**Supplemental Materials and Methods**

**Murine Xenografts.** Four week-old male SCID C.B17 mice were procured from a breeding colony at University of Michigan maintained by our group. Procedures involving mice were approved by the University Committee on Use and Care of Animals (UCUCA) at the University of Michigan and conform to all regulatory standards.

To generate the enzalutamide-resistant prostate cancer tumor model, 4-week-old male mice were surgically castrated and allowed to recover for 2–3 weeks. LNCaP-AR cells suspended in 100μl of PBS with 50% Matrigel (BD Biosciences) were implanted subcutaneously into the dorsal flank on both sides of the mice and once the tumors reached 80–100 mm3, mice were randomized and treated with vehicle control or enzalutamide (10mg/kg) 5 days/week by oral gavage. Vehicle-treated mice were harvested after 27 days of treatment. Growth in tumor volume was recorded using digital calipers and tumor volumes were estimated using the formula (π/6) (L × W2), where L = length of tumor and W = width. For the enzalutamide-resistant VCaP model, VCaP cancer cells were implanted subcutaneously in non-castrated mice. Once the tumors reached a palpable stage (100mm3), the animals were randomized and treated with vehicle control or enzalutamide (30mg/kg) by oral gavage for 5 days a week. Tumor volumes were measured as described above. To study the efficacy of BETi (JQ1 or OTX-015) in combination with anti-androgens (enzalutamide or ARN-509) in a CRPC mouse model, VCaP tumor-bearing mice were castrated when the tumors reached approximately 200mm3 in size and randomized once the tumor grew to the pre-castration size and treated with BETi, anti-androgen, BETi+anti-androgen or vehicle control. JQ1 treatment was administered by I.P. route and OTX-105 was administered orally. Tumor volumes were measured as described above. At the end of the studies, mice were sacrificed post-6hr drug treatment and tumors extracted for further analysis. Patient derived xenofgraft (PDX) MDA PCa 118b and MDA PCa 146-12 used in the study was derived from bone metastases of a patient with castration resistant prostate cancer bone metastases and a bladder extension of a T4N1 castration resistant prostate cancer, respectively (1,2). For PDX experiments, fresh PDX tumors were trypsinized and ficoll-purified to remove dead cells. Five to ten million viable cells were implanted subcutaneously in mice and grown until tumors reached the approximately 100mm3 in size. Xenografted mice were randomized and then received various treatments 5 days/week.

**Cell culture and Cell Viability Assay.** VCaP andLNCaP prostate cancer cells were grown in ATCC recommended media supplemented with 10% FBS (Invitrogen). LNCaP-AR cells were a gift from Dr. Charles Sawyers (Memorial Sloan-Kettering Cancer Center, New York, NY), and were grown in RPMI1640 (Invitrogen) supplemented with 10% FBS. Enzalutamide-resistant tumor derived LNCaP-AR and VCaP cell lines were cultured in their respective media supplemented with 5uM enzalutamide. For short term viability assays, cells were seeded in 96-well plates at 5000-10,000 cells/well (optimum density for growth). Serially diluted compounds were added to the cells 12hr later. Following 96 hr incubation, cell viability was assessed by Cell-Titer GLO (Promega). The values were normalized and IC50s were calculated using GraphPad Prism software.

For long-term colony formation assay, 25,000-50,000 cells/well were seeded in six-well plates and treated with either JQ1 or DMSO. After 12 days, VCaP sub-line cells were counted or LNCaP-AR sub-line cells were fixed with methanol, stained with crystal violet and photographed. For colorimetric assays, the stained wells were treated with 500µl 10% acetic acid and the absorbance was measured at 560nm using a spectrophotometer.

**Quantitative Real-Time PCR.** Primer sequences are provided in the **Table S1**. RNA extraction, cDNA synthesis and QPCRs were performed as previously described (3).

**Western Blot Analyses.** Antibodies used in the study are listed in **Table S2.** All antibodies were employed at dilutions suggested by the manufacturers. Western blot analysis was performed as described previously (3).

**AR-v7 antibody.** The rabbit AR-V7 monoclonal antibody clone (EPR343) used in this study for Western blotting was developed in collaboration with Epitomics Inc (Burlingame, CA, USA). This antibody was generated initially in rabbits as a polyclonal antibody to a peptide that was a portion of the sequence in the cryptic exon CE3b. The polyclonal antibody was subsequently fused to rabbit spleen to generate the rabbit monoclonal antibody EPR343. We have previously published that this antibody specifically detects AR-V7 in the LNCaP 95 cell line on Western blot (4). The band is knocked down with siRNA to the cryptic exon CE3b and exon 1 but is not affected by siRNA to exon7, consistent with the exon structure of AR-V7. In immunocytochemistry using CWR 22Rv1 cells EPR343 stains in the nucleus and the staining is inhibited by siRNA to the AR-V7 specific exon CE3b confirming the specificity of this antibody for AR-V7.

**Reference**

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3. Asangani IA, Dommeti VL, Wang X, Malik R, Cieslik M, Yang R, et al. Therapeutic targeting of BET bromodomain proteins in castration-resistant prostate cancer. Nature 2014;510(7504):278-82.

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