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Supplemental Data

Supplemental Figure legends

Supplemental Fig. 1. A, Proteins features that interact with AR. Extract from PC3wtAR cells was passed through an AR conjugated immuno-column to isolate AR specific interacting proteins. The bound proteins were then eluted for separation on a 2-dimensional gel electrophoresis (2-DE) followed by silver staining. Selected protein features were excised using a sterile 200µl pipette tip and placed in a PCR tube with 5µl of HPLC grade water and sequenced by mass spectrometry. The spot identified as p23 is circled in red. B & C, Protein levels in GST pulldown assays in Figures 1B and 5A. GST- alone or GST- fused to full length p23 or truncated domains was expressed in *E.Coli* BL21 strain by Isopropyl-β-D-thiogalactopyranoside (IPTG) induction using the pGEX-6P1 vector system. Induced GST fused protein was then purified from the culture using glutathione affinity sepharose beads. Comparable amounts of GST control or GST-p23 was then used (as shown in the Coomasie blue-stained SDS-PAGE) for the pulldown assay.

Supplemental Fig. 2. Protein expression levels for cells used in reporter assay (Figure 2A and B) Western blot shows expression levels of AR. 5 µg of total protein was extracted from A, PC3wtAR and B, MCF7 cells following transient transfection with pSG5-p23 for subsequent separation by SDS-PAGE. The Western blot was probed using antibodies specific for AR, p23 and β-actin. Figures are representative blots.

Supplemental Fig. 3. p23 protein expression levels in response to DOX and knockdown treatments. A, LNCaP-TR2-p23 cells (stably transfected with a doxycycline-inducible plasmid expressing V5 tagged p23) were cultured in media containing 100 nM DOX for 48 h prior to starvation for 72 h in the presence of 100 mM DOX. Cells were then treated ± 10 nM MB for 6, 10 or 16 h and harvested. 5 µg of protein

was resolved and probed with antibodies against V5 and β-actin. B, LNCaP cells were transiently transfected with siRNA pool against p23 or scrambled negative control. 48 h later, cells were treated ± 10 nM MB ± 10 nM 17-AAG for 24 h before harvesting and probing 5 μg extract with antibodies against p23 and β-actin.

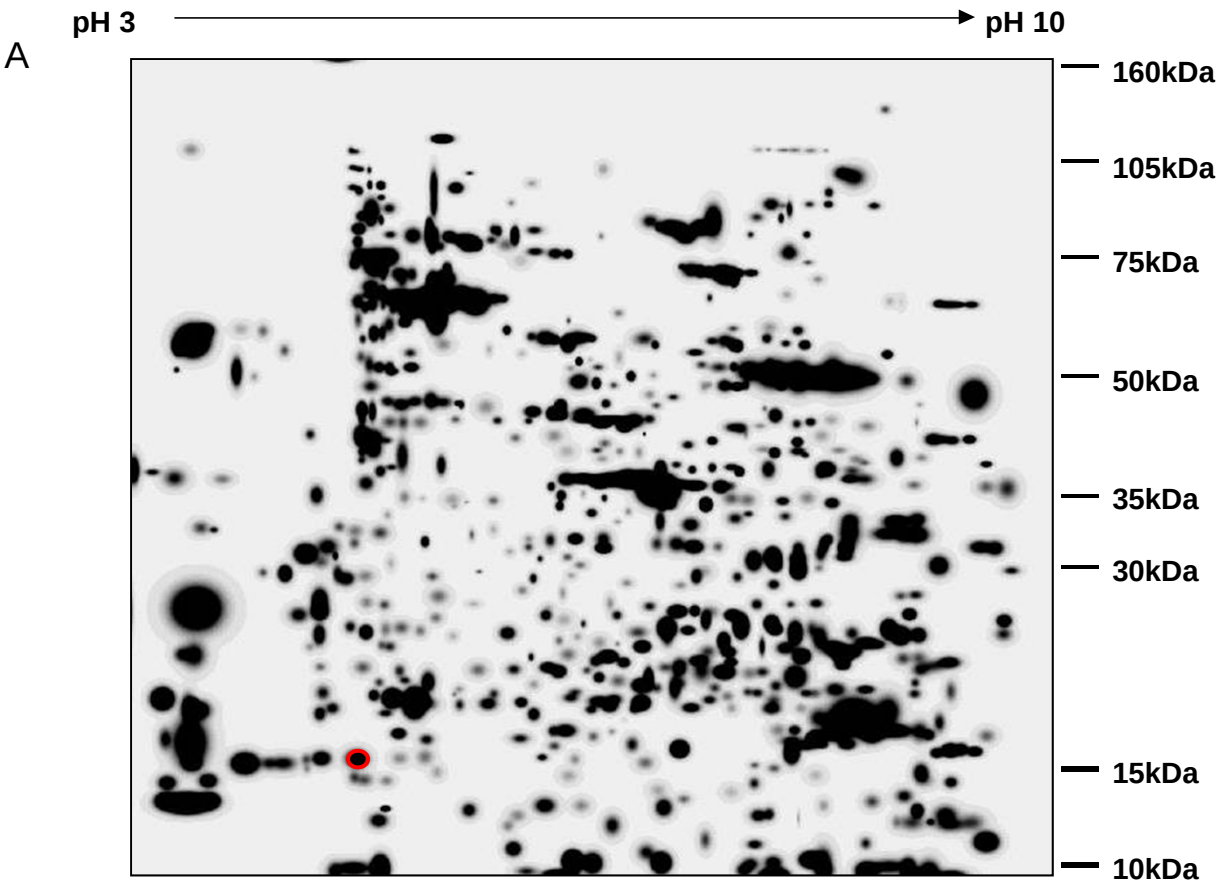
Supplemental Fig. 4. Subcellular localization of p23 and AR. A, PC3wtAR cells were grown on glass coverslips to 70% confluency. Cells were starved for 48 h prior to treatment with 10 nM MB or vehicle control for 2 h. Cells were washed with PBS before fixing in 4% paraformaldehyde (PFA) for 15 min followed by immunostaining for p23 and AR using Alexa-488 or Alexa-596 secondary antibody respectively for visualization by confocal microscopy. B, Chromatin immunoprecipitation was performed on LNCaP cell extracts incubated with 10 nM MB for 2 h. IP was performed using an antibody specific for p23 or control IgG and RT-qPCR performed on the immunoprecipitated DNA to detect the presence of one non-ARE (Negative region) and two specific ARE sequences (Enhancer and Promoter) of the AR target gene, *KLK2*.

Supplemental Fig. 5. The effect of HSP90 inhibitors on cell viability as well as AR and p23 expression levels. . A, LNCaP cells were treated with vehicle (DMSO) or 5 μM 17-AAG for the specified time points. Cell viability was determined by using the MTT viability assay with absorbance readings at 570 nm. B, PC3wtAR cells were treated with ± 10 nM MB, ± geldanamycin (GA) at the indicated concentrations, for 2 h. Immunoprecipitation was performed using anti-p23 antibody and the precipitated complex resolved and probed using antibodies against p23, HSP90 and AR. C, MCF7 cells were grown for 24 h in full media prior to being incubated in starvation media for 24 h. 1, 10 and 100 nM of GA in combination with 10 nM MB was added to the cells for a further 16 h. Cell toxicity was determined by using the sulforhodamine B (SRB) assay with absorbance readings at 495 nm. Gray bars: time = 0 hours, black bars: time = 16 hours.

Supplemental Table 1. RT-qPCR primers used in this study.

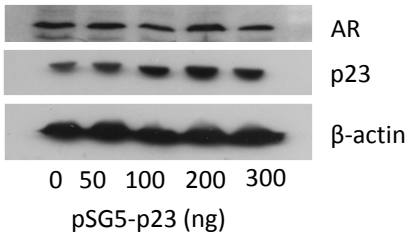
qRT-PCR primer name	Sequence or Applied bioscience reference code
p23	HS00832847_9H
GAPDH	HS99999905_A1
PSA	*HS00426383_A1*
KLK2	*HS00377590_S1*
PSA (Forward)	5'-TTGTCTTCCTCACCCTGTCC- 3'
PSA (Reverse)	5'-AGCTGTGGCTGACCTGAAAT- 3'
KLK2 (Forward)	5'-CCTCACGTTCTGGCATCACTT- 3'
KLK2 (Reverse)	5'-CGGCCAGGTGAGTTCCAA- 3'
DRG1 (Forward)	5'-GCAGCACACACTTCACAAAGC- 3'
DRG1 (Reverse)	5'-CCAGGCACCCGTTTGAAC- 3'
TMPRSS 2 (Forward)	5'-AATCGGTGTGTTCGCCTCTAC- 3'
TMPRSS 2 (Reverse)	5'-AATCGGTGTGTTCGCCTCTAC- 3'
L19 (Forward)	5'-GCGGAAGGGTACAGCCAAT- 3'
L19 (Reverse)	5'-GCAGCCGGCGCAAA- 3'
PSA-negative region (Forward)	5'-TCCACTCCAGCTCTAAGATGGT- 3'
PSA-negative region (Reverse)	5'-CAGGTAAACTCCAAGCACAGTGA- 3'
PSA-Promoter-ARE (Forward)	5'-GTGCATCCAGGGTGATCTAGTAATT- 3'
PSA – Promoter-ARE (Reverse)	5'-CACACCCAGAGCTGTGGAA- 3'
PSA – Enhancer-ARE (Forward)	5'-TGACAGTAAACAAATCTGTTGTAAGAGACA- 3'
PSA – Enhancer-ARE (Reverse)	5'-AGCAGGCATCCTTGCAAGAT- 3'
KLK2 – Negative region (Forward)	5'-TGGGTGATGTGGTTGGATTGG- 3'
KLK2 – Negative region (Reverse)	5'-CCCATGATAACCTCAACCAAAACCT- 3'
KLK2 – Promoter-ARE (Forward)	5'-GCCTCCAGACTGATCTAGTATGTGT- 3'
KLK2 – Promoter-ARE (Reverse)	5'-CACACCCAGAGCTGTGGAA- 3'
KLK2 – Enhancer-ARE (Forward)	5'-TTTATAATTGGGTTGAAAGCAGACCTAC-3'
KLK2 – Enhancer-ARE (Reverse)	5'-AGCAGATTTGTTTACTGTTCAGGACA-3'
TMPRSS2 – Enhancer ARE (Forward)	5'-TCCCAAATCCTGACCCCA-3'
TMPRSS2 – Enhancer ARE (Reverse)	5'-ACCACACAGCCCCTAGGAGA

Supplemental Figure 1



Supplemental Figure 2

A



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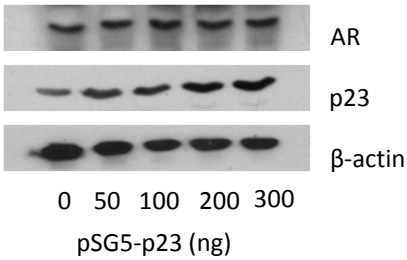
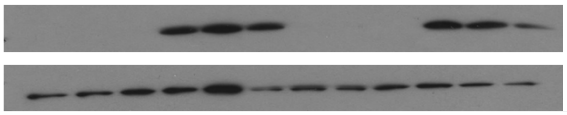


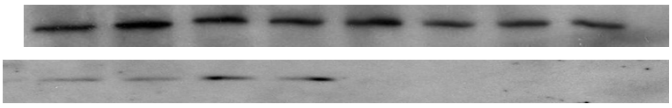
Supplemental Figure 3

A



	6	10	16	6	10	16	6	10	16	6	10	16	treatment (h)
DOX		-			+			-			+		
MB		-			-			+			+		

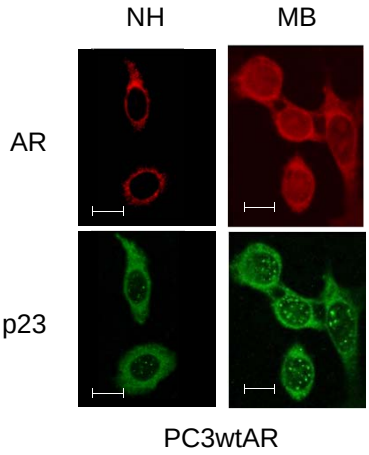
B



MB	-	-	+	+	-	-	+	+
17AAG	-	+	-	+	-	+	-	+
siRNA scrambled	+	+	+	+	-	-	-	-
siRNA p23	-	-	-	-	+	+	+	+

Supplemental Figure 4

A



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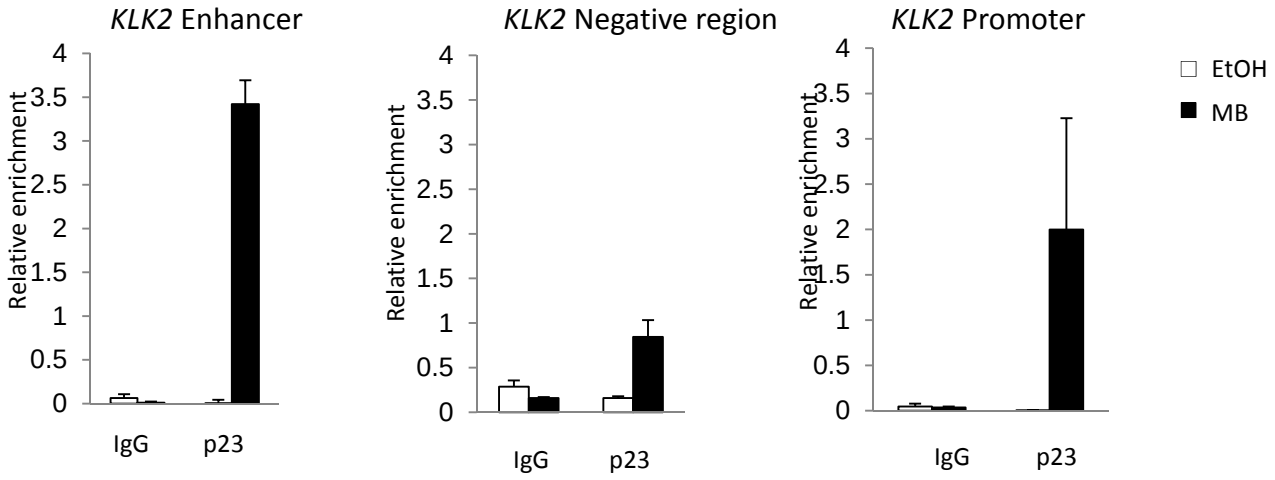

Supplemental Figure 5

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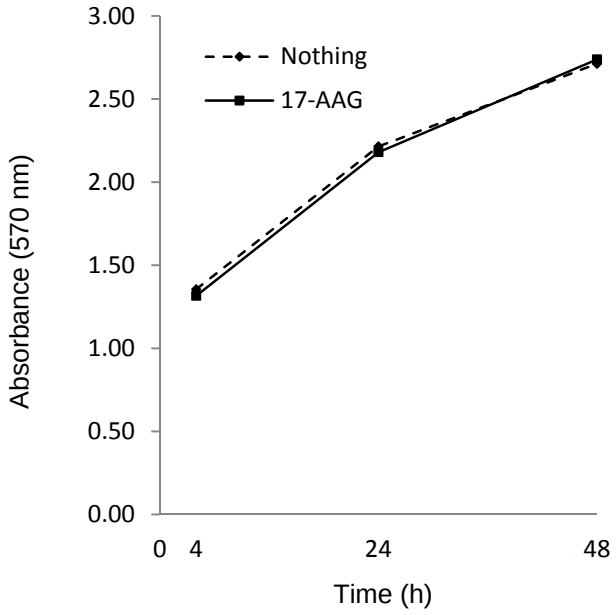

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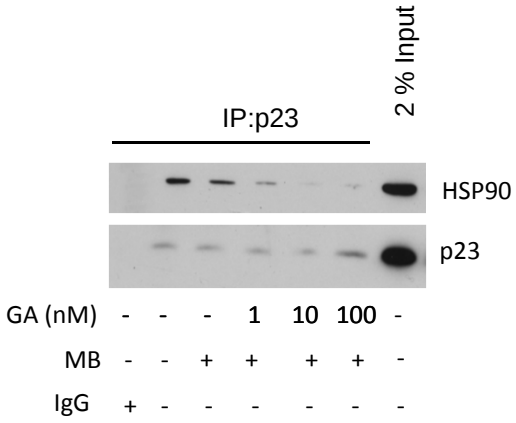

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