Supplementary Figures and Tables

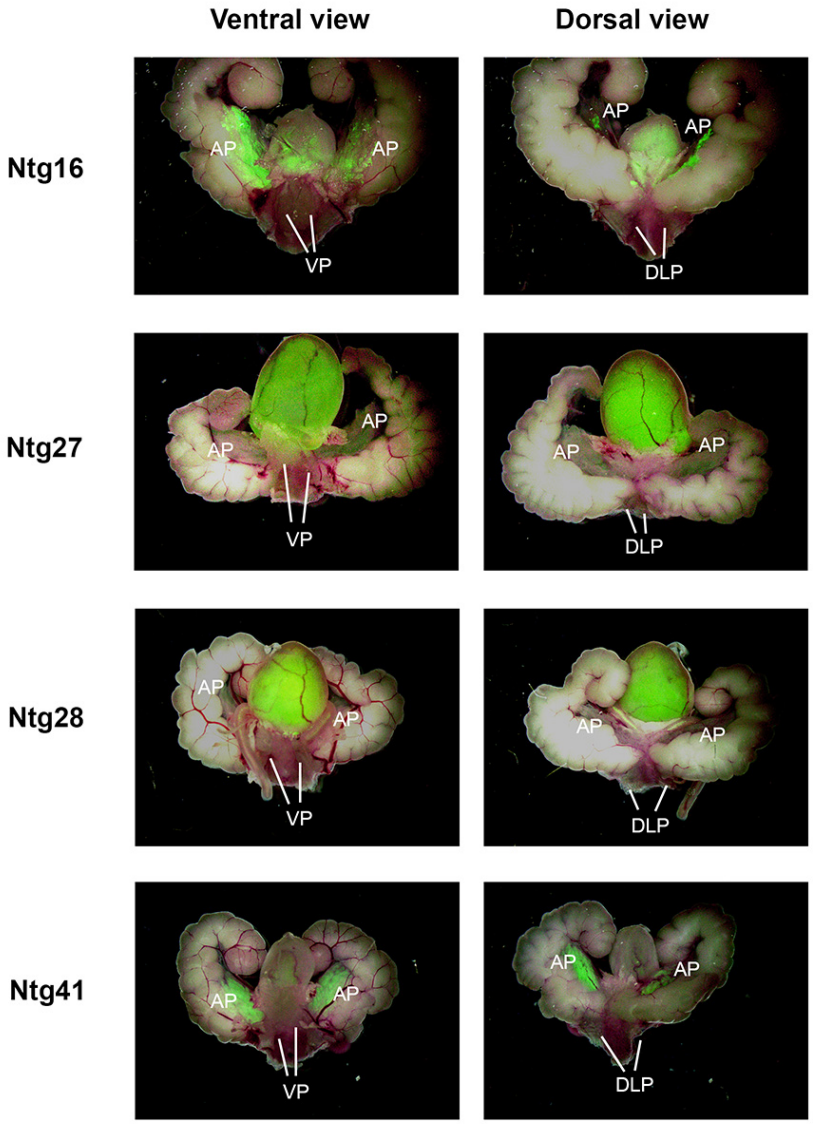

Figure S1. Characterization of other *Nkx3.1(11)-d2EGFP* mouse lines.

White field and GFP channel overlay images of dissected urogenital tissues showing various degrees of GFP expression in prostate lobes for the other four weaker *Nkx3.1(11)-d2EGFP* transgenic lines. The green signal in bladders was due to autofluorescence of urine.

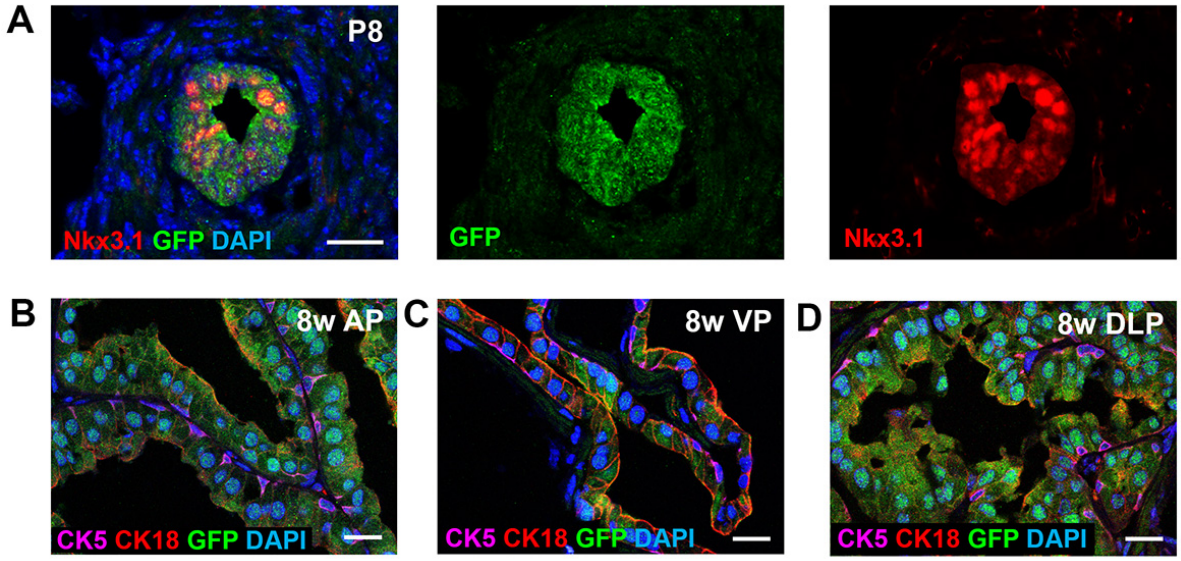

Figure S2. Characterization of *Nkx3.1(11)-d2EGFP* in neonatal prostate and different adult prostate lobes.

(**A**) IF image showing GFP reporter and Nkx3.1 co-localization in the prostate epithelium of mice at stage P8. (**B-D**) Representative IF images showing GFP reporter expression present in luminal cells of adult prostate at 8 weeks of age, with the following order of penetration in different lobes: AP>DLP>VP. Scale bars correspond to 20 microns.

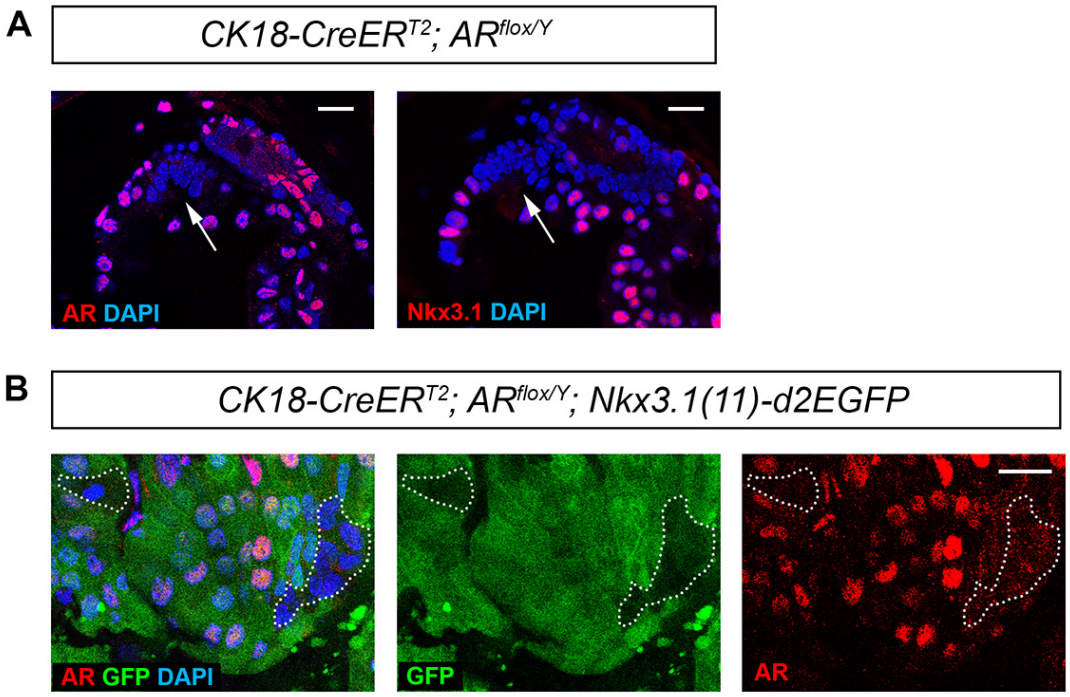

Figure S3. AR activates *Nkx3.1* transcription through the 11-kb in luminal cells.

(**A**) IF staining of adjacent sections of tamoxifen-induced *CK18-CreER^{T2}; AR$^{flox/Y}$* mice showing the absence of Nkx3.1 signal in AR-deleted prostate luminal cells (arrow). (**B**) GFP reporter signal was strongly reduced in *AR*-null luminal cells in the DLP of tamoxifen-induced *CK18-CreER^{T2}; AR$^{flox/Y}$; Nkx3.1(11)-d2EGFP* mice. Scale bars correspond to 20 microns.

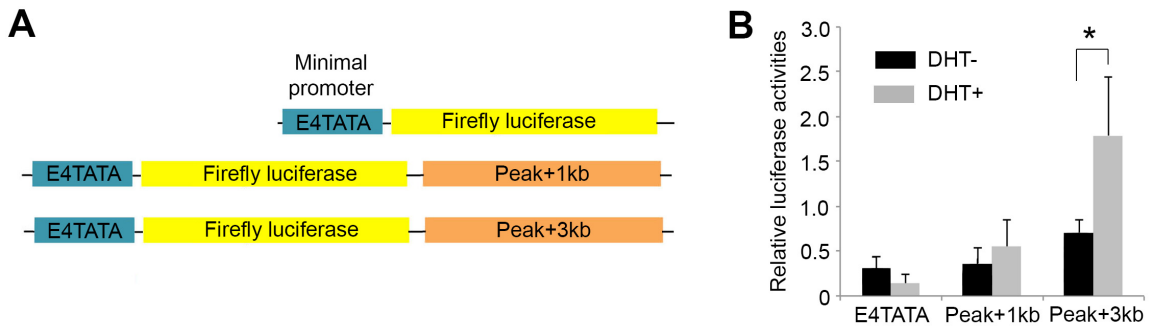

Figure S4. Luciferase assays to test candidate enhancers.

(**A**) Constructs for transient transfection and luciferase assays with the candidate enhancers positioned downstream of the luciferase sequence. (**B**) Luciferase reporter assays comparing the construct with only the minimal promoter to those containing "Peak+1kb" and "Peak+3kb" sequences downstream of the luciferase sequence showing that the "Peak+3kb" region is androgen responsive. Error bars correspond to one s.d. * $p < 0.05$ by student's t test.

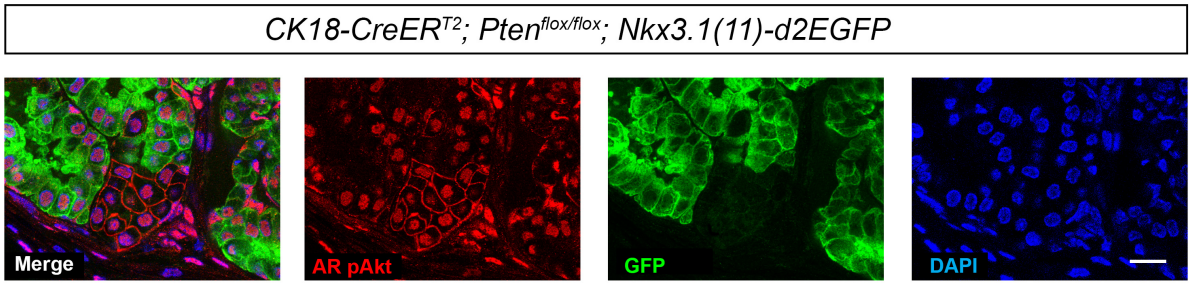

Figure S5. Absence of GFP reporter signal in *Pten*-null luminal cells.

Triple IF staining showing that Pten-null luminal cells, which were identified by pAkt signal (membrane red), still expressed nuclear AR (nuclear red), but lost the GFP reporter expression. Scale bar corresponds to 20 microns.

Table S1. Summary of GFP expression intensity in five *Nkx3.1(11)-d2EGFP* transgenic lines.

Transgenic mouse lines	GFP expression in embryos	GFP expression in adult prostate		
		AP	VP	DLP
Ntg16	negative	strong	weak	weak
Ntg18	negative	strong	strong	strong
Ntg27	negative	moderate	weak	weak
Ntg28	negative	weak	weak	weak
Ntg41	negative	strong	moderate	strong

Table S2. Primers for mouse genotyping.

Allele		Primer sequence
CreER^{T2}	forward	5'-CAGATGGCGCGGCAACACC-3'
	reverse	5'-GCGCGGTCTGGCAGTAAAAAC-3'
AR^{flox}	forward	5'-GTTGATACCTTAACCTCTGC-3'
	reverse	5'-CTTCAGCGGCTCTTTTGAAG-3'
Pten^{flox}	forward	5'-ACTCAAGGCAGGGATGAGC-3'
	reverse	5'-GTCATCTTCACTTAGCCATTGG-3'
R26R-YFP	wild-type forward	5'-GGAGCGGGAGAAATGGATATG-3'
	mutated forward	5'-GCGAAGAGTTTGTCCTCAACC-3'
	reverse	5'-AAAGTCGCTCTGAGTTGTTAT-3'
Nkx3.1	wild-type forward	5'-CTCCGCTACCCTAAGCATCC-3'
	wild-type reverse	5'-GACACTGTCATATTACTTGGACC-3'

Table S3. Antibodies used for immunofluorescence.

Antigen	Supplier	Ig type	Dilution
AR	Sigma #A9853	rabbit IgG	1:400
CK5	Covance #PRB-160P	rabbit IgG	1:500
CK18	Abcam #ab668	mouse IgG1	1:100
Nkx3.1	Kim et al. (2002) PNAS 99: 2884-2889	rabbit IgG	1:2000
Phospho-Akt	Cell Signaling #3787	rabbit IgG	1:50
GFP	Abcam #13970	chick IgY	1:2000
YFP	Abcam #13970	chick IgY	1:2000

Table S4. List of primer sequences for qRT-PCR

Genes	Forward (5' to 3')	Reverse (5' to 3')
Fkbp5	GGGATGTTGTCAGATGGAAAG	TGTCCCAGGCTTTGATAACC
Crabp1	CGCTACAGCCAACACCACT	TCAGCATGGCGTTCACAC
Pgap2	TCCACTTGTCGCCTTCTTCT	CACTGATGGCAGGTAATTGG
Msmb	TGGTGATAGCATCCAAAGCA	AGCATCCATGCAGTCATCAG
Pbsn	ACACGAGTGGCTGGAGTTTT	TCCTCAATGCCCATTTCTTC
Mme	CGAGACCAGTTCCCGATACA	TTTTTGCTTTCTGCACTGCT
Actb	CCAACCGTGAAAAGATGACC	CCATCACAATGCCTGTGGTA

Table S5. List of primer sequences for qChIP

Location	Forward (5' to 3')	Reverse (5' to 3')
-6.5 kb	ACTGGTGGCCAAAGTTCTGA	TGGAAGTAGGGAAACTGAGGTC
-5.5 kb	GCCACCCAGTCAGAGGATAG	ATACAATAGTGCCCCGGTGA
-4.5 kb	CCAAAGAGCAAAGGGACAAG	GTCAGGCCCTTGACTTTCAC
-3.5 kb	GCAGTGCGAACACTCTTGAC	GAAGGAGGGGAGGCTAACAG
-2.5 kb	ATGGCCAGGGCTAATAAGGT	AGCCGCTCTTCTGTGTGACT
-1.3 kb	AGTTTCCCTCCGAAGTTGCT	GCTCTGCAGGGAACCTTGT
-0.2 kb	GAGAGGGAAACCAGGAAAGG	GCTCCAGGTGACCCTCAAG
+1 kb	ACACACATCCAGGATCCACA	ACATTCAGGTCTTAGCGGTTT
+2 kb	ATGGCCCCTTAGATGAGGAT	AAGGGCAGAGAGAAACTCTGG
+3 kb	GGGGTGCTTGTTTTTCATGT	CTCCTTGCTGGTTAGCTTGG
+4 kb	TGCGTGTGGTTTTCAGATTC	AGTGTCCTGTGGGAACATGG
+5 kb	GTGCCTACTTCAGGCAATCC	CATGCTTCTTGCCTGCTACA
+6 kb	TGTGCAAGAGAAAGGGCATA	AAGTCTGCAGAGGAGCCAAA
+7 kb	CCAAGAGAGTGGGTTTTCCA	TCATTGCATGGCAAGAACAT
+8 kb	TATGCCCTGCAGCCATAAG	TAGCTTTGGGCTAGGGTTCC
+9.5 kb	TCTGAGGGAAGGTGTCCTTG	CCACCCTACTTCTCTGGGATG