Supplementary Figure 1

Human AR8 full-length cDNA sequence

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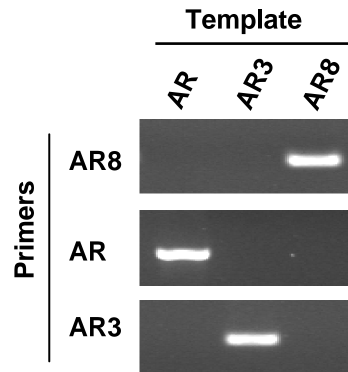
Note: The stop codons upstream of and in-frame with the first putative methionine is in red and underlined. The termination codon is indicated by “-”, and the polyadenylation signal is in red and underlined. The AR8 unique amino acids are in bold and underlined.

A



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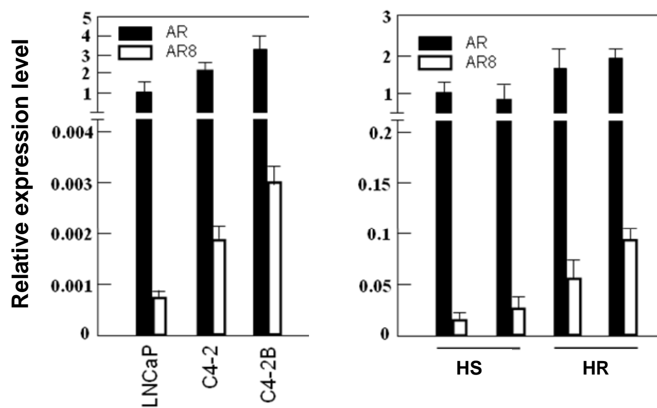

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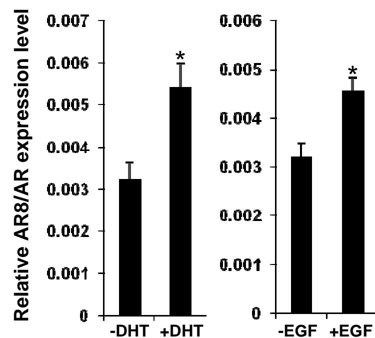
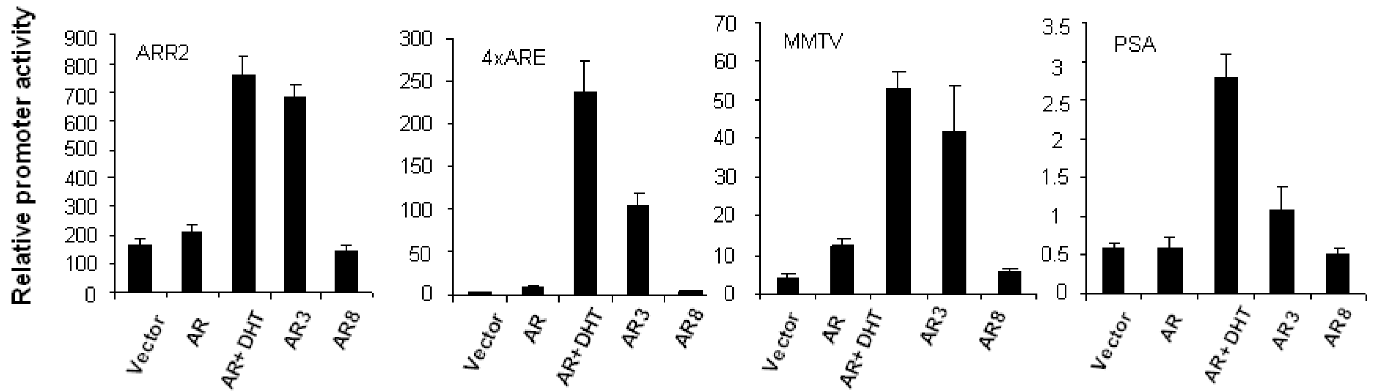


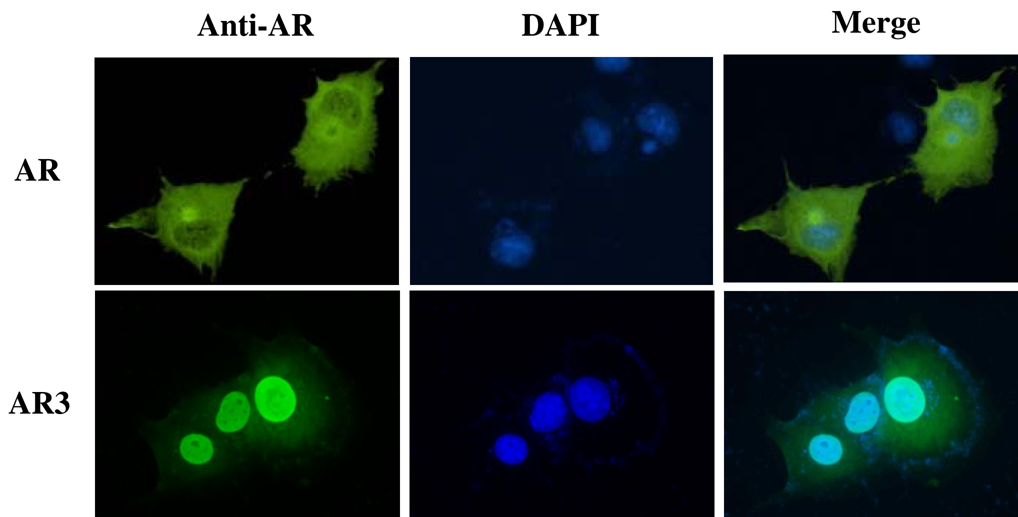
Supplementary Figure 2

A, Specificity of AR8 PCR primers.10ng of AR ,AR3 and AR8 plasmid were used as the PCR template respectively. PCR amplification was carried out using a pair of AR, AR3 or AR8 specific primers as described in the Method. **B,** Relative expression levels of AR and AR8 transcripts in LNCaP,C4-2 and C4-2B (Left) and two pairs of hormone-sensitive (HS) and hormone-resistant (HR) CW22R xenograft tumors were quantified using real-time PCR (Right). **C,** CWR-R1 cells were treated with vehicle or 1nM DHT (Left) or 10 ng/ml EGF (Right) for 12h. The relative expression of AR8 and AR transcript was quantified by real-time RT-PCR. The ratio of AR8 to AR was calculated by using the Pfaffl method. Statistical analysis was performed using a two-tailed Student's t-test. *, $p<0.05$ compared to the untreated control.



Supplementary Figure 3

AR8 did not display detectable transcriptional activity . COS-1 cells were transfected with ARR2-luciferase/4xARE-luciferase/MMTV-luciferase/PSA-luciferase reporters along with AR, AR3, AR8, control vector as indicated. At 24 h post-transfection, cells were treated with DHT or vehicle as indicated for 12h before measuring luciferase activity.



Supplementary Figure 4 COS-1 cells were transfected with AR or AR3 and stained as described in Fig 1D.

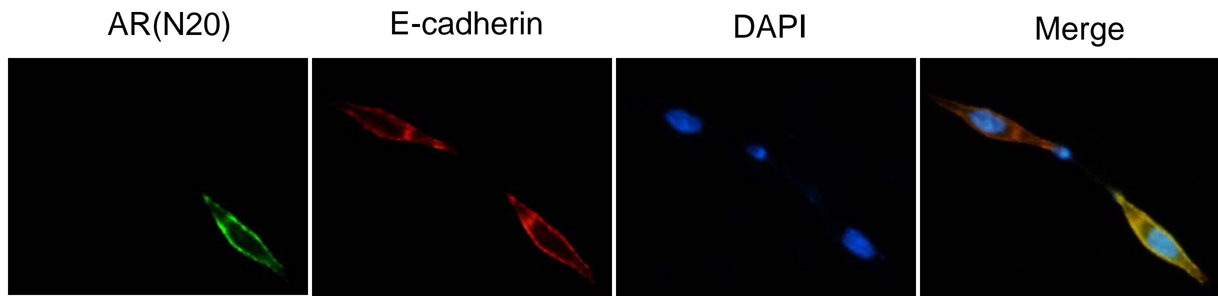
Anti-FLAG DAPI Merge

LNCaP

CWR-R1

Hpr-1

Supplementary Figure 5
Membrane localization of AR8 in prostate cells. LNCaP, CWR-R1 or Hpr-1 cells were infected with lentivirus encoding FLAG-tagged AR8 and stained as Fig 1D.



Supplementary Figure 6
LNCaP cells were infected with lentivirus encoding AR8 and staining with anti-AR (N20) and anti-E-cadherin antibody. Nucleus was visualized using DAPI staining.
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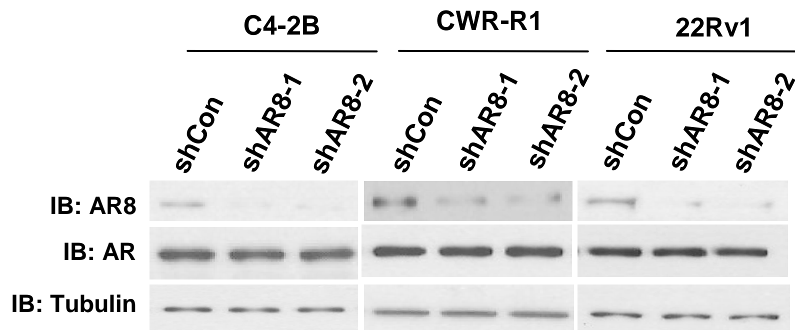
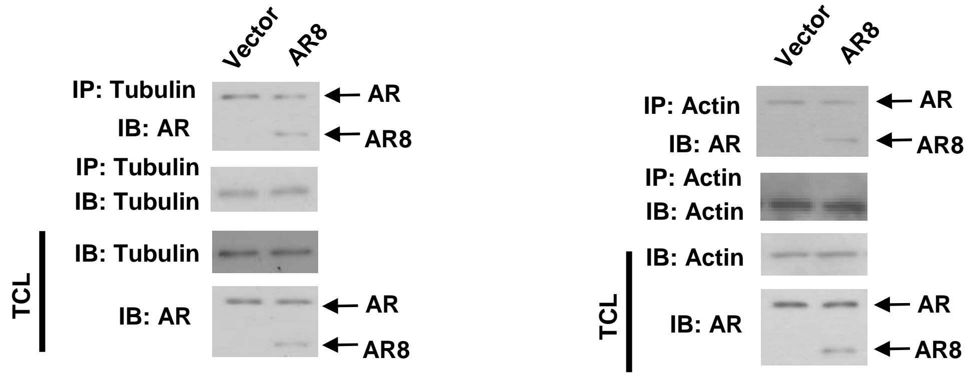


Supplementary Figure 7
The expression level of AR and AR8 protein in the cells used for proliferation assays described in Fig. 5A was determined by Western blot**.**



Supplementary Figure 8
LNCap cells were infected with lentivirus encoding AR8 or control vector. At 48h post-infection,cells lysates were subject to immunoprecipitation with anti-tubulin(left) or anti-Actin(right), foolowed by Western blot using the indicated antibodies.