

# Androgen Receptor and Poly(ADP-ribose) Glycohydrolase Inhibition Increases Efficiency of Androgen Ablation in Prostate Cancer Cells

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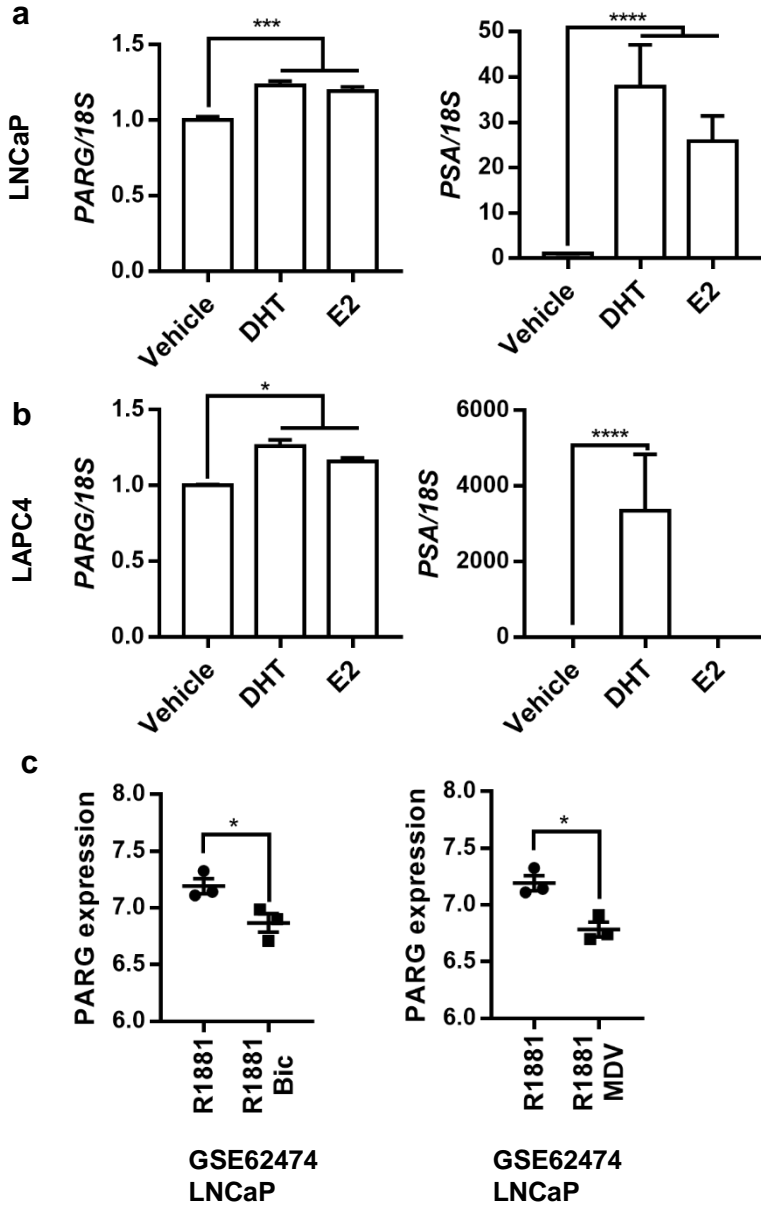
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Gene	Forward	Reverse	Probe
<b>18S</b>	gcaattattccccatgaacg	gggacttaatcaacgcaagc	48
<b>AR</b>	gccttgctctctagcctcaa	gtcgtccacgtgtaagttgc	14
<b>INPP4B</b>	tgtctgatgctgacgctaaga	ccacaaaccaatccagcaa	41
<b>PARG</b>	tctgccaatcacacagtaactattc	taatgtgttgaaaaggtttagga	2
<b>TMPRSS2</b>	ctgatcacaccagccatgat	tatcccctatcagccaccag	4
<b>UGT2B17</b>	tgtcagaagaaagtgccaaca	gccatcaaatctccatagaacc	61
<b>PARG</b>	aaagcgcattacttcaaaaactg	gcaagtcaggatccaagagaa	16
<b>PSA promoter</b>	cccctgttctgtttcatcc	gcctgtagctcatggagac	82

**Supplemental Table 1.** qPCR primer sequences and corresponding probes used to analyze gene expression.

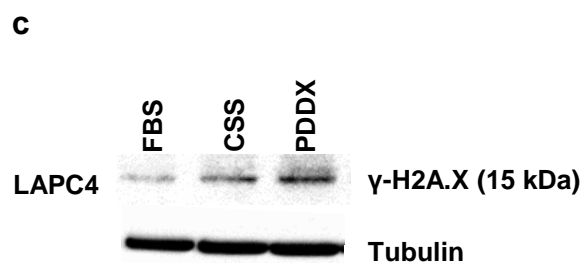
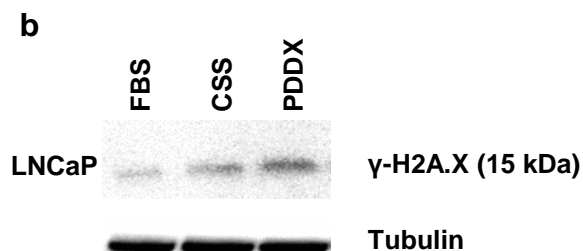
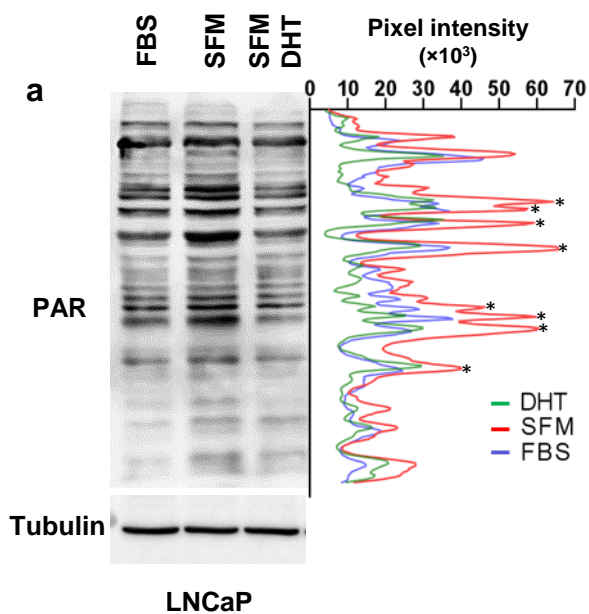


## Supplementary Figure S2



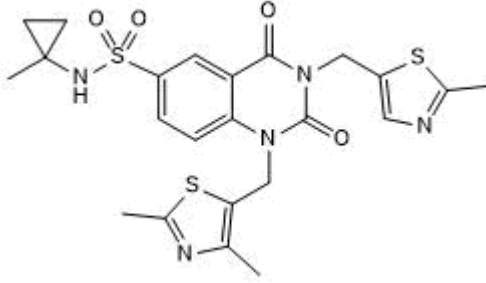
**Supplementary Figure S2.** LNCaP (a) or LAPC4 (b) cells were placed in medium supplemented with 10% CSS. Next day cells were treated with 100 nM dihydrotestosterone (DHT) or 100 nM estradiol (E2) as indicated for additional 72 hours. RNA was analyzed for expression of *PARG* and *PSA*. Expression was normalized to *18S*. Each bar is an average of 3 biological replicates. The experiments were performed 3 times. (c) *PARG* expression in LNCaP cells treated 24 hours with R1881 or R1881 plus bicalutamide (Bic, left) or enzalutamide (MDV, right) for 24 hours. Data were acquired from GSE62474.

## Supplementary Figure S3



**Supplementary Figure S3.** A. LNCaP cells were placed in RPMI medium with 10% FBS or serum-free medium (SFM). Cells were treated with vehicle or 10 nM DHT as indicated for 5 days. Protein levels of PAR and tubulin were assessed by Western blotting and the densitometry profile of PAR is shown. Three measurements of signal intensities were performed along each line and averaged and normalized to signal intensity of the tubulin band. PAR intensities that were significantly changed from FBS control ( $p < 0.05$ ) were marked with an asterisk \*. The experiment was performed 3 times.

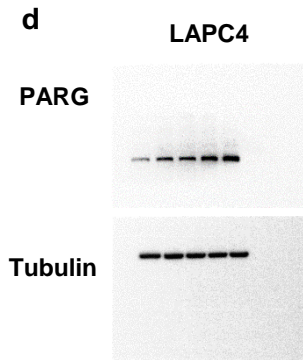
## Supplementary Figure S4

PDD00017272 Properties	
Structure	 <p>The chemical structure of PDD00017272 features a central benzimidazole ring system. It is substituted with a tert-butylsulfonamide group at the 2-position, a 2-methyl-1,3,4-thiazol-5-ylmethyl group at the 4-position, and a 2,4,6-trimethyl-1,3,4-thiazol-5-ylmethyl group at the 5-position. The benzimidazole ring also has a carbonyl group at the 2-position and a methyl group at the 4-position.</p>
Solubility in PBS + 1% DMSO ( $\mu\text{M}$ )	5.2
Solubility in DMSO (mM)	20
PARG Biochemical EC <sub>50</sub> ( $\mu\text{M}$ )	0.0048
PARG Cell EC <sub>50</sub> ( $\mu\text{M}$ )	0.0092
Cytotoxicity ( $\mu\text{M}$ ) (HeLa)	>30
Lipophilicity (XLogP)	4.7
Intrinsic Clearance CL <sub>int</sub> ( $\mu\text{L}/\text{min}/\text{mg}$ ) (human hepatocytes)	135
Half Life (human hepatocytes)	10 min

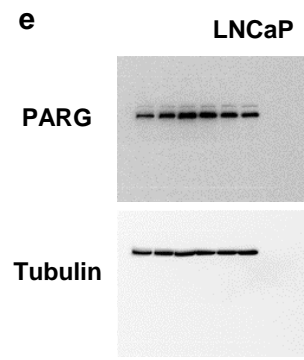
Supplementary Figure S4. Biochemical properties of PDD00017272

# Supplementary Figure S5

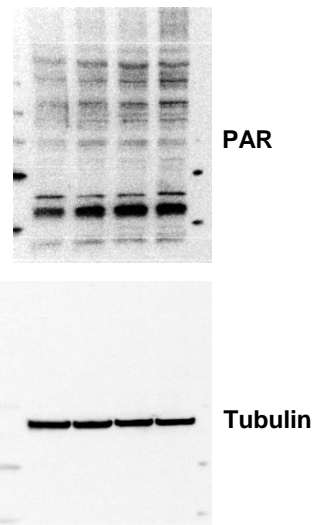
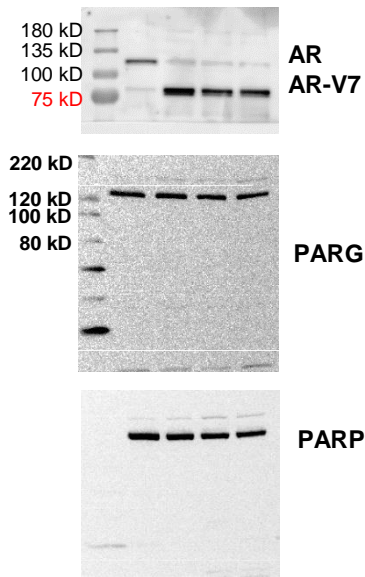
## Figure 1



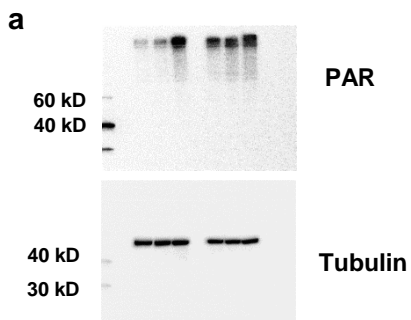
## Figure 1



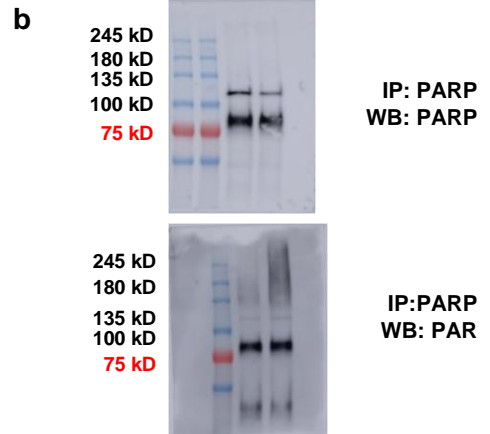
## Figure 2



## Figure 3



## Figure 3



Supplementary Figure S5 continue

Figure 5

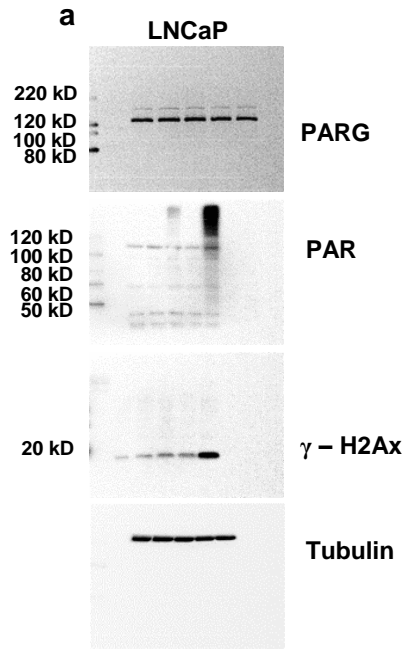
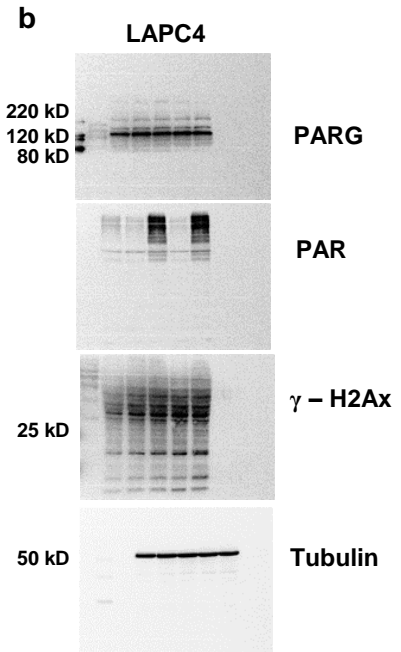


Figure 5



Supplementary Figure S5. Full length images of cropped blots presented in Figures 1, 2, 3 and 5.

## Supplementary Figure S6

Abbreviation	Definition
APE1	Apurinic/pyrimidinic endonuclease 1
AR	Androgen receptor
ARH1	ADP-Ribosylarginine Hydrolase 1
ARH3	ADP-Ribosylarginine Hydrolase 3
BER	Base excision repair
ChIP Assay	Chromatin ImmunoPrecipitation Assay
CRPC	castration-resistant prostate cancer
CSS	Charcoal Stripped Serum
DSB	double stranded breaks
ERG	ETS Transcription Factor
FBS	Fetal Bovine Serum
FoxA1	Forkhead Box A1
KDM4D	Lysine Demethylase 4D
LIG III	DNA Ligase 3
PAR	poly ADP-Ribose
PARG	Poly(ADP-ribose) glycohydrolase
PARP	poly(ADP-ribose) polymerase
Pol $\beta$	polimerase $\beta$
RAR	Retinoic Acid Receptor
SSB	Single Strand Break
TMPRSS2	Transmembrane Serine Protease 2
TMZ	temozolomide
UGT2B17	UDP Glucuronosyltransferase Family 2 Member B17
XRCC1	X-Ray Repair Cross Complementing 1
$\gamma$ H2A.X	Phosphorylated form of H2A histone family member X

Supplementary Figure S6. Abbreviations.