

Supplementary Figure legends

Fig. S1 DHODH inhibitor activates the androgen-AR signaling pathway. **A**, Heatmap displaying the fold changes of gene expression detected by RNA-seq in C42B cells treated with BAY2402234 (25nM and 50nM) for 48 hours, compared to vehicle (DMSO). Genes are displayed in rows, and the standardized count of each sample is displayed in columns. Red indicates up-regulated expression level. AR pathway signature genes that were altered in expression are displayed. **B**, GSEA of the androgen response signatures in C42B cells treated with BAY2402234(25nM). The signature was defined by genes that significant expression changes by androgen stimulation in PCa cells.

Fig. S2 Combine DHODH inhibitor and androgen synthesis inhibitors treatment increases sensitivity of CRPC inhibition. **A**, Vcap-CRPC and 22Rv1 cells were treated with BAY2402234 (25nM or 100nM) or abiraterone (5 μ M) alone or in combination. After 48 hours later, apoptosis was measured by flow cytometry for Annexin and Pi positive cells. Data shown are mean $\pm$ SD. Student's *t*-test, *** $p < 0.005$, **** $p < 0.0001$, $n = 3$. **B**, Vcap-CRPC and 22Rv1 cells were treated with BAY2402234 (25nM or 100nM) or Indomethacin (30 μ M, AKR1C3 inhibitor) alone or in combination. After 96 hours, total cell numbers were counted. Data shown are mean $\pm$ SD. Student's *t*-test, *** $p < 0.005$, **** $p < 0.0001$, $n = 3$. **C**, Vcap-CRPC and 22RV1 cells were treated with BAY2402234 (25nM or 100nM) or Indomethacin (30 μ M) alone or in combination. Indicated proteins were detected by western blot after 48 hours. **D**, H&E staining and immunohistochemical analysis of tumor tissues. H&E images, anti-Ki-67 and anti-cleaved caspase-3 IHC images of tumor sections were shown in above. Scale bars, 20 μ m.