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RESEARCH

Elevated VRK1 levels after androgen deprivation therapy promote prostate cancer progression by upregulating YAP1 expression

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Abstract

Purpose Vaccinia-related kinase 1 (VRK1) is a serine-threonine kinase involved in the proliferation and migration of various cancer cells. However, its role in prostate cancer (PCa), particularly in the development of therapeutic resistance, remains unclear.

Methods We established an androgen-independent PCa cell line derived from LNCaP prostate cancer cells and conducted transcriptome and proteome sequencing together with bioinformatic analyses of large clinical sample databases to investigate the potential role of VRK1 in PCa progression. The correlation between VRK1 and androgen receptor (AR) signaling was evaluated under simulated clinical treatment conditions. The effects of VRK1 on cell proliferation were assessed in vitro and in vivo using Cell Counting Kit-8 and colony formation assays. Additionally, proteome and transcriptome sequencing, combined with rescue experiments were performed to explore VRK1-regulated signaling pathways related to cell proliferation and therapeutic resistance.

Results VRK1 expression was elevated during the progression of androgen-dependent prostate cancer to castration-resistant prostate cancer under therapeutic conditions, and high VRK1 expression was associated with a poor prognosis in patients with PCa. VRK1 was regulated by AR signaling, and its silencing suppressed PCa cell proliferation both in vitro and in vivo. VRK1 drove cell proliferation and therapeutic resistance in PCa by modulating yes-associated protein 1 (YAP1).

Conclusions VRK1 serves as a prognostic marker in PCa, regulated by AR signaling. VRK1 depletion inhibited cell proliferation both in vitro and in vivo, while elevated VRK1 upregulated YAP1, promoting cell proliferation and therapeutic resistance.

Keywords Prostate cancer · Castration-resistant · VRK1 · YAP1 · AR

Abbreviations

PCa	Prostate cancer
ADPC	Androgen-dependent prostate cancer
CRPC	Castration-resistant prostate cancer
AR	Androgen receptor
ADT	Androgen-deprivation therapy
NHT	Novel hormone therapy
ENZ	Enzalutamide
DHT	Dihydrotestosterone
mCRPC	Metastatic castration-resistant prostate cancer
DEGs	Differentially expressed genes
CSS	Charcoal-stripped serum
IHC	Immunohistochemistry
VRK1	Vaccinia-related kinase 1
YAP1	Yes-associated protein 1

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Introduction

Prostate cancer (PCa) is the most common tumor and the second leading cause of cancer-related mortality among men worldwide (Siegel et al. 2024). The progression of PCa is strongly associated with activation of androgen

receptor (AR) signaling, making androgen deprivation therapy (ADT) the primary treatment for these patients (Rebello et al. 2021). While nearly all patients initially respond to hormone therapy, the duration of response varies, typically lasting from a few months to several years. After which this period, the disease frequently progresses to an advanced, treatment-resistant stage known as castration-resistant prostate cancer (CRPC)(Watson et al. 2015).

Recently, novel hormone therapy (NHT), including second-generation anti-androgen drugs such as enzalutamide and abiraterone, has been widely adopted due to its demonstrated survival benefits for patients with CRPC (De Bono et al. 2011; Scher et al. 2012). However, most patients do develop resistance to these therapies eventually. Numerous studies have investigated potential mechanisms of resistance to NHT. AR-targeted therapies can induce AR point mutations, contributing to reactivation of AR signaling; for instance, enzalutamide treatment can promote the AR F877L mutation, which confers resistance to the drug (Balbas et al. 2013). Additionally, the AR splice variant AR-V7 has been linked to primary resistance to enzalutamide (Antonarakis et al. 2014; Efstathiou et al. 2015). Studies on AR bypass signaling have also shown that upregulation of the glucocorticoid receptor in LNCaP xenograft models is associated with enzalutamide resistance (Arora et al. 2013). Additionally, a hypothesis suggests that prostate cancer therapeutic resistance may result from lineage plasticity, whereby drug-sensitive cell types transition into drug-resistant ones (Mu et al. 2017). Neuroendocrine prostate cancer (NEPC) is characterized by low or absent AR expression and the presence of neuroendocrine markers. It progresses rapidly and is generally resistant to AR-targeted endocrine therapies (Beltran et al. 2014). Clinical and genomic analyses indicate that NEPC may originate from a rare population of neuroendocrine cells within adenocarcinoma. Recently, researchers have identified another form of drug-resistant prostate cancer that may arise from lineage transition—AR-null, NE-null double-negative prostate cancer (Cheng et al. 2024). Activation of the FGF/MAPK pathway promotes the survival of double-negative prostate cancer cells under AR-inhibitory conditions (Bluemn et al. 2017). Despite these findings, the mechanisms underlying therapeutic resistance remain incompletely understood, underscoring the urgent need for novel targeted therapies to improve survival outcomes in patients with advanced PCa.

Vaccinia-related kinase 1 (VRK1) is a member of the vaccinia-related kinase family of serine-threonine kinases (Nichols and Traktman 2004); it is primarily localized in the cell nucleus, where it functions as a chromatin-associated kinase involved in cell proliferation, DNA damage repair, transcriptional activation, and histone modification (Aihara et al. 2016; Kang et al. 2008; Salzano et al. 2015; Valbuena et al. 2008). Elevated VRK1 expression has been associated with a poor prognosis in various cancers, including head and neck squamous cell carcinoma, glioma, lung carcinoma, and breast carcinoma (Ben et al. 2018; Mon et al. 2018; Santos et al. 2006; Valbuena et al. 2007). These findings imply that high VRK1 expression may act as a potential oncogenic driver. However, the role of VRK1 in the progression and therapeutic resistance of PCa has not been explored.

Yes-associated protein 1 (YAP1) is a key transcription factor in the Hippo tumor suppressor signaling pathway that plays a critical role in tumor initiation, progression, and metastasis (Zanconato et al. 2016). In PCa, elevated YAP1 expression is considered a marker of tumor progression and has been shown to regulate the proliferation of CRPC cells (Filiz et al. 2017; Jiang et al. 2015). YAP1 is also implicated in therapeutic resistance, contributing to enzalutamide resistance in PCa cells by promoting cancer stemness (Lee et al. 2021). Furthermore, YAP1 interacts with the AR in the nucleus, a relationship associated with the progression of castration resistance (Kuser-Abali et al. 2015).

In this study, we found that elevated VRK1 expression was associated with a poor prognosis in patients with PCa. VRK1 was identified as an AR-regulated gene, and its inhibition suppressed PCa cell proliferation both in vitro and in vivo through YAP1. Furthermore, VRK1 mediated YAP1's involvement in the progression of therapeutic resistance in PCa cells. Together, these findings imply that VRK1 plays a significant role in PCa progression and may serve as a promising therapeutic target for advanced PCa treatment.

Methods

Cell lines and cell culture

Human prostate cancer cell lines LNCaP, 22Rv1, C4-2, and PC3 were obtained from the American Type Culture Collection. The cells were maintained in RPMI1640 medium (L220KJ; BasalMedia) supplemented with 10% fetal bovine serum (F7524; Sigma) at 37 °C in a humidified 5% CO_2 atmosphere. The establishment of androgen-independent (AI) cell lines has been previously described (Zhou et al. 2024). Culture media were replaced every 2–3 days depending on cell condition and density.

Plasmids and lentiviruses

Short hairpin RNA (shRNA) sequences were cloned into the pLKO.1 vector (10,879; Addgene), while the plasmid for overexpressing 3x-Flag-tagged VRK1 was cloned into the pCDH-CMV vector (Generalbiol). A K179E point mutation was introduced via PCR-based mutagenesis. The plasmid for overexpressing 3x-Flag-tagged YAP1 was obtained from Generalbiol. Detailed sequences for shRNAs and cloning

primers are provided in Table S1. Plasmids were transfected into HEK293T cells along with psPAX2 (12260; Addgene), pCMV-VSV-G (8454; Addgene), and PEI 40 K (G1802; Servicebio) following the manufacturer's instructions. After 48 h, the supernatants containing lentiviruses were collected and used to infect target cells. Stable cell transformants were selected by culturing the infected cells in medium containing puromycin (5 μg/ml; Sigma-Aldrich) for 7 days.

Reagents and primary antibodies

Enzalutamide (HY-70002) was purchased from MCE, and DHT (A8380) was obtained from Sigma-Aldrich. The following primary antibodies were used for western blotting: VRK1 (#3307, CST; HPA000660, Sigma-Aldrich); AR (sc-816, Santa Cruz); YAP1 (A21216, ABclonal); PSA (10679-1-AP, Proteintech); Caspase-3 (#3307, CST); Caspase-9 (A2636, ABclonal); β-Tublin (SB-AB2002, ShareBio); Vinculin (A2752, ABclonal); GAPDH (SB-AB0038, ShareBio); and β-actin (GPSG190114AA, GenePharma).

Western blotting

Cells were washed three times with phosphate-buffered saline (PBS) and lysed in lysis buffer. Proteins were separated using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto nitrocellulose membranes (66485, PALL). The membranes were blocked with 5% bovine serum albumin (BSA) in Tris-buffered saline containing Tween-20 (TBST) at room temperature for 2 h. After blocking, membranes were washed three times with TBST (5 min each) and incubated with primary antibodies overnight at 4 °C. The following day, membranes were incubated with appropriate secondary antibodies at room temperature for 40 min and washed three times with TBST (5 min each). Signal intensities were quantified using the Tanon 5200 Imaging System.

Real-time PCR analysis

Total RNA was extracted from cells using the AFTSpin Tissue/Cell Fast RNA Extraction Kit for Animal (RK30120; ABclonal), according to the manufacturer's instructions. RNA was reverse-transcribed into complementary DNA (cDNA) using the 2X Hifair II SuperMix (11120-B; Yeasen). Quantitative PCR (qPCR) was performed using the 2X Universal SYBR Green Fast qPCR Mix (RK21203; ABclonal) on a QuantStudio™ 6 Real-Time PCR System (Software v1.7.1; Applied Biosystems). Relative gene expression levels were calculated using the 2^-ΔΔCt method, with GAPDH as the internal control. The primers used for gene amplification are listed in Table S1.

CCK-8 assay and colony formation assay

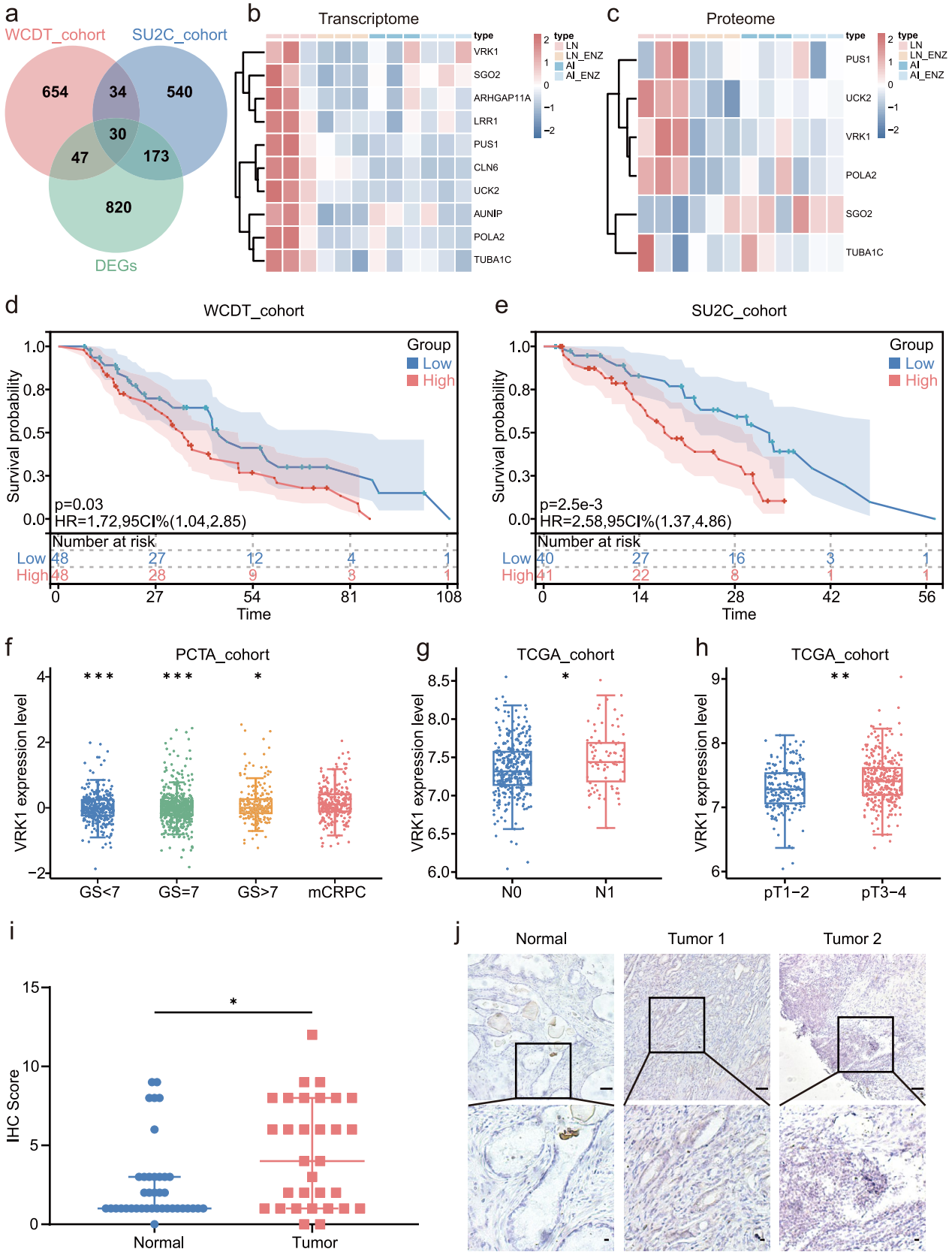
Cell proliferation was assessed using the Cell Counting Kit-8 (CCK-8) assay. Cells were seeded in 96-well plates at a density of 1000–2000 cells per well in RPMI1640 medium. After cell adherence, 10 μL of CCK-8 reagent (SB-CCK8; ShareBio) was added to 90 μL of RPMI1640 medium supplemented with 10% fetal bovine serum (FBS) in each well. Following a 3h incubation at 37 °C, absorbance was measured at 450 nm using i-control 2.0 software (Infinite M200 PRO; TECAN). For colony formation assays, cells (1000–4000 per well) were seeded in complete growth medium in six- or twelve-well plates. After 8–14 days, colonies were fixed with anhydrous methanol for 10 min and subsequently stained with 0.05% crystal violet for 2 h.

Animal experiment

Six-week-old male BALB/c nude mice (SipeiFubiotech) were subcutaneously injected with 5×10^5 lentivirus-infected PC3 or 22Rv1 cells in a 1:1 mixture with Matrigel (abs9492; Absin). Tumor-free status was defined as the absence of a palpable xenograft at the injection site. At the study endpoint, all mice were humanely euthanized using a stepwise CO_2 procedure: exposure to 60% CO_2 for 10 min, followed by 100% CO_2 for 30 min. The resulting xenograft tumors were excised, weighed, photographed, and fixed in paraformaldehyde before being embedded in paraffin and sectioned for further analysis. This animal study was approved by the Experimental Animal Ethics Committee of the Department of Laboratory Animal Science, Fudan University.

Immunohistochemistry

Tissue sections were deparaffinized at 65 °C for 1 h, followed by three 10-min washes in xylene, and then rehydrated through a graded ethanol series. Endogenous peroxidase activity was blocked by incubating the sections in 3% hydrogen peroxide at room temperature for 10 min, protected from light. Antigen retrieval was performed by heating the sections in a citric acid solution (pH 6.0) in a microwave for 25 min. The sections were blocked with a blocking solution for 45 min and subsequently incubated with primary antibodies overnight at 4 °C. IHC staining was performed using an Immunohistochemistry Kit (D601037; Sangon Biotech). The immunohistochemical score for each section was determined by multiplying the intensity score (no staining: 0,


a
WCDT_cohort
SU2C_cohort
654
34
540
30
47
173
820
DEGs
b
Transcriptome
type
LN
LN_ENZ
AI
AI_ENZ
VRK1
SGO2
ARHGAP11A
LRR1
PUS1
CLN6
UCK2
AUNIP
POLA2
TUBA1C
c
Proteome
PUS1
UCK2
VRK1
POLA2
SGO2
TUBA1C
d
WCDT_cohort
Survival probability
Group
Low
High
p=0.03
HR=1.72,95CI%(1.04,2.85)
Number at risk
Low 48 27 12 4 1
High 48 28 9 3 1
Time
e
SU2C_cohort
p=2.5e-3
HR=2.58,95CI%(1.37,4.86)
Low 40 27 16 3 1
High 41 22 8 1 1
f
PCTA_cohort
VRK1 expression level
GS<7
GS=7
GS>7
mCRPC
g
TCGA_cohort
N0
N1
h
TCGA_cohort
pT1−2
pT3−4
i
IHC Score
Normal
Tumor
j
Normal
Tumor 1
Tumor 2

◂**Fig. 1** VRK1 is associated with a poor prognosis in prostate cancer. **a** Venn diagram showing the intersection of genes associated with poor overall survival in the WCDT and SU2C cohorts and differentially expressed genes in LNCaP cells treated with enzalutamide. **b**, **c** Heatmaps displaying the expression patterns of 10 selected genes in the transcriptome and proteome sequencing of LNCaP (LN), enzalutamide-treated LNCaP (LN_ENZ), AI, and enzalutamide-treated AI (AI_ENZ) cells (four genes were not detected in the transcriptome). **d**, **e** Kaplan–Meier analyses comparing overall survival between high and low VRK1 expression groups in the WCDT and SU2C cohorts (log-rank test; groups divided based on the median). **f** Differences in VRK1 expression levels between mCRPC and primary tumors stratified by Gleason score in the PCTA cohort (Mann–Whitney test). **g**, **h** Differences in VRK1 expression levels among patients stratified by lymph node metastasis status and pathological grade in the TCGA cohort (Mann–Whitney test). **i** VRK1 levels in normal prostate and tumor tissues quantified by immunohistochemistry (Mann–Whitney test; error bars represent median and interquartile range). **j** Representative images of prostate samples (scale bar: 100 μm)

weak staining: 1, moderate staining: 2, strong staining: 3) by the percentage score (0%: 0, 1–25%: 1, 26–50%: 2, 51–75%: 3, 76–100%: 4), yielding a final immunohistochemical score.

Transcriptomic sequencing and proteomic sequencing

Total RNA and protein extracted from prostate cancer (PCa) cells were subjected to transcriptomic and proteomic sequencing at Majorbio Biopharm Technology. Expression profiles were subsequently analyzed using the Majorbio Cloud Platform (https://cloud.majorbio.com/). Gene expression datasets for TCGA-PRAD were retrieved from LinkedOmics (https://linkedomics.org/). Data for the SU2C 2019, MSKCC, and FHCRC cohorts were accessed through cBioPortal (http://www.cbioportal.org/), while datasets for the WCDT cohorts were obtained from The Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov/). PCTA cohorts were accessed through Prostate Cancer Transcriptome Atlas (http://www.thepcta.org/) (You et al. 2016).

Statistical analysis

All statistical analyses were performed using GraphPad Prism software (version 9; GraphPad) or R software (version 4.1.0; R Foundation for Statistical Computing). Quantitative data are presented as mean ± standard deviation, with any deviations clarified in the figure legends. Appropriate statistical tests, including t-test, ANOVA, the Mann–Whitney test, and the Kruskal–Wallis test, were applied based on the data distribution. Survival data were analyzed using the Kaplan–Meier method. A p-value of < 0.05 was considered statistically significant ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

Results

VRK1 is a prognostic marker in PCa

To identify genes that contribute to therapeutic resistance, we first performed a univariate Cox regression analysis on two clinical metastatic CRPC (mCRPC) RNA sequencing datasets. We identified 765 and 777 genes in the WCDT and SU2C cohorts, respectively, that were positively correlated with poor overall survival ($p < 0.05$, HR > 1). Next, we analyzed our previously reported LNCaP cell RNA sequencing data (Zhou et al. 2024) and identified 1123 differentially expressed genes (DEGs) that were downregulated in the enzalutamide-treated group ($p < 0.05$, fold change < 0.6). Thirty genes overlapped between DEGs and prognosis-related genes, representing genes downregulated at the onset of NHT but associated with a poor prognosis in patients with advanced prostate cancer (Fig. 1a). We examined the expression patterns of 10 genes not previously reported in PCa using transcriptome and proteome sequencing data from LNCaP cells, enzalutamide-treated LNCaP cells, androgen-independent (AI) cells (Zhou et al. 2024), and enzalutamide-treated AI cells. At both the mRNA and protein levels, after enzalutamide treatment, VRK1 was downregulated in LNCaP cells but upregulated again in AI cells, with no significant change in AI and enzalutamide-treated AI cells (Fig. 1b, c). Therefore, VRK1 was selected for further investigation.

Kaplan–Meier analysis indicated a significant correlation between higher VRK1 expression and shorter survival in patients with mCRPC, in both the WCDT and SU2C cohorts (Fig. 1d, e). Additionally, VRK1 mRNA expression was higher in patients with mCRPC than in those with primary PCa in the PCTA cohort, independent of the Gleason score (Fig. 1f). In TCGA-PARD cohort, patients with regional lymph node metastasis had higher VRK1 mRNA expression compared to those without metastasis (Fig. 1g), and patients with pathological stages T3–4 exhibited higher VRK1 expression than those with stages T1–2 (Fig. 1h). VRK1 expression was further validated in clinical samples through immunohistochemical (IHC) staining; staining scores were significantly higher in PCa

tissues (n = 31) than in adjacent normal tissues (n = 39, Fig. 1i). Representative images are shown in Fig. 1j. Collectively, these results strongly imply that VRK1 is associated with a poor prognosis in patients with PCa and may contribute to therapeutic resistance.

The AR-regulated gene VRK1 contributes to therapeutic resistance in PCa cells

Because VRK1 expression appeared to be influenced by enzalutamide treatment based on sequencing data, we treated LNCaP cells with charcoal-stripped serum (CSS) and enzalutamide to simulate varying levels of clinical treatment targeting androgen, followed by

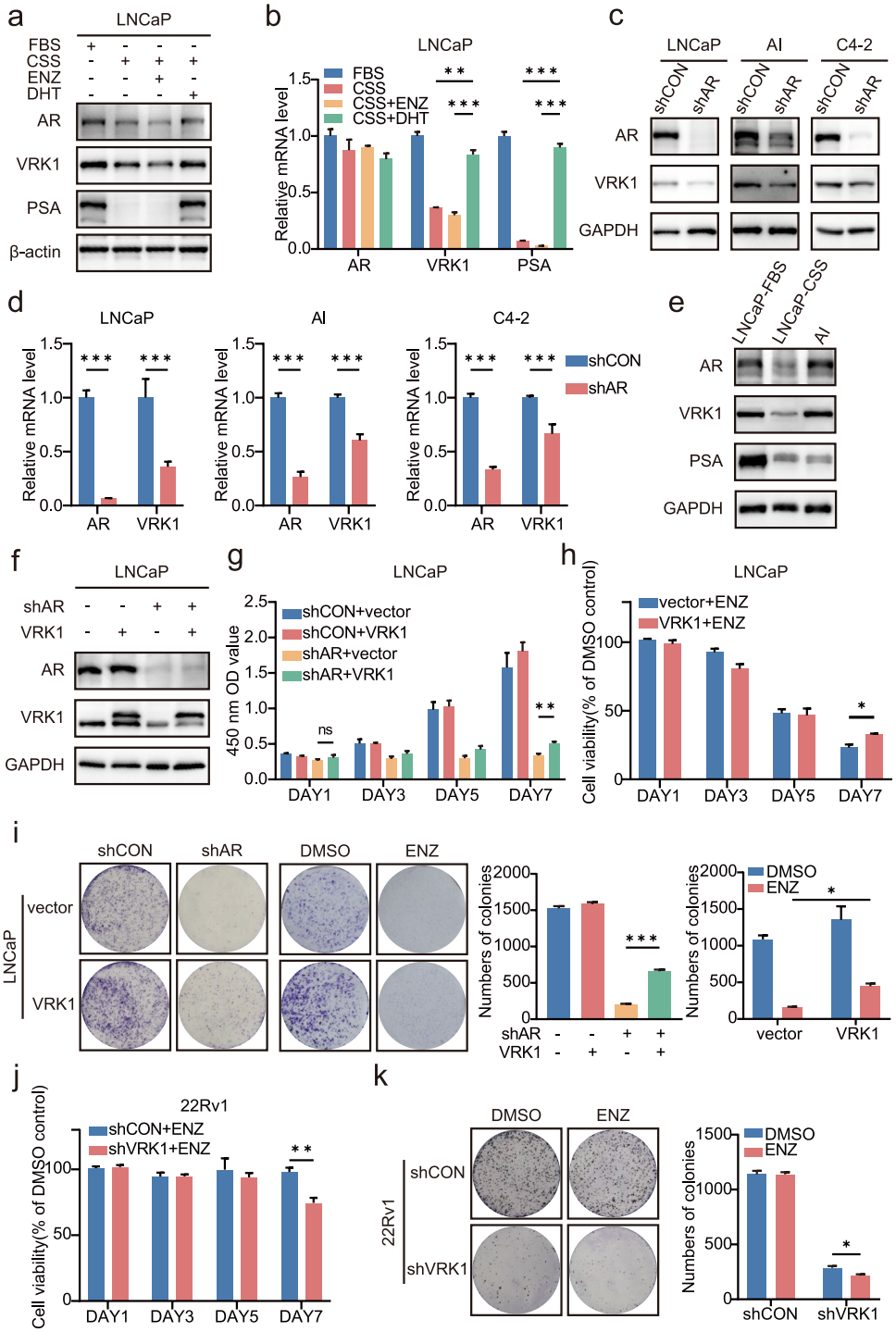


Fig. 2 VRK1 is regulated by AR and influences therapeutic resistance in PCa. **a** Expression of the indicated proteins in LNCaP cells under simulated clinical treatment conditions, detected by western blotting (ENZ: enzalutamide, 10 μM; DHT: dihydrotestosterone, 10 nM). **b** Levels of mRNAs encoding AR, VRK1, and PSA, measured via qPCR (ANOVA). **c** Expression of AR and VRK1 proteins in AR-knockdown LNCaP, AI, and C4-2 cells, detected by western blotting. **d** Levels of mRNAs encoding AR and VRK1 in AR-knockdown LNCaP, AI, and C4-2 cells, measured via qPCR (ANOVA). **e** Expression of VRK1 protein in LNCaP-FBS, LNCaP-CSS, and AI cells, detected by western blotting. **f** Expression of AR and VRK1 in the indicated stably transfected LNCaP cell lines, detected by western blotting. **g** Viability of the indicated stably transfected LNCaP cells, measured via CCK-8 assay (ANOVA). **h** Relative cell viability between the enzalutamide treatment and DMSO control groups in the indicated stably transfected LNCaP cell lines, measured via CCK-8 assay (ANOVA). **i** Colony formation assays performed in the indicated stably transfected LNCaP cells. **j** Relative cell viability between the enzalutamide treatment group and the DMSO control group in the indicated stably transfected 22Rv1 cells, measured by CCK-8 assay (ANOVA). **k** Colony formation assays performed in the indicated stably transfected 22Rv1 cells (ANOVA)

dihydrotestosterone treatment to reactivate androgen signaling. Western blotting showed that VRK1 expression was downregulated in CSS-cultured cells, with enzalutamide treatment further enhancing this downregulation, while dihydrotestosterone restored VRK1 expression (Fig. 2a). Quantitative polymerase chain reaction (qPCR) confirmed that these changes also occurred at the mRNA level (Fig. 2b). These results imply that VRK1 may be a downstream target of AR. To explore this further, we analyzed public RNA sequencing datasets, which revealed a positive correlation between VRK1 mRNA expression and AR signaling signatures in the TCGA-PARD cohort (Fig. S1a). VRK1 was also positively correlated with AR in the MSKCC, FHCRC, and TCGA-PRAD cohorts (Fig. S1b–d). To assess AR's regulation of VRK1, we knocked down AR in LNCaP, AI, and C4-2 cells using shRNA, which resulted in decreased VRK1 expression at both the protein and mRNA levels (Fig. 2c, d). These findings indicate that VRK1 is regulated by AR.

We then investigated the role of VRK1 in therapeutic resistance. VRK1 protein expression was assessed in LNCaP cells cultured in fetal bovine serum (LNCaP-FBS), cultured short-term in CSS (LNCaP-CSS), and in AI cells. VRK1 expression was markedly reduced in LNCaP-CSS compared with LNCaP-FBS, but increased again in AI cells (Fig. 2e), consistent with our sequencing data (Fig. 1b, c). Next, we overexpressed VRK1 in AR-knockdown LNCaP cells and validated protein expression by western blotting (Fig. 2f). Cell Counting Kit-8 (CCK-8) and colony formation assays showed that ectopic VRK1 expression partially rescued the proliferation inhibited by AR knockdown (Fig. 2i). Subsequently, enzalutamide treatment was used to replace AR knockdown in VRK1-overexpressing LNCaP cells. LNCaP cells overexpressing VRK1 exhibited enhanced tolerance to enzalutamide, although the resistance was relatively modest. (Fig. 2i). To verify further the effect of VRK1 on therapeutic resistance, control and VRK1-knockdown 22Rv1 cells were treated with enzalutamide. The results from the CCK-8 and colony formation assays revealed that VRK1 knockdown partially enhanced the sensitivity of 22Rv1 cells to enzalutamide (Fig. 2j, k). These findings imply that VRK1 enhances AR-targeted therapeutic resistance in PCa cell lines.

VRK1 knockdown inhibits PCa cell proliferation in vitro and in vivo

To investigate the role of VRK1 in PCa cell growth, we knocked down VRK1 expression using shRNA in LNCaP, PC3, and 22Rv1 cells. Successful knockdown was confirmed by western blotting (Fig. 3a), and colony formation and CCK-8 assays demonstrated a significant reduction in cell proliferation following VRK1 knockdown (Fig. 3b–h). To confirm that the inhibition of PCa cell proliferation was specifically induced by VRK1 knockdown, we ectopically expressed VRK1 in VRK1-knockdown 22Rv1 and PC3 cell lines, which largely restored proliferation capacity (Fig. 3i–l).

To examine further the in vivo effects of VRK1 on PCa growth, stable VRK1-knockdown and control PC3 cells were subcutaneously injected into 6-week-old male nude mice. After 42 days, the mice were euthanized, and xenografts were collected for analysis. Consistent with the in vitro findings, xenografts from the control group were larger and heavier than those from the VRK1-knockdown group (Fig. 4a, b). Additionally, VRK1 knockdown delayed tumor onset (Fig. 4c). IHC staining confirmed the expression levels of VRK1 in xenograft tissues (Fig. 4d, e). In addition, IHC staining showed that the expression of Ki-67 was significantly decreased in the VRK1-knockdown group (Fig. 4d, f). We also examined the expression of Ki-67 in VRK1 knockdown groups of 22Rv1 and PC3 cell lines using western blotting and found that VRK1 knockdown led to a decrease in Ki-67 expression (Fig. S3a). These results indicate that VRK1 loss significantly inhibited PCa growth both in vitro and in vivo.

VRK1 regulates YAP1 in an AR-independent manner

To investigate the mechanism by which VRK1 knockdown inhibits PCa cell proliferation, we conducted transcriptome sequencing analysis on VRK1-knockdown and control PC3 cells. KEGG enrichment analysis of the differentially expressed genes revealed significant changes in the FoxO, MAPK, and Hippo signaling pathways (Fig. 5a). Subsequently, we performed proteomic sequencing on VRK1-knockdown and control PC3 cells, analyzing the expression patterns of key molecules within these pathways. Our results showed that YAP1 protein expression was significantly downregulated in the VRK1-knockdown group (Fig. 5b).

We further examined YAP1 protein expression in various VRK1-knockdown PCa cell lines, including LNCaP, AI, PC3, and 22Rv1 cells. In all cases, YAP1 expression was reduced following VRK1 inhibition (Fig. 5c). Conversely, VRK1 overexpression in PCa cells led to elevated YAP1 protein expression (Fig. 5d). To confirm that YAP1 elevation is dependent on the functional activity of VRK1 protein, we constructed stable LNCaP cells expressing the VRK1 kinase-inactive mutant K179E. Western blotting showed that overexpression of K179E did not elevate YAP1 expression (Fig. 5e).

Although we confirmed that VRK1 is regulated by AR, Hsiu-Chi et al. reported that YAP1 is an AR-repressed target gene (Lee et al. 2021). However, VRK1 gain- and loss-of-function studies in AR-negative PC3 cells demonstrated that VRK1 regulates YAP1 independently of AR. To confirm this further, we assessed YAP1 expression in LNCaP-FBS,

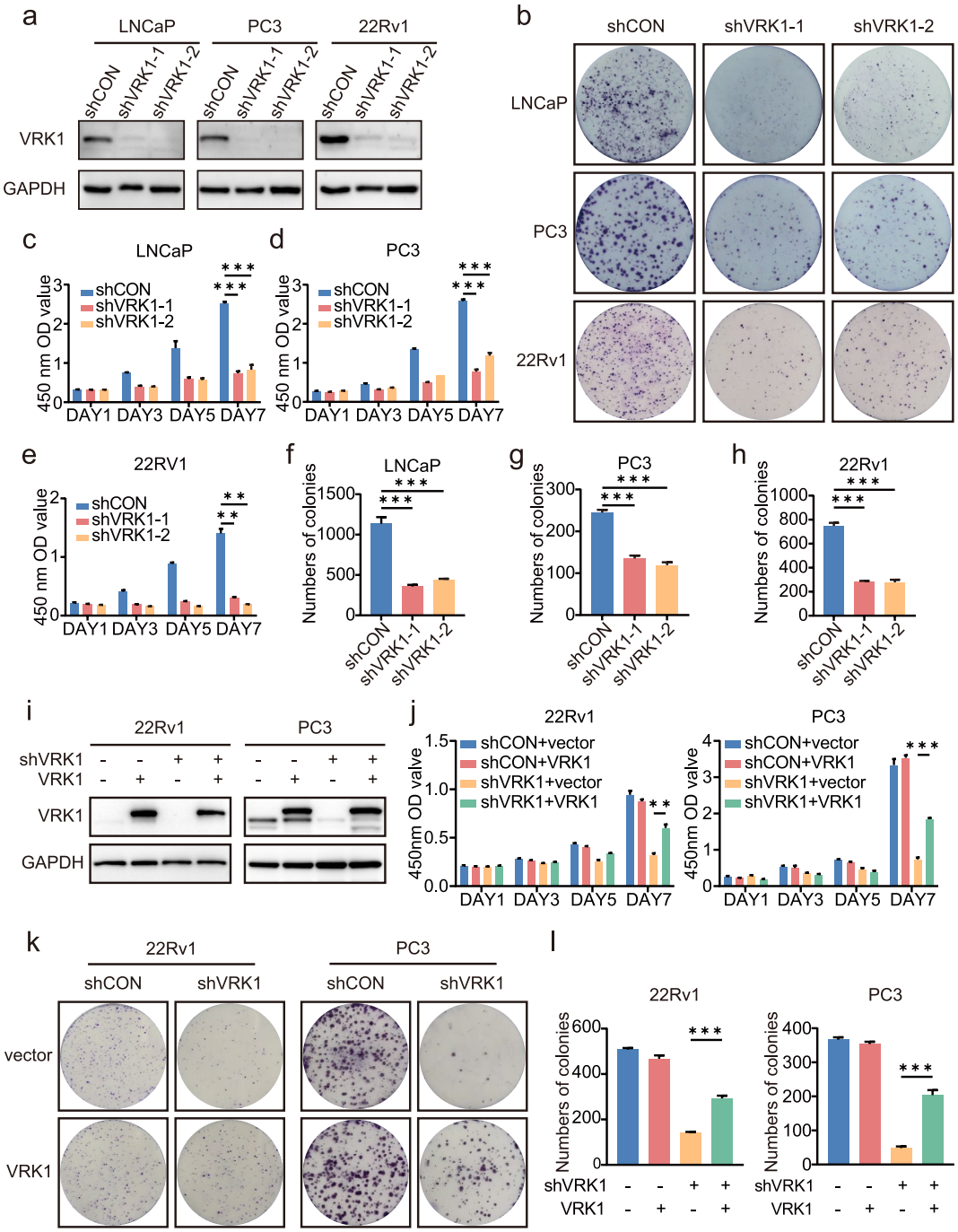


Fig. 3 Disrupting VRK1 impairs PCa proliferation in vitro. **a** Knockdown efficiency in LNCaP, PC3, and 22Rv1 cells, detected by western blotting. **b** Colony formation assays performed in LNCaP, PC3, and 22Rv1 cells transfected with the indicated shRNAs. **c**–**e** Viability of the indicated stable transfected LNCaP, PC3, and 22Rv1 cells, measured by CCK-8 assay (ANOVA). **f**–**h** Colony numbers in each group (ANOVA). **i** Expression of VRK1 protein in the indicated stably transfected 22Rv1 and PC3 cells, evaluated by western blotting. **j** Viability of the indicated stably transfected 22Rv1 and PC3 cells, measured by CCK-8 assay (ANOVA). **k**, **l** 22Rv1 and PC3 cells were transfected with the indicated plasmids, and cell growth was measured by colony formation assay (ANOVA)

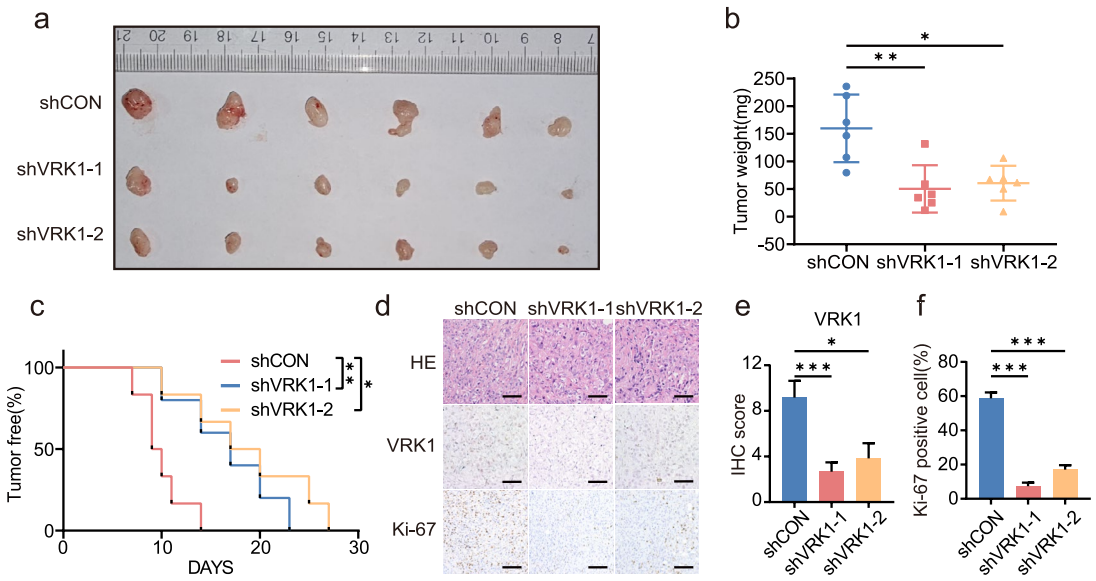


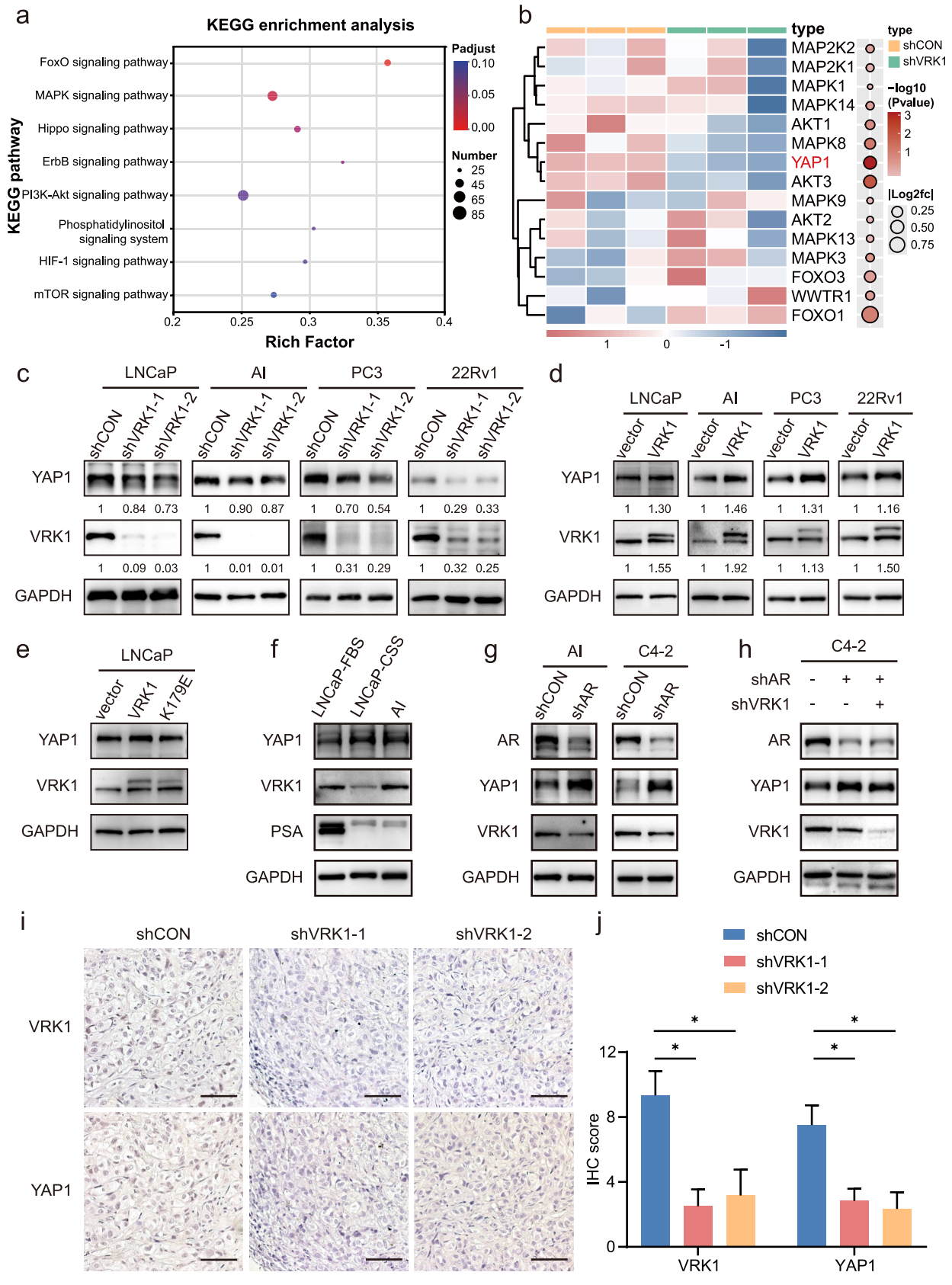
Fig. 4 Disrupting VRK1 impairs PCa proliferation in vivo. **a** Images of subcutaneous xenografts from the PC3 control and VRK1-knockdown groups. **b** Weights of tumor xenografts (Mann–Whitney test; n = 6). **c** Kaplan–Meier analysis of tumor onset (log-rank test). **d** Hematoxylin and eosin staining and IHC staining of VRK1 and Ki-67 in tumor xenografts (scale bar: 100 μm). **e** VRK1 expression shown as an IHC score (Kruskal–Wallis test). **f** Ki-67 expression levels (the numbers of positive cells)

LNCaP-CSS, and AI cells, as well as in AR-knockdown AI and C4-2 cells. The results showed that YAP1 expression increased following AR inhibition, consistent with the findings of Hsiu-Chi et al. (Fig. 5f, g). We then performed additional knockdown of VRK1 in AR-knockdown C4-2 cells and observed decreased YAP1 expression, indicating that the regulation of YAP1 by VRK1 occurs independently of AR (Fig. 5h). Additionally, IHC staining of xenografts for VRK1 and YAP1 revealed decreased YAP1 expression with VRK1 knockdown (Fig. 5i, j). These findings imply that YAP1 is a downstream target of VRK1, and that its regulation is independent of AR.

VRK1 regulates PCa progression and therapeutic resistance via YAP1

Given that VRK1 regulated YAP1 expression, we investigated whether YAP1 influences VRK1-mediated regulation of PCa cell proliferation. YAP1 was overexpressed in 22Rv1 and PC3 cells under VRK1 knockdown conditions, and protein expression was validated by western blotting (Fig. 6a). A CCK-8 assay revealed that YAP1 overexpression partially reversed the proliferation inhibition caused by VRK1 knockdown in vitro (Fig. 6b, c), a finding further confirmed by colony formation assays (Figs. 6d; S2a, b). To evaluate the effects in vivo, we conducted a subcutaneous tumor xenograft experiment in nude mice. Consistent with the in vitro results, xenografts derived from 22Rv1 cell lines with YAP1 overexpression and VRK1 knockdown were larger and heavier than those with VRK1 knockdown alone (Fig. 6e, f). Additionally, tumor formation occurred earlier in the group with YAP1 overexpression after VRK1 knockdown than in the VRK1 knockdown group (Fig. 6g). We also performed Western blotting analysis to investigate whether VRK1 knockdown or YAP1 overexpression affects apoptosis. We observed that VRK1 knockdown significantly downregulated full-length caspase3 protein levels, whereas cleaved-caspase3 showed no significant changes (Fig. S3d). Integrated analysis of transcriptome sequencing and proteomic data revealed that VRK1 knockdown markedly reduced CASP3 mRNA expression and caspase3 protein levels (Fig. S3b, c). Additionally, overexpressing YAP1 in VRK1 knockdown cells did not alter apoptosis-related markers (Fig. S3e, f). Based on these findings, we conclude that neither VRK1 knockdown nor YAP1 overexpression induces apoptosis. These findings imply that VRK1 regulates PCa cell proliferation through YAP1.

To confirm that VRK1-driven therapeutic resistance is mediated by YAP1, we knocked down YAP1 in VRK1-overexpressing LNCaP cells (Fig. 6h) and treated the cells with enzalutamide. Colony formation and CCK-8 assays revealed that the enzalutamide tolerance of VRK1-overexpressing LNCaP cells was eliminated following YAP1 knockdown


a
KEGG enrichment analysis
FoxO signaling pathway
MAPK signaling pathway
Hippo signaling pathway
ErbB signaling pathway
PI3K-Akt signaling pathway
Phosphatidylinositol signaling system
HIF-1 signaling pathway
mTOR signaling pathway
KEGG pathway
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MAP2K2
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AKT3
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MAPK3
FOXO3
WWTR1
FOXO1
c
LNCaP
AI
PC3
22Rv1
shCON
shVRK1-1
shVRK1-2
YAP1
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YAP1
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IHC score

◂**Fig. 5** VRK1 modulates YAP1 independently of AR regulation. **a** KEGG enrichment analysis of differentially expressed genes between the PC3 control and VRK1-knockdown groups. **b** Heatmap illustrating the expression levels of key proteins in the FoxO, MAPK, and Hippo signaling pathways in the proteomic profiles of the PC3 control and VRK1-knockdown groups. **c** Expression of YAP1 protein in the control and VRK1-knockdown groups of LNCaP, AI, PC3, and 22Rv1 cells, detected by western blotting. **d** Expression of YAP1 protein in the control and VRK1-overexpression groups of LNCaP, AI, PC3, and 22Rv1 cells. **e** Expression of YAP1 protein in the control, VRK1-overexpression, and K179E-overexpression groups of LNCaP cells. **f** Expression of YAP1 protein in LNCaP-FBS, LNCaP-CSS, and AI cells. **g** Expression of YAP1 protein in the indicated stably transfected AI and C4-2 cells. **h** Expression of YAP1 protein in the indicated stably transfected C4-2 cells. **i** VRK1 and YAP1 staining in PC3 xenografts with or without VRK1 inhibition (scale bar: 100 μm). **j** VRK1 and YAP1 expression in each group shown as IHC scores (Kruskal–Wallis test)

(Figs. 6i, k; S2c). We further compared the enzalutamide sensitivity of 22Rv1 cells with simultaneous VRK1 knockdown and YAP1 overexpression to that of 22Rv1 cells with VRK1 knockdown alone. Colony formation and CCK-8 assays showed that YAP1 overexpression restored enzalutamide tolerance in VRK1-knockdown 22Rv1 cells (Figs. 6j, l; S2d). Collectively, these results demonstrate that VRK1-driven therapeutic resistance is mediated by YAP1 (Fig. 6m).

Discussion

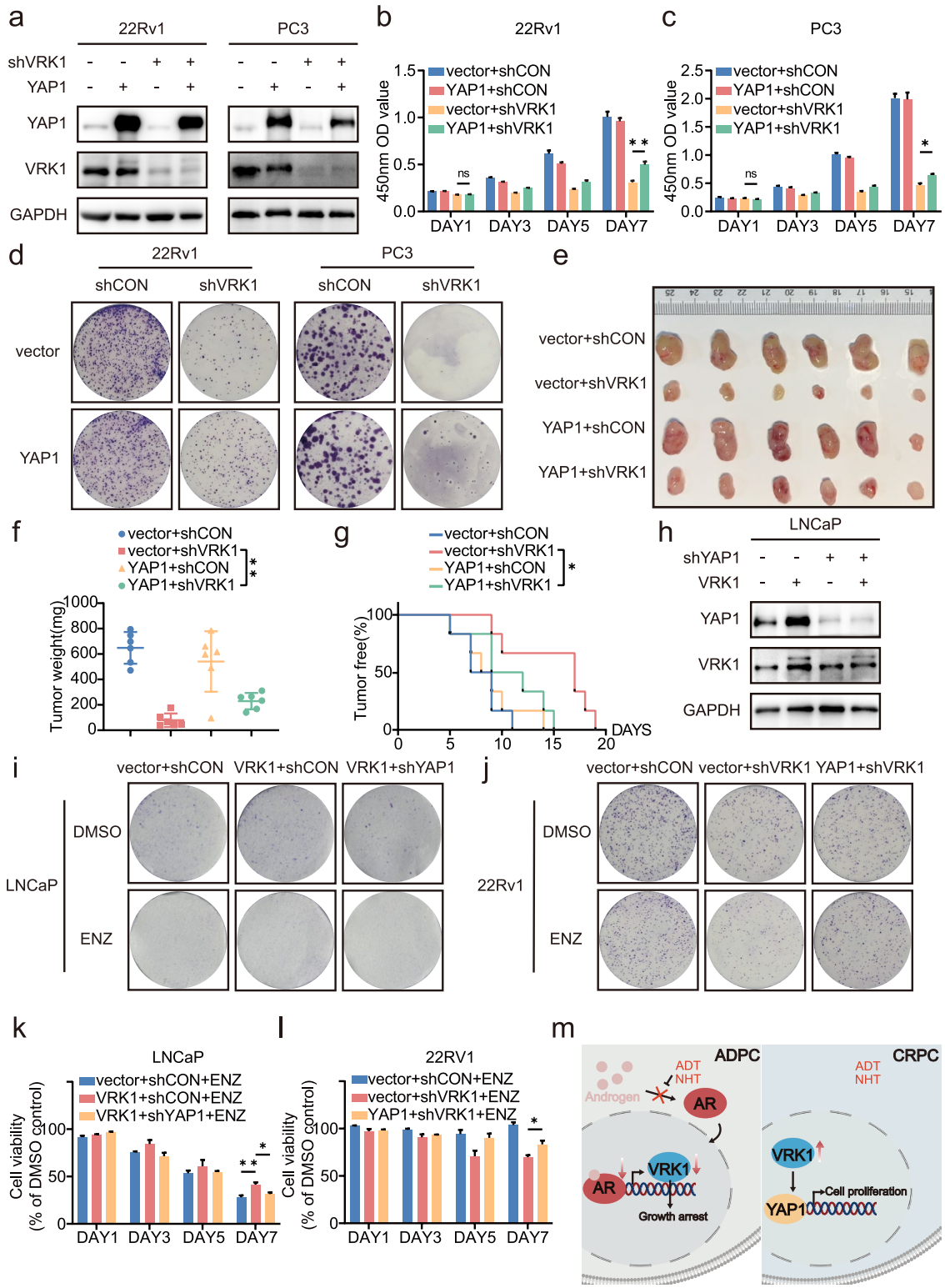
ADT remains the cornerstone treatment for patients with primary PCa because PCa growth is typically androgen-dependent. However, most patients eventually develop resistance to ADT, progressing to CRPC. NHTs, such as the second-generation antiandrogen drug enzalutamide, have demonstrated benefits in extending overall survival and reducing radiographic progression in both patients with mCRPC and those with non-mCRPC (Beer et al. 2014; Scher et al. 2012; Sternberg et al. 2020). Unfortunately, even those who initially respond to NHTs often acquire resistance within months or years (Attard and Antonarakis 2016), underscoring the urgent need to elucidate the mechanisms underlying resistance to AR-targeted therapies.

One mechanism of resistance to AR-targeted therapies is the reactivation of AR signaling. Previous research has shown that numerous AR-responsive genes, including well-known targets such as like KLK2, KLK3, and TMPRSS2, are initially downregulated by AR-targeted therapies but are restored in recurrent tumors (Holzbeierlein et al. 2004; Mousses et al. 2001). Transcriptome sequencing of patients with CRPC has revealed that the gene expression profiles of AI tumors resemble those of untreated androgen-dependent primary cancers rather than those of tumors undergoing androgen deprivation (Holzbeierlein et al. 2004).

In this study, we identified VRK1 as an AR-regulated gene that is downregulated in LNCaP cells undergoing AR-targeted therapies but reactivated in AI cells. Although AR is also highly re-expressed in AI cells, KLK3 remains at a very low level due to the androgen-deprived environment (Fig. 2e). Therefore, we hypothesize that the highly re-expressed AR and VRK1 in AI serve as an alternative pathway to the androgen-activated AR signaling pathway. We also observed that VRK1 overexpression in LNCaP cells partially rescued the proliferation suppression induced by AR knockdown and enzalutamide (Fig. 2f–i). Furthermore, our data show that high VRK1 expression was associated with poor overall survival in patients with advanced PCa (Fig. 1). Collectively, our findings provide strong evidence that VRK1 is involved in the progression of PCa and therapeutic resistance. However, the detailed interaction between AR signaling and VRK1 remains unclear.

VRK1 was originally identified based on its structural similarity to the vaccinia virus B1R kinase (Nezu et al. 1997); it plays important roles in cell cycle regulation. Previous research showed that VRK1 acts as an early response gene in the G1 phase (Valbuena et al. 2008) and phosphorylates cAMP-response element binding protein to promote CCND1 expression (Kang et al. 2008). Additionally, Aihara et al. demonstrated that VRK1 phosphorylates histone H2A at T120 in the CCND1 promoter, thereby activating its transcription (Aihara et al. 2016). VRK1 also participates in the G2/M phase by phosphorylating histone H3 to recruit AURKB for chromatin compaction (Jeong et al. 2013; Kang et al. 2007; Moura et al. 2018). VRK1 is abnormally highly expressed in a variety of cancers. Previous studies have demonstrated that kinases are critical in prostate cancer. Some kinases, such as RIOK1, are aberrantly overexpressed in prostate cancer yet show no correlation with tumor stage (Handle et al. 2023). Our study showed that VRK1 inhibition suppressed proliferation in both ADPC and CRPC cell lines (Figs. 3, 4). Notably, while VRK1 has been shown to regulate CCND1 expression in other contexts, our proteomic analysis of VRK1-knockdown PC3 cells did not reveal noticeable changes in cyclin D1. However, other cell-cycle-related genes such as CCNE1, CCNA1, CDK2, and AURKB showed significant reductions in transcriptome sequencing data.

Our sequencing analyses indicate that VRK1 knockdown induces significant changes in the Hippo signaling pathway. Specifically, VRK1 regulates YAP1 expression in an AR-independent manner (Fig. 5). YAP1 is associated with cell proliferation, survival, and stemness (Basu-Roy et al. 2015; Camargo et al. 2007; Dong et al. 2007), and



dysregulated YAP1 can bypass classical tumor suppressor checkpoints (Harvey et al. 2013). Tumor cells with activated YAP1 exhibit resistance to chemotherapy and radiotherapy, with overexpressed YAP1 providing protection against DNA damage from cisplatin and radiation (Baia et al. 2012; Ciamporcero et al. 2016). Previous studies have linked YAP1 to CRPC progression (Sheng et al. 2015) and enzalutamide resistance by promoting cancer stemness (Lee et al. 2021). In this study, we found that VRK1 overexpression conferred AR-targeted therapeutic resistance to LNCaP

◂**Fig. 6** VRK1 promotes PCa proliferation and therapeutic resistance through YAP1. **a** Expression of VRK1 and YAP1 proteins in the indicated stably transfected 22Rv1 and PC3 cells, evaluated by western blotting. **b**, **c** Viability of the indicated stably transfected 22Rv1 and PC3 cells, measured by CCK-8 assay (ANOVA). **d** 22Rv1 and PC3 cells were transfected with the indicated plasmids, and cell growth was measured by colony formation assay. **e** Images of subcutaneous xenografts from the stably transfected 22Rv1 cells. **f** Weights of the tumor xenografts (Mann–Whitney test; n = 6). **g** Kaplan–Meier analysis of tumor onset (log-rank test). **h** Expression of VRK1 and YAP1 proteins in the indicated stably transfected LNCaP cells. **i**, **j** Colony formation assays performed in each group of LNCaP and 22Rv1 cell lines. **k**, **l** Relative cell viability between the enzalutamide treatment group and the DMSO control group in the indicated stably transfected LNCaP and 22Rv1 cell lines, measured by CCK-8 assay (ANOVA). **m** Schematic summarizing the role of VRK1 in the development of PCa

cells, while VRK1 inhibition restored enzalutamide sensitivity in 22Rv1 cells. This effect was mediated through YAP1 (Fig. 6). However, we did not further explore the specific mechanism by which VRK1 regulates YAP1, highlighting the need for additional investigation.

In summary, our study revealed that the re-activation of AR-regulated gene VRK1 is associated with a poor prognosis in patients with advanced PCa and promotes proliferation and therapeutic resistance via YAP1. VRK1 holds both therapeutic and diagnostic potential for addressing resistance to ADT and AR-targeted therapies. These findings provide promising avenues for developing novel therapeutic strategies for advanced PCa.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00432-025-06168-z.

Author contributions ZJ, YWD, and SGW designed the study. MYB and GJC conducted the experiments. MYB, MHB, and CCC analyzed the data. MYB, ZYH, XYZ, and HXT interpreted the data. MYB and ZC wrote the manuscript. WXL, SGW, YWD, and ZJ reviewed the manuscript. All authors read and approved the final manuscript.

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Data availability Transcriptome data supporting the findings of this study have been deposited in the NCBI Sequence Read Archive (SRA) under the primary accession code PRJNA1187577. Proteomics data have been deposited to the ProteomeXchange Consortium via the iProX (Ma et al. 2019) with the dataset identifier PXD058261 and PXD058205.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Ethical approval All animal procedures were approved by the Experimental Animal Ethics Committee of the Department of Laboratory Animal Science, Fudan University.

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