**Supplementary Figure 1.** A schematic of the classical and ‘backdoor’ steroid biosynthesis pathways. Adapted from reference (20).

**Supplementary Figure 2**. AR transactivation results following treatment with abiraterone or VT-464 at 1, 5 or 10μM for 72h in 10% CSS media (**A**). Results were normalized to protein concentration. Western blotting for PARP and cleaved PARP (~90kD fragment) was performed on MR49C and MR49F cells treated in 10%CSS media with indicated treatments for 6 days, with treatment containing media changed at 72h (**B**). Representative results of duplicate independent experiments +/- SEM are presented.

**Supplementary Figure 3.** Expression of HMGCR and SBREP-1 *in vitro* and *in vivo*. Following treatment with indicated doses of abiraterone and VT-464 in androgen depleted media for 6 days, RT-qPCR was used to assess steroid transcripts in C4-2(**A**), MR49C (**B**) and MR49F cells(**C**). Representative results of triplicate independent experiments +/- SEM are presented.Quantification of intratumoral mRNA transcripts of HMGCR and SBREP-1 from pharmacodynamic study of treatment in MR49F xenografts relative to beta-actin(**D**).

**Supplementary Figure 4.** AR protein degradation is seen on western blotting only at 20uM dose of VT-464 after 48h of treatment in MR49F cells. No AR degradation was seen in MR49C cells. Representative results of duplicate experiments are presented.

**Supplementary Figure 5.** Additional details of *in vivo* study. Mean weight of mice in different treatment arms as a percent of baseline treatment weight (**A**). From in vivo study, RT-qPCR was performed on 4-6 xenograft samples collected per group at the end of the study. Transcript levels were normalized to GAPDH. A relative decrease in AR and AR-dependent mRNA transcripts was seen with VT-464 compared to abiraterone. (**B**). Further, there was a significant decrease in six steroid enzyme transcripts (normalized to beta-actin) in abiraterone and VT-464 treated mice compared to vehicle-treated mice from the same samples (**C**).

**Supplementary Figure 6**. Levels of intratumoral steroid enzyme synthesis mRNA transcripts collected from the pharmacodynamic study. MR49F mice were randomized to treatment with Vehicle, Abiraterone 196mg/kg BID or VT-464 100mg/kg BID as detailed in methods. Tumors were collected after 3-10days of treatment. From tumor samples stored in RNAlater, qRT-PCR was used to amplify indicated SYBR Green primers, with results normalized to beta-actin.