Supplemental Materials and Methods

Plasmid Construction Details

p5H5hAR-A, encoding wild-type human (h)AR was rendered resistant to an AR-targeted small-interference RNA (siRNA) by introducing three silent mutations at AR codons 314, 315, and 316 (GGGGGCTAT, mutant bases underlined) using a QuikChange (Stratagene) site-directed mutagenesis kit (referred to as hAR^{sr}).

To generate the EGFP-expressing AR replacement vector, a cassette encoding AR-targeted short-hairpin RNA (shRNA) was ligated downstream of the histone H1 promoter in the pCMS4-H1p-EGFP vector by annealing sense (5'-gatccccCAAGGGAGGTTACACCAAAttcaagagaTTTGGTGTAACCTCCCTTGtttttggaa a) and antisense (5'-agcttttccaaaaaCAAGGGAGGTTACACCAAAtctcttgaaTTTGGTGTAACCTCCCTTGgg g) fragments and ligating the resultant dsDNA into BglII/HindIII-digested pCMS4-H1p-EGFP. Versions of siRNA-resistant AR were liberated from p5HBhAR-A, and ligated downstream of the CMV promoter in the AR shRNA-containing version of pCMS4-H1p-EGFP.

Point mutant versions of hAR^{sr} and hAR^{Gal4} were generated using QuikChange site-directed mutagenesis coupled with appropriately designed mutagenic primers. Deletion mutant versions of hAR^{Gal4} and NTD^{Gal4} were generated by using QuikChange mutagenesis to create *BssH*II sites flanking the desired deletion, digesting with *BssH*II,

and re-ligating the plasmid. hAR^{Gal4}ΔTAU5/whtlf+, hAR^{Gal4}ΔTAU5/ahtaa+, hAR^{Gal4}ΔTAU5/2Xwhtlf+, and hAR^{Gal4}ΔTAU5/3Xwhtlf+ were generated by inserting synthetic double-stranded cassettes with flanking *BssH*II sites into *BssH*II-digested hAR^{Gal4}ΔTAU5. Fusions between the Gal4 DBD (pM, Clontech) and WHTLF- or AHTAA-containing peptides were constructed using the same strategy.

Details of the primers used for mutagenesis reactions will be made available upon request. All deletion and point-mutant constructs were sequenced to verify their integrity and tested for expression via transient transfection and Western blot with anti-AR and anti-Gal4 antibodies.

Supplemental Figure Legends

Supplemental Figure 1. AR does not display ligand-independent activity in androgen-dependent LNCaP cells. LNCaP cells were transfected with ARE-driven or GAL4-driven, PSA-based reporter constructs along with wild-type (WT) AR^{Gal4} or AR^{Gal4} ΔTAU5 as indicated. Cells were grown in serum-free medium for 48h. Luciferase activity was determined. Data represent the mean +/- S.E. from at least three independent experiments, each performed in duplicate.

Supplemental Figure 2. TAU5 is dispensable for AR activity in response to 1nM mibolerone in ADI C4-2 cells. C4-2 cells were transfected with MMTV-LUC along with wild-type and ΔTAU5 versions of AR^{sr} as indicated and treated with 1nM Mib or

vehicle control (EtOH) for 24h. Luciferase activity was determined. Data represent the mean +/- S.E. from at least three independent experiments, each performed in duplicate.

Supplemental Figure 3. ADI 22Rv1 cells display ligand- and AF-2 independent AR activity. *A*, ADI 22Rv1 cells were grown in serum-free conditions for 48h, and treated with 1nM mibolerone (Mib), 10μM casodex (CDX), or combinations of these compounds for an additional 24h. PSA mRNA levels were determined by Northern blot. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA levels and ethidium bromide staining of ribosomal RNA subunits are shown as controls. AR protein expression was concurrently determined by Western blot. ERK-2 protein expression is shown as a control. *B*, 22Rv1 cells were transfected in androgen-free conditions with separate siRNAs targeted to the AR or a non-targeted control (ctrl) siRNA. Levels of PSA and GAPDH mRNAs, ribosomal RNA subunits, as well as AR and ERK-2 proteins were determined as described for panel A. We have previously shown that casodex selectively inhibits AF-2-dependent but not AF-2 independent AR activity (1). Because ligand-independent PSA expression in 22Rv1 cells is AR dependent (*B*), but resistant to casodex (*A*), we conclude that this activity is AF-2 independent.

Supplemental References

1. Dehm, S. M. and Tindall, D. J. Ligand-independent androgen receptor activity is activation function-2-independent and resistant to antiandrogens in androgen refractory prostate cancer cells. J Biol Chem, *281:* 27882-27893, 2006.