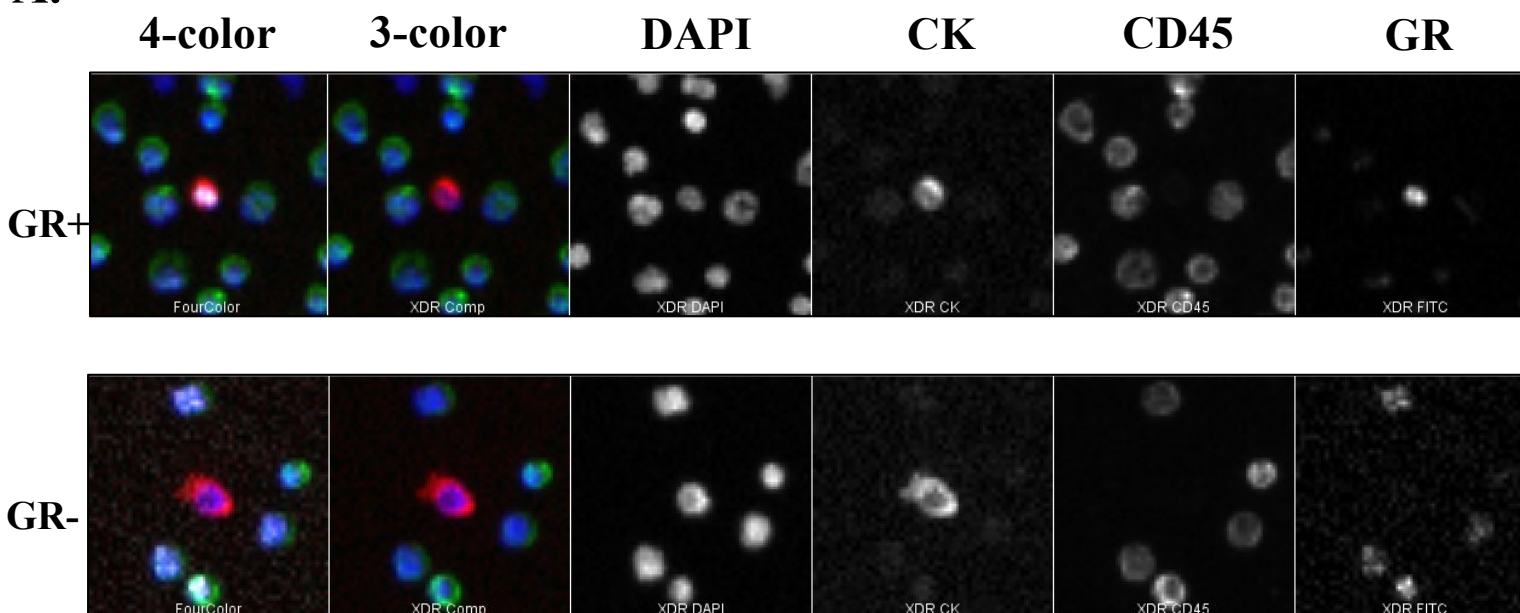


Supplementary Figure 1

A.

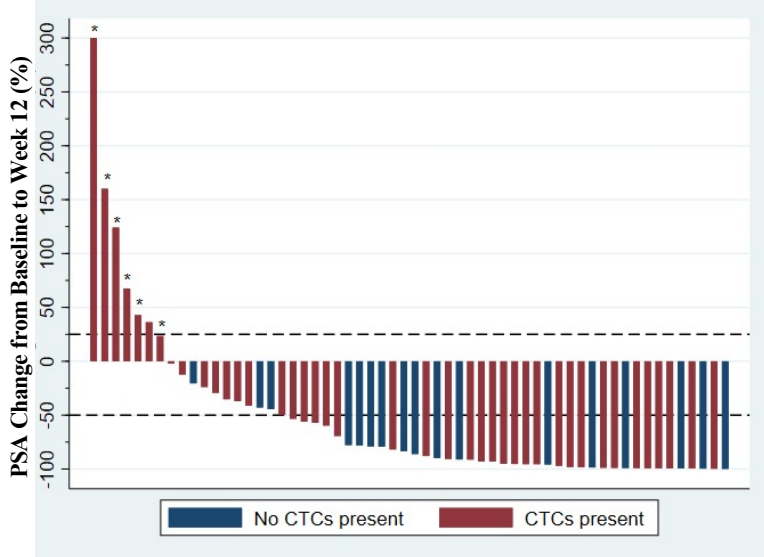


Supplementary Figure 1. Representative Circulating Tumor Cell (CTC) Staining

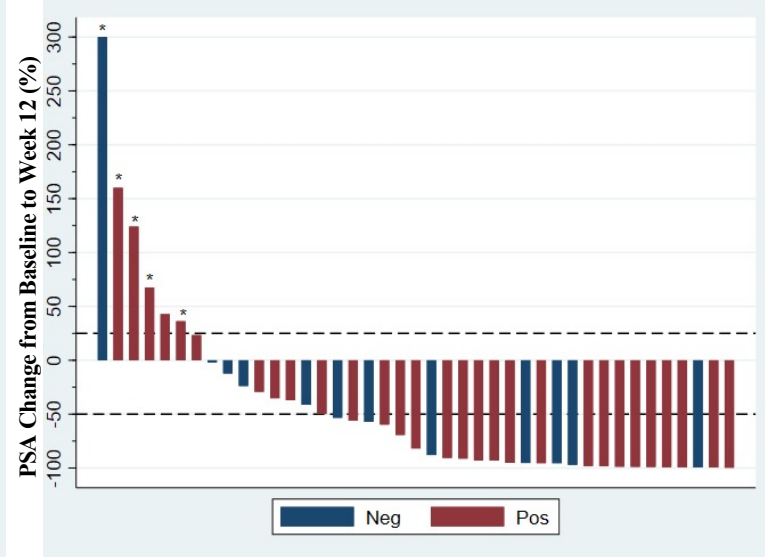
A. Images of CTCs collected from GR positive (+, top panel) and GR negative (-, bottom panel) patients performed at Epic Sciences. Peripheral blood mononuclear cells were deposited on glass slides and probed using a custom 4-color fluorescence in situ hybridization assay (FISH, 4-color) or 3-color FISH assay. Two slides per sample were immunofluorescently stained with a cocktail of antibodies targeting cytokeratins (CK), CD45, and 4',6-Diamidino-2-phenylindole, dihydrochloride (DAPI). A CTC is defined as any CK+, CD45-, DAPI+ cell. CTCs were counted by immunofluorescence following staining with DAPI. Monoclonal antibody (clone D6H2L) specific to the glucocorticoid receptor (GR) C-terminal domain was evaluated on single cell line cells spiked into healthy donor blood and processed as patient samples. LnCaP (GR negative) and DU-145 (GR positive) prostate cancer cell lines were utilized. Due to co-expression of GR on WBCs, only CK+ CTCs could be assessed for GR expression.

Supplementary Figure 1

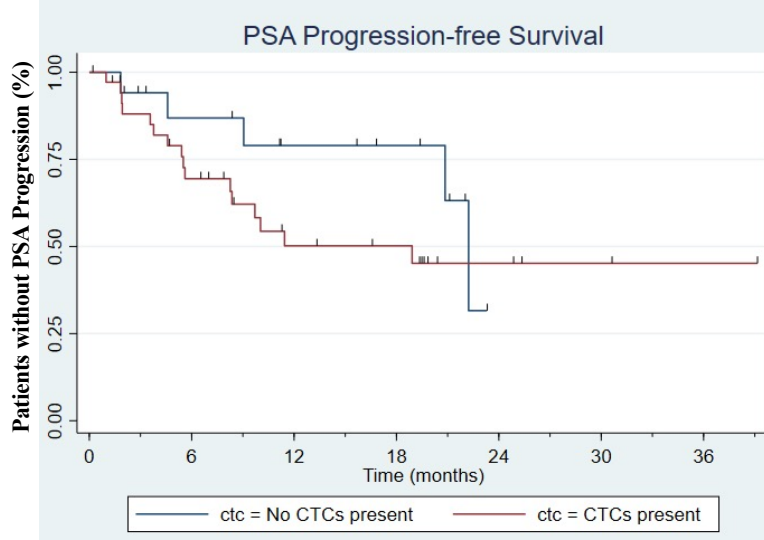
B.



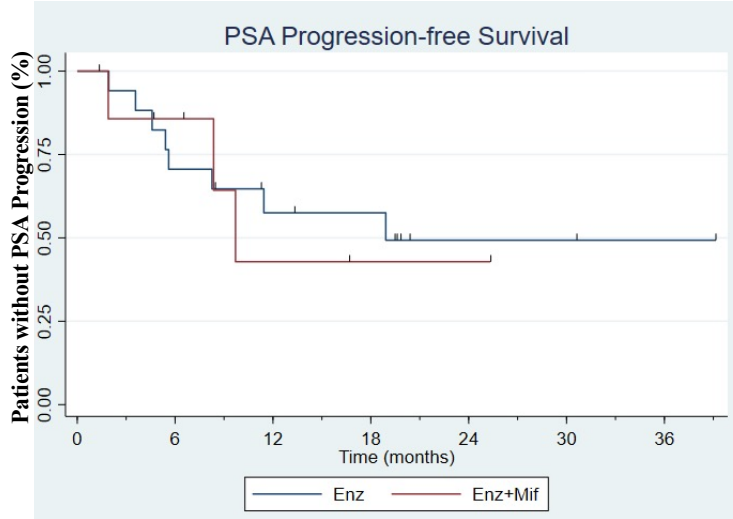
D.



C.



E.



Supplementary Figure 1. Circulating tumor cell analyses

B. Waterfall plot showing percent reduction in PSA from baseline to week 12 comparing patients with CTCs present at week 12 with those who did not have CTCs. Patients who did not proceed to randomization are starred. Note this does not reflect any decreases in PSA from drug combination, but rather decreases in PSA prior to combination dosing. For panel 4B, positive changes are those who increased after 12 weeks.

C. Kaplan-Meier plot of post randomization time to PSA progression comparing those who proceeded to randomization who had CTCs present with those who lacked CTCs at week 12. Plot demonstrates that even among those who randomized, the presence of CTCs at time of randomization was associated with an inferior PSA PFS ($p=0.24$)

D. Waterfall plot showing pre-randomization changes in PSAs comparing those with Week 12 GR positive CTCs (Pos) with those with GR negative CTCs (Neg). Patients who did not proceed to randomization are starred. Note this does not reflect decrease in PSA from drug combination, but rather decrease in PSA prior to combination dosing.

E. Kaplan-Meier plot of time to PSA PFS of those with GR+ CTCs comparing those who were randomized to Enz only vs. those with Enz+Mif. Having GR+ CTCs was not predictive of benefit from the addition of Mif.

Abbreviations: CTCs, circulating tumor cells; Enz, Enzalutamide; Enz alone, Enz 160mg daily after 12-week enzalutamide monotherapy lead in; Enz+Mif, Enz 120mg and Mif 300 mg daily after 12 week Enz lead-in; Mif, Mifepristone; PFS, progression free survival; PSA, prostate specific antigen