Supplementary Material: Dual Inhibitory Action of a Novel AKR1C3 Inhibitor on Both Full-Length AR and the Variant AR-V7 in Enzalutamide Resistant Metastatic Castration Resistant Prostate Cancer

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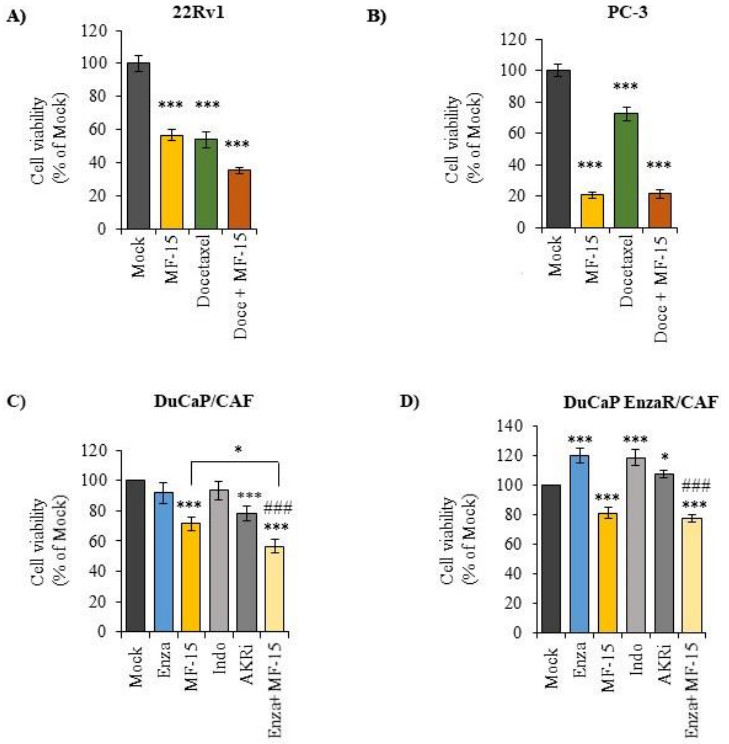

Figure 1. Effects on the viability of 22Rv1 and AR-negative PC-3 cells and spheroid co-cultures. 22Rv1 (**A**) and PC-3 (**B**) cells were seeded into 96 well plates and treated with MF-15 (10 μM), 12nM docetaxel, or a combination of the two drugs over 5 days as described under material and methods. Cell viability was measured through colorimetric MTS cell viability assay (Promega) and indicated as percentage of mock control (DMSO). Co-culture spheroids (**C**, DuCaP/CAF, **D**, DuCaP EnzaR/CAF) were established by seeding equal numbers of tumor cells and CAFs into ULC 96 well plates (Corning). MF-15 (10 μM), indomethacin (indo, 20 μM), AKRi (50 μM), enzalutamide (5μM) and a combination of enzalutamide (5 μM) with MF-15 (10 μM) were added at days 4 and 8 in RPMI + 10% CS-FCS. Cell viability was assessed by colorimetric MTS assay (Promega) and expressed as percentage of mock control. Data represent the mean ±SEM from three independent experiments. Statistical comparisons to the mock control were expressed with an asterisk (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$), comparisons to enzalutamide with a hash key (### $p < 0.001$).