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KIF20A Facilitates Prostate Cancer Proliferation and Docetaxel Resistance via Enhancing USP15-Dependent K63-linked deubiquitination of G3BP1

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Article

Keywords: Prostate cancer, KIF20A, β-catenin, chemotherapy resistance, Deubiquitination

Posted Date: March 11th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-8788398/v1

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Additional Declarations: (Not answered)

Abstract

Although our previous study implied that kinesin family member 20A (KIF20A) is frequently up-regulated in prostate cancer (PCa) and links to poor survival, the roles and mechanism remain largely poor understood. In this study, we further reported that KIF20A overexpressed in PCa and docetaxel resistance cells, and predicted unfavorable prognosis. Moreover, KIF20A accelerated the PCa cells proliferation and conferred resistance to docetaxel in vitro and in vivo. A mechanistic study revealed that KIF20A promotes PCa progression by activating β-catenin pathway. Furthermore, KIF20A physically interacted with USP15, a crucial deubiquitinating enzyme to catalyze K-63 linked deubiquitination of K393 and K495 lysine resident of G3BP1, thus up-regulated G3BP1 activated β-catenin subsequently. Altogether, our study revealed the clinical significance of KIF20A in PCa, and implied that targeting KIF20A might be a promising strategy to delay the PCa proliferation and overcome docetaxel resistance.

INTRODUCTION

Prostate cancer (PCa) is one of the most frequently diagnosed solid tumors of the male urogenital system worldwide[1]. Despite ongoing advances in PCa therapies, approximately 15–35% of patients experienced relapse and distance metastasis within 5–10 years due to the characteristics of aggressive growth, ultimately resulting in compromised efficacy of current therapeutic strategies and unfavored prognosis[2, 3]. Systemic chemotherapy based on docetaxel is considered as first-line chemotherapy agent for the PCa treatment, especially for the advanced stage and late diagnosis patients[4]. However, acquired resistance was inevitably and had become the major obstacle to for PCa patients' prognosis, with a low overall 5-year survival rate[5, 6]. Therefore, in order to provide a theoretical basis for improving our understanding of PCa progression and chemotherapy resistance, it is necessary to identify essential regulatory factors and describe the molecular mechanisms.

Deubiquitination is partially responsible for cancer metastasis, immune invasion, chemoresistance as well as abnormal metabolism in the manner of maintaining proteins homeostasis through catalyzing the removal of ubiquitin molecules from lysine (K) residues[7–9]. Briefly, the complexity of substrate in tumor functionality determines the dual role of deubiquitination. Hence, precise targeting of this natural protein degradation mechanism to stably tumor suppressor proteins or promote the degradation of oncogenic substrates has provided new option for tumor treatment[10]. Ubiquitin-specific protease 15 (USP15) belongs to the largest subfamily of cysteine protease deubiquitinases[11]. It has been reported that USP15 interacted and deubiquitinated of BECN1 to attenuate autophagy for the inhibition of lung cancer progression, whereas increased cancer cells sensitivity to PARP or gemcitabine inhibitors in pancreatic cancer and triple-negative breast cancer[12–14]. It is only recognized that USP15 antagonizes the effect of Nedd4 on IRS-2 in PC-3 cell[15]. Despite that, there are rare literature about the function and molecular mechanisms of USP15 in the process of PCa progression, which needs explored urgently.

Kinesin superfamily (KIFs) contains 45 members that carry out multi-functions in cargo transport, microtubule dynamics and cell division[16]. In terms of tumorigenesis, numerous recent researches have made significant advances in understanding how KIFs usually regarded as scaffold or adaptor in forming functional protein complex[17]. Particularly, we and other laboratory's previous researches report that KIF20A is highly conserved and widely irregular expressed in solid tumors, such as hepatic cancer[18], ovarian cancer[19], esophageal cancer as well as prostate cancer[20, 21], which linked to chemotherapy resistance, metastasis and poor prognosis. Fibrosarcoma patients with high KIF20A expression had a shorter lifespan[22]. Targeting KIF20A rendered cancer cells more sensitive to taxane in breast cancer[23], which raising the possibility of targeting KIF20A as a promising chemotherapeutic strategy. A phase I/II clinical trial led by Yamaue et al also concluded that patients vaccinated with KIF20A-66 peptide had better prognosis in advanced pancreatic cancer[24]. Although these results collectively demonstrated that KIF20A might be an ideal antigen in treatment and diagnosis, they failed to elucidate the specific molecular mechanism of how KIF20A was involved in PCa progression.

In present study, we successfully identified KIF20A as an intriguing tumor driver, that promotes cell growth and chemotherapy resistance in PCa by regulating post-translation modification. Mechanistically, KIF20A recruited USP15 to deubiquitylate G3BP1, moreover, accumulating G3BP1 subsequently activating canonical β-catenin pathway, resulting in the cell proliferation and docetaxel resistance of PCa. These findings uncover a novel mechanism of KIF20A in the context of deubiquitination of G3BP1, highlighting that KIF20A might be a therapeutic target in PCa progression.

MATERIALS AND METHODS

Cell lines and cell culture

Human prostate cancer cells (LNCaP, C4-2, 22Rv1, PC-3 and DU145) and HEK-293T were purchased from ATCC and the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). All cell lines were mycoplasma-free and were authenticated by short tandem repeats (STR) DNA profiling analysis. Cells cultured in Dulbecco’s modified Eagle’s medium (DMEM) or RPMI-1640 supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37°C with 5% CO_2.

Reagents

The information of antibodies for western blot were purchased from Proteintech (Wuhan, China) or Cell Signaling Technology (CST, USA), and the information were listed as follows: anti-GAPDH (1:1000, 10494-1-AP), anti-H3 (1:1000, 17168-1-AP), anti-KIF20A (1:1000, 15911-1-AP), anti-G3BP1 (1:1000, 13057-2-AP), anti-β-catenin (1:1000, 51067-2-AP), anti-USP15 (1:1000, 14354-1-AP), anti-Myc (1:1000, 16286-1-AP), anti-HA (1:1000, 51064-2-AP), anti-Flag (1:1000, 14793T), anti-His (1:1000, 66005-1-Ig), anti-phospho-GSK-3β (1:1000, 67558-1-Ig), anti-c-Myc (1:1000, 10828-1-AP), anti-RHOA (1:1000, 10749-1-AP), anti-PRKCB (1:1000, 68620-1-Ig), anti-ubiquitination (1:1000, 12919-1-AP), anti-cleaved PARP

(1:1000, 9541), anti-cleaved caspase-9 (1:1000, 9509). Docetaxel (HY-B0011), Wnt/β-catenin inhibitor β-catenin-IN-8 (HY-169348) and G3BP1 inhibitor FAZ-3532 (HY-162288) were purchased from MCE.

Immunohistochemistry (IHC)

Subcutaneous tumors were fixed with formalin and then embedded in paraffin. For HE staining, haematoxylin and eosin were used. For the IHC assay, the antibodies were listed as follows: KIF20A (1:200, Proteintech, Wuhan, China), β-catenin (1:200, Service Bio, Wuhan, China) and Ki-67 (1:100, Service Bio, Wuhan, China). All images were captured by a Leica DM2000 microscope (Leica Camera AG). The calculation of the final IHC score is multiply by score of area of staining and score the intensity of staining. The score area of staining ranged from 0 (< 10%), 1 (10–25%), 2 (26–50%), 3 (51–75%) and 4 (> 75%) based on the number of positive cells, and the intensity of staining was scored as 0 (absence of staining), 1 (mild staining), 2 (moderate staining), and 3 (intense staining).

Western blot

Cells were cultured and lysed with RIPA lysis buffer (Beyotime). The concentration of samples was measured by BCA kit according to the protocol. The subsequent steps were performed as previously described[25].

Immunoprecipitation

PCa cells or HEK 293T cells transfected with accordingly plasmid were lysed in IP lysis buffer with proteinase cocktail on the ice for 20 min, centrifuged at 12000 rpm for 20 min at 4℃. Transferred the supernatant to a new tube, added 2 μg of primary antibody or IgG negative control antibody and incubate overnight at 4°C. The lysate mixed with Magnetic Beads (Beyotime, Shanghai, China) and rotated in room temperature for 2 h, the lysate boiled with SDS-loading buffer for 10 min to obtain the protein complexes and analyzed for subsequent experiments.

Mass spectrometry (MS) analysis

KIF20A overexpression PC-3 cell were lysed and co-IP assay by using anti-KIF20A antibody was performed. After confirmed by western blot which KIF20A has been successfully enriched. The purified products were analyzed by LC/MS (Wininnovate bio, Shenzhen, China) subsequently.

Apoptosis assay

Cells digested and harvested, centrifuged with condition medium and washed with pre-cooled 1×PBS for three times. Re-suspended cells with 1× binding buffer for 500 uL/tube, staining cells with 5uL

Annexin/V and 5uL PI for each tube at room temperature in the dark. Detecting cell apoptosis with CytoExpert and analysed apoptosis rate with FlowJo.

Mouse tumor xenograft model

Subcutaneous xenograft models were generated as previously described[25]. Stability knock down PC-3 cells harvested and counted, then 2×10^6 PC-3 cells were subcutaneously injected into mice (Balb/c mice, male, 4 weeks old, 5 mice per group, were blindly and randomly grouped). The volumes of tumors and weight of mice were recorded 1 week after injection. All mice were sacrificed at indicated time to obtain subcutaneous tumors. Samples were fixed in 37% formalin and embedded in paraffin for subsequent histological analysis. For docetaxel treatment, after 7 days of injection, sterile 1× PBS or docetaxel (10 mg/kg) was injected into the mice every 3 days. All the mice were sacrificed at the indicated time and the analysis of results were described above.Animal experiments in this research were approved by the Animal Ethics Committee of Zhujiang Hospital of Southern Medical University and conducted in the Animal Experiment Centre of Zhujiang Hospital (Southern Medical University, Guangzhou, China). Balb/c mice were purchased from Guangdong Animal Center.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 8.0 (GraphPad Software, Inc, San Diego, CA, USA). All experiments were performed in three times, and the results are presented as the means ± SD. Two-tailed Student's t-test and one- or two-way analysis of variance were used to analyze statistical significance as appropriate normally distributed data. Survival analysis and Cox regression analysis were analyzed with the Kaplan–Meier method and evaluated by the log-rank test performed by R (3.6.3 version, www.r-project.org). $P < 0.05$ was considered

Supplementary methods

CCK-8, colony formation, plasmid construction and transfection, ubiquitination assay and qRT-PCR are described in Supplementary file 1.

RESULTS

KIF20A is up-regulated in PCa and indicates poor survival

Although our previous findings have demonstrated that aberrant of KIF20A in PCa patients might be a crucial factor in judging patients' prognosis, suggesting KIF20A maybe involved in PCa progression[26], the precise role of KIF20A in PCa progression and the underlying mechanism in depth remains largely poor understood. Accordingly, the primary objective of this study was to investigate the specific mechanism by which KIF20A accelerates PCa progression.

To ascertain the role of KIF20A in PCa progression, we first detect the KIF20A mRNA expression through the bioinformatic analysis on several independent TCGA or GEO bulk patients' cohort gene expression profile, the KIF20A transcription level was notably raised up in PCa patients' tissue compared with normal tissues (Fig. 1A-D). Meanwhile, in the KIF20A high expression populations, the patients likely to have a worse gleason score (GS ≤ 9), except the GS = 10 (Fig. S1A-C).The further bio-informatics analysis also indicated that KIF20A was an independent risk factor for PCa, and the patients with higher KIF20A expression suffered from a worse biochemical recurrence survival (BCR), yet with no significant change in overall survival (OS)(Fig. 1E-H, Fig. S1E). Receiver operating characteristic curve (ROC) implied KIF20A might be a crucial diagnosed factor (Fig. 1I). For the purpose of exploring the expression of KIF20A in clinical PCa patients' tissues, we found that KIF20A was higher in PCa tissues (Fig. 1J-K), as well as higher in docetaxel resistance tissues (Fig. 1L). All these results indicated that high KIF20A in PCa tissues may result in PCa progression and suggested patients' poor survival.

KIF20A facilitates PCa growth and resistance to docetaxel in vitro

Given that KIF20A was up-regulated in prostate cancer and docetaxel resistance cells. We assumed that ablation of KIF20A might exert the anti-tumor capacity in PCa progression. The KIF20A expression was interfered by transfecting PCa cells with two altered siRNA sequences against KIF20A or overexpression plasmid, then lenti-virus has been constructed subsequently to establish stably KIF20A knock down cells (shNC, shKIF20A-1# and shKIF20A-2#) and overexpression cells (Vector and KIF20A), and the interfering efficiency was detected by qRT-PCR and immunoblot (Fig. 2A-D). A series of functional assay in vitro were carried out for evaluating PCa proliferation ability and tolerance to docetaxel treatment, our results evidently showed that KIF20A knock down significantly inhibited the growth of PCa cells (Fig. 2E-F), while the overexpression of KIF20A remarkably accelerated the proliferation of PCa cells (Fig. 2G-H). Similarity, the oncogenic role of KIF20A also proved by the colony formation assay (Fig. 2I-L). The apoptosis rate was also analyzed and the results showed that KIF20A knock down increased the apoptosis rate in PC-3 and DU145 cells (Fig. 2M-N, Fig. S2A-D). Considering the up-regulated of KIF20A in PC-3 docetaxel resistance cells (PC-3-DR) compared with parental cells, we next attempt to study whether KIF20A regulates PCa cells to docetaxel resistance. After confirming the IC_{50} value in docetaxel-resistant PCa cell line (PC-3-DR) was remarkable arose (Fig. S2E), then we knock down KIF20A expression and detected the IC_{50} value and cell viability, the results found that KIF20A overexpression promoted the resistance of PC-3 parental cells to docetaxel and proliferation, while KIF20A knock down compromised the resistance of PC-3-DR cells to docetaxel and proliferation (Fig. S2F-H). Western blot further detected the markers of apoptosis and found that cleaved-caspase 9 and cleaved- PARP increased when KIF20A inhibited (Fig. S2I). Taken together, our results indicated that inhibition of KIF20A delayed PCa cell growth and attenuated the capacity of resistance to docetaxel.

KIF20A accelerates PCa progression in mice

Previous results have confirmed the oncogenic roles in vitro. We next explored the potential tumorigenic ability of KIF20A in vivo. The subcutaneous tumor xenograft model was constructed by implanting stable KIF20A knock down prostate cancer cells (shNC, shKIF20A-1#) in Balb/c mice, then the volume of transplanted tumors, weight of mice and survival were regularly recorded as schedule (Fig. 3A). Consistent with in vitro findings, the mice with implanting KIF20A stably silencing cells had a smaller subcutaneous tumor, with no significantly change of weight of mice compared with negative control group (Fig. 3B-D, Fig. S3A). Meanwhile, we also found that up-regulated KIF20A in PC3-DR cells remarkably accelerated the growth of tumor (Fig. 3E-G, Fig. S3B). Furthermore, the immunohistochemistry (IHC) staining was performed to analyse the subcutaneous tumor, and results suggested that KIF20A inhibition dramatically reduced the number of Ki-67 positive cells (Fig. 3H, J, Fig. S3C-D), while increased in KIF20A overexpressed subcutaneous tumor (Fig. 3I-J, Fig. S3E-F). These findings indicated that KIF20A promoted PCa cells growth and conferred PCa cells resistance to docetaxel in mice.

KIF20A confers PCa progression by activating β-catenin

To better understand the precise mechanism how increased KIF20A induced PCa progression. The results of GSEA were analyzed, and we found that the hyper-activation of Wnt/β-catenin associated pathway was positive corrected with KIF20A expression, indicating that Wnt/β-catenin might be the downstream target of KIF20A (Fig. 4A-B). Then we hypothesis that KIF20A promotes PCa progression may through activating Wnt/β-catenin pathway. Considering the existence of canonical and non-canonical Wnt/β-catenin in tumor, we selected and detected several representative downstream markers respectively to confirm our hypothesis. After knock down KIF20A in PC-3 and C4-2 cells, we found that the mRNA expression of c-Myc, the crucial marker of canonical Wnt/β-catenin pathway, decreased significantly, indicated that KIF20A may blost PCa progression via canonical β-catenin/c-Myc pathway (Fig. 4C). Hence, we selected the canonical β-catenin pathway for further exploration. After KIF20A knock down or overexpression in PC-3 and C4-2 cells, the western blot verified that the expression of β-catenin and its downstream factor c-Myc were inhibited or up-regulated respectively, with no change of PRKCB and RHOA (Fig. 4D-E). The nucleus and cytoplasm extraction assay further demonstrated that nuclear translocation of β-catenin increased after transfecting PCa cells with KIF20A overexpression plasmid (Fig. 4F). To further determine the role of β-catenin pathway in KIF20A induced tumor progression. The specific inhibitor of β-catenin (HY-169348) was adopted subsequently. Colony formation assay showed that treating PC-3 cells with HY-169348 (10 μM, 24h) led to the significantly growth inhibition, and this suppression phenomenon partly reversed by KIF20A overexpression (Fig. 4G-H). The apoptosis analysis of flow cytometry also suggested that KIF20A overexpression remarkably protect cells from the suffering of HY-169348 (Fig. 4I). Consistence with the molecular function, the PC-3 and C4-2 cells with ectopically expressed KIF20A partially rescued the inhibition of HY-169348 (Fig. 4J-K). Reanalysis of subcutaneous tumor further suggested that β-catenin was an essential downstream target of KIF20A (Fig. S4A-D). All these results elucidated that KIF20A promoted PCa progress via activating canonical β-catenin pathway.

KIF20A stabilizes G3BP1 to promote PCa progression

To ascertain the possible molecular mechanism involved in KIF20A induced activation of β-catenin/c-Myc pathway in promoting tumor growth and docetaxel resistance. GSEA analysis was re-performed and the results showed that ubiquitination might be the crucial manner which KIF20A participated in (Fig. 5A-B). In order to further identify the candidate protein which interacted with KIF20A, mass spectrometry (MS) analysis was carried out and G3BP1 was successfully identified (Fig. 5C). The analysis of mulitriple gene correction in PCa also implied that KIF20A was positive associated with G3BP1 (Fig. S5A). Based on reports from other researchers, G3BP1 serves as a crucial upstream signal for activating the β-catenin pathway and mediates multiple biological processes in solid tumor. Therefore, we assumed that G3BP1 function as a crucial mediator in KIF20A induced PCa progression. The interaction of KIF20A and G3BP1 in PCa cells was studied subsequently, immunoprecipitation suggested that KIF20A endogenous interacted with G3BP1 in PCa cells (Fig. 5D). Moreover, the Flag-labeled KIF20A plasmid and Myc-labeled G3BP1 plasmids were constructed successfully and co-transfecting in HEK-293T cells, the exogenous combination of Flag-KIF20A and Myc-G3BP1 can also be observed (Fig. 5E). To investigate the combination of detailed protein domain, we analyzed the protein structure of KIF20A and three basic domain, N-terminal domain (NTD), Helical domain (HD) and C-terminal domain (CTD) were found totally, then a series of GFP-Flag-labeled trunk plasmid were constructed respectively (Fig. 5F). After co-transfecting HEK-293T cells with GFP-Flag-labeled trunk plasmid and Myc-labeled G3BP1 plasmid, immunoprecipitation analysis was performed and we found that helical domain of KIF20A interacted with G3BP1 (Fig. 5G).

Considering the oncogenic role of G3BP1 in various solid tumor reported by other researchers, the role of G3BP1 in PCa was also investigated. Combination G3BP1 expression with survival, we found that higher G3BP1 expression indicated the poor prognosis in PCa patients (Fig. S5B). Importantly, G3BP1 knock down augmented the apoptosis rate (Fig. S5C-E) and blocked the proliferation of PCa cells (Fig. S5F-G). To explore the detail mechanism in this process, the regulation of KIF20A and G3BP1 in PCa was investigated and we observed that KIF20A up-regulated G3BP1 protein abundance but not effect transcription level through immunoblot and qRT-PCR (Fig. S5H-I). Base on above findings, we hypothesis that KIF20A probably mediated the PCa progression via regulating G3BP1 protein stability. Hence, proteasome inhibitor (MG132) and lysosomal inhibitors (CQ and NH_4Cl) were applied respectively, and we noticed that the severely decreased protein abundance of G3BP1 was partly significantly reversed by MG132, but not by the CQ and NH_4Cl (Fig. 5H-K), indicating that ubiquitin-proteasome system induced protein degradation might be the crucial regulator in KIF20A/G3BP1 axis in PCa. For further investigate whether KIF20A regulated the G3BP1 protein stability, cycloheximide (CHX) was used to block the de-novo synthesis for evaluating the half-time of G3BP1 protein. Consistently with previous results, our results implied that the expression of G3BP1 notably decreased at predetermined time after CHX treatment, while KIF20A overexpression partly delayed the degradation rate of G3BP1 (Fig. 5L-O). These data clearly showed that KIF20A stabilized G3BP1 to promote PCa progression.

KIF20A promotes PCa progression via USP15-mediated G3BP1 K63 deubiquitylation

E3 ligand enzyme is the major effector in ubiquitin-proteasome degradation system. However, rarely researches report that KIF20A is a potential E3 ligand enzyme or have relevant catalytic domain, indicating that it may act as an intermediate factor which plays the catalytic role of E3 ligand enzyme. To better clarify the precise molecular mechanism of KIF20A overexpression induced G3BP1 ubiquitination, we reanalysed the results of MS and we found that USP15 might be the potential deubiquitinase (Fig. S6A), and analysis of correction verified the positive association between USP15 and KIF20A or G3BP1 (Fig. S6B-C). To explore the interaction of KIF20A, USP15 and G3BP1, the His-labeled G3BP1 plasmid was generated. The immunoprecipitation assay was adopted by using anti-USP15 antibody or anti-His antibody respectively, the results suggested that USP15 endogenous interacted with KIF20A and G3BP1 in PCa cells (Fig. 6A, B). Next, we tested whether USP15 impaired the stability of G3BP1 serving as a prominent deubiquitinase. As expected, inhibition of USP15 in PC-3 and C4-2 cells reduced the G3BP1 protein level, without significant change of mRNA level (Fig. 6C, D, Fig. S6D). Furthermore, under the treatment of CHX in PC-3 and C4-2, we found that the half-life of the G3BP1 protein was significantly shorter in USP15 knock down cells than control group (Fig. 6E-H). Further investigation confirmed that MG132 treatment abrogated the inhibition of G3BP1 in USP15 knock down cells (Fig. 6I, J). We then determined whether USP15 is necessary for the interaction between KIF20A and G3BP1. Through immunoprecipitation assay we found that inhibition of KIF20A in PC-3 and C4-2 cells markedly diminished the interaction among USP15 and G3BP1 (Fig. 6K, L), indicating that KIF20A is required for this interaction. Additionally, following simultaneous knock down of KIF20A and USP15 in PC-3 and C4-2 cells, the USP15-mediated suppression of G3BP1 expression was obviously eliminated (Fig. 6M, N), indicating that KIF20A served as a scaffold to recruit USP15 for stabilizing G3BP1. To explore whether KIF20A recruited USP15 to mediate the ubiquitination of G3BP1 in PCa cells, we knocked down KIF20A expression and examined the ubiquitination of G3BP1, the results clearly showed that the ubiquitination level of G3BP1 significantly ascended in KIF20A knock down cells which consistent with previous results (Fig. 6O). More importantly, transfecting HEK-293T cells with exogenous His-labeled USP15 overexpression plasmid, we observed that the previously elevated G3BP1 ubiquitination level was strikingly suppressed, suggesting that USP15 is crucial deubiquitinases for the KIF20A mediated G3BP1 ubiquitination (Fig. 6P).

To gain insight into which kind of ubiquitination is important for the KIF20A. Several ubiquitination plasmids (K63, K48 and K11) were transferring into HEK-293T cells respectively, and the results illustrated that only K-63 ubiquitination level of G3BP1 raised significantly in KIF20A knock down cells (Fig. 6Q). Ubiquitination typically occurs on lysine residues. In order to identify the essential lysines residues involved in this ubiquitination process, we analyzed the amino acid sequence of G3BP1 in proteomic database (https://gpsuber.biocuckoo.cn/) to evaluate the conservation of the amino acid, we found that K5 (Score = 0.7339, cutoff = 0.3566), K393 (Score = 0.5919, cutoff = 0.3566) and K453 (Score = 0.6353, cutoff = 0.3566) were highly conserved among various species (Fig.S6F), and further in vivo

ubiquitinatlytion assay proved that K393 and K453 were essential for KIF20A/USP15 mediated G3BP1 deubiquitination (Fig.S6G).

G3BP1 is required for KIF20A induced PCa progression

To investigate the role of G3BP1 played in the progression of PCa, a series of rescue function experiments were conducted. Briefly, colony formation assays performed in PC-3 and C4-2 cells demonstrated that G3PB1 inhibition not only significantly reduced colony formation efficiency in PCa cells lonely but also eliminated the high colony formation mediated by KIF20A overexpression (Fig. 7A, B). Similarly, analogous to parental prostate cancer cells, the resemble biological characteristics of KIF20A/G3BP1 signaling pathway in docetaxel resistant prostate cancer cells was also observed (Fig. 7C, D). Furthermore, this growth reinforcement by KIF20A overexpression was counteracted by G3BP1 knock down in PCa cells (Fig. 7E, F). Subsequently, we further examined the IC_{50} value of docetaxel resistant prostate cancer cells to evaluate the regulatory effect of the KIF20A/G3BP1 signaling pathway. Consistent with previous findings, the IC_{50} value was maintained at a relatively high level in PC-3-DR cells, and KIF20A overexpression PC-3-DR cells (IC_{50}=248.7 μM) displayed higher IC_{50} (Fig. 7G). However, the suppression of G3BP1 lowered the IC_{50} of PC-3-DR cells (IC_{50}=97.06 μM), and partly fallen the value of KIF20A overexpression PC-3-DR cells (IC_{50}=196.5 μM), indicating that G3BP1 was a critical molecule in the KIF20A mediated docetaxel resistance (Fig. 7G). In addition, flow cytometry revealed that FAZ-3532, a specific inhibitor of G3BP1, exhibited noteworthy anti-tumor ability both in PC-3 and PC-3-DR cells (Fig. 7H).

With the intention of elucidating the PCa cells' biological behavior, the immunoblot in PC-3, C4-2 and PC-3-DR cells manifested that the augment of β-catenin pathway mediated by KIF20A overexpression was enormously offset by G3BP1 knock down (Fig. 7I). Taken together, these findings suggest that G3BP1 was an essential intermediary in KIF20A induced cell growth and docetaxel resistance in PCa.

Inhibition of KIF20A sensitized PCa cells to docetaxel in vivo

The findings in vivo preliminary demonstrated the tumor tumorigenic role of KIF20A in PCa proliferation and docetaxel resistance. To further investigate whether combination KIF20A knock down could overcome the docetaxel resistance in PCa. The docetaxel resistance prostate cancer cells were used to constructed the KIF20A knock down model and implanting into mice, then we treated mice with docetaxel (3mg/kg) alone or DMSO respectively. As we expected, docetaxel has a modulate anti-tumor effect, with the slight decreased of tumor size and weight in docetaxel treatment group (shNC+DMSO versus shNC+Docetaxel), but were remarkably decreased in KIF20A knock down cells (shNC+DMSO versus shKIF20A+DMSO). Importantly, KIF20A knock down combination with docetaxel treatment have a more satisfied tumor inhibition (shNC+Docetaxel versus shKIF20A+Docetaxel) (Fig. 8A-C). The analysis of IHC in subcutaneous tumor also indicated that the ratio of Ki-67 positive cells was decreased, as well as the expression of β-catenin (Fig. 8D-G). All these results showed that targeting KIF20A may delay the PCa progression and overcome the docetaxel resistance in PCa (Fig. 8H).

DISCUSSION

Balance of ubiquitination and deubiquitination alters the biological outcomes of proteins, thereby stimulating tumor development in response to various stresses[27]. In present study, we provide evidence to show that KIF20A exhibited high levels of expression in PCa patients and docetaxel resistance tissues, indicating patients' the worse outcome and oncogenic role in contributing to the proliferation and docetaxel resistance of PCa cells. Mechanistically, KIF20A recruited USP15 for the inhibition of K-63 linked polyubiquitylation of G3BP1 and consequently facilitated the activation of β-catenin pathway. Our study not only identified KIF20A serves as a crucial contributor in PCa docetaxel resistance, but also gained insights into the mechanism behind cell growth and docetaxel resistance in PCa patients, potentially uncovering novel targets for PCa treatment.

Previous studies have shown that KIF20A, one of the members of the kinesin superfamily, was frequent abnormally expressed in numerous solid tumors and associated with multiple biological processes of malignant tumors, PCa included[26, 28]. Hence, it has emerged as a potential prognostic marker and specific therapy target in cancers. Recently, studies reported that up-regulated KIF20A by FOXM1 transcription induced G2/M cell cycle arrest of conjunctival melanoma cells for blocking tumor progression[29]. In addition, activated KIF20A in HCC patients correlated with poor prognosis, and not only promoting glycolysis through competitively inhibiting FBXW7-mediated degradation of c-Myc[30], but also encourage distance metastasis and T cell exhaustion via SPP1-CD44 axis[18, 31]. Importantly, although paprotrain serving as KIF20A specific inhibitor pharmacological inhibiting the tumor progression through intervening the autocrine activation of the androgen receptor in human castration-resistant prostate cancer and serving as downstream factor of FOXM1 in regulating chemoresistance have been reported[21], the relationships between KIF20A and docetaxel resistance and downstream target remains unclear. In our study, consistent with previous results, highly KIF20A was corrected with prostate cancer patients' poor prognosis and higher GS score (GS ≤ 9), yet the phenomenon was not observed in GS = 10 possibly due to the insufficient cases. Further work provided reliable experimental evidence to prove that KIF20A could up-regulate G3BP1 protein through recruiting an essential deubiquitinating enzyme USP15 mediated the inhibition of K-63 linked ubiquitination. Particularly, interfering KIF20A significantly increased the ubiquitination level of the lysine residue of G3BP1 at K393 and K453 site and decreased its stability, causing the inactivation of β-catenin pathway to slow down cell proliferation and sensitized PCa cells response to docetaxel. Therefore, our present study elucidated a previously unrecognized function of KIF20A in regulating target ubiquitination to promote PCa progression, thus suggesting that targeting KIF20A mediated G3BP1 ubiquitination is a potential therapeutic strategy for inhibiting PCa growth and overcome docetaxel resistance.

Deubiquitinases (DUBs) is a reversible and dynamic proofreading mechanism to regulate protein homeostasis for tightly governing malignant biological behavior of tumor cells[32]. Typically, under the catalysis of DUBs, the ubiquitin molecules linked to specific lysine residues of the substrate protein were removed[33]. USP15 is reported as a member of the largest subfamily of cysteine protease DUBs, which

has been considered both as an oncogene and a tumor suppressor in different contexts[11, 34]. USP15 deubiquitinated cGAS to promote DNA-induced oligomerization and phase separation in augmenting innate immune response[35], and catalyzed the inhibition K1299-linked monoubiquitin of TET2 to decrease response to immunotherapy[36]. Additionally, liver specific USP15 knock out mice displayed the reduction of NAFLD phenotype and less inflammation through deubiquitinating FABPs and perilipins[37]. Furthermore, targeting USP15 reduced the deubiquitination of substrate impaired stem and progenitor cell reconstitution potential and leukemic cellular redox in AML[38]. However, the role and corresponding mechanism of USP15 in PCa has rarely been reported. In present study, we recognized that USP15 function as a vital DUBs which may exhibit oncogenic role in PCa which consistent with other researches. A serious of in vitro experiments evidently showed that USP15 participated in KIF20A mediated K63 linked deubiquitination of G3BP1, and the detailed modification site were further identified. Our findings identify a novel binding partner for KIF20A and broaden the understanding of deubiquitination of G3BP1 in PCa.

Recently, the role of G3BP1 has been implicated in various biological processes. For instance, G3BP1 was regarded as a core flexible switch for disassembling stress granule by means of assorted manners within the RNA-protein network, such as phase separation, heat shock and autophagy-dependent elimination[39–41]. Likewise, increasing literature also have customarily disclosed its role of tumor promoter. Targeting G3BP1 compromised immune evasion and sensitizes bladder cancer cells to immune checkpoint inhibitors via restricting PI3Ks mRNA translation[42]. Interestingly, a feed-forward manner between AR and G3BP1 led to a greater aggressiveness behaviors of PCa cells[43], revealed that patients with G3BP1 high expression seemly are more susceptible to AR-targeted therapy[44]. Another study similarity illustrated that G3BP1 collaborated with YTHDF3 to promote the expression of AR[45], thereby counteracting the treatment of AR-targeted inhibitors in PCa. Consistent with previous study, we also further confirmed the tumor-promoting role of G3BP1 in PCa. However, previous study failed to explore the role of G3BP1 in the course of chemotherapy resistance in PCa. In this study, we innovatively discovered that G3BP1 also plays a considerable role in docetaxel treatment. G3BP1 knock down suppressed the proliferation capability of cancer cells and also alleviated tolerance cancer cells to docetaxel treatment via blocking β-catenin pathway activation, which deepen our comprehension of G3BP1 in PCa.

To summary, our study provides sufficient evidences to prove that KIF20A functions as a vital oncogene in the context of PCa growth and docetaxel resistance, and as a critical independent factor in judging patients' prognosis. Specifically, KIF20A acting as a scaffold to recruit USP15 to deubiquitinate the K393 and K453 site of G3BP1 and stabilize it, thus facilitating the chemoresistance and growth of PCa cells via β-catenin pathway. Our study provides novel insights into the mechanisms underlying KIF20A overexpression induced uncontrolled growth and docetaxel resistance and identifies potential therapeutic targets for PCa progression.

Declarations

AUTHOR CONTRIBUTIONS STATEMENT

KXG and ABX conceived and designed the study, KXG QWC and ZHT performed the in vitro experiments, KXG interpreted and analysed data. QWC and RLH performed IHC analysis and animal experiments. ZG performed the public data analysis. KXG made the figures and tables. KXG wrote the manuscript, and YMD and ZG reviewed the manuscript. All authors read and approved the final manuscript.

DECLARATION OF COMPETING INTEREST

The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was performed in accordance with the Declaration of Helsinki, and was approved by the Ethical Review Committee of Zhujiang Hospital, Southern Medical University, and the Animal Ethics Committee of Zhujiang Hospital, Southern Medical University.

FUNDING

This work was supported by National Natural Science Foundation of China (No. 82403228), Guangdong Basic and Applied Basic Research Foundation (Grant No. 2023A1515111040).

ACKNOWLEDGEMENT

We sincerely thanks to the Zhujiang Hospital, Southern Medical University for the support of experimental venues and technical platforms.

DATA AVAILABILITY

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Figures

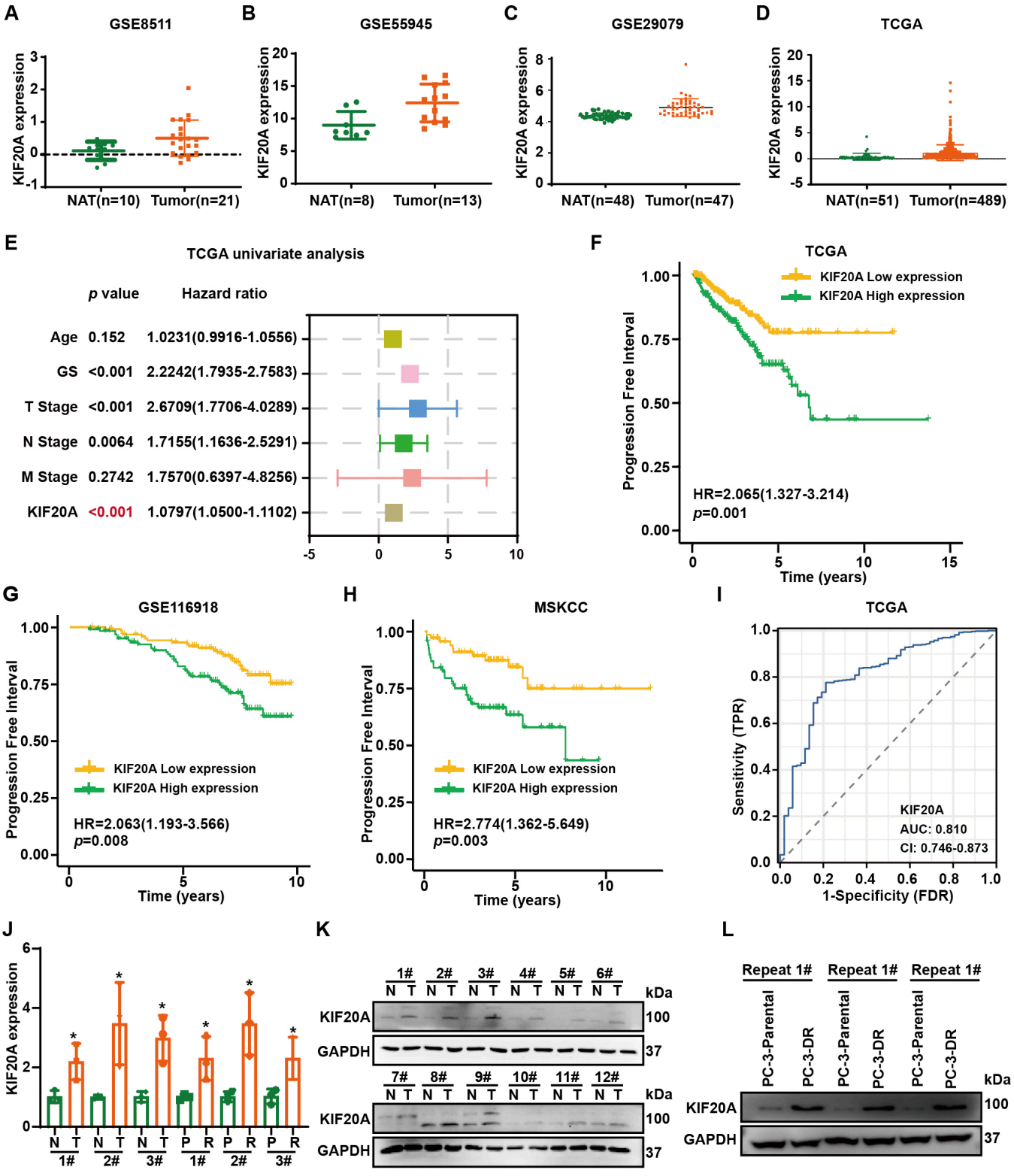


Figure 1

KIF20A is upregulated in PCa and docetaxel resistance tissues and indicates poor survival. A-D KIF20A mRNA expression levels in PCa patients through analyzing public database (GSE8511, GSE55945, GSE29079 and TCGA). **E** Univariate regression analysis derived from TCGA profile was performed to assess the relationship between KIF20A and pathological indicators. **F-H** Kaplan-Meier survival analysis of PCa patients based on the expression of KIF20A to evaluate correlation of KIF20A expression and

biochemical recurrence survival (BCR). **I** Receiver operating characteristic curve (ROC) was used to evaluate the diagnostic effectiveness of the KIF20A in PCa. **J-K** The expression of KIF20A was detected through qRT-PCR (J) and western blot (K) in PCa tissues and docetaxel resistance tissues, N (normal) versus T (tumor), P (parental) versus R (docetaxel resistance), GAPDH used as loading control. **L** the expression of KIF20A was detected through western blot in PC-3 docetaxel resistance cells, GAPDH used as loading control. Data were presented as mean±SD, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

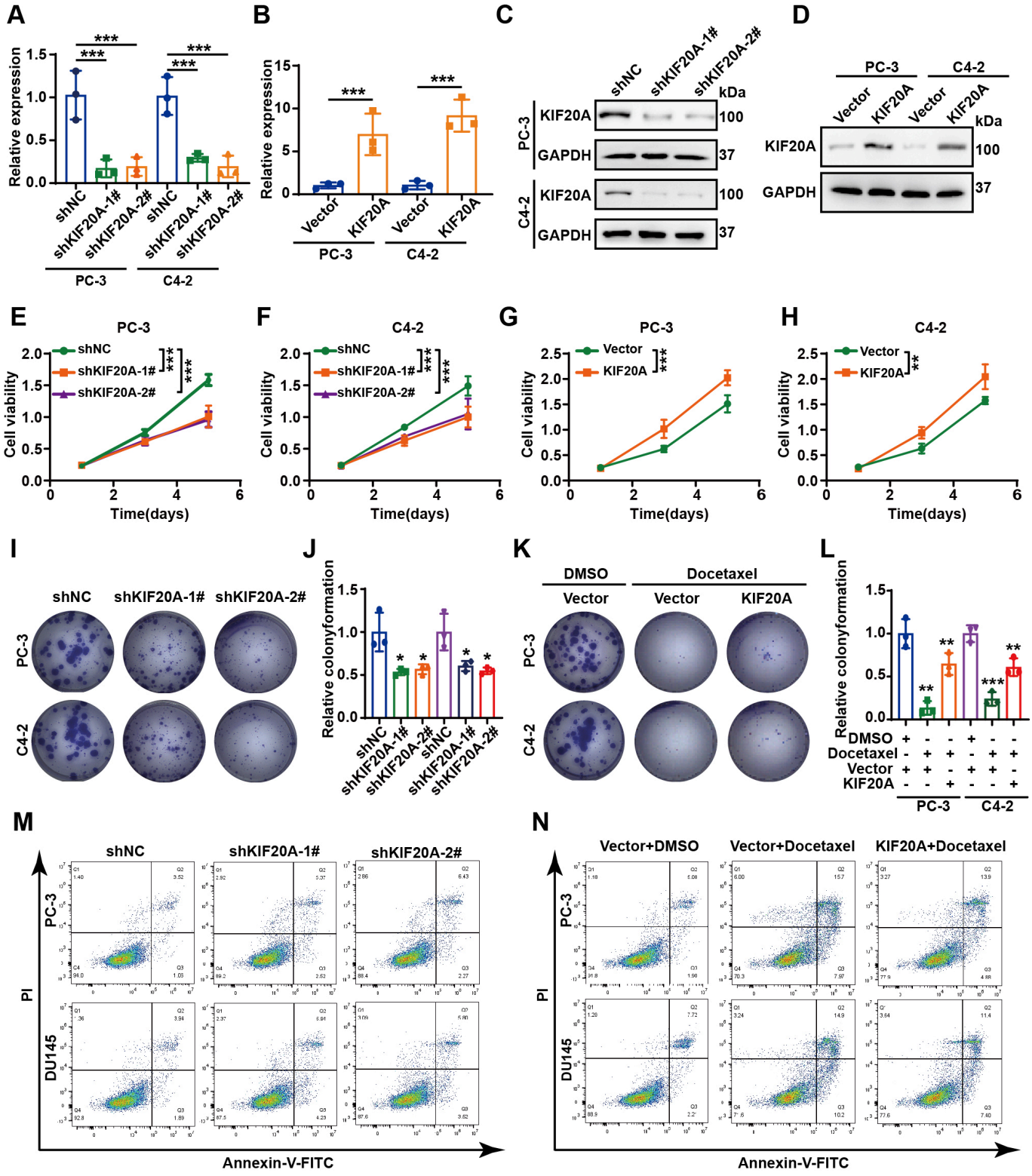


Figure 2

KIF20A promotes the proliferation and doecetaxel resistance of PCa cells in vitro. A-B qRT-PCR was performed to detect the KIF20A mRNA expression in PC-3 and C4-2 cells after KIF20A stable knock down. **C-D**Analysis of KIF20A protein level in PCa knock down and overexpression cells. **E-F**CCK-8 analysis of cell viability in KIF20A knock down cells with KIF20A-specific siRNA (shKIF20A-1#, shKIF20A-2#) or negative control siRNA (shCon). **G-H** CCK-8 analysis of cell viability in PC-3 (G) and C4-2 (H) cells with transfecting KIF20A overexpression plasmid. **I-J** Colony formation assay was conducted to analyze the cell proliferation in KIF20A knock down cells. **K-L** Colony formation assay was conducted to analysis the cell proliferation in KIF20A overexpression cells under the treatment of docetaxel. **M-N** Flow cytometry was used to detect the apoptosis in KIF20A knock down cells or KIF20A overexpression cells. Data were presented as mean±SD, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

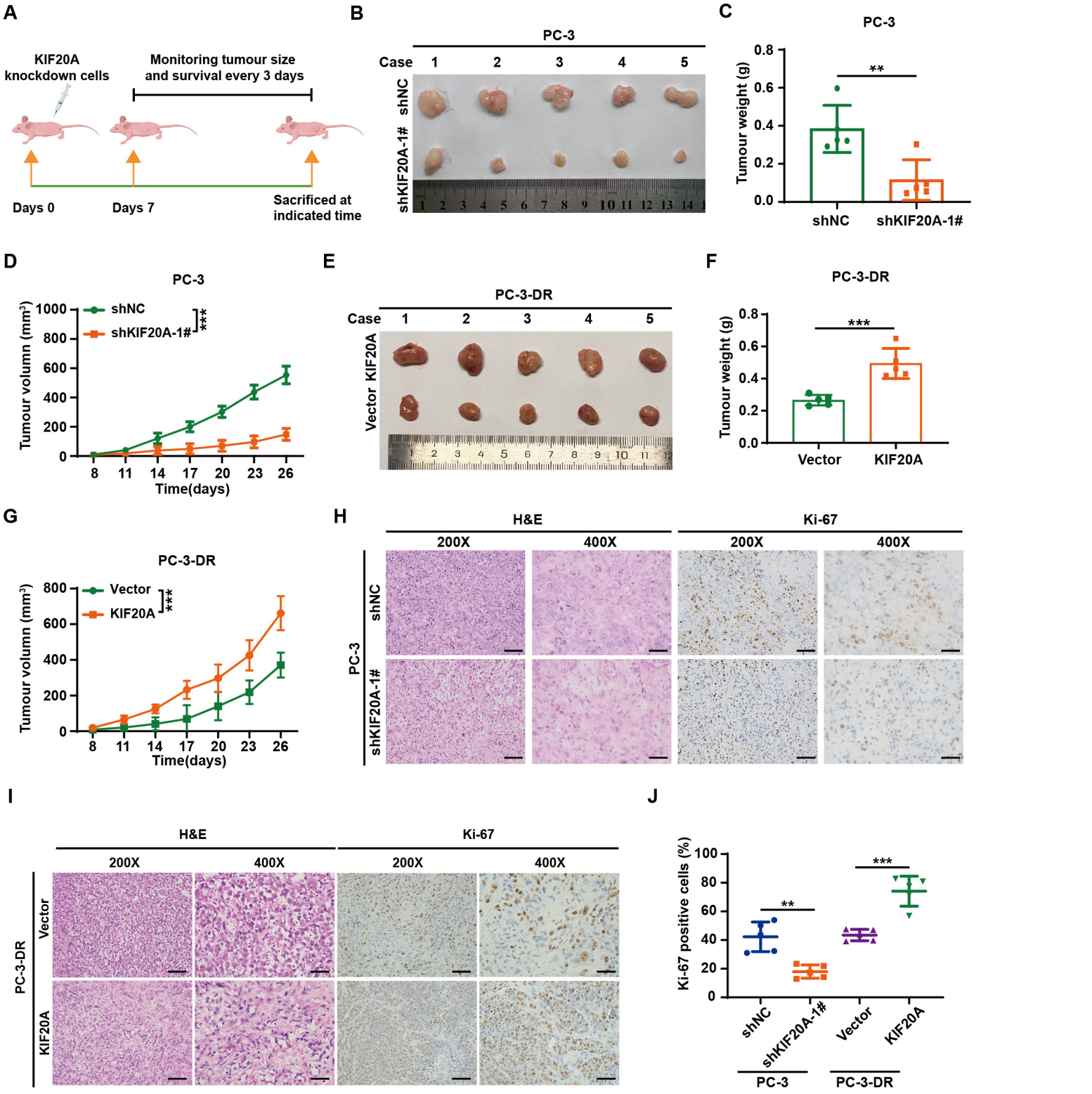


Figure 3

KIF20A promotes the proliferation and doecetaxel resistance of PCa cells in mice. A The schematic diagram of the construction of a subcutaneous tumor model in mice. **B** Gross observation of subcutaneous xenograft tumors derived from mice with injecting KIF20A knkock down PC-3 cells. **C-D** The statistical analysis of weight (C) and volumn (D) of subcutaneous xenograft tumors. **E** Gross observation of subcutaneous xenograft tumors derived from mice with injecting KIF20A overexpression

PC-3 docetacel resistance cells. **F-G** The statistical analysis of weight (F) and volumn (G) of subcutaneous xenograft tumors. **H** Representative images of HE and IHC staining (Ki-67) for PC-3 subcutaneous xenograft tumors at 200×(scale bar: 100 μm) or 400× (scale bar: 50 μm) magnification. **I**Representative images of HE and IHC staining (Ki-67) for PC-3 docetaxel resistance subcutaneous xenograft tumors at 200× (scale bar: 100 μm) or 400× (scale bar: 50 μm) magnification. **J** The statistical analysis of Ki-67 expression in subcutaneous xenograft tumors. Data were presented as mean±SD, **p*< 0.05, ***p* < 0.01, ****p* < 0.001.

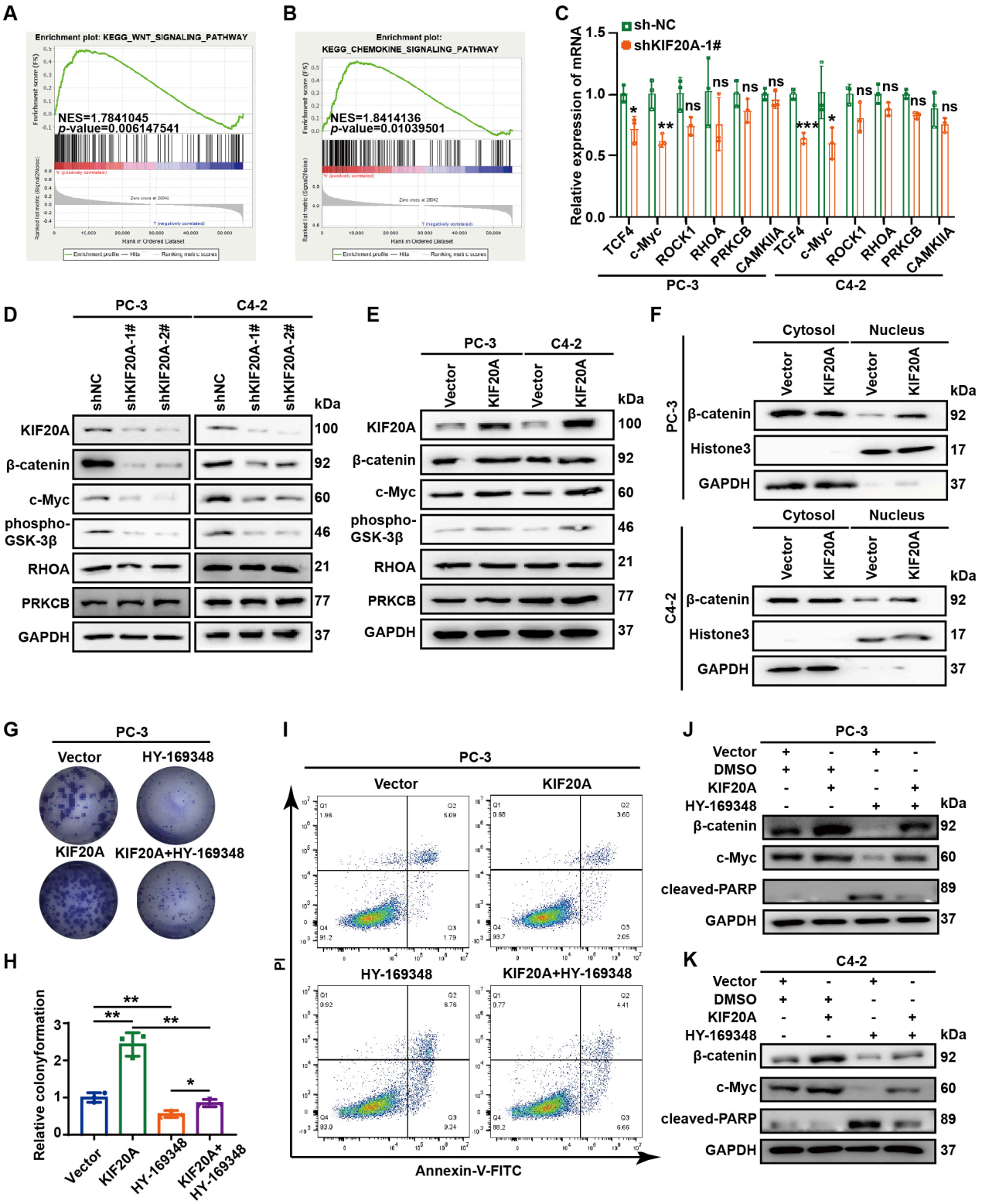


Figure 4

KIF20A activates β-catenin to promote the proliferation and doecetaxel resistance of PCa cells. A-B The GSEA analysis of the TCGA dataset was conducted to plot the positive enrichment of genes associated with Wnt/β-catenin signaling and chemokine signaling. **C**qRT-PCR assay was performed to detect the expression of downstream markers of Wnt pathway. **D-E** Western blot assay was used to detect the activation of canonical wnt/β-catenin pathway in PC-3 and C4-2 cells after KIF20A knock down (D) or overexpression (E). **F** The nucleus and cytoplasm extraction assay was used to detect the nuclear translocation of β-catenin in KIF20A overepxression PC-3 and C4-2 cells. **G-H** The colony formation assay was conducted to analyze the viability of KIF20A overexpression cells with or without β-catenin specfic inhibitos (HY169348). **I** Flow cytometry was used to detect the apoptosis ratio in KIF20A overexpression cells with or without HY169348. **J** Western blot assay was used to analyze the expression of markers of β-catenin pathway and apoptosis in KIF20A overexpression cells with or without HY169348. Data were presented as mean±SD, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

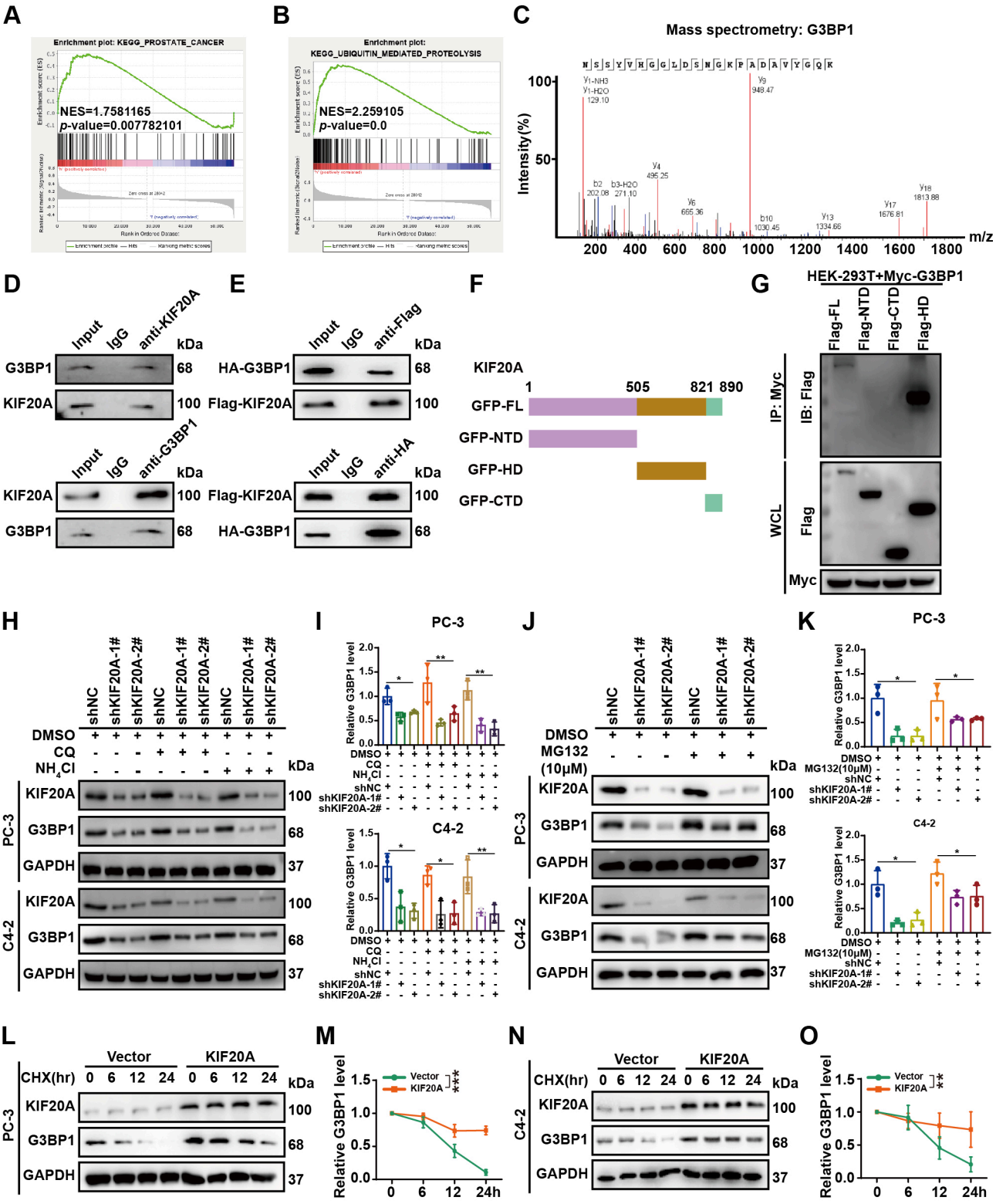


Figure 5

KIF20A stabilizes G3BP1 to promotes PCa progression. A-B GSEA analysis of RNA-seq based on TCGA revealed the relationship between KIF20A expression and PCa and ubiquitin mediated protolysis. **C** Mass spectrometry (MS) experiments were performed to screen and identify potential partner proteins interacted with KIF20A. **D** Co-IP analysis of the endogenous interaction of KIF20A and G3BP1 purified with anti-KIF20A or anti-G3BP1 antibodies respectively in PC-3 cells. **E** Co-IP analysis of the exogenous

interaction of Flag-labelled KIF20A and HA-labelled G3BP1 with anti-Flag or anti-HA respectively in HEK-293T cells. **F** The flow cytometry was used to detect the apoptosis ratio in G3BP1 knock down cells. **G-H** The expression of G3BP1 protein level was detected through western blot assay in KIF20A knock down cells with lysosomal inhibitors (CQ and NH_4Cl) treatment, GAPDH used as loading control. **I-J** The expression of G3BP1 protein level was detected through western blot assay in KIF20A knock down cells with proteasome inhibitor (MG132) treatment, GAPDH used as loading control. **K-N** The stability of G3BP1 protein was evaluated through western blot assay in KIF20A overexpression cells with CHX treatment, GAPDH used as loading control. Data were presented as mean±SD, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

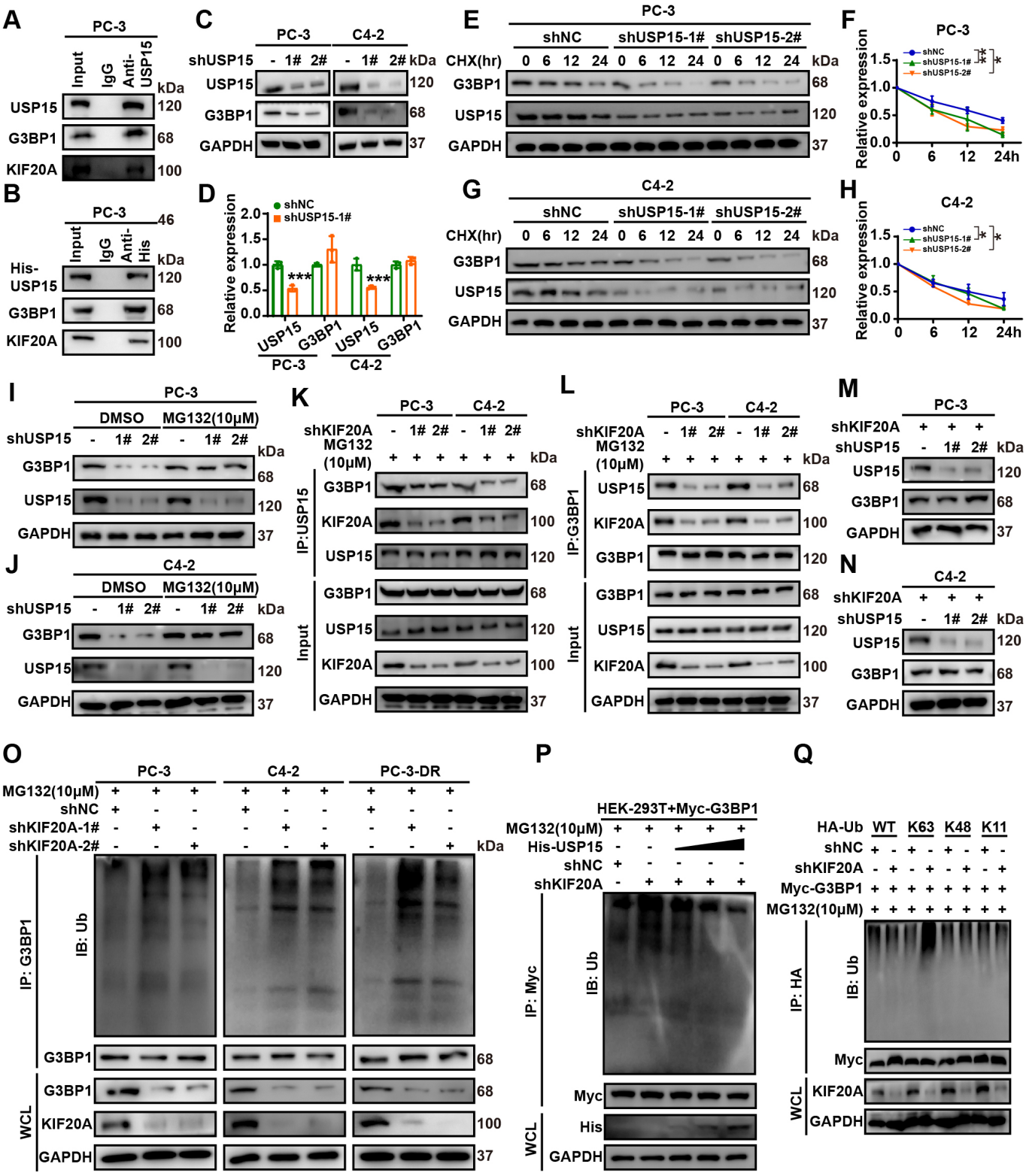


Figure 6

KIF20A recruits USP15 to reinforce K-63 linked deuniquitinated of G3BP1. A-B Co-IP analysis of the endogenous (A) or exogenous (B) interaction of KIF20A, USP15 and G3BP1 purified with anti-USP15 or anti-His antibodies respectively in PC-3 cells. **C-D** The expression of G3BP1 and USP15 was detected through western blot (C) and qRT-PCR (D) in USP15 knock down cells, GAPDH used as loading control. **E-H** The expression of G3BP1 and USP15 was detected through western blot in USP15 knock down cells

after treating with CHX at indicated time, GAPDH used as loading control. **I-J** The expression of G3BP1 and USP15 was detected through western blot in USP15 knock down cells after treating with MG132 (10 μM, 8h). **K-L**Co-IP (purified with anti-USP15 or anti-G3BP1 antibody) was conducted to detect the expression of G3BP1, KIF20A and USP15 in PC-3 and C4-2 cells with KIF20A knock down following MG132 treatment (10 μM, 8h). **M-N** The expression of G3BP1 and USP15 was detected through western blot in PC-3 and C4-2 KIF20A knock down cells with transfecting sh-NC, shUSP15-1# or shUSP15-2# respectively. **O**The ubiquitination level of G3BP1 was detected through IB of anti-ubuiquitination antibody after purified with anti-G3BP1 in KIF20A knock down cells (PC-3, C4-2, PC-3-DR) following MG132 treatment (10 μM, 8h). **P**The ubiquitination level of G3BP1 was detected through IB of anti-ubuiquitination antibody after purified with anti-Myc in HEK-293T KIF20A knock down cells co-transfecting Myc-lablled G3BP1 plasmid and increasing concentration of His-lablled USP15 plasmid following MG132 treatment (10 μM, 8h). **Q** The ubiquitination level was detected through IB of anti-ubuiquitination antibody after purified with anti-HA in HEK-293T KIF20A knock down cells co-transfecting with Myc-lablled G3BP1 plasmid and the indicated ubiquitin Lys 11/48/63-only mutant plasmids respectively following MG132 treatment (10 μM, 8h). Data were presented as mean±SD, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

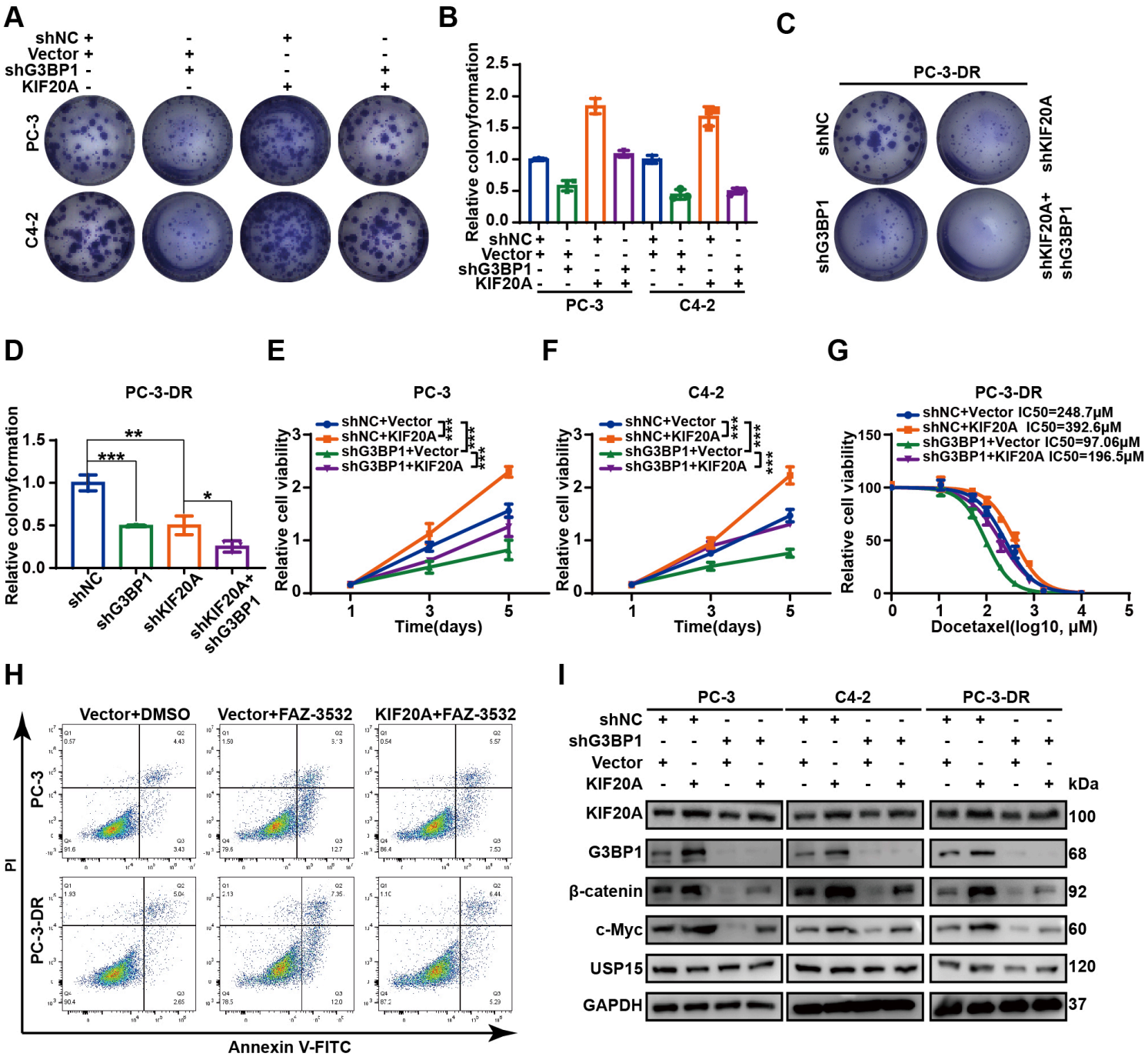


Figure 7

KIF20A facilitates PCa progression partial dependent G3BP1. A-B Colony formation assay was performed to evaluate the proliferation of KIF20A overexpression PC-3 and C4-2 cells after transfecting with shNC or shG3BP1 respectively. **C-D** Colony formation assay was performed to evaluate the proliferation of KIF20A overexpression PC-3-DR cells after transfecting with shNC or shG3BP1. **E-F** The cell viability was measured through CCK-8 assay in KIF20A overexpression or control PC-3 and C4-2 cells after transfecting with shNC or shG3BP1 respectively. G The IC_{50} value of cells in response to docetaxel were measured in KIF20A overexpression or control PC-3-DR cells after transfecting with shNC or shG3BP1 respectively. **H** Flow cytometry was used to detect the apoptosis ratio in KIF20A

overexpression cells with or without G3BP1 inhibitor (HY169348). The expression of G3BP1, KIF20A, USP15 and downstream markers of β-catenin pathway (β-catenin and c-Myc) were detected through western blot assay in KIF20A overexpression cell transfected with shG3BP1. Data were presented as mean±SD, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

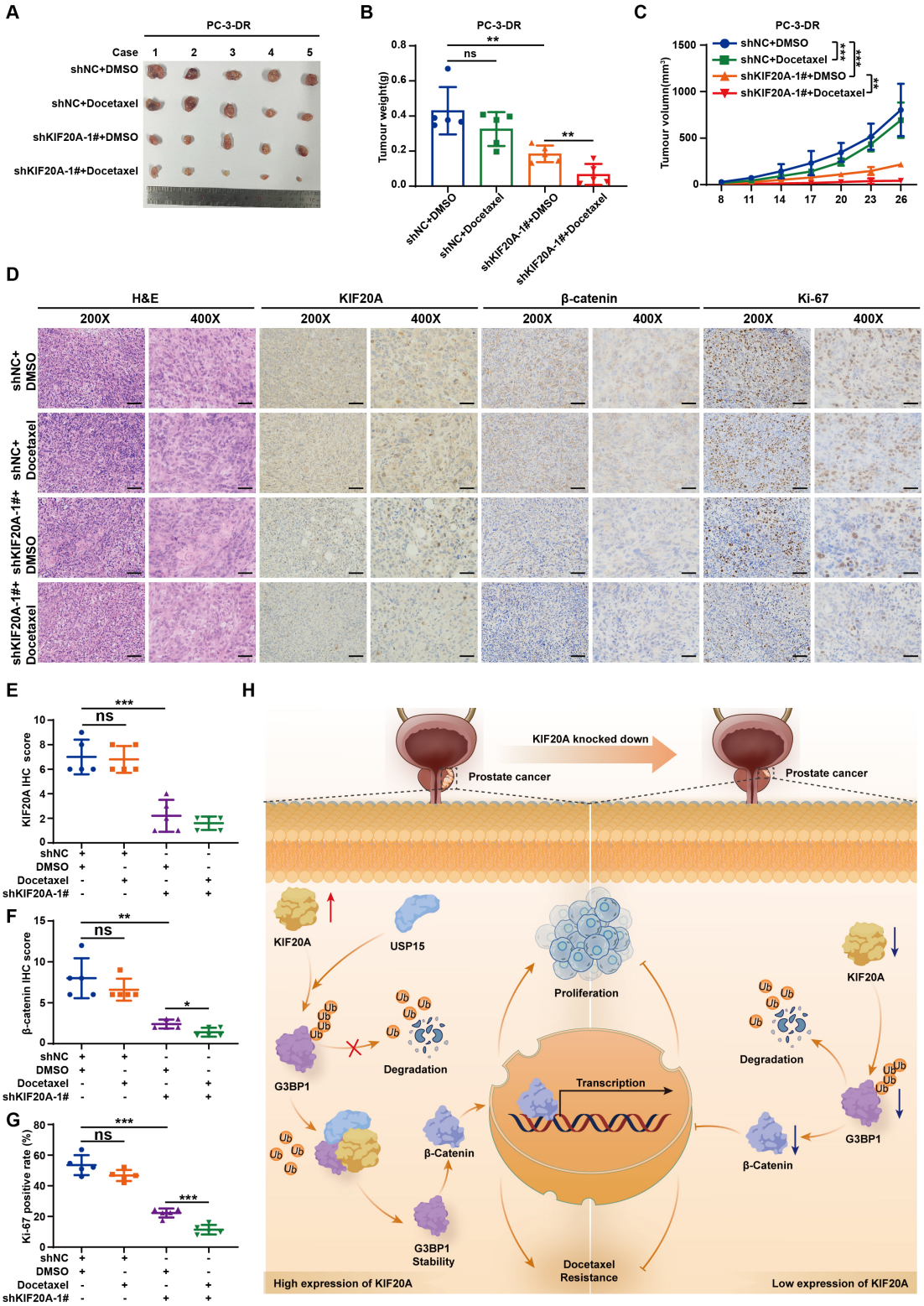


Figure 8

Loss of KIF20A sentizes PCa cells to docetaxel treatment. A Gross observation of subcutaneous xenograft tumors derived from mice with injecting KIF20A knkock down PC-3-DR cells with or without docetaxel treatment, DMSO used as control. **B-C** The statistical analysis of weight (B) and volumn (C) of subcutaneous xenograft tumors. **D-G**Representative images and corresponding statistical analysis of HE and IHC staining (Ki-67, KIF20A, β-catenin) for PC-3-DR subcutaneous xenograft tumors at 200× (scale bar: 100 μm) or 400× (scale bar: 50 μm) magnification. **H**Proposed working model of the molecular mechanism of KIF20A in PCa progression. The up-regulated KIF20A in PCa tissues and docetaxel resistance tissues functions as a scaffold to recruit USP15 for deubiquitylation and stabilization of G3BP1 via K-63 linked deubiquitination at K393 and K453 site, thus facilitating activation of β-catenin pathway and promoting the proliferation and chemoresistance of PCa cells. Data were presented as mean±SD, *p< 0.05, **p < 0.01, ***p < 0.001, ns: not significantly.

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