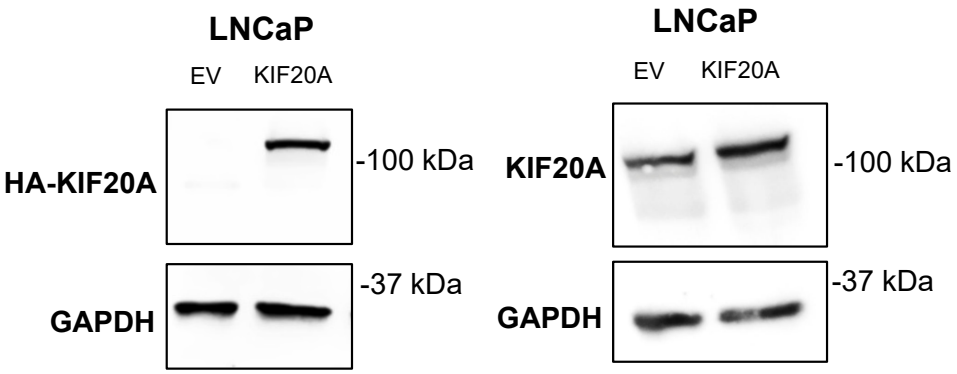
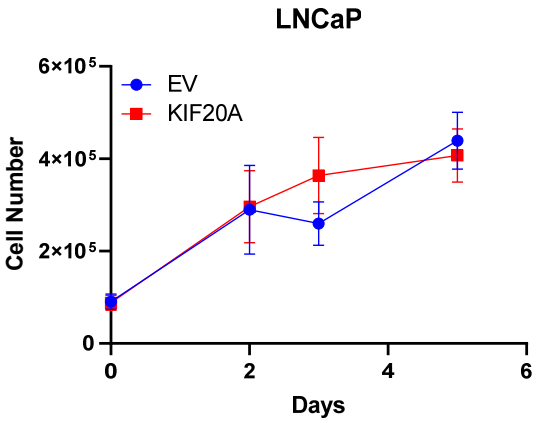
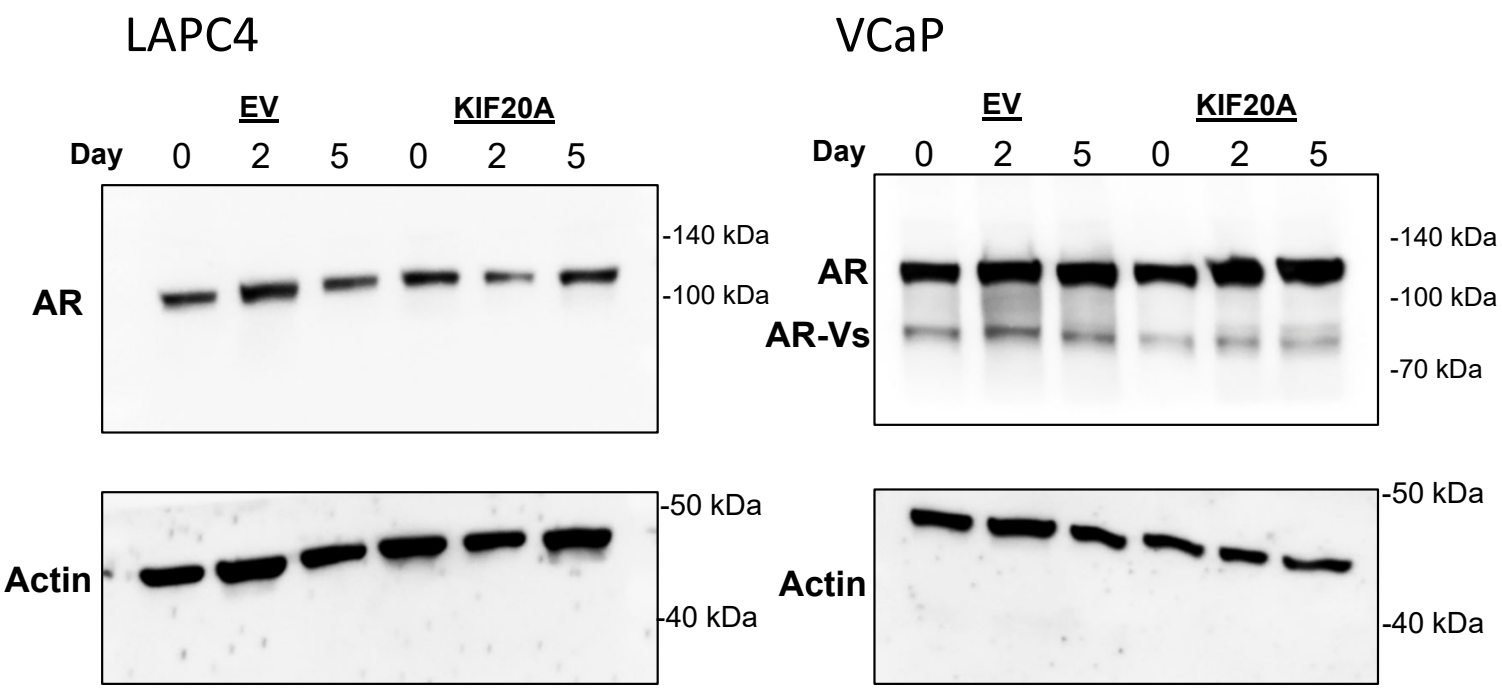


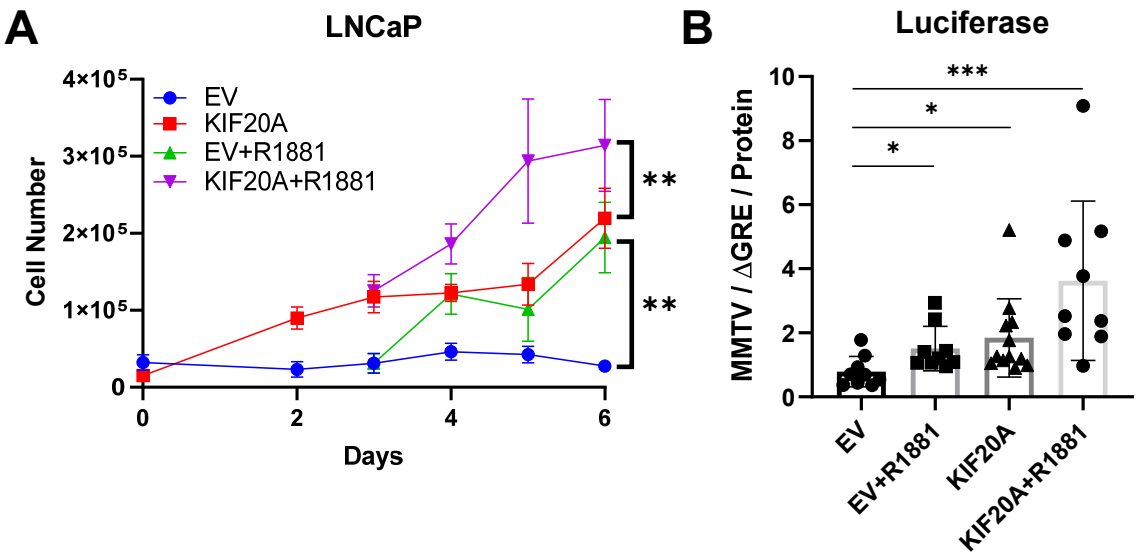
<u>Supplemental Figure 1</u>. Stable expression of KIF20A in androgen-dependent prostate cancer cell lines. Western blot analysis shows KIF20A expression, using antibodies that probe for the HA-tag (left panel) (Santa-Cruz, #SC-805) or KIF20A (right panel) (Bethyl Labs, #A300-879A).



<u>Supplemental Figure 2</u>. KIF20A does not confer a proliferation advantage to androgen-dependent PC cells in androgen-replete media. Proliferation curve of LNCaP EV or KIF20A cells plated in androgen-replete conditions (10% FBS) (N = 3). Unpaired t test did not measure a statistical difference.



Supplemental Figure 3. AR levels are similar in androgen-dependent prostate cancer cell lines expressing ectopic KIF20A compared to EV controls. Western blot analysis of AR and AR variants, using an antibody that targets the AR N-terminal domain (Millipore, #06-680), in LAPC4 (left) and VCaP (right) KIF20A or EV cells growing in 5% CSS.



<u>Supplemental Figure 4</u>. LNCaP KIF20A cells are hypersensitive to androgens. (**A**) LNCaP KIF20A and EV cell proliferation was evaluated following treatment with R1881 (0.1nM) or vehicle 0.01% ethanol (N = 3) administered on Day 3. Area Under the Curve calculations were used to test for statistical significance. (**B**) Luciferase assay (N = 3) of LNCaP KIF20A or EV cells incubated in 5% CSS with 0.1 nM R1881 or ethanol with MMTV (containing androgen / glucocorticoid response elements) or ΔGRE (lacking response elements) plasmids. The ratios of luciferase values (MMTV/ ΔGRE) were normalized to protein concentration. (The EV and KIF20A luciferase datapoints are the same as those shown in Figure 4E). Dunnett's multiple comparisons test was used to test differences in luciferase signal compared to EV. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Error bars = SEM

Supplemental Data

Material & Methods

Stable Expression of KIF20A in Cell Lines

For viral production, GP2-293 and Lenti-X cells were seeded at 60% confluence in 10 cm dishes (Corning, #430165) and used to package viral particles for retroviral transfection. For KIF20A cDNA expression, GP2-293 cells were transiently transfected with 4 µg pQCXIN EV or pQCXIN HA-KIF20A, 2 µg VSVG, and 18 µL X-tremeGENE 9 DNA Transfection Reagent. For KIF20A depletion, Lenti-X cells were transiently transfected with 4 µg pLKO.1 shGFP or shKIF20A, 400 ng VSVG, 4 µg Δ8.2, and 18 µL X-tremeGENE 9 DNA Transfection Reagent. After 48 hours, viral supernatant was collected and filtered to remove cellular debris using a syringe attached to a 0.45 µm cellulose acetate filter (VWR, #28145-481).

LNCaP, LAPC4, and VCaP cells were seeded into 10 cm dishes at 40-60% confluence and allowed to attach for 24 hours in fully supplemented media. The cells were transfected with retrovirus, containing pQCXIN HA-KIF20A or pQCXIN EV, and 4 µg/mL polybrene (Sigma-Aldrich, #TR-1003-G). The following day, media were replaced with fresh media, and incubated for 24 hours. This process was repeated with the same batch of retrovirus. Cells were then selected with the antibiotic, G-418 (Sigma-Aldrich, #4727878001), at 600 µg/mL until a “canary” plate showed 90-100% cell death (usually 6-8 days). The selected cells were maintained in 100 µg/mL G-418. Stable cell lines were routinely generated for experiments and were not passaged beyond 5 times.

Cell Proliferation Assays

LNCaP or LAPC4 KIF20A or EV cells were seeded in 24-well plates (Corning #353047) at $3.8*10^4$ cells per well in fully supplemented media (10% FBS). 24 hours later the cell cultures underwent an androgen deprivation protocol consisting of one wash with 37°C-warmed DPBS [Dulbecco's Phosphate Buffered Saline; HyClone, #SH30028.02]; followed by two 1-hour incubations at 37C in media without supplements [serum, antibiotics]). Cells were then cultured in fresh media containing 5% CSS and half the media were replaced with fresh media containing 5% CSS every 4 days. At each timepoint, cells were trypsinized and re-suspended in media containing trypan blue dye (Sigma-Aldrich, #T8154). Cell Countess II (ThermoFisher) was used to quantify live cells (excluding trypan blue).

VCaP KIF20A or EV cells were seeded (Corning, #353046) at $3.5*10^5$ cells per well in 6-well plates in fully supplemented media. After 24 hours, cells underwent androgen deprivation and were then incubated in media supplemented with 5% CSS for 14 days. The cells were washed with DPBS and fixed with ice cold methanol with 5% acetic acid at -20 °C for 10 minutes, then washed twice with DPBS. Cells were stained with 0.1% crystal violet (SigmaAldrich, #V5265) in 10% ethanol (Pharmco, #111000200CSPP) for 15 minutes at room temperature. The stain was eluted with 1% SDS (ThermoFisher, #24730020) and absorbance at 560 nm was measured using the GloMax Explorer (Promega).

Conditioned media (CM) was harvested from KIF20A or EV cells cultured in 5% CSS in 24-well plates as stated above. The CM was collected on Day 4 and filtered through 0.45 µm cellulose acetate (Corning, #430625) to remove cellular debris. KIF20A and EV CM amounts were normalized to cell number (measured on Day 4). CM were used the

same day or stored at -20 °C. During proliferation experiments, half the media was replaced with fresh CM every 4 days.

For knockdown experiments, 22Rv1 or C4-2B cells were transduced by overnight incubation with lentiviral preparations (pLKO.1 shGFP or shKIF20A) and 4 µg/mL polybrene, followed by 48 hour selection with 2.5 µg/mL puromycin (ThermoFisher, #A1113803). The cells were plated and counted similarly to the stable expression lines. Cells treated with Enzalutamide, paprotrain (Tocris, #4813), or GW4869 (Tocris, # 6741) were seeded into 24-well plates as stated previously. On Day 3, the media were replaced with fresh media (or CM), containing the drug (or DMSO control).

Sample Size Justification for Subcutaneous Xenografts

The main objective of the study was to measure the impact of KIF20A in the LNCaP and VCaP cell line in the mouse model. There were two groups for each experiment (A: LNCaP/VCaP EV and B: LNCaP/VCaP KIF20A). A total of 16 mice (8 per group) was needed for the LNCaP experiment, and a total of 14 mice (4 mice in A and 10 mice in B) was needed for the VCaP experiment. The proposed number of animals in this study provided enough power for significant statistical analysis of investigation to evaluate efficacy of enzalutamide compared to vehicle group. We expected that tumor size of mice in group A increased from 100mm^3 to 1,000mm^3 over about 8 weeks and that of mice in the group B increased at a slower pace. As an illustration of power, we conducted a Monte Carlo simulation (via 5,000 repetitions) based on adjusted area-under-the-curve (aAUC) model using tumor size measurements [18]. Key parameters of the aAUC model were growth rate for each group and s for the measure of departure from the growth curve. The LNCaP growth rates were l_A=0.047 and l_B=0.01, and the

VCaP growth rates were l_A=0.05 and l_B=0.032. We set s=0.025 for the LNCaP model and s=0.01 for the VCaP model. The relative tumor volume for each group was defined as tumor volume divided by that of the date of injection. We assumed that the experiment duration after injection is 8 weeks and exponential growth curves for each group. Relative tumor volume measurement was quantified by adjusted area-under-the-curve (aAUC). The above LNCaP growth rates gave 46% increase for group A and 8.7% increase for group B in terms of relative tumor volume. For the VCaP growth rates, group A would increase 49.2%, and groub B would increase 29.3%. We consider ratio of aAUCs: $aAUC_A/aAUC_B$ for comparing group A vs. group B. The ratio is to examine effect of Enzalutamide on KIF20A tumor growth. In the LNCaP xenograft cohort, statistical powers, to test whether $aAUC_A/aAUC_B$ is less than 1, was 93.1% based on 95% two-sided confidence interval of the ratio of aAUCs with 8 mice per each group. In the VCaP xenograft cohort, statistical powers, to test whether $aAUC_{B1}/aAUC_{B2}$ is less than 1, was 86.6% based on 95% two-sided confidence interval of the ratio of aAUCs with 5 mice per each group in KIF20A.

Supplemental Figure Legends

Supplemental Figure 1. Stable expression of KIF20A in androgen-dependent prostate cancer cell lines. Western blot analysis shows KIF20A expression, using antibodies that probe for the HA-tag (left panel) (Santa-Cruz, #SC-805) or KIF20A (right panel) (Bethyl Labs, #A300-879A).

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