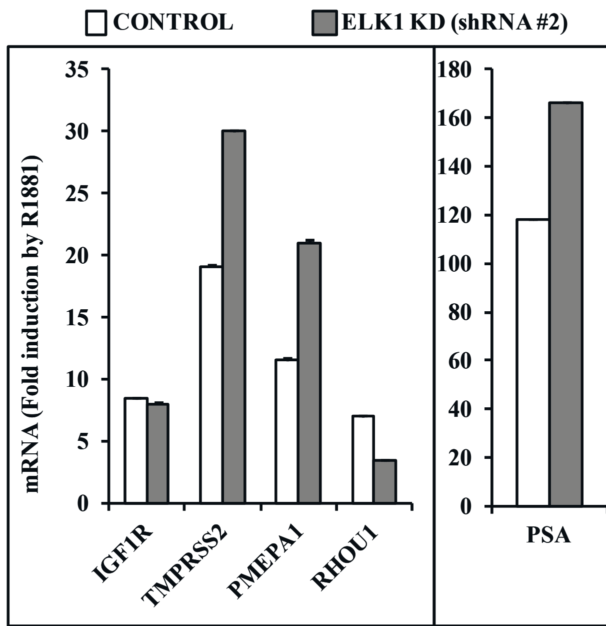
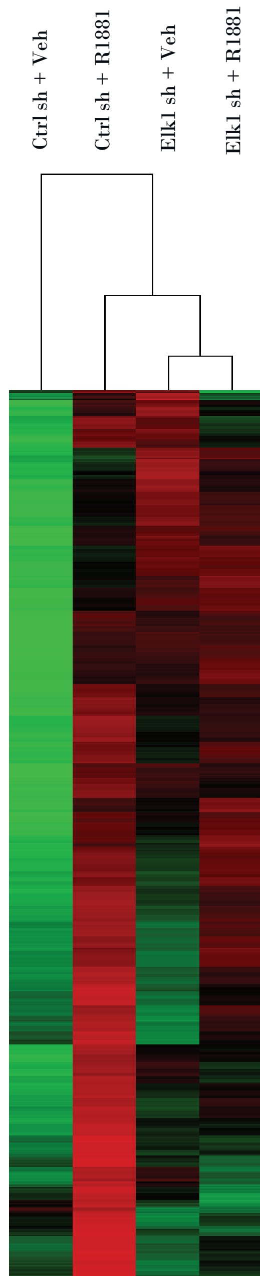
Supplemental Figure 1



Supplemental Figure 1. Hormone depleted LNCaP cells were infected with Elk1 shRNA #2 lentivirus or control shRNA lentivirus. Following treatment with vehicle or R1881 (1nM), cells were harvested at 48 h of treatment to measure mRNA levels for the indicated androgen activated genes by quantitative real-time PCR.

Supplemental Figure 2

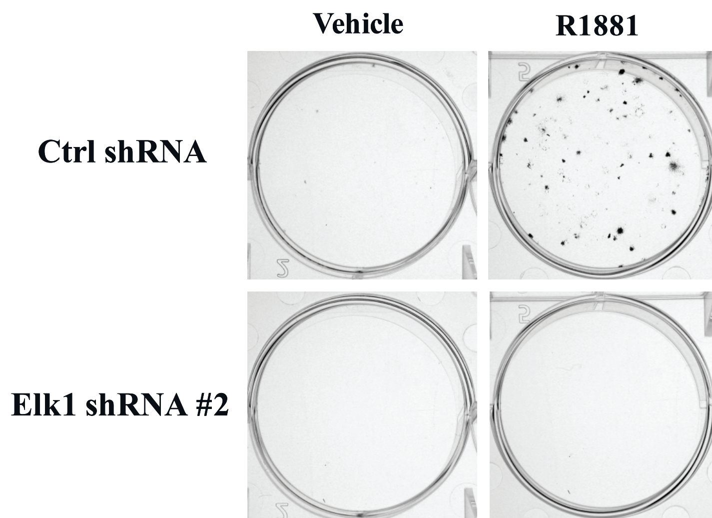


Genes strongly repressed by Elk1. Androgen opposes repression by Elk1 but does not independently activate these genes.

Elk1 is required for optimal activation gene activation by androgen. Androgen is at least partially dependent upon the presence of Elk1 to activate these genes.

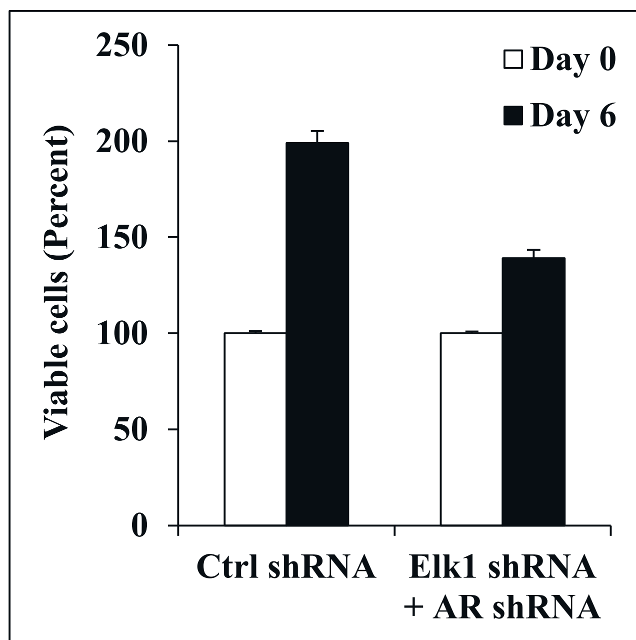
Supplemental Figure 2. Genes that are partially or fully dependent upon the presence of Elk1 for activation by R1881. Heat map of Affymetrix DNA microarray analysis in LNCaP cells. The average values from replicate samples are indicated.

Supplemental Figure 3



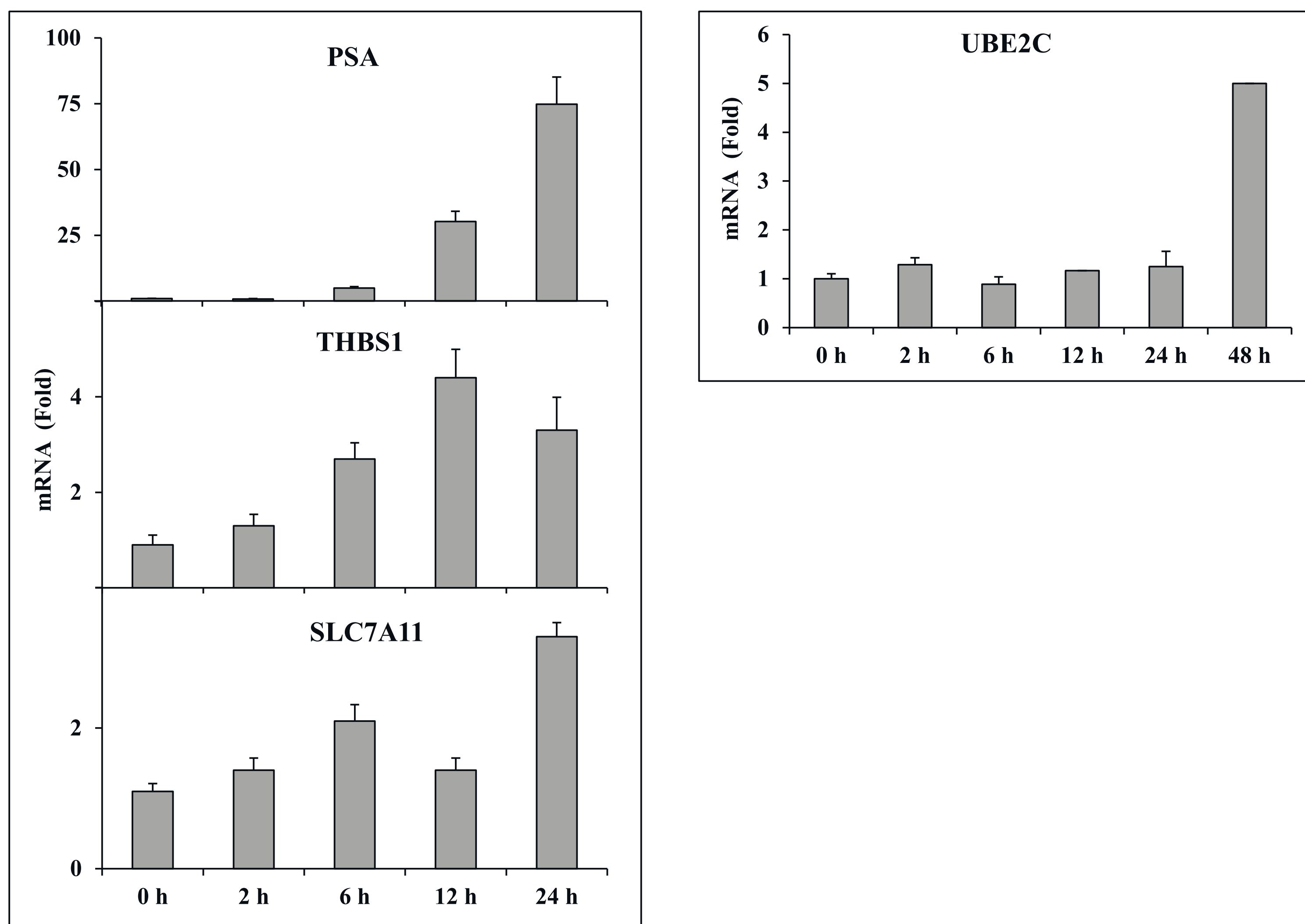
Supplemental Figure 3. Hormone-depleted C4-2 cells were infected with Elk shRNA #2 lentivirus or control shRNA lentivirus. Anchorage-dependent colony formation was measured following treatment with vehicle or R1881 (1nM). At the end of 2 weeks, colonies were stained with crystal violet.

Supplemental Figure 4



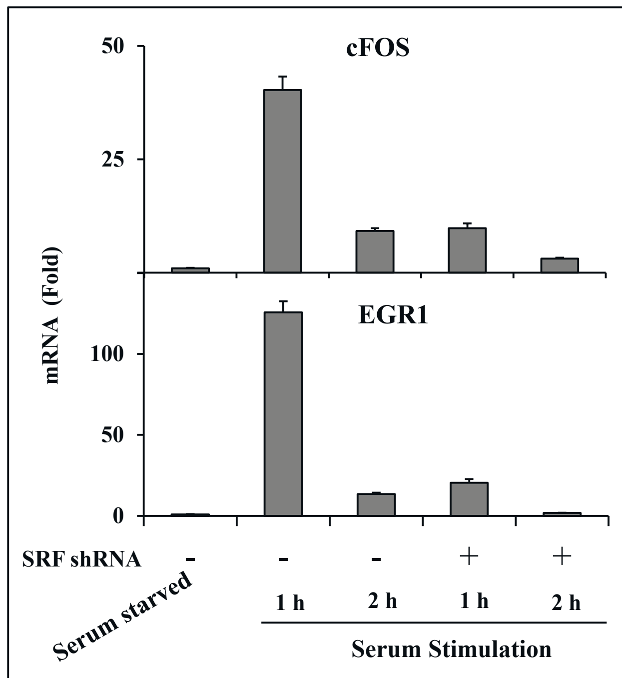
Supplemental Figure 4. Hormone depleted C4-2 cells were infected either with Elk1 shRNA and AR shRNA lentivirus or control lentivirus. 72 hours later (after knockdown had occured) cell growth was monitored in hormone depleted media.

Supplemental Figure 5



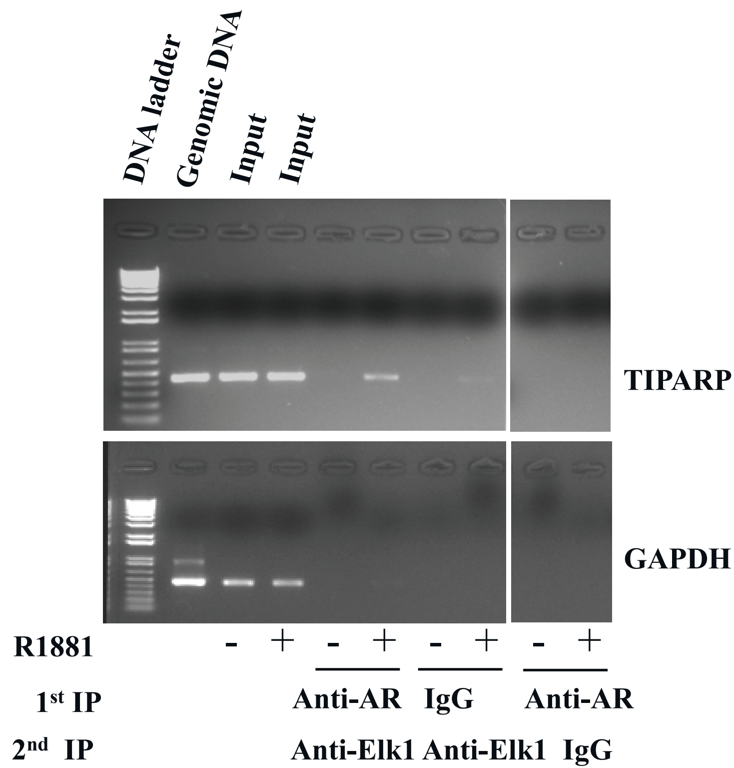
Supplemental Figure 5. Time course of induction of additional AR-Elk1 target genes bvy R1881. Hormone depleted LNCaP cells were treated with vehicle or R1881 for the indicated times. RNA was harvested and the induction of endogenous PSA, THBS1, SLC7A11 (*left panel*) and UBE2C (*right panel*) by R1881 was measured by real-time PCR. The Y-axis shows R1881 treated values relative to the vehicle control.

Supplemental Figure 6



Supplemental Figure 6. Control showing abrogation of the immediate early gene response due to depletion of SRF. Hormone depleted LNCaP cells were infected with SRF shRNA lentivirus or control lentivirus. 72 h after infection, the cells were serum starved for 24 h and then stimulated with 20%FBS for the indicated times. RNA was harvested and the induction of endogenous cFOS and EGR1 by serum was measured by real-time PCR.

Supplemental Figure 7



Supplemental Figure 7. Hormone-depleted LNCaP cells were treated with either vehicle or R1881 (1nM) for 2 hours. Cells were harvested and subjected to ChIP using either anti-AR antibody or normal rabbit IgG. The chromatin complexes from the first ChIP were subjected to re-ChIP using anti-Elk1 antibody. The immunoprecipitated complexes were amplified by PCR using primers specific for TIPARP gene promoter or a genomic sequence of GAPDH (non-target control). PCR products were analyzed by agarose gel electrophoresis on a 1% gel. Untreated genomic DNA was used as a control to ensure the specificity of the PCR amplification.

Supplement 8

The shRNA sequences are:

AR shRNA: (TRCN0000003718, MISSION™TRC shRNA Target Set, Sigma)
CCGGCACCAATGTCAACTCCAGGATCTCGAGCTCCTGGAGTTGACATTGGTGTTTTT

ELK1 (shRNA #1): (TRCN0000007450, MISSION™TRC shRNA Target Set, Sigma)
CCGGCCCAAGAGTAACTCTCATTATCTCGAGATAATGAGAGTTACTCTTGGGTTTTT

ELK1 (shRNA #2): (TRCN0000007453, MISSION™TRC shRNA Target Set, Sigma)
CCGGCCTGCTTCCTACGCATACATTCTCGAGAATGTATGCGTAGGAAGCAGGTTTTT

SRF shRNA: (TRCN0000003981, MISSION™TRC shRNA Target Set, Sigma)
CCGGGAGACCGGCAAGGCACTGATTCTCGAGAATCAGTGCCTTGCCGGTCTCTTTTT

Non-target control shRNA: (MISSION™ Non-Target shRNA Control Vector, Sigma)
CCGGCAACAAGATGAAGAGCACCAACTCGAGTTGGTGCTCTTCA TCTTGTTGTTTTT

Real time PCR primer and TaqMan probe sequences for ChIP experiments:

The optimal target sequences chosen to amplify the genomic sequences by real-time qRT-PCR were as follows:
Major ARE enhancer region of the PSA promoter (-4366 to -3874 nt):
Forward primer: 5'-GCCTGGATCTGAGAGAGATATCATC-3'
Reverse primer: 5'-ACACCTTTTTTTTTCTGGATTGTTG-3'
Probe: 5'-/56-FAM/TGCAAGGATGCCTGCTTTACAAAC/36-TAMSp/-3'

Chromosome 5 (43075562-8194):
Forward primer: 5'-GAAACTGGCGCGTTGAACTTAGCA-3'
Reverse primer: 5'-TTAGGTGTGGAAGCACCGCTCTTA-3'
Probe: 5'-/56-FAM/TAGCGGATAGCGCTGGTATTGCCAAA/36-TAMSp/-3'

Chromosome 6 (91353148-5086):
Forward primer: 5'-ATTAAAGTGCGCGAACGGAAGTGG-3'
Reverse primer: 5'-GGCCAAGACATATTTCACGCAGCA-3'
Probe: 5'-/56-FAM/TCCCTTAAGGACCAGCGGGAAAGATA/36-TAMSp/-3'

Chromosome 1 (144218176-9723):
Forward primer: 5'-ACACTTCCGGTATCTTTCCGCACT-3'
Reverse primer: 5'-CGCCAGTCAAGCTGACCAATCAAA-3'
Probe: 5'-/56-FAM/TTACCGTGCAGAGGGAGGGATTTAGA/36-TAMSp/-3'

TIPARP gene promoter:
Forward primer: 5'-GACTGCCCTTTGTACCGAATTCAC-3'
Reverse primer: 5'-GGCTCTAACTGCCTAAGAAGTGT-3'
Probe: 5'-/56-FAM/CCGGTCAGCATGAAGATCGAATTGGT/36-TAMSp/-3'

PCR primer sequences for Re-ChIP experiments:

The forward and reverse primer sequences for amplifying the 407bp fragment of the promoter region of the TIPARP gene are:
5’-GCTACAGGTACCCCACTTAAAGCAGAGATCGAGGTGACAGGCGAGC-3’ and
5’- GCTACAGCTAGCCCATCTTCATGCTGACGGGACCCGACTGAATTCG-3’

The forward and reverse primers for amplifying the 478bp genomic fragment within the GAPDH gene are:
5’-TTCCCGTCCTCCTTATCCCCAGCTGGGTTGCAACCAAATTGCCAGAG-3’ and
5’- GACAAAGTGATGGCAACAGTAAAAAGATCAGGGGTTGTGGGGAGAGAGG -3’