.U., Polyak, K., 2019. Insights into molecular classifications of triple-negative breast cancer: improving patient selection for treatment. *Cancer Discov.* 9, 176–198.
- Ge, Y., Zhou, M., Chen, C., Wu, X., Wang, X., 2022. Role of AMPK mediated pathways in autophagy and aging. *Biochimie* 195, 100–113.
- Guo, J., Qi, C., Liu, Y., Guo, X., Meng, Y., Zhao, J., Fu, J., Di, T., Zhang, L., Guo, X., Liu, Q., Wang, Y., Li, P., Wang, Y., 2022. Terrestrosin D ameliorates skin lesions in an imiquimod-induced psoriasis-like murine model by inhibiting the interaction between substance P and dendritic cells. *Phytomedicine* 95, 153864.
- Kato, T., Murata, D., Anders, R.A., Sesaki, H., Iijima, M., 2021. Nuclear PTEN and p53 suppress stress-induced liver cancer through distinct mechanisms. *Biochem. Biophys. Res. Commun.* 549, 83–90.
- Lamouille, S., Xu, J., Deryck, R., 2014. Molecular mechanisms of epithelial-mesenchymal transition. *Nat. Rev. Mol. Cell Biol.* 15, 178–196.
- Lehmann, B.D., Bauer, J.A., Chen, X., Sanders, M.E., Chakravarthy, A.B., Shyr, Y., Pietenpol, J.A., 2011. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. *J. Clin. Invest.* 121, 2750–2767.
- Levy, J., Towers, C.G., Thorburn, A., 2017. Targeting autophagy in cancer. *Nat. Rev. Cancer* 17, 528–542.
- Lorin, S., Pierron, G., Ryan, K.M., Codogno, P., Djavaheri-Mergny, M., 2010. Evidence for the interplay between JNK and p53-DRAM signalling pathways in the regulation of autophagy. *Autophagy* 6, 153–154.
- Luo, Y.D., Fang, L., Yu, H.Q., Zhang, J., Lin, X.T., Liu, X.Y., Wu, D., Li, G.X., Huang, D., Zhang, Y.J., Chen, S., Jiang, Y., Shuai, L., He, Y., Zhang, L.D., Bie, P., Xie, C.M., 2021. p53 Haploinsufficiency and increased mTOR signalling define a subset of aggressive hepatocellular carcinoma. *J. Hepatol.* 74, 96–108.
- Mariño, G., Niso-Santano, M., Baehrecke, E.H., Kroemer, G., 2014. Self-consumption: the interplay of autophagy and apoptosis. *Nat. Rev. Mol. Cell Biol.* 15, 81–94.
- Park, H.C., Kim, H., Kim, J.Y., Lee, H.Y., Lee, J., Cha, W., Ahn, S.H., Jeong, W.J., 2023. PSMD1 as a prognostic marker and potential target in oropharyngeal cancer. *BMC Cancer* 23, 1242.
- Qiu, M., An, M., Bian, M., Yu, S., Liu, C., Liu, Q., 2019. Terrestrosin D from *Tribulus terrestris* attenuates bleomycin-induced inflammation and suppresses fibrotic changes in the lungs of mice. *Pharm. Biol.* 57, 694–700.
- Sun, X.C., Song, X., Guo, F., Yuan, Y.H., Wang, S.Y., Wang, S., Liu, K.L., Lv, X.Y., Han, B., Zhang, C., Liu, J.T., 2022. Terrestrosin D, a spirostanol saponin from *Tribulus terrestris* L. with potential hepatoprotective toxicity. *J. Ethnopharmacol.* 283, 114716.
- Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A., Bray, F., 2021. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* 71, 209–249.
- Tasdemir, E., Chiara Maiuri, M., Morselli, E., Criollo, A., D'Amelio, M., Djavaheri-Mergny, M., Cecconi, F., Tavernarakis, N., Kroemer, G., 2008. A dual role of p53 in the control of autophagy. *Autophagy* 4, 810–814.
- Thoreen, C.C., Sabatini, D.M., 2005. AMPK and p53 help cells through lean times. *Cell Metab.* 1, 287–288.
- Wang, E.S., 2023. MDM2 and BCL-2: to p53 or not to p53. *Blood* 141, 1237–1238.
- Wang, S., Zhang, J., Wang, Y., Chen, M., 2016. Hyaluronic acid-coated PEI-PLGA nanoparticles mediated co-delivery of doxorubicin and miR-542-3p for triple negative breast cancer therapy. *Nanomedicine* 12, 411–420.
- Wei, S., Fukuhara, H., Chen, G., Kawada, C., Kurabayashi, A., Furukata, M., Inoue, K., Shuin, T., 2014. Terrestrosin D, a steroid saponin from *Tribulus terrestris* L., inhibits growth and angiogenesis of human prostate cancer in vitro and in vivo. *Pathobiology* 81, 123–132.
- Wu, X., Deng, Y., 2002. Bax and BH3-domain-only proteins in p53-mediated apoptosis. *Front. Biosci.* 7, d151–d156.
- Yang, X., Zhao, G., Bo, Y., Yang, D., Dong, Z., Wu, G., Xu, N., An, M., Zhao, L., 2022. Mechanism exploration of terrestrosin D on pulmonary fibrosis based on plasma metabolomics and network pharmacology. *Biomed. Chromatogr.* 36, e5441.
- Yin, L., Duan, J.J., Bian, X.W., Yu, S.C., 2020. Triple-negative breast cancer molecular subtyping and treatment progress. *Breast. Cancer Res.* 22, 61.
- Zhang, J., Xie, Y., Fan, Q., Wang, C., 2021. Effects of karanjin on dimethylhydrazine induced colon carcinoma and aberrant crypt foci are facilitated by alteration of the p53/Bcl2/BAX pathway for apoptosis. *Biotech. Histochem.* 96, 202–212.