

FBXL22, a circadian-regulated immune biomarker with pan-cancer prognostic value and therapeutic potential in prostate cancer

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Abstract. F-box and leucine-rich repeat protein 22 (FBXL22) has been implicated in breast and prostate cancer types; however, comprehensive pan-cancer analyses and detailed investigations of its role in cancer development are still needed. To address this, the present study used The Cancer Genome Atlas, Genotype-Tissue Expression, University of Alabama at Birmingham CANcer data analysis Portal, Kaplan-Meier-Plotter and cBioPortal databases to analyze the correlation between FBXL22 expression and prognosis, gene mutations, DNA methylation, immune cell infiltration and immune-related gene regulation in several types of cancer. The findings of the present study demonstrated that FBXL22 was predominantly downregulated in several cancer types and may serve as a prognostic and diagnostic marker in certain cancer types, particularly prostate cancer. Strong correlations between FBXL22 expression and immune checkpoint genes were observed, which suggests a role in the tumor immune

microenvironment. Additionally, FBXL22 expression was associated with infiltration of cancer-associated fibroblasts and endothelial cells, which may affect immunotherapy outcomes. Mechanistically, FBXL22 was involved in circadian rhythm regulation, ubiquitin-mediated proteolysis, focal adhesion signaling and cGMP-protein kinase G signaling. In prostate cancer, FBXL22 upregulation suppressed cell viability, invasion and metastasis, while promoting apoptosis, potentially through modulation of the polo-like kinase 1 pathway. To the best of our knowledge, the present study provides the first comprehensive pan-cancer analysis of FBXL22, which highlights its potential as an immune biomarker and therapeutic target for multiple cancer types with implications for precision medicine.

Introduction

The circadian rhythm, an intrinsic biological process with a period of ~24 h, regulates a wide array of physiological functions such as sleep-wake cycles, metabolism, hormone secretion and immune responses (1-3). Disruptions in this rhythm, collectively termed circadian rhythm disorders (CRDs), have been associated with various health issues, including an increased risk of developing cancer. The impact of CRDs on cancer is multifaceted, influencing not only the proliferation and survival of cancer cells but also the tumor immune microenvironment (TME) (4), which serves a key role in tumor progression and response to therapy (5-7).

F-box and leucine-rich repeat protein 22 (FBXL22), an F-box protein-coding gene, is known to participate in the ubiquitin-proteasome system, which is central to the regulation of protein turnover and cellular homeostasis (8,9). Emerging evidence suggests that FBXL22 also serves a role in cancer development and progression, although the exact mechanism remains unclear (8,10). Previous bioinformatics analysis has indicated that FBXL22 is associated with breast cancer and acts as a favorable factor for relapse-free survival in prostate cancer (PCa) (11-13). However, a comprehensive pan-cancer analysis of FBXL22, particularly in the context of the TME and its interactions with the circadian rhythm, is lacking.

Due to the importance of circadian rhythms in oncology and the potential role of FBXL22 in cancer, the present study

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Abbreviations: BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; HRD, homologous recombination deficiency; LAML, acute myeloid leukemia; LIHC, liver hepatocellular carcinoma; PAAD, pancreatic adenocarcinoma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; STAD, stomach adenocarcinoma; THCA, thyroid carcinoma; TMB, tumor mutation burden; UCEC, uterine corpus endometrial carcinoma; UVM, uveal melanoma

Key words: F-box and leucine-rich repeat protein 22, pan-cancer, tumor immune microenvironment, biomarkers, prostate cancer

aimed to perform an extensive analysis of FBXL22 across various cancer types. By leveraging data from The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) databases, the present study explored gene expression, genetic alterations and DNA methylation patterns of FBXL22. The present analysis aimed to reveal the correlation of these patterns with patient prognosis, immune cell infiltration and TME, thereby providing insights into the potential of FBXL22 as a biomarker and therapeutic target in cancer.

Materials and methods

Data collection, normalization and batch effect correction. Pan-cancer and normal tissue expression data for FBXL22 were extracted from GTEx (<https://www.gtexportal.org/home/datasets>) and TCGA datasets (TCGA-PANCAN, <https://portal.gdc.cancer.gov/>) of University of California Santa Cruz XENA (<https://xenabrowser.net/datapages/>). University of Alabama at Birmingham CANcer data analysis Portal (<https://ualcan.path.uab.edu/>) and cBioPortal (<https://www.cbioportal.org/>). RNA sequencing expression data from TCGA and GTEx were harmonized to ensure cross-dataset comparability. Raw read counts from TCGA were normalized to transcripts per million (TPM) using the 'edgeR' R package (R Development Core Team, version 3.46.0; <https://bioconductor.org/packages/release/bioc/html/edgeR.html>). GTEx data were processed using the same pipeline to maintain consistency. Batch effects arising from differences in sequencing platforms and study cohorts were corrected using the ComBat algorithm (implemented via the 'sva' R package; version, 3.52.0; <https://bioconductor.org/packages/release/bioc/html/sva.html>), with 'study cohort' (TCGA vs. GTEx) as the batch variable. Low-quality samples (read depth <10 million) and genes with zero expression in >90% of the samples were excluded prior to analysis.

Diagnostic receiver operating characteristic (ROC) curve and immune infiltration analysis. The predictive accuracy of FBXL22 gene expression in TCGA cancer types was assessed using ROC analysis with the 'pROC' package (Version, 1.18.0; <https://cran.r-project.org/web/packages/pROC/>) in R software (R Development Core Team; Version, 4.4.2). To assess immune cell infiltration using the 'ssGSEA' algorithm, predefined gene sets were employed from the Molecular Signatures Database (MSigDB; <https://www.gsea-msigdb.org/gsea/msigdb/index.jsp>) hallmark collection, specifically the hallmark gene sets related to the immune response. The cell-type marker genes used for immune infiltration analysis were obtained from the ImmPort database (version, DR35; <https://www.immport.org/>) and published literature, ensuring their relevance and specificity for the immune cell types under investigation. The ssGSEA algorithm was run with 1,000 permutations to assess statistical significance and normalized enrichment scores were used to quantify the relative abundance of immune cells in each sample. For the Tumor Immune Estimation Resource 2.0 (TIMER2) tool (version 2.0; <http://timer.cistrome.org>), the present study used the default settings with the immune gene module, which includes a comprehensive set of immune-related genes and their corresponding cell type-specific markers.

Correlation analysis between FBXL22 expression and immune cell infiltration was performed using Spearman's correlation coefficient to account for nonlinear relationships.

Functional enrichment analysis of FBXL22-related genes. Top 100 target genes associated with FBXL22 expression were identified using the 'Similar Gene Detection' module of Gene Expression Profiling Interactive Analysis 2 (<http://gepia2.cancer-pku.cn/>). Heatmap data for the top 10 genes were generated using the 'Gene_Corr' module of TIMER2. Protein-protein interaction networks were constructed using the Search Tool for the Retrieval of Interacting Genes/Proteins database (STRING: functional protein association networks; <https://cn.string-db.org/>). Functional enrichment analysis was performed using the 'clusterProfiler' package in the R software (version 4.10.0; <https://bioconductor.org/packages/clusterProfiler/>). Gene ontology (GO, <https://www.geneontology.org/>) and Kyoto Encyclopedia of Genes and Genomes (KEGG, <https://www.genome.jp/kegg>) enrichment analyses (14) were performed to explore the biological processes and pathways associated with FBXL22. Significant terms were defined as those with an adjusted P-value <0.05 (Benjamini-Hochberg correction) and a minimum gene count of 5 per term.

Subgroup survival prognosis analysis for PCa and expression analysis in disease subtypes. RNA sequencing data from TCGA-prostate adenocarcinoma (TCGA-PRAD; <https://portal.gdc.cancer.gov/projects/TCGA-PRAD>) project were downloaded and organized using TCGA database (<https://portal.gdc.cancer.gov>). Clinical outcome data were obtained from a publication on general cancer prognosis (15). The Prostate Cancer Transcriptome Atlas (PCTA) database (version, 1.0.1; <http://www.thepcta.org/>) was used to analyze FBXL22 expression and its association with disease progression in different subtypes of PCa. Patients were stratified based on clinical features, such as Gleason score (GS) (16) and clinical stage and survival analysis was performed using the Kaplan-Meier method with log-rank tests (<https://kmplot.com/analysis/>).

Immuno-infiltration and immune checkpoint gene co-expression analysis in PCa. Infiltration of the 24 immune cell types was quantified using the 'ssGSEA' algorithm in the 'GSVA' R package (R Development Core Team; version 1.52.0; <https://bioconductor.org/packages/release/bioc/html/GSVA.html>). Spearman's correlation analysis was performed to examine the relationship between FBXL22 expression and immune cell infiltration matrix data as well as immune checkpoint protein expression in the dataset.

Functional enrichment analysis of FBXL22 in PCa. Patients were classified into high and low FBXL22 expression groups (low, 0-50%; high, 50-100%) for enrichment analysis using the R package 'clusterProfiler' (version 4.10.0; R Development Core Team; <https://bioconductor.org/packages/clusterProfiler/>). Gene set enrichment analysis (GSEA) was performed using the 'clusterProfiler' and 'msigdb' packages (version 7.5.1; <https://cran.r-project.org/web/packages/msigdb/index.html>) and the MSigDB gene collection database. For GO/KEGG analysis of differentially expressed genes, significance thresholds were

an adjusted P-value <0.05 and a minimum gene count of 5. For GSEA, gene sets with a false discovery rate <0.25 and a nominal P-value <0.05 were considered statistically significant.

Cell culture and transfection. Human normal prostate epithelial cells (RWPE-1) and PCa cell lines (DU145 and PC3) were obtained from Procell Life Science and Technology Co., Ltd. and cultured in DMEM (cat. no. D6429; MilliporeSigma) or F-12K medium (cat. no. 21127022; Thermo Fisher Scientific, Inc.), supplemented with 10% FBS (cat. no. A5670701; Thermo Fisher Scientific, Inc.) under standard conditions (37°C; 5% CO₂). Human natural killer (NK) cells (cat. no. CP-H168), also from Procell Life Science and Technology Co., Ltd., were maintained in RPMI-1640 medium (cat. no. 11875093; Thermo Fisher Scientific, Inc.) with 10% FBS under the same conditions. The human FBXL22 gene was amplified using forward (F) primer 5'-TACCGAGCTCGGATCCATGTGGCCACTCTGCACCATG-3' and reverse (R) primer 5'-GATATCTGCAGAATTCTCACTGCGGGAACGCATCCG-3', then subcloned into a pcDNA3.1 (+) mammalian expression vector (cat. no. V79020; Invitrogen; Thermo Fisher Scientific, Inc.) to generate the recombinant plasmid pcDNA3.1-FBXL22. The empty vector pcDNA3.1 served as a negative control (NC) and both were provided by ABclonal Biotech Co., Ltd. (cat. no. RM09014P). For transfection, cells were seeded into 24-well plates at a density of 5x10⁴ cells per well and cultured until they reached 80% confluence. Lipofectamine® 3000 (cat. no. L3000001; Thermo Fisher Scientific, Inc.) was used as the transfection reagent. A mixture of 25 µl Opti-MEM I medium and 0.5 µl Lipofectamine® 3000 reagent was prepared. Meanwhile, 1 µl P3000™ reagent and 25 µl Opti-MEM I medium were added to 2 µg pcDNA3.1-FBXL22 or pcDNA3.1-NC plasmid DNA. The two solutions were subsequently combined and incubated at 25°C for 10 min. The resulting 50 µl complex was added to the cell culture medium, followed by a 48 h incubation at 37°C. After 48 h, the medium was replaced with fresh complete medium. All functional assays were performed 48 h post-transfection. Successful transfection was confirmed using reverse transcription (RT)-quantitative (q)PCR and western blotting.

Cell viability, invasion and migration assays. At 48 h post-transfection, cell viability was assessed using a Cell Counting Kit (CCK-8; cat. no. A50298; Thermo Fisher Scientific, Inc.) assay following a 24 h incubation with the CCK-8 reagent. Transwell invasion assays were performed using 24-well chambers (cat. no. 3378; Corning, Inc.). The polycarbonate membrane of the upper chamber was coated with 100 µl Matrigel (cat. no. M8370; Beijing Solarbio Science & Technology Co., Ltd.) diluted 1:8 in serum-free DMEM at 4°C and polymerized for 0.5-1 h at 37°C. The upper chamber was seeded with ~5x10⁵ cells in 200 µl serum-free DMEM. The lower chamber contained 500 µl medium with 20% FBS. Cells were incubated for 24 h at 37°C in 5% CO₂. The membrane was fixed with 600 µl of 4% tissue cell fixative (cat. no. P1110; Beijing Solarbio Science & Technology Co., Ltd.) for 20-30 min at room temperature. After discarding the fixative, cells were stained with 0.1-0.2% crystal violet (cat. no. G1062; Beijing Solarbio Science & Technology Co., Ltd.) for 5-10 min at room temperature. Excess stain was rinsed with PBS. Invasive cells

in the lower membrane surface were imaged using an inverted light microscope (model ICX41; Ningbo Sunny Instruments Co., Ltd.) with a 50 µm scale bar. For the wound healing assay, prostate cancer cell lines (DU145 and PC3) were seeded in 6-well plates (Shanghai Kejin Biotechnology Co., Ltd.) at a density of 5x10⁵ cells per well and cultured until a confluent monolayer had formed. Vertical scratches were created across the monolayer using a sterile 20 µl pipette tip, aligned with pre-marked horizontal lines on the back of the plate to ensure consistent observation points. After scratching, cells were washed with PBS (cat. no. C3580-0500; VivaCell; Shanghai XP Biomed Ltd.) 2-3 times to remove detached cells and then incubated in serum-free DMEM. Images of the scratches were captured immediately at 0 h using an inverted light microscope (model ICX41; Ningbo Sunny Instruments) at 40x magnification. Cells were further cultured at 37°C in a 5% CO₂ incubator, with additional images taken at 24 h under the same 40x magnification. The healing rate was quantified by measuring the change in scratch width using ImageJ 1.5.2a software (National Institutes of Health).

NK cell co-culture system with DU145 and PC3 PCa cell lines. NK cells were labeled with CellTracker™ Green CMFDA (Thermo Fisher Scientific, Inc.) and co-cultured with FBXL22-overexpressing or control DU145/PC3 cells in a Transwell system (Corning, Inc.) with a pore size of 0.4 µm. The upper chamber contained 1x10⁵ NK cells in RPMI-1640 medium without FBS and the lower chamber contained 2x10⁵ PCa cells in RPMI-1640 medium supplemented with 10% FBS. After 48 h of co-culture, the Transwell membranes were first fixed with 600 µl of 4% tissue cell fixative (cat. no. P1110; Beijing Solarbio Science & Technology Co., Ltd.) at room temperature for 20-30 min. Following fixation, the membranes were stained with 0.1-0.2% crystal violet (cat. no. G1062; Beijing Solarbio Science & Technology Co., Ltd.) at room temperature for 5-10 min. Excess stain was removed by rinsing with PBS three times. Analyzed for NK cell migration and cytotoxic activity against PCa cells. For Transwell assays assessing NK cell migration, the upper chamber contained 1x10⁵ NK cells in RPMI-1640 medium without FBS, while the lower chamber contained RPMI-1640 medium with 10% FBS. After 48 h, the membranes were processed for analysis.

Flow cytometry. Apoptosis was analyzed using an Annexin V-FITC/PI Apoptosis Detection Kit (cat. no. CA1020; Solarbio Science & Technology Co., Ltd.) according to the manufacturer's instructions. Briefly, transfected DU145 and PC3 cells were harvested by trypsinization with EDTA-free trypsin (cat. no. C0205; Beyotime Institute of Biotechnology), centrifuged at 100 x g for 5 min at 4°C, and washed twice with cold PBS. The cell pellet was resuspended in 1x binding buffer (10x binding buffer diluted 1:9 with deionized water) to a density of 1-5x10⁶ cells/ml. A 100 µl aliquot of the cell suspension was incubated with 5 µl Annexin V-FITC in the dark at room temperature (25°C) for 15 min. Subsequently, 5 µl PI solution and 400 µl PBS were added and samples were immediately analyzed using a FACSCanto™ flow cytometer (BD Biosciences). Data were analyzed using the FlowJo software (v10.8.1; FlowJo LLC; BD Biosciences).

Gene expression. FBXL22 mRNA was quantified by reverse transcription-quantitative PCR (RT-qPCR). RNA was extracted from transfected DU145, PC3, and RWPE-1 cells using TRNzol (cat. no. DP424; Tiangen Biotech Co., Ltd.). Concentration/purity was measured via NanoDrop One/OneC (cat. no. 840-317400; Thermo Fisher Scientific, Inc.; $A_{260/280}=1.8-2.0$). cDNA was synthesized with FastKing One-Step SuperMix (cat. no. KR118; Tiangen Biotech Co., Ltd.; 20 μ l; 4 μ l 5x SuperMix; 2 μ g RNA; RNase-free water). The thermal cycling protocol was conducted as follows: An initial step at 42°C for 15 min, followed by heat inactivation at 95°C for 3 min and a final hold at 4°C for 30 min. RT-qPCR was performed using a StepOnePlus™ Real-Time PCR System (cat. no. 4376600; Thermo Fisher Scientific, Inc.) with SYBR™ Green Master Mix (cat. no. G3320-15; Wuhan Servicebio Technology Co., Ltd.) or SuperReal Premix Plus (cat. no. FP205; Tiangen Biotech Co., Ltd.). Each 20 μ l reaction mixture contained 10 μ l 2x Premix, 0.6 μ l forward and reverse primer each (10 μ M), 2 μ l of cDNA template, and was made up to the final volume with nuclease-free water. The following thermocycling conditions were used: 95°C 15 min; 40x (95°C 10 sec; 60°C 32 sec). A melting curve analysis was performed immediately after the amplification cycles to confirm the specificity of the PCR products and the absence of primer-dimers. Expression calculated via $2^{-\Delta\Delta C_q}$ (17) with β -actin control. Primers sequences were as follows: FBXL22, Forward 5'-CCATGCACATAACCCAGCTCA-3'; Reverse (R), 5'-CCGAGGTGATTTCCGGTCCAAC-3'; β -actin F, 5'-CATGTACGTTGCTATCCAGGC-3'; R, 5'-CTCCTTAATGTCACGCACGAT-3'.

Western blotting. Protein levels were determined using western blotting. Total protein was extracted from cells using RIPA lysis buffer (cat. no. R0010; Beijing Solarbio Science & Technology Co., Ltd.) and quantified with a BCA assay kit (cat. no. BL521A; Biosharp Life Sciences). An equal amount (30 μ g) of protein per sample was separated by 10% SDS-PAGE and then electrophoretically transferred to a PVDF membrane. The membrane was blocked with 5% non-fat milk in TBST (containing 0.1% Tween 20), for 2 h at room temperature. After primary antibody incubation (4°C; overnight; Table SI) and three washes with TBST, membranes were incubated with secondary antibodies: Goat anti-rabbit IgG H and L (HRP) (cat. no. ab97051; Abcam) or HRP-conjugated goat anti-mouse IgG (H+L) (cat. no. AS003; ABclonal Biotech Co., Ltd.), both at a 1:10,000 dilution, for 1 h at room temperature. Signals were detected using an enhanced chemiluminescence kit (cat. no. P10300; Suzhou Xinsaimai Biotechnology Co., Ltd) and visualized with a multi-functional imaging system (SH-CUTE523; Zhehiang ICP; Table SII).

Co-immunoprecipitation (Co-IP). An immunoprecipitation kit (cat. no. P2179S) purchased from Beyotime Institute of Biotechnology was used for the experiment. Transfected PC3 and DU145 cells (1×10^7) were collected and lysed on ice with 500 μ l lysis buffer (provided in the kit) for 30 min. Lysates were centrifuged at 12,000 x g for 10 min at 4°C to pellet cellular debris and the protein concentration of the supernatants was determined using a BCA assay (cat. no. BL521A; Biosharp Life Sciences). A small amount of lysate (50 μ l) was incubated with

20 μ l protein A/G agarose beads (pre-treated with lysis buffer) at 4°C for 1 h to block non-specific binding. For immunoprecipitation, 500 μ l lysate (containing ~500 μ g total protein) was incubated with 1 μ g anti-FBXL22 primary antibody (cat. no. ab223059; Abcam) at 4°C overnight. Subsequently, 40 μ l pre-treated protein A/G agarose beads were added and incubated at 4°C for 2 h. The samples were centrifuged at 1,000 x g at 4°C for 3 min and the beads were collected and rinsed with elution buffer. Finally, the bound proteins were eluted by boiling the beads in 2X SDS-PAGE loading buffer at 95°C for 10 min. Western blotting was performed on the eluent to analyze the co-precipitated proteins.

Statistical analysis. Statistical analyses were performed using the R software and GraphPad Prism 9.5.1(Dotmatics). Gene expression differences were compared using Wilcoxon rank-sum (for two-group comparisons) and Kruskal-Wallis tests (for multi-group comparisons). For significant results using Kruskal-Wallis tests ($P < 0.05$), post hoc pairwise comparisons were performed using Dunn's test with Bonferroni correction. Correlations were assessed using Spearman's or Pearson's correlation analysis. Survival characteristics were compared using Kaplan-Meier curves and Cox regression analysis. For the KM plotter, mRNA expression data were used; and patients were stratified into high and low FBXL22 expression groups using the 50th percentile (median) as the cut-off (low, 0-50%; high, 50-100%). Data were represented as mean $\pm$ standard deviation (SD) * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$ were considered to indicate a statistically significant difference.

Results

Pan-cancer expression and epigenetic regulation of FBXL22. FBXL22 was differentially expressed in 33 cancer types when compared with corresponding normal tissues. FBXL22 was significantly downregulated in 19 cancer types, including uterine carcinosarcoma ($P < 0.0001$), endometrial carcinoma (UCEC; $P < 0.0001$) and colon adenocarcinoma (COAD; $P < 0.0001$), but overexpressed in seven cancer types such as cholangiocarcinoma (CHOL; $P < 0.0001$) and acute myeloid leukemia (LAML; $P < 0.0001$) (Fig. 1A and B). When comparing tumor tissues with the corresponding normal tissues from TCGA and GTEx databases, significant variations in FBXL22 expression were observed in 26 cancer types ($P < 0.05$), with the exception of kidney chromophobe, pheochromocytoma and paraganglioma and thyroid carcinoma (THCA) (Fig. 1B). This comparison highlights the distinct expression levels of FBXL22 in neoplastic vs. non-neoplastic tissues and provides a foundation for understanding its potential role in cancer. However, it should be noted that expression levels in normal tissues were based on the GTEx database and these data should be interpreted with caution because of the potential differences in tissue collection and processing methods between TCGA and GTEx.

A strong negative correlation between FBXL22 expression and promoter methylation was observed ($P < 0.0001$, Spearman's $\rho = -0.27$; Pearson's $r = -0.31$; Fig. 1C). Elevated methylation levels in tumors vs. normal tissues were prominent in kidney renal papillary cell carcinoma, breast invasive carcinoma (BRCA), THCA and PRAD ($P < 0.0001$), whereas reduced methylation

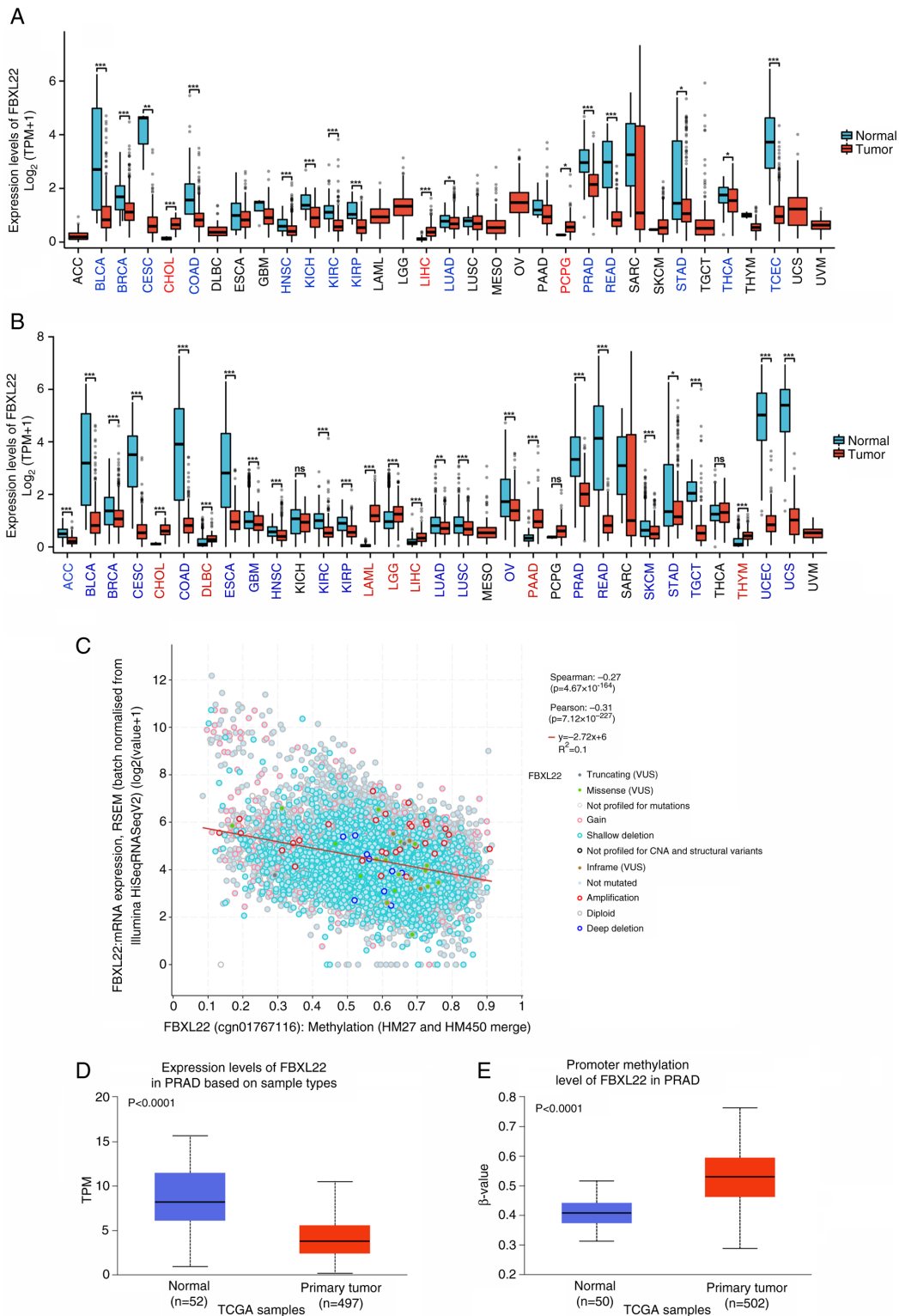


Figure 1. Expression levels of FBXL22 in pan-cancer. (A) The expression status of FBXL22 in different cancer tissues and para-cancerous tissues in TCGA database. Error bars represent standard deviation. (B) Expression status of FBXL22 in matched cancer tissues and normal tissues in TCGA and GTEx databases. Cancer names in red text indicate high FBXL22 expression in this cancer, while blue text indicates low FBXL22 expression in this cancer. (C) Correlation between FBXL22 expression and methylation in TCGA database (D) Promoter methylation levels of FBXL22 in PRAD samples. (E) Expression differences of FBXL22 in PRAD samples based on sample types. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, $P \geq 0.05$. FBXL22, F-box and leucine rich repeat protein 22; ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, brain lower grade glioma; LIHC, Liver hepatocellular carcinoma; LUAD, Lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; ns, not significant; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TCGA, The Cancer Genome Atlas; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; TMB, tumor mutation burden; TPM, transcripts per million; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma.

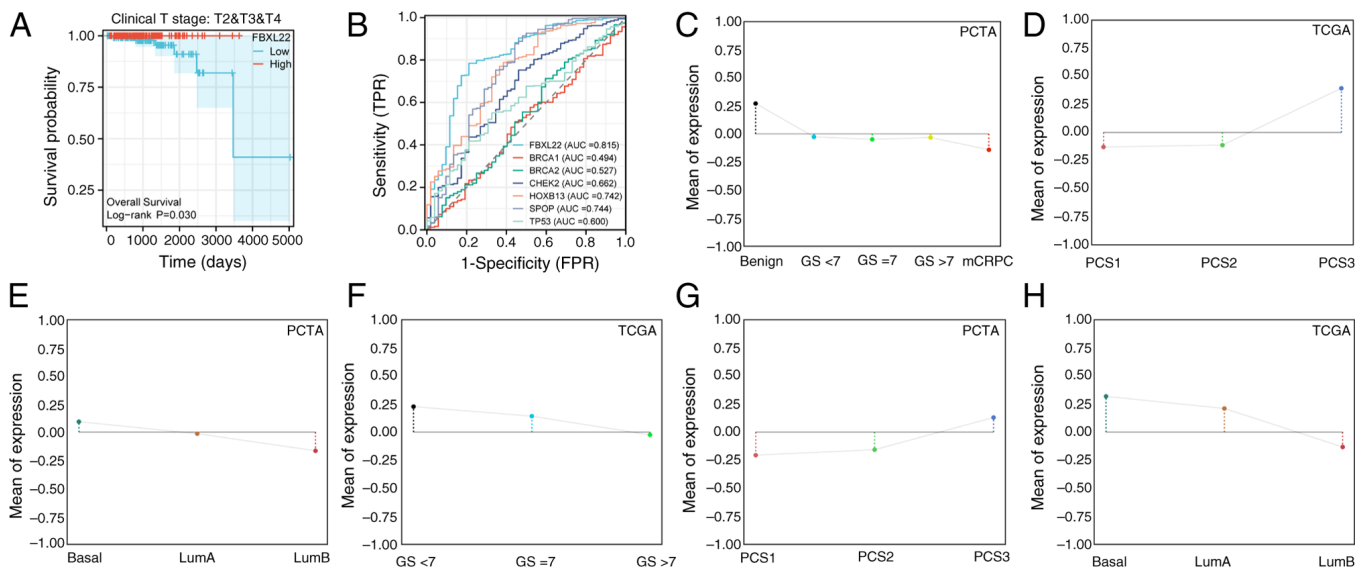


Figure 2. Clinical diagnosis and prognostic implications of FBXL22 in PCa. (A) Kaplan-Meier curve of PFI in patients with intermediate to advanced disease stage (Clinical T stage, T2, T3 and T4) in PCa by FBXL22-expression groups. (B) Diagnostic ROC curves of FBXL22 and other genes such as SPOP, HOXB13, CHEK2, TP53, BRCA2 and BRCA1 in PCa. (C) FBXL22 expression across PCa disease course subtypes. (D) FBXL22 expression in PCS subtypes. (E) FBXL22 expression in basal, LumA and LumB subtypes. (F) FBXL22 expression across GS subgroups. (G) FBXL22 expression in PCS subtypes. (H) FBXL22 expression in basal, LumA and LumB subtypes. FBXL22, F-box and leucine rich repeat protein 22; PCa, prostate cancer; PFI, progression-free interval; ROC, receiver operating characteristic; SPOP, Speckle-type pox virus and zinc finger protein; HOXB13, homeobox B13; CHEK2, checkpoint kinase 2; PAM50, Prediction Analysis of Microarray 50; PCTA, Prostate Cancer Transcriptome Atlas; TCGA, The Cancer Genome Atlas; PCS, PCa subtypes; ROC, receiver operating characteristic; Lum, luminal; GS, Gleason score; TPR, true positive rate; FPR, false positive rate; AUC, area under the curve.

was observed in glioblastoma multiforme, liver hepatocellular carcinoma (LIHC) and pancreatic adenocarcinoma (PAAD) (Figs. 1D and S1). In PCa, FBXL22 demonstrated significant promoter hypermethylation ($P < 0.0001$) and downregulation ($P < 0.0001$) (Figs. 1D, E and S2).

Genomic alterations and mutation frequency of FBXL22. Genomic alterations in FBXL22 were observed in 26 cases across 12 cancer types, with a somatic mutation frequency of 0.2% (Fig. S3). Among the various cancer types, UCEC exhibited the highest mutation frequency of 1.7% (9/529 cases), followed by stomach adenocarcinoma [STAD; 1.14% (5/440 cases)] (Fig. S3). Missense mutations were the predominant type of genetic alteration observed. However, no significant correlation was found between FBXL22 expression levels and mutation types or copy number alteration (Fig. S4; Table SIII; $P > 0.05$). Although genomic alterations of FBXL22 were identified across several cancer types, the overall mutation frequency was relatively low. This suggested that while genetic mutations can occur in FBXL22, they may not be the primary driver of its expression changes in most cancer types.

Survival analysis and prognostic implications of FBXL22. Survival analysis revealed that elevated FBXL22 expression was significantly associated with worse overall survival (OS) in bladder cancer (BLCA; $P < 0.01$) and STAD ($P < 0.05$), whereas low expression predicted unfavorable recurrence-free survival in THCA ($P < 0.05$) (Fig. 2A and B). Kaplan-Meier validation across 21 cancer types ($n = 7,489$) further demonstrated that low FBXL22 expression correlated with worse OS in esophageal adenocarcinoma ($P < 0.05$) and PAAD ($P < 0.05$), whereas high expression remained a prognostic factor for poor OS in BLCA ($P < 0.05$) and STAD ($P < 0.05$) (Fig. S5). Notably, FBXL22

expression exhibited significant associations with PCa clinico-pathological features: Higher levels were observed in patients with $GS \leq 7$ (Fig. 2C and F; $P < 0.001$) and advanced clinical stages (Fig. 2D; $P < 0.05$) and metastatic castration-resistant PCa (Fig. 2E-G, $P < 0.001$) and luminal B Prediction Analysis of Microarray 50 subtypes (Fig. 2H, $P < 0.001$). Collectively, these findings underscore the key relationship between FBXL22 expression and PCa progression markers (GS, staging and molecular subtypes), which suggests it has a potential role as a biomarker and functional driver in disease progression.

FBXL22 immune activation and TME. Comprehensive analyses revealed a significant association between FBXL22 expression and TME dynamics (Figs. 3 and S6). Violin plots demonstrated that FBXL22-high tumors exhibited markedly elevated enrichment scores across diverse immune cell populations ($P < 0.05$; Wilcoxon test), particularly in cytotoxic subsets, including NK and $CD8^+$ T cells (Fig. 3A and I). Consistent with this immune-activated phenotype, key immunoregulatory markers [programmed cell death 1 (PDCD1), CD274 and PDCD1 ligand 2] demonstrated 2.1- to 3.4-fold higher expression in FBXL22-high samples [$\log_2(\text{TPM}+1)$; $P < 0.01$; Fig. 3B]. Heatmap analysis further demonstrated strong co-expression patterns between FBXL22 and immune checkpoint regulators (Pearson's $r = 0.62-0.71$; Z-score normalized; Fig. 3C). Quantitative correlation analyses revealed dose-dependent relationships between FBXL22 expression and infiltration densities of antitumor immune subsets, including significant associations with NK cells (Spearman's $\rho = 0.57$; $P < 0.00001$), $CD8^+$ T cells ($r = 0.49$; $P < 0.001$), Th1 cells ($r = 0.43$; $P < 0.001$) and dendritic cells ($r = 0.38$; $P < 0.01$; Fig. 3D-H). These multimodal findings collectively suggest that FBXL22 is a potential immunomodulatory hub with high expression levels

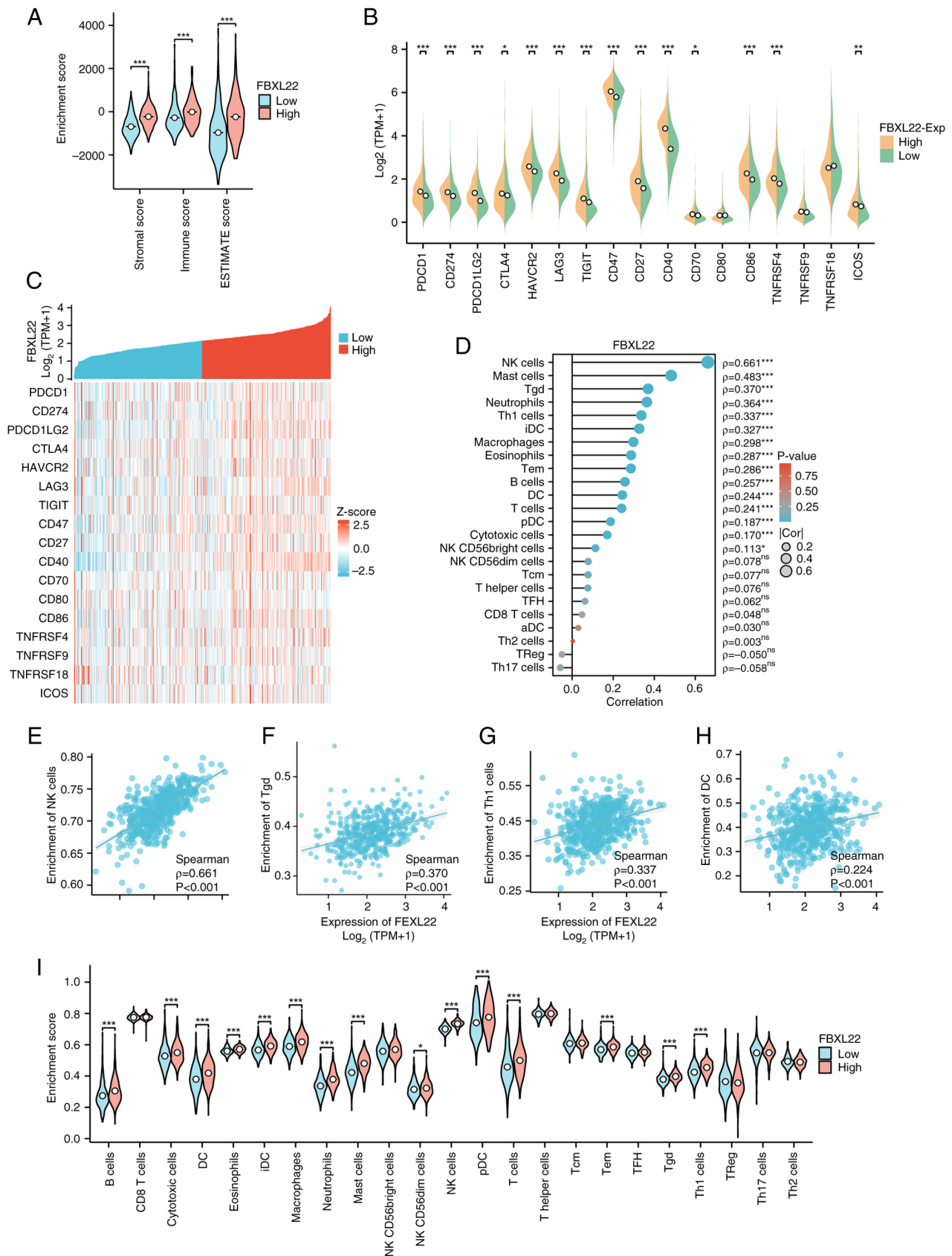


Figure 3. Association of FBXL22 with immune-infiltration in PCa. (A) The immune score of FBXL22-expression groups. (B) Expression levels of the 17 immune checkpoint proteins by FBXL22-expression group. (C) Heatmap illustrating correlations between FBXL22 expression and 17 immune checkpoint proteins. (D) Correlation between FBXL22 expression and the infiltration levels of various immune cells. Scatter plot of correlation between FBXL22 expression and the infiltration levels of (E) NK, (F) Tgd, (G) Th1 and (H) DC cells. (I) Infiltration levels of 23 types of immune cells were assessed by FBXL22-expression group using ssGSEA. * $P<0.05$, ** $P<0.01$ and *** $P<0.001$; ns, $P\geq 0.05$. FBXL22, F-box and leucine rich repeat protein 22; PCa, prostate cancer; NK, natural killer; DC, dendritic cells; Tgd cells, $\gamma\delta$ T cells; Th1 cells, T helper type 1 cells; Treg, regulatory T cells; ssGSEA, single-sample gene set enrichment analysis; ESTIMATE, Estimation of STromal and Immune cells in MAlignant Tumor tissues using Expression data; TPM, transcripts per million; ns, not significant.

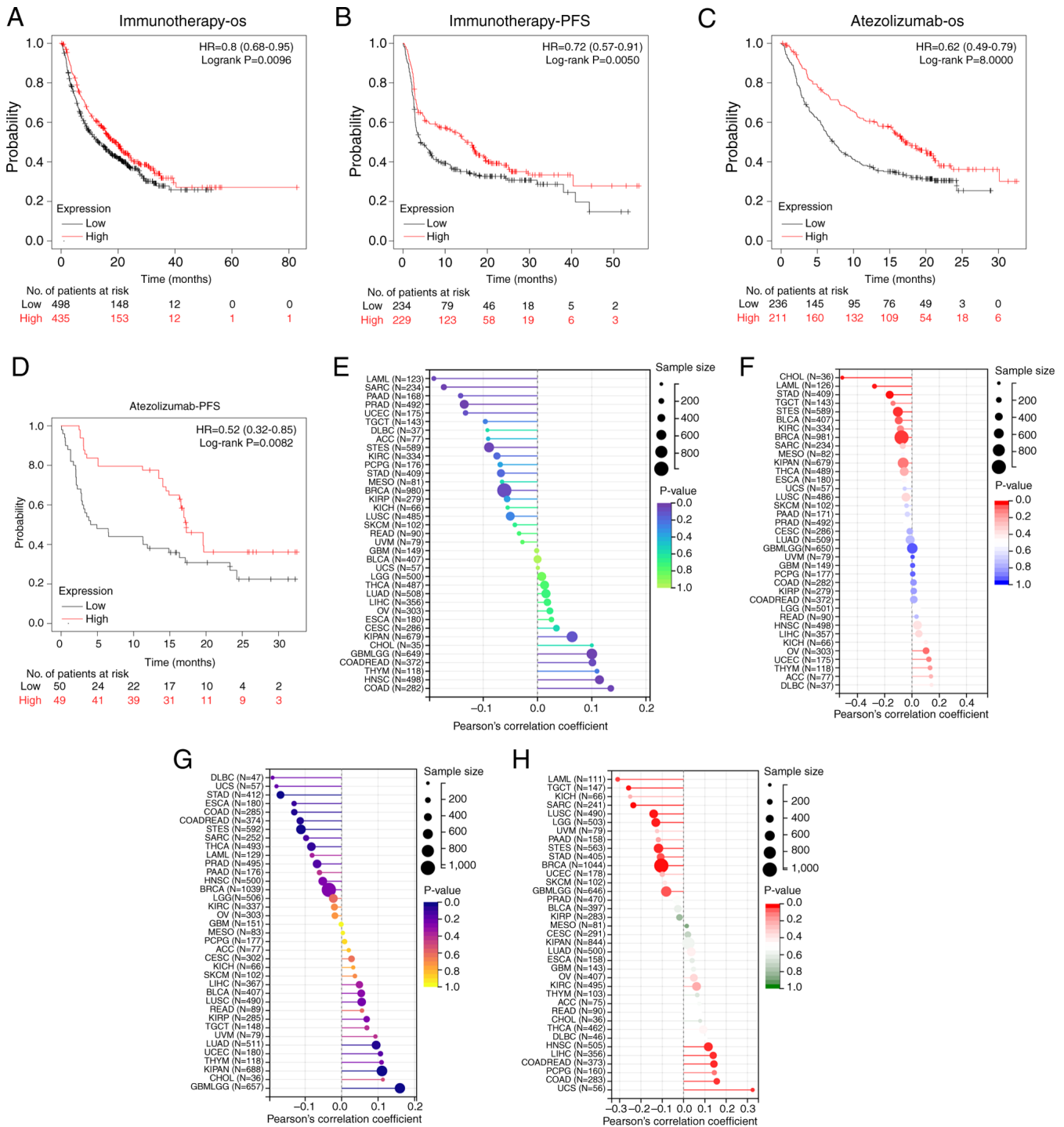


Figure 4. Predictive effects of therapeutic response for FBXL22 in pan-cancer. Kaplan-Meier curves of patients treated with immunotherapy by FBXL22 expression for (A) OS and (B) PFS. Kaplan-Meier curves for (C) OS and (D) PFS of patients treated with atezolizumab by FBXL22 expression. Correlation between FBXL22 expression and (E) MATH, (F) TMB, (G) MSI and (H) HRD. FBXL22, F-box and leucine rich repeat protein 22; MATH, mutant allele tumor heterogeneity; TMB, tumor mutation burden; MSI, microsatellite instability; HRD, homologous recombination deficiency; OS, overall survival; PFS; progression-free survival; HR, hazard ratio.

correlating with enhanced immune infiltration and checkpoint activation, which suggests it has functional involvement in shaping antitumor immune responses.

Therapeutic response prediction based on FBXL22 expression. Low FBXL22 expression was associated with worse OS ($P<0.01$) and progression-free survival (PFS; $P=0.005$) in immunotherapy-treated patients, whereas high expression

predicted improved outcomes in atezolizumab-treated cohorts ($P<0.001$ for OS; $P<0.01$ for PFS) (Fig. 4A-D). This suggests that FBXL22 expression may be a predictive biomarker of therapeutic responses to specific cancer treatments. Notably, FBXL22 expression was negatively correlated with tumor mutation burden (TMB) in LAML, BRCA and STAD, and positively correlated with homologous recombination deficiency (HRD) in COAD and LIHC (Fig. 4E-H; all $P<0.05$).

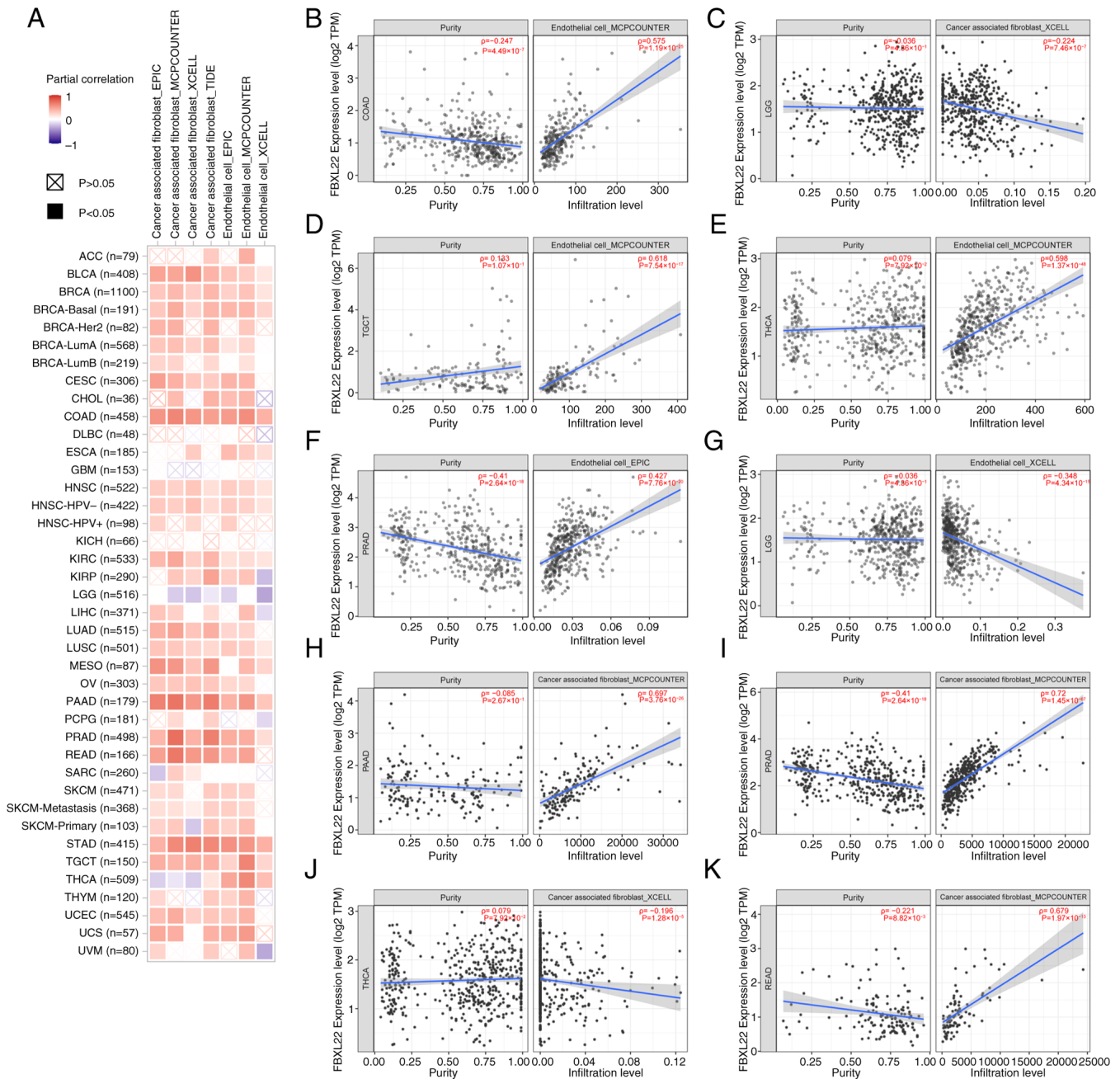


Figure 5. Correlation analysis between FBXL22 expression and immune infiltration of CAFs and ECs across all types of cancer in TCGA. (A) Corresponding heatmap and (B) COAD, (C) LGG, (D) TGCT, (E) THCA, (F) PRAD, (G) LGG, (H) PAAD, (I) PRAD, (J) THCA, (K) READ. Each scatter plot includes data points representing FBXL22 expression levels (x-axis) and infiltration levels of CAFs or ECs (y-axis), with a trend line indicating the correlation direction. Spearman's correlation coefficient (p) and corresponding P-value are annotated for each plot to quantify the strength and significance of the association. FBXL22, F-box and leucine rich repeat protein 22; CAFs, cancer-associated fibroblasts; ECs, endothelial cells; TCGA, The Cancer Genome Atlas; TPM, transcripts per million; COAD, colon adenocarcinoma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; LGG, lower grade glioma.

The negative correlation between FBXL22 expression and TMB in certain cancer types and the positive correlation with HRD in others further emphasizes its potential as a biomarker for guiding personalized treatment decisions.

Immune regulatory gene analysis. FBXL22 expression was significantly correlated with immune checkpoint pathway genes across multiple cancer types, including positive associations with inhibitory genes [for example, programmed death-ligand 1 (PD-L1) and cytotoxic T-lymphocyte

associated protein 4 (CTLA4)] and stimulatory genes (for example, CD40 and inducible T-cell co-stimulator) (Fig. S7; all P<0.05). Additionally, FBXL22 correlated with immunomodulation-related genes such as chemokines and major histocompatibility complex molecules in uveal melanoma, READ and PRAD (Fig. S8; all P<0.05). FBXL22 expression positively correlated with cancer-associated fibroblast (CAF) and endothelial cell (EC) infiltration in most cancer types, except brain lower grade glioma (Fig. 5A-K). In THCA, FBXL22 demonstrated a negative correlation with CAFs

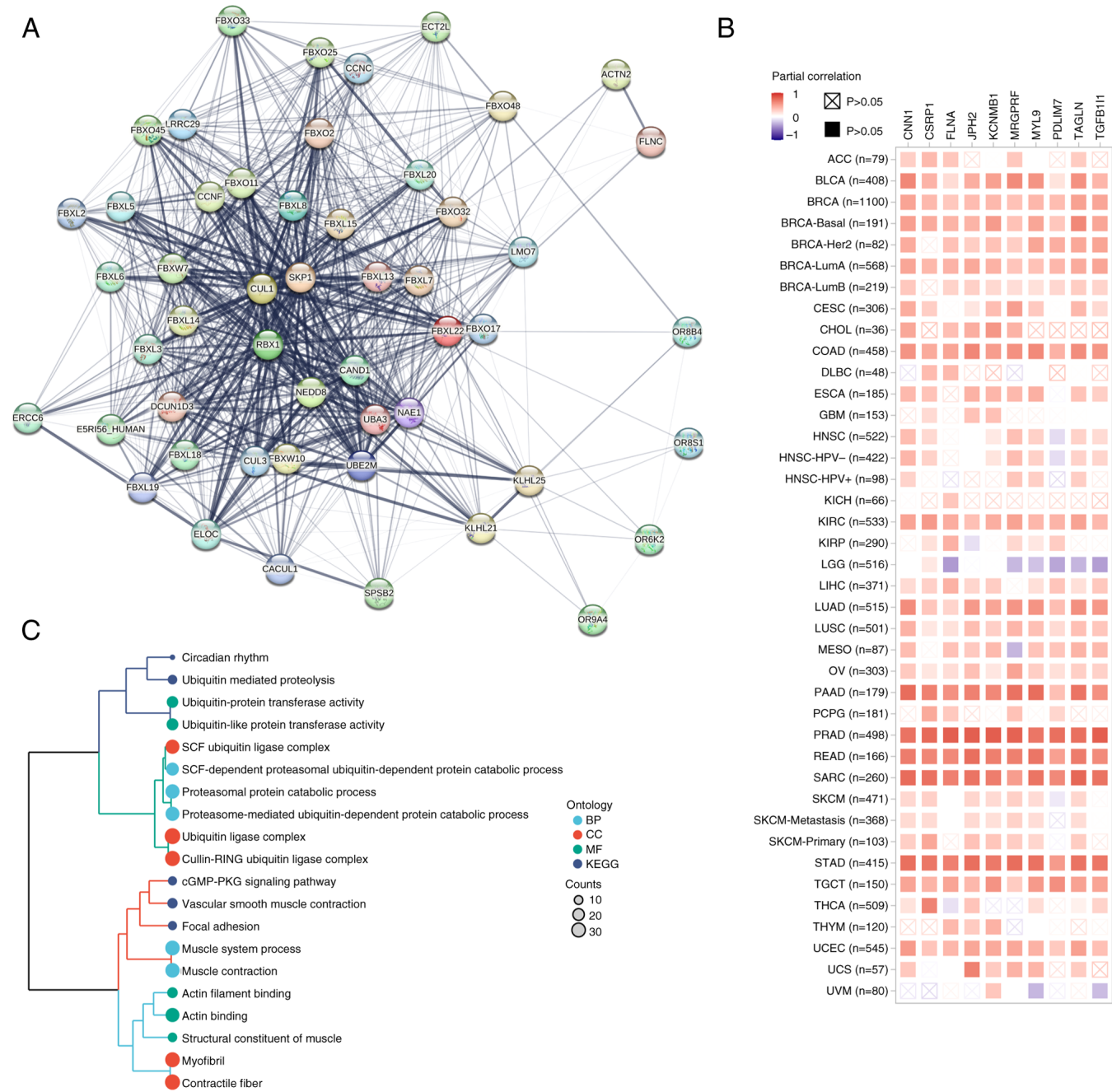


Figure 6. FBXL22-related gene enrichment analysis in pan-cancer. (A) Network of interactions among FBXL22-binding proteins using the STRING tool. (B) Heatmap of expression correlations between FBXL22 and the top 10 FBXL22-correlated genes in different cancer types of TCGA using the GEPIA2 tools, including MRGPRF, KCNMB1, PDLIM7, TAGLN, TGFBI1, JPH2, CNN1, FLNA, CSRPI and MYL9. (C) KEGG and GO enrichment analyses are visualized by cluster tree diagram. FBXL22, F-box and leucine rich repeat protein 22; STRING, Search Tool for the Retrieval of Interacting Genes/Proteins; TCGA, The Cancer Genome Atlas; GEPIA2, Gene Expression Profiling Interactive Analysis 2; MRGPRF, Mas-related G protein-coupled receptor F; KCNMB1, potassium calcium-activated channel subfamily M regulatory β subunit 1; PDLIM7L, PDZ and LIM domain 7; TAGLN, Transgelin; TGFBI1, transforming growth factor β 1 induced 1; JPH2, junctophilin 2; CNN1, calponin 1; FLNA, filamin A; CSRPI, cysteine and glycine rich protein 1; MYL9, myosin light chain 9; KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene ontology; BP, Biological process; CC, Cellular component; MF, Molecular function.

($\rho=-0.1961$; $P<0.0001$) but a positive association with ECs ($\rho=0.5977$; $P<0.0001$) (Fig. 5E, J and K).

Functional enrichment analysis of FBXL22-related genes. Protein-protein interaction analysis identified 50 FBXL22-binding partners, including filamin C (Fig. 6A). The top 100 FBXL22-correlated genes, MRGPRF ($r=0.83$) and KCNMB1 ($r=0.81$), were enriched in pathways such as ‘Circadian rhythm’, ‘Ubiquitin-mediated proteolysis’ and

‘cGMP-PKG signaling’ (Figs. 6B, C and S9; Table SIV and SV). These findings provide insight into the molecular mechanisms by which FBXL22 exerts its effects on cancer cells and the TME.

Differential expression and functional enrichment of FBXL22 in Pca. In Pca, differential expression analysis identified 1,243 upregulated and 1,017 downregulated genes in the high-FBXL22 groups. These genes were enriched in pathways

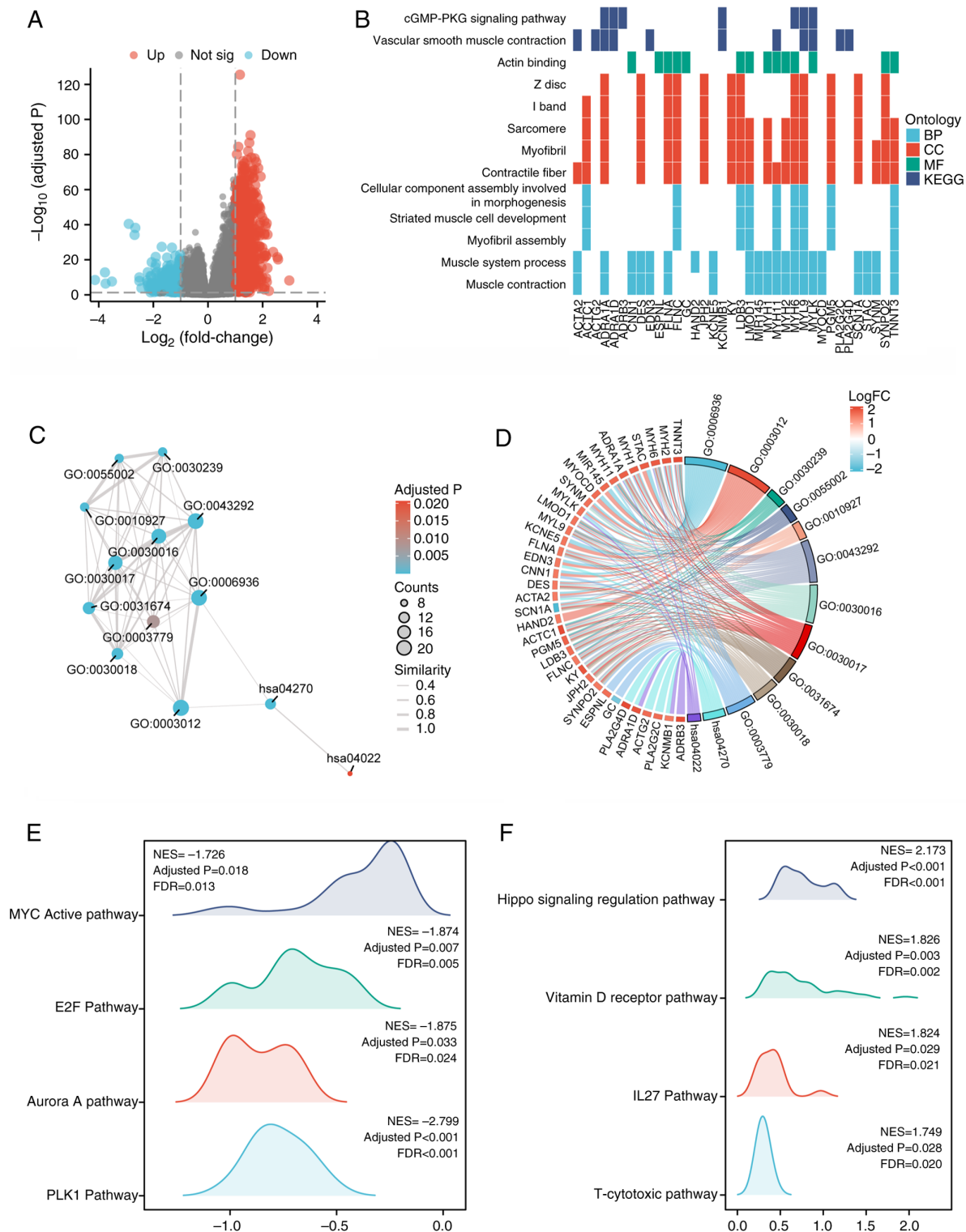


Figure 7. Differential expression and functional enrichment analysis of FBXL22-associated genes in prostate cancer. (A) Volcano plot illustrating differential expression analysis between high-FBXL22 and low-FBXL22 expression groups in PCa, which highlights the genes that were significantly upregulated or downregulated in association with FBXL22 expression levels. The results of KEGG and GO enrichment analysis are depicted using (B) heat map and (C) Enrichment Map Analysis Platform, respectively. (D) The analysis of GO/KEGG combined log(fold change) is represented by a chordal diagram. The mountain maps display the pathways (E) significantly negative or (F) significantly positive associated with expression levels of FBXL22 in GSEA analysis results. Error bars represent standard deviation. FBXL22, F-box and leucine rich repeat protein 22; PCa, prostate cancer; KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology; GSEA, Gene Set Enrichment Analysis; E2F, E2 DNA-binding factor; PLK1, polo-like kinase 1; NES, nuclear export sequence; FDR, false discovery rate; BP, biological process; CC, cellular component; MF, molecular function.

such as ‘Muscle contraction’ and ‘cGMP-PKG signaling’ (Fig. 7A-D, Table SVI and SVII). GSEA revealed that FBXL22 negatively regulated pro-tumor pathways, such as

'Myc active', 'E2F' and 'PLK1', and were positively associated with tumor-suppressive pathways, such as 'Hippo signaling regulation' and 'Vitamin D receptor' (Fig. 7E-F). These

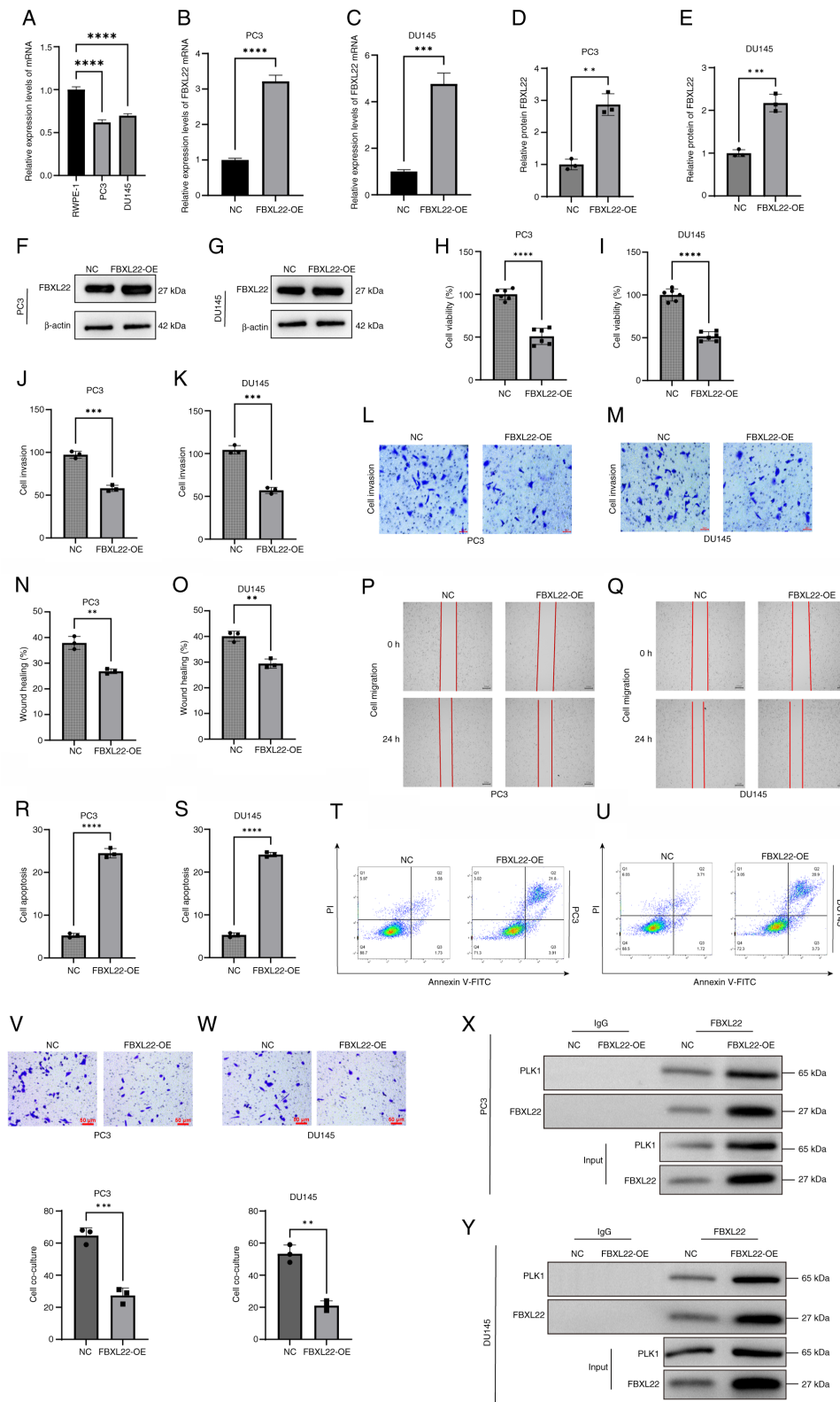


Figure 8. Effects of FBXL22 on viability, invasion, migration and apoptosis of PCa cells. (A) The relative expression levels of FBXL22 in normal prostate epithelial cell lines RWPE-1 and PCa cell lines PC3 and DU145. The relative expression levels of FBXL22 in NC and FBXL22-OE in (B) PC3 and (C) DU145 cell lines. Semi-quantification of relative protein levels of FBXL22 in NC and FBXL22-OE groups in (D) PC3 and (E) DU145 cells. Western blot of FBXL22 in NC and FBXL22-OE groups in (F) PC3 and (G) DU145 cells. Cell viability was assessed using the CCK-8 assay in (H) PC3 and (I) DU145 cells. (J-M) The Transwell chamber was utilized to evaluate cell invasive ability (scale bar, 50 μ m). (N-Q) Cell migratory ability was examined using a wound healing assay (scale bar, 250 μ m). The histograms demonstrate the relative percentage of wound healing. (R) Quantification of apoptosis rates in PC3 cells transfected with FBXL22-OE compared with the NC group, shown as a bar graph. (S) Quantification of apoptosis rates in DU145 cells under the same conditions. (T) Representative flow cytometry dot plots for PC3 cells, detailing the distribution of cells in different apoptosis stages. (U) Representative flow cytometry dot plots for DU145 cells, using annexin V-FITC and PI staining to delineate cell viability and apoptosis stages. NK cell migration and cytotoxicity against FBXL22-OE (V) PC3 and (W) DU145 cells in co-culture Transwell experiments (scale bar, 50 μ m). Co-immunoprecipitation analysis confirming the interaction between FBXL22 and PLK1 in (X) PC3 and (Y) DU145 cells. Error bars represent standard deviation. ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$; ns, $P \geq 0.05$. PCa, prostate cancer; FBXL22, F-box and leucine rich repeat protein 22; OE, overexpression; NC, negative control; ns, not significant; NK, natural killer.

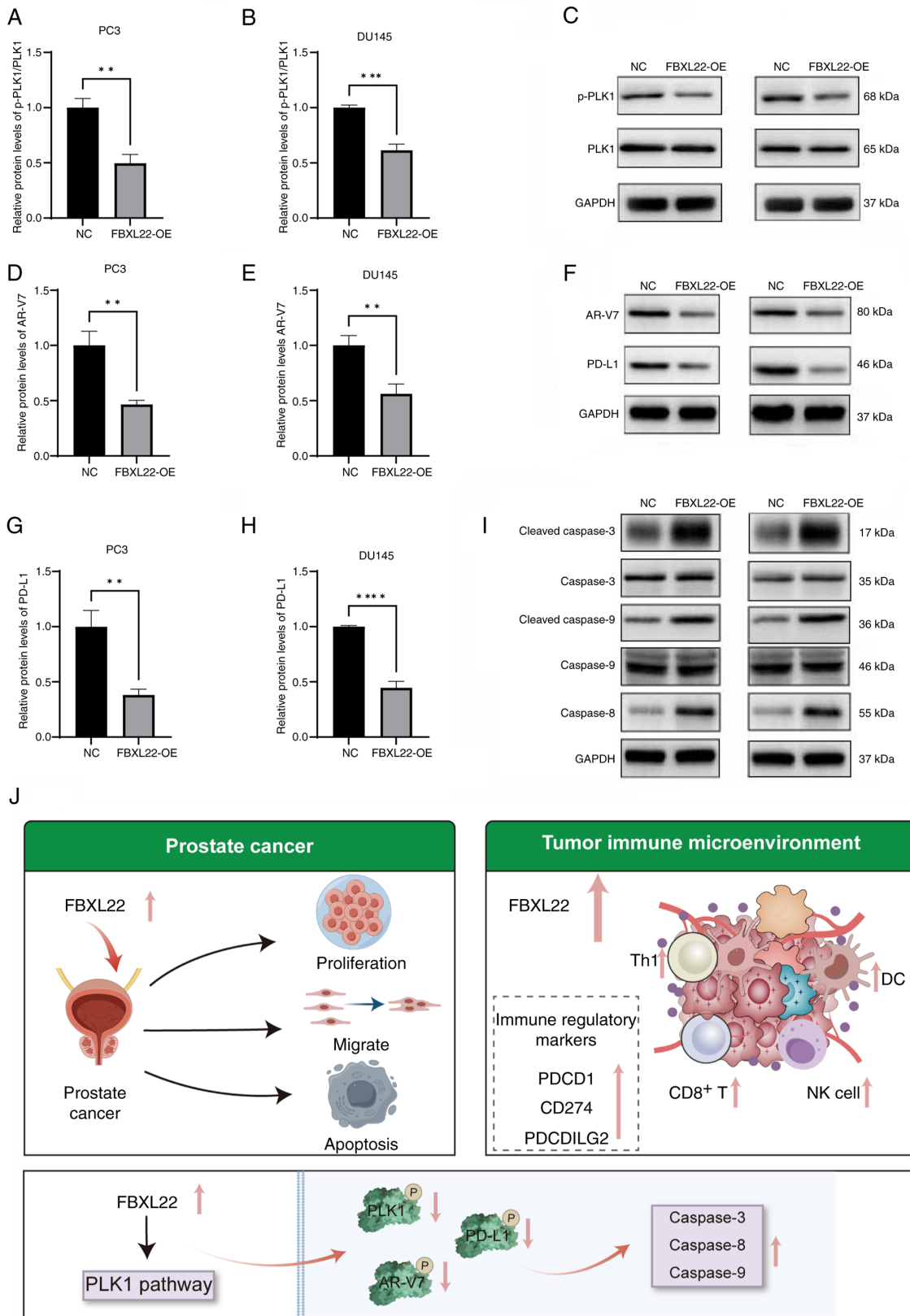


Figure 9. Possible mechanism underlying the impact of FBXL22 on PCa cell function. Western blot analysis demonstrated the relative levels of p-PLK1 and PLK1 in (A) PC3 and (B) DU145 cells transfected with FBXL22-OE compared with the NC group. (C) Representative western blot images for p-PLK1, PLK1 and GAPDH in PC3 and DU145 cells. Western blot analysis indicated the relative levels of AR-V7 in (D) PC3 and (E) DU145 cells. (F) Representative western blot images for AR-V7, PD-L1 and GAPDH in PC3 and DU145 cells. Western blot analysis indicated the relative levels of PD-L1 in (G) PC3 and (H) DU145 cells. (I) Representative western blot images for cleaved-caspase-3, caspase-3, cleaved-caspase-9, caspase-9, caspase-8 and GAPDH in PC3 and DU145 cells. (J) Mechanism by which FBXL22 functions in PCa cells. Error bars represent standard deviation. The data indicates that FBXL22 overexpression reduces p-PLK1, AR-V7 and PD-L1 levels while increasing cleaved caspase-3, -8 and -9 levels, which suggests modulation of the PLK1 pathway and promotion of apoptosis. ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$. PCa, prostate cancer; FBXL22, F-box and leucine rich repeat protein 22; OE, overexpression; NC, negative control; PLK1, polo-like kinase 1; AR-V7, androgen receptor splice variant 7; PD-L1, programmed death-ligand 1; PDCD1, programmed cell death 1; PDCDILG2, programmed cell death 1 Ligand 2.

results suggested that FBXL22 serves a tumor-suppressive role in PCa by modulating specific signaling pathways.

Inhibition of malignant behavior of PCa cells by FBXL22. Analysis revealed that FBXL22 expression was significantly decreased in PC3 and DU145 cells compared with normal prostate epithelial cells (RWPE-1; Fig. 8A). Compared with NC cells, FBXL22 overexpression markedly increased both mRNA and protein levels of FBXL22 in PC3 and DU145 cells (Fig. 8B-G). CCK-8 assays revealed that FBXL22 overexpression significantly reduced the cell viability of PC3 and DU145 cells (Fig. 8H and I). Transwell assays indicated that cell invasion was significantly decreased in FBXL22-overexpressing cells compared with NC cells (Fig. 8J-M). Wound healing assays demonstrated that FBXL22 overexpression substantially inhibited cell migration (Fig. 8N-Q). Flow cytometric analysis revealed that FBXL22 overexpression significantly increased the apoptosis rate in PC3 and DU145 cell lines (Fig. 8R-U). Co-culture experiments with NK cells revealed enhanced migration and cytotoxic activity of NK cells against FBXL22-overexpressing PCa cells (Fig. 8V and W). Co-IP experiments confirmed the interaction between FBXL22 and PLK1 (Fig. 8X and Y). Collectively, these findings indicate that FBXL22 overexpression suppresses proliferation, invasion and migration of PCa cells while promoting apoptosis and enhancing immune cell activity.

Mechanism of FBXL22 in PCa. FBXL22 upregulation resulted in reduced levels of phosphorylated PLK1, AR-V7 and PD-L1 ($P < 0.01$), as well as increased levels of cleaved caspases-3, -8 and -9 ($P < 0.01$) (Figs. 9A-I, S10). These findings suggest that the modulation of the PLK1 pathway may be a key mechanism by which FBXL22 exerts tumor-suppressive effects in PCa. These results provide a basis for the further exploration of FBXL22 as a therapeutic target in PCa.

Discussion

The present study results demonstrated that FBXL22 upregulation suppresses cell viability, invasion and metastasis while promoting apoptosis, potentially through the modulation of the PLK1 pathway. Our comprehensive pan-cancer analysis of FBXL22 revealed its role as a circadian rhythm-regulated immune biomarker with notable implications for cancer prognosis and therapy. These findings indicate that FBXL22 is predominantly downregulated across a multitude of cancer types and may serve as a prognostic and diagnostic marker in specific cancer types, notably in PCa. This downregulation was associated with poor OS in certain cancer types such as BLCA and STAD, which highlights the potential utility of FBXL22 as a prognostic biomarker (5-7).

Pan-cancer analysis of FBXL22 expression in the present study revealed a complex and heterogeneous pattern. Notably, FBXL22 was predominantly downregulated in most cancer types compared with the corresponding normal tissues from the GTEx database. However, significant overexpression was detected in CHOL and LAML. This heterogeneity implies that FBXL22 may exert diverse functions in different cancer contexts, which highlights the importance of considering

tissue-specific factors when interpreting the role of FBXL22 in cancer. In cancer types such as BLCA and STAD, elevated FBXL22 expression was associated with poor prognosis, which appears to contradict its potential tumor-suppressive role, as suggested by its low expression in other cancer types. To reconcile this apparent discrepancy, we hypothesized that the role of FBXL22 may be intricately associated with the specific molecular landscape and TME of each cancer type. For instance, in BLCA and STAD, FBXL22 overexpression might be associated with the activation of alternative signaling pathways that drive tumor progression, such as the PLK1 pathway, which has been modulated by FBXL22 in PCa. In addition, tissue-specific factors and genetic alterations can differentially influence the function of FBXL22. In CHOL and LAML, the overexpression of FBXL22 might reflect its involvement in distinct pathophysiological processes relevant to these cancer types. Further research is warranted to delineate the specific mechanisms by which FBXL22 contributes to tumorigenesis in a cancer-specific manner, including functional studies in CHOL and LAML models, as well as a more detailed analysis of the molecular context in which FBXL22 exerts its effects.

The present study results demonstrated a positive correlation between high FBXL22 expression and immune checkpoint genes such as PD-L1 and CTLA4, which suggests a potential role for FBXL22 in immune suppression. However, high FBXL22 expression was also associated with an improved response to immunotherapy, which creates an apparent paradox. To address this contradiction, several hypotheses were proposed that warrant further investigation. First, the relationship between FBXL22 and immune checkpoint genes may be context dependent. In some cancer types, FBXL22-induced immune checkpoint expression may represent a feedback mechanism that limits excessive immune activation, thereby maintaining immune homeostasis while still permitting an overall immune-active environment conducive to the immunotherapy response. This hypothesis was supported by the observed co-expression patterns of FBXL22 with both inhibitory and stimulatory immune checkpoint genes across multiple cancer types, which suggests complex modulation of immune responses. Second, the expression levels of immune checkpoint genes, while correlated with FBXL22, may not solely determine the therapeutic outcome but rather reflect a broader immune landscape. High FBXL22 expression was also associated with increased infiltration of antitumor immune cells, such as NK and CD8⁺ T cells, which could outweigh the potential immunosuppressive effects of checkpoint molecules, leading to enhanced immunotherapy responses. Third, the TMB and the spectrum of antigenic mutations present in tumors with high FBXL22 expression may enhance the efficacy of immune checkpoint blockade therapy, even in the presence of elevated checkpoint expression. This could be particularly relevant in cancer types in which FBXL22 expression is positively correlated with TMB, such as certain subtypes of bladder and stomach cancer types. To fully understand these interactions, future studies should incorporate *in vivo* models to evaluate the direct impact of FBXL22 on immune cell function within the TME and investigate how FBXL22 modulates the balance between immune activation and suppression. Additionally, analyzing the correlation among FBXL22 expression, immune checkpoint usage and clinical outcomes across diverse cancer

types and immunotherapy regimens could provide further insights into the role of FBXL22 as a biomarker of treatment response.

The mechanisms underlying the role of FBXL22 in cancer appear to be multifaceted and involve circadian rhythm regulation, ubiquitin-mediated proteolysis, focal adhesion signaling and cGMP-PKG signaling (18). The present analysis also revealed strong correlations between FBXL22 expression and immune checkpoint genes, which suggests their involvement in the TME (19). This is further supported by the observation that FBXL22 expression is associated with the infiltration of CAFs and ECs, which may affect immunotherapy outcomes (20,21).

In PCa, the present study provided evidence that FBXL22 upregulation suppresses cell viability, invasion and metastasis while promoting apoptosis, potentially by modulating the PLK1 pathway (22). This suggests that FBXL22 is a promising target for therapeutic interventions in PCa.

The additional experiments further elucidate the role of FBXL22 in PCa and the TME. The co-culture experiments with NK cells provide evidence that FBXL22 overexpression enhances immune cell activity, reinforcing its potential as an immunomodulatory hub. This is supported by the observed increase in NK cell migration and cytotoxicity, which suggests that FBXL22 may promote antitumor immune responses by modulating the TME. Furthermore, the Co-IP validation of the FBXL22-PLK1 interaction strengthens the mechanistic basis for the tumor-suppressive effects of FBXL22, likely mediated through PLK1 pathway modulation. These findings collectively enhance current understanding of the dual role of FBXL22 in regulating cancer cell behavior and immune cell function, which underscores its therapeutic potential in PCa.

The correlation between FBXL22 expression and TMB, microsatellite instability (MSI) and HRD across various cancer types further underscores its potential as a predictive biomarker of therapeutic response (23). The findings of the present study indicate that FBXL22 can serve as a biomarker to guide individualized cancer treatment decisions, particularly in the context of immunotherapy and targeted therapies (24).

TME, a complex network of cells and molecules that surrounds a tumor, serves a key role in tumor biology (25). Based on TCGA data, the present analysis suggested that FBXL22 was positively correlated with the presence of CAFs and ECs in tumors (26). This correlation, along with the notable associations between FBXL22 expression and genes associated with immune checkpoint pathways and immunomodulation across multiple cancer types, suggests that FBXL22 may function as an oncogene involved in regulating the TME to promote an active state or inflammatory response conducive to a robust response to immune checkpoint inhibitor drugs (27).

Enrichment analysis integrating information about FBXL22-binding protein genes and FBXL22 expression-related genes across all tumor types revealed associations with processes such as Skp1-Cullin-F-box-dependent, proteasomal, ubiquitin-dependent, protein catabolic processes and muscle contraction (28). KEGG analysis suggested that the pan-cancer role of FBXL22 may involve pathways, such as the circadian rhythm, ubiquitin-mediated proteolysis, cGMP-PKG signaling pathway and focal adhesion, which are closely associated with tumor occurrence and development (29).

Therefore, to the best of our knowledge, the present study provided the first comprehensive pan-cancer analysis of the circadian rhythm gene FBXL22 and revealed its close association with clinical prognosis, DNA methylation, immune regulation, immune cell infiltration, TMB and MSI. The findings of the present study suggest that FBXL22 is a promising novel marker of cancer recurrence risk and immuno-infiltration in various tumor types, particularly in PCa. These results contribute to current understanding of the potential role and molecular mechanisms of FBXL22 in tumor development and the TME based on clinical tumor samples (30). Further research is warranted to explore the mechanisms by which FBXL22 affects the development and immune microenvironment of PCa and to validate its potential as a therapeutic target.

In conclusion, the pan-cancer analysis of the circadian rhythm gene FBXL22 revealed close associations between its expression and clinical prognosis, DNA methylation, immune regulation, immune cell infiltration, TMB and MSI. Furthermore, preliminary investigations were performed into the mechanisms underlying the potential ability of FBXL22 to inhibit PCa and enhance the efficacy of immunotherapy. The aforementioned comprehensive multi-omics analysis demonstrated that FBXL22 has notable potential as a novel marker for cancer recurrence risk and immuno-infiltration in various tumor types, particularly PCa. These results contribute to current understanding of the potential role and molecular mechanisms of FBXL22 in tumor development and TIM, based on clinical tumor samples.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

JL, WL and YS supervised the project and designed the present study. JL, MF and JZ performed the data analysis and prepared all the figures and tables. MF, SY and JZ performed the experiments. JL and SY drafted the manuscript. WL and YS revised the manuscript. JL obtained funding for the study. All authors read and approved the final version of the manuscript. JL and WL confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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