

TXNL1 Knockout Suppresses Pancreatic Cancer Cell Proliferation, Invasion, and EMT via Concomitant Inhibition of MEK/ERK and PI3K/AKT Signaling

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Article

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Abstract

Objective

This study aimed to investigate the expression and biological functions of TXNL1 in pancreatic ductal adenocarcinoma (PDAC) and to explore its underlying molecular mechanisms.

Methods

We first evaluated TXNL1 expression using public databases (TCGA and GTEx) and tissue microarrays from PDAC patients. Subcellular localization was examined by immunofluorescence. In vitro functional assays were performed in Mia PaCa-2 and PANC-1 cells following TXNL1 knockdown using CRISPR-CAS9. Cell viability, proliferation, cell cycle distribution, migration, invasion, and epithelial–mesenchymal transition (EMT) were assessed via CCK-8, colony formation, flow cytometry, Transwell, and Western blot analyses, respectively. In vivo tumor growth was evaluated in a xenograft mouse model. The activation of MEK/ERK and PI3K/AKT pathways was examined by detecting phosphorylated and total ERK and AKT proteins.

Results

TXNL1 was significantly upregulated in PDAC tissues and cell lines, and its high expression correlated with advanced clinical stage. Immunofluorescence revealed both cytoplasmic and nuclear localization of TXNL1 in cancer cells. Knockdown of TXNL1 markedly suppressed cell proliferation, induced G1 phase arrest, and reduced colony formation. It also inhibited cell migration and invasion, accompanied by increased E-cadherin and decreased Vimentin expression, indicating reversal of EMT. In vivo, TXNL1 silencing significantly impaired tumor growth. Mechanistically, TXNL1 downregulation led to reduced phosphorylation of ERK and AKT, while total protein levels remained unchanged, suggesting that TXNL1 modulates the MEK/ERK and PI3K/AKT signaling axes.

Conclusion

Our findings demonstrate that TXNL1 is overexpressed in PDAC and promotes malignant progression by enhancing proliferation, migration, invasion, EMT, and in vivo tumorigenicity, potentially through activation of the MEK/ERK and PI3K/AKT pathways. TXNL1 may serve as a promising prognostic biomarker and a novel therapeutic target for pancreatic cancer.

1. Introduction

Pancreatic cancer is the most common malignancy of the pancreas, with a median survival of approximately 4 months and a 5-year survival rate of only 13%. It is projected to become the second

leading cause of cancer-related deaths in the United States by 2030 [1, 2]. Pancreatic cancer is characterized by high metastatic potential, strong invasiveness, and intrinsic chemoresistance. Approximately 90% of pancreatic cancers are pancreatic ductal adenocarcinoma (PDAC). Currently, the mainstay of treatment remains a combination of chemotherapy and surgery [3]. However, due to the insidious onset of early symptoms and the lack of effective screening tools, over 80% of patients are diagnosed at an unresectable stage, precluding curative surgery [4, 5]. Therefore, current research efforts are focused on identifying novel therapeutic targets to improve the clinical management of pancreatic cancer.

At the cellular level, epithelial–mesenchymal transition (EMT) is a critical process by which pancreatic cancer cells acquire migratory and invasive capabilities. During EMT, cells lose epithelial characteristics, with downregulation of epithelial markers such as E-cadherin and upregulation of mesenchymal markers including Vimentin and N-cadherin, along with increased expression of related transcription factors, leading to enhanced cell motility and matrix invasion [6, 7]. Meanwhile, the PI3K–AKT and RAF–MEK–ERK signaling axes are constitutively activated in pancreatic cancer, promoting proliferation, survival, migration, EMT, and therapeutic resistance [8]. Thus, identifying upstream molecules that link proliferation, invasive phenotypes, and ERK/AKT signaling may provide deeper insights into the mechanisms underlying pancreatic cancer progression.

TXNL1 is a conserved thioredoxin-fold protein expressed in all cell types. It is a component of the 26S proteasome [9, 10] and can stably interact with the proteasome. TXNL1 is broadly involved in various biological processes, including cell signal transduction, cell cycle regulation, protein synthesis, post-translational modification and degradation, transcriptional regulation, and oxidative stress responses [11], and has been implicated in the development of human tumors. Studies have shown that TXNL1 functions both as a redox-active thioredoxin and as an ATP- and redox-independent molecular chaperone [12]. Moreover, the expression of TXNL1 and XRCC1 may play an important role in gastric cancer cells responding to oxidative stress, thereby contributing to adjuvant chemotherapy outcomes [13]. In pancreatic cancer cells, rapid downregulation of 17 short-lived proteins, including TXNL1, has been reported to be associated with the induction of acute apoptosis [14]. However, the specific role of TXNL1 in pancreatic cancer has not been fully elucidated. Therefore, the present study aims to investigate the expression of TXNL1 in pancreatic cancer and its contribution to the initiation and progression of the disease.

2. Materials and Methods

1.1 Patients and tissue specimens

Pancreatic cancer tissue specimens were collected from 7 patients who underwent surgical resection at Liaocheng People's Hospital. Based on histological grading, there were 3 cases of well-differentiated adenocarcinoma, 3 cases of moderately differentiated adenocarcinoma, and 1 case of poorly differentiated adenocarcinoma. None of the patients had received radiotherapy, chemotherapy, or

immunosuppressive therapy prior to surgery. Adjacent non-cancerous pancreatic tissues, resected at least 1 cm away from the tumor margin, were used as normal controls. All animal experiments were performed in accordance with the relevant guidelines and regulations. The study protocol was reviewed and approved by the Research Ethics Committee of the Second Hospital of Shandong University .

1.2 Cell lines, reagents and instruments

Human pancreatic cancer cell lines Mia PaCa-2, PANC-1, CFPAC-1, Capan-2, human normal pancreatic epithelial cell line HPDE, and human embryonic kidney cell line 293T were all purchased from the Cell Bank of the Chinese Academy of Sciences.

Reagents

The Lenti-X Tet-On 3G CRISPR/Cas9 Kit was obtained from Takara Bio Inc. (Shiga, Japan). CCK-8 reagent was purchased from Sigma-Aldrich (St. Louis, MO, USA). DMEM medium and fetal bovine serum (FBS) were purchased from Gibco (Grand Island, NY, USA). Transwell chambers were obtained from JET Bio-Filtration (Guangzhou, China). Matrigel was purchased from Yeasen Biotechnology (Shanghai, China). Rabbit anti-phospho-Akt(S473) polyclonal antibody, rabbit anti-phospho-AktT308 polyclonal antibody, rabbit anti-ERK1/2 polyclonal antibody, rabbit anti-phospho-ERK1/2 polyclonal antibody, and rabbit anti-TXNL1 polyclonal antibody were obtained from Proteintech Group (Wuhan, China). Rabbit anti-Akt polyclonal antibody, rabbit anti-Ki-67 polyclonal antibody, rabbit anti-PCNA polyclonal antibody, and mouse anti-Vimentin polyclonal antibody were purchased from Cell Signaling Technology (Danvers, MA, USA). Mouse anti- β -actin monoclonal antibody, HRP-conjugated goat anti-mouse and anti-rabbit IgG secondary antibodies, and FITC-conjugated goat anti-mouse and anti-rabbit IgG secondary antibodies were obtained from ZSGB-BIO (Beijing, China).

Instruments

The electrophoresis system was purchased from Bio-Rad (Hercules, CA, USA). The digital slide scanner was a NanoZoomer S60 (Hamamatsu, Hamamatsu, Japan). Fluorescence microscopy was performed using an Olympus BX53 (Olympus, Tokyo, Japan). Flow cytometry was conducted on a CytoFLEX system (Beckman Coulter, Brea, CA, USA). The microplate reader was a Cytation 5 (BioTek, Winooski, VT, USA).

1.3 Immunofluorescence and immunohistochemistry

For tissue immunofluorescence, pancreatic cancer tissues were fixed with 4% paraformaldehyde to preserve antigen structure, embedded in paraffin, and cut into 4- μ m-thick sections mounted on glass slides. The sections were deparaffinized with xylene and rehydrated through a graded ethanol series. Antigen retrieval was performed by microwave heating in antigen retrieval solution to unmask epitopes. After blocking with blocking solution for 1 h at room temperature, the sections were incubated with primary antibodies overnight at 4°C. The next day, after washing with PBST, the sections were incubated with fluorescence-conjugated secondary antibodies for 1 h at room temperature in the dark. Nuclei were counterstained with DAPI, and the sections were mounted with anti-fade mounting medium. Images

were captured using a fluorescence microscope. For tissue microarray immunofluorescence, the tissue microarray (TMA) was designed and constructed by Shanghai Outdo Biotech Co., Ltd. (Shanghai, China). Staining was performed as described above. The fluorescence intensity of 60 specimens on the TMA was evaluated independently by two pathologists. Fluorescence intensity was graded as +, ++, +++, or +++, with + defined as negative. Intensity grades of + and ++ were classified as low expression, while +++ and ++++ were classified as high expression. Clinicopathological correlation analyses were performed based on the TMA scoring results. For cellular immunofluorescence, coverslips were placed in 24-well plates, and cells were seeded at a density of 1×10^4 cells per well. After reaching an appropriate density, cells were fixed and permeabilized, followed by blocking, primary antibody incubation, and secondary antibody incubation. DAPI was then added for nuclear staining at room temperature for 5 min. After washing, coverslips were mounted onto glass slides with anti-fade mounting medium, sealed, and observed under a fluorescence microscope. For immunohistochemistry, paraffin-embedded sections (4 μ m) were deparaffinized, rehydrated, subjected to antigen retrieval, and treated with 3% hydrogen peroxide to block endogenous peroxidase activity. After blocking, sections were incubated with primary antibodies overnight at 4°C, followed by HRP-conjugated secondary antibodies for 30 min at 37°C. Signals were visualized using DAB substrate, and sections were counterstained with hematoxylin. Images were captured, and the percentage of positive cells was calculated as the number of positive cells divided by the total number of cells in the entire visual field, quantified using ImageJ software. Informed consent was obtained from all participants and/or their legal guardians.

1.4 Construction of conditional TXNL1 knockout Mia PaCa-2 and PANC-1 cell lines using CRISPR/Cas9 technology

Three 20-bp sgRNAs targeting TXNL1 were designed: sgRNA1: 5'-caccGAAGCCCGTCGGGAGCGACC-3', sgRNA2: 5'-caccGTATCAACCTACCCCGATCTA-3', and sgRNA3: 5'-caccGTTCCCTAGAATTGGCAAGT-3'. The CRISPR Nuclease Vector-sgRNA plasmids were successfully constructed and verified by sequencing (Sangon Biotech, Shanghai, China). Subsequently, 293T cells were co-transfected with PLVX-hyg-sgRNA, VSV-G, and lentiviral packaging plasmids. Supernatants were collected at 24 h and 48 h post-transfection, purified, and stored at -80°C. Mia PaCa-2 and PANC-1 target cells were infected with the lentivirus, and monoclonal cell lines were selected using the Tet-On 3G system combined with G418 selection. These Tet-On 3G-expressing pancreatic cancer cells were then infected with Cas9 lentivirus and selected with puromycin. Subsequently, TXNL1-sgRNA lentivirus was introduced, and stable cell lines were selected with hygromycin. Finally, well-growing Mia PaCa-2 and PANC-1 monoclonal cells were treated with 5 μ g/mL doxycycline to induce TXNL1 knockout.

1.5 CCK-8 cell proliferation assay

After 5 days of doxycycline treatment, cells and control cells were seeded into 96-well plates at a density of 3,000 cells per well, with three replicate wells per group. The time of cell attachment was recorded as 0 h. OD values were measured at 0, 24, 48, and 72 h. At each time point, the original medium was discarded, and 100 μ L of pre-prepared CCK-8 working solution was added to each well. After incubation

at 37°C for 2–4 h, the absorbance at 450 nm was measured using a microplate reader. Data were recorded and plotted as line graphs.

1.6 Wound healing assay

Cells in the logarithmic growth phase were trypsinized and seeded into 6-well plates. When the cells reached confluence, a straight scratch was made in each well using a 200-μL pipette tip guided by a ruler, maintaining consistent pressure, with three scratches per well. After scratching, the wells were gently washed three times with PBS, and 2 mL of serum-free medium was added. Images were captured at 0 h and 24 h post-scratching.

1.7 Transwell assay

For the migration assay, Transwell chambers were placed into 24-well plates. Cells in the logarithmic growth phase were trypsinized and resuspended in serum-free medium. A total of 1×10^5 cells in 200 μL of serum-free medium were added to the upper chamber, while the lower chamber contained 700 μL of medium supplemented with 20% FBS. After incubation at 37°C for 36 h, the medium was discarded, and the chambers were washed once with PBS. Cells were fixed with 4% paraformaldehyde for 20 min, washed with PBS, and stained with 0.1% crystal violet at 4°C for 30 min. After washing with distilled water, cells on the upper surface of the membrane were carefully removed using a cotton swab. The membranes were placed on glass slides with a drop of distilled water, and cells on the lower surface were counted under a microscope. For the invasion assay, Matrigel was diluted and coated onto the upper surface of the Transwell membrane in a 24-well plate. The remaining steps were performed as described for the migration assay.

1.8 Colony formation assay

Cells in the logarithmic growth phase were seeded into 6-well plates at a density of 1,000 cells per well and cultured at 37°C for 14 days, with medium changed every 3 days. After colony formation, cells were washed with PBS, fixed with 4% paraformaldehyde for 20 min at room temperature, and stained with 0.1% crystal violet for 30 min. After washing with distilled water and air-drying, colonies containing more than 50 cells were counted under a microscope. The colony formation rate was calculated as (number of colonies / number of seeded cells) $\times$ 100%.

1.9 Western blot analysis

Total cellular proteins were extracted using RIPA lysis buffer containing protease and phosphatase inhibitors. After sonication, samples were heated at 99°C for 5 min in a metal bath. Proteins were separated by SDS-PAGE using a 10% separating gel under the following conditions: 80 V for 30 min through the stacking gel, followed by 120 V for 1 h through the separating gel. Proteins were then transferred onto NC membranes at a constant current of 240 mA for 90 min. After blocking with 5% skim milk, membranes were incubated with primary antibodies overnight at 4°C. The following primary antibodies were used: rabbit anti-TXNL1 polyclonal antibody (Proteintech; 1:1000), rabbit anti-phospho-Akt polyclonal antibody (Proteintech; 1:1000), rabbit anti-phospho-ERK1/2 polyclonal antibody

(Proteintech; 1:1000), rabbit anti-Akt polyclonal antibody (CST; 1:1000), rabbit anti-Ki-67 polyclonal antibody (CST; 1:1000), rabbit anti-PCNA polyclonal antibody (CST; 1:1000), mouse anti-Vimentin polyclonal antibody (CST; 1:1000), and mouse anti- β -actin monoclonal antibody (ZSGB-BIO; 1:2000). After washing, membranes were incubated with HRP-conjugated goat anti-mouse IgG (ZSGB-BIO; 1:2000) or HRP-conjugated goat anti-rabbit IgG (ZSGB-BIO; 1:2000) secondary antibodies for 1 h at room temperature. Membranes were washed with TBST for 30 min, and signals were detected using ECL reagent and imaged. Band densitometry was quantified using ImageJ software, with β -actin as the loading control.

1.10 Flow cytometric analysis of cell cycle

Cells in the logarithmic growth phase were collected, washed twice with PBS, and fixed with pre-chilled 70% ethanol overnight at 4°C. The next day, cells were centrifuged, the fixative was discarded, and cells were washed twice with PBS. Cells were then incubated with staining solution containing 50 μ g/mL propidium iodide (PI) and 100 μ g/mL RNase A for 30 min at 37°C in the dark. After filtration through a 300-mesh nylon mesh, cells were analyzed using a flow cytometer. Cell cycle distribution was analyzed using ModFit LT software.

1.11 Subcutaneous xenograft tumor model in nude mice

Female nude mice aged 6 weeks were randomly divided into control and experimental groups ($n = 5$ per group) and housed under specific pathogen-free (SPF) conditions. Mia PaCa-2 cells were cultured in 10-cm culture dishes. When cells reached the appropriate number, they were trypsinized and resuspended at a density of 7×10^6 cells/mL. A total of 150 μ L (approximately 1.05×10^6 cells) of the cell suspension was injected subcutaneously into the right axilla of each mouse. After tumor formation, tumor size was measured regularly, and the volume was calculated as $(\text{length} \times \text{width}^2)/2$. Body weights were also recorded regularly. Five weeks after injection, mice were anesthetized and euthanized. Tumors were dissected, photographed, weighed, and fixed in 4% paraformaldehyde for subsequent immunohistochemical analysis. All mice were anesthetized via isoflurane inhalation. Anesthesia was induced with 4–5% isoflurane in oxygen at a flow rate of 1.5 L/min and maintained with 1.5–2% isoflurane in oxygen at 1.0 L/min delivered through a nose cone. Anesthesia depth was monitored by toe pinch reflex. At the end of the experiment, mice were euthanized by cervical dislocation under deep anesthesia. All methods were performed in accordance with the relevant guidelines and regulations.

1.12 Bioinformatics analysis

TXNL1 expression data were obtained from the TCGA and GTEx databases. The Sangerbox 3.0 platform (<http://vip.sangerbox.com/home.html>) was used to analyze TXNL1 mRNA expression levels across 34 tumor and normal tissue types. The Human Protein Atlas (HPA, <https://www.proteinatlas.org/>) was used to visualize TXNL1 protein expression in pancreatic cancer and normal pancreatic tissues. The GEPIA3.0 database was used to analyze TXNL1 mRNA expression levels in pancreatic cancer versus normal tissues, as well as the correlation between TXNL1 and six common immune checkpoint molecules. The TIMER algorithm was employed to evaluate the correlation between TXNL1 expression and immune cell

infiltration scores for three immune cell types, and the "IOBR" R package was used to generate heatmaps. The "pROC" R package was used to generate ROC curves. Kaplan-Meier survival curves were plotted using the KM-plotter online database (<https://www.kmplot.com/>).

1.13 Statistical analysis

Statistical analyses were performed using R software (version 4.5.2) and GraphPad Prism 10.0. Quantitative data are presented as mean $\pm$ standard deviation (SD). All in vitro experiments were performed with at least three independent biological replicates (n = 3).

Data normality was assessed using the Shapiro–Wilk test. For normally distributed data, comparisons between two groups were performed using independent samples t-test, and comparisons among multiple groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. For non-normally distributed data, the Mann–Whitney U test was used for two-group comparisons, and the Kruskal–Wallis test was used for multiple-group comparisons.

For CCK-8 growth curves and xenograft tumor growth curves, which involved repeated measurements over time, repeated-measures ANOVA (or a mixed-effects model) was applied, followed by Bonferroni's post-hoc test. Clinicopathological characteristics in Table 1 were analyzed using the Chi-square test (or Fisher's exact test when the expected frequency in any cell was less than 5). Correlation analyses were performed using Pearson correlation for normally distributed variables and Spearman rank correlation for non-normally distributed variables.

Table 1
Association between TXNL1 expression and clinicopathological characteristics in patients with pancreatic ductal adenocarcinoma

Clinicopathological parameter	Total(n)	Low TXNL1 (n)	High TXNL1 (n)	P value
Age				
> 60 years	34	18	16	0.265
≤ 60years	26	10	16	
SEX				
Male	34	15	19	0.651
Female	26	13	13	
Clinical stage				
1A-2A	18	12	6	0.042
2B-4	42	16	26	
Organ involvement				
Yes	31	11	20	0.073
No	29	17	12	
Invasion status				
Yes	9	3	6	0.355
No	51	25	26	

A P value < 0.05 was considered statistically significant. All statistical tests were two-sided. The multiple-comparison corrections were applied as described above to control for family-wise error rates.

3. Results

3.1 TXNL1 is upregulated in PDAC with distinct subcellular redistribution

To assess the clinical relevance of TXNL1, we first interrogated its mRNA expression across 34 cancer types using the Sangerbox3.0 platform. This pan-cancer analysis revealed that TXNL1 was significantly upregulated in 25 tumour types relative to their normal counterparts, including pancreatic adenocarcinoma ($P < 0.05$). This finding was independently corroborated by GEPIA3 analysis, which confirmed elevated TXNL1 transcript levels in PDAC tissues compared with normal pancreatic controls ($P < 0.05$). At the protein level, immunohistochemical data from the Human Protein Atlas (HPA) further supported the marked overexpression of TXNL1 in PDAC specimens.

To precisely localise TXNL1 expression within the tumour microenvironment, we performed immunofluorescence staining on seven paired PDAC and adjacent non-tumorous tissues. In normal pancreatic parenchyma, TXNL1 immunoreactivity was weak and predominantly confined to the cytoplasm of pancreatic stellate cells, small ductal cells and islet cells (Fig. 1a). In contrast, PDAC cells across all differentiation grades exhibited robust TXNL1 staining distributed in both the cytoplasmic and nuclear compartments (Fig. 1b). Notably, tumour stromal cells demonstrated only negligible staining (Fig. 1c). Collectively, these data indicate that TXNL1 is upregulated in PDAC at both the transcriptional and translational levels and undergoes a distinct subcellular redistribution in malignant cells.

Figure 1. TXNL1 is significantly upregulated in pancreatic cancer and shows aberrant subcellular localization.

(A) Pan-cancer mRNA expression profiling of TXNL1 across 34 tumour types and paired normal tissues from the TCGA and GTEx databases, as analysed by Sangerbox3.0. (B) Relative TXNL1 mRNA levels in PDAC tissues versus normal pancreatic controls, analysed using GEPIA3.0 ($P < 0.05$). (C) Representative immunohistochemical images of TXNL1 protein expression in PDAC tissues from the HPA database. (D) Representative immunohistochemical images of TXNL1 in normal pancreatic tissues from the HPA database. (E) Immunofluorescence staining of TXNL1 (green) and DAPI (blue) in paired PDAC and adjacent non-tumorous tissues from seven clinical specimens. Data are presented as means $\pm$ SD. $P < 0.05$, * $P < 0.01$, ** $P < 0.001$, *** $P < 0.0001$; n.s., not significant.

3.2 TXNL1 expression correlates with immune infiltration and immune checkpoint signatures in PDAC

To explore the potential immunomodulatory role of TXNL1, we evaluated its association with tumour-infiltrating immune cells using the TIMER algorithm. TXNL1 expression in PDAC was found to be positively correlated with the infiltration levels of neutrophils, macrophages and CD8⁺ T cells. Furthermore, we analysed the relationship between TXNL1 expression and six canonical immune checkpoint genes using the GEPIA3.0 database. This analysis revealed that TXNL1 was significantly correlated with CTLA4, PDCD1LG2 (PD-L2), HAVCR2 (TIM-3), CD274 (PD-L1), TIGIT and SIGLEC15 (all $P < 0.05$). These immunogenomic findings suggest that TXNL1 may be involved in the tumour immune microenvironment in PDAC (Fig. 2).

(A) Heatmap showing the correlation between TXNL1 expression and infiltration levels of neutrophils, macrophages and CD8⁺ T cells in PDAC, as evaluated by the TIMER algorithm. (B) Scatter plots depicting the correlations between TXNL1 expression and five immune checkpoint genes—CTLA4, PDCD1LG2 (PD-L2), HAVCR2 (TIM-3), CD274 (PD-L1), TIGIT and SIGLEC15—in pancreatic cancer, analysed using GEPIA3.0. Data are presented as means $\pm$ SD. $P < 0.05$, * $P < 0.01$, ** $P < 0.001$, *** $P < 0.0001$.

3.3 High TXNL1 expression is associated with poor prognosis and advanced clinical stage

We next evaluated the prognostic significance of TXNL1 using the Kaplan–Meier plotter database. Patients with high TXNL1 expression exhibited significantly shorter overall survival (OS) compared with those with low expression ($P < 0.05$), indicating that elevated TXNL1 levels are associated with an unfavourable clinical outcome in PDAC. To further explore the clinicopathological relevance, we performed immunofluorescence staining on a tissue microarray comprising 60 PDAC specimens. Correlation analysis revealed that high TXNL1 expression was significantly associated with advanced clinical stage ($P < 0.05$, Table 1). In addition, ROC curve analysis suggested that TXNL1 expression levels may hold diagnostic potential for discriminating PDAC from normal pancreatic tissues (Fig. 3). These findings identify TXNL1 as a candidate prognostic biomarker for pancreatic adenocarcinoma.

(A) Kaplan–Meier survival curves showing overall survival (OS) of PDAC patients stratified by high versus low TXNL1 expression, analysed using the Kaplan–Meier plotter database. (B) Receiver operating characteristic (ROC) curve evaluating the diagnostic performance of TXNL1 expression for distinguishing PDAC tissues from normal pancreatic controls, based on tissue microarray immunofluorescence data. Data are presented as means $\pm$ SD. $P < 0.05$.

3.4 TXNL1 is associated with PDAC cell proliferation and cell-cycle regulation in vitro

We first examined TXNL1 protein expression across a panel of PDAC cell lines (MiaPaCa-2, PANC-1, CFPAC-1 and Capan-2) and normal pancreatic ductal epithelial cells (HPDE). TXNL1 was consistently upregulated in all four PDAC lines relative to HPDE (Fig. 4a). To investigate its functional role, we employed the CRISPR–Cas9 system to establish stable TXNL1-knockout (KO) clones in MiaPaCa-2 and PANC-1 cells (Fig. 4b). Loss of TXNL1 significantly attenuated the proliferative capacity and colony-forming ability of both cell lines, as assessed by CCK-8 and colony formation assays ($P < 0.05$, Fig. 4c–d). Cell-cycle analysis by flow cytometry further revealed that TXNL1 depletion induced G1-phase accumulation accompanied by a reduction in the G2-phase fraction in both cell lines; in PANC-1 cells, a marked decrease in the S-phase population was also observed ($P < 0.05$, Fig. 4e). Consistently, TXNL1 knockout was associated with downregulated expression of key G1/S transition regulators, including CDK2, CDK4, Cyclin D1 and Cyclin E1 (Fig. 4f). These data suggest that TXNL1 is associated with a pro-proliferative phenotype in PDAC cells, possibly through facilitating cell-cycle progression and sustaining core cell-cycle machinery.

Figure 4. TXNL1 knockout is associated with suppressed PDAC cell proliferation and cell-cycle progression.

(A) Western blot analysis of TXNL1 protein expression in HPDE and four PDAC cell lines. (B) Validation of CRISPR–Cas9-mediated TXNL1 knockout efficiency and establishment of monoclonal knockout cell lines. (C) CCK-8 proliferation assays showing growth curves of TXNL1-KO and control cells. (D) Representative images of colony formation assays. (E) Flow cytometric analysis of cell-cycle distribution. (F) Immunoblots showing expression levels of CDK2, CDK4, Cyclin D1 and Cyclin E1. Data are presented as means $\pm$ SD. $P < 0.05$, * $P < 0.01$, ** $P < 0.001$.

3.5 TXNL1 correlates with PDAC cell migration, invasion and EMT markers in vitro

We next assessed the impact of TXNL1 on the migratory and invasive capacities of PDAC cells. Wound-healing assays revealed that TXNL1 knockout retarded the closure rate of both MiaPaCa-2 and PANC-1 monolayers compared with control cells. Consistently, Transwell migration and Matrigel invasion assays demonstrated that TXNL1 depletion reduced the number of migrated and invaded cells in both lines ($P < 0.05$, Fig. 5a–b).

At the molecular level, immunoblotting showed that TXNL1 knockout was associated with reduced phosphorylation levels of ERK and AKT (Fig. 5c). Furthermore, TXNL1 depletion led to increased E-cadherin expression and decreased Vimentin expression in both cell lines ($P < 0.05$, Fig. 5d). These findings were independently validated by immunofluorescence staining, which recapitulated the same reciprocal expression patterns of E-cadherin and Vimentin upon TXNL1 ablation (Fig. 5e). Collectively, these data suggest that TXNL1 is associated with PDAC cell migration and invasion, potentially involving EMT reprogramming and altered ERK/AKT phosphorylation.

(A) Representative images of wound-healing assays at 0 and 24 hours. (B) Transwell migration and Matrigel invasion assays. (C) Western blot analysis of p-ERK, ERK, p-AKT and AKT. (D) Western blot analysis of E-cadherin and Vimentin. (E) Immunofluorescence images of E-cadherin and Vimentin expression. Data are presented as means $\pm$ SD. $P < 0.05$, * $P < 0.01$, ** $P < 0.001$.

3.6 TXNL1 is associated with PDAC tumour growth in vivo

To determine whether the phenotypic effects observed in vitro could be reproduced in an in vivo setting, we subcutaneously injected TXNL1-KO and control MiaPaCa-2 cells into nude mice. TXNL1 ablation significantly reduced both tumour volume and tumour weight compared with the control group ($P < 0.05$, Fig. 6a–c). Immunohistochemical staining of excised tumour xenografts revealed markedly lower staining intensity and positive rates of the proliferation markers PCNA and Ki-67 in the TXNL1-KO group ($P < 0.05$, Fig. 6d). These in vivo findings corroborate our in vitro observations and support the association between TXNL1 and PDAC growth in a xenograft model.

Figure 6. TXNL1 ablation is associated with suppressed PDAC tumour growth in vivo.

(A) Representative images of xenograft tumours. (B) Quantitative comparison of tumour weights. (C) Tumour volume growth curves and body weight measurements. (D) Immunohistochemical staining and quantification of Ki-67 and PCNA. Data are presented as means $\pm$ SD. $P < 0.05$, * $P < 0.01$.

4. Discussion

Pancreatic ductal adenocarcinoma (PDAC) is characterized by high aggressiveness and poor prognosis. Its progression is not only influenced by classical driver genes and the tumor microenvironment, but also

closely associated with tumor cell proliferation, migration, invasion, and phenotypic plasticity [15, 16]. In this study, we investigated the expression characteristics and biological functions of TXNL1 in pancreatic cancer. Our results showed that TXNL1 was highly expressed in pancreatic cancer tissues and cell lines, and its expression correlated with clinical stage. Knockdown of TXNL1 significantly inhibited proliferation, cell cycle progression, migration, invasion, and epithelial–mesenchymal transition (EMT) phenotypes in pancreatic cancer cells, and also reduced tumor growth in vivo. Furthermore, TXNL1 inhibition was accompanied by decreased levels of p-ERK and p-AKT. These findings suggest that TXNL1 may act as a pro-tumorigenic molecule in pancreatic cancer progression, potentially through the MEK/ERK and PI3K-AKT signaling pathways.

We first validated the high expression of TXNL1 in pancreatic cancer using both public database and clinical tissue specimens. Tissue microarray analysis revealed that high TXNL1 expression was significantly correlated with clinical stage, suggesting that aberrant upregulation of TXNL1 may be associated with disease progression. Notably, immunofluorescence staining showed that TXNL1 was localized not only in the cytoplasm but also in the nucleus of pancreatic cancer tissues. Given that TXNL1 has been reported to be involved in redox homeostasis, protein quality control, and proteasome function [12, 17, 18], its abnormal expression and altered subcellular distribution may reflect the adaptation of tumor cells to sustained proliferation, stress conditions, and protein homeostasis demands.

Functional experiments further demonstrated that TXNL1 promotes the malignant growth of pancreatic cancer cells. Following TXNL1 knockdown, the proliferation rate and colony-forming ability of Mia PaCa-2 and PANC-1 cells were reduced, accompanied by an increased proportion of cells in the G1 phase, indicating cell cycle arrest [19, 20]. Changes in cell cycle-related proteins further corroborated the flow cytometry results. As a thioredoxin-like protein, TXNL1 may support pancreatic cancer cell growth by maintaining cellular homeostasis or modulating pro-proliferative signaling.

Local invasion and distant metastasis are major causes of treatment failure in pancreatic cancer, and EMT is a key mechanism by which tumor cells acquire migratory and invasive capabilities [6]. In our study, TXNL1 knockdown led to a decrease in the number of migrating and invading cells in Transwell assays, along with increased E-cadherin and decreased Vimentin expression. These results extend the role of TXNL1 beyond proliferation to invasion and metastasis-related phenotypes, suggesting that TXNL1 may exert broad functional effects on malignant progression in pancreatic cancer.

The MEK/ERK and PI3K/AKT pathways are important oncogenic signaling axes in pancreatic cancer, participating in the regulation of proliferation, survival, migration, invasion, and EMT [21–23]. Our study found that TXNL1 knockdown reduced the levels of p-ERK and p-AKT, while total ERK and total AKT remained largely unchanged, indicating that TXNL1 may be associated with the activation status of these pathways. Combined with the observed effects on proliferation, invasion, and EMT, it is plausible that TXNL1 contributes to the maintenance of malignant phenotypes in pancreatic cancer through modulation of the MEK/ERK and PI3K/AKT pathways.

We acknowledge certain limitations in this study. The sample size of the tissue microarray was limited, and the prognostic value of TXNL1 needs to be validated in larger independent cohorts with complete follow-up data. In addition, the functional experiments were primarily based on TXNL1 knockdown models; rescue experiments with TXNL1 overexpression would further strengthen the causal evidence.

In conclusion, our study demonstrates that TXNL1 is highly expressed in pancreatic cancer and correlates with clinical progression. Knockdown of TXNL1 suppresses proliferation, cell cycle progression, migration, invasion, EMT, and in vivo tumor growth, along with reduced activation of the MEK/ERK and PI3K/AKT pathways. These findings suggest that TXNL1 may function as a pro-tumorigenic molecule involved in disease progression and holds potential value as a candidate biomarker or therapeutic target in pancreatic cancer.

5. Conclusions

In summary, this study demonstrates that TXNL1 is significantly upregulated in pancreatic ductal adenocarcinoma and promotes malignant progression by enhancing proliferation, cell cycle progression, migration, invasion, and epithelial–mesenchymal transition, while also facilitating in vivo tumor growth. Mechanistically, TXNL1 exerts its pro-tumorigenic effects at least in part through activation of the MEK/ERK and PI3K/AKT signaling pathways. Our findings provide a mechanistic basis for considering TXNL1 as a potential prognostic biomarker and therapeutic target for pancreatic cancer, warranting further preclinical and clinical evaluation.

Declarations

Acknowledgments

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Authors' contributions:

Concept and design: NK and YN. Methodology: NK and YMa. Data curation: MMo, YMa, TN, KM, YMa, MMu, MT, YY, and TH. Software: NK. Formal analysis: NK and YMa. Writing the original draft: NK. Writing, review, and editing: NK, YY, and YN. All the authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate:

This study was conducted in accordance with the ethical standards of the Declaration of Helsinki of 1964 and its later amendments (Fortaleza, 2013). This study was approved by the Ethics Committee of Kagawa University, Japan (approval numbers: 2026-024), and the requirement for written informed consent was waived because of the retrospective observational study design.

Consent for publication:

Not applicable.

Disclosure/Competing interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Availability of data and material:

The datasets used and analyzed in the current study are available from the corresponding author upon reasonable request.

References

1. STOOPT F, JAVED A A, OBA, A. et al. *Pancreat. cancer [J] Lancet*, **405**(10485): 1182–1202. (2025).
2. SMITH B D, R. A. H. I. B. L. et al. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States [J]. *Cancer Res.* **74** (11), 2913–2921 (2014).
3. KRAUß, L. & SCHNEIDER, C. Epigenetic control of pancreatic cancer metastasis [J]. *Cancer Metastasis Rev.* **42** (4), 1113–1131 (2023).
4. REN, B. et al. Tumor microenvironment participates in metastasis of pancreatic cancer [J]. *Mol. Cancer.* **17** (1), 108 (2018).
5. RAMESH R P G, YASMIN, H. et al. Phenotypic heterogeneity and tumor immune microenvironment directed therapeutic strategies in pancreatic ductal adenocarcinoma [J]. *Front. Immunol.* **16**, 1573522 (2025).
6. WANG, S., HUANG, S. & SUN, Y. L. Epithelial-Mesenchymal Transition in Pancreatic Cancer: A Review [J]. *Biomed Res Int*, 2017: 2646148. (2017).
7. DONG, D. et al. STN1 facilitates metastasis by promoting transcription of EMT-activator ZEB1 in pancreatic cancer [J]. *Nat. Commun.* **16** (1), 7815 (2025).
8. MORTAZAVI, M. et al. Prospects of targeting PI3K/AKT/mTOR pathway in pancreatic cancer [J]. *Crit. Rev. Oncol. Hematol.* **176**, 103749 (2022).
9. ARKINSON, C. et al. Structural landscape of the degrading 26S proteasome reveals conformation-specific binding of TXNL1 [J]. *Nat. Struct. Mol. Biol.* **32** (12), 2403–2415 (2025).
10. ANDERSEN K M, MADSEN, L. et al. Thioredoxin Txnl1/TRP32 is a redox-active cofactor of the 26 S proteasome [J]. *J. Biol. Chem.* **284** (22), 15246–15254 (2009).

11. ZHAO, J. M. & QI T, G. The role of TXNL1 in disease: treatment strategies for cancer and diseases with oxidative stress [J]. *Mol. Biol. Rep.* **48** (3), 2929–2934 (2021).
12. ANDOR, A. et al. TXNL1 has dual functions as a redox active thioredoxin-like protein as well as an ATP- and redox-independent chaperone [J]. *Redox Biol.* **67**, 102897 (2023).
13. XU, W. et al. TXNL1-XRCC1 pathway regulates cisplatin-induced cell death and contributes to resistance in human gastric cancer [J]. *Cell. Death Dis.* **5** (2), e1055 (2014).
14. QI, T. et al. Proteomic profiling identified multiple short-lived members of the central proteome as the direct targets of the addicted oncogenes in cancer cells [J]. *Mol. Cell. Proteom.* **13** (1), 49–62 (2014).
15. SINGH N K, AHMAD, F. Molecular dissection of pancreatic cancer signaling: Toward targeted therapies for KRAS, MDM2-TP53, EGFR, and PI3K/AKT/mTOR [J]. *Pathol. Res. Pract.* **283**, 156506 (2026).
16. YING, H. et al. Genetics and biology of pancreatic ductal adenocarcinoma [J]. *Genes Dev.* **39** (1–2), 36–63 (2025).
17. GAO, J. et al. Structure of the TXNL1-bound proteasome [J]. *Nat. Struct. Mol. Biol.* **32** (12), 2398–2402 (2025).
18. LEE, K. et al. Structures of dynamic interactors at native proteasomes by PhIX-MS and cryo-electron microscopy [J]. *Mol. Cell.* **86** (15), 3067–85e13 (2026).
19. CHIEN T L et al. PNU-74654 Induces Cell Cycle Arrest and Inhibits EMT Progression in Pancreatic Cancer [J]. *Med. (Kaunas)*, **59**(9). (2023).
20. LIU, Y. & ELSAWA S F, ALMADA, L. L. Primers on molecular pathways - cycling toward pancreatic cancer [J]. *Pancreatology* **10** (1), 6–13 (2010).
21. LI, O. et al. Biological characteristics of pancreatic ductal adenocarcinoma: Initiation to malignancy, intracellular to extracellular [J]. *Cancer Lett.* **574**, 216391 (2023).
22. ZENG, J. et al. EPLIN, a prospective oncogenic molecule with contribution to growth, migration and drug resistance in pancreatic cancer [J]. *Sci. Rep.* **14** (1), 30850 (2024).
23. XU, C. et al. CLT-003 exerts anti-tumor activity in pancreatic cancer by blocking the PI3K/AKT/HIF-1 α pathway [J]. *Cancer Biol. Med.* **22** (12), 1558–1577 (2025).

Figures

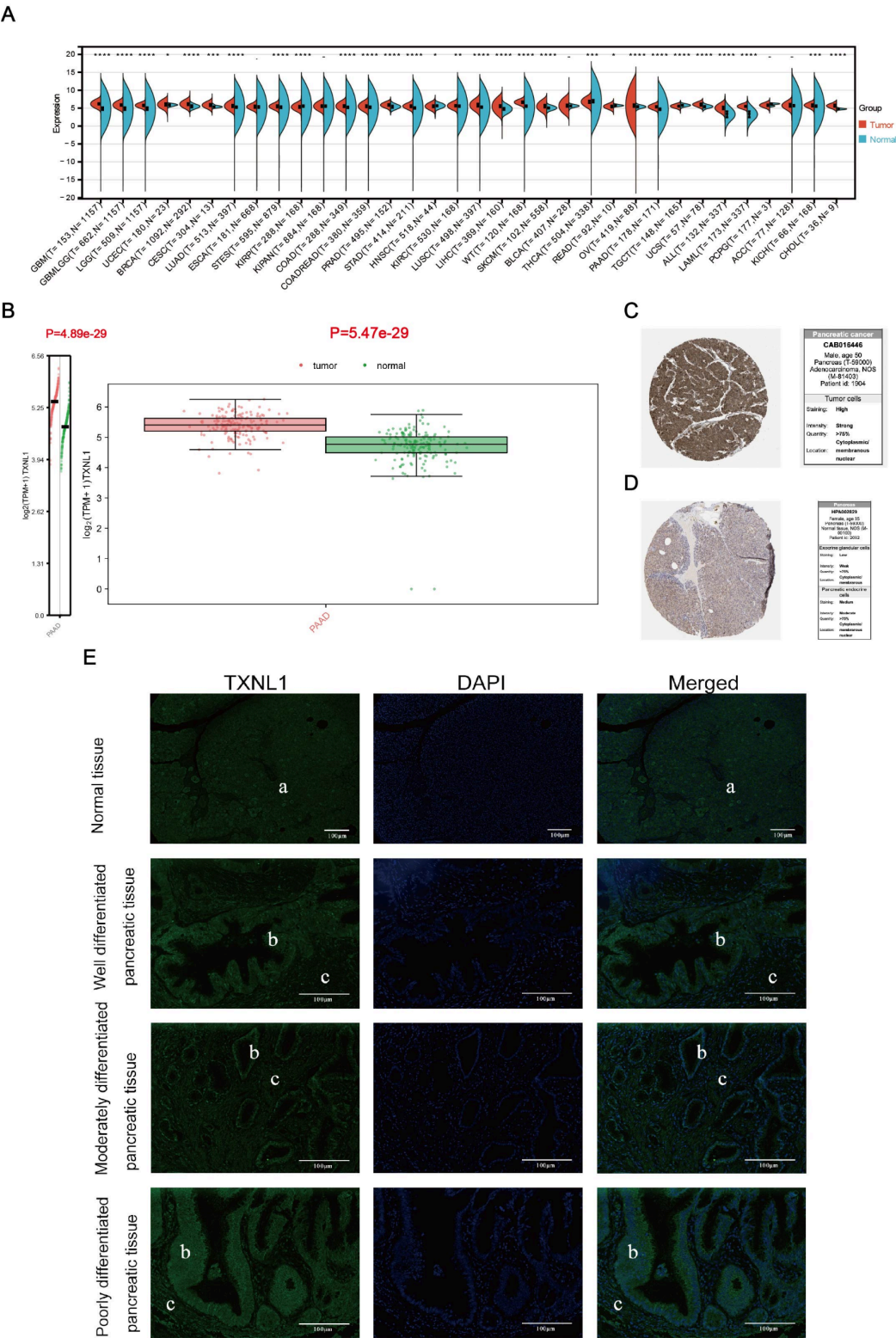


Figure 1

TXNL1 is significantly upregulated in pancreatic cancer and shows aberrant subcellular localization. (A) Pan-cancer mRNA expression profiling of TXNL1 across 34 tumour types and paired normal tissues from the TCGA and GTEx databases, as analysed by Sangerbox3.0. (B) Relative TXNL1 mRNA levels in PDAC tissues versus normal pancreatic controls, analysed using GEPIA3.0 ($P < 0.05$). (C) Representative immunohistochemical images of TXNL1 protein expression in PDAC tissues from the HPA database. (D)

Representative immunohistochemical images of TXNL1 in normal pancreatic tissues from the HPA database. (E) Immunofluorescence staining of TXNL1 (green) and DAPI (blue) in paired PDAC and adjacent non-tumorous tissues from seven clinical specimens. Data are presented as means $\pm$ SD. $P < 0.05$, * $P < 0.01$, ** $P < 0.001$, *** $P < 0.0001$; n.s., not significant.

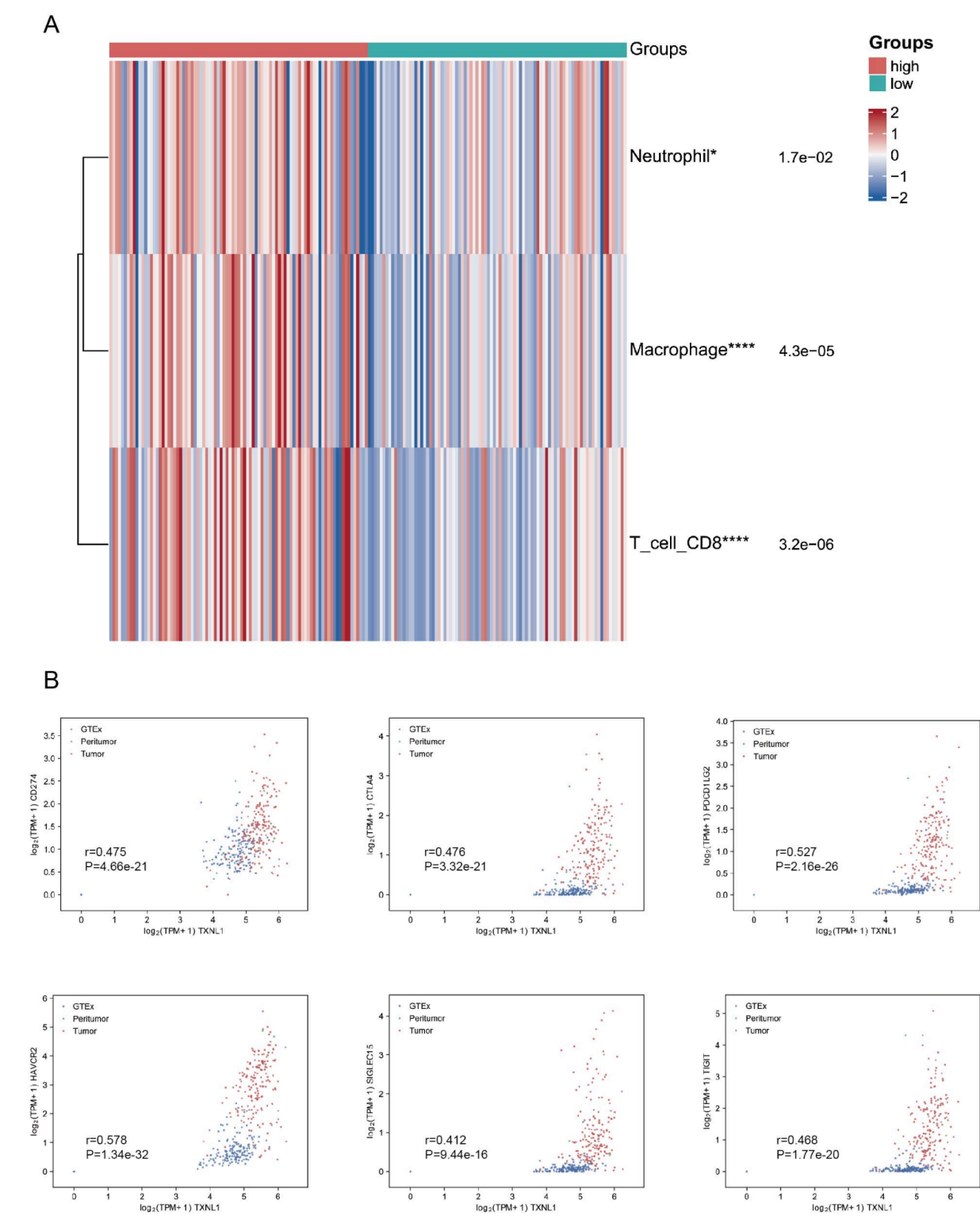


Figure 2

TXNL1 expression positively correlates with immune cell infiltration and immune checkpoint molecules in PDAC.

(A) Heatmap showing the correlation between TXNL1 expression and infiltration levels of neutrophils, macrophages and CD8⁺ T cells in PDAC, as evaluated by the TIMER algorithm. (B) Scatter plots depicting the correlations between TXNL1 expression and five immune checkpoint genes—CTLA4, PDCD1LG2 (PD-L2), HAVCR2 (TIM-3), CD274 (PD-L1), TIGIT and SIGLEC15—in pancreatic cancer, analysed using GEPIA3.0. Data are presented as means ± SD. P < 0.05, *P < 0.01, **P < 0.001, ***P < 0.0001.

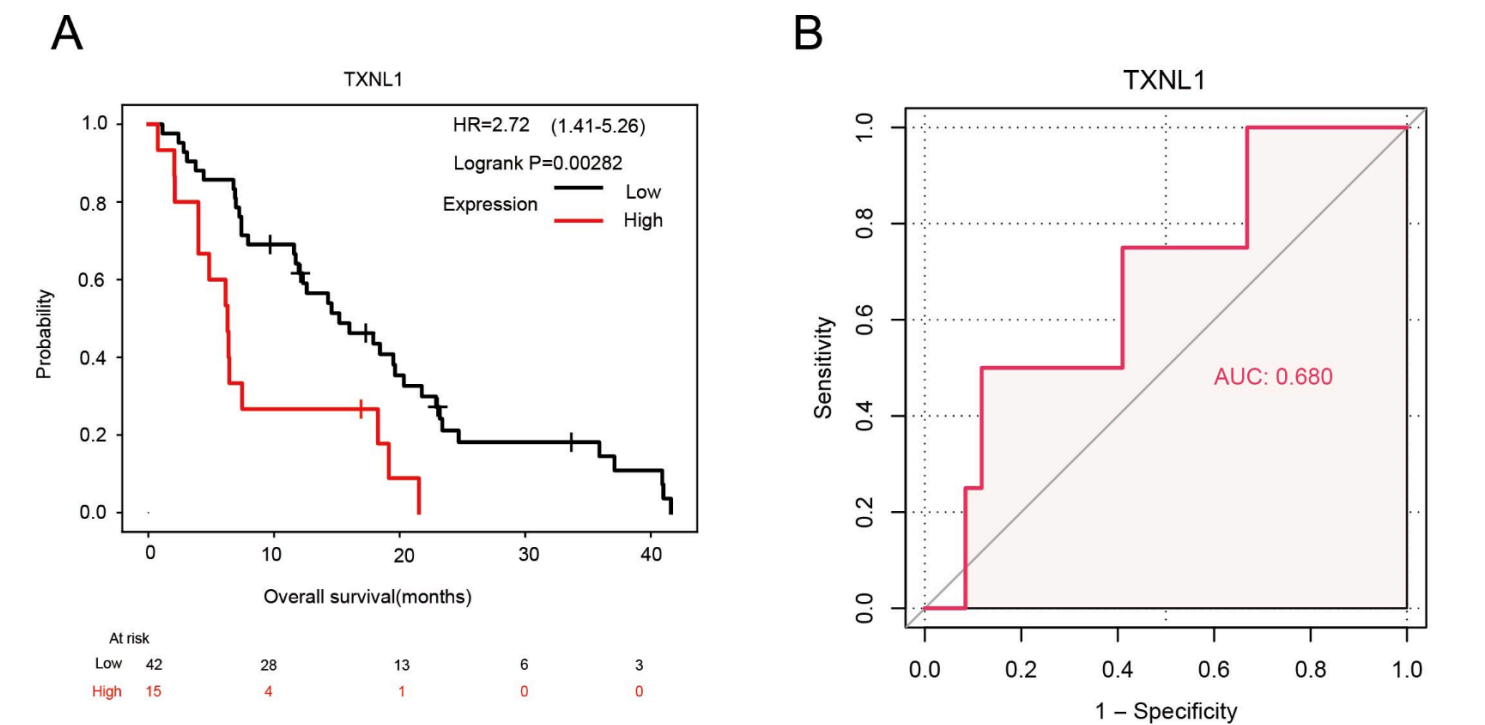


Figure 3

High TXNL1 expression is associated with poor overall survival and shows diagnostic potential in PDAC.

(A) Kaplan–Meier survival curves showing overall survival (OS) of PDAC patients stratified by high versus low TXNL1 expression, analysed using the Kaplan–Meier plotter database. (B) Receiver operating characteristic (ROC) curve evaluating the diagnostic performance of TXNL1 expression for distinguishing PDAC tissues from normal pancreatic controls, based on tissue microarray immunofluorescence data. Data are presented as means ± SD. P < 0.05.

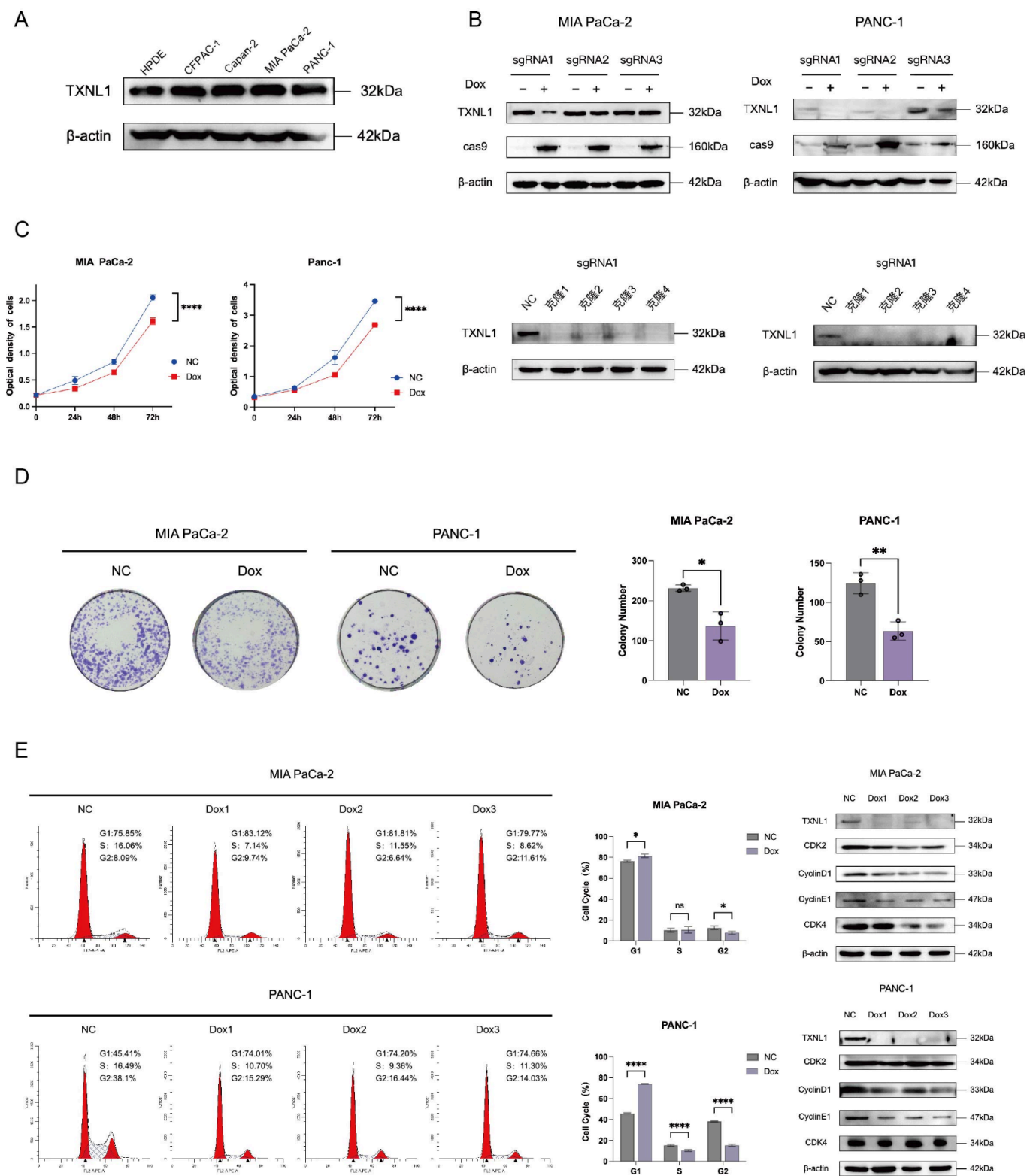


Figure 4

TXNL1 knockout is associated with suppressed PDAC cell proliferation and cell-cycle progression. (A) Western blot analysis of TXNL1 protein expression in HPDE and four PDAC cell lines. (B) Validation of CRISPR-Cas9-mediated TXNL1 knockout efficiency and establishment of monoclonal knockout cell lines. (C) CCK-8 proliferation assays showing growth curves of TXNL1-KO and control cells. (D) Representative images of colony formation assays. (E) Flow cytometric analysis of cell-cycle

distribution. (F) Immunoblots showing expression levels of CDK2, CDK4, Cyclin D1 and Cyclin E1. Data are presented as means $\pm$ SD. $P < 0.05$, * $P < 0.01$, ** $P < 0.001$.

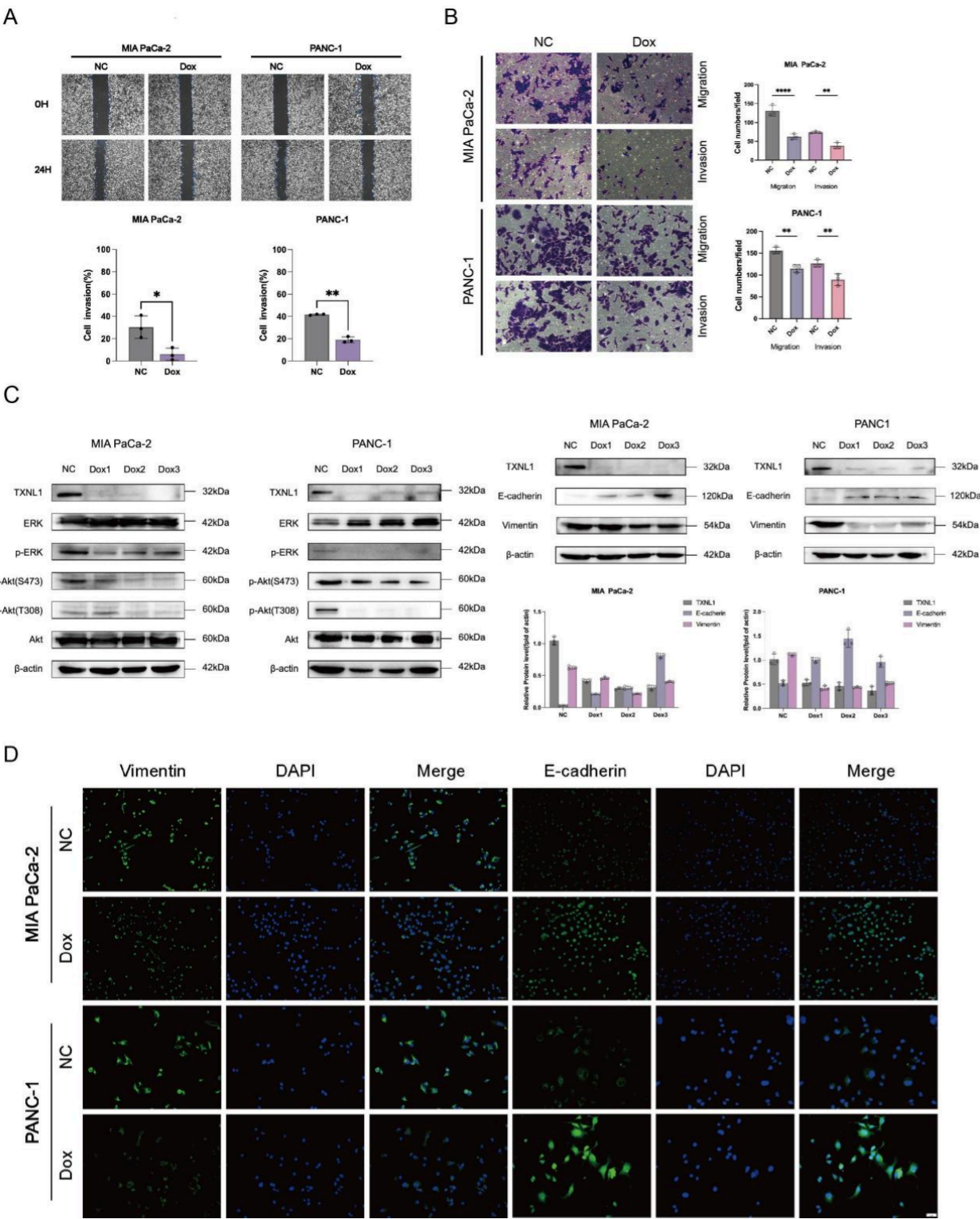


Figure 5

TXNL1 knockout is associated with impaired PDAC cell migration, invasion and EMT marker reversal. (A) Representative images of wound-healing assays at 0 and 24 hours. (B) Transwell migration and

Matrigel invasion assays. (C) Western blot analysis of p-ERK, ERK, p-AKT and AKT. (D) Western blot analysis of E-cadherin and Vimentin. (E) Immunofluorescence images of E-cadherin and Vimentin expression. Data are presented as means $\pm$ SD. $P < 0.05$, * $P < 0.01$, ** $P < 0.001$.

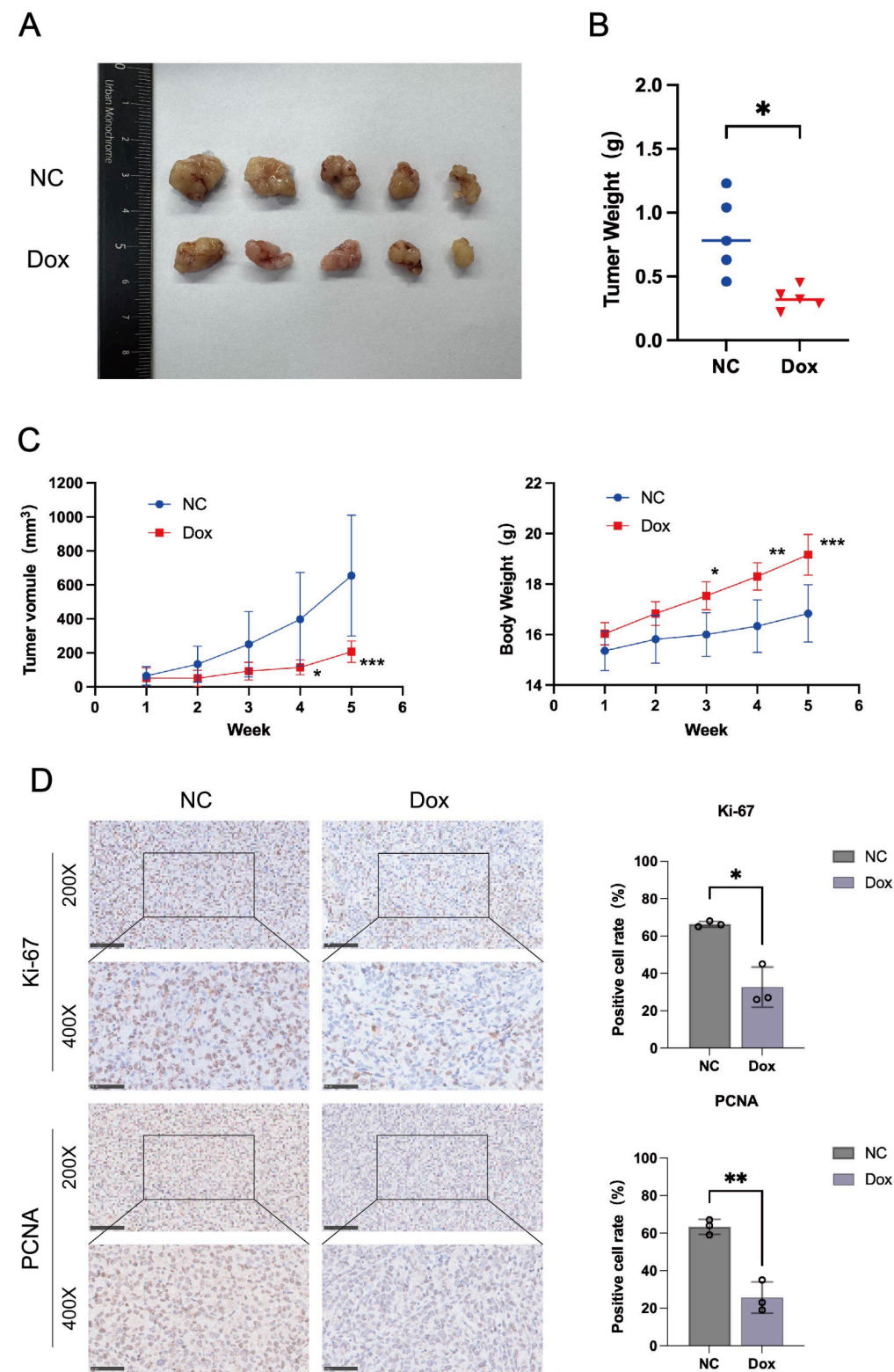


Figure 6

TXNL1 ablation is associated with suppressed PDAC tumour growth in vivo.

(A) Representative images of xenograft tumours. (B) Quantitative comparison of tumour weights. (C) Tumour volume growth curves and body weight measurements. (D) Immunohistochemical staining and quantification of Ki-67 and PCNA. Data are presented as means $\pm$ SD. $P < 0.05$, * $P < 0.01$.