



LncRNA PVT1 promotes cuproptosis through transcriptional activation of FDX1 in colorectal cancer

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ABSTRACT

Background: Cuproptosis, a copper-dependent form of regulated cell death, has emerged as a redox-sensitive vulnerability in cancer. However, the molecular basis by which this process is initiated and sustained in tumors remains poorly defined.

Methods: We investigated the functional role of FDX1 in cuproptosis in colorectal cancer through a series of *in vivo* and *in vitro* assays. Differential gene expression analysis and correlation studies were employed to identify long noncoding RNAs (lncRNAs) that regulate FDX1. Techniques such as molecular docking simulations, chromatin isolation by RNA purification (ChIRP), chromatin immunoprecipitation (ChIP), luciferase reporter assays, and bioinformatics analysis have elucidated the interactions and mechanisms between PVT1 and FDX1. The therapeutic potential of the PVT1-FDX1 axis was evaluated in a mouse xenograft model.

Results: FDX1 is upregulated in colorectal cancer and is indispensable for cuproptosis both *in vitro* and *in vivo*. The cuproptosis-related lncRNA PVT1 acts as a novel upstream regulator of FDX1. Mechanistically, PVT1 directly binds to the FDX1 promoter, increasing H3K27ac deposition and activating FDX1 transcription. Our findings also revealed that 35/98 nt of PVT1 bind to the −104/−41 bp region of the FDX1 promoter. Additionally, PVT1 was found to recruit SF1 to the FDX1 promoter, further enhancing FDX1 expression, leading to proteotoxic stress and ultimately triggering copper-dependent cell death. Clinically, PVT1 increases tumor sensitivity to cuproptosis by promoting FDX1 transcription.

Conclusions: We identify a novel regulatory axis in which PVT1 promotes cuproptosis by epigenetically activating FDX1 in colorectal cancer. Targeting the PVT1-FDX1 axis may offer an effective anticancer strategy, particularly given the widespread overexpression of PVT1 and its role in therapy resistance.

1. Background

Accumulating evidence has revealed that programmed cell death (PCD) subroutines are critical features of tumorigenesis and may ultimately lead to the establishment of different therapeutic strategies [1]. Recently, Tsvetkov et al. reported a novel PCD pathway induced by copper and its chelator, elesclomol, termed cuproptosis [2]. Cuproptosis is primarily induced by intracellular copper accumulation, which triggers the abnormal aggregation of mitochondrial lipoylated proteins and the destabilization of iron-sulfur (Fe-S) cluster proteins in the tricarboxylic acid (TCA) cycle, ultimately leading to proteotoxic stress and

cell death [2–4]. With the demonstration of cuproptosis, studies on cuproptosis have focused mainly on bioinformatics analysis and the development of biomaterial delivery systems for antitumor therapy based on cuproptosis [5–12]. These findings tentatively suggest the potential of cuproptosis as an effective antitumor therapy. However, given the presence of tumor heterogeneity, cancer therapy based on cuproptosis still lacks biological evidence and experimental validation.

Ferredoxin 1 (FDX1), an upstream regulator of protein lipoylation, is a vital gene that promotes cuproptosis [2]. Specifically, FDX1 is the direct target of elesclomol, which mediates cuproptosis by regulating mitochondrial protein lipoylation and reducing Cu²⁺ to its more toxic

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form, Cu^+ [2,13]. Previous studies have shown that FDX1 is critical for Fe-S cluster biosynthesis and steroid synthesis [14,15]. Sun et al. reported that lactylation of the non-histone protein METTL16 promoted the methylation of FDX1 mRNA, which mediated cuproptosis in gastric cancer [16]. FDX1 is also stabilized by ferroptosis inducers (FINS) and promotes cuproptosis in liver cancer cells [17]. Nevertheless, the regulatory mechanism of FDX1 is still inconclusive, and whether the upstream regulators of FDX1 can be used to formulate individualized cuproptosis-based tumor therapies needs to be explored.

Emerging evidence suggests that long noncoding RNAs (lncRNAs) play important roles in tumor onset, development, and metastasis [18, 19]. The lncRNA plasmacytoma variant translocation 1 (PVT1) is frequently amplified and associated with tumorigenesis in various types of cancer [20–25]. In colorectal cancer (CRC), PVT1 is significantly upregulated and strongly correlated with poor prognosis [26]. PVT1 may also broadly affect the expression and function of essential genes in the TGF β /SMAD and Wnt/ β -catenin pathways, affecting cancer cell proliferation, migration, and invasion [27]. PVT1 could be a promising prognostic biomarker and a potential therapeutic target for CRC therapy [28]. However, limited research has been conducted on PVT1 regulation by mitochondrial ferredoxin. Whether PVT1 can modulate FDX1 and thus influence cuproptosis in CRC remains unknown, and the detailed molecular regulatory mechanisms still need to be investigated.

Here, we demonstrate that PVT1 is a critical mediator of cuproptosis in colorectal cancer and that the PVT1-FDX1 axis is a sensitive indicator of cuproptosis-based therapy. Moreover, the lncRNA PVT1, a novel upstream target of FDX1, mediates cuproptosis by transcriptionally activating FDX1. In detail, PVT1 recruits the transcriptional regulator SF1 to the FDX1 promoter and increases H3K27 acetylation to initiate the transcription of FDX1. Notably, our study revealed an innovative PVT1-FDX1 regulatory axis in cuproptosis, which is essential for regulating copper homeostasis within cancer cells, and its dysregulation contributes to tumorigenesis. The utilization of cuproptosis to treat cancers characterized by high PVT1 expression holds significant promise as a therapeutic strategy.

2. Methods

2.1. Cell lines and clinical samples

The human colorectal cancer cell lines HCT116 cat. CCL-247, HCT8 cat. CCL-244, SW480 cat. CCL-228, SW620 cat. CCL-227, DLD-1 cat. CCL-221, HT-29 cat. HTB-38 and the immortalized colon epithelial cell lines NCM460 cat. CP-H04. and FHC cat. CRL-1831 were obtained from the ATCC (Manassas, VA). All cell lines were subjected to short tandem repeat (STR) analysis and mycoplasma testing. These cell lines were cultured in DMEM (Gibco, Carlsbad, CA, USA) or RPMI 1640 (Gibco, Carlsbad, CA, USA) supplemented with 10 % fetal bovine serum (FBS, Gibco) and antibiotics. All these cells were maintained at 37 °C with 5 % CO_2 . Colorectal cancer patient samples were collected from the First Affiliated Hospital of Zhengzhou University (Henan Province, China). Clinical specimens were frozen in liquid nitrogen for subsequent use immediately after excision.

2.2. Antibodies and reagents

HSP70 (Proteintech, Cat #10995-1-AP, 1:2000), FDX1 (Proteintech, Cat #12592-1-AP, 1:1000), LIAS (Proteintech, Cat #11577-1-AP, 1:2000), DLAT (Proteintech, Cat #13426-1-AP, 1:2000), β -actin (Proteintech, Cat #81115-1-RR, 1:5000), Lipoic Acid (Santa Cruz Biotechnology, sc-101354, 1:1000), Caspase3 (Proteintech, Cat #19677-1-AP, 1:2000), SDHB (Solarbio, Cat #K107471P, 1:8000), H3K27ac (ABclonal, A7253, 1:2000) were used in our experiments.

Elesclomol (MedChemExpress, Cat #HY-12040), Etoposide (MedChemExpress, Cat #HY-13629), DMSO (Sigma-Aldrich, Cat #D2650), tetrathiomolybdate (TTM) (Sigma-aldrich, Cat#323446), z-VAD-FMK

(MedChemExpress, Cat #HY-16658B), Ferrostatin-1 (MedChemExpress, Cat #HY-100579), Chloroquine (CQ) (Selleck, Cat #S6999), MG149 (Selleck, Cat #S7476), CuCl_2 (Sigma-Aldrich, Cat #751944) were used in our experiments.

2.3. Plasmid construction and transfection

The plasmid of shRNA targeting PVT1, shRNA targeting SF1, pcDNA3.1-PVT1 and empty vector were purchased from YouBio (Changsha, China). The plasmid of pcDNA3.1-PVT1 del, pcDNA3.1-FDX1, shRNA targeting FDX1 and their control were ordered from HanBio Technology (Shanghai, China). For luciferase reporter assay, the plasmid of pRL-TK, pGL3-basic-NC, pGL3-FDX1, pcDNA3.1-NC, pGL3-FDX1 del were ordered from HanBio Technology (Shanghai, China).

Transfections were performed with Lipofectamine™ 2000 reagent (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's protocol. The medium was replaced with a fresh culture medium after 5 h. Cells were then examined 24–48 h after transfection.

2.4. Cell viability and apoptosis assay

A CCK8 assay kit (Vazyme Biotech, Nanjing, China) was used to measure cell viability. The cells in the logarithmic growth phase were seeded in a 96-well plate at a density of 5000 cells per well. The indicated concentrations of the test compounds were added at least 24 h after plating in triplicate for each condition. CCK-8 solution was added directly to the cells. To estimate the viability of the cells, the absorbance at 450 nm (OD_{450}) was measured with a 96-well plate reader (PerkinElmer, Waltham, MA).

For the colony formation assays, equal numbers of cells after different treatments were plated into 6-well plates. Colonies were visualized by crystal violet staining 14 days after plating. Then, the colonies were fixed with 4 % paraformaldehyde and stained with crystal violet (Beyotime, Shanghai, China). The percent area of crystal violet staining was quantified with ImageJ, or the number of colonies was assessed manually. The pictures were imported into the software, and the color and parameters were set. Finally, the software was used to calculate the specific number of colonies. The relative colony number was calculated as the number of colonies divided by the average number of clones in the control group.

2.5. Immunofluorescence

The cells were seeded on slides for 24 h and treated with 4 % paraformaldehyde in PBS for 15 min. After three times washing with PBS, 0.2 % Triton X-100 was added and incubated for 20 min to permeabilize cells. After blocking for 1 h, membranes were incubated with primary antibody at 4 °C overnight, followed by incubation with corresponding secondary antibody (rabbit anti-mouse IgG labelled with FITC) for 90 min. Next, cell nuclei were stained with DAPI solution (Beyotime, Shanghai, China) for 5 min under dark conditions. Fluorescence signals were observed using a fluorescence microscope with appropriate filters (LSM880 FastAiry, Carl Zeiss).

2.6. Real-time quantitative PCR (real-time PCR)

Total RNA was extracted from CRC tissues and cells using Trizol reagent (Vazyme, Nanjing, China) following the instructions of the manufacturer. Approximately 1 μg of total RNA was reverse transcribed to cDNA using a reverse transcriptase cDNA synthesis kit (Novoprotein, Shanghai, China). Subsequently, quantitative PCR reactions were performed using SYBR Green PCR Master Mix (Vazyme, Nanjing, China). The quantitative PCR primer sequences were presented in [Supplementary Table 1](#).

2.7. Dual-luciferase reporter assay

293T cells were seeded in 96-well plates and grown to 70 %–80 % confluence. The cells were transiently transfected with plasmids. After 48h, the cells were lysed, and the firefly and Renilla luciferase activities were determined using the Dual-Luciferase® Reporter Assay System (HanBio Technology, Shanghai, China) following the instructions of the manufacturer. The luciferase activity was normalized to pRL-TK activity. Data are represented as fold induction after normalizing the luciferase activity of the tested sample to the corresponding control sample.

2.8. Subcellular fractionation

Nuclear and cytoplasmic protein extraction was performed with the Nuclear and Cytoplasmic Protein Extraction Kit (Beyotime, Shanghai, China) in accordance with manufacturer's instructions. Wash and digest the adherent cells with PBS, centrifuge and discard the medium and supernatant, and retain the cell pellet. Add 200 μ L of cytoplasmic and nuclear protein extraction reagent A containing RNase Inhibitor to each 20 μ L of cell pellet, vortex for 5 s and ice bath. Add 10 μ L of cytoplasmic and nuclear protein extraction reagent B, vortex for 5 s and ice bath, centrifuge at 4 °C. Take the supernatant as the cytoplasmic fraction, retain the pellet as the nuclear fraction, and extract RNA with Trizol. Add equal amounts of cytoplasmic RNA and nuclear RNA to Real-Time PCR, use optimal primers and simplified formula to analyze the results. nuclear % = $2^{-\text{nuclear CT value}} / (2^{-\text{cytoplasmic CT value}} + 2^{-\text{nuclear CT value}})$, cytoplasmic % = 1 - nuclear %.

2.9. DNA and RNA molecular docking simulation

PyMOL v2.5.4 was used to predict double-stranded DNA 3D structures. 3dRNA/DNA Server (<http://biophy.hust.edu.cn/new/3dRNA/>) was used to predict RNA 3D structures [29]. HNADOCK [30] was applied for PVT1-FDX1 molecular docking. The binding interface of model 1 (Fig. 4C), the best binding model for FDX1-PVT1 complexes, was fully characterized and systematically analyzed, and the interaction-related details were supplemented by PyMOL v2.5.4 to form a 3D interaction map.

2.10. Immunohistochemistry staining

For IHC, slides were retrieved by EDTA-Citrate Antigen Retrieval Solution (Beyotime, Shanghai, China). Briefly, 1 % hydrogen peroxide was used to block the endogenous peroxidase activity. The slides were incubated with primary antibodies anti-FDX1 (1:100), anti-LIAS (1:100), anti-SDHB (1:200), anti-Lip-DLAT (1:500), anti-HSP70 (1:100) overnight at 4 °C and then incubated with secondary antibodies conjugated to peroxidase-labelled dextran polymer according to the manufacturer's instructions. A light microscope was used to observe the slides. All IHC results were read blindly by two independent researchers.

2.11. Chromatin isolation by RNA purification (CHIRP) assay

First, the cells were crosslinked, lysed on ice and sonicated in an ice bath for 3 s on, 5 s off, and 35 % power was applied until the sample was clear. Agarose beads were added to the sonicated sample, which was subsequently incubated at 4 °C for binding. The mixture was subsequently centrifuged, and the supernatant was removed for precleaning. The probe and magnetic beads were prepared according to the manufacturer's instructions. Finally, hybridization buffer and DTT, protease inhibitor, or RNase inhibitor were added to the sample, which was denatured at 65 °C and hybridized at 37 °C. The mixture was added to the magnetic beads, which were incubated at room temperature for binding, and subsequent experiments were performed.

2.12. RNA immunoprecipitation (RIP) assay

The RIP assays were performed via the BersinBio RIP Kit (BersinBio, Guangzhou, China) according to the manufacturer's instructions. Briefly, the cells were lysed in lysis buffer containing a protease inhibitor cocktail and RNase inhibitor, and the lysates were immunoprecipitated with antibodies against H3K27ac, SF1 or normal rabbit IgG (Proteintech, Wuhan, China). Finally, the retrieved RNAs were quantified via qPCR. The PVT1 primers used for amplification are listed in [Supplementary Table 1](#).

2.13. Chromatin immunoprecipitation (ChIP) assay

ChIP assays were performed via a ChIP assay kit (Beyotime, Shanghai, China) following the provided reagents and protocol. The colorectal cells were crosslinked with formaldehyde for 10 min, which was terminated with glycine solution for 5 min at room temperature. The cells were subsequently incubated on ice before being lysed in SDS lysis buffer. NaCl was added to the cell lysates at 65 °C for 4 h to remove cross-links between protein and genomic DNA. The immunoprecipitates were probed with antibodies against H3K27ac, SF1 or normal rabbit IgG (Proteintech, Wuhan, China) overnight at 4 °C and protein A + G agarose/salmon sperm DNA. We subsequently washed the DNA-protein complex three times at 4 °C. The sequences of the primers used for FDX1 in this study are listed in [Supplementary Table 1](#).

2.14. Bioinformatic analysis

The expression of FDX1 in normal colorectal tissues and colorectal cancer (CRC) tissues, as well as the correlation between FDX1 expression and prognosis in CRC patients, were analyzed using the GEPIA and Kaplan-Meier Plotter databases.

For the cuproptosis-related lncRNAs, transcriptomic data from 473 colon adenocarcinoma (COAD) tumors and 41 normal samples were obtained from TCGA (<http://cancergenome.nih.gov/>). A total of 14,036 lncRNAs and 19,173 mRNAs were separated using Strawberry Perl (<http://www.perl.org/>). After data merging, 401 COAD patients were included in the study. We collected 18 cuproptosis-related mRNAs (FDX1, DLD, PDHA1, PDHB, MTF1, GLS, CDKN2A, DLAT, LIAS, LIPT1, LIPT2, ATP7A, ATP7B, SLC31A1, SLC31A2, DLST, NFE2L2, and NLRP3) from existing literature, and extracted the expression levels of these cuproptosis-related genes (CRGs) in COAD samples. After the Pearson correlation algorithm with the filter of $|\text{coefficient}| > 0.2$ and $p < 0.001$, we selected lncRNAs that were related with CRGs and considered as cuproptosis-related lncRNAs (CRLs). The differential expression of cuproptosis-associated lncRNAs between 473 colorectal cancer tumors and 41 normal tissues was then compared using differential analysis by R package "limma", resulting in the identification of 1042 differentially expressed cuproptosis-associated lncRNAs ($|\text{Log2 fold change (FC)}| > 1$, false discovery rate (FDR) < 0.05).

For the prognostic lncRNAs associated with colon cancer, univariate Cox regression and LASSO regression analyses were performed to identify those with prognostic significance.

CHIPbase database was used to analyze co-expression analysis of PVT1 and FDX1. The localization of PVT1 was predicted using the lncATLAS database. CatRAPID and PROMO databases were used to predict the proteins that bind to PVT1 and the transcription factors that regulate FDX1. LongTarget was used to predict lncRNA-DNA interactions and RPISeq for predicting lncRNA-protein interactions.

2.15. Xenograft studies

Briefly, we performed two groups of xenograft tumor experiments. The first group included 20 BALB/c female nude mice aged 4–5 weeks. HCT116 WT- or shVector- or shFDX1-transfected HCT116 cells were injected subcutaneously into the flank region of the mice (5 mice per

group, 1×10^6 cells/100 μ L per flank). The second group included 15 BALB/c mice subjected to the same conditions described above. Stably transfected PVT1-wt/del or PVT1-wt + shFDX1 HCT116 cells were injected into the flank region of the mice (5 mice per group, 1×10^6 cells/100 μ L per flank). PBS (100 μ L) or Elesclomol + CuCl₂ (10 mg/kg, Cu²⁺ 0.1 mg/kg) was peritumorally injected into the mice every 2 days for 14 days when the tumor volume reached approximately 100 mm³. The weights and volumes of the tumors were measured and calculated after collection. Tumors were resected, photographed, and weighed at the end of the experiments.

2.16. Statistical analysis

The data represent the means $\pm$ SDs of triplicate experiments. Statistical analyses were performed via GraphPad Prism 9.0 software (GraphPad, San Diego, USA). Comparisons between two groups were performed via unpaired two-tailed Student's *t* tests. Comparisons among three or more groups were performed via ordinary one-way analysis of variance (ANOVA). Differences were regarded as statistically significant when the *P* value was less than 0.05.

3. Results

3.1. Elesclomol and CuCl₂ induced FDX1-dependent cuproptosis in colorectal cancer cells

To test whether elesclomol combined with CuCl₂ can induce colon cancer cuproptosis, a group of colon cancer cell lines (HCT116, HCT8, SW480, SW620, DLD1 and HT29) were exposed to different concentrations of elesclomol for different time intervals to assess their viability. We first determined the optimal concentration of elesclomol-CuCl₂ for treatment by testing different doses on six colon cancer cell lines. We found that elesclomol-CuCl₂ reduced cell viability in a dose- and time-dependent manner (Fig. 1A and B). Additionally, our results demonstrated that HCT116 and HCT8 cells were sensitive to the treatment (Fig. 1A). These two cell lines were selected as representative examples for further analysis. In addition, treatment with different concentrations of elesclomol and 1 μ M CuCl₂ decreased the colony-forming ability of HCT116 and HCT8 cells to reduce cell proliferation (Fig. 1C). Furthermore, we confirmed that elesclomol-CuCl₂ indeed induced cuproptosis in colorectal cancer cells by detecting the hallmarks of cuproptosis (Supplementary Fig. S1A–G). In addition, elesclomol-induced cell death did not involve either caspase 3 cleavage or activation (Supplementary Fig. S1F). Furthermore, treatment with the pancaspase inhibitor Z-VAD-FMK, ferroptosis inhibitor ferrostatin-1, and autophagy inhibitor CQ failed to prevent cell death, indicating that elesclomol-induced cell death is distinct from other known cell death pathways (Fig. 1D and E). However, this effect was reversed when cells were treated with the copper ion chelator TTM. Together, these data suggest that elesclomol-CuCl₂ induces colon cancer cell death via cuproptosis.

In a paper published in Science, Tsvetkov et al. reported that the knockout of seven genes, including FDX1, rescued the killing effect of elesclomol and disulfiram. In our study, we found that FDX1 expression was markedly increased in colon cancer tissue samples compared with adjacent normal tissue samples from patients (Fig. 1G). FDX1 expression was upregulated in the GEPIA database (Fig. 1F). Furthermore, FDX1 expression was higher in human colon cancer cell lines (HCT116, HCT8, SW480, SW620, DLD1, and HT29) than in normal colonic epithelial cell lines (FHC and NCM460) at both the mRNA and protein levels (Fig. 1H, Supplementary Fig. S1G and S1H).

Next, we further investigated the essential role of FDX1 in the process of cuproptosis in colorectal cancer. We found that FDX1 knockdown significantly reversed the elesclomol- and CuCl₂-induced decrease in cell viability (Fig. 1I and J), whereas FDX1 overexpression markedly reduced this decrease (Supplementary Fig. S1I and S1J). FDX1 deficiency abolished the copper-induced downregulation of the Fe-S cluster

protein and abrogated the lipoylation and oligomerization of DLAT (Fig. 1K–M). Intriguingly, we also corroborated these results by immunofluorescence, revealing that elesclomol treatment markedly induced the formation of DLAT foci, whereas such foci were diminished in FDX1-silenced cells (Fig. 1N). Moreover, HSP70, the protein toxicity stress marker, was markedly inhibited by FDX1 knockdown (Fig. 1O and P, Supplementary Fig. S1K). These changes are characteristic features of cuproptosis and indicate that FDX1 deficiency attenuated the cuproptotic process. Overall, these findings indicate that FDX1 is an indispensable regulator of cuproptosis in colon cancer cells.

3.2. Loss of FDX1 *in vivo* impairs cuproptosis in colorectal cancer

To validate the therapeutic effects of FDX1-mediated cuproptosis *in vivo*, a nude mouse xenograft model was established via the subcutaneous injection of shFDX1 HCT116 cells. As shown in Fig. 2A–C, tumor growth was markedly inhibited in the ES-Cu²⁺ and ES-Cu²⁺+shVector groups but not in the ES-Cu²⁺+shFDX1 group, as indicated by a reduction in tumor volume and weight. In addition, the body weights of the nude mice tended to be stable, with no significant changes in the organs, indicating the biosafety of cancer therapy based on cuproptosis (Fig. 2D).

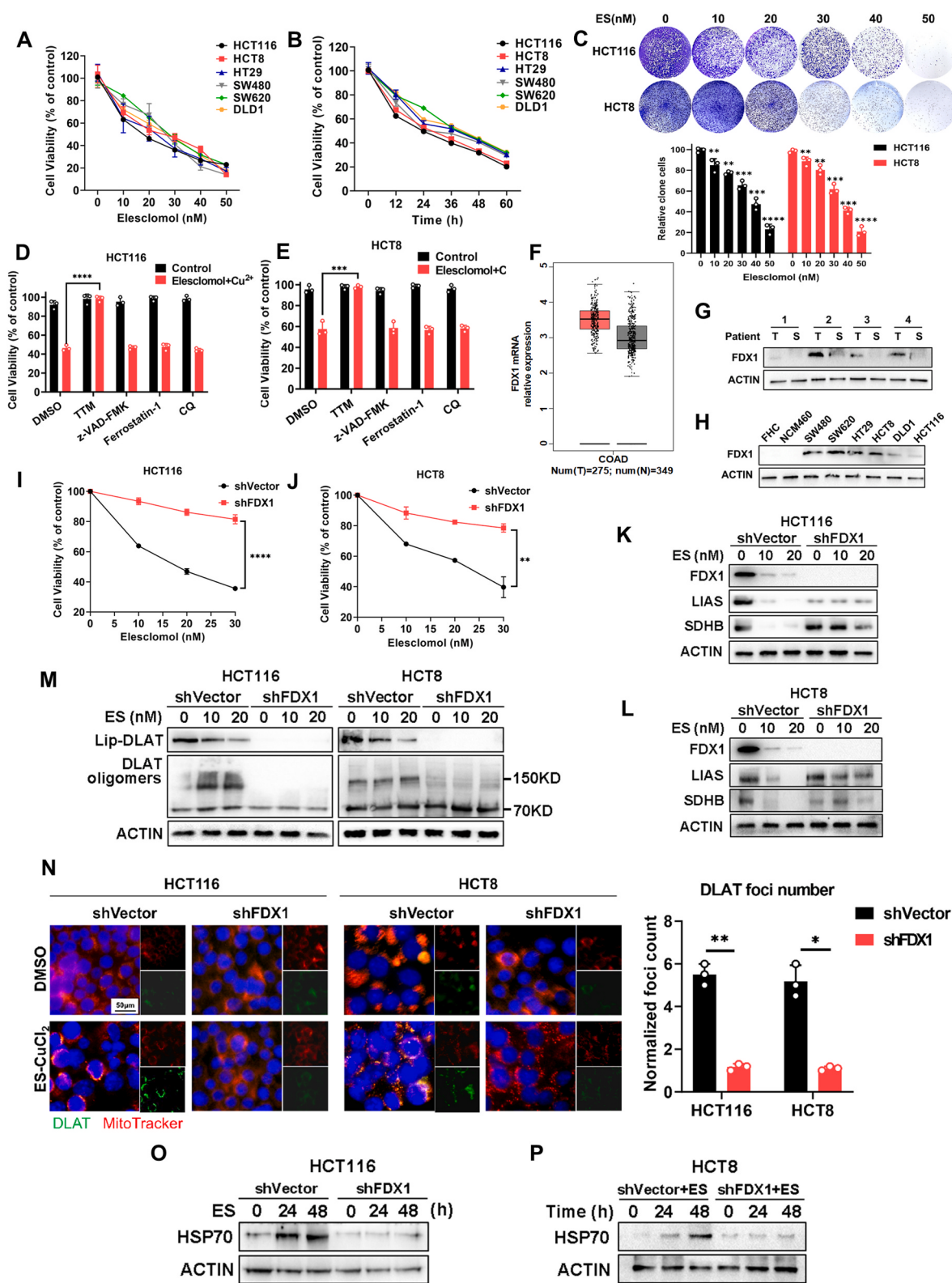
Next, we explored the changes in the expression of the hallmark molecules of cuproptosis. Consistent with our *in vitro* experiments, elesclomol-CuCl₂ treatment resulted in decreased expression of Fe-S cluster proteins (LIAS, SDHB) and lipoylated DLAT, whereas HSP70 was elevated (Fig. 2E–F, Supplementary Fig. S2A) in the tumors. In contrast, FDX1 knockdown counteracted this effect. The IHC results further confirmed these results (Fig. 2G–H, Supplementary Fig. S2B). Collectively, these results suggest that the cuproptosis induced by elesclomol and copper has promising anticancer therapeutic effects, further confirming that FDX1 is a critical mediator of cuproptosis.

3.3. PVT1 promoted cuproptosis in CRC by regulating FDX1 transcription

In light of the aforementioned findings, we further investigated the upstream regulatory mechanisms of FDX1. Studies have revealed that lncRNAs play pivotal roles in regulating diverse gene expression and cellular processes [31]. Therefore, as described in the Materials and Methods section, we screened 1042 differentially expressed cuproptosis-related lncRNAs (CRLs) in colorectal cancer, and the top 5 up- and downregulated CRLs were identified (Fig. 3A, Supplementary data 3). By intersecting the differentially expressed CRL set and 23 prognostic lncRNAs in CRC, we determined that PVT1 and AL035071.2 may be mediators of cuproptosis in CRC (Fig. 3B, Supplementary data 4). To further verify this result, the CHIPBase database was used to analyze the correlation between these two potential lncRNAs and FDX1 expression. We detected a positive correlation between PVT1 and FDX1, whereas AL035071.2 was not significantly correlated with FDX1 (Fig. 3C). Moreover, the expression levels of PVT1 and FDX1 were significantly greater in 14 pairs of CRC tissues than in normal colorectal tissues (Supplementary Fig. S3A and S3B), and there was a positive association between PVT1 and FDX1 (Fig. 3D). Therefore, PVT1, as a potential regulator of FDX1, influences the occurrence of cuproptosis in CRC.

Next, we further investigated the role of PVT1 in cuproptosis. As shown in Fig. 3E and F, PVT1 overexpression resulted in a more significant decrease in LIAS and Lip-DLAT with elesclomol-CuCl₂ treatment, and the copper ion chelator TTM alleviated this effect. Moreover, PVT1 overexpression enhanced DLAT oligomerization following elesclomol and CuCl₂ treatment (Fig. 3G). These results indicated that PVT1 promoted cuproptosis in CRC cells.

To further explore how PVT1 regulates FDX1 and thus influences cuproptosis, we knocked down PVT1 in HCT116 and HCT8 cells and examined the expression level of FDX1. As shown in Fig. 3H–J and Supplementary Fig. S3D, PVT1, but not AL035071.2, knockdown



(caption on next page)

Fig. 1. FDX1 is indispensable in cuproptosis in CRC cells. **A and B**, Cell viability of CRC cell lines treated with 1 μM Cu^{2+} and different concentrations of elesclomol (**A**) or different time intervals of 20 nM elesclomol (**B**) was measured by CCK-8 assay. **C**, Colony formation assay of HCT116 and HCT8 cells treated with different concentrations of elesclomol and 1 μM Cu^{2+} . Quantification of crystal violet staining of (**A**). **D and E**, CCK-8 assay of cell viability of HCT116 (**D**) and HCT8 (**E**) cells treated with different cell death inhibitors. **F**, Expression status of FDX1 in colorectal cancer. The expression levels of FDX1 in colorectal cancer (COAD) and normal colon tissues (Normal) were retrieved from the GEPIA database. Data are presented as box plots. **G**, Expression levels of FDX1 protein in patient tissues. **H**, Expression levels of FDX1 protein in various cell lines. **I and J**, Effect of FDX1 knockdown on cell viability of HCT116 (**I**) and HCT8 cells (**J**). The x-axis shows the different concentrations (nM) of elesclomol treatment, and the y-axis shows the relative cell viability (%). **K and L**, Effect of FDX1 overexpression on the level of iron-sulfur cluster proteins in HCT116 (**K**) and HCT8 cells (**L**) treated with different concentrations of elesclomol and 1 μM CuCl_2 . **M**, Western blot analysis of lipoylated DLAT and oligomerized DLAT in HCT116 cells and HCT8 cells with the same treatment as (K and L). **N**, shVector/shFDX1 HCT116 and HCT8 cells were treated with 20 nM elesclomol and 1 μM CuCl_2 ; DLAT protein oligomerization was analyzed after 24 h by immunofluorescence images (DLAT - green, Mitotracker-red). Foci were segmented and quantified in each condition. **O and P**, Western blot analysis of HSP70 in shVector/shFDX1 HCT116 (**O**) and HCT8 cells (**P**) treated with different concentrations of elesclomol and 1 μM CuCl_2 . Statistical data presented in this figure show mean values $\pm$ SD of three times of independent experiments. Statistical significance was determined by Two-tailed *t*-test, *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

significantly reduced the protein and mRNA levels of FDX1. In contrast, PVT1 overexpression reversed the decrease in FDX1 (Supplementary Fig. S3C and S3E). Next, a dual-luciferase reporter assay revealed that PVT1 knockdown markedly decreased the luciferase activity of the FDX1 promoter-containing pGL3 reporter vector, whereas PVT1 overexpression increased it (Fig. 3K and Supplementary Fig. S3F). In addition, the transcriptional inhibitor ActD significantly suppressed the increase in FDX1 expression caused by PVT1 overexpression (Fig. 3L and M). Collectively, these data suggest that PVT1 promotes cuproptosis by transcriptionally activating FDX1.

3.4. PVT1 directly bound to the -104/-41 bp region of the FDX1 promoter and initiated transcription

To further clarify the potential mechanisms underlying the regulation of FDX1 by PVT1, we conducted a nuclear-cytoplasmic fractionation assay to determine the subcellular location of PVT1 in HCT116 cells. As shown in Fig. 4A–B and Supplementary Fig. S4A, PVT1 was located mainly in the nucleus. Using the LongTarget tool, we found that -104 to -41 bp (R1 region) of the FDX1 promoter sequence was the potential binding region with PVT1 (Fig. 4C). ChIRP assays subsequently revealed that biotin-labelled PVT1 could capture the R1 region (-104/-41 bp) of the FDX1 promoter in particular (Supplementary Fig. S4B and Fig. 4D). Furthermore, we generated an FDX1 promoter R1 region deletion-containing pGL3B reporter vector (FDX1 del) and cotransfected it with PVT1 ectopic expression into 293T cells. The results revealed that the stimulatory effects of PVT1 on luciferase activity were attenuated when the binding sequences in the promoter area were deleted (Fig. 4E).

We next explored the sites where PVT1 binds to the FDX1 R1 region and their effects. According to predictions made by LongTarget, the 35/98 nt region of PVT1 is regarded as the site of binding to the FDX1 R1 region. As shown in Fig. 4F–I, deleting the binding region (35/98 nt) in PVT1 eliminated FDX1 luciferase activity and mRNA expression. These data indicate that 35/98 nt of PVT1 bound to the -104/-41 bp region of the FDX1 promoter and enhanced FDX1 transcription.

In addition, molecular docking simulations were performed for binding site confirmation. The results suggested that there are more than one hundred potential patterns of 35/98 nt PVT1 binding to the R1 region of FDX1. The top ten models and docking scores are shown in Supplementary Table 2. The top three binding models were selected on the basis of their docking scores for hydrogen bonding and hydrophobic interactions from the docking results and are displayed in Fig. 4J. Here, we present a model with the highest docking score, which further confirmed the prediction and experimental results of the binding mode of PVT1 to FDX1 (Fig. 4K).

3.5. H3K27 acetylation is required for PVT1-driven FDX1 transcription

Moreover, we elucidated how PVT1 enhances the transcriptional activity of FDX1. RIP and ChIRP assays revealed that PVT1 bound to histone H3, which suggests that PVT1 is localized in the nucleus to function with chromatin (Fig. 5A–B). Acetylation of lysine 27 of histone

H3 (H3K27ac) is known to be an active genetic enhancer. Here, we found that H3K27ac bound to PVT1, which implied that PVT1 may activate transcription by increasing H3K27ac modification in the FDX1 promoter region (Fig. 5B–C, Supplementary Fig. S5A). A ChIP assay with H3K27ac antibodies confirmed that H3K27ac was deposited in the FDX1 promoter region (Fig. 5D). In addition, we found that PVT1 knockdown reduced the enrichment of H3K27ac at the FDX1 promoter (Fig. 5E). Furthermore, MG149, a general inhibitor of histone acetyltransferases, suppressed FDX1 mRNA levels and inhibited the increase in FDX1-luciferase activity caused by PVT1 overexpression (Fig. 5F–H). These results revealed that PVT1 promotes FDX1 transcription by enhancing the acetylation of H3K27 in the FDX1 promoter, which in turn activates the transcription of FDX1.

3.6. PVT1 increased FDX1 transcription via the recruitment of SF1

To further elucidate the transcription factors involved in PVT1 regulation of FDX1 transcription, the catRAPID and PROMO databases were used to predict the proteins that bind to PVT1 and the transcription factors that regulate FDX1 (Supplementary data 5 and Supplementary data 6). The analysis revealed that SF1 is a potential transcription factor associated with PVT1-driven regulation of FDX1 (Fig. 6A). Moreover, we observed the upregulation of SF1 expression in colon cancer, as evidenced by the GEPIA database, and this upregulation was associated with poor prognosis in colon cancer patients (Supplementary Fig. S6A and S6B). The ChIPBase database also revealed a positive correlation between PVT1 and SF1 (Supplementary Fig. S6C). Additionally, the RPISeq database predicted a high probability of interaction between PVT1 and SF1 (Fig. 6B).

To further validate the interaction between PVT1 and SF1, we employed two methods. First, the ChIRP assay, followed by silver staining and western blot analysis, demonstrated the interaction between the SF1 protein and the PVT1 antisense probes. Second, the RIP assay revealed that a significantly greater amount of PVT1 precipitated in the SF1 antibody group than in the IgG group (Fig. 6D). On the basis of these findings, we hypothesized that SF1 is recruited by PVT1 as a transcription factor that participates in FDX1 transcription.

To gain further insights into the role of SF1 in the regulation of FDX1 and the involvement of PVT1 in this process, SF1-knockdown cell lines were established to assess FDX1 expression. The results revealed a decrease in both the mRNA and protein levels of FDX1 upon SF1 knockdown (Fig. 6E–G). Furthermore, SF1 knockdown significantly reduced FDX1-luciferase activity, suggesting that SF1 activates FDX1 gene transcription (Fig. 6I). In the ChIP assay, we observed a remarkable enrichment of the FDX1 promoter in the SF1-bound complex (Fig. 6H). Moreover, PVT1 knockdown led to a significant reduction in SF1 enrichment at the FDX1 promoter, whereas PVT1 overexpression reversed this reduction (Fig. 6J–K). Collectively, these findings provide evidence that PVT1 recruits the transcription factor SF1 to initiate FDX1 transcription.

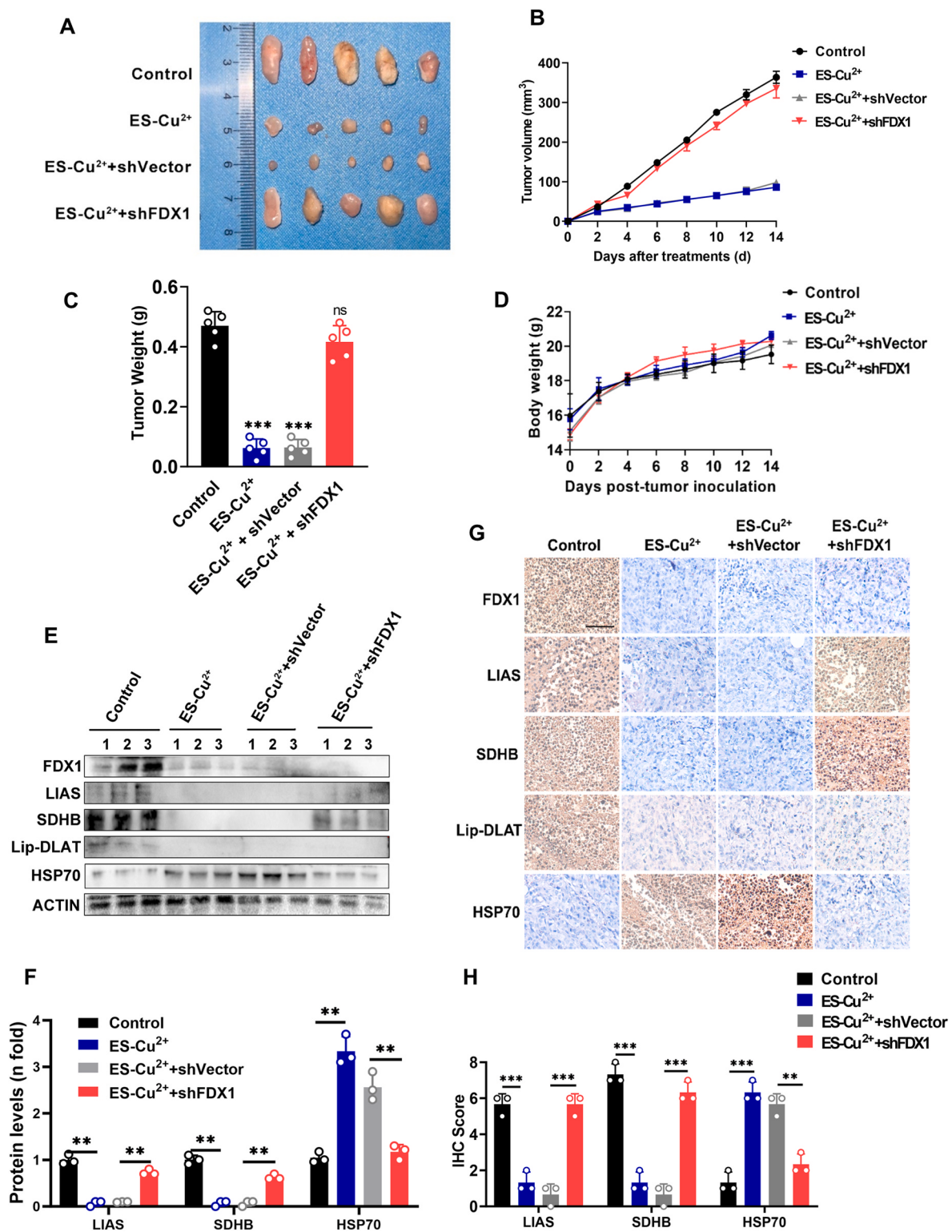


Fig. 2. Potential tumor therapeutic role of FDX1-dependent cuproptosis *in vivo*. **A and B**, (A) Representative mouse pictures, (B) tumor volume, (C) tumor weight and (D) body weights of the mouse at the indicated time points after different treatments ($n = 5$). **E**, The expression of cuproptosis-related proteins in tumor tissues. **F**, Quantitative analysis of LIAS, SDHB and HSP70 expression in Fig. 2G. **G and H**, Representative images of immunohistochemical (IHC) staining of cuproptosis-related protein in tumor tissues (G) and quantification analysis of LIAS, SDHB and HSP70 in (G), scale bar, 50 μ m. Statistical data presented in this figure show mean values $\pm$ SD of three times of independent experiments. Statistical significance was determined by Two-tailed *t*-test, *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

3.7. PVT1-SF1-FDX1 axis-mediated cuproptosis is a promising therapeutic strategy

We aimed to further investigate the role of the PVT1-SF1-FDX1 axis

in cuproptosis in CRC cells. To investigate the role of PVT1 in cuproptosis, we knocked down PVT1 in HCT116 and HCT8 cells and treated them with elesclomol-CuCl₂. As expected, PVT1 knockdown restored cell viability compared with that in the shVector group (Fig. 7A–B).

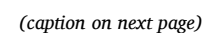


Fig. 3. PVT1 transcriptionally activates FDX1 expression and promotes cuproptosis. **A**, The volcano plot of cuproptosis-related lncRNAs (CRLs) in COAD, in which the red dots indicated upregulated lncRNAs, the green dots indicated down-regulated lncRNAs and black dots indicated lncRNAs that were no significant difference (left panel). The top 5 up/down-regulated CRLs were listed in the right panel. **B**, An overlap between 1040 CRLs and 21 prognostic lncRNAs in CRC. **C**, Pearson correlation between PVT1 or AL035071.2 and FDX1 in CRC tissue analyzed by the CHIPbase database. **D**, Pearson's correlation analysis was applied to assess the correlation between PVT1 expression and FDX1 expression in CRC tissues ($n = 14$). **E–G**, Impact of PVT1 overexpression on cuproptosis. In panels **E** and **F**, PVT1 was overexpressed in HCT116 (**E**) and HCT8 (**F**) cells treated with 20 nM elesclomol and 1 μ M CuCl₂, with or without 20 μ M TTM for 24 h. LIAS and Lip-DLAT were analyzed by western blot. Panel **G** shows DLAT protein oligomerization in similarly treated HCT116 and HCT8 cells, assessed by immunofluorescence (DLAT-green, Mitotracker-red). **H**, Effect of PVT1 knockdown on the level of FDX1 proteins. **I** and **J**, Effect of PVT1 knockdown on the expression of FDX1 mRNA. The mRNA level of FDX1 was measured by RT-qPCR after knockdown of PVT1 in HCT116 (**I**) and HCT8 (**J**) cells. **K**, Luciferase reporter plasmid containing the FDX1 promoter was co-transfected with PVT1 knockdown plasmid in 293T cells for 24 h followed by dual luciferase activity assay. **L** and **M**, Impact of transcriptional inhibitor on PVT1's regulation of FDX1. In **L**, HCT116 and HCT8 cells transfected with or without a PVT1 overexpression plasmid for 24 h were treated with or without actinomycin D (1 mg/ml) for 12h before Western blot analysis of FDX1. In **M**, after 24 h of PVT1 overexpression, cells were treated with actinomycin D; FDX1 mRNA levels were then assessed at various times by RT-qPCR. Statistical data presented in this figure show mean values $\pm$ SD of three times of independent experiments. Statistical significance was determined by Two-tailed *t*-test, *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Moreover, the deletion of PVT1 inhibited the loss of iron–sulfur cluster proteins, such as LIAS and SDHB, while suppressing DLAT oligomerization and lipid acylation (Fig. 7C–D, Supplementary Fig. S7A and S7B). Furthermore, we investigated the role of SF1 in cuproptosis by knocking down SF1. As depicted in Fig. 7E–F, the knockdown of PVT1-recruited SF1 rendered CRC cells insensitive to cuproptosis. Consistently, we also observed a reversal of the key molecules involved in cuproptosis after SF1 knockdown (Fig. 7G–Supplementary S7C and S7D). Finally, we conducted rescue experiments by overexpressing FDX1 in PVT1- or SF1-knockdown cells. Importantly, we found that overexpressing FDX1 restored cell viability (Fig. 7H–I) and reduced DLAT oligomerization following the deletion of PVT1 or SF1. Notably, simultaneous overexpression of FDX1 reversed this effect (Fig. 7J–K). Taken together, these findings indicate that the PVT1-SF1-FDX1 axis is an effective target for cancer therapy involving cuproptosis.

3.8. The PVT1-FDX1 axis is a sensitivity indicator for cuproptosis-based therapies

To investigate whether PVT1 regulates FDX1 transcription and promotes FDX1-dependent cuproptosis-mediated antitumor effects, we conducted xenograft experiments in nude mice treated with elesclomol and CuCl₂. Following elesclomol and CuCl₂ treatment, tumors derived from cells transfected with full-length PVT1 (PVT1 wt) exhibited reduced volume and weight, accompanied by lower Ki67 staining (Fig. 8A–D). To confirm whether this effect results from PVT1 binding to the FDX1 promoter and subsequently upregulating its transcription, we introduced a deletion construct lacking the FDX1 promoter-binding region (PVT1 35/98 nt del). As shown in Fig. 8A–D, the absence of 35/98 nt of PVT1 abolished the growth-suppressive effect under the same treatment conditions, as reflected by increased tumor volume and elevated Ki67 positivity. In addition, to determine whether this regulatory effect is specifically mediated through FDX1, FDX1 was simultaneously silenced in cells from the PVT1 wt group (PVT1 wt+shFDX1). Silencing FDX1 expression abolished the tumor-suppressive effect in PVT1 wt group, supporting the notion that FDX1 is essential for PVT1-mediated sensitivity to cuproptosis. These results demonstrated that PVT1, through its binding to the FDX1 promoter, promoted tumor sensitivity to elesclomol and CuCl₂ treatment.

In addition, we found that the expression of lip-DLAT and FDX1, the hallmarks of cuproptosis, was lower in the PVT1 35/98 nt del and PVT1 wt + shFDX1 groups than in the PVT1 wt group (Fig. 8C–E). These findings suggest that the tumor inhibitory effect mediated by the PVT1-FDX1 axis is dependent on cuproptosis. Moreover, compared with that in the PVT1 wt group, FDX1 mRNA in tumor tissues was clearly decreased in the PVT1 35/98 nt del group (Fig. 8F). ChIRP experiments also revealed that only PVT1 in the PVT1 wt group exhibited a significant capacity to bind the FDX1 promoter but not in the PVT1 35/98 nt del group (Fig. 8G–H and Supplementary S8A–D). These results further support the notion that PVT1 facilitates the transcription of FDX1 and enhances tumor sensitivity to cuproptosis-based therapy.

4. Discussion

Cuproptosis, a recently described copper-dependent form of regulated cell death, represents a promising target in oncology due to elevated copper levels observed in various tumors [32–35]. In this study, we identified the oncogenic lncRNA PVT1 as a key upstream regulator of cuproptosis in CRC. Mechanistically, PVT1 facilitates H3K27 acetylation at the FDX1 promoter region, which in turn promotes the recruitment of the transcription factor SF1, ultimately leading to transcriptional activation of FDX1. Functionally, tumors with high PVT1 expression exhibit increased sensitivity to cuproptosis, indicating that PVT1 may serve as a biomarker for cuproptosis responsiveness. Therefore, exploiting the endogenous overexpression of PVT1 in tumors may serve as a promising strategy to identify malignancies with increased sensitivity to cuproptosis-based therapy.

FDX1 has been identified as a core mediator of cuproptosis [2]. Our results confirm that FDX1 is essential for cuproptosis in CRC, aligning with findings from previous studies [16,36]. This finding prompted us to explore its upstream regulatory mechanisms. FDX1 was initially reported to be regulated by the NR5A and cAMP signaling pathways [37]. With recent advances in cuproptosis research, the complex regulatory network of FDX1 has been revealed. Transcriptionally, the transcription factor YY1 strongly suppresses FDX1 expression in ovarian and liver cancers [38,39]. Post-transcriptionally, non-coding RNAs, such as miR-21-5p, circKIAA1797, and LINC02362 modulate FDX1 mRNA stability in renal, lung, and liver cancers [40–42], while METTL16 promotes mRNA stabilization through m⁶A methylation [16]. At the protein level, AKT1 modifies FDX1 through phosphorylation and lipoylation [43], and ferroptosis inducers affect FDX1 protein stability [17]. In our study, we identified a novel transcriptional regulation of FDX1 in CRC during cuproptosis, with the oncogenic lncRNA PVT1 functioning as the key regulator (Fig. 3). PVT1 binds directly to the –104/–41 bp region of the FDX1 promoter. This is accompanied by increased H3K27 acetylation at the promoter (Figs. 4 and 5).

We next sought to identify transcription factors responsible for PVT1-driven FDX1 transcription. In this study, we first identified steroidogenic factor 1 (SF1) as a transcription factor that activates FDX1 transcription, thereby promoting cuproptosis in CRC (Fig. 6). Notably, SF1 is a member of the NR5A family that regulates steroid hormone biosynthesis [44–47]. FDX1 is also involved in mitochondrial steroid biosynthesis [37]. This functional consistency supports the plausibility of SF1 as a regulator of FDX1. The PVT1-mediated regulation of FDX1 transcription involves a complex and multilayered mechanism, some aspects of which remain to be further elucidated. Future research will focus on identifying histone acetyltransferases and H3K4me1-marked enhancers to uncover additional potential enzyme therapeutic targets and improve our understanding of the spatial organization of gene regulation. In addition, investigation of the cis-regulatory motifs mediating the interaction between SF1 and the FDX1 promoter may help refine our understanding of the precise transcription factor binding landscape.

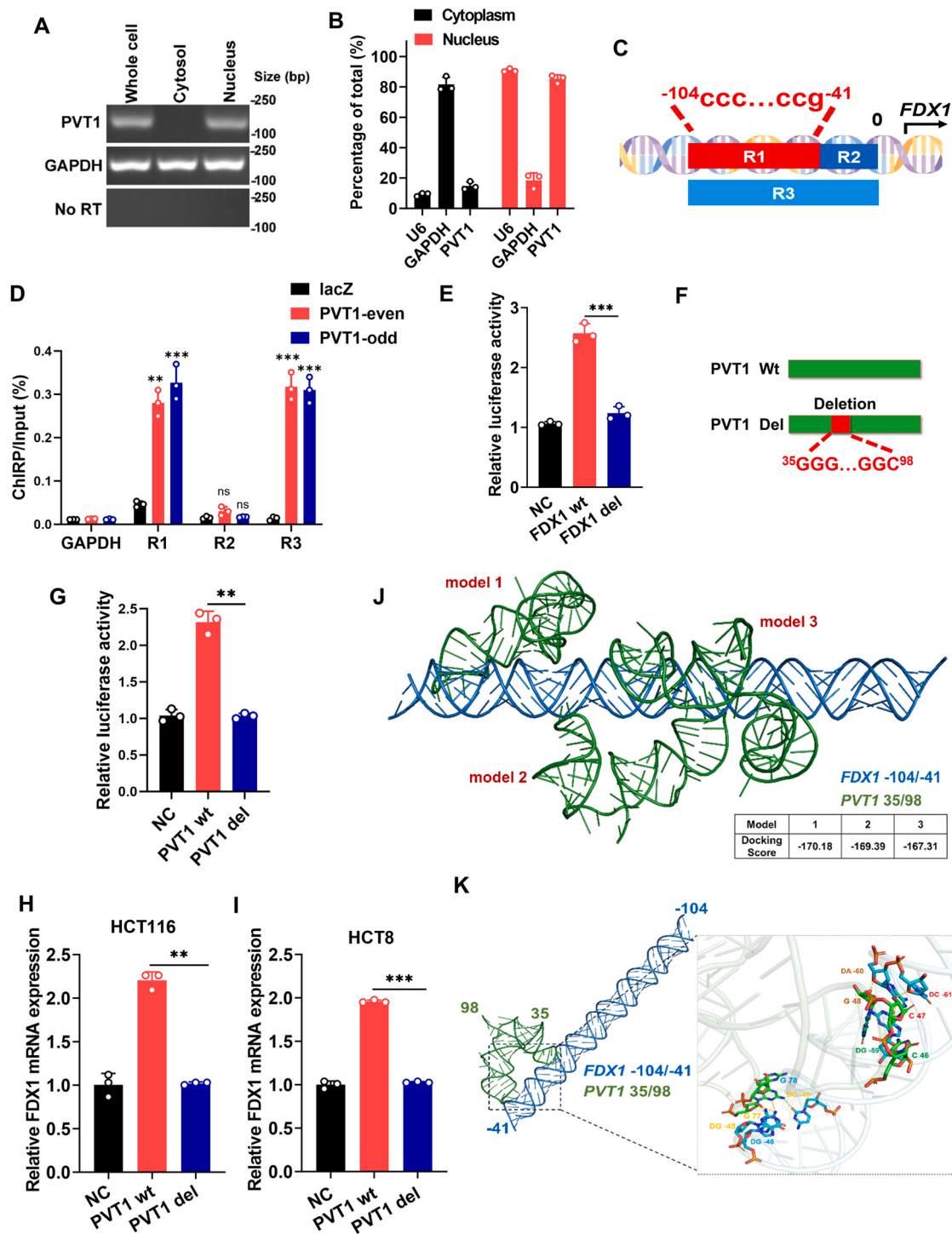


Fig. 4. PVT1 binds to the FDX1 promoter and promotes FDX1 transcription. **A and B,** A nuclear-cytoplasmic fractionation assay was conducted to determine the subcellular distribution of PVT1 in HCT116 cells, with levels of U6, GAPDH, and PVT1 assessed via qRT-PCR in both fractions. U6 served as the nuclear RNA control, while GAPDH was the cytoplasmic RNA control. **C,** Schematic representation of PVT1 binding sites in FDX1 promoter predicted by Longtarget database. **D,** ChIP-qPCR analysis of PVT1 enrichment on the FDX1 promoter region. PVT1 bound to the FDX1 promoter R1 region (−104 to −41 bp). **E,** Wild-type FDX1-luc (FDX1 wt-luc) or its truncated form with the potential PVT1 binding site deleted (FDX1 del-luc) were transfected into 293T cells with PVT1 overexpression vector co-transfection followed by luciferase activity measurement. **F,** Schematic representation of the PVT1 deletion binding sites predicted by Longtarget database. **G,** The dual luciferase reporter assay was performed to detect the luciferase activity of the FDX1 promoter after transfection of PVT1 wild type (PVT1 wt) or PVT1 deletion mutant (PVT1 del) into 293T cells. **H and I,** HCT116 (**H**) and HCT8 cells (**I**) transfected with PVT1 wt or PVT1 del were subjected to RT-qPCR analysis to examine the FDX1 mRNA level. **J,** HANDOCK server predictions for the representative models of PVT1 (35/98 nt) combined with R1 region of FDX1. The top three models and their docking scores were displayed. **K,** Optimal model of PVT1 (35/98 nt) bound to FDX1's R1 region predicted by the HANDOCK server is shown, with PVT1 in green and FDX1 in blue. Yellow dashed lines highlight hydrogen bonds between DNA and RNA bases, with matching colors indicating binding sites. Statistical data presented in this figure show mean values ± SD of three times of independent experiments. Statistical significance was determined by Two-tailed *t*-test, *, *p* < 0.05; **, *p* < 0.01; ***, *p* < 0.001.

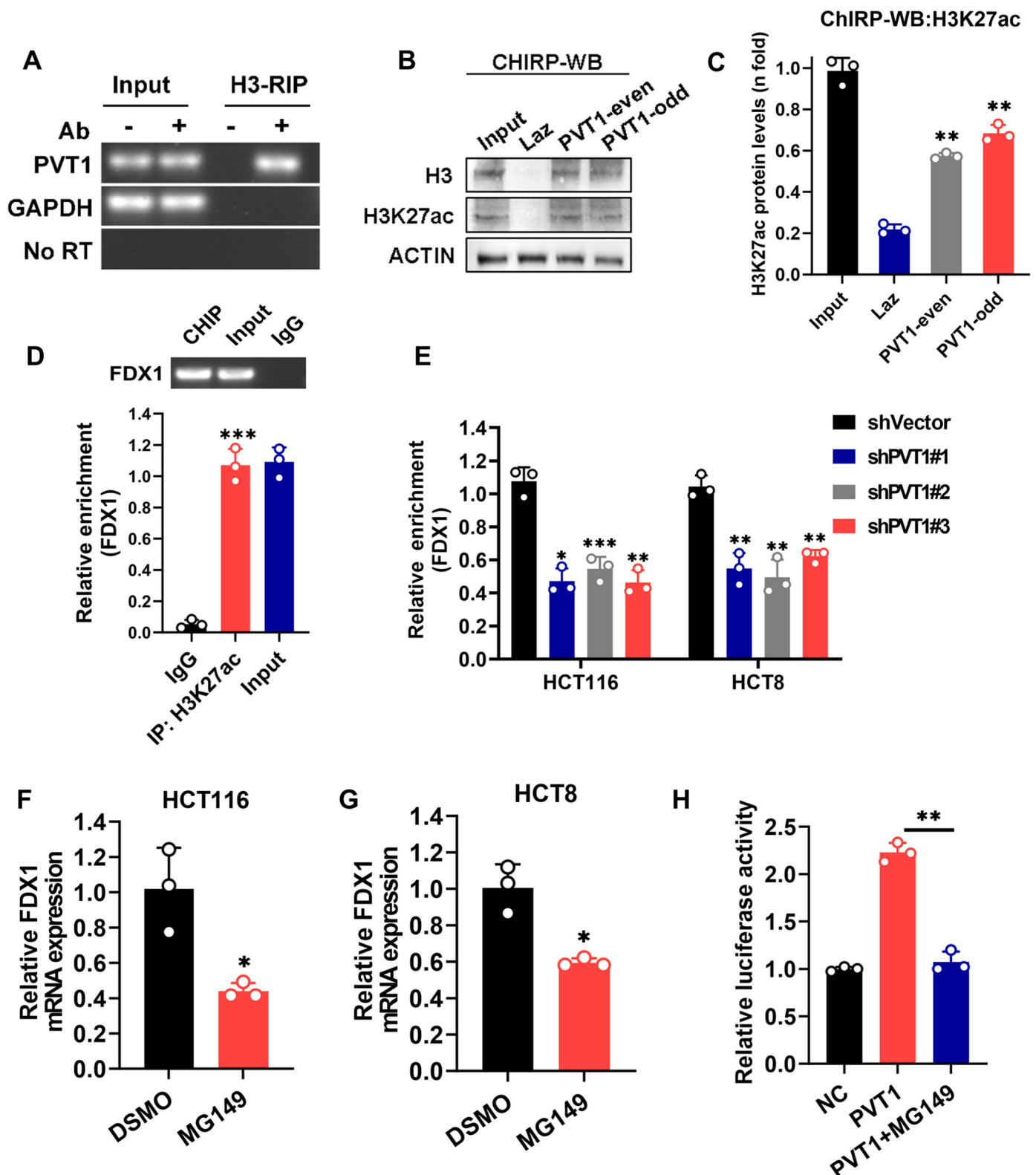


Fig. 5. PVT1 increases histone H3K27ac level on the FDX1 promoter. **A**, RIP experiments were performed using the histone 3 (H3) antibody, and specific primers were used to detect the enrichment of PVT1. **B**, ChIRP-WB assay was performed to detect the binding effect between PVT1 and H3 and H3K27ac. **C**, Quantitative analysis of H3K27ac expression in Fig. 5B. The error bars represent $\pm$ SEM across, $n = 3$ replicates. **C and E**, ChIP-qPCR assay for FDX1 was performed with antibody against H3K27ac or control IgG with (E) or without (C) PVT1 knockdown. **F and J**, HCT116 (F) and HCT8 (J) cells were treated with the inhibitor of histone acetyltransferases MG149 (1 μ M) or DMSO for 24 h. FDX1 mRNA levels were measured by RT-qPCR and normalized to DMSO-treated cells. **H**, With or without MG149 treatment for 24h, relative luciferase expression in 293T cells transfected with a PVT1 overexpression vector and either a vector containing luciferase only. Statistical data presented in this figure show mean values $\pm$ SD of three times of independent experiments. Statistical significance was determined by Two-tailed t -test, *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

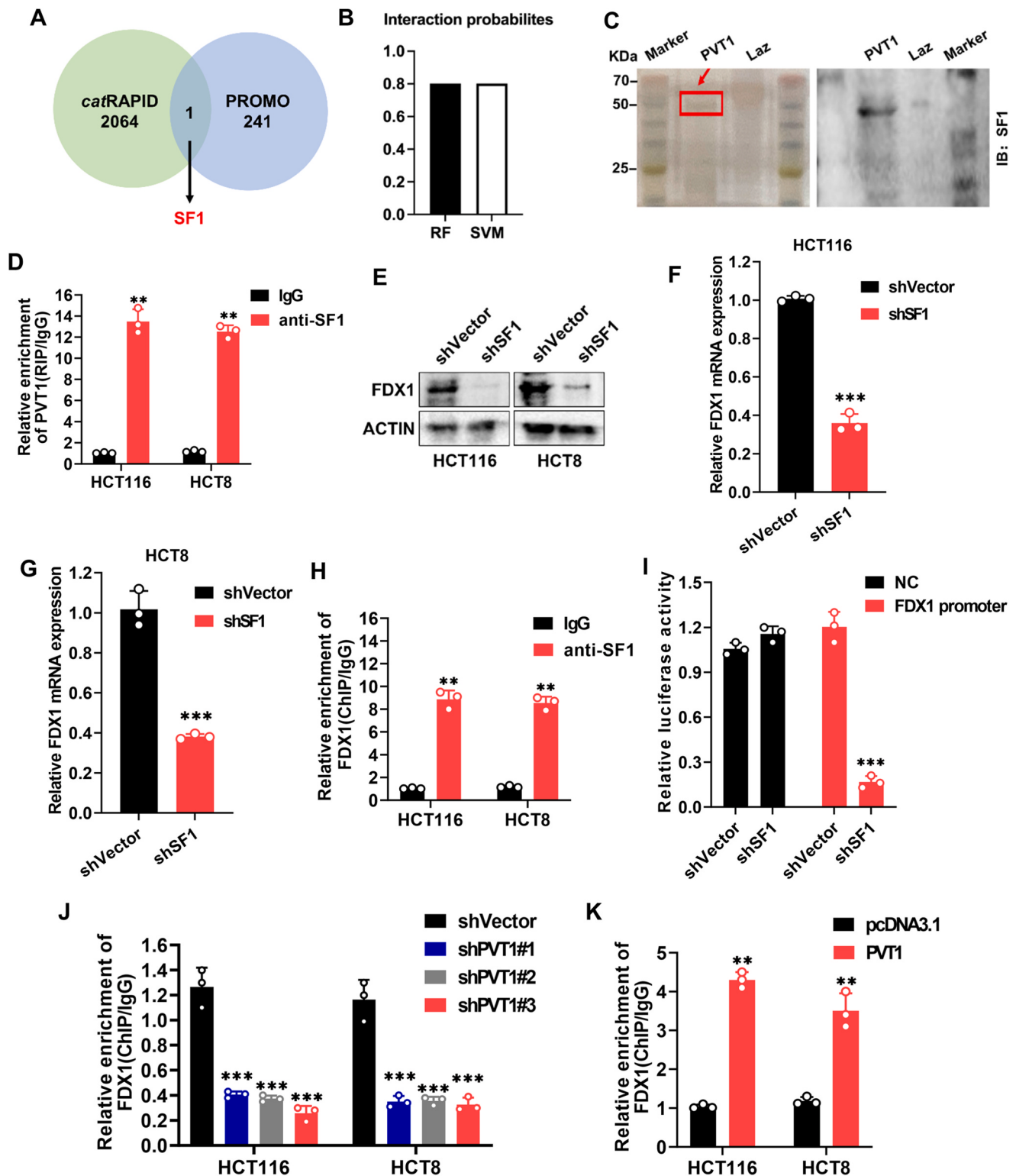


Fig. 6. PVT1 recruits the transcription factor SF1 to participate in FDX1 transcription. **A**, The Venn diagram showed the overlap of the proteins binding to PVT1 by catRAPID and transcription factors of FDX1 predicted by PROMO ChIPBase. **B**, The interaction probabilities between PVT1 and SF1 were predicted by the RNA-Protein Interaction Prediction (RPISeq) website. **C**, Biotinylated antisense oligonucleotide probes captured PVT1 and its binding proteins, and WB and silver staining detected the binding protein products. **D**, RIP experiments were performed using the SF1 antibody, and specific primers were used to detect the enrichment of PVT1. **E-G**, The protein level of FDX1 was detected by western blot after the knockdown of SF1 (**E**); The mRNA level of FDX1 was measured by qRT-PCR after the knockdown of SF1 in HCT116 (**F**) and HCT8 (**G**) cells. **H**, ChIP-qPCR assay was performed with antibody against SF1 or control IgG in HCT116 and HCT8 cells. Immunoprecipitated FDX1 was analyzed by RT-qPCR. **I**, Luciferase reporter plasmid containing the FDX1 promoter was co-transfected with SF1 knockdown plasmid in 293T cells for 24 h followed by dual luciferase activity assay. **J and K**, ChIP-qPCR assay for FDX1 was performed with antibodies against SF1 or control IgG in HCT116 and HCT8 cells with PVT1 knockdown (**J**) or overexpression (**K**). Statistical data presented in this figure show mean values $\pm$ SD of three times of independent experiments. Statistical significance was determined by Two-tailed *t*-test, *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

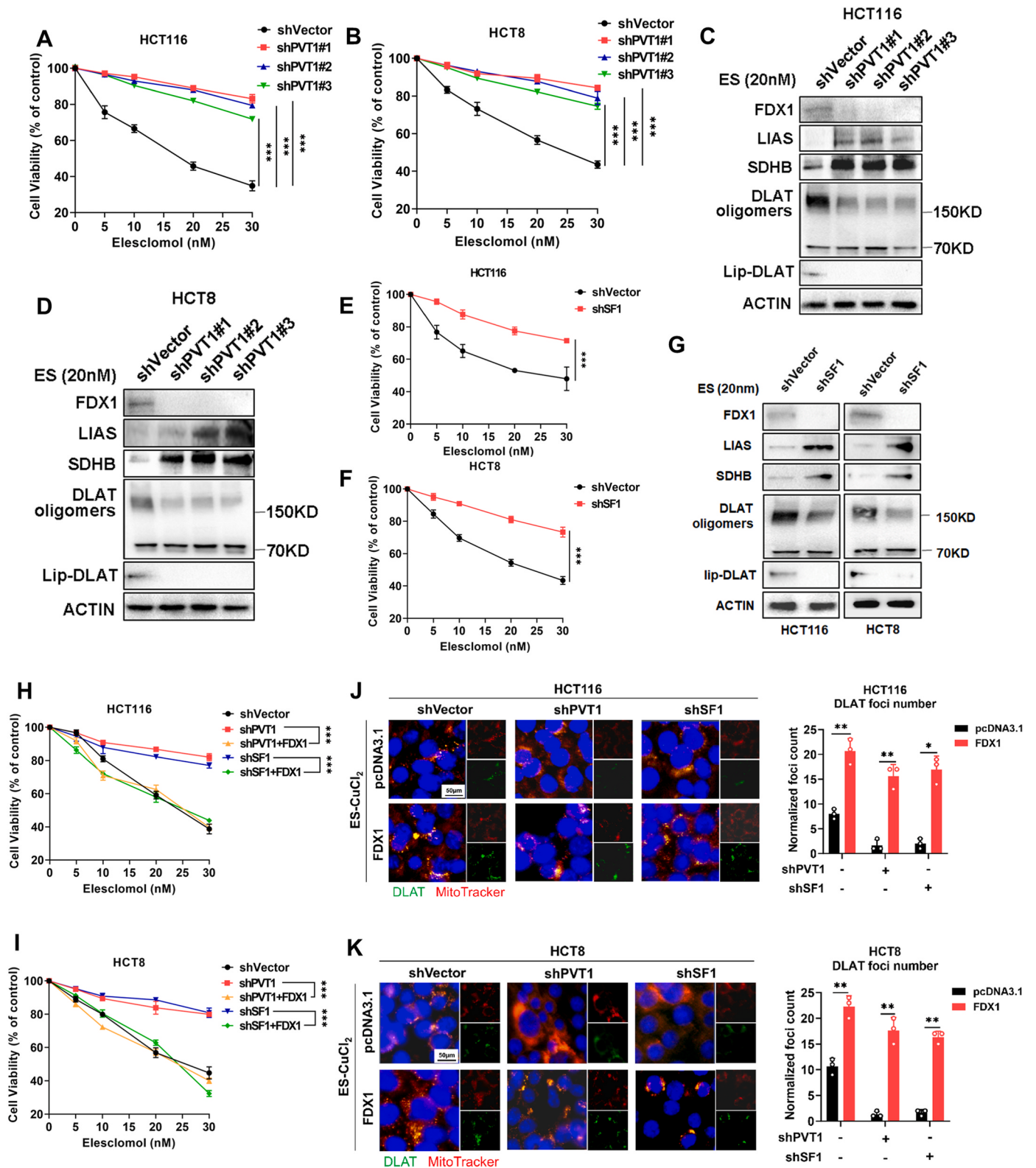


Fig. 7. PVT1/FDX1 axis mediates cuproptosis in colorectal cancer cells. **A and B, E and F,** The role of PVT1 or SF1 in cuproptosis. HCT116 and HCT8 cells transfected with shPVT1 (**A and B**) or shSF1 (**E and F**) plasmid or empty vector were treated with different concentrations of elesclomol and 1 μ M CuCl₂. The cell viability was evaluated by CCK8 assay after 24 h. **C, D and G,** HCT116 and HCT8 cells with PVT1 (**C and D**) or SF1 (**G**) knockdown were treated with 20 nM elesclomol and 1 μ M CuCl₂ for 24 h. WB assays were performed to detect iron-sulfur cluster proteins and DLAT levels. **H and I,** HCT 116 (**H**) or HCT8 (**I**) cells were treated for 24h with different concentrations of elesclomol and 1 μ M CuCl₂ after overexpressing FDX1 and knocking down PVT1 or SF1, cell viability was evaluated by CCK-8 assay. **J and K,** HCT116 (**J**) and HCT8 (**K**) cells were treated with 20 nM elesclomol and 1 μ M CuCl₂ after overexpressing FDX1, knocking down SF1/PVT1 or both in combination; DLAT protein oligomerization was analyzed after 24 h by immunofluorescence images (DLAT - green, Mitotracker-red). Statistical data presented in this figure show mean values $\pm$ SD of three times of independent experiments. Statistical significance was determined by Two-tailed *t*-test, *, *p* < 0.05; **, *p* < 0.01; ***, *p* < 0.001.

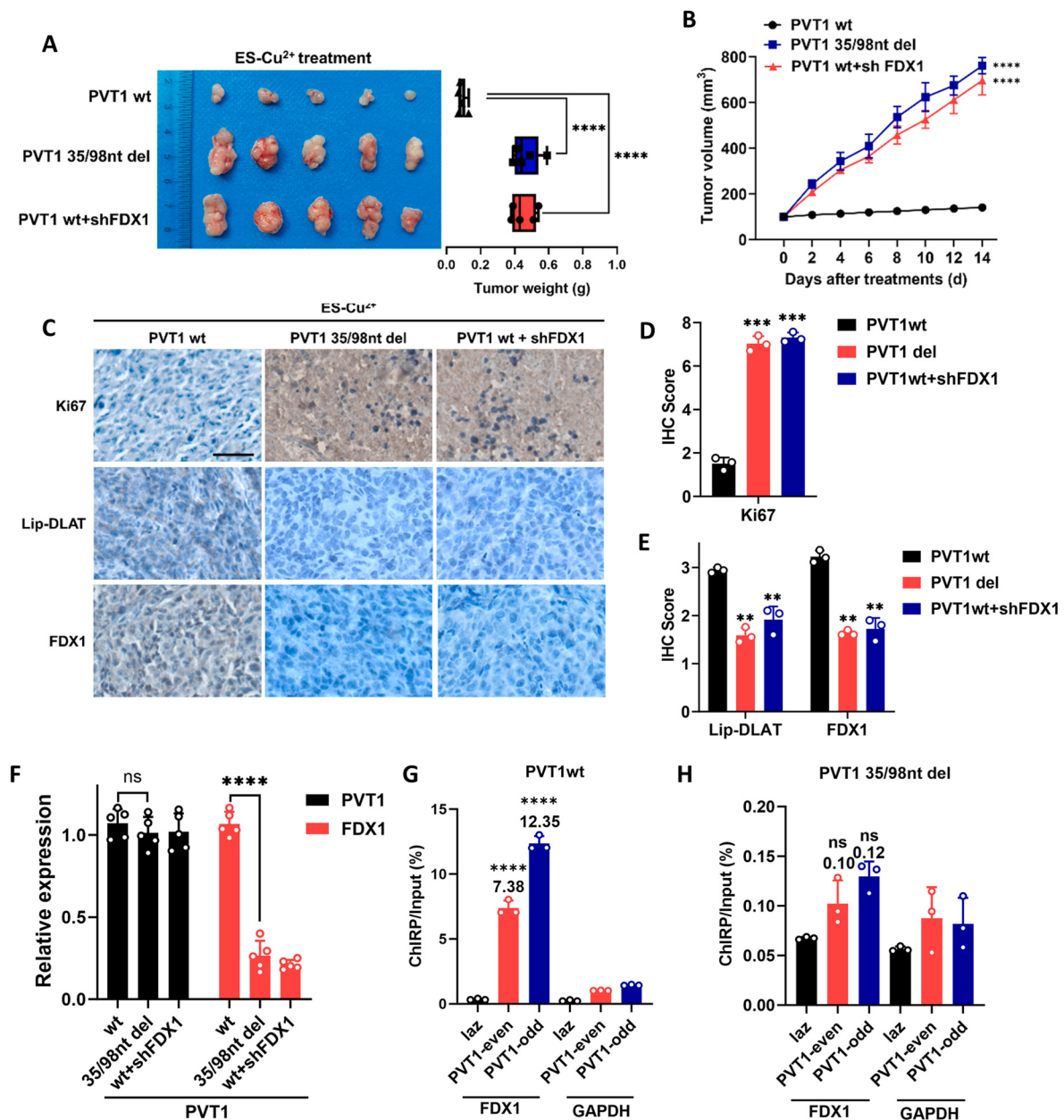


Fig. 8. The PVT1-FDX1 axis serves as a biomarker for the sensitivity of cuproptosis-targeted therapies. **A and B**, Xenograft experiments were performed by subcutaneously injecting HCT116 cells transfected with PVT1 wt, PVT1 del, or PVT1 wt + shFDX1 into nude mice ($n = 5$ per group), followed by treatment with elesclomol-CuCl₂. Tumors were collected and photographed (**A**, left). The Tumor weights (**A**, right) and the growth curve (**B**) were measured with indicated treatment. **C-E**, Representative images and quantification analysis of immunohistochemical images of ki67 and cuproptosis-related protein in tumor tissues (scale bar, 50 μ m). **F**, The expression of FDX1 and PVT1 in implanted tumor was measured by qRT-PCR. **G and H**, The combination of FDX1 and PVT1 in implanted tumor was verified by ChIRP assay. Statistical data presented in this figure show mean values $\pm$ SD of three times of independent experiments. Statistical significance was determined by Two-tailed t -test, ** $p < 0.01$, ****, $p < 0.0001$.

Notably, our study uncovers a novel regulatory mechanism by which the oncogenic lncRNA PVT1 promotes cuproptosis through epigenetic activation of FDX1 in CRC. The PVT1-FDX1-cuproptosis axis identified in CRC may have broader therapeutic implications, given the widespread overexpression of PVT1 across tumors and the essential role of FDX1 in mediating cuproptosis. Leveraging this intrinsic molecular feature provides a rationale for targeting the axis to induce cuproptosis without artificially manipulating gene expression. However, given the heterogeneous expression and context-dependent role of FDX1 across tumor types [36,48–50], further studies are needed to validate the broader therapeutic applicability of this strategy beyond CRC. Nevertheless, our findings provide a mechanistic framework for exploring

lncRNA-driven regulation of cuproptosis in other cancer models. Future studies may clarify whether this axis contributes to copper-based therapeutic responses in malignancies with high PVT1 expression.

This approach may be particularly useful in overcoming chemotherapy resistance, as PVT1 overexpression has been associated with reduced responsiveness to agents such as 5-FU [51]. Combining copper ionophores with standard treatments could help restore treatment efficacy in PVT1-high, treatment-refractory tumors, offering a potential strategy for precision cancer therapy. To further improve safety and precision, we are developing targeted delivery systems, such as in situ injectable copper-coordinated hydrogels, for localized and sustained copper ion release. Additional strategies, including dose optimization,

the use of alternative low-toxicity copper carriers, and combination with other therapeutic modalities (e.g., immunotherapy or targeted therapy), may enhance the translational potential of cuproptosis-based therapies.

Collectively, this study identifies lncRNA PVT1 as a novel upstream regulator of cuproptosis in CRC. PVT1, which is highly expressed in CRC, transcriptionally activates FDX1, thereby sensitizing cells to elesclomol-CuCl₂. These results establish PVT1 as a biomarker for predicting cuproptosis therapeutic sensitivity in CRC. Furthermore, our study provides a mechanistic rationale for copper-based treatment strategies in CRC patients with high PVT1 expression, particularly those exhibiting PVT1-mediated chemotherapy resistance.

CRediT authorship contribution statement

Jinyan Ma: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Yingjie Zhang:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Zhuoran Sun:** Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Hui Guo:** Visualization, Formal analysis, Data curation. **Xiang Li:** Validation, Formal analysis. **Jueting Cai:** Validation, Formal analysis. **Meichen Zhang:** Visualization, Formal analysis, Data curation. **Mengmeng Chen:** Visualization, Formal analysis, Data curation. **Jingjing Jiang:** Validation, Formal analysis. **Lingling Zhang:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition.

Ethics approval and consent to participate

All animal experiments were performed in accordance with procedures approved by the ethics committee of Central South University (approval no. CSU-2022-0514). The local ethics committee follows the rules of the Basel Declaration.

Clinical trial number

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The bioinformatics analysis and database-generated data can be found in this article and its supplementary information files. All the raw data generated in this study are available upon request from the corresponding author.

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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.

Acknowledgments

Not applicable.

Abbreviations

CRC: Colorectal Cancer; ES: Elesclomol; lncRNA: Long noncoding RNA; PVT1: plasmacytoma variant translocation 1; FDX1: Ferredoxin 1; SF1: Steroidogenic factor 1; DLAT: Dihydrolipoamide acetyltransferase; HSP70: heat shock protein 70; Lip-DLAT: lipid molecule of dihydrolipoamide acetyltransferase; TTM: Tetrathiomolybdate; CQ: Chloroquine; Z-VAD-FMK: Benzylloxycarbonyl-valyl-alanyl-aspartyl fluoromethyl ketone; RIP: RNA immunoprecipitation; CHIP: Chromatin immunoprecipitation; CHIRP: Chromatin isolation by RNA purification; H3K27ac: Histone H3 lysine 27 acetylation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.redox.2025.103722>.

Data availability

Data will be made available on request.

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