PNO1 promotes cell proliferation in prostate cancer

Jianpeng Hu
Affiliateed People's of Jiangsu University https://orcid.org/0000-0002-6511-6448
Feilun Cui (pdcuifeilun@163.com)
Affiliated People's Hospital of Jiangsu University
Zhipeng Xv
Affiliated People's of Jiangsu University
Jian Tan
Affiated People's Hospital of Jiangsu University
Zhengyu Wang
Affiated People Hospital of Jiangsu University

Research article

Keywords: prostate cancer, PNO1, prognosis, proliferation, biomarker

Posted Date: February 13th, 2020

DOI: https://doi.org/10.21203/rs.2.17848/v2

Abstract

Background

Prostate cancer (PCa) is one of the most commonly diagnosed cancers. The functions of PNO1 in yeasts were involved in regulating ribosome and proteasome biogenesis. Human PNO1 is crucial to the site 3 cleavage at the 3′-end of 18S pre-rRNA. Previous studies indicated that PNO1 may be related to the progression of cancers. However, the functions of PNO1 in PCa remained unclear.

Methods

The present study evaluated the expression levels of PNO1 in PCa by using GSE45016, GSE55945 and GSE17951 datasets. Then, in vivo and in vitro assays were conducted to detect the biological functions of PNO1 in PCa. Microarray and bioinformatic analysis were carried out to detect the downstream targets and pathways regulated by PNO1.

Results

The present study for the first time demonstrated PNO1 was up-regulated in PCa samples compared to normal tissues. ShRNA mediated knockdown of PNO1 significantly suppressed PCa proliferation and clone formation, however, induced PCa apoptosis. Microarray analysis and bioinformatics analysis revealed PNO1 was involved in regulating multiple cancer related biological processes, such as regulation of DNA repair, single organismal cell-cell adhesion, translational initiation, RNA splicing, transcription, and positive regulation of mRNA catabolic process. OF note, in vivo results showed PNO1 knockdown remarkably reduced the PCa growth rate.

Conclusions

Despite more in-depth research is still required, this study showed PNO1 could serve as a potential biomarker for PCa.

Background

Prostate cancer (PCa) is one of the most commonly diagnosed cancers and caused about 9% of cancer-related deaths in males in US [1]. PCa is a highly heterogeneous cancer. Although there has been a series of studies showed Androgen Receptor signaling [2], PI3K/mTOR signaling [3, 4] and TGF-β signaling [5, 6] played crucial roles in PCa progression, the detail mechanisms underlying PCa tumorigenesis had not been fully understood. Identifying novel PCa progression regulators are critical to improve the understanding of the biological progression of PCa and to provide novel biomarkers for PCa.

PNO1 localized on chromosome 2p14, which contained seven exons [7]. The functions of PNO1 in yeasts were mainly involved in regulating ribosome and proteasome biogenesis [8]. However, the functions of PNO1 in mammalian cells remained unclear. knockout of PNO1 in mice caused early lethality by stopping

embryo development before compaction stage [8]. Human PNO1 is crucial to the site 3 cleavage at the 3′-end of 18S pre-rRNA [9]. Zhou et al. found PNO1 was expressed in liver, lung, spleen and kidney, but not in heart, brain, skeletal muscle, placenta, pancreas, and prostate [7]. NOB1 was identified as a PNO1 binding partner in both yeasts and human cells [10]. Previous studies showed NOB1 played as an oncogenetic gene, whose expression was found to up-regulated in multiple cancer types, including laryngeal cancer[11], and ovarian cancer[12]. Silencing of NOB1 suppressed laryngeal cancer [11] and oral cancer proliferation[13]. These results suggested the potential functions roles of PNO1 in human cancers. Very interestingly, several studies demonstrated that PNO1 was also related to the progression of bladder cancer[14], hepatocellular carcinoma[15], and colorectal cancer[16]. However, the expression levels and molecular functions of PNO1 in PCa remained unknown.

In this study, we aimed to investigate the potential functions of PNO1 in PCa using loss-of-function assays and explore the molecular mechanism of PNO1 using microarray and bioinformatics analysis. This study for the first time showed PNO1 played as a tumor promotor in PCa. Our results showed PNO1 may be a potential therapeutic and diagnostic marker for PCa.

Methods

Cell culture.

The human PCa cell lines, DU145 and PC-3, were purchased from the American Type Culture Collection (Manassas, VA, USA). All cells were cultured in RPMI-1640 medium (HyClone Laboratories; USA) supplemented with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc. USA) at 37°C in a humidified atmosphere with 5% CO_2.

Lentiviral constructs and transfections.

The PNO1 shRNA sequences were got from GeneChem Co., Ltd. (Shanghai, China). Recombinant lentiviral vectors carrying PNO1 shRNA were constructed in accordance to manufacturer's instruction [17]. PNO1 shRNA-1 was CCGGCCCATGATTGACCAGTCAAATTTCAAGAGAATTTGACTGGTCAATCATGGGTTTTTG.

RT-qPCR.

Total RNA was extracted from PCa cells using TRIzol reagent (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Reverse transcription was performed using the RevertAid First Strand cDNA Synthesis kit (Promega Corporation, USA), according to the manufacturer's protocol. RT-qPCR was performed using SYBR Master mix (Takara Biotechnology Co., Ltd., Dalian). The thermal cycle included: 95°C for 15 sec, 45 cycles of 95°C for 5 sec, and 60°C for 30 sec. The following primers were used for qPCR: CEBPA-F, CCAGAAAGCTAGGTCGTGGGT and -R, TGGACTGATCGTGCTTCGTGT; GAPDH-F, ATGGTCAGGTTATACTCCTCCTC and -R, CACATGCCACTTGATACAATCC. The $2^{-\Delta\Delta Ct}$ method was conducted to calculate the relative expression levels of targets.

Celigo analysis

Celigo® Image Cytometer (Nexcelom, Lawrence, MA, USA) was used to evaluate the number of cells by scanning green fluorescence daily for 5 days at room temperature according to our previous reports [18].

CCK-8 assay

CCK-8 assay was used to analyze the effect of PNO1 knockdown on cell proliferation according to our previous reports [18].

FACS analysis

Fluorescence-activated cell sorting (FACS) was used to analyze cell apoptosis according to our previous reports [18]. Briefly, following transfection for 48 h, cells were harvested and washed with phosphate-buffered saline (PBS) three times. For the apoptosis assay, cells were assayed with an Annexin V-APC Apoptosis Detection kit (eBioscience; Thermo Fisher Scientific, Inc.) and were analyzed using a flow cytometer (BD Biosciences, San Jose, CA, USA) .

In vivo tumorigenicity

shPNO1-transfected or control PC-3 cells were suspended in phosphate-buffered saline (PBS) and injected subcutaneously into the backs of six-week-old male BALB/c nude mice (Shanghai SLAC Laboratory Animals Co., Ltd. Shanghai, China). A luciferase-expressing DU145 cell line transfected with shPNO1 or shNC were constructed to race tumors in live animals. Bioluminescence imaging (BLI) was conducted to monitor in vivo tumor growth using an in vivo imaging system-Lumina II (Perkin Elmer). Each mouse was intraperitoneally injected with 150 mg/kg luciferin potassium salt (115144-35-9; Fanbo). Ten minutes later, the mice were anesthetized using 1.5% isoflurane. To maintain body temperature, mice were placed on a thermostatically controlled heating pad (37°C) during imaging. Acquisition binning and duration were set according to tumor activity. Signal intensity was quantified as the total flux (photons/s) within regions of interest drawn manually around the tumor area using Living Image 4.0 software (Perkin Elmer). Tumor growth was measured using calipers over the course of 49 days. Tumor volume was calculated according to the formula: volume = 0.5 × length × width2. The mice were euthanized with CO2 and sacrificed on day 49. Tumor weight was measured and compared between two groups. All in vivo studies protocols were approved by the Shanghai Medical Experimental Animal Care Commission (Approval ID: ShCI-14-008).

Western blot analysis

In accordance with standard Western-blot protocols, proteins were separated in 10% SDS-PAGE and transferred to PVDF membrane through Bio-Rad systems (Bio-Rad, Hercules CA, USA). Rabbit anti-PNO1, and mouse anti-GAPDH antibodies were used in this study. The Quantity One software package (Bio-Rad, USA) was used for the quantitation of signal intensities.

Statistical analysis

The SPSS 19.0 software (IBM Corp, Armonk, NY, USA) was used to perform statistical analysis. Each experiment was performed for three times. Differences between 2 groups was calculated using student' T-test. For more groups, one-way ANOVA followed by Newman–Keuls posthoc test was used. $P<0.05$ was considered to indicate a statistically significant difference.

Results

PNO1 was overexpressed in PCa samples.

Three independent GEO datasets, GSE45016 [19], GSE55945 [20] and GSE17951 [21], were used to determine the expression levels of PNO1 in PCa samples. Our results revealed PNO1 was markedly up-regulated in PCa compared to normal tissues (Figure 1A-C). Moreover, we detected PNO1 expression in PCa cell lines, including LNCaP, PC-3 and DU145 cells, and found PNO1 was highly expressed in metastatic cell lines PC-3 and DU145 than that in LNCaP (Figure 1D).

Silencing of PNO1 suppressed PCa cell proliferation

In present study, we used lentiviral vectors mediated knockdown to suppress PNO1 expression in PC-3 and DU145 cells. By using RT- qPCR, we found that 80% and 65% PNO1 endogenous expression was knockdown in PC-3 (Figure 2A) and DU145 (Figure 2C) cells, respectively. western blot assay also showed the protein levels of PNO1 in PC-3 (Figure 2B) and DU145 (Figure 2D) cells were also successfully knockdown using a special shRNA.

Celigo analysis was used to detect the effect of PNO1 knockdown on PCa proliferation. Five days' post transfection, we found the cell numbers in PNO1 knockdown groups decreased by 88.3% and 65% compared to control group in both PC-3 (Figure 2E-F) and DU145 (Figure 2G-H) cells. Similar with Celigo analysis results, the CCK-8 assay also demonstrated that PNO1 knockdown suppressed proliferation compared to control ($P<0.01$; Fig. 2I-J).

In addition, we found that knockdown of PNO1 suppressed PC-3 (Figure 3A and B) and DU145 (Figure 3C and D) cell colony formation. The relative colony number in PNO1 knockdown decreased by 85- and 78-percent in DU145 and PC-3 cells, respectively.

PNO1 knockdown induced apoptosis in PCa cells.

To investigate the effect of PNO1 knockdown on PCa cell apoptosis, cells transfected with shPNO1 and shCtrl were subjected to FACS analysis. We revealed the apoptosis of PC-3 (Figure 4A and B) and DU145 (Figure 4C and D) cells was significantly increased after PNO1 knockdown compared with control groups. These results suggested that PNO1 suppressed PCa cell apoptosis.

Bioinformatics analysis revealed the targets regulated by PNO1 in PCa.

Microarray analysis was conducted to identify PNO1 targets in PCa. Totally, 291 genes were found to be up-regulated and 498 genes were found to be suppressed after PNO1 knockdown in PC-3 cells (Figure 5A). The top 10 up- and down-regulated genes were shown in Table1. Bioinformatics analysis revealed PNO1 induced genes was involved in regulating translational initiation, RNA splicing, transcription, DNA-templated, positive regulation of mRNA catabolic process, regulation of energy homeostasis, cellular response to hypoxia, rRNA processing, mRNA processing, energy reserve metabolic process, and response to unfolded protein (Figure 5B). Meanwhile, the PNO1 reduced genes were involved in regulating positive regulation of DNA repair, single organismal cell-cell adhesion, cellular amino acid metabolic process, response to stress, preassembly of GPI anchor in ER membrane, membrane to membrane docking, regulation of angiogenesis, viral process, post-translational protein modification, and response to oxidative stress (Figure 5C).

Identification of key targets of PNO1 in PCa using PPI network analysis

Furthermore, PPI networks were constructed to reveal the protein-protein interaction among PNO1 induced and reduced genes using String database. A Mcode plugin in Cytoscape was used to identify hub genes in these networks. As shown in Figure 6 and 7, we showed the top 3 PNO1 up-regulated (Figure 6A-C) and down-regulated (Figure 7A-C) genes mediated hub networks. Five PNO1 up-regulated genes (PRPF8, CDC5L, RPL36, RPL23, RPL28) and 10 PNO1 down-regulated genes (TNF, EGFR, RNF213, CLTCL1, AP2B1, CXCL1, KLHL5, UBE2J1, CXCL8, PLAU) were identified as key targets of PNO1 in PCa. These genes interacted with more than 10 nodes in PPI network.

Furthermore, cytoscape plugin clueGO was used for functional enrichment analysis of these key genes in PCa. These results suggested that PNO1 correlates to translational silencing of Ceruloplasmin expression and GTP hydrolysis and joining of the 60S ribosomal subunit through suppressing EIF4A2, EIF2S1, EIF3M, and RPSA (Figure 6D). PNO1 was involved in regulating Spliceosome via down-regulating GCFC2, PRPF8, PRPF3, RBM22, SART1, RBM25, SNRNP200, CDC5L, and SMNDC1 (Figure 6D). PNO1 was involved in regulating Glycosylphosphatidylinositol (GPI)-anchor biosynthesis through enhancing PIGN, GPAA1 and PIGP, involved in regulating ubiquitin conjugating enzyme activity through promoting UBE2Z, UBE2N, UBE2J1 and UBE2G1, involved in regulating Clathrin-mediated endocytosis via promoting AP2B1, CLTCL1, AGFG1, STAM2 and EGFR (Figure 7D).

Ubiquitin conjugating enzymes played an important role in regulating degradation of unfolded protein. In order to provide several clues to validate the effects of PNO1 on these proteins in PCa, we conduction co-expression analysis between PNO1 and these ubiquitin conjugating enzymes using GEPIA database. The results showed PNO1 was positively correlated to the expression of UBE2Z (Figure 8A), UBE2N (Figure 8B), UBE2J1 (Figure 8C) and UBE2G1 (Figure 8D).

Knockdown of PNO1 suppressed prostate cancer growth in vivo

We further conducted in vivo tumor growth assay to determine the effect of PNO1 on tumor growth. A luciferase-expressing DU145 cell line transfected with shPNO1 or shNC were constructed to race tumors

in live animals. In vivo tumor growth were detected using caliper measurements. The tumor growth curve analysis showed the PNO1 knockdown tumor xenografts had an obvious reduction of tumor volume relative to that in control groups (Figure 9A). On day 41, the luciferase expression in all mice were detected and the results showed the luciferase levels in shPNO1 group was down-regulated compared to normal group (Figure 9B and E). Then mice were sacrificed and the tumor xenografts were excised and weighed according to the manufacturer's instruction. It was observed that the weight of tumor xenografts in shPNO1 group was significantly lower than that of shNC group (Figure 9C-D, $P<0.05$).

Discussion

The functions of PNO1 in human cancers remained unclear. Previous studies showed PNO1 was involved in regulating ribosome and proteasome biogenesis [8]. NOB1, a PNO1 cofactor, was overexpressed in multiple cancer types, including laryngeal cancer [11], and ovarian cancer [22]. These reports suggested PNO1 may be also involved in regulating progression of human cancers. In present study, we for the first time demonstrated PNO1 was up-regulated in PCa samples compared to normal tissues. ShRNA mediated knockdown of PNO1 significantly suppressed PCa proliferation and clone formation. Moreover, our results showed PNO1 knockdown induced PCa apoptosis. OF note, in vivo results showed PNO1 knockdown remarkably reduced the PCa growth rate. Until to now, this is the first study revealed PNO1 played as an oncogene in PCa. Very interestingly, we found knockdown of PNO1 had no significantly effects on cell cycle regulators by analyzing microarray data. Moreover, we found that knockdown of PNO1 did not affect the cell cycle progression using flow cytometer (data not shown). Based on these findings, we thought the regulation of PNO1 on PCa cell proliferation may not depend on cell cycle process. Despite we validated that PNO1 could suppress cell apoptosis, the mechanisms of PNO1 regulating PCa proliferation remained to be further investigated.

Due to that emerging studies demonstrated that the functions of PNO1 were involved in regulating ribosome and proteasome biogenesis in yeast and the site 3 cleavage at the 3′-end of 18S pre-rRNA in human, we thought PNO1 may play its functions in PCa cells through affecting a series of targets. With the development of high-through methods, microarray and RNA-sequence were widely used to identify functions and molecular mechanism of novel genes in human cancers. For example, Yao Li et al conducted microarray to reveal downstream targets of Androgen Receptor (AR) [23]. Sunkel et al identified CREB1/FoxA1 transcriptional targets using ChIP-seq and RNA-seq method[24]. Thus, in present study, we investigated the mechanisms of PNO1 in PCa using high-throughput method and bioinformatics analysis. In this study, we identified 291 up-regulated and 498 down-regulated targets of PNO1 in PCa. Bioinformatics analysis showed PNO1 was involved in regulating multiple cancer related pathways, such as DNA repair [25], regulation of angiogenesis[26, 27], translational initiation[28, 29], RNA splicing [30, 31], and cellular response to hypoxia[32, 33]. Emerging studies showed these pathways played crucial roles in PCa progression and treatment. Defective DNA repair induced tumour evolution and progression. DNA repair pathway was widely observed to be mutated in primary and advanced-stage PCa[34]. Angiogenesis was considered one of the hallmarks of tumor initiation, growth and development. Emerging studies had demonstrated angiogenesis also plays a fundamental role for PCa growth [35].

Changes in mRNA splicing patterns have been associated with key pathological mechanisms in prostate cancer progression[36].

A few regulators were identified as key targets of PNO1, including PRPF8, CDC5L, RPL36, RPL23, RPL28, TNF, EGFR, RNF213, CLTCL1, AP2B1, CXCL1, KLHL5, UBE2J1, CXCL8, and PLAU. PRPF8 was a spliceosomal protein [37]. RPL36, RPL23, and RPL28 were ribosomal proteins, which were involved in regulating protein synthesis. TNF was involved in regulating cancer apoptosis through multiple kinases. Including ASK1 and MEK4 [38]. EGFR played crucial roles in regulating PCa proliferation and apoptosis [39, 40]. Targeting EGFR was considered as a potential therapeutic target. CXCL1 was increased in high-grade PCa. In PCa, CXCL1 was reported to induce cell growth and metastasis through activating a secretory network [41]. KLHL5 knockdown was able to increase the sensitivity of cancer cells to anticancer drugs[42]. These reports and our finding showed PNO1 played key roles in PCa through regulating these important cancer regulators.

In this study, there also existed some limitations. Firstly, we evaluated the potential mechanisms of PNO1 in PCa based on microarray analysis and bioinformatics analysis. These findings should be further validated using experimental assays. Secondly, the bioinformatics analysis demonstrated that PNO1 was involved in regulating angiogenesis. Exploring the roles of PNO1 in regulating angiogenesis could broad our understanding about the functional importance of this gene in PCa. Thirdly, despite bioinformatics analysis and functional validation showed PNO1 was related to angiogenesis, RNA splicing and apoptosis. However, whether PNO1 affect PCa proliferation through these pathways remained to be further investigated in the near future.

In conclusion, this study for the first time showed PNO1 was up-regulated in PCa. PNO1 played its oncogenetic role in PCa progression through inducing cell proliferation and reducing cell apoptosis. Despite more in-depth research is still required, this study showed PNO1 could serve as a potential biomarker for PCa.

Abbreviations

PCa, prostate cancer

shRNA, Short hairpin RNA

PNO1, Partner Of NOB1 Homolog

FACS, Fluorescence-activated cell sorting

CCK-8, cell counting kit-8.

Declarations

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

FEILUN CUI conceived the experiments and drafted the manuscript. JIANPENG HU,ZHIPENG XV,JIAN TANconducted the experiments. ZHENGYU WANG helped to analyze and interpret the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

None.

Funding

Social Development Plan of Jiangsu Province-Standardization of key disease diagnosis and treatment project, BE2016715. Jiangsu Province youth medical key talent program, QNRC2016457. Natural Science Foundation of Jiangsu Province BK20191221. Jiangsu Provincial Health Commission Project H2017089.

References

1. Mahal BA, Alshalalfa M, Spratt DE, Davicioni E, Zhao SG, Feng FY, Rebbeck TR, Nguyen PL, Huang FW: **Prostate Cancer Genomic-risk Differences Between African-American and White Men Across Gleason Scores**. *Eur Urol* 2019.
2. Takuwa H, Tsuji W, Shintaku M, Yotsumoto F: **Hormone signaling via androgen receptor affects breast cancer and prostate cancer in a male patient: A case report**. *BMC Cancer* 2018, **18**(1):1282.
3. Tang KD, Ling MT: **Targeting drug-resistant prostate cancer with dual PI3K/mTOR inhibition**. *Curr Med Chem* 2014, **21**(26):3048-3056.
4. Yasumizu Y, Miyajima A, Kosaka T, Miyazaki Y, Kikuchi E, Oya M: **Dual PI3K/mTOR inhibitor NVP-BEZ235 sensitizes docetaxel in castration resistant prostate cancer**. *J Urol* 2014, **191**(1):227-234.

5. Collazo J, Zhu B, Larkin S, Martin SK, Pu H, Horbinski C, Koochekpour S, Kyprianou N: **Cofilin drives cell-invasive and metastatic responses to TGF-beta in prostate cancer**. *Cancer Res* 2014, **74**(8):2362-2373.
6. Lin PH, Pan Z, Zheng L, Li N, Danielpour D, Ma JJ: **Overexpression of Bax sensitizes prostate cancer cells to TGF-beta induced apoptosis**. *Cell Res* 2005, **15**(3):160-166.
7. Zhou GJ, Zhang Y, Wang J, Guo JH, Ni J, Zhong ZM, Wang LQ, Dang YJ, Dai JF, Yu L: **Cloning and characterization of a novel human RNA binding protein gene PNO1**. *DNA Seq* 2004, **15**(3):219-224.
8. Wang X, Wu T, Hu Y, Marcinkiewicz M, Qi S, Valderrama-Carvajal H, Luo H, Wu J: **Pno1 tissue-specific expression and its functions related to the immune responses and proteasome activities**. *PLoS One* 2012, **7**(9):e46093.
9. Woolls HA, Lamanna AC, Karbstein K: **Roles of Dim2 in ribosome assembly**. *J Biol Chem* 2011, **286**(4):2578-2586.
10. Raoelijaona F, Thore S, Fribourg S: **Domain definition and interaction mapping for the endonuclease complex hNob1/hPno1**. *RNA Biol* 2018, **15**(9):1174-1180.
11. Gao X, Wang J, Bai W, Ji W, Wang L: **NOB1 silencing inhibits the growth and metastasis of laryngeal cancer cells through the regulation of JNK signaling pathway**. *Oncol Rep* 2016, **35**(6):3313-3320.
12. Lin Y, Jin Y, Xu T, Zhou S, Cui M: **MicroRNA-215 targets NOB1 and inhibits growth and invasion of epithelial ovarian cancer**. *Am J Transl Res* 2017, **9**(2):466-477.
13. Yin J, Wang J, Jiang Y, Wang L, Wu H, Liu H: **Downregulation of NOB1 inhibits proliferation and promotes apoptosis in human oral squamous cell carcinoma**. *Oncol Rep* 2015, **34**(6):3077-3087.
14. Lin C, Yuan H, Wang W, Zhu Z, Lu Y, Wang J, Feng F, Wu J: **Importance of PNO1 for growth and survival of urinary bladder carcinoma: Role in core-regulatory circuitry**. *J Cell Mol Med* 2020, **24**(2):1504-1515.
15. Dai H, Zhang S, Ma R, Pan L: **Celecoxib Inhibits Hepatocellular Carcinoma Cell Growth and Migration by Targeting PNO1**. *Med Sci Monit* 2019, **25**:7351-7360.
16. Shen A, Chen Y, Liu L, Huang Y, Chen H, Qi F, Lin J, Shen Z, Wu X, Wu M *et al*: **EBF1-Mediated Upregulation of Ribosome Assembly Factor PNO1 Contributes to Cancer Progression by Negatively Regulating the p53 Signaling Pathway**. *Cancer Res* 2019, **79**(9):2257-2270.
17. Wang L, Ouyang L: **Effects of EIF3B gene downregulation on apoptosis and proliferation of human ovarian cancer SKOV3 and HO-8910 cells**. *Biomed Pharmacother* 2019, **109**:831-837.
18. Cui F, Hu J, Fan Y, Tan J, Tang H: **Knockdown of spindle pole body component 25 homolog inhibits cell proliferation and cycle progression in prostate cancer**. *Oncol Lett* 2018, **15**(4):5712-5720.
19. Satake H, Tamura K, Furihata M, Anchi T, Sakoda H, Kawada C, Iiyama T, Ashida S, Shuin T: **The ubiquitin-like molecule interferon-stimulated gene 15 is overexpressed in human prostate cancer**. *Oncol Rep* 2010, **23**(1):11-16.
20. Arredouani MS, Lu B, Bhasin M, Eljanne M, Yue W, Mosquera JM, Bubley GJ, Li V, Rubin MA, Libermann TA *et al*: **Identification of the transcription factor single-minded homologue 2 as a**

potential biomarker and immunotherapy target in prostate cancer. *Clin Cancer Res* 2009, **15**(18):5794-5802.

21. Jia Z, Wang Y, Sawyers A, Yao H, Rahmatpanah F, Xia XQ, Xu Q, Pio R, Turan T, Koziol JA *et al*: **Diagnosis of prostate cancer using differentially expressed genes in stroma**. *Cancer Res* 2011, **71**(7):2476-2487.
22. Lin Y, Peng S, Yu H, Teng H, Cui M: **RNAi-mediated downregulation of NOB1 suppresses the growth and colony-formation ability of human ovarian cancer cells**. *Med Oncol* 2012, **29**(1):311-317.
23. Mo W, Zhang J, Li X, Meng D, Gao Y, Yang S, Wan X, Zhou C, Guo F, Huang Y *et al*: **Identification of novel AR-targeted microRNAs mediating androgen signalling through critical pathways to regulate cell viability in prostate cancer**. *PLoS One* 2013, **8**(2):e56592.
24. Sunkel B, Wu D, Chen Z, Wang CM, Liu X, Ye Z, Horning AM, Liu J, Mahalingam D, Lopez-Nicora H *et al*: **Integrative analysis identifies targetable CREB1/FoxA1 transcriptional co-regulation as a predictor of prostate cancer recurrence**. *Nucleic Acids Res* 2016, **44**(9):4105-4122.
25. Wang JY, Ho T, Trojanek J, Chintapalli J, Grabacka M, Stoklosa T, Garcia FU, Skorski T, Reiss K: **Impaired homologous recombination DNA repair and enhanced sensitivity to DNA damage in prostate cancer cells exposed to anchorage-independence**. *Oncogene* 2005, **24**(23):3748-3758.
26. Stifter S, Dordevic G: **Prostate cancer and new insights in angiogenesis**. *Front Oncol* 2014, **4**:243.
27. Aragon-Ching JB, Dahut WL: **The role of angiogenesis inhibitors in prostate cancer**. *Cancer J* 2008, **14**(1):20-25.
28. Garrido MF, Martin NJ, Bertrand M, Gaudin C, Commo F, El Kalaany N, Al Nakouzi N, Fazli L, Del Nery E, Camonis J *et al*: **Regulation of eIF4F Translation Initiation Complex by the Peptidyl Prolyl Isomerase FKBP7 in Taxane-resistant Prostate Cancer**. *Clin Cancer Res* 2019, **25**(2):710-723.
29. Marques N, Sese M, Canovas V, Valente F, Bermudo R, de Torres I, Fernandez Y, Abasolo I, Fernandez PL, Contreras H *et al*: **Regulation of protein translation and c-Jun expression by prostate tumor overexpressed 1**. *Oncogene* 2014, **33**(9):1124-1134.
30. Li Y, Xie N, Chen R, Lee AR, Lovnicki J, Morrison EA, Fazli L, Zhang Q, Musselman CA, Wang Y *et al*: **RNA Splicing of the BHC80 Gene Contributes to Neuroendocrine Prostate Cancer Progression**. *Eur Urol* 2019, **76**(2):157-166.
31. Munkley J, Livermore K, Rajan P, Elliott DJ: **RNA splicing and splicing regulator changes in prostate cancer pathology**. *Hum Genet* 2017, **136**(9):1143-1154.
32. Dal Pra A, Milosevic M, Hill R, Wouters B, Warde P, Bristow RG: **Hypoxia, androgen deprivation and systemic metastases in prostate cancer (in response to "Antivascular effects of neoadjuvant androgen deprivation for prostate cancer: an in vivo human study using susceptibility and relaxitivity dynamic MRI": in regard to Alonzi R et al. (Int J Radiat Oncol Biol Phys 2011;80(3):721-727)**. *Int J Radiat Oncol Biol Phys* 2012, **82**(4):1319.
33. Anastasiadis AG, Bemis DL, Stisser BC, Salomon L, Ghafar MA, Buttyan R: **Tumor cell hypoxia and the hypoxia-response signaling system as a target for prostate cancer therapy**. *Curr Drug Targets* 2003, **4**(3):191-196.

34. Warner EW, Yip SM, Chi KN, Wyatt AW: **DNA repair defects in prostate cancer: impact for screening, prognostication and treatment**. *BJU Int* 2018.
35. Russo G, Mischi M, Scheepens W, De la Rosette JJ, Wijkstra H: **Angiogenesis in prostate cancer: onset, progression and imaging**. *BJU Int* 2012, **110**(11 Pt C):E794-808.
36. Lapuk AV, Volik SV, Wang Y, Collins CC: **The role of mRNA splicing in prostate cancer**. *Asian J Androl* 2014, **16**(4):515-521.
37. Onyango DO, Lee G, Stark JM: **PRPF8 is important for BRCA1-mediated homologous recombination**. *Oncotarget* 2017, **8**(55):93319-93337.
38. Royuela M, Rodriguez-Berriguete G, Fraile B, Paniagua R: **TNF-alpha/IL-1/NF-kappaB transduction pathway in human cancer prostate**. *Histol Histopathol* 2008, **23**(10):1279-1290.
39. Giannoni E, Fiaschi T, Ramponi G, Chiarugi P: **Redox regulation of anoikis resistance of metastatic prostate cancer cells: key role for Src and EGFR-mediated pro-survival signals**. *Oncogene* 2009, **28**(20):2074-2086.
40. Le Page C, Koumakpayi IH, Lessard L, Mes-Masson AM, Saad F: **EGFR and Her-2 regulate the constitutive activation of NF-kappaB in PC-3 prostate cancer cells**. *Prostate* 2005, **65**(2):130-140.
41. Benelli R, Stigliani S, Minghelli S, Carlone S, Ferrari N: **Impact of CXCL1 overexpression on growth and invasion of prostate cancer cell**. *Prostate* 2013, **73**(9):941-951.
42. Schleifer RJ, Li S, Nechtman W, Miller E, Bai S, Sharma A, She JX: **KLHL5 knockdown increases cellular sensitivity to anticancer drugs**. *Oncotarget* 2018, **9**(100):37429-37438.

Table

Table 1. The top 10 up- and down-regulated genes after knockdown of PNO1.

Gene	NC-1	NC-2	NC-3	KD-1	KD-2	KD-3	Fold Change	Regulation	FDR
RRM2	11.94	11.96	11.96	10.22	10.23	10.22	-3.32	down	5.7E-17
LSM6	10.44	10.55	10.52	8.88	8.96	8.87	-3.04	down	1.9E-14
TNFAIP6	9.73	9.84	9.63	8.28	8.33	8.26	-2.71	down	1.15E-12
KIF1B	9.21	9.16	9.09	7.77	7.87	7.58	-2.67	down	2.84E-11
PNO1	9.48	9.49	9.51	8.04	8.11	8.14	-2.64	down	3.05E-13
TINAG	8.85	8.93	8.88	7.52	7.50	7.47	-2.62	down	1.63E-12
GLO1	11.87	11.81	11.85	10.50	10.52	10.57	-2.49	down	3.88E-15
MTMR2	10.04	10.04	10.07	8.78	8.82	8.64	-2.46	down	7.84E-13
MSMP	9.67	9.70	9.78	8.43	8.51	8.38	-2.42	down	1.38E-12
ZNF468	7.70	7.73	7.32	6.32	6.15	6.47	-2.41	down	2.42E-08
INTS4	6.38	6.34	6.14	7.50	7.54	7.53	2.36	up	6.26E-10
MBD2	6.82	6.75	6.74	7.83	8.18	8.14	2.43	up	1.35E-09
GDAP2	8.94	9.05	8.97	10.34	10.24	10.23	2.43	up	3.57E-13
NRG1	10.33	10.33	10.32	11.60	11.66	11.62	2.46	up	3.72E-15
MICAL3	6.71	6.71	6.81	8.07	7.97	8.12	2.48	up	6.62E-11
HMOX1	10.66	10.77	10.76	12.08	12.08	12.07	2.55	up	5.02E-15
EMSY	6.97	7.20	7.34	8.59	8.58	8.62	2.69	up	1.31E-10
RAP1A	12.24	12.34	12.35	13.76	13.75	13.74	2.71	up	1.08E-15
CDC23	8.37	8.53	8.43	10.03	9.86	9.96	2.85	up	6.85E-13
HS2ST1	6.64	6.39	6.66	8.54	8.59	8.64	4.07	up	1.61E-12

Figures

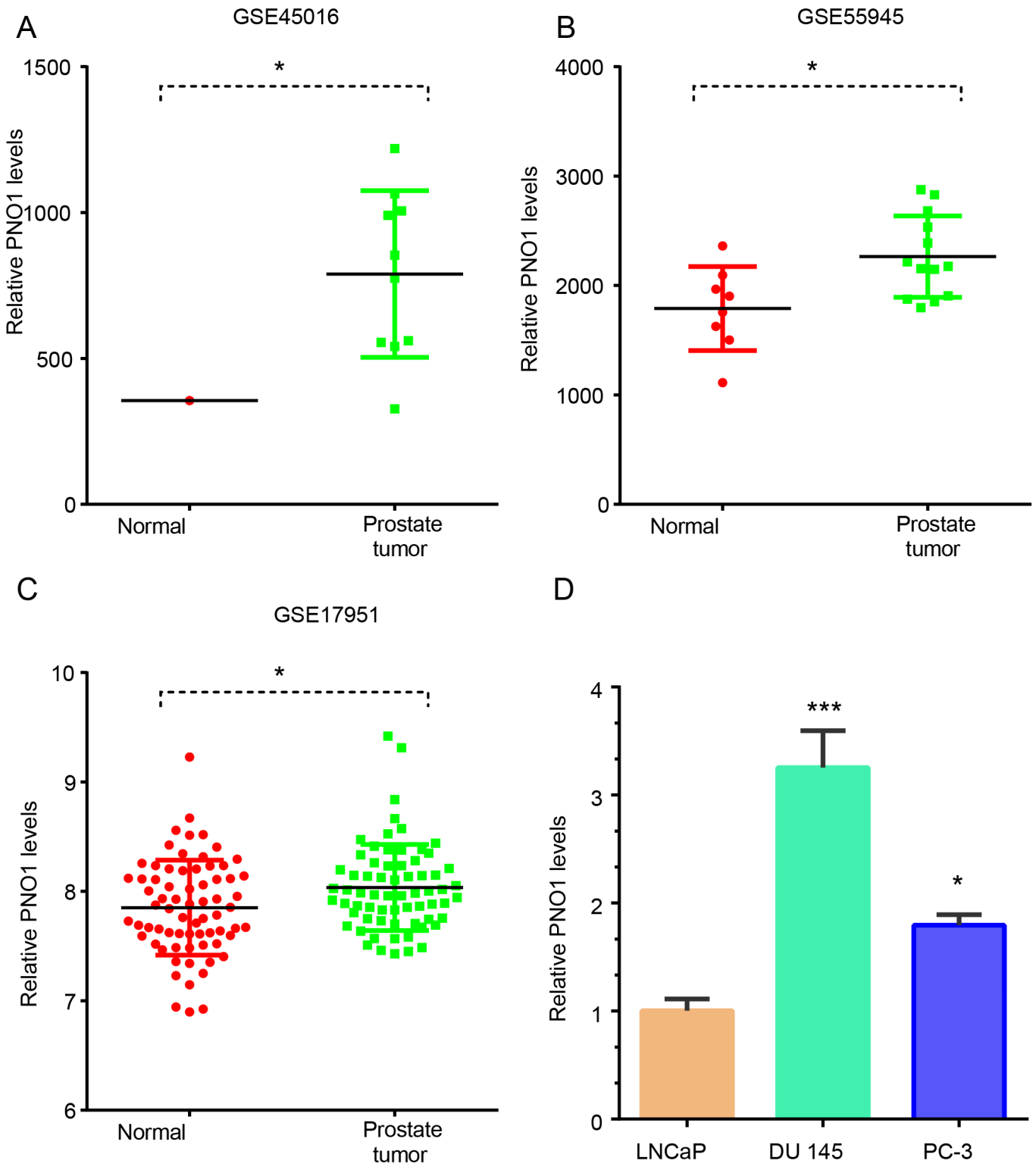

Figure 1

PNO1 was up-regulated in prostate cancer. (A-C) PNO1 was significantly overexpressed in PCa compared to normal samples using GSE45016 (A), GSE55945 (B), GSE17951 (C). (D) The expression pattern of PNO1 in LNCaP, PC-3 and DU145 cells. Differences between 2 groups was calculated using student' T-test. For more groups, one-way ANOVA followed by Newman–Keuls posthoc test was used. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

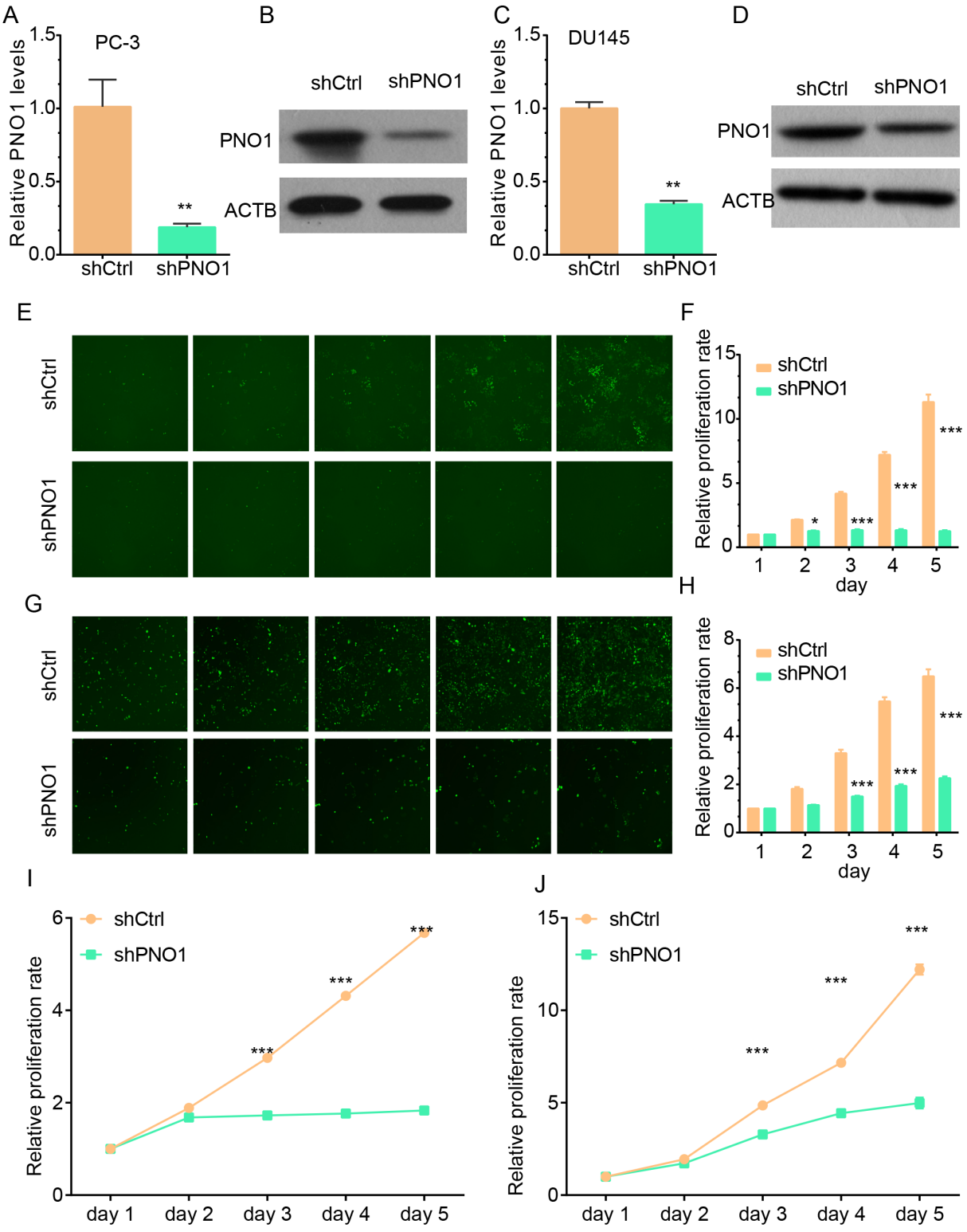

Figure 2

Knockdown of PNO1 suppresses cell proliferation in PC-3 and DU145 cells. (A-B) RT-PCR and western blot assay were used to detect the mRNA (A) and protein (B) levels of PNO1 after transfecting with shRNA or NC in PC-3 cells. The results showed RNA (A) and protein (B) levels of PNO1 were decreased after transfecting with shRNA or NC in PC-3 cells. (C-D) RT-PCR and western blot assay were used to detect the mRNA (C) and protein (D) levels of PNO1 after transfecting with shRNA or NC in DU145 cells. The results

showed RNA (A) and protein (B) levels of PNO1 were decreased after transfecting with shRNA or NC in DU145 cells. (E-F) The Celigo® system showed knockdown of PNO1 inhibited cell proliferation in PC-3 cells. (G-H) The Celigo® system showed knockdown of PNO1 inhibited cell proliferation in DU145 cells. (I-J) The CCK-8 assay showed knockdown of PNO1 inhibited cell proliferation in PC-3 and DU145 cells. Differences between 2 groups was calculated using student' T-test. For more groups, one-way ANOVA followed by Newman–Keuls posthoc test was used. $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

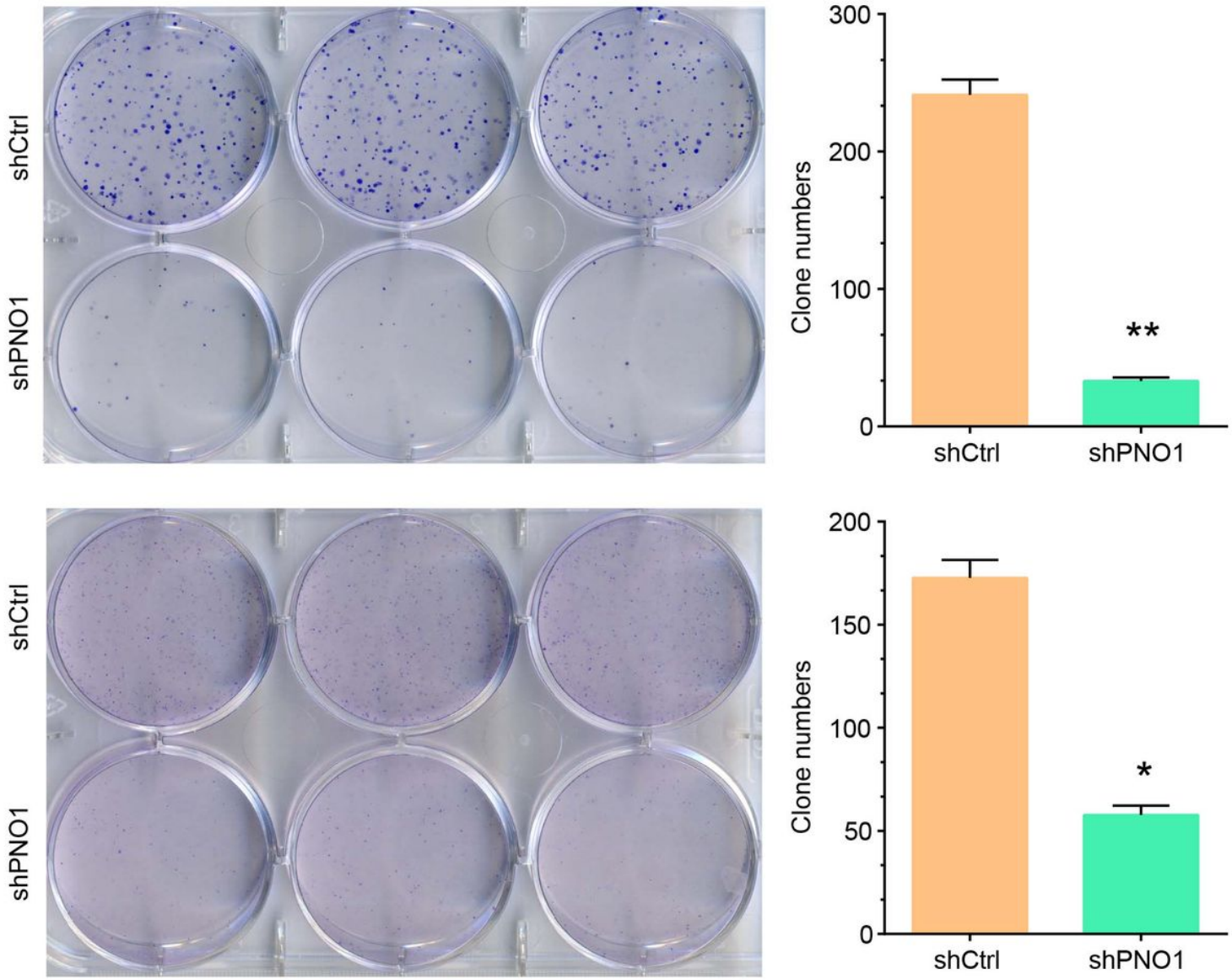

Figure 3

PNO1 knockdown inhibits cell colony formation in PCa cell lines. (A-B) knockdown of PNO1 inhibited cell colony formation after transfecting with shRNA or NC in PC-3 cells. (C-D) knockdown of PNO1 inhibited cell colony formation after transfecting with shRNA or NC in DU145 cells. The results presented as mean ± SD (n = 3). Differences between 2 groups was calculated using student' T-test. Significance was defined as $p < 0.05$ (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

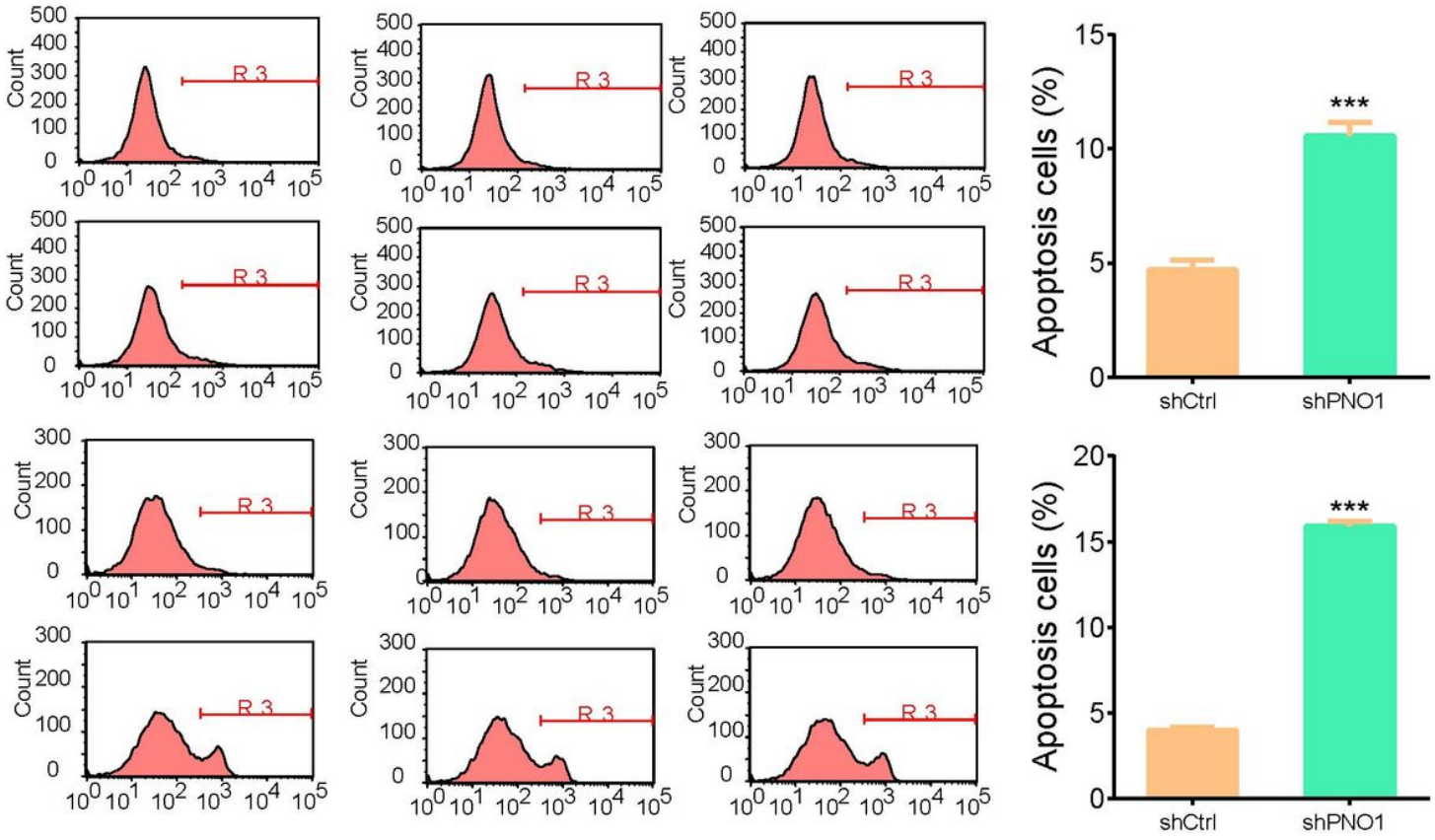

Figure 4

PNO1 knockdown promotes apoptosis of prostate cancer cells. (A-B) Treatment with shPNO1 promotes apoptosis of PC-3 cells after transfecting with shRNA or NC. (C-D) Treatment with shPNO1 promotes apoptosis of DU145 cells after transfecting with shRNA or NC. The cell apoptosis analysis was conducted with an Annexin V-APC Apoptosis Detection kit using a flow cytometer. The cell apoptosis analysis results presented as mean ± SD (n = 3). Differences between 2 groups was calculated using student' T-test. Significance was defined as $p<0.05$ (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

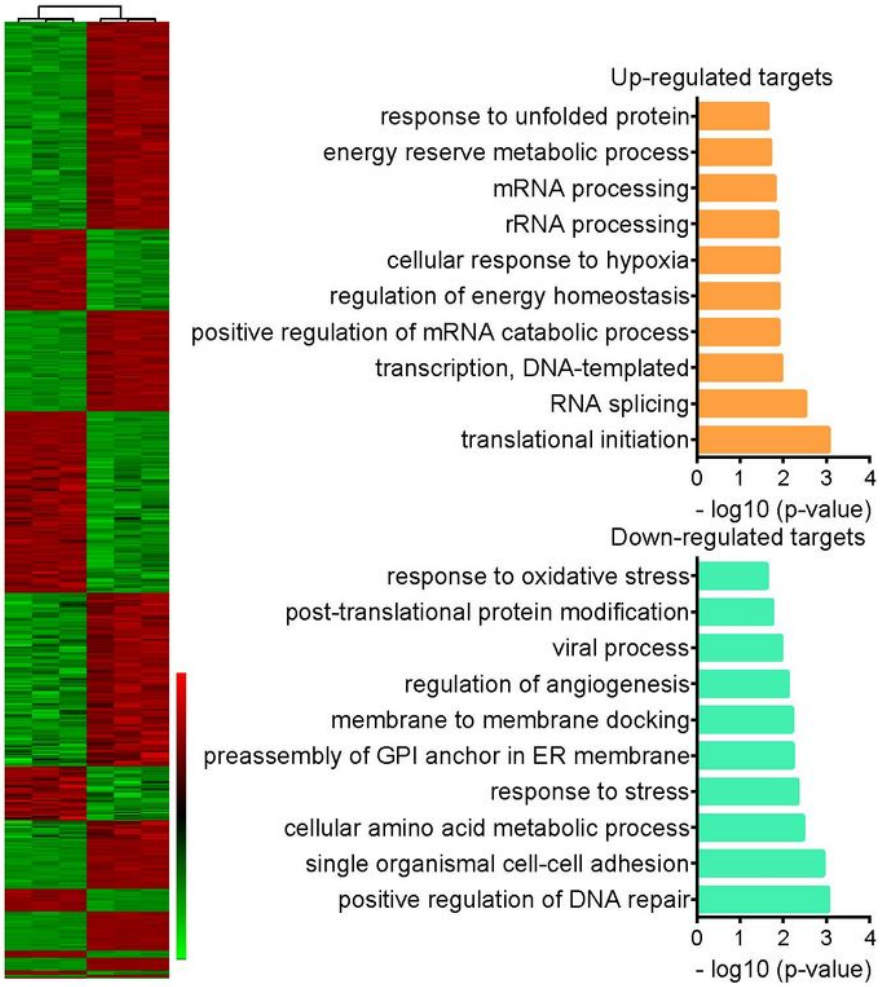

Figure 5

Bioinformatics analysis revealed multiple functional roles of PNO1 in PCa. (A) Heat map shows differential genes expression after PNO1 knockdown. (B-C) GO analysis revealed the potential pathways regulated by PNO1 up-regulated genes and down-regulated genes in PCa.

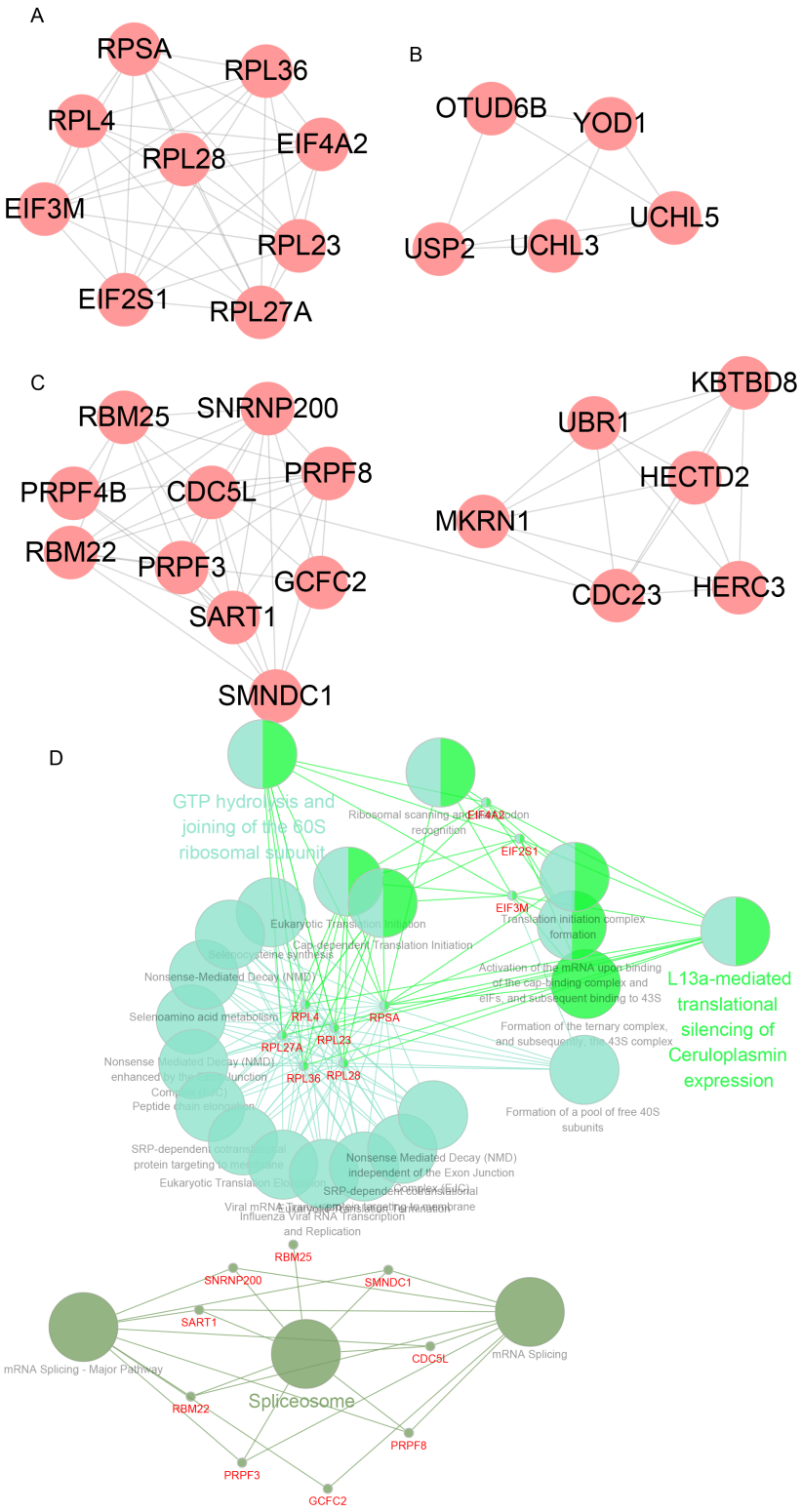

Figure 6

Identification of key up-regulated targets after PNO1 knockdown in PCa. (A-C) The top 3 hub networks were identified in PNO1 up-regulated genes mediated PPI networks. (D) enrichment analysis results of key up-regulated targets after PNO1 knockdown in PCa. A larger node represents a functional term, and the size of the node is inversely proportional to the enrichment significance P-value. Smaller nodes represent

differentially expressed genes. The color of the nodes reflects the functional correlation between the pathways (kappa > 0.4), and the same color indicates participation in related biological functions.

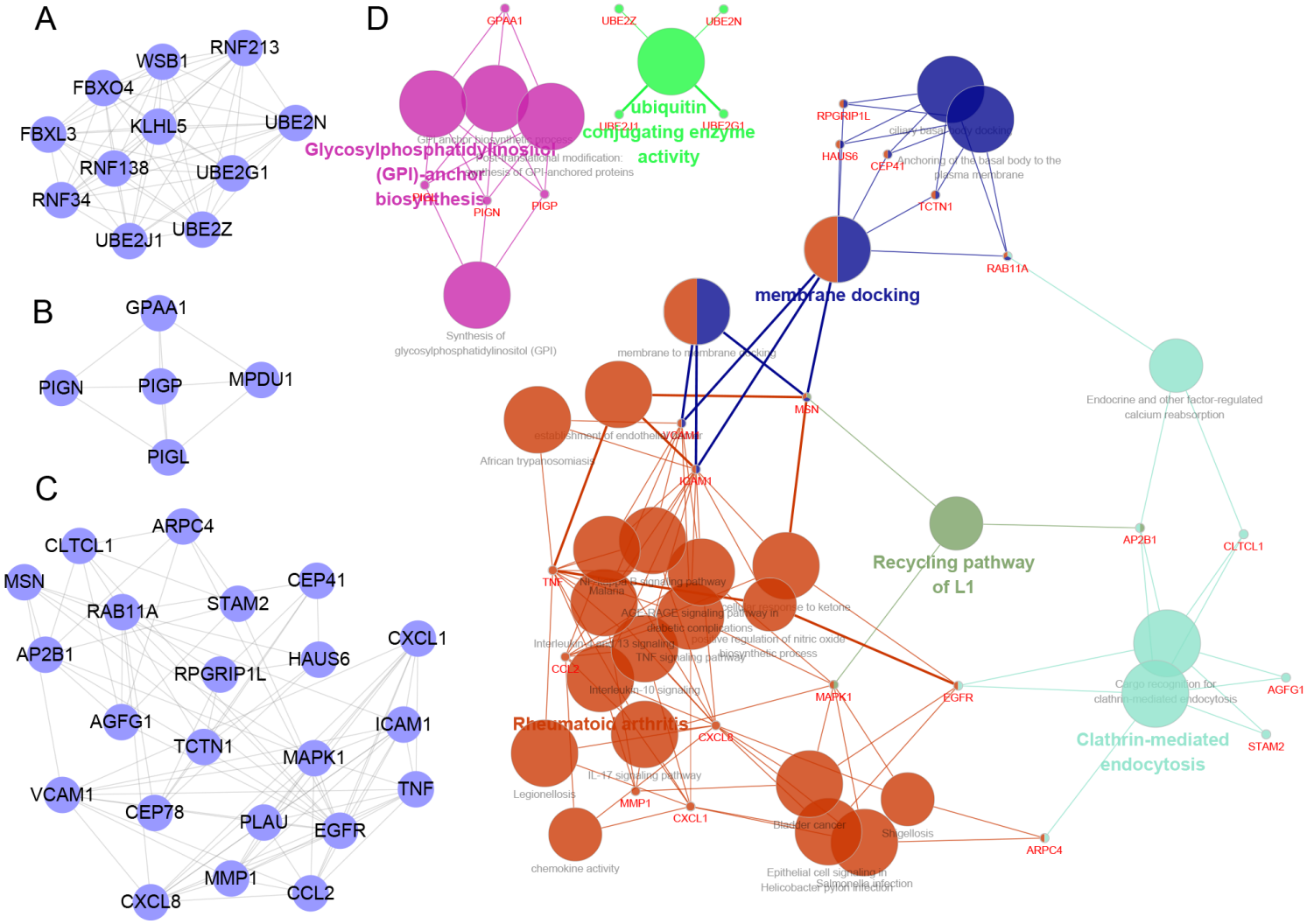

Figure 7

Identification of key down-regulated targets after PNO1 knockdown in PCa. (A-C) The top 3 hub networks were identified in PNO1 down-regulated genes mediated PPI networks. (D) enrichment analysis results of key down-regulated targets after PNO1 knockdown in PCa. A larger node represents a functional term, and the size of the node is inversely proportional to the enrichment significance P-value. Smaller nodes represent differentially expressed genes. The color of the nodes reflects the functional correlation between the pathways (kappa > 0.4), and the same color indicates participation in related biological functions.

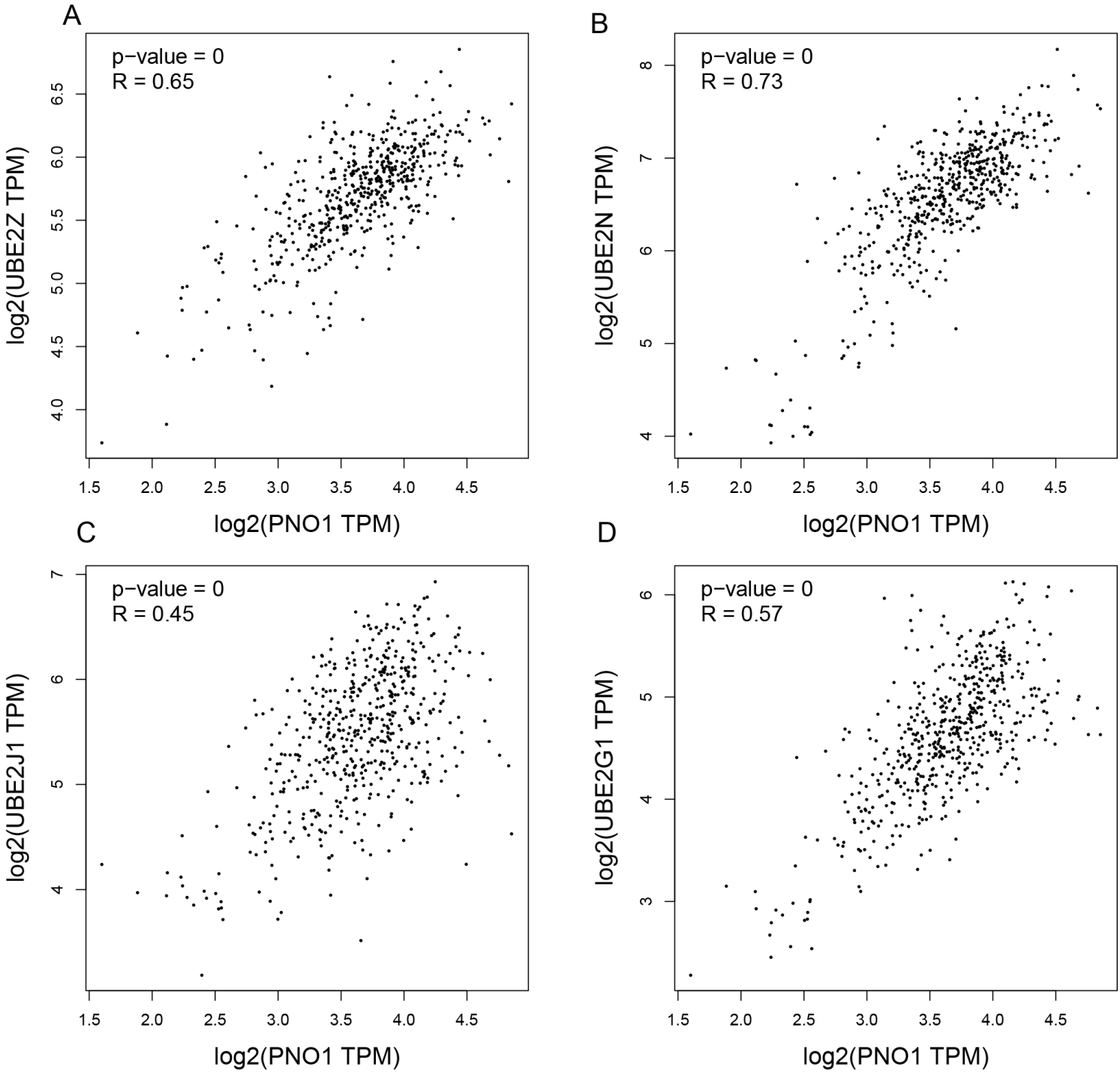

Figure 8

PNO1 was positively correlated to ubiquitin conjugating enzymes in PCa. PNO1 was positively correlated to the expression of UBE2Z (A), UBE2N (B), UBE2J1 (C) and UBE2G1 (D) in PCa by using GEPIA database (http://gepia.cancer-pku.cn/index.html).

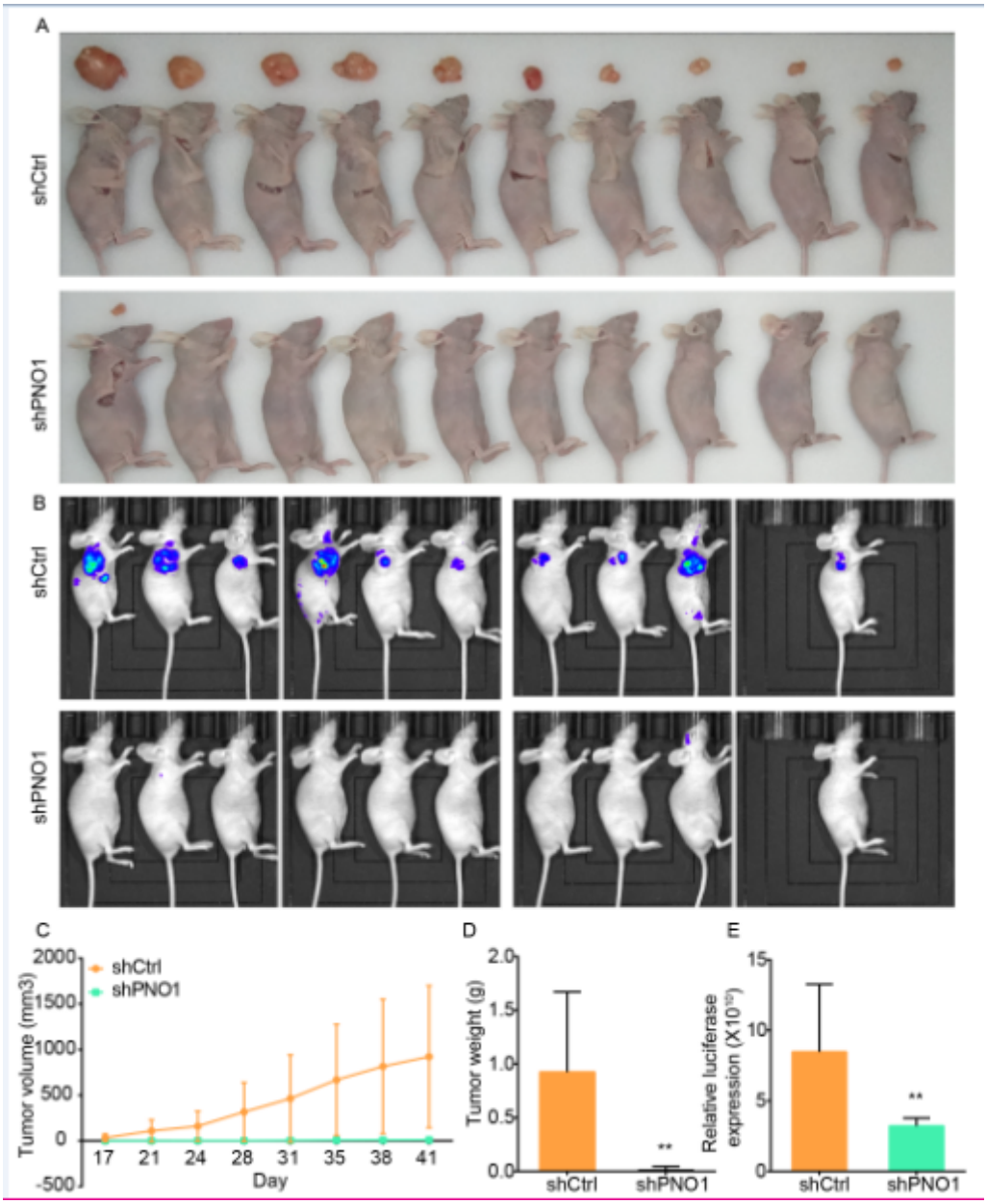

Figure 9

PNO1 knockdown inhibits prostate cancer growth in vivo. (A, D) PNO1 knockdown significantly inhibits PCa growth in vivo. (B, E) The luciferase signaling in PNO1 knockdown group was significantly lower than that in control groups. (C) The growth curve revealed PNO1 knockdown significantly inhibits PCa growth in vivo. Differences between 2 groups was calculated using student' T-test. Data are presented as the mean ± SD (n = 3) *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- NC3RsARRIVEGuidelinesChecklistfillable.pdf

- SupplementaryFigure1.tif