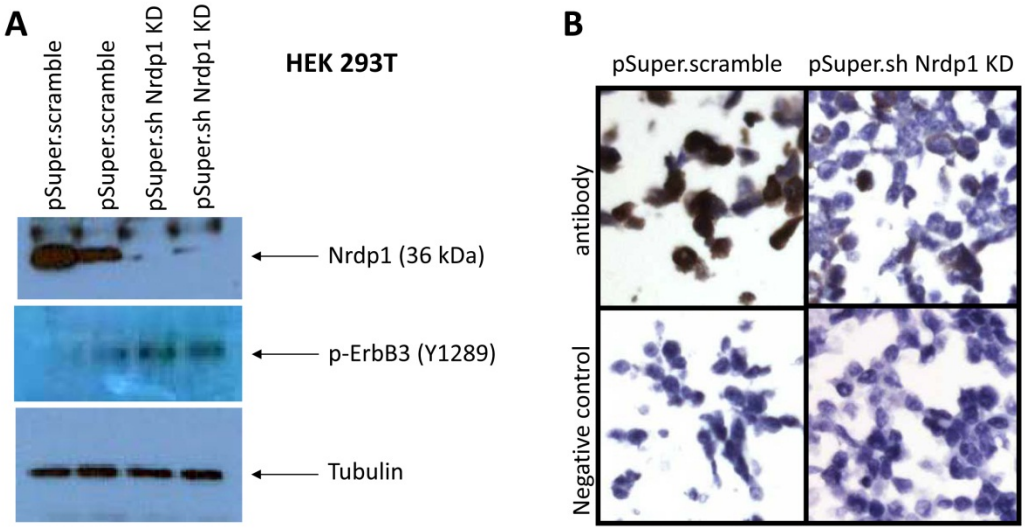
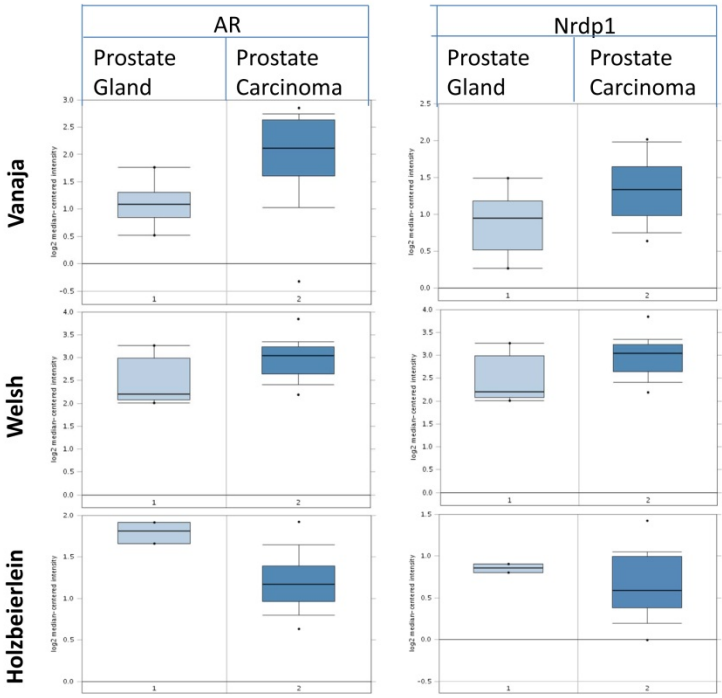
SUPPLEMENTARY MATERIAL

Transcription of *Nrdp1* by the androgen receptor is regulated by nuclear Filamin A in prostate cancer

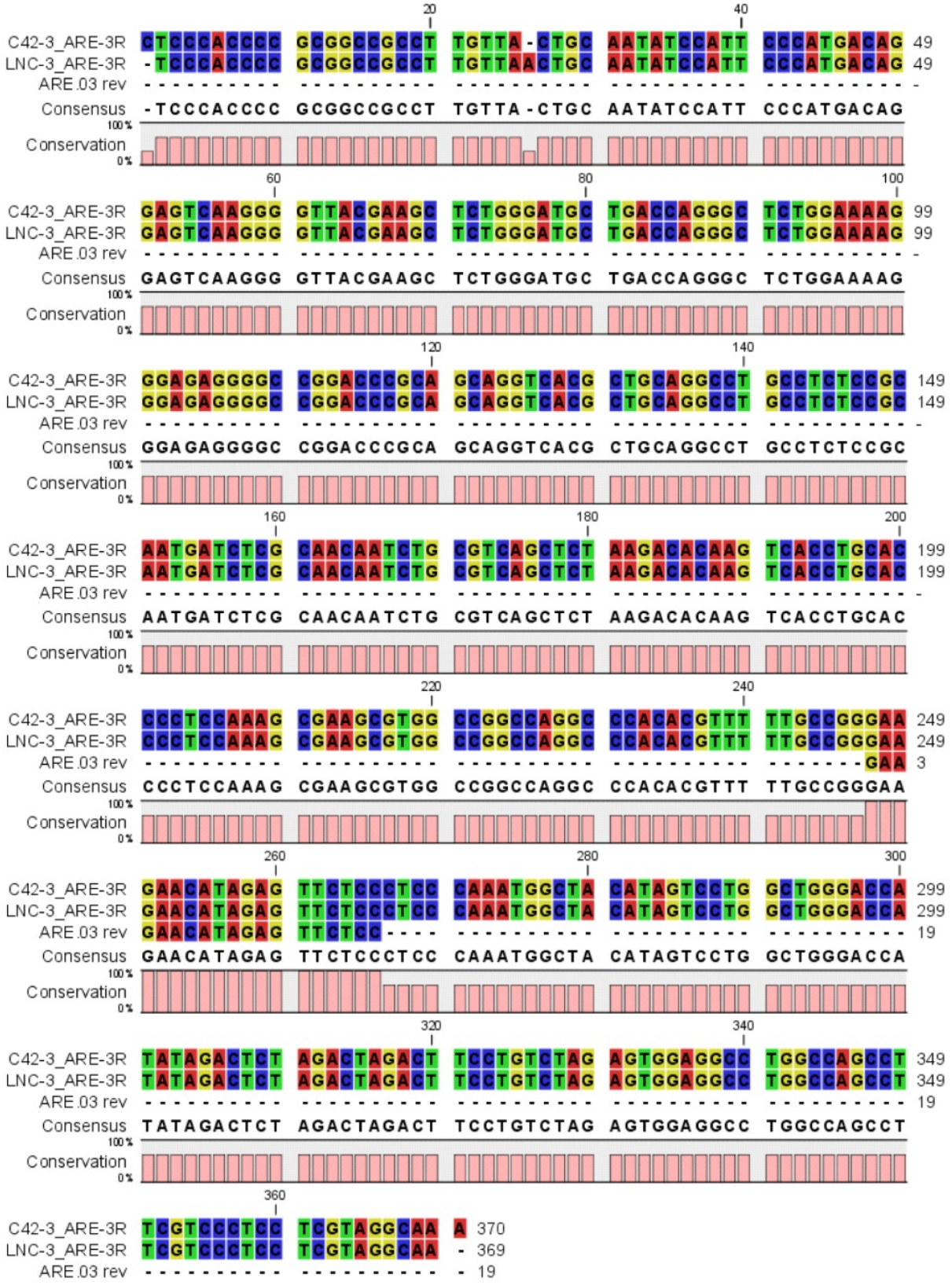
Supplementary Figure 1. Nrdp1 Antibody Characterization. 293T HEK cells were transfected with control or Nrdp1 shRNA to show the specificity of a rabbit polyclonal anti-Nrdp1 antibody. **(A)** Western blot analysis showed Nrdp1 staining as a 36 KDa band. Specificity of the band was determined by knockdown of Nrdp1 upon shRNA use, and a corresponding increase of p-ErbB3. 293T HEK cells were grown in FBS media. Cell lystaes were immunoblotted with andti-Nrdp1, anti-p-Erb3 and anti-tubulin antibodies. **(B)** 293T HEK cell pellets (similarly treated) were paraffin embedded, sectioned and stained with the same Nrdp1 antibody. Lack of background staining in the shRNA-treated cells denotes that the IHC staining was more specific.



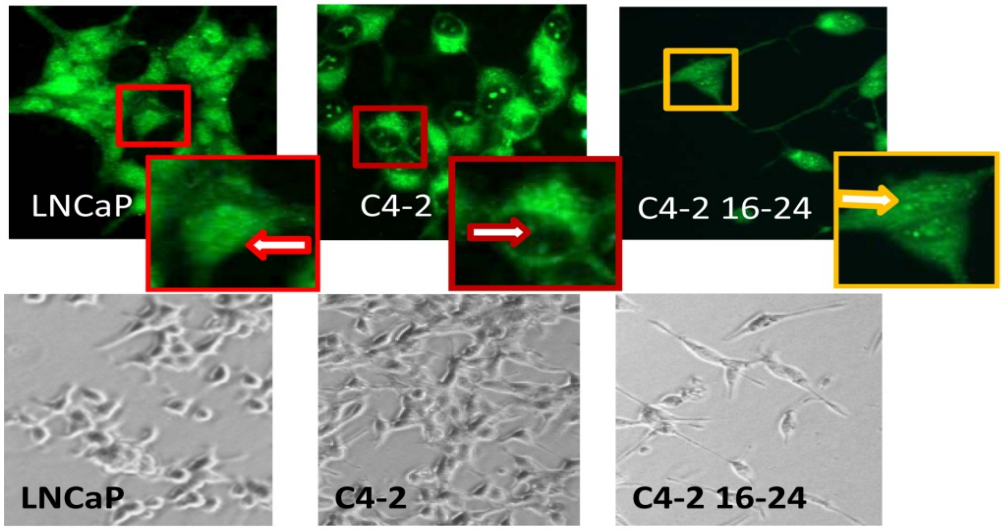


Supplementary Figure 2. AR and Nrdp1 expression in cancer and normal cells. Confirmation of the up regulation of Nrdp1 and AR in cancer vs. normal prostate and the correlation between three Oncomine datasets. (For AR, p = 3.26 x 10^-8; for Nrdp1, p = 1.62 x 10^-6; values calculated using Fisher's combined probability test). Box plots shown are from studies in the Oncomine database representative of overall trend. Note that Nrdp1 levels correspond to AR values.

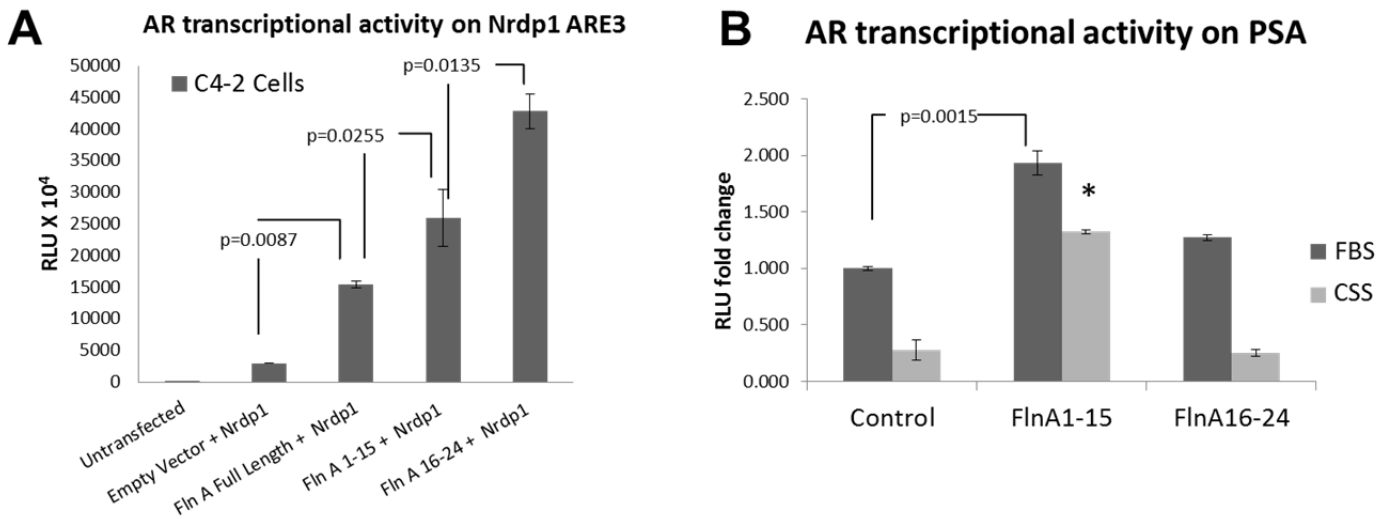
Supplementary Figure 3. Nrdp1 ARE.03 Genomic Sequence is not different in LNCaP and C4-2 cells despite altered AR binding. Nucleotide comparison of the Nrdp1 ARE.03 sequence in LNCaP and C4-2 showing no mutation in the sequence despite a change in AR binding in the two cell lines.



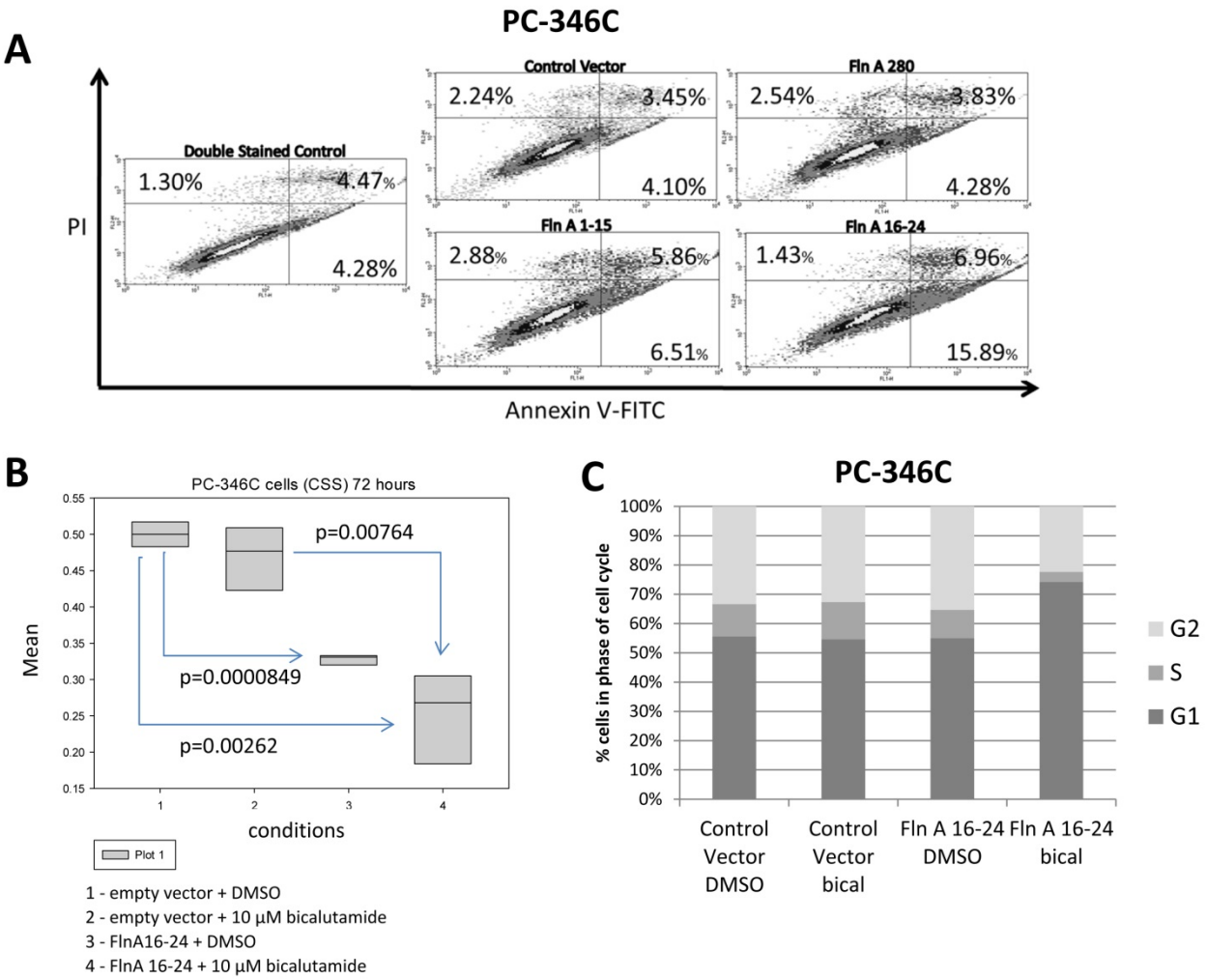
Supplementary Figure 4. Localization of FlnA expression in PCa cells. ***(upper panel)*** LNCaP and C4-2 cells were cultured in FBS and stained with anti-FlnA antibody. Note that in the LNCaP cells, FlnA is mostly nuclear (arrow points to region of FlnA staining (green) in the nucleus, while in the C4-2 cells, the staining is mostly cytoplasmic (arrow points to dark nuclei in the center, indicating lack of nuclear FlnA, though the nucleolus appears to stain green). Transfection of FlnA 16-24 restored FlnA nuclear localization in the C4-2 cells (see the green nucleus). ***(lower panel)*** phase contrast images of the same cells to show the number of cells. Note that FlnA16-24 expression significantly reduces the number of cells.



Supplementary Figure 5. Comparison of the effects of nuclear and cytoplasmic FlnA on Nrdp1 and PSA transcription by the AR and on apoptosis in PCa cells. (A) FlnA restores AR transcriptional activity on Nrdp1 ARE3 in androgen independent cells. C4-2 cells were cultured in FBS medium and transfected with full-length FlnA, FlnA repeats 1-15, or FlnA repeats 16-24, and AR transcriptional activity was measured by luciferase assay using a reporter construct containing *Nrdp1* ARE3. **(B)** Nuclear FlnA (FlnA16-24) induced AR transcriptional activity is androgen-sensitive whereas that by cytoplasmic FlnA (FlnA1-15) is androgen-resistant. C4-2 cells were grown in FBS or CSS-containing media and transfected with empty vector, FlnA 1-15, or FlnA 16-24. AR transcriptional activities on a PSA promoter construct containing ARE-I and ARE-II was measured by luciferase assay. In both FBS (p=0.0015) and CSS-containing media ("*"p<0.0001) FlnA 1-15 increased AR transcriptional activity at the PSA promoter, indicating hormone-insensitive upregulation of AR transcriptional activity, while in cells transfected with FlnA 16-24, AR transcriptional activity was not significantly altered.



Supplementary Figure 6. PC-346C cells isolated from a primary prostatectomy specimen responds to bicalutamide in the presence of FlnA 16-24. The following experiments were conducted in media containing charcoal stripped serum (CSS) as PC-346C cells do not express nuclear FlnA when cultured for prolonged periods in CSS. **(A)** Flow cytometric analysis in Annexin-V/PI-stained PC346C cells to determine the effect of the FlnA isoforms on apoptosis. Cells were cultured in FBS and transfected with either empty vector, full length FlnA, FlnA 1-15, or FlnA 16-24. At the end of 48 hours, the cells were harvested and analyzed for the onset of apoptosis using staining with Annexin V as an indicator of early and propidium iodide (PI) as as indicator of late apoptosis/cell death. Note that full length FlnA and FlnA 1-15 had little to no effect on apoptosis, while FlnA 16-24 increased the rate of apoptosis 3.7 fold (4.28 – 15.89%). **(B)** PC-346C cells were transfected with an empty vector or FlnA 16-24 and cultured for 72 hours in CSS. MTT assay showed that despite the fact that the cells were isolated from a primary tumor, those transfected with the empty vector had little response to 10 μM bicalutamide; however, when transfected with FlnA 16-24, the rate of cell growth slowed considerably and the cells significantly responded to the same dose of bicalutamide. **(C)** Cell cycle analysis by flow cytometry of PC-346C cells. The cells were transfected with an empty vector (pCMV) or FlnA 16-24 and treated with DMSO or 10 μM bicalutamide for 48 hours, at the end of which they were fixed in cold ethanol, stained with PI and the PI-stained fixed cells subjected to flow cytometry to identify the percentage of cells in each phase of the cell cycle (G1, S and G2/M). A decrease in the percentage of cells in S-phase in this case is an indicator of growth arrest. Note that growth arrest was observed only in cells transfected with FlnA 16-24 and treated with bicalutamide.



Supplementary Table 1: Primers used for qPCR

β-Actin	forward	5'-TCACCCACACTGTGCCCATCTACGA-3'
	reverse	5'-CAGCGGAACCGCTCATTGCCAATGG-3'
Nrdp1	forward	5′-ATCGCAGAGCTGGAGAAGACGT-3'
	reverse	5'-TGTCTCCTCCAGGTTCTGAAGG-3'
TMPRSS2	forward	5′-CCTGCATCAACCCCTCTAACTG-3′
	reverse	5′-AGGCGAACACACCGATTCTC-3′
AR	forward	5’-GACGACCAGATGGCTGTCATT-3’
	reverse	5’-GGGCGAAGTAGAGCATCCT-3’
FlnA	forward	5′-GTGAAC TCTGCCCGCTTCTT-3′
	reverse	5′-CCCTGCCAGGCATCG-3′
GDF15	forward	5’-AGAGATACGCAGGTGCAGGT-3’
	reverse	5’-AGCTGGGAAGATTCGAACAC-3’
IL32	forward	5’-CCTGAGCAGAAGTAGGGAGG-3’
	reverse	5’-TTGGCTCCTTGAACTTTTGG-3’
BHLHE40	forward	5'-GACGGGGAATAAAGCGGAGC-3'
	reverse	5'-CCGGTCACGTCTCTTTTTCTC-3'
HMOX1	forward	5'-AAGACTGCGTTCCTGCTCAAC-3'
	reverse	5'-AAAGCCCTACAGCAACTGTCG-3'

Supplementary Table 2: Primers used for ChIP analysis (AR IP or input):

Nrdp1 ARE3	forward	5’- CAACAATCTGCGTCAGCTCTA-3'
	reverse	5'-GCCAGGACTATGTAGCCATTT-3'
Znf333 (neg control)	forward	5'-TGAAGACACATCTGCGAACC-3'
	reverse	5'-TCGCGCACTCATACAGTTTC-3'
ARarfneg (neg control)	forward	5’- TTGCTGAGTCAAAGGGTGTATATAAG-3’
	reverse	5’- GACACACACAAATAATGTATGGAAA-3’

Supplementary Table 3: Androgen response elements identified in FlnA-regulated genes:

	Gene	**ARE**
1	IL-32	CG**TCACCT**TCC**TGTACT**CA
		GA**GAGGCT**GCG**TGTGCT**TT
		TT**TGGCCG**CCA**TGTGCT**TC
2	HMOX1	AT**GATTCA**TAC**AGTCCT**TT
		TC**CATTCG**TTT**TGTCCT**CT
3	GDF15	CC**ATGACC**TTC**TGTCCT**TT
		AG**TTTTCA**TCT**TGTCCT**TG
4	BHLHE40	GG**GTTTCT**TTC**CGTCCT**GG
5	TMPRSS2	TC**TGGTCA**GGC**TGTCCT**GG
		GA**GATTCT**TCT**AGTTCT**TT