**Supplementary Table 1. NCI/NIH FDA-approved anticancer “Approved Oncology Drugs Set”**

**Supplementary Table 2. Antibodies used for western blot and IHC**

**Supplementary Table 3. TaqMan probes for qPCR analysis of gene expression**

**Supplementary Table 4. PSMA fold change normalized to Vinculin and divided by overall DMSO**

**Supplementary Table 5. Mutations identified in patient derived xenograft CP286 through next generation sequencing**

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| **Supplementary Figure 1 PSMA NCI/NIH Oncology Set screen results, 1 µM.** Drug screen: LNCaP95 cells were treated with 1 µM of 147 compounds available with the NIH FDA-approved anticancer collection for 48-hours Lysates were then probed for PSMA and vinculin (VINC) expression, the latter used to normalize PSMA expression per sample. |

**Supplementary Figure 2 PSMA NCI/NIH Oncology Set screen results, 100 nM.** Drug screen: LNCaP95 cells were treated with 100 nM of 147 compounds available with the NIH FDA-approved anticancer collection for 48-hours. Lysates were then probed for PSMA and vinculin (VINC) expression, the latter used to normalize PSMA expression per sample.

**Supplementary Figure 3. Daunorubicin and mitoxantrone fold change.** LNCaP, LNCaP95 and 22Rv1 cell lines were plated in 96-well plates and allowed to adhere overnight. Media was replaced with increasing concentrations of daunorubicin (DAUN) or mitoxantrone (MIT) and differences in cell viability measured on day 5 (A) or day 2 (B). Treatment wells were normalized to vehicle (DMSO) controls.

**Supplementary Figure 4. Benign lines and mRNA change after radiation.** LNCaP, LNCaP95 and 22Rv1 cell lines were subjected to cumulative radiation doses of 6 Gy or 12 Gy over 6 fractions of 1 Gy and 2 Gy, respectively. Samples were taken for qPCR analysis of *FOLH1* change 72-hours after the last dose of radiation (A). Benign lines, WPE-1 and RWPE-1 were subjected to cumulative radiation doses of 6 Gy, 12 Gy or 18 Gy over 6 fractions (6Gy/6#, 12Gy/6#, 18Gy/6#). Western blot analysis evaluated PSMA protein change after 2-weeks of fractionated radiation (B). qPCR in treatment versus control groups were subjected to Student’s t-tests to detect significance. Mean and standard deviation are shown. Normality was assumed. Statistical significance was considered at p < 0.05 (p < 0.05 : \*, p < 0.01 : \*\*, p < 0.001 : \*\*\*, p < 0.0001 : \*\*\*\*).

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**Supplementary Figure 5. CP286 development from patient and genomic description.** CP286, a PDX model with heterogeneous expression of PSMA, was derived from an mCRPC patient lymph node biopsy from a patient whose tumor had mismatch repair defects and PTEN loss and had progressed through treatment with ADT, docetaxel and enzalutamide. Targeted next generation sequencing (NGS) tumor profiling of this patient revealed these defects, along with other mutations outlined in ***Supplementary Table 5***. After 3 passages in NSG mice, the CP286 PDX was considered established. During this time IHC had been conducted on passaged tumor cores identifying heterogeneous expression of PSMA expression. DAUN = Daunorubicin; MIT = Mitoxantrone; RR = Repeat radiation.

**Supplementary Figure 6. Mitoxantrone and PSMA cell positivity in CP286 PDXs.** CP286 tumor pieces were implanted into NSG mice and allowed to grow to sufficient size (400mm2) before randomization and treatment with either vehicle (PBS), or 0.5mg/kg or 1mg/kg of mitoxantrone (MIT). Tumor samples were subsequently taken on Day 8 and investigated for changes in PSMA expression through IHC and western blot analysis. Three tumor cores from each treatment group were fixed and underwent PSMA IHC (A). Representative images of mitoxantrone treated tumors at 10x and 20x are displayed in and black scale bars represent 100 µm (top row) and 40 µm (bottom row) (A). IHC of tumor cores were scanned and analyzed by HALO® image analysis software. PSMA positivity (percentage) in all cells and cytoplasm vs membrane were compared between treatment groups (B). Average optical density (OD), a read out for intensity, was also investigated for changes in cytoplasmic and membranous PSMA (C). Lysates of tumor cores were also analyzed by western blot and densitometry undertaken to measure change in PSMA (D). PSMA was normalized to the house keeping gene vinculin. Changes in various measures of PSMA in treatment versus vehicle groups were subjected to two-tailed, unpaired Student’s t tests to detect significance (E). Median and 95% CI are shown. Normality was assumed. Statistical significance was considered p<0.05 (p<0.05 = \*, p<0.01 = \*\*, p <0.001 = \*\*\*, p<0.0001 = \*\*\*\*).

**Supplementary Figure 7. Topoisomerase inhibitor treatment induces a reduction in TOP2A mRNA expression but not FOLH1.** Tumor core samples (n=4 per condition) were taken after 1 week treatment with 1 or 2 mg/kg (cumulative dose) of mitoxantrone (Mit) or daunorubicin (Daun). Cores were investigated for changes in PSMA (FOLH1) and TOP2A expression via qPCR. TOP2A and FOLH1 were normalized to housekeeping genes RPLP0 and GAPDH. Changes in FOLH1 (ns) and TOP2A (mitoxantrone p=0.02, daunorubicin p=0.02) RNA in treatment versus vehicle groups were subjected to two-tailed, unpaired Student’s t tests to detect significance. Mean and standard deviation are shown. Normality was assumed. Statistical significance was considered p<0.05 (p<0.05 = \*, p<0.01 = \*\*, p <0.001 = \*\*\*, p<0.0001 = \*\*\*\*).

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| **Supplementary Figure 8. Schematic of PSMA upregulation in combination with PSMA targeted therapy.** Treatment with a DNA damaging agent (1), leads to upregulation of PSMA protein (2). If utilized prior to targeted therapy (3) this may lead to improved efficacy of PSMA targeting therapy (4) reducing overall tumor burden for patients. |