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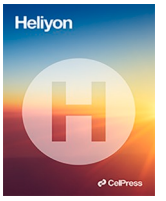

Research article

TRIM59-mediated ferroptosis enhances neuroblastoma development and chemosensitivity through p53 ubiquitination and degradation

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ABSTRACT

Neuroblastoma, predominantly afflicting young individuals, is characterized as an embryonal tumor, with poor prognosis primarily attributed to chemoresistance. This study delved into the impact of tripartite motif (TRIM) 59, an E3 ligase, on neuroblastoma development and chemosensitivity through mediating ferroptosis and the involvement of the tumor suppressor p53. Clinical samples were assessed for TRIM59 and p53 levels to explore their correlation with neuroblastoma differentiation. In neuroblastoma cells, modulation of TRIM59 expression, either through overexpression or knockdown, was coupled with doxorubicin hydrochloride (DOX) or ferrostatin-1 (Fer-1) therapy. In vivo assessments examined the influence of TRIM59 knockdown on neuroblastoma chemosensitivity to DOX. Co-immunoprecipitation and ubiquitination assays investigated the association between TRIM59 and p53. Proliferation was gauged with Cell Counting Kit-8, lipid reactive oxygen species (ROS) were assessed via flow cytometry, and protein levels were determined by Western blotting. TRIM59 expression was inversely correlated with neuroblastoma differentiation and positively linked to cell proliferation in response to DOX. Moreover, TRIM59 impeded lipid ROS generation and ferroptosis by directly interacting with p53, promoting its ubiquitination and degradation in DOX-exposed neuroblastoma cells. Fer-1 countered the impact of TRIM59 knockdown on neuroblastoma, while TRIM59 knockdown enhanced the therapeutic efficacy of DOX in xenograph mice. This study underscores TRIM59 as an oncogene in neuroblastoma, fostering growth and chemoresistance by suppressing ferroptosis through p53 ubiquitination and degradation. TRIM59 emerges as a potential strategy for neuroblastoma therapy.

1. Introduction

Neuroblastoma is an embryonal tumor of the sympathetic nervous system, predominantly impacting young children [1]. It

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constitutes around 8% of all malignant tumors in childhood and 15% of pediatric cancers [2,3]. Patients with low- and intermediate-risk neuroblastoma exhibit a high 5-year survival, while those with high-risk neuroblastoma face a 40% survival rate [4,5]. Chemoradiotherapy stands as the primary mainstay strategy for neuroblastoma patients with a poor prognosis [4]. The primary challenges in this field revolve around devising innovative strategies to mitigate or overcome chemoresistance and enhance the sensitivity of neuroblastoma to chemotherapy.

Neuroblastoma originates from sympathetic nervous system cells, specifically sympathoadrenal progenitor cells, and progresses to sympathetic ganglion and adrenal chromaffin cells [6]. The differentiation of sympathoadrenal precursor cells is linked to alterations in various genetic factors, including TP53 (the gene encoding p53) deletions, NGF and MYCN amplification, ALK amplifications or mutations, and ATRX deletions or mutations [6–9]. p53, a well-established tumor suppressor protein, plays a significant role in the cellular response to diverse stressors such as malnutrition, oncogene activation, hypoxia, and DNA damage [10]. p53 modulates survival and death in response to stress through acetylation, phosphorylation, ubiquitination, and other modifications [11]. Furthermore, p53 inactivation is pivotal in relapsed neuroblastoma and may contribute to chemoresistance [12]. Recent studies have unveiled p53 as a novel regulator of ferroptosis in malignancies both *in vitro* and *in vivo* [13–15]. However, this aspect not been explored in neuroblastoma, and the precise underlying mechanism remains unknown.

Ferroptosis is a form of regulated cell death characterized by iron accumulation and lipid peroxidation [16]. Mechanistically, the inhibition of GPX4, essential for clearing ROS, triggers ferroptosis. p53's interference with cysteine uptake sensitizes cells to ferroptosis by downregulating SLC7A11 and GPX4 expression, thereby reducing antioxidant capacity and ROS accumulation [17]. Diverse oxidative and antioxidant systems converge on cellular degradation machinery to influence the ferroptotic response [18]. The induction of ferroptosis emerged as a potential therapeutic strategy to induce cell death in brain tumors [19,20]. Recent findings indicate a connection between ferroptosis and neuroblastoma prognosis. In stage 1 neuroblastoma, as opposed to stage 4, a majority of ferroptosis-related genes (26 out of 27) exhibited increased expression and correlated with overall survival (OS) [21]. Moreover, the suppression of ferroptosis is linked to chemoresistance and the progression of various tumor types [22,23]. Stimulating ferroptosis, such as through PTC596 or PKCα inhibition, has been reported to enhance multidrug resistance in neuroblastoma [24,25].

The ubiquitin proteasome system (UPS) serves as the primary mechanism for degrading proteins labeled with ubiquitin. However, the key pathway through which UPS facilitates ferroptotic cell death is not well understood [26]. Recently, there has been growing interest in TRIM and TRIM-like proteins, which function as E3 ligases. TRIM proteins play roles in various physiological functions, including antimicrobial immunity, oncogenesis, cell death regulation, signal transduction, and gene regulation [27]. A study has demonstrated that TRIM members act as oncogenes in neuroblastoma by stimulating the Wnt/β-catenin signaling pathway [28]. Furthermore, multiple studies have highlighted the regulatory effects of TRIM59 and p53 on various tumor processes, including clone formation, cell proliferation, migration and invasion, and chemosensitivity [29–31]. Furthermore, TRIM59-mediated ferroptosis has been implicated in nonalcoholic non-alcoholic fatty liver disease and pancreatic cancer development [32,33]. However, in neuroblastoma, no evidence of an association between TRIM59 and p53 has been found. Our research reveals that TRIM59 regulates ferroptosis in neuroblastoma by ubiquitinating and degrading p53. Notably, TRIM59 knockdown enhances neuroblastoma susceptibility to DOX. These findings contribute to the understanding of TRIM59 as a potential therapeutic target for neuroblastoma.

2. Materials and methods

2.1. Clinical samples

Six adjacent mediastinal, six moderately differentiated, and 12 poorly differentiated mediastinal neuroblastoma tumor tissues were acquired from the patients at Fudan University's Children's Hospital. Written informed consent was obtained from the patients. Protein content evaluation of the samples was conducted through Western blotting.

2.2. Cells

The human neuroblastoma cell lines SH-SY5Y, SK-N-BE2, and SK-N-SH were obtained from the Chinese Academy of Sciences Cell Bank (Shanghai, China). Cell cultivation followed the procedures outlined in a prior study [28].

2.3. Lentivirus

TRIM59 overexpression and knockdown lentiviruses, as well as p53 overexpression lentiviruses were generated by GeneChem Company (Shanghai, China). The sequences and primers used are detailed in Table 1. Lentivirus injection into wells was based on

Table 1
Sequences or primers used for vector construction.

Vectors	Sequences or primers
shTRIM59–1	**5′-GGAAGCTGTTCTCCAGTAT-3′**
shTRIM59–2	**5′-GAAGAGTCTCCACTTAAAT-3′**
P53(CDS), Forward	**5′-CCCAAGCTTATGGAGGAGCCGCAGTCAG-3′**
P53(CDS), Reverse	**5′-CGGAATTCTCAGTCTGAGTCAGGCCCTTC-3′**

colony-forming units. Subsequently, cells were seeded onto 6-well plates for 12 h and then cultivated for 48 h with or without medications. Infection efficiency in cells was assessed using Western blotting.

2.4. Cell viability assays

A total of 3×10^4 cells were seeded into 96-well plates and exposed to DOX/ferrostatin-1 (Fer-1) for 48 h. Cell proliferation was assessed at different time points (0, 12, 24, and 48 h) using the Cell Counting Kit-8 (CCK-8) assay kit (Beyotime, Shanghai, China) according to the manufacturer's instructions. The optical density at 450 nm was then measured using a microplate reader (BioTek, VT, USA).

2.5. Lipid reactive oxygen species (ROS) measurement

Cultured cell lipid ROS levels were determined using a Reactive Oxygen Species Assay Kit (S0033S, Beyotime, Shanghai, China) in accordance with the manufacturer's instructions. Flow cytometry (BD Biosciences, NJ, USA) was employed to analyze the cell sample with an excitation wavelength of 480 nm and an emission wavelength of 525 nm.

2.6. Co-immunoprecipitation (CO-IP) and ubiquitination assays

The interaction between TRIM59 and p53 was examined using a co-immunoprecipitation (Co-IP) assay following previously described procedures [34]. IP was carried out using anti-IgG (Santa Cruz Biotechnology, sc-2027, CA, USA), anti-TRIM59 (Novus Biologicals, NBP1-59777, Beijing, China), and anti-p53 (CST #2527, MA, USA) antibodies. Western blotting was performed using anti-TRIM59 (Proteintech, 28575-1-AP, IL, USA), anti-p53 (Proteintech, 60283-2-Ig, IL, USA), and anti-ubiquitin (Abcam, ab7780, Shanghai, China) antibodies.

2.7. Real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR)

For the measurement of p53 mRNA expression, qRT-PCR was employed, utilizing the primers specified in our previous research (Table 2) [28]. The expression of the target gene was normalized to ACTB and calculated using the $2^{-\Delta\Delta}$Ct method.

2.8. Western blotting

Total protein was extracted from cell lysate, and the protein concentration was determined using a BCA protein assay kit (Thermo Fisher Scientific, MA, USA). Following established procedures [28], 35 μg of protein from each sample was separated through sodium dodecyl sulfate polyacrylamide gel electrophoresis. Protein levels were assessed using primary antibodies: anti-TRIM59 (1:800, Proteintech, 28575-1-AP, IL, USA), anti-p53 (1:1000, CST #2527), anti-SLC7A11 (1:2000, Abcam, ab37185), anti-GPX4 (1:1000, Abcam, ab125066), and anti-β-actin (1:2000, Abcam, ab8227). Lastly, the protein bands were analyzed and digitized using ImageJ software (NIH, USA).

2.9. Xenograph mouse model

Male BALB/c nude mice, 7 weeks old (n = 96 mice), were provided by the Shanghai Experimental Animal Center (Shanghai, China). Approval for this study was obtained from the Animal Ethics Committee of our hospital. Subcutaneous injection of neuroblastoma SH-SY5Y cells (1×10^6) into the left posterior flanks of the mice was carried out. DOX (8 mg/kg, twice weekly) was administered to the mice for 4 weeks, and mouse specimens were sacrificed (six mice per group). Throughout this period, the growth curve of the subcutaneous tumor was documented. TUNEL labeling, following established procedures, was used to assess tumor cell death [35], with the examination of tumor cells conducted using a fluorescence microscope (Olympus, Japan). Furthermore, the survival rate of 90-day-old nude mice was analyzed (16 mice per group).

2.10. Statistical analysis

The data are expressed as means ± standard deviations. Group differences were assessed using one-way analysis of variance, Tukey's post hoc test, and Student's *t*. Statistical significance was defined by p values < 0.05.

Table 2
qPCR primers.

Gene	primers
P53, Forward	**5′-GCTGCTCAGATAGCGATGG 3′**
P53, Reverse	**5′-CCCAGGACAGGCACAAAC-3′**
beta actin, Forward	**5′-GTGGACATCCGCAAAGAC-3′**
beta actin, Reverse	**5′-GAAGGTGGACAGCGAGGC-3′**

3. Results

3.1. TRIM59 is positively related to the viability of neuroblastoma cells exposed to DOX

We observed higher TRIM59 abundance in SH-SY5Y and SK-N-SH cells but lower levels in SK-N-BE2 cells [28]. Initial exploration involved evaluating the impact of TRIM59 shRNA (two shRNA sites, sh#1, and sh#2) on SH-SY5Y and SK-N-SH cell proliferation, followed by examining the effects of TRIM59 overexpression (OE) on SK-N-BE2 cell proliferation. CCK-8 assay results demonstrated that DOX treatment (2, 5, and 10 μg/mL) suppressed cell proliferation (Fig. 1). Notably, TRIM59 knockdown intensified the inhibitory effect of DOX on cell proliferation (Fig. 1A and B), while TRIM59 overexpression attenuated it (Fig. 1C). This indicates a positive association between TRIM59 and the viability of neuroblastoma cells exposed to DOX.

3.2. TRIM59 knockdown promotes ferroptosis in neuroblastoma cells

We also explored the effects of TRIM59 shRNA (sh#1 and sh#2) on ferroptosis in SH-SY5Y and SK-N-SH cells. Transfecting TRIM59 shRNA lentivirus into these cells led to reduced growth (Fig. 2A and B). Flow cytometry revealed that TRIM59 shRNA induced lipid ROS production in both SH-SY5Y and SK-N-SH cells (Fig. 2C–F). Notably, TRIM59 shRNA downregulated the protein levels of TRIM59, GPX4, and SLC7A11, key ferroptosis repressors [18]. Moreover, TRIM59 shRNA treatment induced p53 expression in SH-SY5Y and SK-N-SH cells (Fig. 2G–P).

3.3. TRIM59 overexpression inhibits ferroptosis in neuroblastoma cells

SK-N-BE2 cells were transfected with TRIM59 overexpression lentivirus, and cultured for 48 h. Subsequent examinations included assessing cell proliferation, lipid ROS levels, and protein levels of p53, TRIM59, SLC7A11, and GPX4. As illustrated in Fig. 3, TRIM59

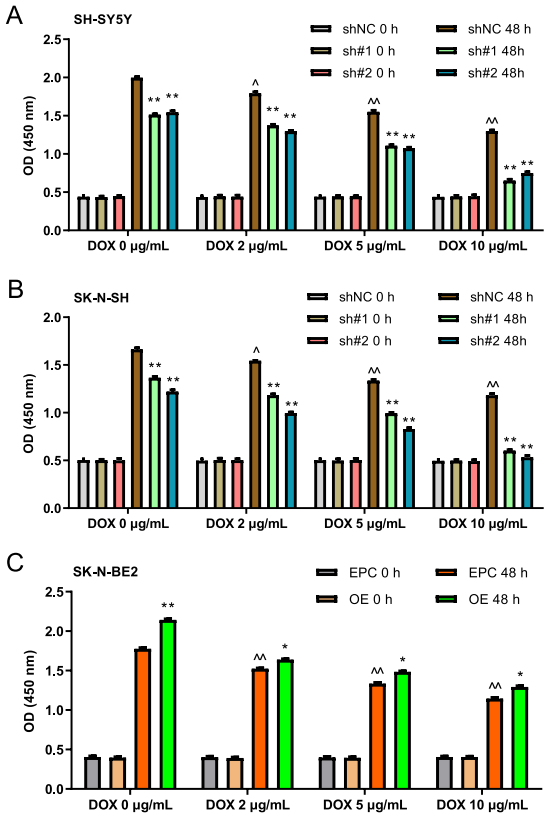

Fig. 1. Impact of TRIM59 on neuroblastoma cell proliferation under DOX exposure. TRIM59 knockdown inhibits the proliferation of SH-SY5Y (A) and SK-N-SH (B) cells exposed to DOX. (C) TRIM59 overexpression increases the proliferation of SK-N-BE2 cells exposed to DOX. shNC, shRNA null control. sh#1, TRIM59 shRNA site 1. sh#2, TRIM59 shRNA site 2. One-way ANOVA and Tukey's post hoc test were used to test the differences among the 48-h treatment groups. EPC, empty plasmid control; OE, TRIM59 overexpression. $\hat{}p < 0.05$, $\hat{}\hat{}p < 0.01$ data in DOX (2, 5, and 10 μg/mL) compared to DOX (0 μg/mL), $*p < 0.05$, $**p < 0.01$ data in sh#1 and sh#2 or OE compared to shNC or EPC exposed to the same DOX concentration ($n = 3$).

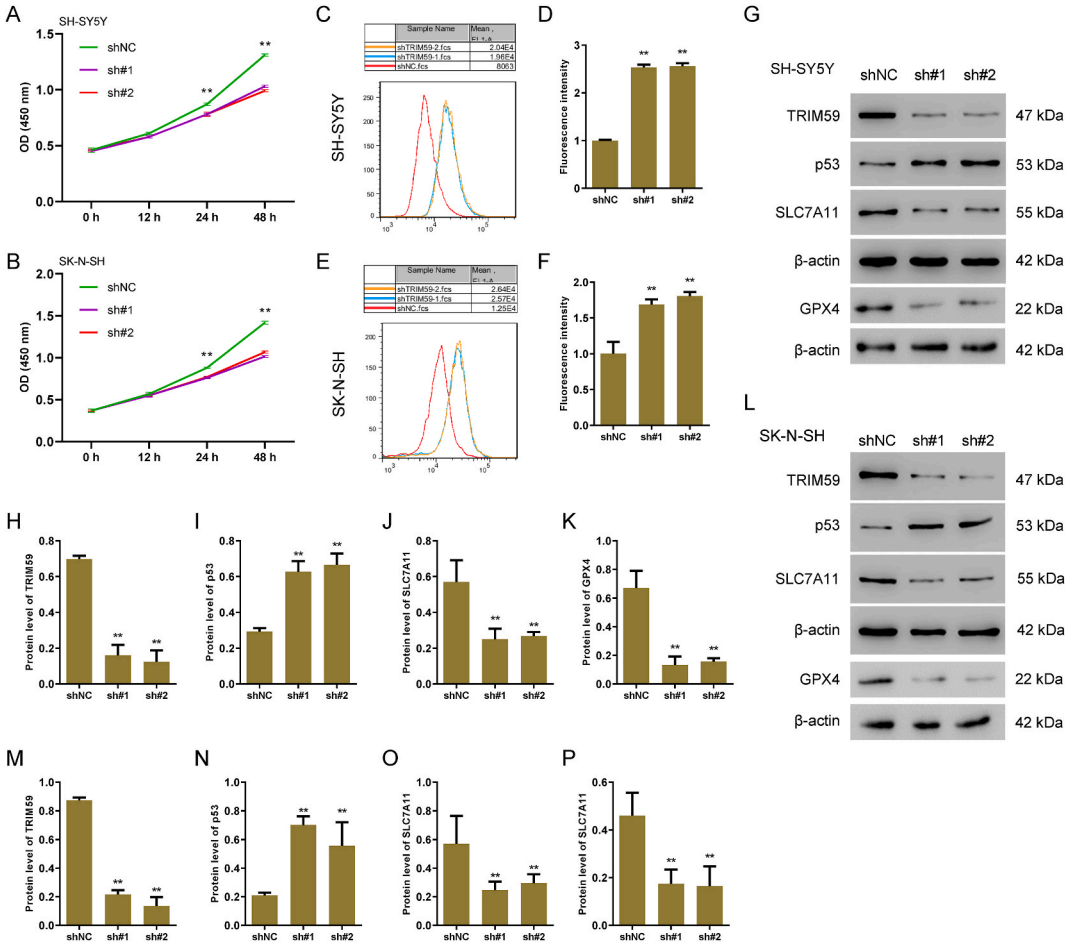

Fig. 2. Promotion of ferroptosis by TRIM59 knockdown in neuroblastoma cells. TRIM59 knockdown inhibits the proliferation of SH-SY5Y (A) and SK-N-SH (B) cells. TRIM59 knockdown induces the lipid ROS production in SH-SY5Y (C and D) and SK-N-SH (E and F) cells. (G–K) TRIM59 knockdown enhances the protein level of p53 and decreases TRIM59, SLC7A11, and GPX4 levels in SH-SY5Y cells. (L–P) TRIM59 knockdown elevates p53 protein levels and reduces TRIM59, SLC7A11, and GPX4 levels in SK-N-SH cells. sh#1, TRIM59 shRNA site 1. sh#2, TRIM59 shRNA site 2. ANOVA and Tukey's post hoc test were utilized for statistical analysis among the groups. shNC, shRNA null control. $^{**}p < 0.01$ data in sh#1 and sh#2 compared to shNC ($n = 3$).

overexpression significantly enhanced the proliferation of SK-N-BE2 cells compared to the empty plasmid vector (EPC) at 24 and 48 h post treatment (Fig. 3A). Moreover, TRIM59 overexpression led to a reduction in lipid ROS levels (Fig. 3B and C). Furthermore, TRIM59 overexpression increased the protein levels of TRIM59, GPX4, and SLC7A11 while decreasing the protein levels of p53 (Fig. 3D–H). This collectively indicates that TRIM59 overexpression mitigates ferroptosis in SK-N-BE2 cells.

3.4. *TRIM59 induces degradation of p53 by ubiquitination*

We proceeded to examine the association between TRIM59 and p53 expression and its correlation with neuroblastoma cell differentiation. The interaction between TRIM59 and p53 was also investigated. As shown in Fig. 4, TRIM59 protein levels were lower in adjacent mediastinal (AM) tissues and higher in moderately differentiated (MD) neuroblastoma tumor tissues (Fig. 4A). Conversely, p53 showed an inverse relationship with TRIM59 (Fig. 4A). Furthermore, TRIM59 knockdown in SH-SY5Y and SK-N-SH cells and TRIM59 overexpression in SK-N-BE2 cells did not affect mRNA expression of p53 (Fig. 4B–D). Therefore, we investigated the protein interaction between p53 and TRIM59. The Co-IP assay conducted on SH-SY5Y cells confirmed the interaction between TRIM59 and p53 proteins (Fig. 4E and F). Furthermore, we investigated p53 ubiquitination degradation in SH-SY5Y cells with TRIM59 interference, revealing that TRIM59 shRNA inhibited p53 ubiquitination degradation (Fig. 4G).

3.5. *TRIM59 and p53 are involved in the sensitivity of neuroblastoma cells to DOX*

In this section, we explored the effects of TRIM59 and p53 overexpression (OE) on SK-N-BE2 cells treated with DOX (5 μg/mL).

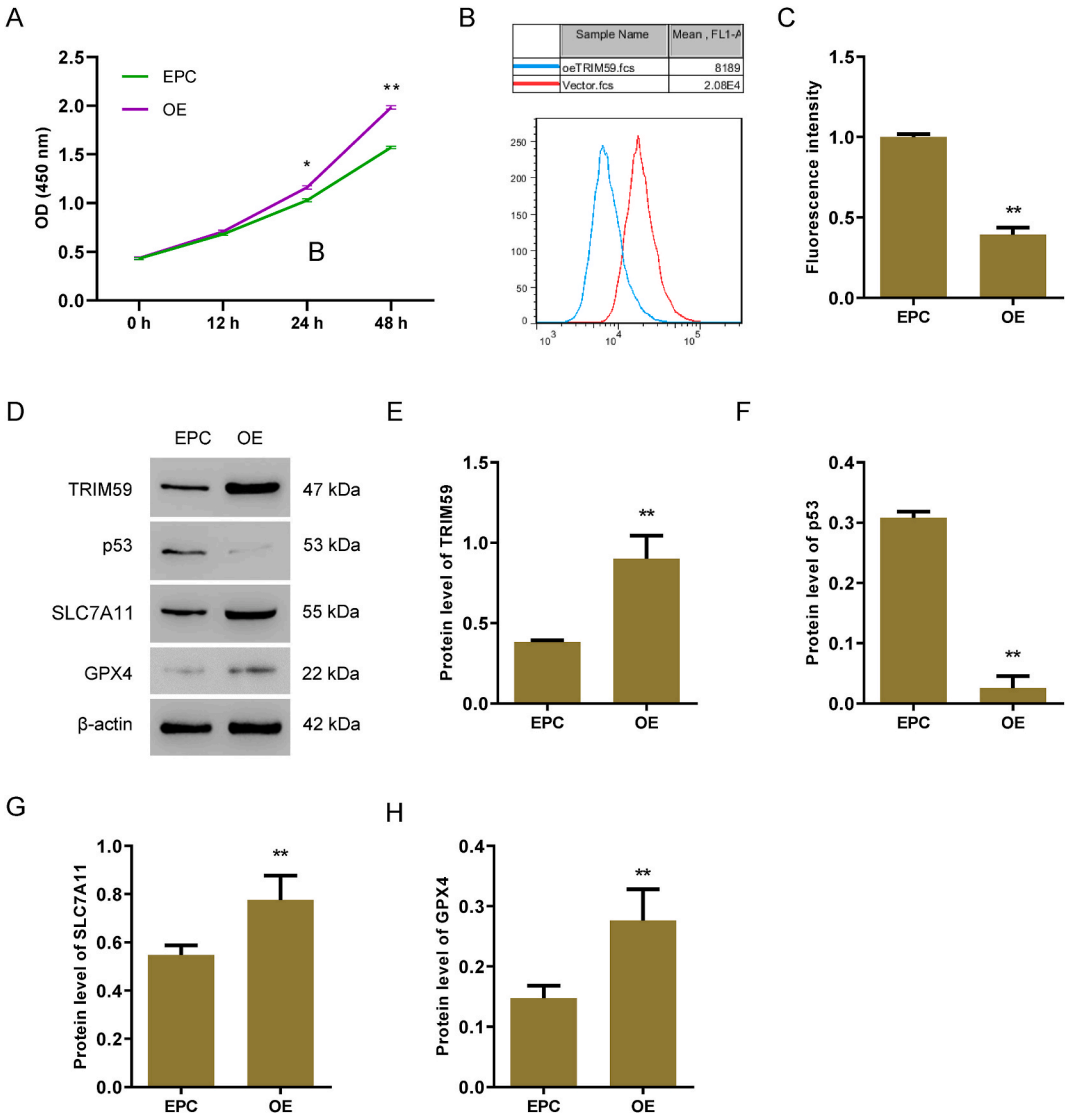

Fig. 3. Suppression of ferroptosis by TRIM59 overexpression in neuroblastomas. (A) TRIM59 overexpression enhanced the proliferation of SK-N-BE2 cells. (B and C) TRIM59 overexpression decreases lipid ROS levels in SK-N-BE2 cells. (D–H) TRIM59 overexpression increases the protein levels of TRIM59, SLC7A11, and GPX4 and decreases p53. *t*-test was used for statistical analysis between the two groups. EPC, empty plasmid control; OE, TRIM59 overexpression. $*p < 0.05$, $**p < 0.01$ compared to EPC ($n = 3$).

Following transfection into SK-N-BE2 cells, which were then cultured for 24 h, the efficacy of the p53 overexpression vector was tested using qRT-PCR and Western blotting (Fig. 5A–C). Our findings indicate that under DOX exposure, TRIM59 overexpression increased the proliferation of SK-N-BE2 cells, while p53 overexpression had the opposite effect at 24 and 48 h (Fig. 5D). Furthermore, DOX increased lipid ROS levels, further induced by p53 overexpression. In contrast, TRIM59 overexpression reduced lipid ROS levels, irrespective of the absence or presence of p53 (Fig. 5E and F). Furthermore, SLC7A11 and GPX4 protein levels were upregulated by TRIM59 overexpression but downregulated by p53 overexpression. Concurrently, p53 protein levels exhibited an opposing trend (Fig. 5G–J). Moreover, p53 overexpression significantly reversed the changes induced by TRIM59 overexpression in SK-N-BE2 cells exposed to DOX (Fig. 5D–J).

3.6. TRIM59 knockdown promotes ferroptosis and increases neuroblastoma cell sensitivity to DOX

We investigated the impact of TRIM59 knockdown on the sensitivity of SH-SY5Y cells to DOX. SH-SY5Y cells were transfected with either TRIM59 shRNA or shNC and then subjected to DOX (5 μg/mL) and Fer-1 (10 μM) treatment for 48 h. Subsequent assessments involved examining the proliferation and lipid ROS levels of SH-SY5Y cells. The results presented in Fig. 6 indicate that the proliferation of SH-SY5Y cells was reduced by DOX. Additionally, Fer-1 enhanced the proliferation of SH-SY5Y cells exposed to DOX, while

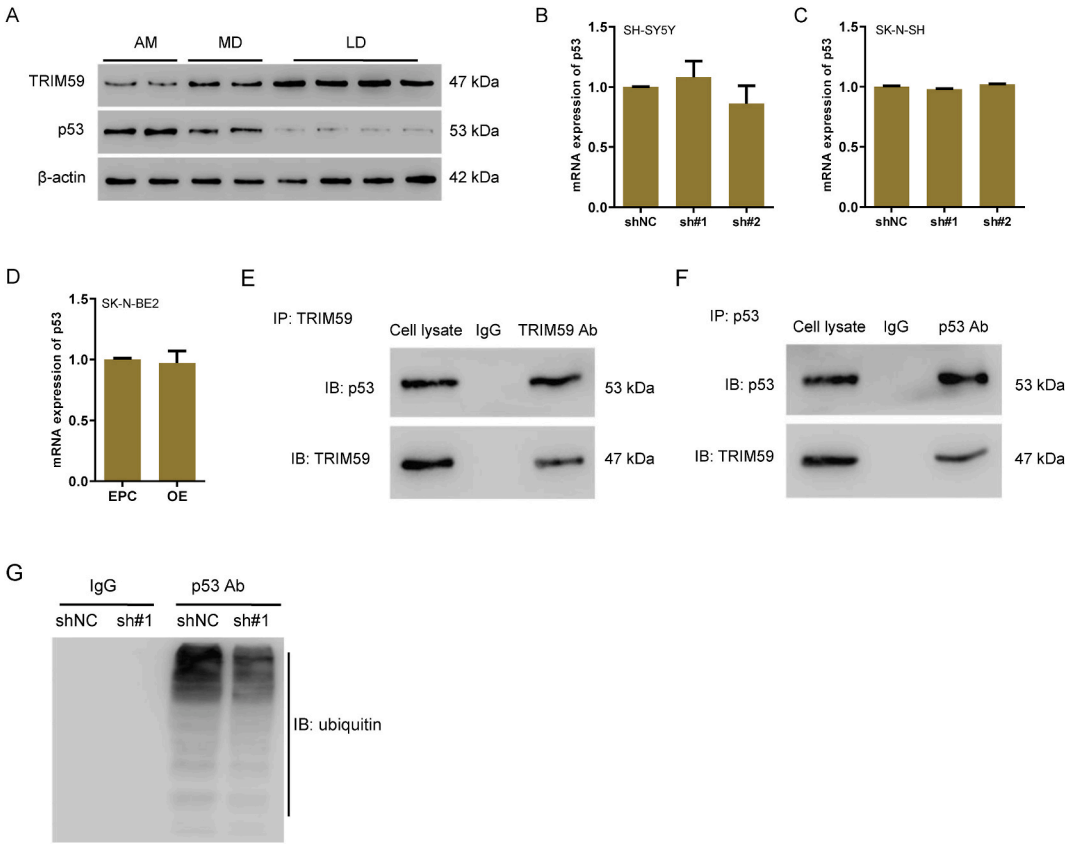

Fig. 4. Induction of degradation through ubiquitination by TRIM59. (A) Protein levels of TRIM59 and p53 in adjacent mediastinal (AM) ($n = 6$), moderately differentiated (MD) neuroblastoma tumor ($n = 6$), and poorly differentiated neuroblastoma tumor tissues ($n = 12$). (B–D) Unaltered mRNA expression in neuroblastoma cells despite TRIM59 knockdown or overexpression ($n = 3$). (E and F) Co-IP assays confirming a direct interaction between the TRIM59 and p53 proteins. (D) Reduction in ubiquitination and p53 degradation attributed to TRIM59 shRNA.

TRIM59 shRNA exerted an inhibitory effect. Notably, Fer-1 treatment counteracted the impact of TRIM59 shRNA on SH-SY5Y cells (Fig. 6A). Furthermore, the lipid ROS content displayed an inverse relationship with cell proliferation (Fig. 6B and C).

3.7. TRIM59 knockdown increases neuroblastoma cell sensitivity to DOX

In this section, we initiated TRIM59 knockdown in SH-SY5Y cells and established a xenograft mouse model. As illustrated in Fig. 7, both TRIM59 knockdown and DOX treatment contributed to a reduction in tumor growth (Fig. 7A and B). Furthermore, the combined effect of TRIM59 knockdown and DOX treatment improved the survival rates of 90-day nude mice (Fig. 7C). Moreover, tumor cell death was observed following TRIM59 knockdown and DOX treatment (Fig. 7D and E). In addition, TRIM59 knockdown resulted in an increase in p53 protein levels and a decrease in SLC7A11 and GPX4 protein levels (Fig. 7F–I). These results suggest that TRIM59 knockdown increases the sensitivity of neuroblastoma cells to DOX.

4. Discussion

Neuroblastoma stands out as one of the prevalent solid tumors in children, and ferroptosis, a distinct iron-dependent cell death pathway, deviates from apoptosis. Generally, antitumor medications induce ferroptosis as part of their therapeutic action [36]. This investigation reveals the pivotal role of TRIM59 in modulating the susceptibility of neuroblastoma cells to DOX through the mediation of ferroptosis. TRIM59 expression exerts a beneficial influence on neuroblastoma cell proliferation. Furthermore, TRIM59 overexpression demonstrated a capacity to reduce lipid ROS levels, whereas TRIM59 knockdown led to their elevation. Notably, TRIM59 protein levels exhibited an inverse correlation with neuroblastoma differentiation. Furthermore, our observations indicated that cell ferroptosis suppressing proteins SLC7A11 and GPX4 were negatively regulated by TRIM59. The downregulation of TRIM59 increased neuroblastoma sensitivity to DOX, both *in vitro* and *in vivo*. This mechanism is intricately linked to the inhibition of p53 ubiquitination and degradation.

TRIM proteins, functioning as E3 ligases, are implicated in diverse biological activities [27]. Their crucial roles in the regulating of cellular biological processes in tumor cells have been established through various studies [37]. Several studies reveal a reciprocal

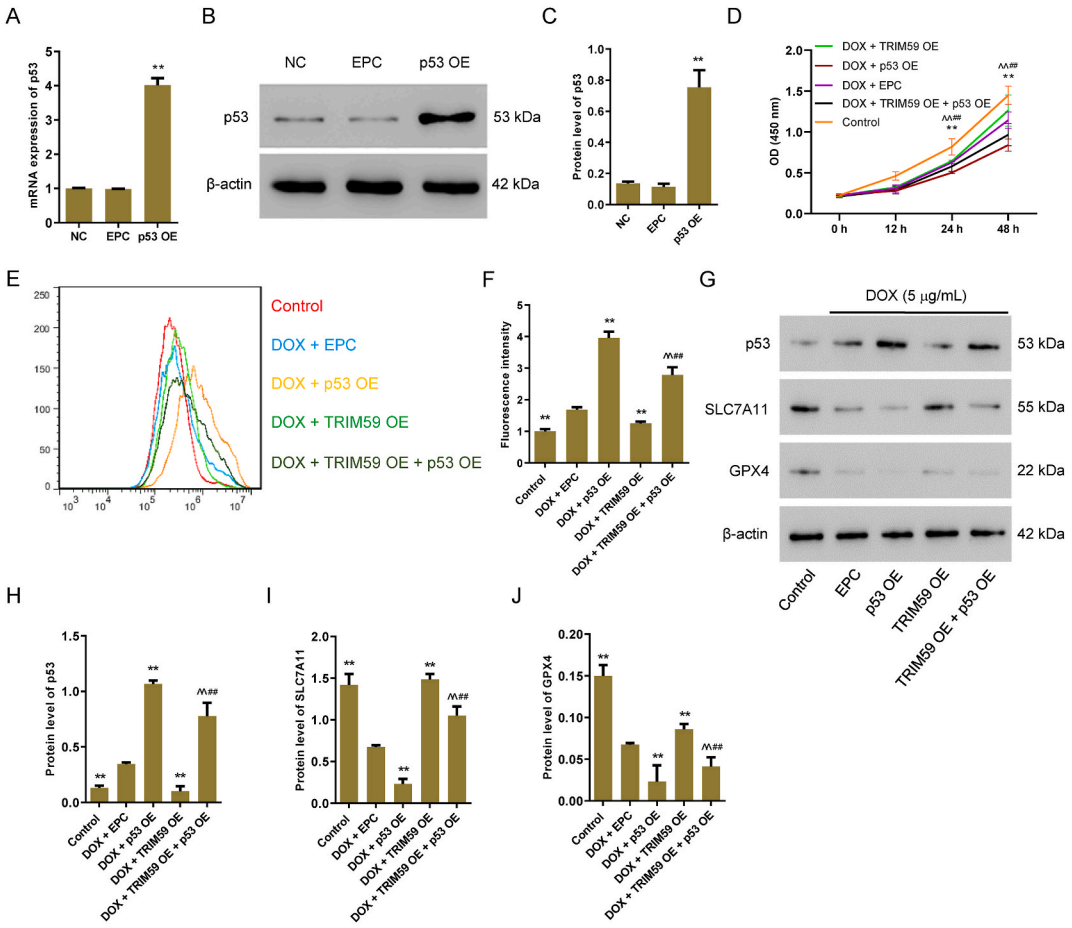

Fig. 5. Involvement of TRIM59 and p53 in neuroblastoma sensitivity to DOX. Evaluation of p53 overexpression vector efficiency through RT-PCR (A) and Western blotting (B and C). (D) Increased proliferation of SK-N-BE2 cells exposed to DOX (5 μg/mL) by TRIM59 overexpression and decreased proliferation by p53 overexpression. The efficiency of TRIM59 overexpression was partly abolished by p53 overexpression. (E and F) Induction of lipid ROS production in SK-N-BE2 exposed to DOX (5 μg/mL) induced by p53 overexpression and reduction by TRIM59 overexpression. The efficiency of TRIM59 overexpression is partly abolished by p53 overexpression. (G–J) Protein levels of p53, SLC7A11, and GPX4 in SK-N-BE2 cells. ANOVA and Tukey's post hoc test were used for statistical analysis among the groups. EPC, empty plasmid control; NC, normal control; p53 OE, p53 overexpression; TRIM59 OE, TRIM59 overexpression.$^{**}p < 0.01$ compared to EPC/(DOX + EPC). $^{\wedge\wedge}p < 0.01$ data in DOX + TRIM59 OE + p53 OE compared to DOX + TRIM59 OE. $^{\#\#}p < 0.01$ data in DOX + TRIM59 OE + p53 OE compared to DOX + p53 OE ($n = 3$).

relationship between p53 and TRIM proteins: p53 regulates the expression of certain TRIM proteins, while TRIM proteins modulate regulate p53 activity and levels. This regulatory interplay plays a pivotal role in influencing the biological behaviors of tumors and other diseases [38–40]. The inactivation of p53 signaling is a pivotal event in the formation and development of human cancers, making it an attractive target for cancer treatment [41]. TRIM59, identified as an oncogene in numerous tumor types, including breast cancer [42], non-small cell lung cancer [43], ovarian cancer [44], and retinoblastoma [45], and is implicated in various signaling pathways. The findings from this study elucidate that TRIM59 ubiquitinates and degrades the tumor suppressor p53. TRIM59 knockdown induces ferroptosis in neuroblastoma cells by enhancing p53 expression and lipid ROS generation, while decreasing the expression of SLC7A11 and GPX4. Interestingly, TRIM59 exhibits a dual role in neuroblastoma, being associated with neuroblastoma differentiation and promoting its development by reducing ferroptosis. However, TRIM59 knockdown induces ferroptosis and enhances neuroblastoma susceptibility to DOX. Furthermore, the combined treatment of TRIM59 knockdown and DOX exhibits improved therapeutic effects in xenograft mice.

Ferroptosis, a relatively recent form of cell death, is characterized by its dependence on iron and the accumulation of lipid peroxide [15]. Various pathways, including Nrf2, P53, heme oxygenase-1, BECN1, and FANCD2 regulate ferroptosis in tumors [46]. p53, a crucial regulator of ferroptosis, is known to be involved in various tumor processes, encompassing cell cycle arrest, senescence, apoptosis, genome stability maintenance, metabolism modulation, autophagy, and the recently identified ferroptosis [47]. p53 plays dual roles in ferroptosis induction under different conditions [13]. In this study, TRIM59 was found to directly interact with p53, promoting its ubiquitination and degradation. This interaction subsequently induced SLC7A11 and GPX4 expression, inhibiting ferroptosis. SLC7A11, recognized as a ferroptosis inhibitor, is overexpressed in numerous cancers. It functions as a cystine importer,

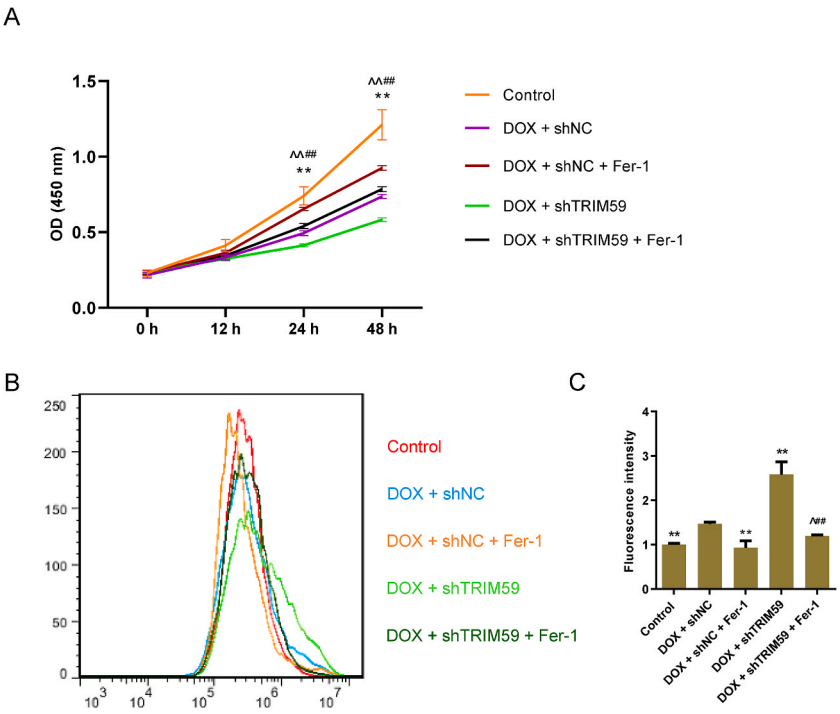

Fig. 6. TRIM59 knockdown increases ferroptosis and sensitivity of neuroblastoma to DOX. (A) Increased proliferation of SH-SY5Y cells exposed to DOX (5 μg/mL) with Fer-1 (10 μM) and decreased proliferation with TRIM59 knockdown. Partial abolition TRIM59 knockdown efficiency by Fer-1. (B and C) Reduction in lipid ROS levels in SH-SY5Y cells exposed to DOX (5 μg/mL) with Fer-1 (10 μM) and further decrease with TRIM59 knockdown. ANOVA and Tukey's post hoc test were used for statistical analysis among the groups. shNC, shRNA null control; shTRIM59, TRIM59 shRNA site 1. $^{**}p < 0.01$ compared to DOX + shNC, $^{\wedge\wedge}p < 0.01$ data in DOX + shTRIM59 + Fer-1 compared to DOX + shTRIM59. $^{\#\#}p < 0.01$ data in DOX + shTRIM59 + Fer-1 compared to DOX + shNC + Fer-1 ($n = 3$).

enhancing antioxidant defense and glutathione production [48]. SLC7A11 upregulation was associated with tumor formation through both ferroptosis-independent and ferroptosis-dependent mechanisms, and inhibiting SLC7A11 inhibition has been suggested as an effective tumor suppression strategy [49]. The present study demonstrated a reduction in the ferroptosis suppressors SLC7A11 and GPX4 by shTRIM59 in neuroblastoma, both *in vitro* and *in vivo*. Moreover, shTRIM59 improved neuroblastoma chemosensitivity to DOX, with the mechanism associated with the decreased ubiquitination and degradation of p53 and the induction of cell ferroptosis.

5. Conclusion

In summary, this study highlights the significance of TRIM59 in the advancement of neuroblastoma. Higher TRIM59 expression correlates with both tumor progression and chemoresistance in neuroblastoma. Diminishing TRIM59 levels may enhance neuroblastoma ferroptosis, thereby augmenting the sensitivity of neuroblastoma cells to DOX. Consequently, targeting TRIM59 emerges as a potentially valuable strategy in treating neuroblastoma.

Ethics approval statement

This study was approved by the Ethics Committee of the Children's Hospital of Fudan University (NO. 2021270). All methods were performed in accordance with the Declarations of Helsinki. Written informed consent was obtained from the parents.

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Data availability statement

The data used to support this work are available from the corresponding authors upon reasonable request.

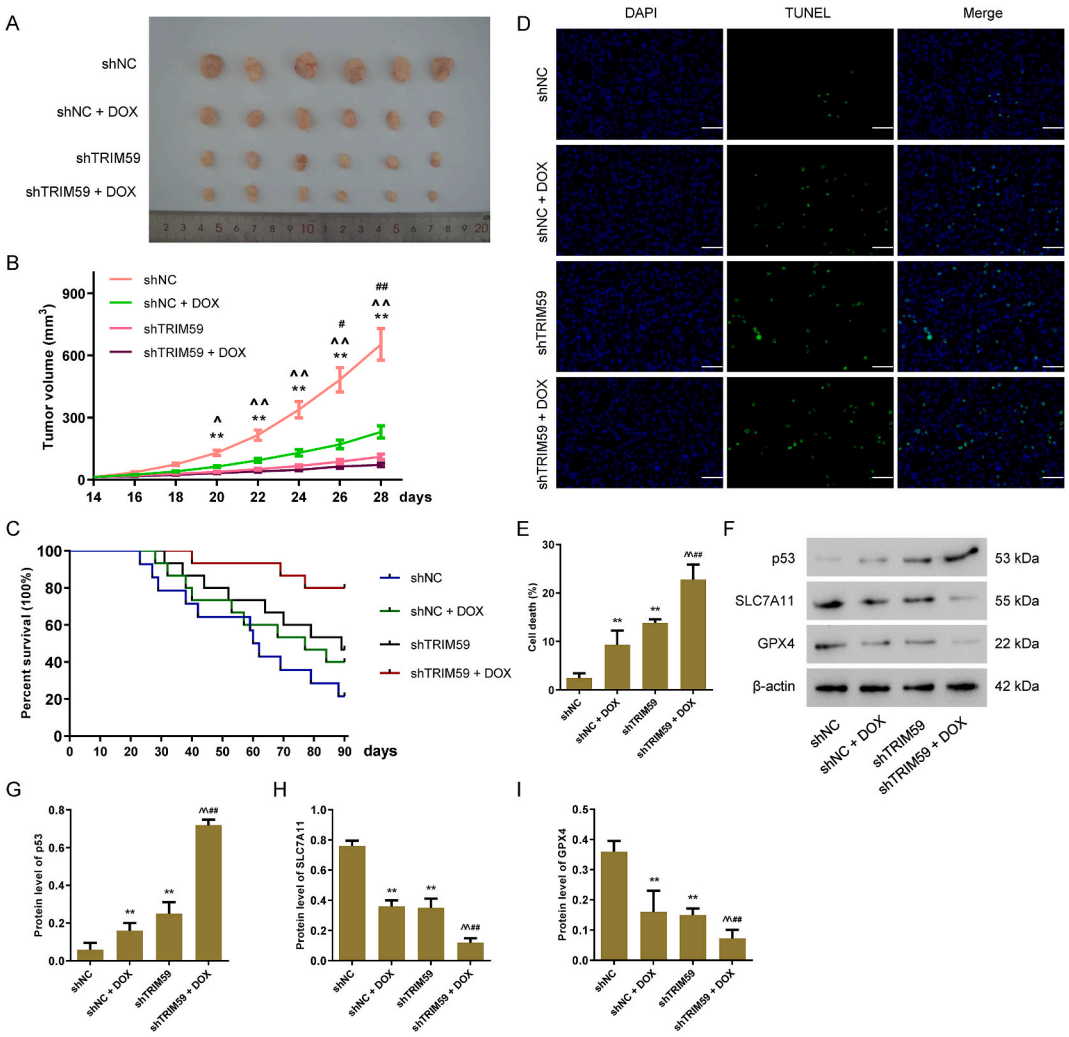

Fig. 7. Enhanced neuroblastoma sensitivity to DOX *in vivo* with TRIM59 knockdown. (A and B) Inhibition of tumor growth with TRIM59 knockdown and DOX treatment ($n = 6$). (C) Improved survival rates of 90-day nude mice with TRIM59 knockdown and DOX treatment ($n = 16$). (D and E) Induction of tumor cell apoptosis with TRIM59 knockdown and DOX treatment. Scale bar, 50 μm ($n = 6$). (F–I) The protein levels of p53, SLC7A11, and GPX4 in tumors. ANOVA and Tukey's post hoc test were used for statistical analysis among the groups. shNC, shRNA null control; shTRIM59, TRIM59 shRNA site 1. $\hat{p} < 0.05$, $\hat{\hat{p}} < 0.01$ data in shTRIM59 + DOX compared to shNC + DOX. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ data in shTRIM59 + DOX compared to shTRIM59.

CRediT authorship contribution statement

Yingbei Liu: Writing – review & editing, Writing – original draft, Project administration, Investigation, Formal analysis, Data curation. **Na Jiang:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Weicheng Chen:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Wenbo Zhang:** Software, Methodology, Formal analysis, Data curation. **Xiao Shen:** Writing – review & editing, Writing – original draft, Visualization, Software, Methodology. **Bing Jia:** Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Data curation, Conceptualization. **Gang Chen:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e26014.

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