Supplemental Experimental Procedures

Immunoblot and protein analysis- Whole cell extracts were prepared by scraping the cells from the dish with a rubber spatula, washing the cell pellet with 1X cold PBS, extracting with lysis buffer at 4°C for 1 hour followed by the removal of cell debris by centrifugation at 14,000 x g for 20 min at 4°C. Protein concentrations were determined using the Bio-Rad protein assay system (Bio-Rad Laboratories, Richmond, CA). Equal amounts of protein were resolved by SDS-PAGE, transferred to PVDF membrane and stained with SYPRO Ruby. Membranes were blocked for 1hr at room temperature or 4°C overnight with 5% non-fat dry in TBS-T (10 mM Tris, pH 7.4 + 0.05% Tween-20). After treatment with the appropriate primary and secondary antibodies in 5% milk in TBS-T, enhanced chemiluminescence was performed (Amersham, Piscataway, NJ). The following antibodies (clone, dilution) were used: anti-androgen receptor (clone F39.4.1, BioGenenix, San Ramon, CA, 1:400), anti-α-tubulin (clone B-5-1-2, Sigma, St. Louis, MO; 1:3000), anti-β-actin (clone AC-15, Abcam, Cambridge, MA, 1:5000), anti-PARP (clone F-2, Santa Cruz Biotechnology, 1:200), anti-pro-caspase-3 (clone E-8, Santa Cruz Biotechnology 1:200), anti-Bcl-xL (clone H-62, Santa Cruz Biotechnology, 1:200), and anti-AR (Millipore, Temecula, CA, 1:500), anti-XIAP (clone 2F-1, Abcam, Cambridge, MA, 1:1000). Quantitation of protein expression was determined using Image J analysis. Representative western blots of at least 3 independent experiments are depicted in the figures.

Apoptosis analysis by Annexin V staining- Cells treated with the indicated concentrations of docetaxel and/or TOK-001 for 5 days were collected by brief trypsinization, resuspended in media containing 10% FBS and returned to the humidified 5% CO_2 incubator for 30-45 minutes. Apoptotic cells were measured with the Annexin V-FITC Apoptosis Detection Kit (BD Biosciences, San Jose, CA). Propidium iodide (1 mg/mL) was added just prior to flow cytometric analysis (Becton Dickinson FACScan). Additional samples were prepared from vehicle-treated cells and were unstained, stained with PI alone, or stained with Annexin V-FITC alone. Ten thousand cells per sample were analyzed. All apoptosis studies were repeated a minimum of two times.

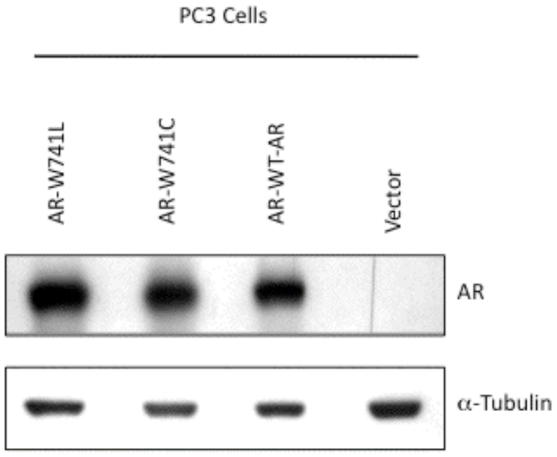
Supplementary Figure Legends

Supplementary Fig. 1. Expression of the wild type and mutant AR proteins in PC-3 cells. PC3 (AR null) prostate cancer cells were transiently transfected with separate expression plasmids encoding either the wild type (WT-AR), W741C mutant AR, or W741L mutant AR. Whole cell extracts were collected 24 hours post-transfected and subjected to Western blot analysis. AR protein levels are depicted (_-tubulin control).

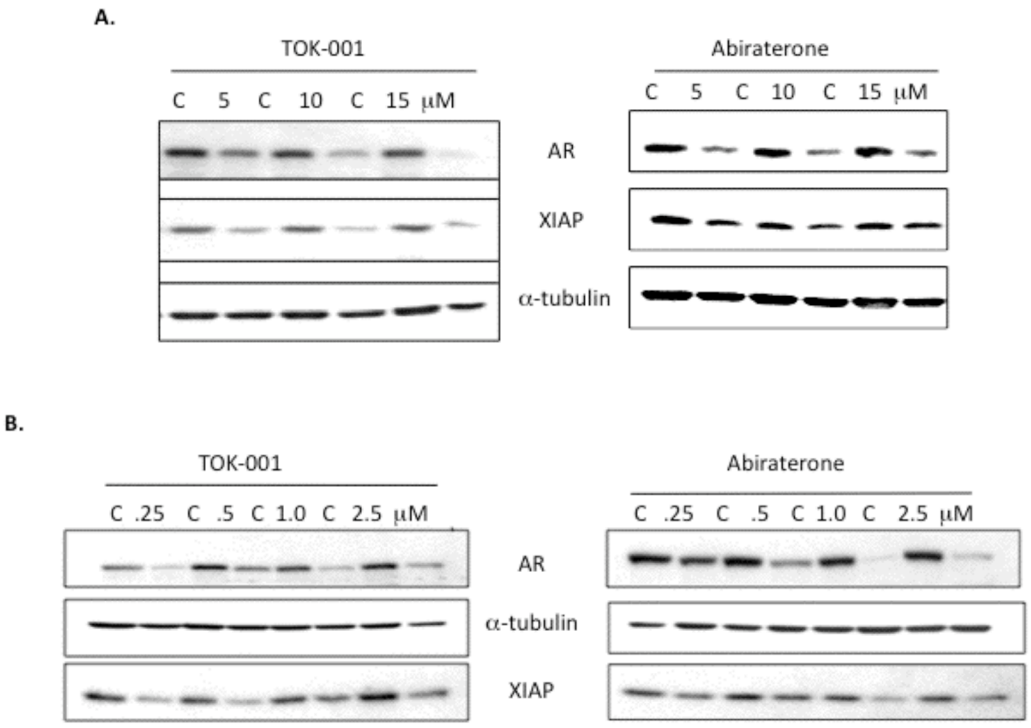
Supplementary Fig. 2. The effect of 17-HASs on the expression of the IRES-dependent gene XIAP. LNCaP (A) or LAPC-4 (B) cells were cultured in CSS media and treated with the indicated concentrations of either TOK-001 or abiraterone alcohol for 5 days. Western blot analysis was performed on whole cell extracts for AR, a-tubulin, and XIAP protein levels.

Supplementary Fig. 3. TOK-001 does not promote apoptosis in LNCaP cells. (A). Western blot for the AR and apoptosis-related proteins in LNCaP cells treated with the indicated concentration of TOK-001 (5-15 _M) for 1 and 3 days. Control (C) cells were treated with an equal volume of DMSO. TOK-001 did not alter the levels of the apoptosis-related proteins Bcl-xL and Bax, nor does TOK-001 increase cleavage of PARP-1 or pro-caspase-3. (B). TOK-001 does not promote apoptosis in LNCaP cells. LNCaP cells were treated with taxotere alone (1-10 _M), TOK-001 alone, or a combination of taxotere and TOK-001 for 5 days. Apoptosis was quantified by FITC-Annexin V/PI staining and flow cytometry using a minimum of 10,000 cells after gating the different populations of cells using the untreated control. Live: Annexin V-negative/PI-negative; Necrotic: Annexin V-negative/PI-positive; Early apoptotic: Annexin V-positive/PI-negative; Late apoptotic: Annexin V-positive/PI-positive.

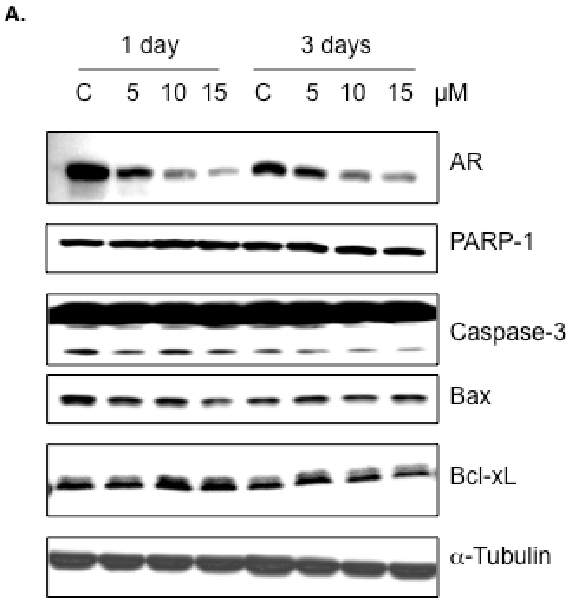
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3A



Supplementary Figure 3B