AR-v7 protein expression is regulated by protein kinase and phosphatase

Supplementary Material

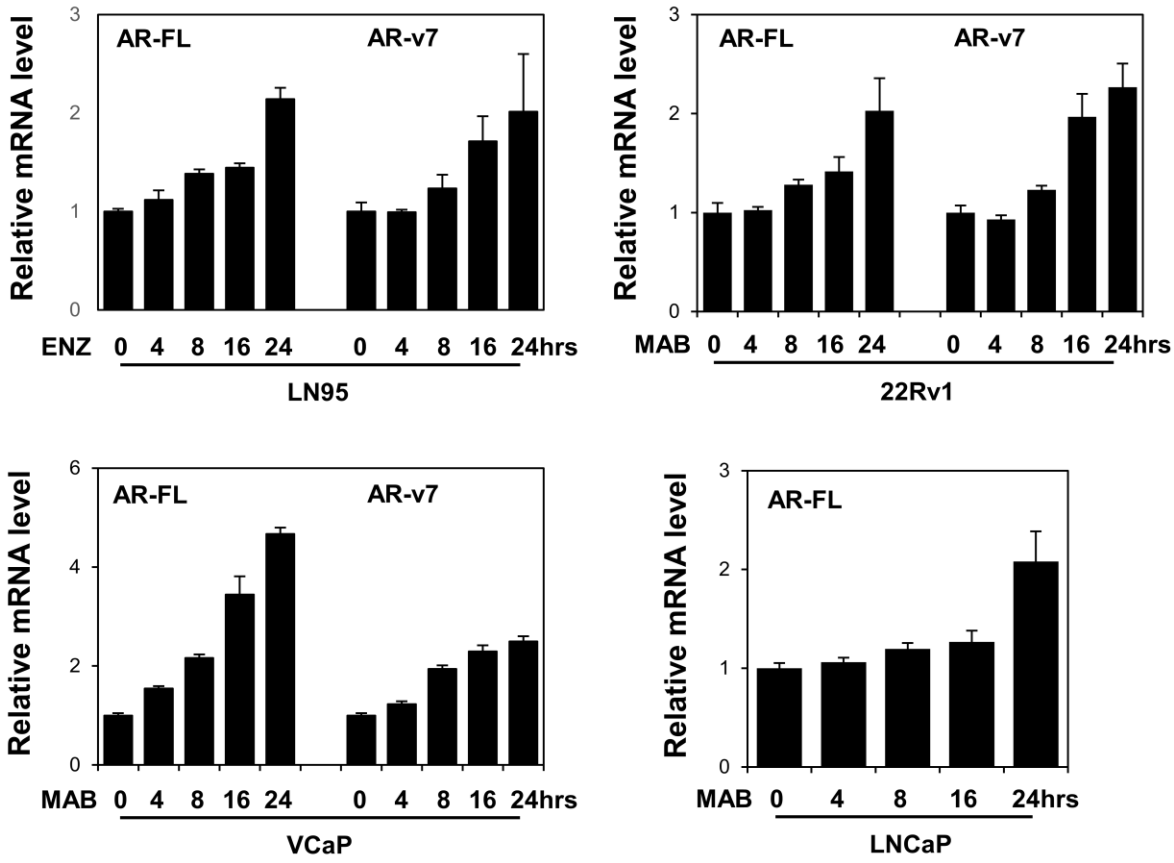

Figure S1

LNCaP, 22Rv1 and VCaP cells were cultured in medium containing 5% FBS and LNCaP95 cells in medium containing 5% charcoal stripped serum (CSS) for 24 hours before replaced with medium containing 5% CSS and 5μM of enzalutamide (ENZ) for 0-24 hours. Total mRNA was extracted. AR-FL and AR-v7 mRNA levels relative to 18S rRNA were determined by real-time qPCR.

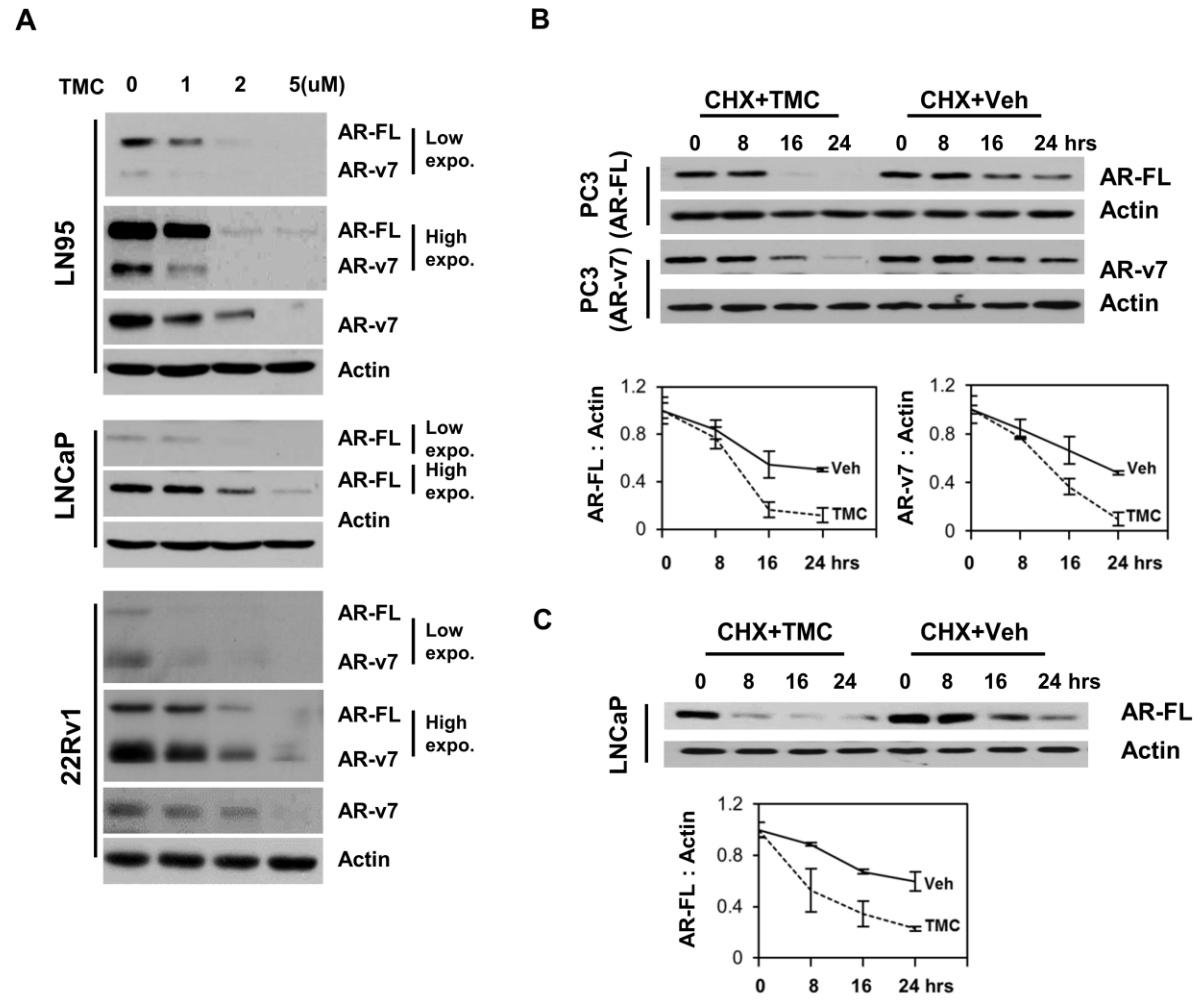

Figure S2

LNCaP, PC-3 and 22Rv1 cells were cultured in medium containing 5% FBS. LNCaP95 cells were in medium containing 5% charcoal stripped serum (CSS). **(A)**. LNCaP95, LNCaP and 22Rv1 cells were treated with 0, 1, 2, and 5μM of tautomycetin (TMC) for 0-24 hours. AR-FL, AR-v7 and β-actin protein levels were measured by Western blotting. (**B**) PC3(AR-FL) and PC3(AR-v7) cells and (**C**) LNCaP cells were treated with 50μg/ml cycloheximide (CHX) plus either vehicle or 5μM of TMC for 0-24 hours. AR-FL and AR-v7 protein levels were measured by Western blotting. Densitometry analyses of AR-FL and AR-v7 levels were normalized to β-actin. Results were from triplicate experiments. Data in line graphs represent means ± SD.

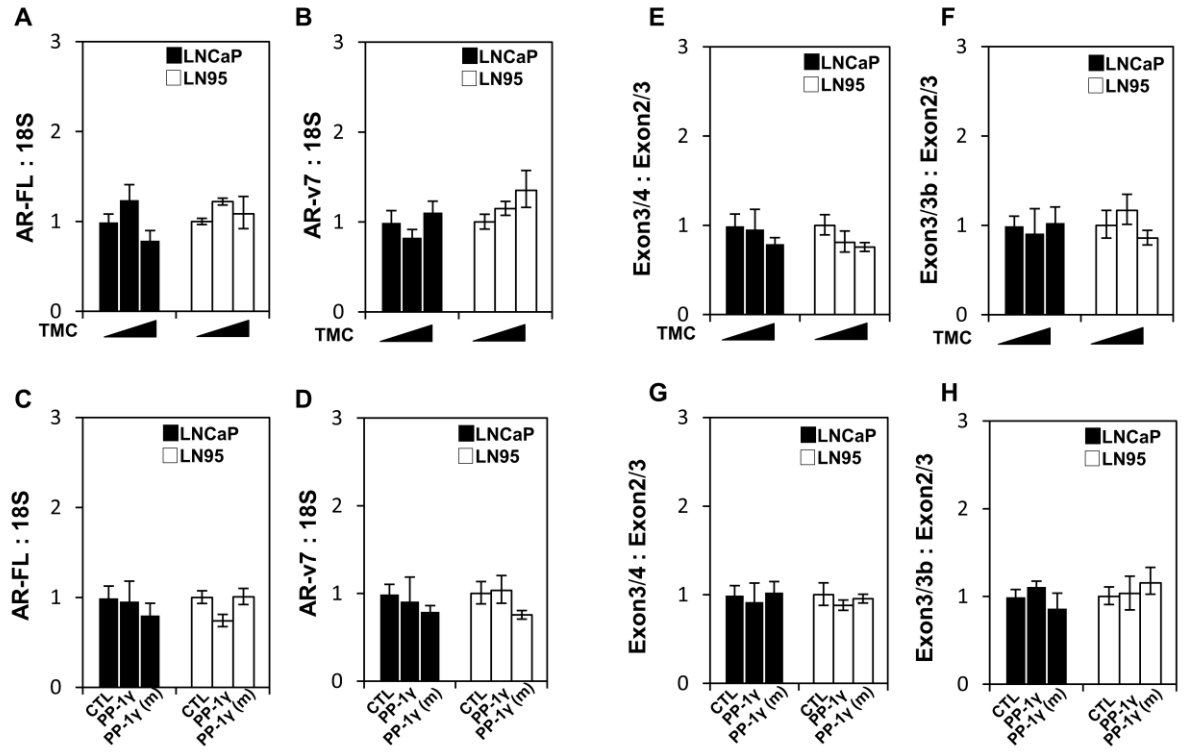

Figure S3

LNCaP cells were cultured in medium containing 5% FBS, while LNCaP95 cells were in medium containing 5% charcoal stripped serum (CSS). LNCaP and LNCaP95 cells were treated with 0, 2, 5μM of TMC (**A, B, E and F**) or transfected with expression vector of control, PP-1γ or PP-1γ(H125A) (**C, D, G and H**) for 24 hours. Real-time PCR measured AR-FL and AR-v7 mRNA levels relative to 18S (**A-D**). Real-time PCR measured AR-FL splicing (**E and G**) and AR-v7 splicing (**F and H**) rates relative to all AR transcripts. Primers were designed to cover the junctions of exons 3 and 4 for AR-FL, exons 3 and 3b for AR-v7 and exons 2 and 3 for total AR transcripts. Results were from three independent experiments. Data in line graphs represent means ± SD.

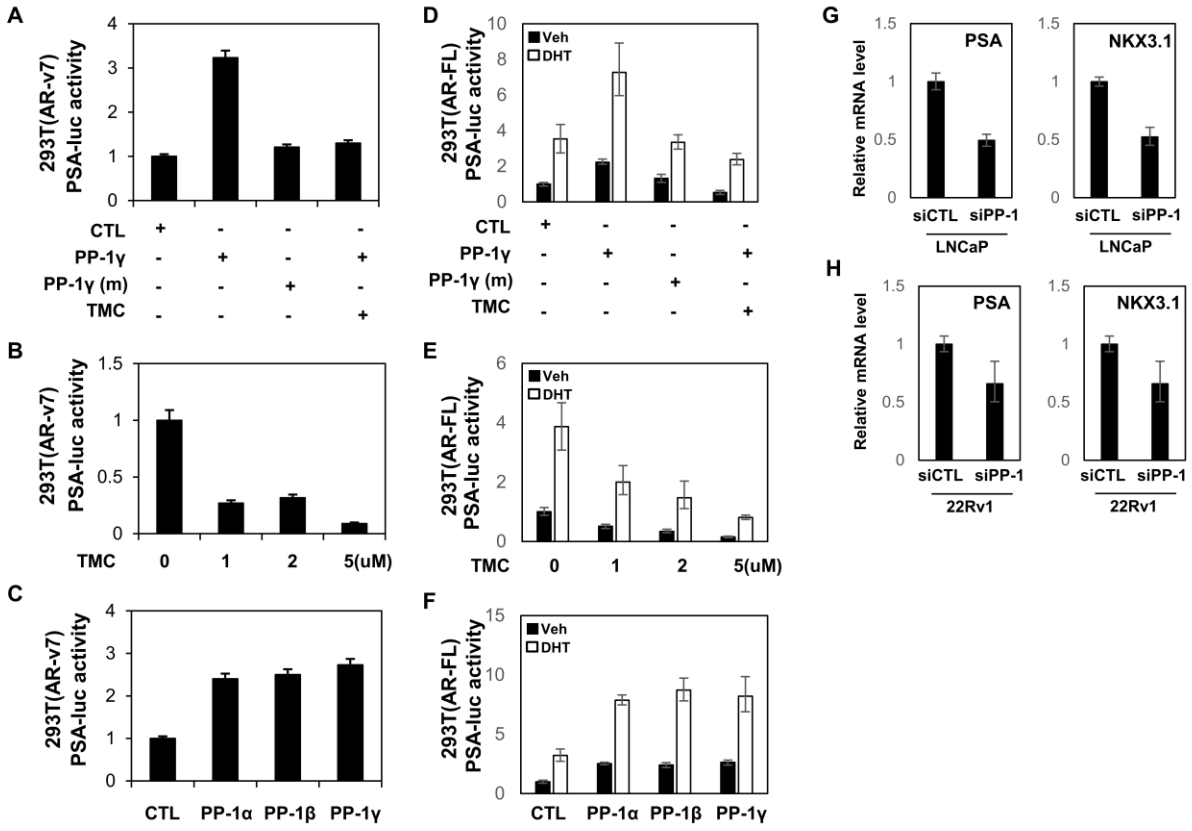

Figure S4

293T cells were transfected with AR-v7 (**A, B and C**) or AR-FL (**D, E and F**) expression vector, PSA-luciferase reporter and a renilla reporter. (**A and D**) Cells were also transfected with control, PP-1γ or PP-1γ(m) plasmid and treated with either vehicle or 5μM of TMC for 24h. (**B and E**) Cells were treated with 0, 1, 2, 5μM of TMC for 24 hours. (**C and F**) Cells were co-transfected with control, PP-1α, PP-1β or PP-1γ for 24 hours. Luciferase assays were performed as described in *Material and Method* section. Data in line graphs represent means ± SD. (**G and H**) (G) LNCaP and (H) 22Rv1 cells were transfected with control siRNA or pooled siRNA against PP-1α, PP-1β or PP-1γ. Total mRNA was collected after 48 hours post transfection. Real-time PCR measured PSA and NKX3.1 mRNA levels relative to 18S.

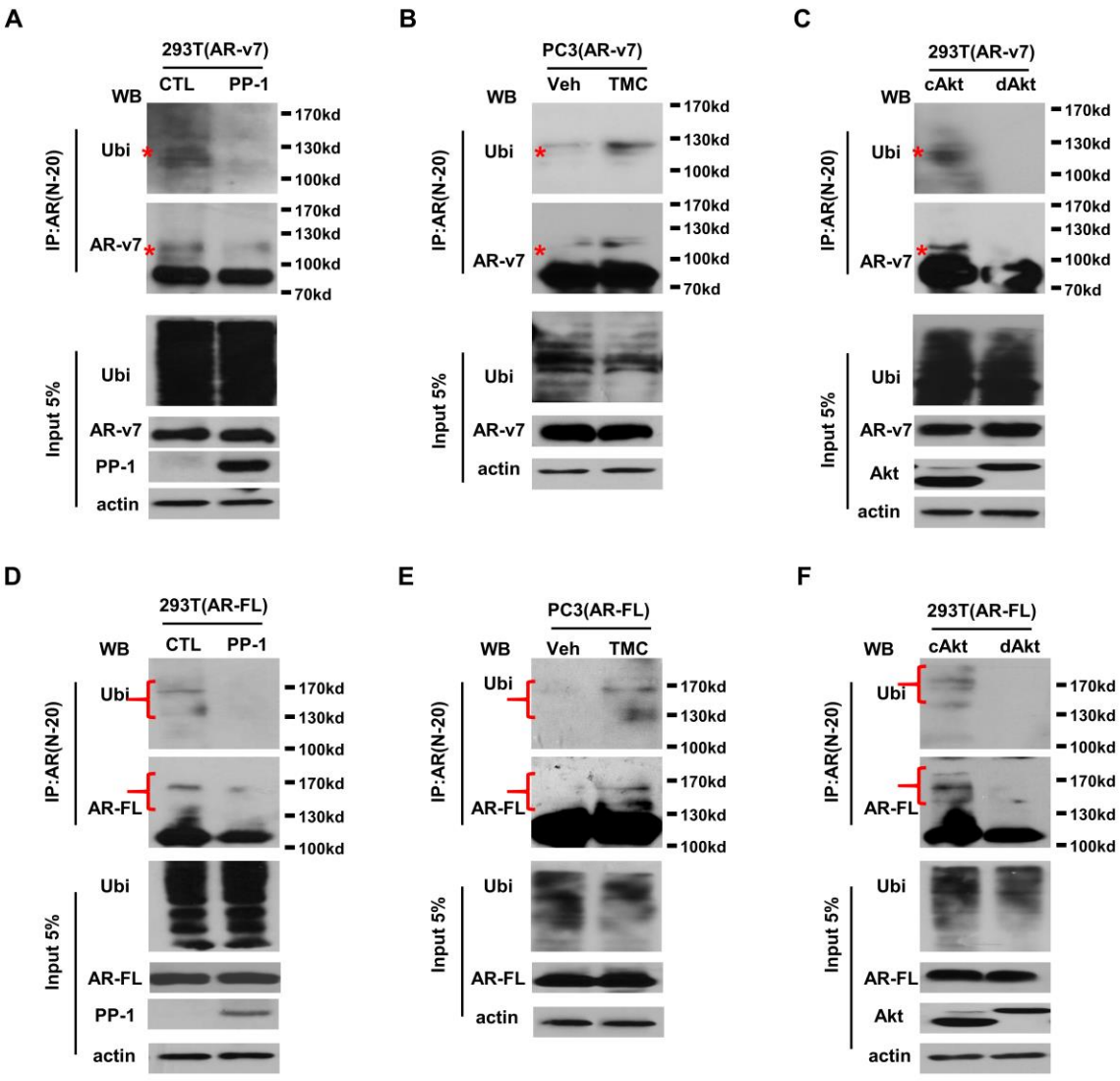

Figure S5.

(**A and C**) 293T cells were transfected with AR-v7 plasmid. (**B**) PC3(AR-v7) cells were stably expressing AR-v7. (**D and F**) 293T cell was transfected with AR-FL plasmid. (**E**) PC3(AR-FL) cells were stably expressing AR-FL. (**A and D**) Cells were also transfected with either control or PP-1α expression vector. (**B and E**) Cells were also treated with vehicle or 5μM of TMC. (**C and F**) Cells were also transfected with cAkt or dAkt expression vector. Cells were also treated with 2μg/ml of MG132 for another 16 hours. *In vivo* ubiquitination assays were performed as described in *Material and Method* section. All experiments were repeated at least three times with one set of results shown in the figure.

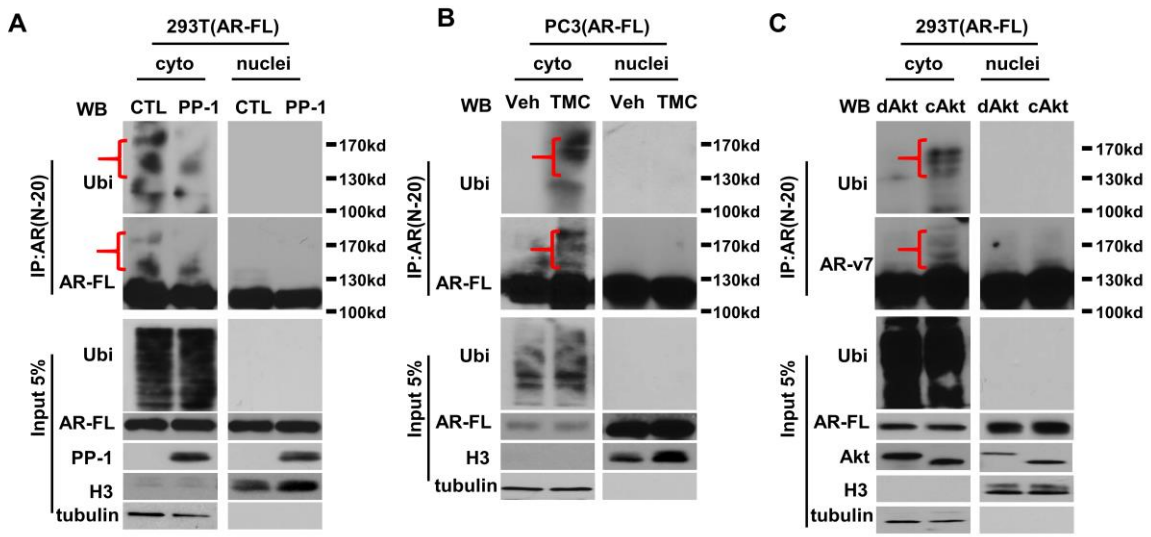

Figure S6.

293T and PC-3 cells were cultured in medium containing 5% FBS. 293T cells were transfected with AR-FL (**A and C**) plasmid. PC3 cells were stably introduced AR-FL (**B**). Both cell lines were culture in medium containing 5% FBS. Cells were co-transfected with either control or PP-1α plasmid (**A**), or treated with vehicle or 5μM of TMC (**B**) or transfected with dAkt or cAkt expression vector for 24 hours (**C**). Cells were also treated with 2μg/ml of MG132 for another 16 hours. Cytoplasmic and nuclear fractions of protein lysis were extracted. Histone 3 (H3) and tubulin were detected by Western blotting and used as markers to confirm the efficacy of the nuclear fraction extraction. *In vivo* ubiquitination assays were performed as described in *Material and Method* section. All experiments were repeated at least three times with one set of results shown in the figure.

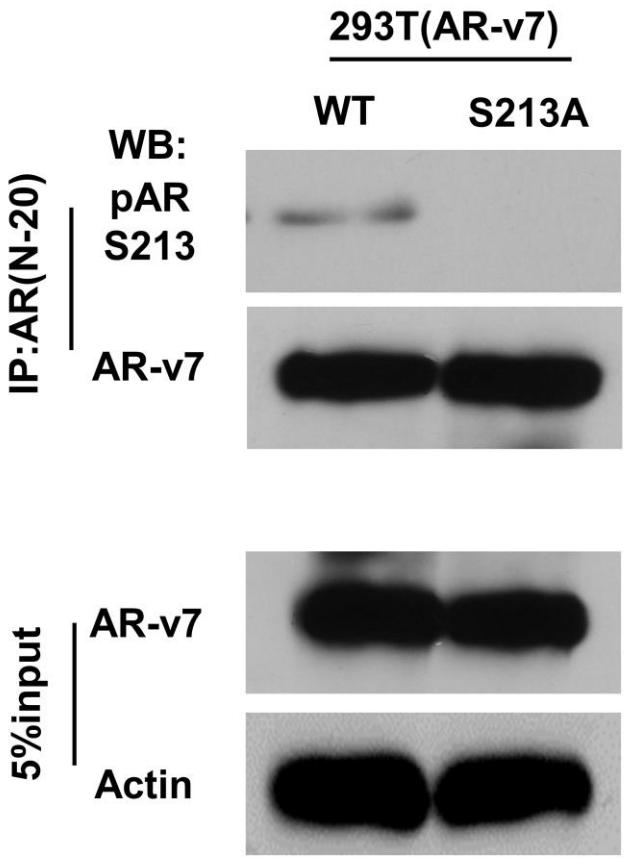

Figure S7
293T cells were transfected with wild type or mutant AR-v7 expression vector (S213A) for 24 hours. Protein lysates were and co-immunoprecipitated with AR (N-20) antibody and immunoblotted with phosphor-AR(Ser213) and AR(N-20) antibodies.

SUPPLEMENTARY MATERIALS

Table S1 Reagents

Name	Cat No.	Company
Enzalutamide	HY-70002	Haoyuan Chemexpress
Tautomycetin	2305	Tocris Bioscience
MG132	13697	Cayman Chemical
Epoxomicin	10007806	Cayman Chemical
Cycloheximide	14126	Cedarlanelab

Table S2 Real-time qPCR primers

Name and location	Sequences
AR-FL(Exon 3) F:	5'-TCTTGTCGTCTTCGGAAATGT-3'
AR-FL(Exon 4) R:	5'-AAGCCTCTCCTTCCTCCTGTA-3'
AR-v7(Exon 3/3b) F:	5'-CAGGGATGACTCTGGGAGAA-3'
AR-v7(3’ UTR) R:	5'-GCCCTCTAGAGCCCTCATTT-3'
18S rRNA F:	5′-TTGACGGAAGGGCACCACCAG-3′
18S rRNA R:	5′-GCACCACCACCCACGGAATCG-3’
PSA F:	5'-AGTGCGAGAAGCATTCCCAAC-3'
PSA R:	5'-CCAGCAAGATCACGCTTTTGTT-3'
NKx3.1 F:	5'-CCCACACTCAGGTGATCGAG-3'
NKx3.1 R:	5'-GAGCTGCTTTCGCTTAGTCTT-3'
AR total(Exon 2) F:	5'-GTGGAAGCTGCAAGGTCTTC-3'
AR total(Exon 3) R:	5'-GGCGCACAGGTACTTCTGTT-3'

Table S3 siRNAs

Name	Cat No.	Company
siCTL	sc-37007	Santa Cruz
siMdm2	L-003279-00-0005	Dharmacon
siPP-1α	sc-36299	Santa Cruz
siPP-1β	sc-36295	Santa Cruz
siPP-1γ	sc-36297	Santa Cruz
siAkt1/2	sc-43609	Santa Cruz

Table S4 Antibodies

Name	Description	Cat No.	Company
AR	N-20	Sc-816	Santa Cruz
AR-v7		AG10008	Precision Antibody
pAR	Ser213	Sc-135635	Santa Cruz
Akt		9272	Cell signaling
pAkt	Ser473	9271	Cell signaling
PP-1α	C-19	Sc-6104	Santa Cruz
PP-1γ	C-19	Sc-6108	Santa Cruz
Mdm2	SMP14	Sc-965	Santa Cruz
pMdm2	Ser166	Sc-293105	Santa Cruz
Actin	Ac-15	A5441	Sigma
Flag-tag	M5	F4042	Sigma
HA-tag		Sc-7392	Santa Cruz
Ubiquitin	P4D1	3936	Cell signaling
Histone H3		Ab-1791	Abcam
Tubulin	TU02	Sc-8035	Santa Cruz