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Original Research

FOS-Like Antigen 1 Regulates Proliferating Cell Nuclear Antigen Expression and Pancreatic Cancer Cell Proliferation and Migration

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Abstract

Background: Pancreatic cancer ranks among the leading causes of cancer-related death worldwide, and novel therapeutic strategies are urgently needed for advanced or metastatic disease states. Notably, targeted therapy remains a particularly promising approach. The transcription factor FOS-like antigen 1 (*FOSL1*) is overexpressed in refractory pancreatic cancers and drives tumor progression, correlating with unfavorable clinical outcomes. Thus, this study aimed to investigate the molecular mechanisms through which FOSL1 and proliferating cell nuclear antigen (PCNA) promote pancreatic cancer progression by regulating DNA repair, invasion, and migration. **Methods:** We evaluated the role of *FOSL1* in pancreatic cancer cell proliferation, DNA repair, stemness, invasion, and migration, and identified downstream targets and pathways through transcriptomic analysis. Mechanistically, we validated the FOSL1–PCNA interaction, FOSL1 binding to the *PCNA* promoter, and the role of FOSL1 in regulating PCNAubiquitination. Finally, we assessed whether FOSL1 functions through PCNA and explored the effects of combined FOSL1 knockdown and PCNA inhibition. **Results:** *FOSL1* depletion altered cell proliferation, steady-state DNA damage, stemness-associated markers, invasion, and migration. *PCNA* overexpression partially rescued the reduction in colony formation caused by *FOSL1* knockdown. Mechanistically, FOSL1 binds the *PCNA* promoter, and FOSL1 knockdown reduced *PCNA* mRNA expression. Moreover, chromatin immunoprecipitation–quantitative PCR (ChIP–qPCR) showed that FOSL1 occupies the *PCNA* promoter region. Conversely, PCNA knockdown suppressed pancreatic cancer cell proliferation and impaired the expression of genes involved in DNA repair, stemness, and epithelial-mesenchymal transition (EMT)-mediated invasion and migration. **Conclusion:** Our findings indicate that FOSL1 promotes pancreatic cancer progression, at least in part by upregulating PCNA expression. We hypothesize that combined inhibition of FOSL1 and PCNA may represent a therapeutic strategy for advanced pancreatic cancer.

Keywords: pancreatic neoplasms; fos-related antigen 1; proliferating cell nuclear antigen; DNA repair; epithelial-mesenchymal transition

1. Introduction

Novel therapeutic strategies are urgently needed to address advanced and metastatic malignancies, which constitute predominant contributor to global cancer-related mortality [1,2,3]. Targeted therapy represents one of the most promising methods in current cancer treatment [4]. Nevertheless, currently available targeted agents encounter substantial clinical challenges, most notably the development of therapeutic resistance, highlighting an urgent need to discover novel therapeutic targets and devise effective strategies for managing refractory cancers [4,5]. The transcription factor Activator protein-1 (AP-1), constituted by Jun/Fos heterodimeric complexes, governs multiple events such as proliferation, migration, and invasion [6]. Aberrant AP-1 signaling is mechanistically linked to tumor progression, invasion, metastasis, and therapeutic resistance, thereby representing a promising molecular target for anticancer intervention [6,7]. FOS-like antigen 1 (*FOSL1*), a principal component of the AP-1 transcription factor complex, exhibits significant overexpression in multiple malignancies, most notably pancreatic cancer [8,9]. Its expression level is associated with aggressive disease features and poor patient prognosis [8,9]. Mechanistically, FOSL1 regulates oncogenic processes including cell metastasis, stemness, and chemoresistance through transcriptional regulation of downstream effector genes [8,9,10,11,12,13]. Studies have shown that *FOSL1* plays a functional role in promoting epithelial-mesenchymal transition (EMT) in head and neck squamous cell carcinoma, maintaining stem cell populations in glioblastoma, mediating 5-fluorouracil resistance in colorectal adenocarcinoma, and conferring therapeutic resistance in breast carcinoma [11,12,13]. Further-

more, *FOSL1* has been shown to critically drive progression and unfavorable prognosis in mutant Kirsten rat sarcoma viral oncogene homolog (KRAS)-driven pancreatic cancers [8]. Notably, *FOSL1* represents a viable molecular target for precision oncology interventions, with particular therapeutic relevance in the management of pancreatic cancer.

Pancreatic cancer remains among the most aggressive and lethal cancers, marked by frequent late-stage diagnosis, aggressive biological behavior, and profound therapeutic resistance [14]. The disease carries a dismal prognosis, with fewer than 10% of patients achieving five-year survival, and only 10–15% presenting with surgically resectable tumors at diagnosis [14,15]. Surgery remains the primary approach for patients with localized pancreatic cancer lesions. Moreover, recent innovations in minimally invasive techniques and perioperative adjuncts have significantly improved procedural safety and outcomes. However, the limitations of surgical intervention alone have necessitated increased dependence on systemic therapies [14,15]. For unresectable and metastatic cancers, systemic chemotherapy remains the primary treatment method [14,15]. While concurrent administration of systemic chemotherapy and radiotherapy can yield meaningful prognostic improvements for pancreatic cancer patients, the inevitable development of chemoresistance in most patients ultimately compromises treatment efficacy and survival outcomes. Additionally, the substantial toxicity profiles of these systemic agents frequently impair the quality of life of patients [16]. Advances in molecular characterization have revealed the remarkable genomic complexity of pancreatic cancer, enabling precision medicine approaches guided by actionable molecular alterations [17,18]. Tumors harboring defects in DNA damage repair pathways, particularly those involving breast cancer susceptibility gene 1 (*BRCA1*), breast cancer susceptibility gene 2 (*BRCA2*), and partner and localizer of BRCA2 (*PALB2*), exhibit enhanced sensitivity to platinum-based agents and demonstrate durable responses to poly(ADP-ribose) polymerase (*PARP*) inhibitors like Olaparib [17]. Similarly, therapeutic breakthroughs have emerged targeting *KRAS*, which is mutated in roughly 95% of pancreatic cancer cases. The KRAS G12C inhibitor sotorasib has shown clinical efficacy in patients harboring this specific mutation [18]. Notwithstanding these advances, current targeted therapies remain constrained by narrow applicability and the inevitable emergence of resistance mechanisms [19]. Hence, identifying novel molecular targets and developing innovative therapeutic approaches represent pressing unmet needs in the management of pancreatic cancer.

Our prior investigations have demonstrated that suppression of *FOSL1* significantly curtails the aggressive growth of pancreatic tumor cells, thereby nominating it as a promising therapeutic target [20]. In this investigation, we explore for the first time the molecular underpinnings of FOSL1's influence on the progression of pancreatic malignancy through the specific lens of DNA lesion repair. Compelling evidence establishes that DNA damage repair dysregulation fundamentally contributes to the occurrence, progression, and therapeutic resistance in multiple cancers [21,22,23,24]. The integrity of DNA repair pathways is critical for genomic stability maintenance and cell survival. Functional impairments or regulatory disturbances in DNA damage repair can lead to mutational accumulation, and chromosomal rearrangements, ultimately driving tumorigenesis [21]. In contrast to normal cells, cancer cells frequently acquire DNA damage repair adaptations that confer proliferative advantages, enhanced invasive potential, and immune evasion capabilities [22,23]. Notably, resistance to multiple DNA damage-inducing anticancer agents frequently arises through either hyperactivation of canonical DNA damage repair signaling or engagement of alternative repair mechanisms [24]. This augmented DNA damage repair capacity enables rapid repair of therapy-induced DNA lesions, driving acquired chemoresistance [24]. Consequently, pharmacological disruption of DNA repair to promote the accumulation of damaged DNA has become a crucial therapeutic approach for cancer treatment [25]. The accumulation of extensive DNA damage fragments not only induces chromosomal instability and tumor cell death, but may also stimulate the cytosolic cyclic GMP–AMP synthase (cGAS)/stimulator of interferon genes (STING) signaling pathway, thereby facilitating type I interferon-mediated antitumor immunity [26]. Presently, targeting core proteins in regulating DNA damage repair has emerged as a critical frontier in precision cancer therapy [17,25]. Delineating the mechanism through which FOSL1 modulates DNA damage resolution will uncover novel paths for therapeutic intervention in pancreatic cancer.

In this study, we examined the functional contribution of *FOSL1* to the regulation of cellular growth, genomic repair fidelity, stem-like attributes, and tissue invasion in pancreatic cancer cells. Beyond its established functions in maintaining stemness and facilitating invasion and migration, we present novel evidence that FOSL1 accelerates cancer progression by augmenting the efficiency of DNA damage correction. Integrative analysis of RNA sequencing (RNA-seq) and co-immunoprecipitation-mass spectrometry (Co-IP-MS) datasets revealed proliferating cell nuclear antigen (PCNA) as a physical binding counterpart of FOSL1. Chromatin immunoprecipitation quantitative PCR (ChIP-qPCR) assays validated FOSL1 occupancy at the –821 bp region of the *PCNA* promoter, corresponding to the motif tttgactcagc. Our data showed that FOSL1 knockdown reduced PCNA expression and was associated with increased ubiquitin signals in PCNA immunoprecipitates, concomitant with decreased pancreatic cancer cell proliferation. PCNA is a ring-shaped homotrimeric protein that regulates DNA replication and repair, cell cycle pro-

gression, cell stemness, and invasion [27,28]. Silencing of PCNA inhibited pancreatic cancer cell proliferation, stemness, invasion, and migration, while promoting DNA damage and apoptosis. Moreover, PCNA promoted cancer cell DNA repair, stemness, and invasion by regulating genes related to DNA damage repair, stemness maintenance, and EMT regulation. Combined *FOSL1* and *PCNA* inhibition correlated with decreased pancreatic cancer cell proliferation. Our findings provide novel mechanistic insights into pancreatic cancer pathogenesis and propose pharmacological disruption of the *FOSL1* and *PCNA* may represent a potential therapeutic strategy for advanced and metastatic pancreatic cancer.

2. Materials and Methods

2.1 Bioinformatics Analysis

Disparities in gene transcript levels between pancreatic adenocarcinoma (PAAD) specimens and noncancerous counterparts within The Cancer Genome Atlas (TCGA) repository were analyzed using the Gene Expression Profiling Interactive Analysis (GEPIA) web platform (http://gepia2.cancer-pku.cn/#index). Statistical significance was derived employing one-way ANOVA approach. The influence of gene expression on patient survival was assessed using Kaplan-Meier method and compared with the log-rank test. Patients were divided into high- and low-expression groups based on the median transcript level. Survival analysis was performed to evaluate the prognostic significance of FOSL1 and PCNA. Overall survival and disease-free survival were analyzed using Kaplan–Meier survival curves, and statistical significance was assessed using the log-rank test. Correlation analyses were performed using Pearson's correlation for normally distributed variables. Primary clinical datasets encompassing RNA-seq profiles from 350 pancreatic cancer subjects were retrieved from TCGA. Diagnostic discrimination were scrutinized via receiver operating characteristic (ROC) curve assessment. An additional cohort with RNA-seq data derived from 183 pancreatic cancer cases was likewise acquired from TCGA. ROC plots and corresponding area under the curve (AUC) metrics were produced using the pROC R package (https://www.R-project.org/) and rendered with ggplot2 (https://ggplot2.tidyverse.org/). AUC interpretation followed these guidelines: values between 0.5 and 0.7 denote moderate predictive strength, 0.7 to 0.9 indicate robust performance, and figures exceeding 0.9 signify exceptional discriminatory power. All computational analyses and data visualizations were executed within R version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria; https://www.R-project.org/). Transcript co-expression associations among pivotal genes were evaluated via the GEPIA2 interface. The distribution of *FOSL1* transcript levels across diverse pancreatic cancer lines was sourced from the Cancer Cell Line Encyclopedia (CCLE) portal (https://portals.broadinstitute.org/ccle). Evaluations were performed using R v4.0.3 (https://www.R-project.org/) with ggplot2 package version v3.3.3 (https://ggplot2.tidyverse.org/). *FOSL1* ChIP-seq datasets were retrieved via the Cistrome Data Browser (http://cistrome.org/db/#/). Shared differentially expressed genes (DEGs) between negative control knockdown (NC KD) and *FOSL1* KD conditions were analyzed using the BioVenn online tool (https://www.biovenn.nl/). Functional categorization and pathway involvement of shared genes were explored by Gene Ontology (GO) annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway using the Omicsmart analytical suite [20,29].

2.2 Lentivirus Transfection

Lentiviral constructs carrying human full-length *FOSL1* cDNA (pGV341-cFOSL1), full-length *PCNA* cDNA (pGV341-cPCNA), or a scrambled sequence (pGV341-cNC) were generated, alongside lentiviral shRNA vectors targeting PCNA (pGV112-shPCNA), FOSL1 (pGV112-shFOSL1), or a scrambled control (pGV112-shNC). For transduction, PANC-1 and SW1990 cells were exposed to these lentiviruses using HitransG P reagent (GeneChem, Shanghai, China), following the supplier's instructions. After 2–3 days of exposure, the medium containing viral particles was discarded. The transduced cells were then grown for an additional 2–3 days and subsequently treated with puromycin to enrich transduced cells. For rescue experiments, cells were initially transduced with one lentiviral vector for 3 days. After removing the virus-containing medium, they were transduced with the second vector for another 3 days, followed again by removal of the viral medium. Cells co-transfected with both vectors were cultured for 2–3 days prior to downstream assays. All transduced cell populations were confirmed by RT-qPCR and Western blot [20,29], and the primer sequences used for RT-qPCR are listed in **Supplementary Table 1**. The shRNA target sequences used were: for *FOSL1*, 5′-CTGTACCTTGTATCTCCCTTT-3′; for *PCNA*, 5′-AAGCCACTCCACTCTCTTCAA-3′; and for the negative control, 5′-CATTCTCCGAACGTGTCACGT-3′.

2.3 Cell Culture

All cell lines were validated for their identity by Short Tandem Repeat (STR) genotyping and tested negative for mycoplasma. PANC-1 cultures were propagated in DMEM enriched with 8% fetal bovine serum (FBS). SW1990 cells were cultivated in Leibovitz's L-15 formulation supplemented with 10% FBS. BxPC3 cells were grown using RPMI-1640 base medium containing 12% FBS. Incubation conditions for all cell lines were set at 37 °C in a humidified environment, with 5% CO_2 for PANC-1 and BxPC3 cells and 100% air for SW1990 cells [29].

2.4 RNA-seq Analysis and DEG Processing

The effects of *FOSL1* shRNA or NC shRNA on the gene expression profile of PANC-1 cells were analyzed by RNA sequencing (RNA-seq), which was performed by Gene Denovo Biotechnology Co., Ltd. DEGs distinguishing the NC shRNA (NC KD) and *FOSL1* shRNA (FOSL1 KD) groups were defined using a significance filter of $|\log_2 FC| > 1.0$ and an adjusted p-value < 0.05. Transcripts exhibiting $\log_2 FC$ values below –1.0 were categorized as suppressed, while those exceeding 1.0 were deemed elevated. The unprocessed sequencing datasets have been deposited in the Sequence Read Archive (SRA) repository under accession identifier PRJNA1217410.

2.5 Cell Proliferation Assays

The influence of *FOSL1* modulation, *PCNA* perturbation, and AOH1996 exposure on the expansion of PANC-1 and SW1990 cells was measured using an MTT assay. For reconstitution analyses, SW1990 or PANC-1 cells underwent primary infection with *FOSL1* shRNA lentivirus for 3 days, after which the viral supernatant was substituted. These cells received a subsequent infection with a *FOSL1* cDNA expression vector for an extra 3 days interval. Following the elimination of viral remnants, cells were distributed at a concentration of 1×10^3 cells/mL into 96-well assay plates and allowed to proliferate for 1, 2, 3, or 5 days prior to analysis. In AOH1996 intervention studies, SW1990 cells were plated at 1×10^3 cells/mL in 96-well plates and subjected to 0.6 µM AOH1996 for intervals of 1, 2, 3, or 5 days. Parallel cultures lacking drug exposure were maintained as baseline controls. After the respective treatments, MTT reagent was introduced to individual wells, and plates were held at 37 °C for 4 h. The liquid medium was then aspirated, and resultant formazan precipitates were solubilized using DMSO. The viability of cells was gauged by optical density readings at 490 nm using a Synergy NEO_2 plate reader (BioTek, Winooski, VT, USA). The relative MTT metric was derived using the formula: relative ratio = $A_{Group,day}/A_{Control,1}$, wherein $A_{Group,day}$ denotes absorbance from treated groups at designated timepoints, and $A_{Control,1}$ represents the absorbance measured from untreated controls following the initial 24-hour culture period [20].

2.6 Alkaline Comet Assay

DNA damage in cancer cells following FOSL1 shRNA, PCNA shRNA, or AOH1996 treatment was evaluated using an alkaline comet assay kit [29]. Briefly, cells in each group were harvested, resuspended at a density of 1×10^4/mL, and combined with low-melting-point agarose at a 1:10 volume ratio. The cell-agarose suspension was then spread onto slides and incubated in lysis buffer at 4 °C for 2 h. The slides were then immersed in alkaline unwinding solution for 40 min at room temperature before electrophoresis. After electrophoresis, the samples were stained with propidium iodide (PI) for 15 min and examined under a Nikon ECLIPSE 80i inverted fluorescence microscope (Tokyo, Japan). For each slide, five random fields were photographed.

2.7 Sphere Formation Assay

Sphere-forming capacity of PANC-1 cells was examined as previously reported [20]. After treatment, cells in each group were seeded into ultra-low attachment plates at a density of 1×10^4 cells/mL, and then cultured in serum-free DMEM/F12 supplemented with B27, 20 ng/mL epidermal growth factor (EGF), and 10 ng/mL basic fibroblast growth factor (bFGF). The cultures were maintained for 14 days under these conditions. At the end of the incubation period, spheroids were visualized using a BioTek Cytation 5 live-cell imaging system (Winooski, VT, USA).

2.8 Co-IP-MS Assay

PANC-1 cells were lysed using the lysis and wash buffers supplied with the Protein A/G Magnetic IP/Co-IP kit. Anti-FOSL1 (Rabbit, 1:50, CST, Danvers, MA, USA), Anti-PCNA (Rabbit, 1:50, CST), or IgG (Rabbit, 1:50, CST) were pre-bound to Protein A/G beads overnight at 4 °C to generate the corresponding immunoprecipitation complexes. After protein quantification and normalization, cell lysates were incubated with the prepared beads for 2 h. After incubation, beads were collected and washed thoroughly with immunoprecipitation (IP) buffer to remove nonspecifically bound proteins. The immunoprecipitated proteins were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), and visible bands were excised for mass spectrometric analysis. For liquid chromatography–tandem mass spectrometry (LC-MS/MS), gel pieces were first dehydrated with acetonitrile for 10 min, followed by reduction with dithiothreitol and alkylation with iodoacetamide. The proteins were digested using trypsin, and the resulting peptides were analyzed by LC-MS/MS.

2.9 ChIP-qPCR Assay

Chromatin immunoprecipitation (ChIP) was carried out using the Simple ChIP Plus Sonication Chromatin IP Kit [20]. PANC-1 cells were fixed with 1% formaldehyde for 15 min, and crosslinking was terminated by glycine. After washing, cells were lysed in ChIP Sonication Cell Lysis Buffer. The nuclear fraction was collected and sonicated to generate fragmented chromatin. The sheared chromatin was then incubated with antibodies. The following antibodies were used for ChIP: anti-FOSL1 (Rabbit, 1:50, CST), anti-Histone H3 (Rabbit, 1:50, CST) as a positive control, and rabbit IgG (1:50, CST) as a negative control. Immune complexes were captured using ChIP-Grade Protein G beads, and the associated DNA was purified after reversal of crosslinking with 5.0 M NaCl and Proteinase K at 65 °C for 2 h. The

recovered DNA was subjected to qPCR analysis. Potential FOSL1-binding regions in the *PCNA* promoter were identified using the Java Architecture for Scanner and Profile of Regulatory Regions (JASPAR) database, including sites located at –821 bp (TTTGACTCAGC) and –1154 bp (GGTGGCTCACG). In addition, the –1621 bp (ATTACAATTGA) region was selected as a control. The primer sequences used for ChIP-qPCR were as follows: –821 forward GCAGGAGACTCACTTGAACC, reverse CTGAGTCAAAGAGCGTACACA; –1154 forward TAGAAATGCCCTTTCCTGTTCCC; reverse ATTCTTTATCTCTGGAATGCCTT; –1621 forward GACAGTTTTCTTGCTTGTGCT; reverse TCTTTATCTCTGGAATGCCTT.

2.10 Animal Care and Treatment

All animal procedures were performed in accordance with institutional guidelines for the care and use of laboratory animals and were approved by the Animal Experiment Ethics Committee of Lanzhou University (Lanzhou, Gansu Province, China) (Approval No. Lzujcyxy20250122). Six- to seven-week-old female athymic BALB/c-nude mice weighing 16–18 g were provided by the Animal House of the Model Animal Institute at Nanjing University (Nanjing, Jiangsu, China) and housed at 25°C with 60–70% relative humidity under a 12-h light/dark cycle. Each mouse was considered the experimental unit. A total of 20 mice were randomly assigned to four groups, with five mice per group: PANC-1 cells transduced with NC shRNA, PANC-1 cells transduced with FOSL1 shRNA, SW1990 cells transduced with NC shRNA, and SW1990 cells transduced with FOSL1 shRNA. Randomisation was performed using a random number table generated by an investigator not involved in subsequent experiments. The sample size was determined based on previous xenograft studies with similar designs and the expected variability in tumor growth. Cells in each group at a concentration of 1×10^7 cells/mL were injected subcutaneously into the right flank of each mouse. Tumor growth and body weight were monitored once the mean tumor volume in the NC KD group reached 50 mm^3. Tumor dimensions were measured every 3 days with a vernier caliper by investigators blinded to group allocation, and tumor volume was calculated using the formula $1/2 \times a \times b^2$, where a and b denote tumor length and width, respectively. The primary outcome was tumor growth, assessed by tumor volume throughout the observation period and tumor weight at the endpoint. Body weight was monitored as an indicator of general animal health. Predefined exclusion criteria included unsuccessful tumor engraftment, defined as a tumor volume <50 mm^3 14 days after tumor cell inoculation, or death during the observation period. No animals met these criteria, and no animals or data points were excluded. After 35 days of follow-up, the mice were anesthetized with 5% isoflurane and euthanized by cervical dislocation after adequate anesthesia was confirmed for tumor collection and weight measurement. Tumors were excised and weighed to assess tumor burden. All animal procedures were conducted in accordance with the ARRIVE guidelines.

2.11 Statistical Analysis

Data analysis was performed using SPSS 19.0 (https://www.ibm.com/spss), and data are presented as the mean ± SD, as indicated in the figure legends. Statistical analyses were conducted using a linear mixed-effects model with repeated measures for longitudinal measurements, one-way ANOVA for comparisons among more than two groups, and Student's *t*-test for comparisons between two groups. Correlation analyses were performed using Pearson's correlation for normally distributed variables. p value < 0.05 was considered statistically significant.

3. Results

3.1 FOSL1 Promotes the Growth of Pancreatic Cancers

The FOS transcription factor family, comprising *FOS*, *FOSB*, *FOSL1*, and FOS like antigen 2 (*FOSL2*), has been extensively implicated in the pathogenesis of various cancers [6,7,8]. To methodically probe the link between FOS family constituents and pancreatic cancer advancement, we began by examining their differential transcript profiles in tumor relative to adjacent non-malignant tissues utilizing TCGA datasets (Fig. 1A). Notably, *FOSL1*, *FOSL2*, *FOS*, and *FOSB* exhibited significant overexpression in PAAD (Fig. 1A). ROC curve analysis was performed to evaluate the diagnostic discrimination of FOS transcription factor family members between pancreatic tumor and normal tissues, as reflected by the AUC values. The AUC metrics for *FOSL1* and *FOSL2* were 0.97 and 0.91, respectively (Fig. 1B), whereas those for FOS and FOSB were 0.79 and 0.83, respectively (**Supplementary Fig. 1A**). Ensuing survival analyses confirmed that increased *FOSL1* correlated with markedly curtailed overall survival (OS) and disease-free survival(DFS), whereas high *FOSL2* expression specifically predicted shorter DFS (Fig. 1C). In contrast, survival analyses of FOS and FOSB showed no clear association with patient prognosis (**Supplementary Fig. 1B**). These findings position *FOSL1* as the most clinically relevant family member in progression of pancreatic cancer. Proceeding from these results, we charted the distribution of *FOSL1* mRNA across a panel of pancreatic cancer lines. The findings indicated relatively high transcript levels in PANC-1 and SW1990 models (Fig. 1D and **Supplementary Fig. 1C**). Subsequent RT-qPCR and Western blot analyses further corroborated the elevated FOSL1 protein and mRNA in these two specific lines (Fig. 1E,F, and **Supplementary Fig. 1D**). Thus, we selected these two cell lines for further research. Next, we systematically evaluated the effect of FOSL1 on the proliferation of PANC-1 and SW1990 cells. In PANC-1 cells, FOSL1 knockdown significantly suppressed cell proliferation, whereas FOSL1

overexpression reversed this inhibitory effect (Fig. 1G,H). Consistently, colony formation assays showed that *FOSL1* silencing reduced the clonogenic capacity of PANC-1 cells, which was rescued by *FOSL1* overexpression (Fig. 1I, **Supplementary Fig. 1E**). Similar results were observed in SW1990 cells, where FOSL1 knockdown significantly inhibited cell proliferation and colony formation, and FOSL1 overexpression attenuated these suppressive effects (Fig. 1J–L, **Supplementary Fig. 1E**). Similarly, apoptosis assays revealed that *FOSL1* knockdown promoted apoptosis in both cell lines. In PANC-1 cells, *FOSL1* silencing increased the apoptosis rate from 3.98% to 39.5% compared to the NC KD group, which was reduced to 5.2% after *FOSL1* overexpression in the rescue group (Fig. 1M, **Supplementary Fig. 1F**). In SW1990 cells, *FOSL1* knockdown elevated the apoptosis rate from 6.39% to 31.59%, while rescue experiments reduced it to 5.8% (Fig. 1N, **Supplementary Fig. 1F**).

Subsequently, we established stable *FOSL1*-knockdown PANC-1 and SW1990 cell lines to investigate the role of FOSL1 in pancreatic cancer growth *in vivo* (Fig. 2). Xenograft tumors were established using *FOSL1*-knockdown PANC-1 cells or the corresponding NC-knockdown controls (Fig. 2A–F). During the 35-day monitoring period, *FOSL1* silencing did not alter mouse body weight or induce detectable histopathological changes in the kidney, lung, spleen, liver, or heart (Fig. 2A,B). In contrast, tumor growth was markedly attenuated in the *FOSL1* KD group, as reflected by significantly decreased tumor volume and weight (Fig. 2C–E). Relative to the NC KD group, tumor volume and tumor weight were reduced by 56.4% and 50.2%, respectively (Fig. 2F). Consistently, immunohistochemistry (IHC) staining revealed a pronounced decrease in Ki-67 expression, indicating impaired proliferative activity in *FOSL1*-depleted PANC-1 xenografts (Fig. 2G, **Supplementary Fig. 2A**). A similar pattern was observed in the SW1990 xenograft model (Fig. 2H–M). Compared with NC KD controls, *FOSL1* knockdown had no obvious impact on body weight or organ morphology, but significantly suppressed tumor growth, leading to reductions in both tumor volume and weight (Fig. 2H–L). The corresponding inhibition rates were 44.6% for tumor volume and 41.5% for tumor weight (Fig. 2M). Ki-67 staining was decreased in the *FOSL1* KD tumors, further supporting an inhibitory effect of *FOSL1* depletion on SW1990 xenograft growth (Fig. 2N, **Supplementary Fig. 2B**).

3.2 FOSL1 Regulates Genes Associated With DNA Repair, Stemness, and EMT

To explore the mechanism by which *FOSL1* contributes to pancreatic cancer cell proliferation, RNA-seq was performed in PANC-1 cells after *FOSL1* knockdown (Fig. 3A–C). *FOSL1* depletion resulted in 2144 upregulated and 305 downregulated DEGs. GO and KEGG enrichment analyses suggested that these *FOSL1*-responsive genes were predominantly involved in DNA repair, stemness, and EMT (Fig. 3A). Consistently, Gene Set Enrichment Analysis (GSEA) further supported the involvement of *FOSL1* in DNA repair and cell migration-related pathways (Fig. 3B). Intersection analysis between the NC KD and *FOSL1* KD groups identified 14 shared DEGs (Fig. 3C). Among these, six were linked to DNA repair, four were associated with stemness, and four were related to EMT. Analysis of TCGA PAAD datasets showed that *RAD51*, *PCNA*, and X-ray repair cross-complementing protein 1 (*XRCC1*) were significantly elevated in pancreatic cancer. In addition, the stemness-associated genes B lymphoma Mo-MLV insertion region 1 homolog (*BMI1*), high mobility group A1 (*HMGA1*), and snail family transcriptional repressor 1 (*SNAIL*) were upregulated, and EMT-related markers including vimentin (*VIM*), β-catenin (*CTNNB1*), matrix metalloproteinase 1 (*MMP1*), and E-cadherin (*CDH1*) were also aberrantly expressed in PAAD (Fig. 3D). Notably, *CDH1* expression is generally associated with reduced migratory potential, whereas *VIM*, *CTNNB1*, and *MMP1* are linked to enhanced migration, underscoring the complexity of EMT regulation in pancreatic cancer. Correlation analysis further revealed that *FOSL1* expression was positively associated with *XRCC2*, *RAD51*, *BRCA2*, *PCNA*, *XRCC1*, *CD44*, *HMGA1*, *SNAIL*, and *CTNNB1* in pancreatic cancer cells (Fig. 3E). Collectively, these findings indicate that *FOSL1* may facilitate pancreatic cancer progression by modulating networks involved in DNA damage repair, stemness maintenance, and EMT.

3.3 FOSL1 shRNA Inhibits DNA Repair, Stemness, Invasion, and Migration in Pancreatic Cancer Cells

Guided by the RNA-seq findings, we assessed whether *FOSL1* influences DNA damage repair, stemness, invasion, and migration in pancreatic cancer cells. Comet assay results showed that *FOSL1* knockdown markedly increased DNA damage in both PANC-1 and SW1990 cells, as indicated by longer comet tails and a higher proportion of tail-positive cells (Fig. 4A) [30]. Because phosphorylated histone H2AX (*γ-H2AX)* is a marker of DNA double-strand breaks and accumulates in response to DNA damage, its expression was also examined. Immunofluorescence (IF) assays suggested that FOSL1 shRNA significantly induced γ-H2AX expression (Fig. 4B). RT-qPCR results showed that silencing *FOSL1* in PANC-1 cells significantly inhibited *PARP1* and *XRCC1* expression, thereby suppressing DNA single-strand breaks repair. Concurrently, knockdown of FOSL1 reduced X-ray repair cross-complementing protein 2 (*XRCC2*), *BRCA1*, *BRCA2*, and *RAD51* expression, impairing DNA double-strand breaks repair. Additionally, inhibition of *FOSL1* impaired *PCNA* expression, disrupting DNA replication and repair. The rescue experiments demonstrated that the overexpression of *FOSL1* could reverse the expression of these genes (Fig. 4C). Western

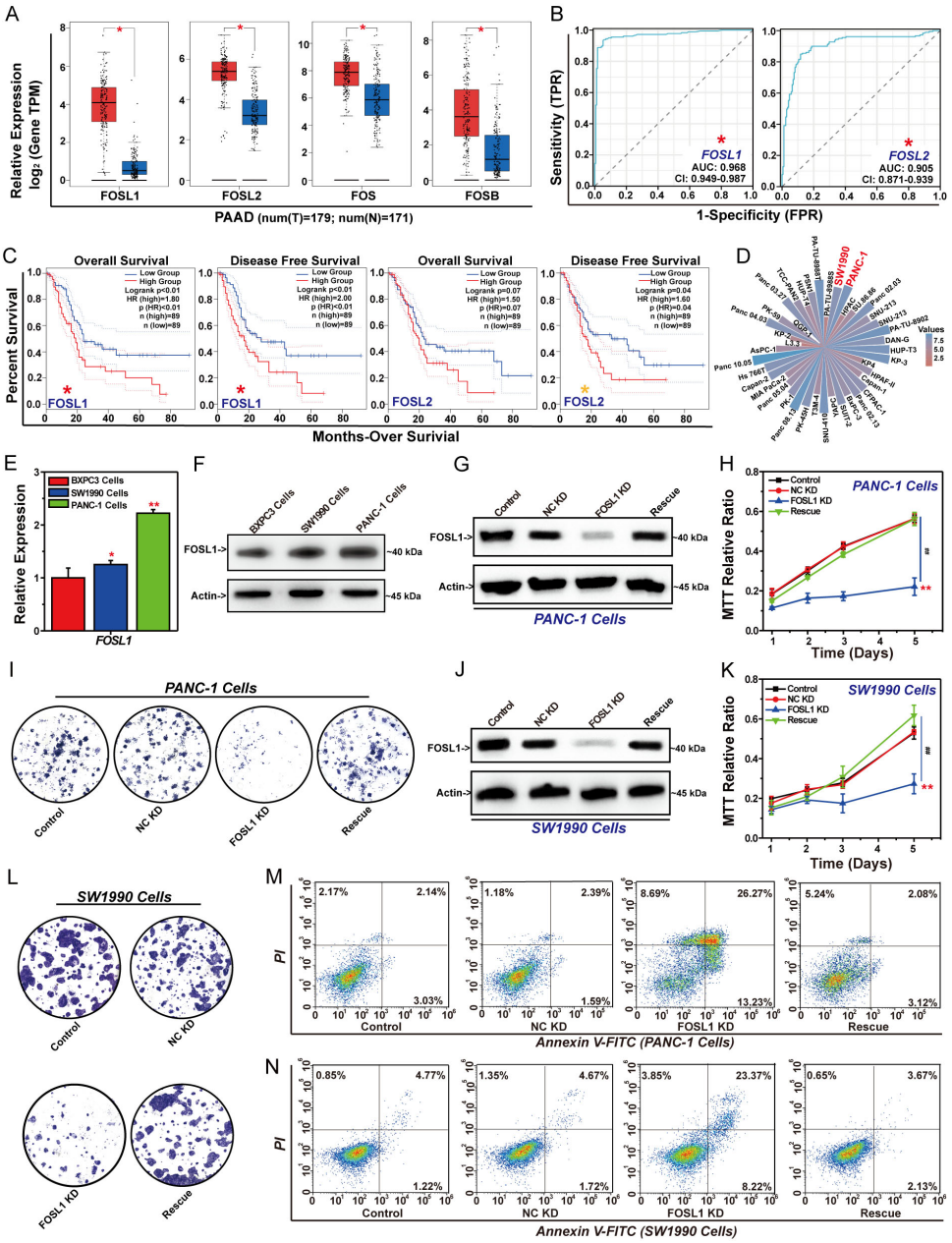

Fig. 1. FOSL1 promotes pancreatic cancer cell proliferation. (A) Differential expression of FOS family members between PAAD and adjacent normal tissues in TCGA.T, tumor tissues; N, adjacent normal tissues. (B) ROC curves showing the diagnostic discrimination of *FOSL1* and *FOSL2* expression between pancreatic tumor and normal tissues. (C) Correlation between *FOSL1* or *FOSL2* expression and survival outcomes in patients with pancreatic cancer. (D) Distribution of *FOSL1* mRNA across pancreatic cancer cell lines. (E) RT-qPCR analysis of *FOSL1* expression in BxPC3, SW1990, and PANC-1 cells. (F) Western blot analysis of FOSL1 expression in three cell lines. (G) Validation of FOSL1 knockdown and rescue efficiency in PANC-1 cells. (H) Relative viability of PANC-1 cells determined by MTT assay. (I) Effect of FOSL1 on colony formation in PANC-1 cells. (J) Validation of FOSL1 knockdown and rescue efficiency in SW1990 cells. (K) Relative viability of SW1990 cells determined by MTT assay. (L) Effect of *FOSL1* on colony formation in SW1990 cells. (M) Effect of *FOSL1* on apoptosis in PANC-1 cells. (N) Effect of *FOSL1* on apoptosis in SW1990 cells. Representative images were randomly selected from five replicates. Survival curves were generated using the Kaplan–Meier method and compared by the log-rank test. Correlation analyses were performed using Pearson's correlation for normally distributed variables. MTT data were conducted using a linear mixed-effects model with repeated measures. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. control group; $^{\#\#}p < 0.01$ vs. FOSL1 KD group. FOSL1, FOS-like antigen 1; PAAD, pancreatic adenocarcinoma; TCGA, The Cancer Genome Atlas; ROC, receiver operating characteristic; KD, knockdown.

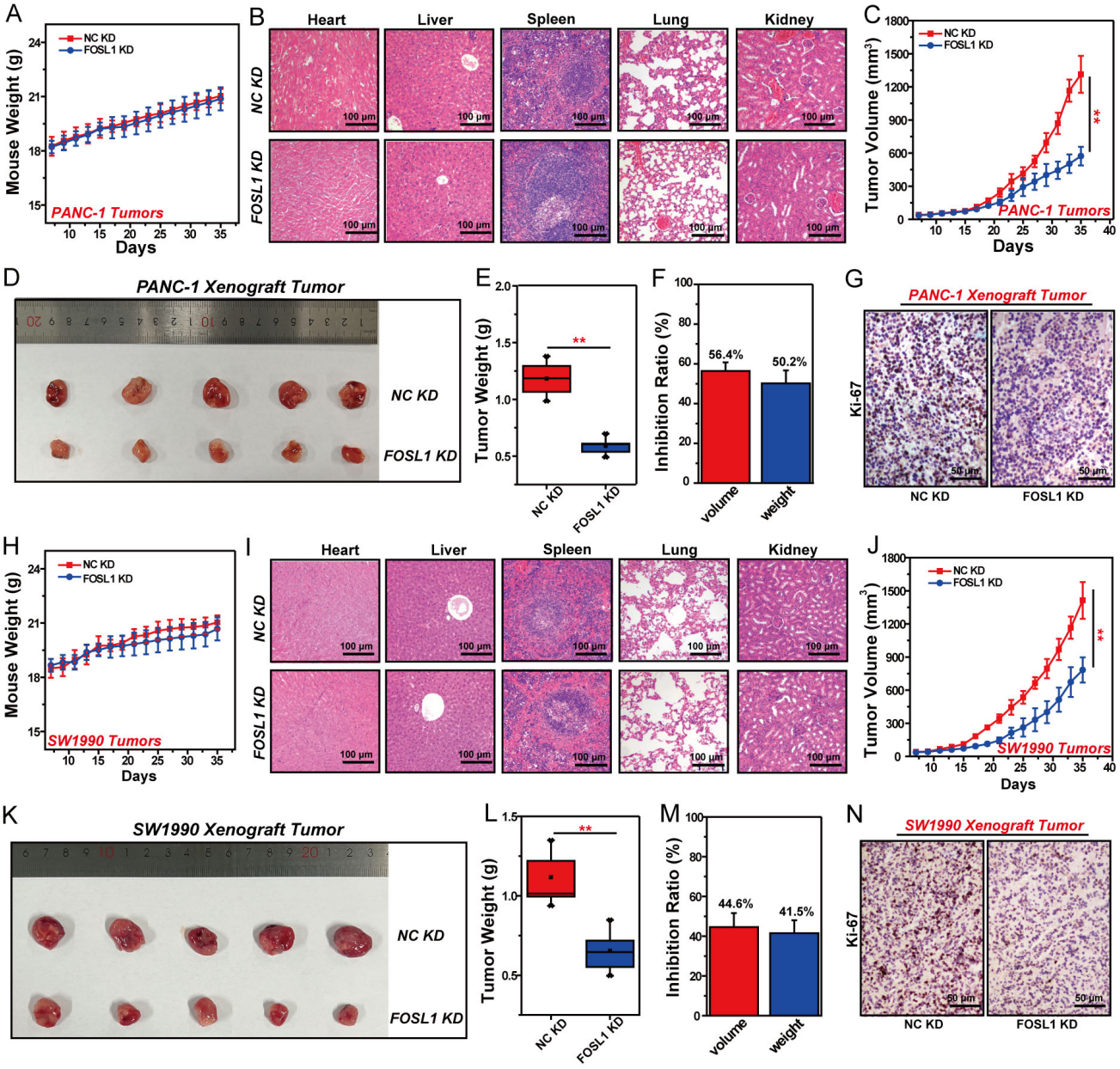

Fig. 2. FOSL1 knockdown inhibits the growth of PANC-1 and SW1990 xenograft tumors. (A) Mouse weight of mice in the PANC-1 xenograft tumor model. (B) Histological examination of the heart, liver, spleen, lungs, and kidneys of mice (scale bar: 100 μm). (C) Volume of PANC-1 xenograft tumors. (D) Images of PANC-1 xenograft tumors from each group. (E) Weights of PANC-1 xenograft tumors. (F) Inhibition rates of *FOSL1* knockdown on PANC-1 xenograft tumor volume and weight. (G) Ki-67 expression in PANC-1 xenograft tumors assessed by IHC (scale bar: 50 μm). (H) Mouse weight in the SW1990 xenograft tumor model. (I) H&E staining of the heart, liver, spleen, lungs, and kidneys in the SW1990 xenograft tumor model (scale bar: 100 μm). (J) Volume of SW1990 xenograft tumors. (K) Images of SW1990 xenograft tumors from each group. (L) Weight of SW1990 xenograft tumors. (M) Inhibition rates of FOSL1 knockdown on SW1990 xenograft tumor volume and weight. (N) Ki-67 expression in SW1990 xenograft tumors assessed by IHC (scale bar: 50 μm). Representative images were randomly selected from five replicates. Tumor growth data were analyzed using a linear mixed-effects model with repeated measures. $^{**}p < 0.01$ vs. NC KD group. IHC, Immunohistochemistry.

blot confirmed that FOSL1 shRNA inhibited DNA damage repair-related targets, leading to the accumulation of damaged DNA in pancreatic cancer cells (Fig. 4D, **Supplementary Fig. 3A**). We further examined the impact of *FOSL1* on pancreatic cancer cell stemness. IF analysis showed that CD44 expression was markedly lower in both PANC-1 and SW1990 cells after FOSL1 knockdown, indicating that FOSL1 plays a role in maintaining cancer cell stem-like properties (Fig. 4E). Subsequently, we performed sphere formation assay to confirm the role

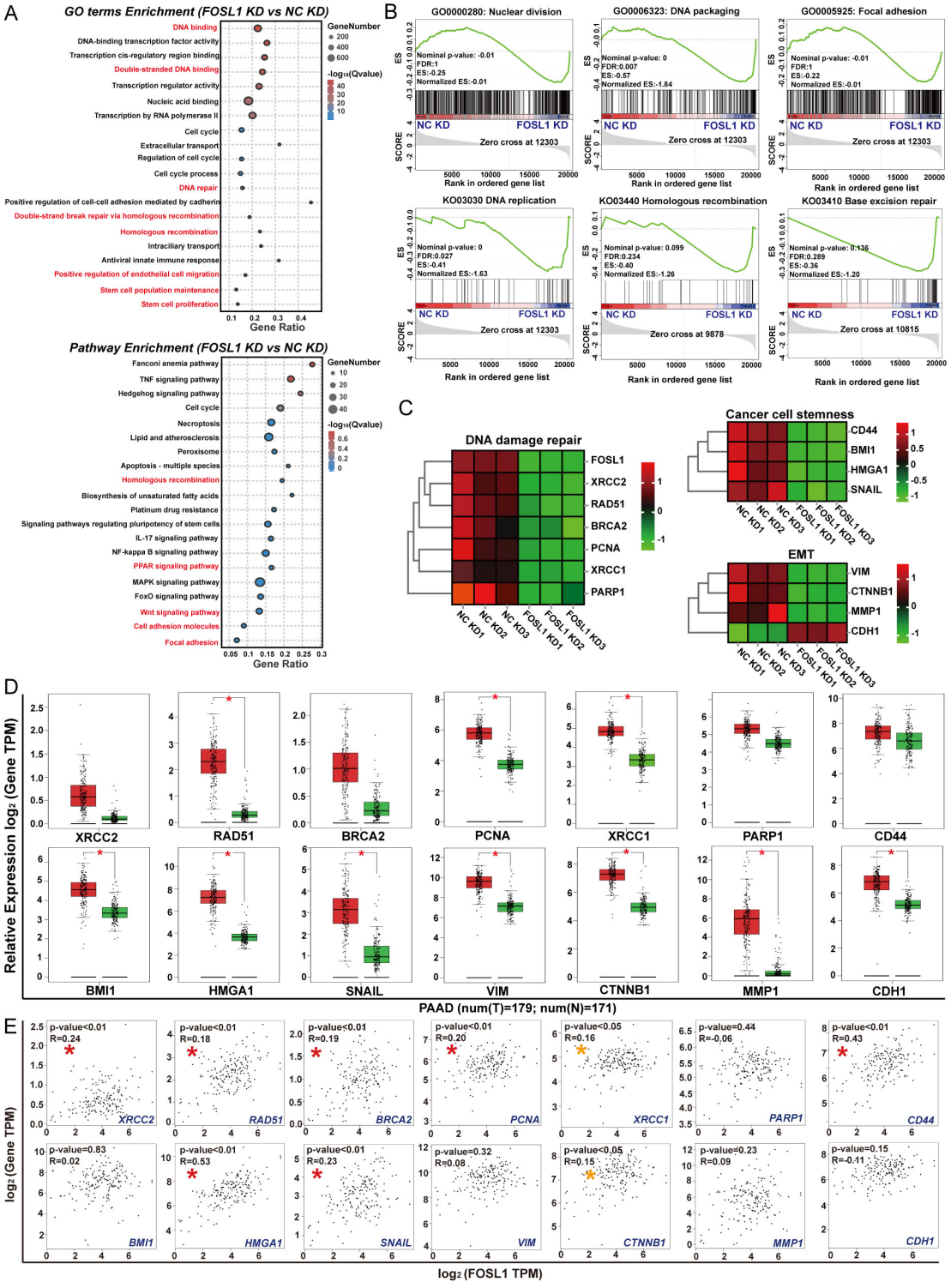

Fig. 3. FOSL1-regulated genes are involved in DNA repair, cell stemness, and EMT. (A) GO and KEGG enrichment analyses of DEGs between *FOSL1* KD and NC KD groups. (B) GSEA of *FOSL1*-regulated DEGs. (C) Heatmap showing differentially expressed genes related to DNA repair, cell stemness, invasion, and migration identified by RNA seq. (D) Differential expression of key genes in pancreatic cancer tissues versus adjacent normal tissues in TCGA.T, tumor tissues; N, adjacent normal tissues. (E) Correlation analysis between *FOSL1* and key genes. Differential expression was determined using one-way ANOVA approach. Correlation analyses were performed using Pearson's correlation for normally distributed variables. $^{*}p < 0.05$ was considered statistically significant. EMT, epithelial-mesenchymal transition; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; DEGs, differentially expressed genes; NC, negative control; GSEA, Gene Set Enrichment Analysis.

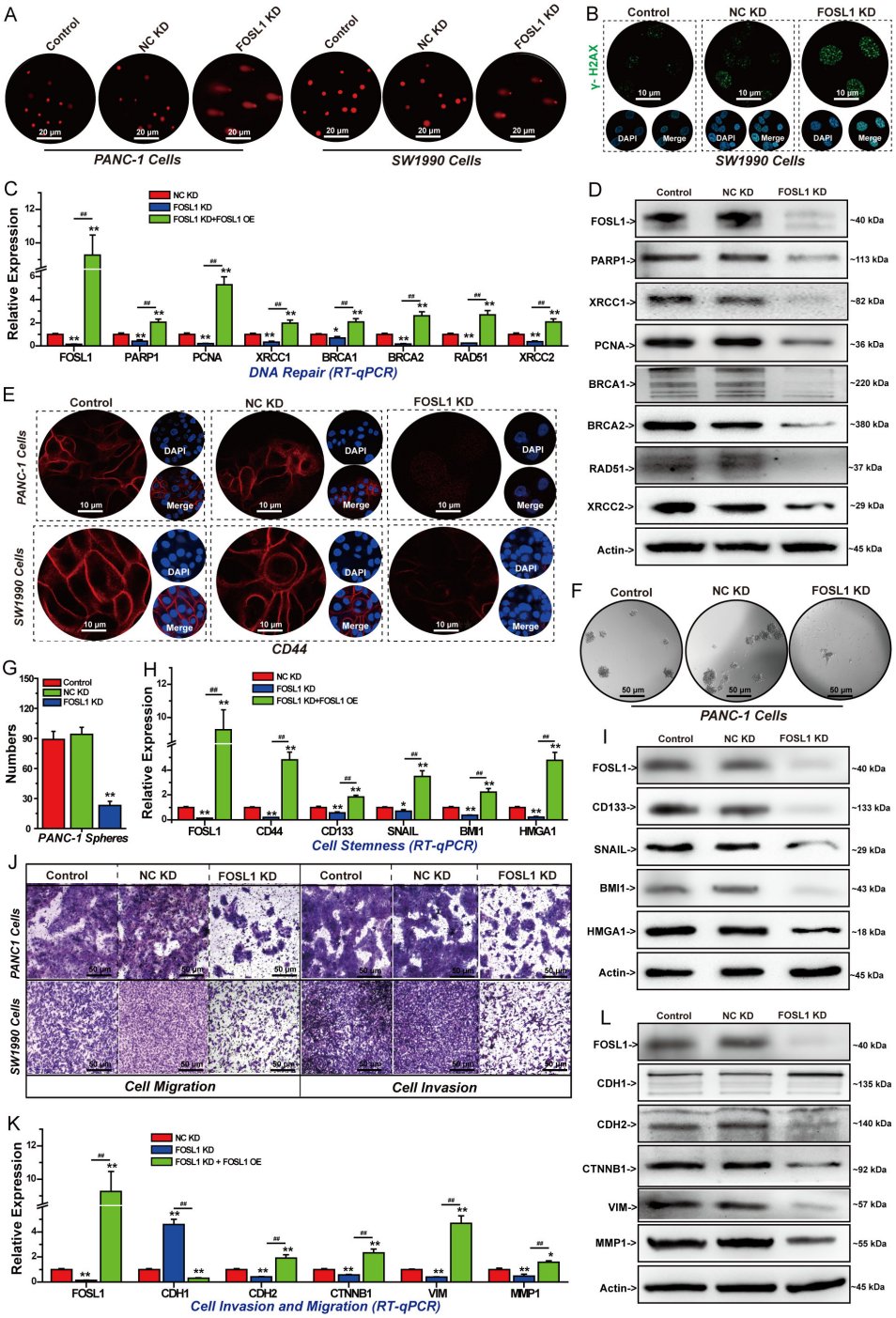

Fig. 4. FOSL1 promotes pancreatic cancer cell DNA repair, stemness, invasion, and migration. (A) Effects of *FOSL1* on DNA damage in pancreatic cancer cells assessed by comet assay (scale bar: 20 μm). (B) Effects of FOSL1 on the expression of γ-H2AX assessed by IF (scale bar: 10 μm). (C) RT-qPCR analysis of *FOSL1*-mediated changes in the expression of DNA repair-related genes. (D) Western blot analysis of DNA damage repair-associated proteins. (E) Effects of FOSL1 on CD44 expression assessed by IF (scale bar: 10 μm). (F) Effects of FOSL1 on three-dimensional sphere formation (scale bar: 50 μm). (G) Number of three-dimensional spheres formed by PANC-1 cells in each group. (H) RT-qPCR analysis of *FOSL1*-mediated changes in the expression of stemness-related genes in PANC-1 cells. (I) Western blot analysis of stemness-related proteins. (J) Effects of *FOSL1* on the invasion and migration of pancreatic cancer cells (scale bar: 50 μm). (K) RT-qPCR analysis of *FOSL1*-mediated changes in the expression of EMT-related genes in PANC-1 cells. (L) Western blot analysis of EMT-related proteins. Representative images were randomly selected from five replicates. Data from three independent experiments were analyzed using one-way ANOVA followed by Tukey's post hoc test. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. NC KD group; $^{\#\#}p < 0.01$ vs. FOSL1 KD group. γ-H2AX, phosphorylated histone H2AX; IF, Immunofluorescence.

of *FOSL1* in promoting cell stemness. This assay is commonly used to assess cancer cell stemness, with a greater number of three-dimensional spheres reflecting higher proliferative capacity and stronger stem-like properties in the corresponding cells [31]. Compared with the control group and the NC shRNA group, PANC-1 spheres were significantly reduced in the FOSL1 KD group (Fig. 4F,G, **Supplementary Fig. 3B**). RT-qPCR results further confirmed that knockdown of *FOSL1* suppressed the expression of stem-related genes such as *BMI1*, *SNAIL*, *HMGA1*, and cluster of differentiation 133 (*CD133*). Rescue assays further showed that *FOSL1* overexpression restored the expression of these genes (Fig. 4H). In addition, Western blot indicated that FOSL1 shRNA inhibited the abundance of stemness-associated proteins, supporting a role for FOSL1 in maintaining pancreatic cancer cell stem-like properties (Fig. 4I, **Supplementary Fig. 3C**). We analyzed the impact of *FOSL1* on pancreatic cancer cell invasion and migration. The results showed that silencing *FOSL1* in PANC-1 and SW1990 cells could significantly inhibit tumor cell invasion and migration (Fig. 4J, **Supplementary Fig. 3D**). RT-qPCR results revealed that *FOSL1* shRNA suppressed the expression of N-cadherin (*CDH2*), *CTNNB1*, *VIM*, and *MMP1* while promoting *CDH1* expression, thereby inhibiting EMT. Overexpression of *FOSL1* reversed the expression of EMT-related genes (Fig. 4K). Western blot results confirmed that suppression of FOSL1 blocked EMT-regulated tumor cell invasion and migration (Fig. 4L, **Supplementary Fig. 3E**). In summary, FOSL1 is a crucial target that promotes pancreatic cancer cell proliferation, DNA damage repair, stemness, as well as invasion and migration.

3.4 FOSL1 Promotes the PCNA Expression to Drive Pancreatic Cancer Cell Proliferation

To further elucidate the molecular mechanisms by which FOSL1 promotes pancreatic cancer progression, we performed Co-IP-MS to identify proteins that interact with FOSL1 in PANC-1 cells. A total of 810 proteins were identified as potential interactors of FOSL1. Integrative analysis of RNA-seq and Co-IP-MS data revealed 70 common genes (Fig. 5A, **Supplementary Table 2**). GO and KEGG enrichment analyses indicated that these common genes are primarily involved in DNA repair, apoptosis, and cell migration-related processes (Fig. 5B,C). Among them, PCNA emerged as a potential downstream target of FOSL1 (Fig. 5A). Co-IP assays further confirmed that FOSL1 and PCNA may reside within the same protein complex in PANC-1 cells (Fig. 5D). As a key transcription factor, FOSL1 is known to regulate downstream targets at the transcriptional level. To determine whether PCNA is transcriptionally regulated by FOSL1, we analyzed FOSL1 ChIP-seq data from the Cistrome Data Browser. Distinct enrichment peaks were observed in the *PCNA* promoter region, suggesting FOSL1 occupancy at this locus (**Supplementary Fig. 4A**). Consistently, ChIP-qPCR further verified that FOSL1 binds to the PCNA promoter at –821 bp (sequence: tttgactcagc), and FOSL1 knockdown reduced *PCNA* mRNA expression (Fig. 5E,F). Previous studies have reported that PCNA undergoes ubiquitination [32,33]. Therefore, we assessed whether FOSL1 influences PCNA ubiquitination. Although FOSL1 knockdown did not alter global ubiquitination levels in PANC-1 cells, it significantly increased ubiquitination of PCNA (Fig. 5G,H). PCNA pull-down assays confirmed increased ubiquitin immunoreactivity in the FOSL1 knockdown group compared with the NC KD group (Fig. 5G,H). K48-linked ubiquitination has been reported to be associated with PCNA ubiquitination [33]. PCNA monoubiquitination typically occurs at Lys164 and is mediated by the E3 ligase Rad18. However, this modification does not trigger PCNA protein loss but instead is involved in DNA damage repair [34]. Therefore, we further examined whether FOSL1 knockdown detectably altered PCNA monoubiquitination at Lys164. Ubiquitination-specific antibody analysis showed that PCNA monoubiquitination at Lys164 was not detectably altered upon FOSL1 knockdown (Fig. 5I,J). These results suggest that FOSL1 knockdown was associated with increased ubiquitin signals in PCNA immunoprecipitates. We next explored the reciprocal regulatory relationship between FOSL1 and PCNA. RT-qPCR and Western blot analyses showed that FOSL1 knockdown significantly reduced PCNA expression at both the mRNA and protein levels. Conversely, PCNA knockdown also significantly decreased FOSL1 expression at both levels (Fig. 5K,L, **Supplementary Fig. 4B**). These findings suggest that FOSL1 and PCNA regulate each other and function together. Similar to *FOSL1*, *PCNA* is also highly expressed in pancreatic cancer and is closely associated with poor patient prognosis. Increased expression of PCNA significantly reduced both OS and DFS in pancreatic cancer patients (Fig. 5M). Meanwhile, ROC curve analysis demonstrated the diagnostic discrimination of *PCNA* expression between pancreatic tumor and normal tissues (Fig. 5N). In addition, PCNA was highly expressed in PANC-1 and SW1990 cells (Fig. 5O–Q, **Supplementary Fig. 4C**). Finally, rescue experiments were performed to assess the functional role of PCNA in FOSL1-mediated effects. The results of RT-qPCR and Western blot showed that PCNA overexpression reversed the suppressive effects of FOSL1 knockdown on both FOSL1 and PCNA expression (Fig. 5R,S; **Supplementary Fig. 4D**). Colony formation assays further demonstrated that *PCNA* overexpression partially restored the proliferative capacity of PANC-1 cells with silenced *FOSL1* (Fig. 5T, **Supplementary Fig. 4E**). Collectively, these findings indicate that FOSL1 promotes the malignant proliferation of pancreatic cancer cells, at least in part by facilitating the expression of PCNA.

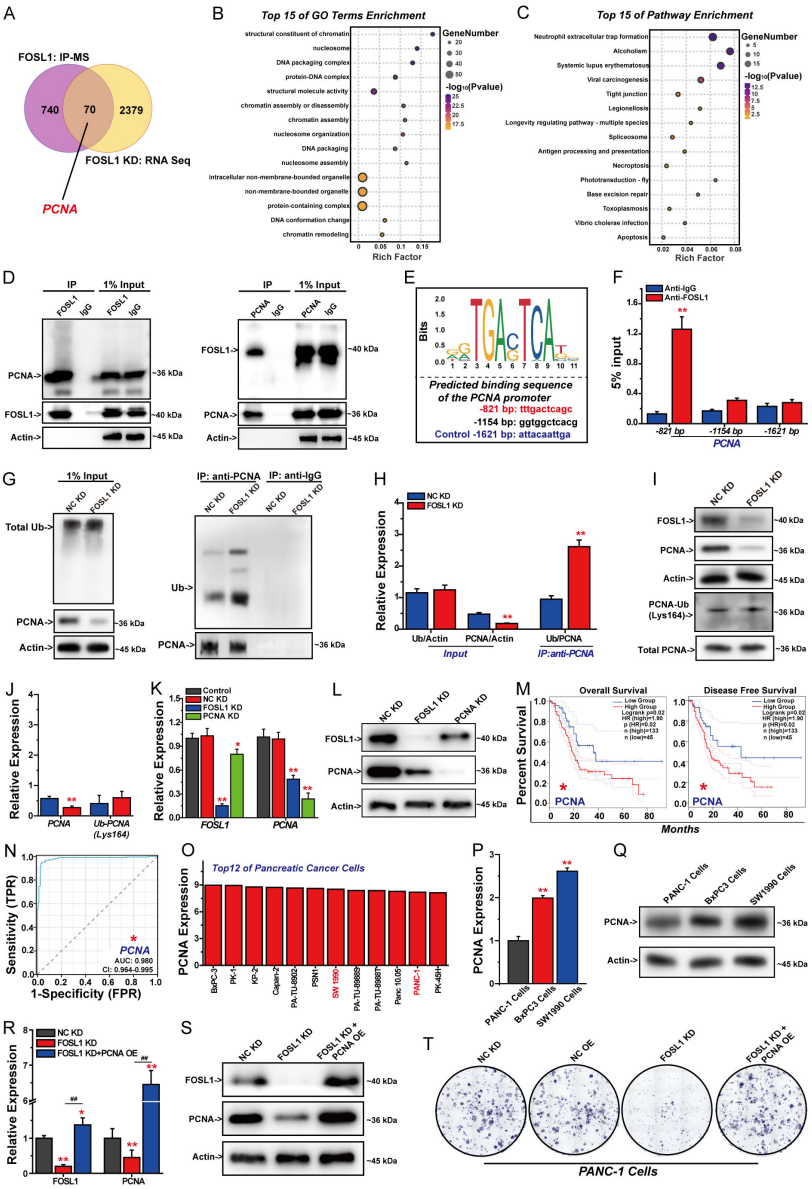

Fig. 5. FOSL1 regulates PCNA expression and ubiquitin-associated signals in PCNA immunoprecipitates. (A) Venn diagram showing the overlap between genes identified by RNA seq and Co-IP mass spectrometry. (B) GO enrichment analysis of the overlapping genes. (C) KEGG pathway enrichment analysis of the overlapping genes. (D) The interaction between FOSL1 and PCNA in PANC-1 cells analyzed by Co-IP. (E) Schematic representation of the conserved *PCNA* promoter binding site predicted by the JASPAR database. (F) ChIP-qPCR analysis of FOSL1 binding to the *PCNA* promoter region in PANC-1 cells. (G) PCNA pulldown assay assessing the effect of FOSL1 on PCNA ubiquitination. (H) Relative ubiquitin levels. (I) Western blot analysis of FOSL1-mediated regulation of PCNA expression and ubiquitination. (J) Relative expression of PCNA and ubiquitinated PCNA. (K) RT-qPCR analysis of *FOSL1* and *PCNA* expression. (L) Western blot analysis of FOSL1 and PCNA expression. (M) Association between *PCNA* expression and survival in pancreatic cancer patients. (N) ROC curves evaluating the diagnostic discrimination of *PCNA* expression between pancreatic tumor and normal tissues. (O) Distribution of *PCNA* expression across pancreatic cancer cell lines. (P) RT-qPCR analysis of *PCNA* expression in pancreatic cancer cells. (Q) Western blot analysis of PCNA expression distribution. (R) RT-qPCR analysis of FOSL1 and PCNA expression following rescue experiments. (S) Western blot analysis of FOSL1 and PCNA expression following rescue experiments. (T) Effect of *PCNA* on the inhibitory effect of FOSL1 knockdown in PANC-1 colony formation. Representative images were randomly selected from five replicates. Survival curves were generated using the Kaplan–Meier method and compared by the log-rank test. Data from three independent experiments were analyzed by one-way ANOVA followed by Tukey's post hoc test. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. NC KD group; $^{\#\#}p < 0.01$ vs. FOSL1 KD group. PCNA, proliferating cell nuclear antigen; Co-IP, co-immunoprecipitation; ChIP-qPCR, chromatin immunoprecipitation quantitative PCR; JASPAR, Java Architecture for Scanner and Profile of Regulatory Regions.

3.5 Inhibiting PCNA Suppresses DNA Repair, Cell Stemness, Invasion, and Migration in Pancreatic Cancer Cells

As a downstream target of *FOSL1*, *PCNA* has been implicated in the progression of pancreatic cancer. To further clarify the functional role of *PCNA*, we investigated its effects on pancreatic cancer cell proliferation, DNA damage repair, cell stemness, invasion, and migration. Western blot analysis showed that treatment with the PCNA inhibitor AOH1996 had no significant impact on PCNA expression (Fig. 6A, **Supplementary Fig. 5A**). Both *PCNA* knockdown and pharmacological inhibition significantly suppressed SW1990 cell proliferation (Fig. 6B). Similarly, colony formation assays demonstrated that the number of colonies formed by SW1990 cells was significantly reduced in the *PCNA* KD and AOH1996-treated groups compared with the control and NC KD groups (Fig. 6C, **Supplementary Fig. 5B**). Apoptosis assays further revealed that both PCNA silencing and functional inhibition significantly promoted apoptosis in SW1990 cells (Fig. 6D,E). To evaluate the role of *PCNA* in DNA damage repair, comet assays were performed. Compared with the control and NC KD groups, the *PCNA* KD and AOH1996-treated groups exhibited a significantly increased number and length of tail cells in both PANC-1 and SW1990 cells, indicating enhanced DNA damage on *PCNA* suppression or inhibition (Fig. 6F). Consistently, RT-qPCR analysis showed that inhibition of *PCNA* expression or function significantly reduced the expression of multiple DNA damage repair-related genes, including *PARP1*, *XRCC1*, *BRCA1*, *BRCA2*, *RAD51*, and *XRCC2* (Fig. 6G). We next examined the effects of *PCNA* on pancreatic cancer stemness. Sphere formation assays demonstrated that both *PCNA* shRNA and AOH1996 significantly inhibited sphere-forming capacity in PANC-1 cells (Fig. 6H, **Supplementary Fig. 5C**). In parallel, IF and RT-qPCR analyses showed that silencing *PCNA* or blocking its activity significantly reduced the expression of stemness-associated markers such as CD44, suggesting that PCNA contributes to the maintenance of pancreatic cancer cell stemness (Fig. 6I,J). Invasion and migration assays further indicated that *PCNA* knockdown or inhibition significantly impaired the invasive and migratory abilities of SW1990 and PANC-1 cells (Fig. 6K,L, **Supplementary Fig. 5D,E**). Subsequent RT-qPCR analysis revealed that *PCNA* regulates EMT-related gene expression, including *CDH1* and *VIM*, thereby facilitating EMT-associated invasion and migration (Fig. 6M). Moreover, Pearson correlation analysis demonstrated a positive association between *PCNA* expression and genes involved in DNA repair, stemness, invasion, and migration, with the exception of *SNAIL*, *CDH2*, and *MMP1* (Fig. 6N). Collectively, these findings suggest that *PCNA* promotes pancreatic cancer progression by enhancing cell proliferation, suppressing apoptosis, facilitating DNA repair, and facilitating stemness, invasion, and migration.

3.6 Combined FOSL1 Knockdown and AOH1996 Treatment Produces Greater Effects Than Either Treatment Alone

FOSL1 promotes cell proliferation, at least in part, by upregulating *PCNA*, highlighting the critical role of combined *FOSL1* and *PCNA* inhibition as a therapeutic strategy for pancreatic cancer. To further explore this possibility, we examined whether FOSL1 knockdown affects the response of pancreatic cancer cells to the PCNA inhibitor AOH1996. The combination of *FOSL1* knockdown and AOH1996 treatment resulted in fewer colonies than either treatment alone in both SW1990 and PANC-1 cells following 14 days of exposure to 0.6 μM AOH1996 (Fig. 7A, **Supplementary Fig. 6A**). Similarly, the combination group showed more apoptotic cells than either single-treatment group in PANC-1 cells (Fig. 7B, **Supplementary Fig. 6B**). Comet assay analysis further indicated that the combined treatment of *FOSL1* shRNA and AOH1996 significantly increased DNA damage in PANC-1 cells (Fig. 7C). Western blot analysis showed that AOH1996 alone did not significantly alter the expression of PCNA or FOSL1. However, the combination of AOH1996 and FOSL1 shRNA showed greater suppression of DNA repair-related proteins than either single-treatment group. Specifically, AOH1996 alone significantly reduced the expression of BRCA1, BRCA2, RAD51, and XRCC2. In parallel, FOSL1 silencing suppressed key DNA damage repair regulator expression, including PCNA, and the combined treatment exerted an even stronger inhibitory effect on the expression of these DNA repair-associated proteins (Fig. 7D, **Supplementary Fig. 6C**). In addition, IF and Western blot analyses demonstrated that AOH1996 significantly decreased the expression of stemness-related markers, including CD44, CD133, SNAIL, and HMGA1. The combination group showed greater suppressive effect of AOH1996 on these stemness-associated proteins (Fig. 7E,F, **Supplementary Fig. 6D**). Moreover, compared with either FOSL1 shRNA or AOH1996 treatment alone, the combination treatment more effectively inhibited the PANC-1 cell invasion and migration (Fig. 7G, **Supplementary Fig. 6E**). Mechanistically, AOH1996 significantly downregulated CDH2 and VIM while upregulating CDH1 expression. FOSL1 silencing not only altered the expression of these EMT-related proteins but also significantly reduced CTNNB1 expression (Fig. 7H, **Supplementary Fig. 6F**). In summary, combined FOSL1 knockdown and AOH1996 treatment suppressed pancreatic cancer cell proliferation, DNA repair, and stemness, indicating a promising combination strategy for further evaluation.

4. Discussion

Pancreatic cancer is the aggressive and lethal malignancy, and it remains highly resistant to most therapeutic modalities [14,15,16,17,18,19]. Therefore, develop novel and more effective treatment strategies is urgent. With the

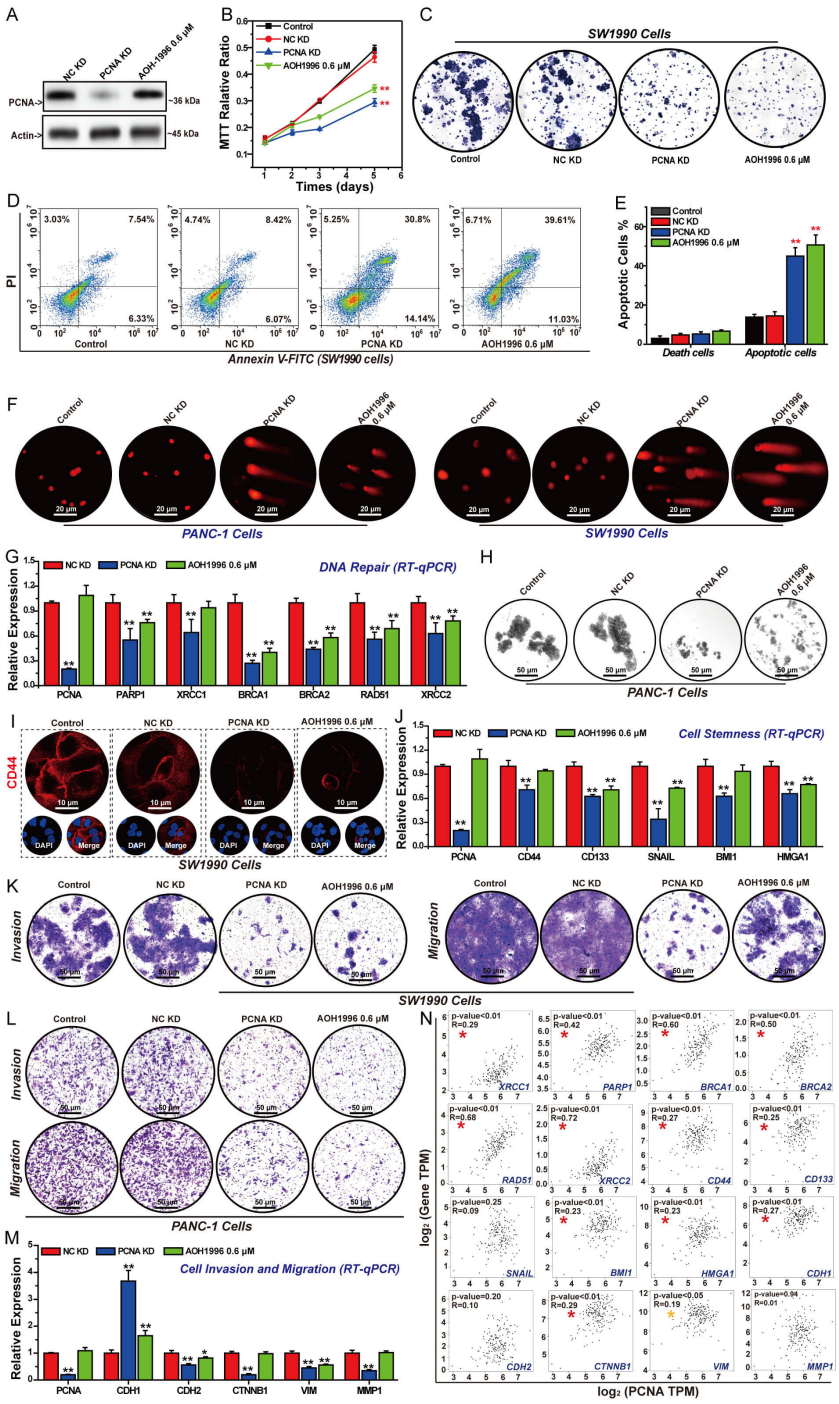

Fig. 6. Inhibiting PCNA suppresses pancreatic cancer cell proliferation, DNA repair, stemness, invasion, and migration. (A) Western blot analysis of PCNA expression. (B) Effects of *PCNA* on SW1990 cell viability. (C) Effects of *PCNA* on SW1990 colony formation. (D) Effects of *PCNA* on SW1990 cell apoptosis. (E) Quantification of dead cells and apoptotic cells. (F) Effects of *PCNA* on DNA damage analyzed by comet assay (scale bar: 20 μm). (G) RT-qPCR analysis of *PCNA*-mediated changes in the expression of DNA repair-related genes. (H) Effects of *PCNA* on PANC-1 sphere formation (scale bar: 50 μm). (I) Effects of PCNA on CD44 expression in SW1990 cells assessed by IF (scale bar: 10 μm). (J) RT-qPCR analysis of *PCNA*-mediated changes in the expression of stemness-related genes in SW1990 cells. (K) Effects of *PCNA* on SW1990 cell invasion and migration (scale bar: 50 μm). (L) Effects of *PCNA* on PANC-1 cell invasion and migration (scale bar: 50 μm). (M) RT-qPCR analysis of *PCNA*-mediated changes in the expression of EMT-related genes in SW1990 cells. (N) Pearson correlation analysis between *PCNA* and key genes in PAAD. Representative images were randomly selected from five replicates. MTT data were conducted using a linear mixed-effects model with repeated measures. Other data from three independent experiments were analyzed by one-way ANOVA followed by Tukey's post hoc test. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. NC KD group.

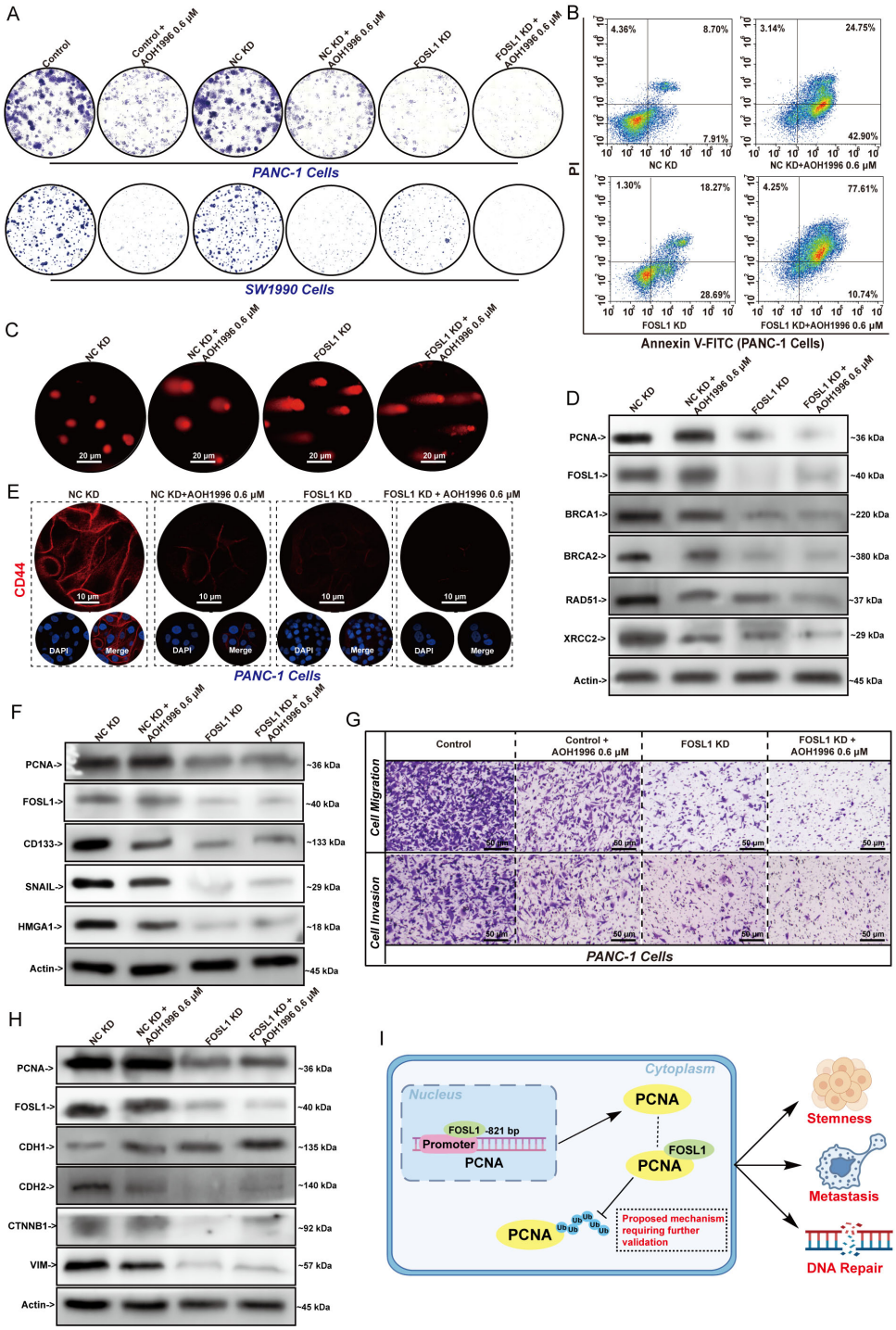

Fig. 7. Combined FOSL1 knockdown and AOH1996 treatment produces greater effects than either treatment alone at the tested concentrations. (A) Effect of *FOSL1* knockdown on AOH1996-mediated inhibition of colony formation. (B) Effect of *FOSL1* knockdown on AOH1996-induced apoptosis. (C) Effect of *FOSL1* knockdown on AOH1996-induced DNA damage (scale bar: 20 μm). (D) Western blot analysis of the effects of FOSL1 knockdown and AOH1996 on DNA repair-related protein expression. (E) IF analysis of the effects of FOSL1 knockdown and AOH1996 on CD44 expression (scale bar: 10 μm). (F) Western blot analysis of the effects of FOSL1 knockdown and AOH1996 on stemness-related protein expression. (G) Effect of *FOSL1* knockdown on AOH1996-mediated suppression of pancreatic cancer cell invasion and migration (scale bar: 50 μm). (H) Western blot analysis of the effects of FOSL1 knockdown and AOH1996 on EMT-related protein expression. (I) Graphical summary illustrating the potential role of FOSL1 in pancreatic cancer progression through the regulation of PCNA expression and ubiquitin-associated signals. Representative images were randomly selected from three independent experiments.

advancement of molecular targeted therapy, mechanism-guided combination strategies have emerged as a particularly promising option for patients with pancreatic cancer [17,18]. *FOSL1*, a transcription factor that is frequently overexpressed in multiple cancers, has been consistently associated with malignant progression [8,9,10,11,12,13,35]. Accumulating evidence indicates that *FOSL1* promotes cancer cell proliferation, stemness, and metastasis, underscoring its promise as a therapeutic target [10,11,12,13]. However, its role in DNA damage repair and the underlying mechanisms remain incompletely understood. In the present study, we investigated the function of *FOSL1* in pancreatic cancer progression from the perspective of DNA damage repair and demonstrated that *FOSL1* exerts protumorigenic effects partially by upregulating PCNA expression.

FOSL1, the key member of AP-1 family, has been reported to be closely related to poor prognosis in multiple cancers, including pancreatic cancer, primarily due to its aberrant overexpression [6,7,8,9]. Previous studies have demonstrated that *FOSL1* promotes cancer cell proliferation, stemness, and metastasis mainly by regulating downstream target expression [10,11,12,13]. In particular, *FOSL1*-driven tumor stemness has been recognized as an important contributor to chemoresistance in several cancers [13]. However, the regulatory role of *FOSL1* in DNA damage repair of cancer cells is still not fully understood. We found that *FOSL1* overexpression in pancreatic cancer is associated with shorter survival and poorer prognosis. Moreover, depletion of *FOSL1* significantly inhibited pancreatic cancer cell proliferation *in vitro* and suppressed xenograft tumor growth *in vivo*. In addition to genes associated with stemness and EMT, *FOSL1* also regulates pathways involved in DNA replication and repair. These findings suggest that *FOSL1* promotes pancreatic cancer progression through multiple mechanisms, including regulation of DNA damage repair, stemness, and metastasis. When repair capacity is inhibited, unrepaired damaged DNA accumulates as DNA fragments, which is directly reflected by increased tail DNA and tail length in the comet assay [29]. Comet assay showed that *FOSL1* shRNA led to damaged DNA accumulation and was accompanied by increased γ-H2AX expression. Since *H2AX* is phosphorylated to *γ-H2AX* in response to accumulated DNA double-strand breaks, *FOSL1* participates in the DNA damage response and repair process [36]. Mechanistically, silencing *FOSL1* significantly reduced the expression of *PCNA*, *PARP1*, *XRCC1*, *BRCA1*, *BRCA2*, *RAD51*, and *XRCC2* in pancreatic cancer cells. *PCNA* plays a central role in DNA replication and DNA repair [27]. *PARP1* is a key mediator of single-strand break repair through the transfer of ADP-ribose units from NAD^+ to targets [37]. XRCC1 is essential for base excision repair and facilitates the repair of single-strand breaks and direct DNA damage [38]. *BRCA1*, *BRCA2*, *RAD51*, and *XRCC2* cooperate to ensure the repair of DNA double-strand breaks via homologous recombination [39,40]. The downregulation of these repair-related factors provides direct evidence that FOSL1 knockdown and PCNA inhibition impair DNA repair in pancreatic cancer cells, including both single-strand break repair and double-strand break repair. *FOSL1* exerts its oncogenic effects, at least in part, by regulating cancer cell stemness and metastasis [8,9,10,11]. Our research has revealed that inhibiting the expression of *FOSL1* suppressed three-dimensional sphere formation and reduced stemness-associated marker expression, including CD44, CD133, BMI1, SNAIL, and HMGA1. Enhanced sphere-forming capacity is generally associated with increased proliferative potential and stem-like properties of cancer cells [20]. CD44 and CD133 are closely related to cancer cell stemness [41]. SNAIL is a key transcription factor that regulates chemoresistance, stemness, and metastasis in multiple tumors [42]. BMI1 promotes cancer cell proliferation and stemness by regulating multiple pathways [43]. HMGA1 has been involved in cancer stemness, invasion, and drug resistance [44]. These findings suggest that *FOSL1* promotes pancreatic cancer stemness by upregulating stemness-related gene network. We further examined the effects of *FOSL1* on pancreatic cancer cell invasion, migration, and EMT-related gene expression. The experimental results showed that *FOSL1* knockdown significantly inhibited invasion and migration, while increasing *CDH1* expression and decreasing the expression of *CDH2*, *VIM*, *CTNNB1*, and *MMP1*. *CDH1*, *CDH2*, and *VIM* are key regulators of EMT [45]. EMT is a dynamic biological process, and its dysregulation can drive cancer invasion and metastasis [46]. Reduced expression of the epithelial marker *CDH1*, together with increased expression of *VIM* and *CDH2*, has been shown to facilitate tumor invasion and dissemination [45]. *CTNNB1* is a central component of the Wnt/β-catenin pathway and is closely associated with tumor metastasis [47]. *MMP1* contributes to cancer progression and poor prognosis by promoting invasion, metastasis, and angiogenesis [48]. The regulatory effects of *FOSL1* on these genes indicate that it may promote pancreatic cancer cell invasion and migration through EMT-associated mechanisms. The roles of *FOSL1* in DNA repair, stemness, invasion, and migration suggest that it functions as a key driver. Therefore, *FOSL1* may serve as a promising therapeutic target to improve survival and quality of life in patients with pancreatic cancer.

We employed an integrated analysis of RNA-seq and Co-IP-MS to identify the key downstream target of *FOSL1* involved in pancreatic cancer progression. Among the candidate targets regulated by *FOSL1*, PCNA was found to be overexpressed in pancreatic cancer, and its elevated expression was associated with poorer patient survival and an unfavorable prognosis. ChIP-qPCR confirmed that *FOSL1* binds to the *PCNA* promoter, with ChIP-qPCR confirming enrichment at the –821 bp region corresponding to the sequence tttgactcagc. In addition, Co-IP assays revealed

an interaction between FOSL1 and PCNA. Functionally, our data showed that FOSL1 knockdown was associated with increased ubiquitin signals in PCNA immunoprecipitates. Further analysis indicated that PCNA monoubiquitination at Lys164 was not detectably altered upon FOSL1 knockdown, consistent with the known role of this modification in DNA damage repair [34]. Additional studies are required to investigate the functional implications of the increased ubiquitin signals observed in PCNA immunoprecipitates upon FOSL1 knockdown. Together, these results indicate that FOSL1 knockdown reduced *PCNA* mRNA expression and was associated with increased ubiquitin signals in PCNA immunoprecipitates. The results of RT-qPCR and Western blot showed that FOSL1 regulates PCNA expression at both the mRNA and protein levels. This confirmed that PCNA is a downstream effector of FOSL1. PCNA overexpression partially reversed the proliferative inhibition induced by FOSL1 silencing, further suggesting that FOSL1 promotes pancreatic cancer cell proliferation, at least in part, through upregulation of PCNA expression. Collectively, these results indicate that *FOSL1* drives pancreatic cancer progression partly by enhancing *PCNA* expression. As a critical downstream target of *FOSL1*, *PCNA* is essential for DNA replication and repair. Previous studies have demonstrated that PCNA is often overexpressed in variety of cancers and is linked to malignant progression and unfavorable prognosis [27]. In addition, *PCNA* has been implicated in the regulation of cancer cell stemness and invasion [28]. In our study, silencing of *PCNA* or pharmacological inhibition with AOH1996 significantly suppressed pancreatic cancer cell proliferation, DNA repair, stemness, invasion, and migration, while markedly promoting apoptosis. These findings suggest that the *FOSL1* and *PCNA* plays a pivotal role in driving pancreatic cancer progression and may represent a potential therapeutic target. To further evaluate the effects of combined *FOSL1* and *PCNA* inhibition on pancreatic cancer cell growth, we examined the combination of *FOSL1* knockdown and AOH1996 treatment. We found that the combined use of *FOSL1* shRNA and the *PCNA* inhibitor AOH1996 significantly inhibited the proliferation of pancreatic cancer cells. Mechanistically, dual inhibition of *FOSL1* and *PCNA* more effectively suppressed pancreatic cancer cell growth, DNA repair, stemness, invasion and migration by regulating the expression of genes associated with DNA damage repair, stemness, and EMT. However, given that *PCNA* is a critical regulator of DNA replication and repair in normal proliferating cells, its inhibition may also affect nonmalignant tissues with rapid turnover [27,28]. Therefore, further studies are needed to comprehensively evaluate the toxicity and safety of targeting *FOSL1* and *PCNA*. Collectively, combined inhibition of *FOSL1* and *PCNA* may provide a potential strategy for inhibiting pancreatic cancer progression.

5. Limitations

However, several limitations should be acknowledged in this study. First, the *in vivo* antitumor effects of targeting *PCNA* and *FOSL1* were not assessed, and no orthotopic models were used to evaluate their roles in metastatic progression. Second, although our data indicate that *FOSL1* occupies the *PCNA* promoter and may transcriptionally regulate *PCNA*, luciferase reporter assays are needed for direct validation. Third, while our findings indicate that FOSL1 knockdown was associated with increased ubiquitin signals in PCNA immunoprecipitates, CHX chase and MG132 rescue assays are required to further investigate the functional consequences of the increased ubiquitin signals in PCNA immunoprecipitates. The specific E3 ligase mediating PCNA ubiquitination also remains unidentified. Fourth, rescue groups were not included in the Western blot analysis used to assess the effects of *FOSL1* on the expression of stemness-related and DNA repair-related factors. Finally, because clinical samples are difficult to obtain, our study lacks adequate validation in patient-derived samples.

6. Conclusion

In summary, we investigated the molecular mechanisms by which *FOSL1* promotes pancreatic cancer progression from the perspective of DNA damage repair, stemness, and EMT-mediated migration and invasion. Our findings demonstrate that *FOSL1* enhances pancreatic cancer cell proliferation, DNA damage repair, stemness, invasion, and migration. Mechanistically, *PCNA* was identified as a key downstream effector of *FOSL1*. *FOSL1* knockdown reduced *PCNA* mRNA expression, and ChIP-qPCR showed *FOSL1* occupancy at the *PCNA* promoter region. In addition, *FOSL1* interacts with *PCNA* and *FOSL1* knockdown was associated with increased ubiquitin signals in PCNA immunoprecipitates. These findings suggest that *FOSL1* may be involved in the transcriptional regulation of *PCNA*. As a critical effector downstream of *FOSL1*, PCNA also contributes to the regulation of DNA repair, stemness, invasion, and migration. Collectively, *FOSL1* promotes pancreatic cancer progression partially by increasing *PCNA* expression (Fig. 7I). Moreover, simultaneous inhibition of *FOSL1* and *PCNA* significantly suppresses pancreatic cancer cell proliferation, DNA repair, stemness, invasion, and migration. These findings suggest that the *FOSL1*-*PCNA* interaction is essential for pancreatic cancer progression. Combined inhibition of *FOSL1* and *PCNA* may represent a potential therapeutic strategy.

Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request. The raw RNA-seq sequencing data have been deposited in the SRA database under accession number PRJNA1217410.

Author Contributions

XZ and YA: Conceptualization, Data curation, Investigation, Formal Analysis, Methodology, Writing and Editing. JL: Formal Analysis, Methodology, Validation and Writing. YC and TZ: Methodology, and Visualization. SW: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing original draft, and Editing. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

All animal experiments were conducted in compliance with institutional guidelines for the care and use of laboratory animals and complied with the Chinese national standards for laboratory animal welfare and ethics, including GB/T 35892-2018 (Guideline for Ethical Review of Laboratory Animal Welfare) and GB/T 42011-2022 (General Code of Laboratory Animal Welfare). The animal experiments were approved by the Animal Experiment Ethics Committee of Lanzhou University (Lanzhou, Gansu Province, China) (Approval No. Lzujcyxy20250122). Approval project: Investigate the molecular mechanism of FOSL1 in regulating DNA repair and immune response to drive the malignant progression and therapeutic resistance of pancreatic cancer, February 26, 2025. All experiments were conducted and reported in accordance with the ARRIVE 2.0 guideline.

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Conflicts of interest

The authors declare no conflicts of interest. The judgments in data interpretation and writing were not influenced by any external relationship.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/FBL52523.

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