Supplemental Figure 1. Equal protein expression and transfection efficiency of AR DNA (WT, T877A, D879G, W741C, M749L, R629Q) in PC-3 cells. PC-3 cells were co-transfected with PEGFP-C2 and each mutant. Protein expression for AR, GFP and GAPDH were analyzed by western blot.

Supplemental Figure 2. The effect of eplerenone and spironolactone on W741C and D879G mutant AR activity. PC-3 cells were co-transfected with ARE3-luciferase and W741C or D879G mutant AR. Cells were treated with the range of concentrations shown of R1881, eplerenone or spironolactone alone or in combination with 10µM bicalutamide for 16 hours and then analyzed for luciferase activity. Fold change from the DMSO control was then calculated. Data shown are the mean and SEM of 3 independent experiments of 16 replicates.

Supplemental Figure 3. The effect of abiraterone on WT and mutant AR activity in the absence of R1881. PC-3 cells were co-transfected with ARE3-luciferase and WT or mutant AR (T877A, W741C, D879G, M749L, R629Q). Cells were treated with DMSO or 0.1 - 25µM abiraterone for 16 hours and then analyzed for luciferase activity. Fold change from the DMSO control was then calculated.

Supplemental Figure 4. PC-3 and DU145 cells were treated with abiraterone, MDV3100 and bicalutamide for 72 hours and then analyzed for cell viability. Fold change from the DMSO control was then calculated and plotted. No inhibition of proliferation was observed. Data shown are the mean (error bars, standard error of the mean, SEM) of 3 independent experiments in duplicate.

Supplemental Figure 5. Displacement of [3H] R1881 by eplerenone and abiraterone in LNCaP cells. LNCaP cells were treated with CSS media containing 5nM of [3H] R1881 in combination with cold R1881, spironolactone, eplerenone, abiraterone or bicalutamide at the concentrations shown for 2 hours. Abiraterone was insoluble in cell media at concentrations greater than 25µM. Cell-associated radioactivity was measured and the data analyzed by nonlinear regression to determine the K_i for each test compound (GraphPad Prism). Data shown are the mean and SEM of three independent experiments with six replicates for percentage (%) [3H]-R1881 bound versus log10 of concentration (µM) of cold competitor. K_i and 95% confidence intervals are given.

Supplemental Figure 6. VCaP cells were treated with 0.1µM spironolactone in combination with DMSO, 10µM bicalutamide, 10µM MDV3100 or 5µM abiraterone for 5 hours. RNA was extracted and cDNA synthesized for analysis by quantitative PCR to determine relative levels of PSA and TMPRSS2 mRNA expression. Data shown are the mean and SEM of 3 independent experiments in duplicate.
Supplemental Figure 7. Increased DHT levels reduce AR inhibition by MDV3100. PC-3 cells were co-transfected with ARE3-luciferase and WT-AR. Cells were treated with DHT in combination with DMSO or MDV3100 at the concentrations indicated for 16 hours and then analyzed for luciferase activity. Fold change from the DMSO control was calculated. Data shown are from three independent experiments and represent mean and SEM of 24 replicates. ***, P< 0.01 relative to R1881 or DHT control with DMSO (one-way ANOVA with Bonferroni correction).