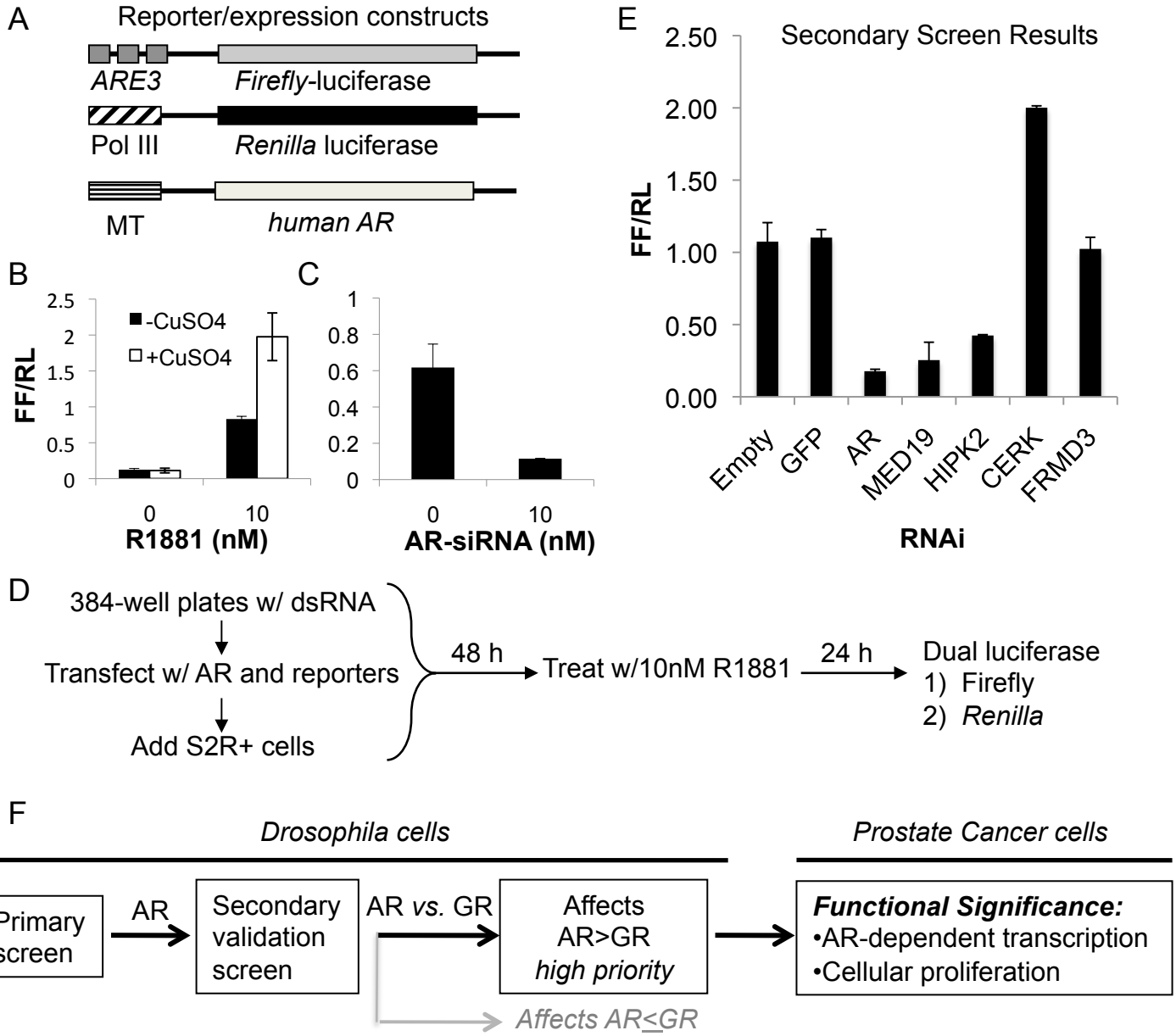
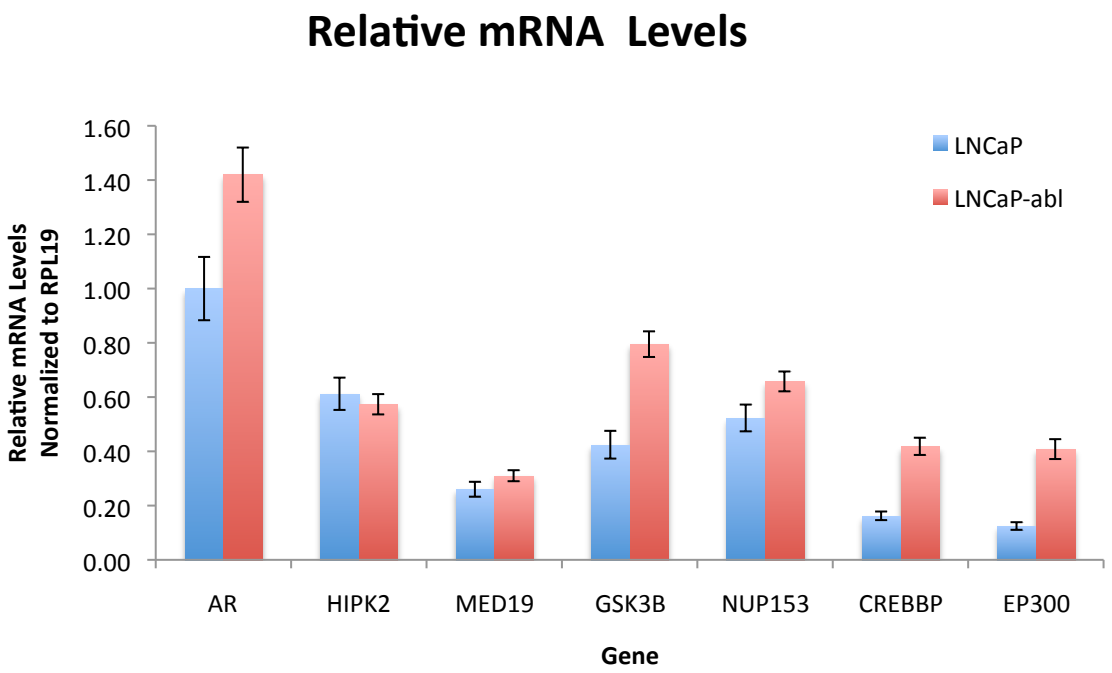
Imberg-Kazdan-Supplementary Figure 1



Supplementary Figure 1. Screen for regulators of AR-mediated transcriptional activation A) Reconstitution of human AR signaling in Drosophila cells. Relevant portions of the vectors are shown: an AR reporter gene consisting of three copies of an AR-binding site upstream of a minimal Drosophila alcohol dehydrogenase promoter driving Firefly luciferase; a PolIII-*Renilla* luciferase vector a control for transfection efficiency; an expression vector encoding human AR cDNA under the control of the Drosophila metallothionein promoter (MT). B) Drosophila S2R+ cells were transfected with the expression and reporter constructs and AR-dependent transcriptional activation was quantified as a ratio of Firefly luciferase (FL) to *Renilla* luciferase (RL) in the absence and presence of 10 nM of the synthetic androgen R1881. The assay was performed in both the absence (-) and presence (+) of induction of the MT promoter by copper sulfate (CuSO4). Given that AR was sufficiently active in the absence of CuSO4, the screen was performed under this condition. C) AR activity is reduced by siRNA against AR. Cells were transfected as in A, along with a control scrambled siRNA (0 nM siRNA-AR) or AR directed siRNA (10 nM AR-siRNA) and luciferase activity (FL/RL) was determined. D) Outline of screen design. E) Examples of the results from the secondary screen. Compared to no siRNA (Empty) or siRNA against GFP (negative control), the depletion of AR (positive control), MED19 and HIPK2 reduced AR activity, whereas depletion of CERK increased AR activity and depletion of FRMD3 had no effect on AR activity. F) Prioritization scheme. Factors identified in the primary screen that reduced AR activity were validated in a secondary screen and tested for specificity by comparing to the glucocorticoid receptor (GR). Factors showing AR selectivity were assigned a higher priority, and effects on AR-mediated transcription and cell proliferation in prostate cancer cells were evaluated by siRNA depletion of the human homologue.



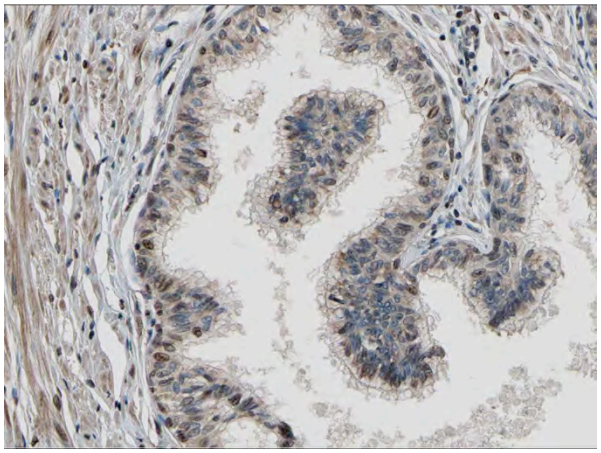
Supplementary Figure 2. Expression of select AR regulators in LNCaP and LNCaP-abl cells. mRNA expression was determined relative to RPL19 and expressed relative to AR in LNCaP cells which was arbitrarily set to 1. The average cycle number for RPL19 was virtually identical between the two cell types. RPL19 average Ct values: LNCaP=15.81± 0.27; LNCaP-abl=15.90 ± 0.15.

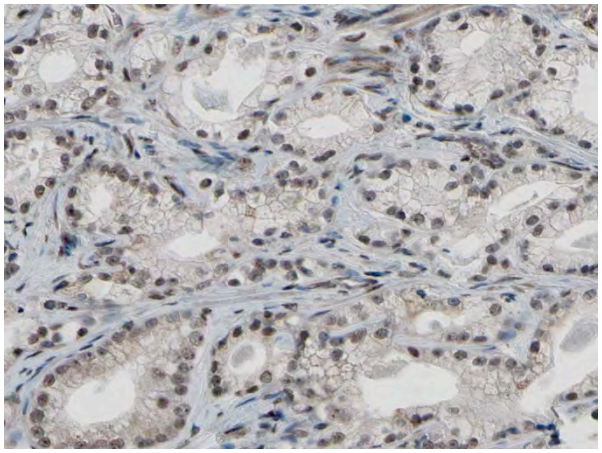
HIPK2

Benign

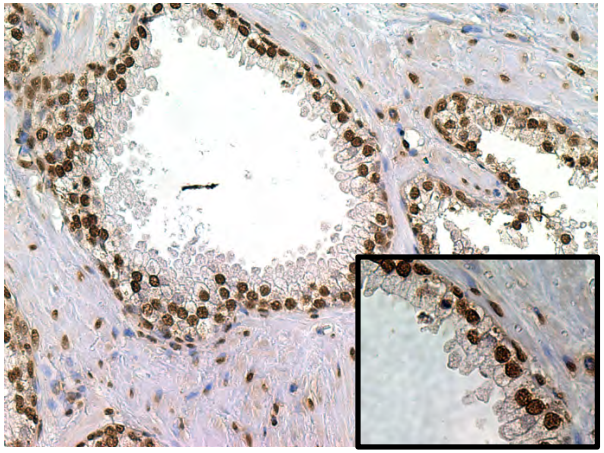
Malignant

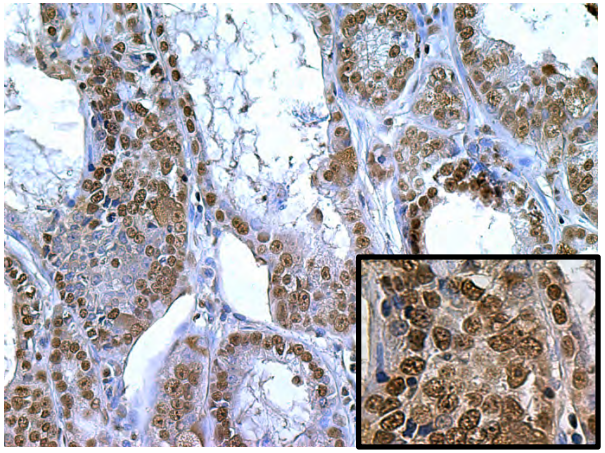
Human Protein Atlas



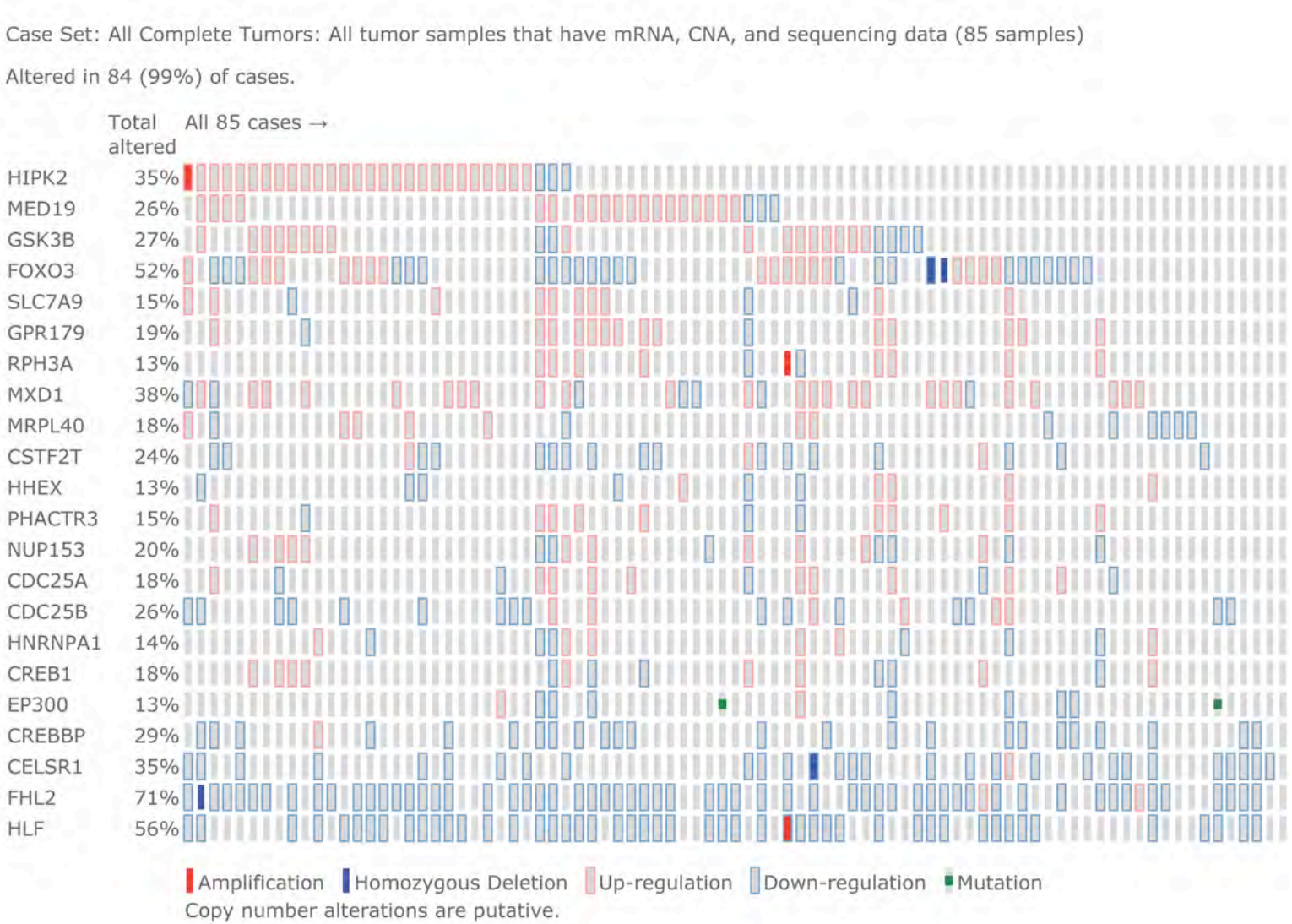


Prostate TMA

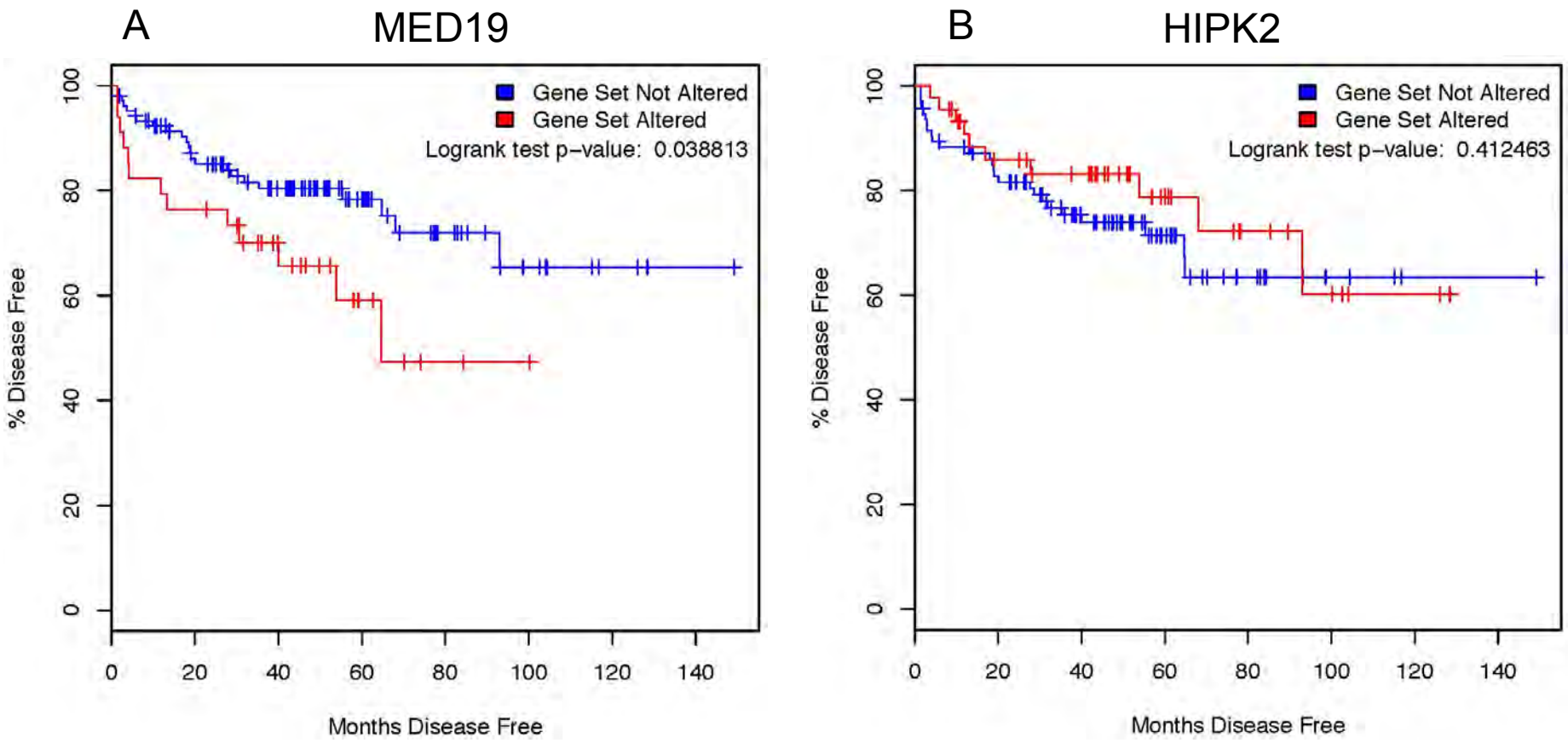




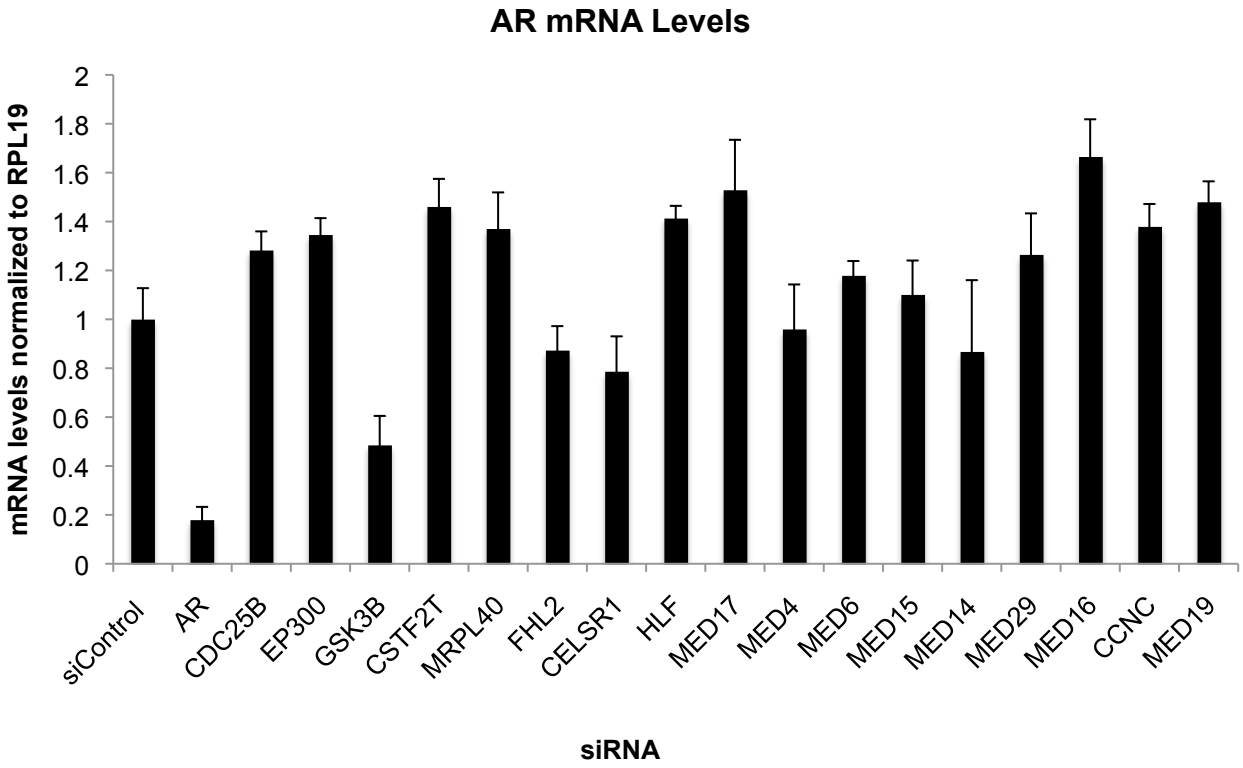
Supplementary Figure 3. HIPK2 expression in benign and malignant prostate tissue. Immunohistochemical staining of HIPK2 from the Human Protein Atlas (www.proteinatlas.org) and from representative specimens from a prostate tissue microarray (TMA) generated by the Prostate Cancer Biorepostiory Network and stained at the NYU School of Medicine. Bottom images are at 20x magnification, and inserts are at 40x magnification.



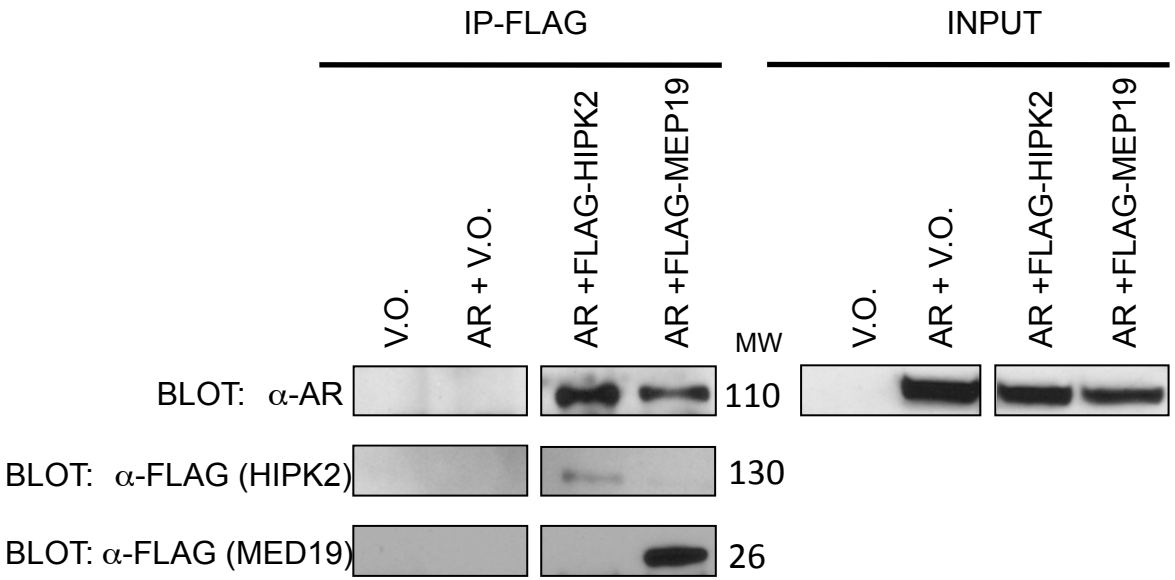
Supplementary Figure 4. Expression of AR regulators in human prostate cancers
Oncoplot diagram of expression of the AR regulators from 85 human prostate cancer specimens from the Memorial Sloan Kettering cancer genome portal (http://www.cbioportal.org/public-portal Taylor et al Cancer Cell 2010 18, pp. 11-22). Percentage of cases with expression changes of 1.5-fold or greater are shown. For example, HIPK2 expression is altered in 35% of the cases and upregulated in 32% of the cases.



Supplementary Figure 5. MED19 and HIPK2 mRNA expression and clinical outcome in human prostate cancer. Kaplan-Meier curves plotting percent of patients disease free *versus* Months Disease free. The diagrams are generated from expression analysis of 151 tumors with expression changes of 1.5-fold or greater (data are from Taylor et al Cancer Cell 2010 18, pp. 11-22 and analyzed using http://www.cbioportal.org/public-portal/). A) Patients with changes in MED19 mRNA expression (red line) have lower rates of survival, compared to those cases where MED19 expression does not change (blue line). B) Changes in HIPK2 expression not linked to changes in long-term survival.

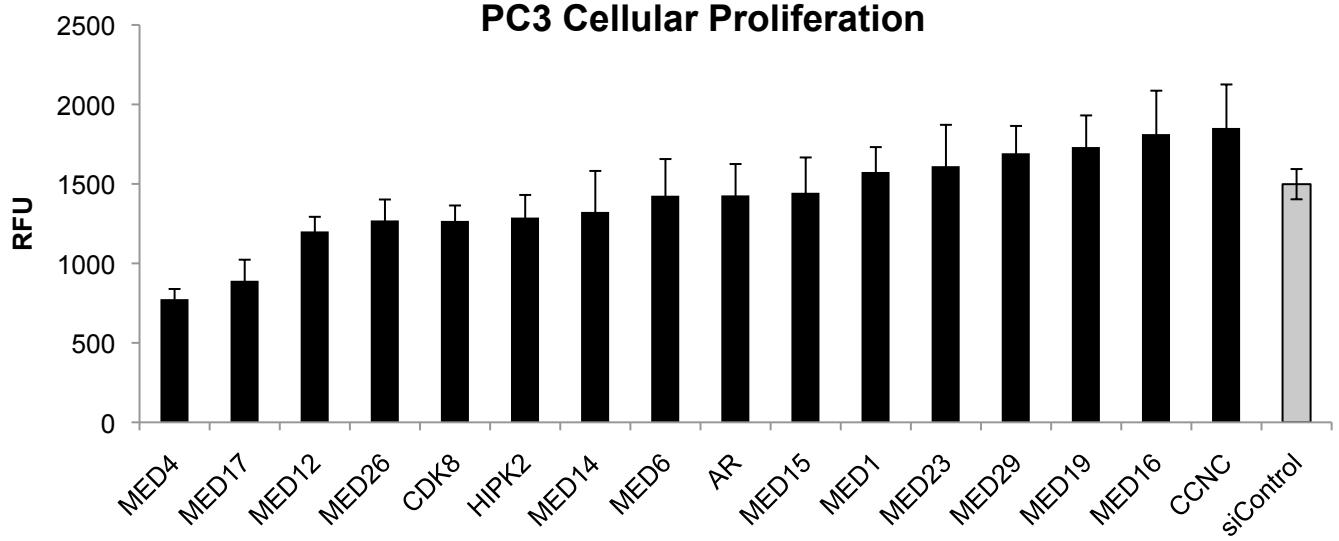


Supplementary Figure 6. Depletion of AR regulators has little impact on AR mRNA expression
LNCaP-abl cells were transfected with siRNAs against the indicated AR regulator or a control siRNA and AR mRNA expression was measured relative to RPL19. Note that only depletion of GSK3β reduced AR mRNA by ~50%.

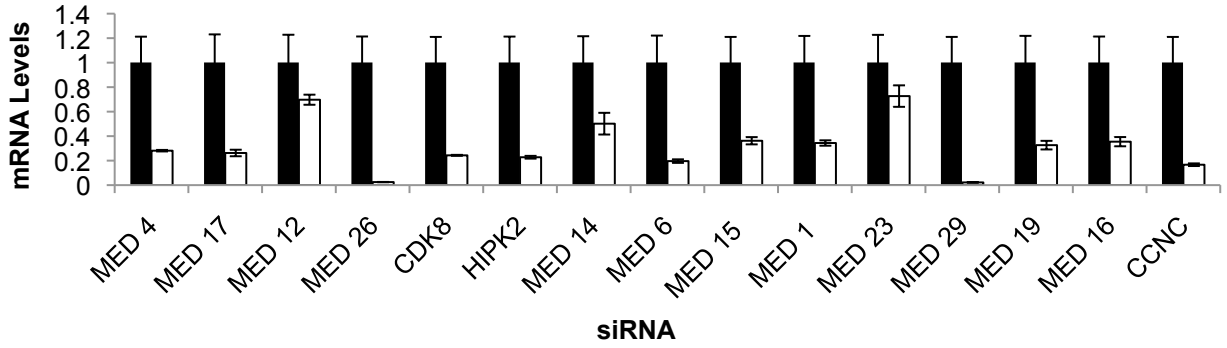


Supplementary Figure 7. Association of HIPK2 and MED19 with AR. HEK293 cells were transfected with expression vectors for AR, along with FLAG-tagged versions of HIPK2, MED19, or vector only (v.o.) control and immunoprecipitated with FLAG from whole-cell extracts, and associated proteins were resolved by SDS-PAGE. AR associated with HIPK2 and MED19 was detected by western blotting with an antibody against AR. HIPK2 and MED19 immunoprecipitations are detected with an antibody against FLAG. The right panel shows the expression of AR prior to immunoprecipitation (input), and the top left panel reveals that AR was immunoprecipitated with HIPK2 and MED19 (FLAG-IP). Molecular weight markers in kDa are shown. The lanes shown from either the IP or INPUT are from the same gel with the relevant portions excerpted and shown in boxes. The experiment was repeated twice with similar results.

A

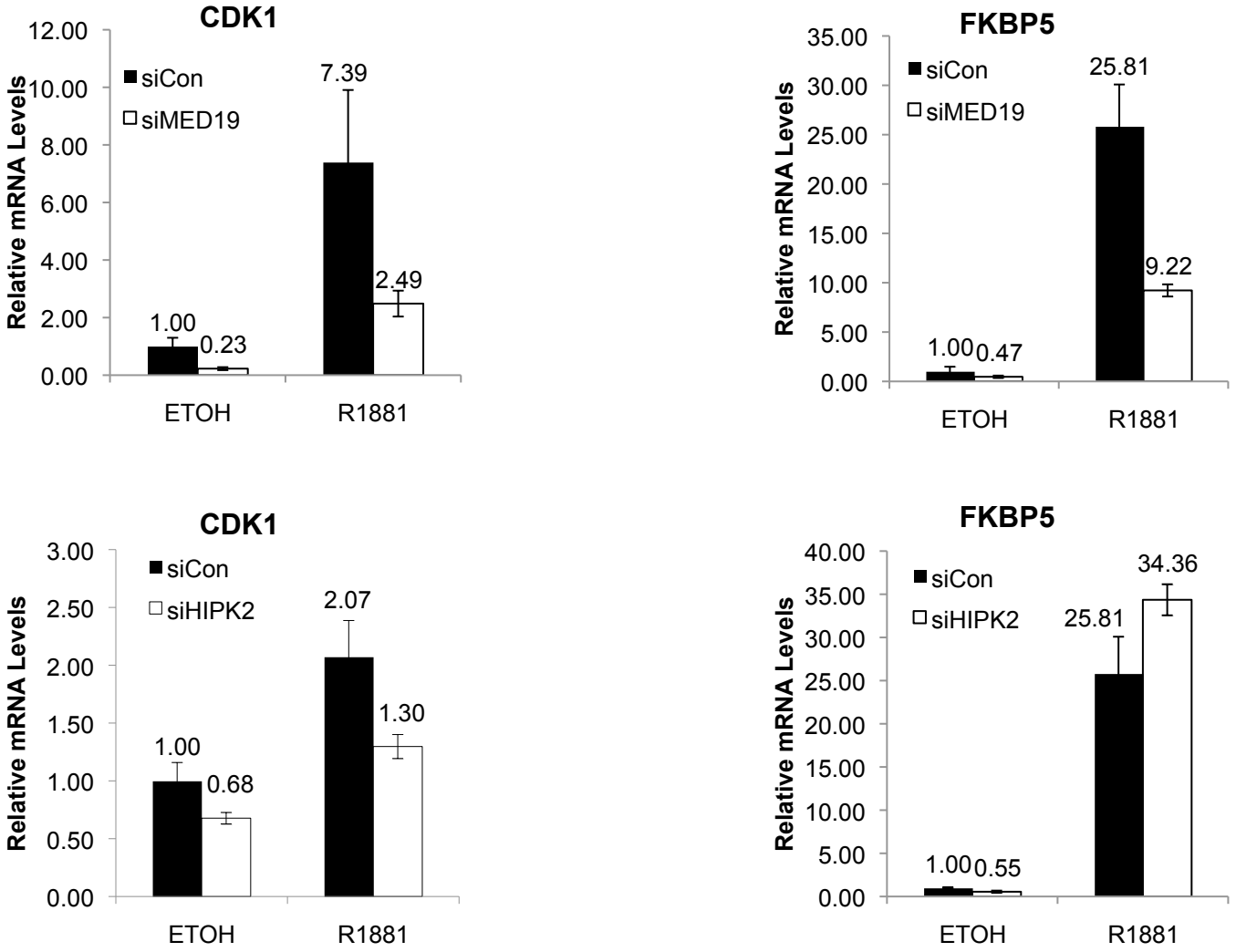


B



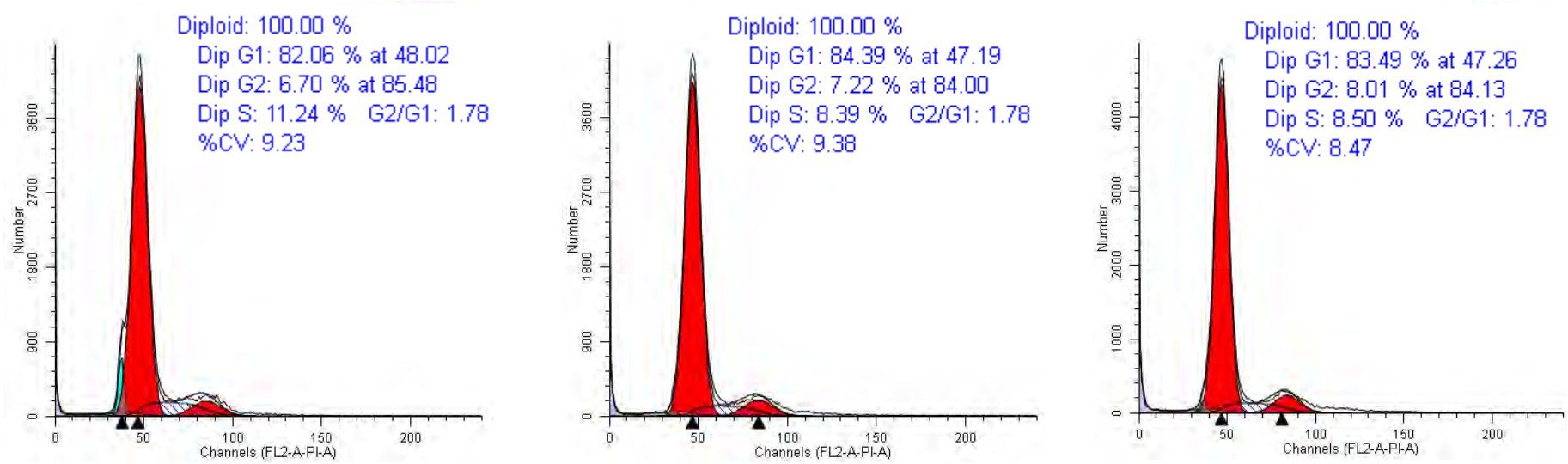
Supplementary Figure 8. Depletion of Mediator subunits differentially affect AR-deficient PC3 prostate cancer cell proliferation

A) PC3 cells were transfected with siRNAs against the indicated Mediator complex subunits or a control siRNA and cell proliferation was measured as in Figure 1 and shown as Relative Fluorescence Units (RFU). B) The efficiency of knock down was monitored at the mRNA level for each factor relative to RLP19. Black bars, control siRNA, while bars, Mediator subunit siRNAs. Error bars = Standard deviations from triplicate samples.

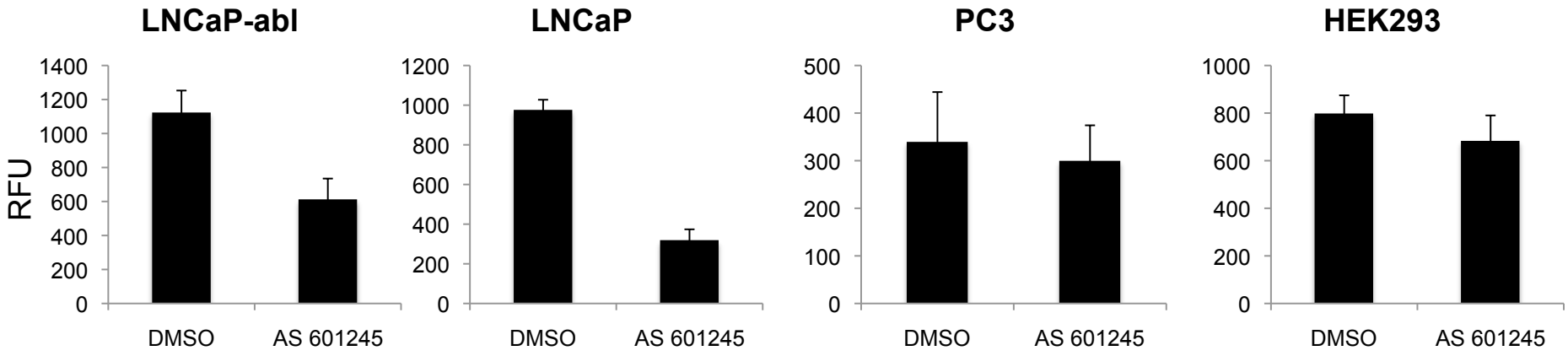


Supplementary Figure 9. MED19 and HIPK2 depletion differentially affect AR-hormone-dependent target gene expression in LNCaP-abl cells

LNCaP-abl cells were transfected with control siRNA (siCon),MED19 siRNA (siMED19) or HIPK2 siRNA (siHIPK2) and either androgen deprived for 24h and then treated with 10nM R1881 for 4h for CDK1 siHIPK2 and 24h for all others. Relative mRNA levels of the indicated genes were analyzed by qPCR and normalized to RPL19, and expression levels denoted about each column. Each assay was performed in duplicate, with error bars representing the range of the mean.



Supplementary Figure 10. Cell cycle analysis of LNCaP-abl cells as a function of HIPK2 and MED19.
LNCaP-abl cells were transfected with either control siRNA (siControl), or siRNAs target HIPK2 (siHIPK2) or MED19 (siRNA). After 24 hours, cell were harvested, stained with propidium iodide and analyzed by flow cytometry. Note that compared to control, depletion with either factor resulted in only a small decrease in the percentage of cells in S-phase of the cell cycle.



Supplementary Figure 11. A HIPK2 kinase inhibitor (AS 601245) reduces AR-expressing prostate cancer cell proliferation

LNCaP-abl, LNCaP, PC3 and HEK293 cells were treated with 5μM AS 601245 and cell proliferation was measured after 72 hours as in Figure 1.

Supplementary Table 1A

Selective AR regulators indentified in the secondary validation screen

(+)Hormone		(-)Hormone	
Decreased: (21)	**Increased: (8)**	**Decreased: (3)**	**Increased: (7)**
BAT1	CERK	ROS1	BMPR2
CBP/p300	DDR2	MLXIPL	DDR2
CDC25A/CDC25B	CDC7	SAP130	FOXO3
CELSR1	FAM166B		SGSM1
CREB1	FST		SNUPN
CSTF2T	NAPG		TNRC6C
DGKI	PASK		UCKL1
FHL2	UCKL1		
FOXO3			
GPR179			
GSK3B			
HHEX			
HIPK2			
HLF			
HNRNPA1			
MED19			
MRPL40			
MXD1			
NUP153			
PHACTR3			
RPH3A			

Supplementary Table 1B

Non-selective AR regulators indentified in the secondary validation screen

(+)Hormone		(-)Hormone	
Decreased: (23)	Increased: (5)	Decreased: (8)	Increased: (29)
ATP2B3	ADCK1	AURKA	ADCK1
AURKA	CSNK1A1	CCNA2 / CCNA1	ATPBD3
CCNA1	SFRS3	HDAC5	CBP / p300
CCNA2	SFRS8	MRPL40	CCDC124
CHRNA3		MYCBP2 / PAM	CCNT1
COQ6		ODC1	CDC2L2 / CDK11
CTTNBP2NL		PRKCE	CDK7
FZD8			CHMP6
HDAC5			CSNK1A1
LBXCOR1			CDC7
MAP3K12			DUSP7 DUSP6
MKL2			FAM166B
ODC1			FST
PATL1			KCNQ5
PPP1R10			LARP1
PRKCE			MAN1A2
PRPF39			MAPK14
PSD3			NAPG
SNUPN			PASK
SOCS1			PPP1R3C
TADA1L			RPS7
TFDP2			SFRS3
UBAP2			SFRS8
WTIP			SLC4A3
			SOX1 SRY
			STK17A
			UBE2Q2
			UBR4 / p600

Supplementary Table 2

Expression of AR regulators in prostate cancer cells and tissues and interaction with AR

Gene	*AR Interaction	# protein expression normal	tumor
1. HIPK2		E+/S +	E++/S+
2. GSK3B	yes	E+/S	E++/S+
3. DGKI		E-/S++	E-/S+
4. CDC25A	yes	E++/S-	E+++/S-
5. CDC25B	yes	E+/S-	E++/S-
6. CDC2			
7. MED19		E++/S++	E+++/S+++
8. HLF			
9. MXD1		E++/S+	E++/S+
10. PHACTR3			
11. FOXO3	yes	E++/S++	E++/S++
12. HHEX			
13. NUP153		E++/S++	E++/S++
14. BAT1		E+++/S++	E+++/S++
15. CSTF2T		E-/S-	E-/S-
16. HNRNPA1	yes	E+++/S+++	E+++/S+++
17. CREBBP	yes	E++/S++	E++/S++
18. EP300	yes	E++/S++	E++/S++
19. CREB1		E+++/S+++	E+++/S+++
20. GPR179		E+/S-	E+/S-
21. CELSR1			
22. MRPL40		E++/S++	E+++/S+++
23. FHL2	yes	E++/S++	E+/S+
24. AR		E+++/S+++	E++/S-

*Previously demonstrated interaction of AR regulators identified in the siRNA screen with AR: data complied from the Androgen Receptor-Interacting Proteins database (http://androgendb.mcgill.ca/ARinteract.pdf).
Protein expression of the AR regulators in normal and malignant human prostate tissue: data collected from the Human Protein Atlas (http://www.proteinatlas.org/): KEY: E= prostate epithelial cells; S= prostate stromal cells: Staining Intensity: none -; low +; moderate++: high ++++. Blanks have not been determined.

Supplementary Table 3

Gene expression changes (>1.5-fold) in common upon depletion of HIPK2 and AR, and MED19 and AR from LNCaP-abl cells

siHIPK2 and siAR

1. JPH1
2. CDKL1
3. KLK2
4. LOC440896
5. LOC283761
6. GCFC1-AS1
7. HSPC081
8. CLVS2
9. LOC283454
10. SPIRE2
11. VMP1
12. KCNA4
13. SLC34A1
14. SMG1
15. ONECUT1
16. C6orf145
17. ZNF440
18. C3orf67
19. ZNF818P
20. GLIPR1L1
21. C8orf12
22. NLRP10
23. WIPI2
24. PRSS2
25. IL2RA
26. CACNG3
27. MLANA
28. CH25H
29. CASP5
30. C9orf38
31. RARRES2
32. OR2F2
33. LINC00202
34. H1FNT
35. HORMAD2

siMED19 and siAR

1. SPIN3
2. TAS1R1
3. MLL3
4. EFHB
5. RAB6A
6. TPPP2
7. LOC100506314
8. HSPC081
9. CHGA
10. ARHGEF4
11. PLAG1
12. MICAL2
13. KCNA4
14. NRTN
15. ADRA1A
16. CERS4
17. ZNF771
18. EFHC2
19. H2AFJ
20. KRTDAP
21. SLC38A5
22. ZNF818P
23. GLIPR1L1
24. C8orf12
25. TSPEAR
26. GATA4
27. CHRM3
28. NBPF4
29. LOC100130950
30. LOC283392
31. CACNG3
32. SLITRK3
33. TNFRSF9
34. C9orf38
35. CCRL2
36. OR2F2
37. RTP4
38. PLAC9
39. LOC401022
40. DNM1P41
41. GSDMC

Supplementary Table 4: Drosophila gene symbols and amplicon identification (Id) numbers examined in the secondary validation screen

Official Gene Symbol	Amplicon Id
Mkp3	DRSC10084
CG32506	DRSC20540
CG32264	DRSC08061
XLL1	DRSC19959
Abl	DRSC23306
nAcRalpha-96Aa	DRSC13670
CG32297	DRSC22781
CycA	DRSC09132
drongo	DRSC00814
foxo	DRSC13017
fs(1)h	DRSC29017
CG34422	DRSC27508
HDAC4	DRSC25237
MED19	DRSC10516
Taf7	DRSC26720
CG6621	DRSC26979
PEK	DRSC12361
dor	DRSC27554
dlp	DRSC10472
Patj	DRSC28714
gammaSnap	DRSC28381
Pfrx	DRSC19315
Mnt	DRSC17894
CG12054	DRSC23137
fs	DRSC06259
Nup153	DRSC19904
Cdk8	DRSC28684
CG5359	DRSC15780
Ipp	DRSC26058
CG10881	DRSC28960
CG34380	DRSC26352
CG15254	DRSC29568
Paps	DRSC27101
CG31019	DRSC13994
Hrs	DRSC00549
gw	DRSC17135
CG8635	DRSC07226
CG7028	DRSC08144
lig	DRSC07246
fz2	DRSC10087
CG12452	DRSC11674
Rbp1	DRSC16817
CG6643	DRSC16082
nej	DRSC18801
Gprk2	DRSC16682
MTA1-Like	DRSC22022
CG8147	DRSC16390

Official Gene Symbol	Amplicon Id
KCNQ	DRSC06121
CG9053	DRSC20141
CycE	DRSC37016
Pde11	DRSC02460
BM-40-SPARC	DRSC38983
hiw	DRSC20339
CG3264	DRSC04353
muskelin	DRSC07273
CG8271	DRSC07133
sgg	DRSC23946
ATPCL	DRSC07147
CG7900	DRSC16336
Hr51	DRSC06627
CstF-64	DRSC16616
SP2353	DRSC07545
CG10924	DRSC06013
Dgk	DRSC05064
Sema-5c	DRSC11279
Pi4KIIalpha	DRSC12323
mRpL40	DRSC15749
CG32666	DRSC28871
Rad17	DRSC16811
CG7056	DRSC16186
Mbs	DRSC10553
CG31158	DRSC16141
cdc2	DRSC03504
CG3253	DRSC04350
larp	DRSC16984
dnc	DRSC17911
Odc2	DRSC26384
Sbf	DRSC16852
CG18048	DRSC29053
CG3812	DRSC19879
l(1)G0148	DRSC18429
dlg1	DRSC19767
CG3105	DRSC28829
CG4611	DRSC26382
CG8726	DRSC07250
CG11006	DRSC28079
Abl	DRSC09661
CG33276	DRSC29463
Pitslre	DRSC23346
CG10362	DRSC19355
CG34422	DRSC20029
CycT	DRSC11124
su(w[a])	DRSC18840
Ack	DRSC37045

Supplementary Table 4: Drosophila gene symbols and amplicon identification (Id) numbers examined in the secondary validation screen

scrt	DRSC23001
DNApol-delta	DRSC11138
Mhcl	DRSC14216
cic	DRSC16918
PNUTS	DRSC00638
CycB	DRSC04605
CG11590	DRSC28405
CG33275	DRSC10866
CG9238	DRSC11055
Mob2	DRSC09117
CG11253	DRSC28996
Oatp30B	DRSC02727
cdc2	DRSC03504
Dbp73D	DRSC11142
CG16708	DRSC12285
CG8397	DRSC07162
CG11722	DRSC14358
CG34404	DRSC15335
CG32850	DRSC21574
pho	DRSC37622
CG1646	DRSC15133
CycA	DRSC11123
CG34347	DRSC14305
CG10809	DRSC29041
gammaSnap	DRSC04686
nub	DRSC28839
CG10721	DRSC02098
CG8078	DRSC07069
stg	DRSC37007
CG8630	DRSC16441
CG15738	DRSC19704
nej	DRSC26774
CG8677	DRSC03107
CG34439	DRSC06364
CG31638	DRSC02269
CG18335	DRSC06723
hiw	DRSC20338
CG6540	DRSC19999
hh	DRSC29242
CG31367	DRSC22275
Cdk7	DRSC23327
MESK2	DRSC04259
Pp4-19C	DRSC21251
Hel25E	DRSC28348
cdc2c	DRSC36625
CG10915	DRSC06469
alc	DRSC07062
CG5656	DRSC11781
drl	DRSC28564

CG5705	DRSC29576
sev	DRSC39109
Pdp1	DRSC08897
CkIalpha	DRSC25564
CG32533	DRSC39093
CG9492	DRSC16503
sbr	DRSC20368
drl	DRSC03523
CG31140	DRSC14603
rdgA	DRSC39133
sti	DRSC09739
stan	DRSC05234
put	DRSC17039
CG2218	DRSC28089
CG3608	DRSC04394
wnd	DRSC11046
RpS7	DRSC28468
Ada1-2	DRSC01222
CG7277	DRSC03040
gw	DRSC17160
CG11063	DRSC19367
CG31064	DRSC15943
CG11093	DRSC21452
Pkc98E	DRSC28311
CrebB-17A	DRSC20232
Socs36E	DRSC02455
Btk29A	DRSC02666
CG1344	DRSC04837
CG1513	DRSC06553
wit	DRSC26091
CG30438	DRSC21419
nonA	DRSC27455
Mio	DRSC22006
Cdk7	DRSC18451
Dp	DRSC07402
stg	DRSC37007
CG31660	DRSC23053
syt	DRSC00157
CG34420	DRSC10133
mts	DRSC03574
tomosyn	DRSC19365
Lk6	DRSC24029
CG13390	DRSC39014
nub	DRSC39102
twf	DRSC15466
cg34380	DRSC02318
CG14804	DRSC18555
ix	DRSC06325
Fs	DRSC06258

Supplementary Table 4: Drosophila gene symbols and amplicon identification (Id) numbers examined in the secondary validation screen

Mrtf	DRSC08410
TyrRII	DRSC15150
sgg	DRSC18832
Dok	DRSC18204
Rph	DRSC17790
CG5214	DRSC15739
flw	DRSC25161
CG17723	DRSC21989
Antp	DRSC23104
Cklalpha	DRSC20231
Gprk2	DRSC23336
MED19	DRSC39059
rdgC	DRSC11387
Mpk2	DRSC16743
Pitslre	DRSC23346
PNUTS	DRSC23503
aur	DRSC27171
CG11105	DRSC19380
trio	DRSC08740
CG1907	DRSC15399
mbt	DRSC23020
Vps20	DRSC04448
CG9619	DRSC11081
Lmpt	DRSC09450
CG6013	DRSC15947
RpS24	DRSC28412
CycA	DRSC36992
Patr-1	DRSC14159
CG33275	DRSC10865
hipk	DRSC07885
CG8635	DRSC29143
CG5004	DRSC19930
CG32297	DRSC08277
CG5022	DRSC02802
Slbp	DRSC16863
ia2	DRSC00652
alpha-Man-I	DRSC17642
PPP4R2r	DRSC27825
Hsp70Aa	DRSC29327
mspo	DRSC07652
CG9855	DRSC12344
futsch	DRSC17952
CG3573	DRSC18576
Hrb98DE	DRSC24508
CG2924	DRSC28336
CG8177	DRSC08997
Dgk	DRSC06782
HLH3B	DRSC18669
CG8213	DRSC07109

Tbp	DRSC23111
ball	DRSC14145
Cirl	DRSC23235
PMCA	DRSC38802
ham	DRSC02512
l(2)k01209	DRSC29673
SoxN	DRSC03440
poe	DRSC28537

Supplementary Table 5– qPCR primers list

Gene	Forward Primer	Reverse Primer
PHACTR3	GCGCTGAACGACTCCATTAT	GCCGGTTCCTTAGCTTCAC
MXD1	GTGCCTGGAGAAGTTGAAGG	AGCTGGTCGATTTGGTGAAC
CELSR1	CGCTCATGGAGGTGTCTGT	CTGTTGGTCAGCATGTCGTC
HIPK2	CTGGCGGACTGGAGAAATAC	CCACATTTAAGGGCTGTGCT
MED19	CTGTGGCCCTTTTTACCTCA	GCTTCTCCTTCACCTTCTTCC
NUP153	TATATGCCGATGAGGAGAGC	AGATGGCTCCGATGAAGAGA
GSK3B	ACTGGTCGCCATCAAGAAAG	GAAGAAATAACGCAATCGGACT
DDX39B	TCTGGCTTTCGTGACTTCCT	GCACTCATGCTGGACTTCTG
HLF	AAATGTTTGACCCTCGCAAA	TGCCATGTTGTTCTTTCTGC
CDC25A	ACTGTCGCCTGTCACCAAC	GCTCATAATCACTGCCCAGA
CDC25B	GATTCCCCCAGCCCTATG	CCTGGATGGCCTGTTCAA
GPR179	GCACTCAGGCTCCTACCTTG	TGGACCTCTAGCTGGGCATA
FOXO3	GCAAAGCAGACCCTCAAACT	CGGTATCACTGTCCACTTGC
CSTF2T	GGATGCAGGGGGCAGGCATACA	CTAGGCTGGCTTCCACCTTGCTTG
MRPL40	AGCGAGCGTCATTGTTGTCT	TCGGATCTTCCTTTTCAAGC
HHEX	TGGGCAAACCTCTACTCTGG	GGTCTGGTCGTTGGAGAATC
CREBBP	GTGCTGGCTGAGACCCTAAC	CCCAGTTATTCCCATCTTGG
EP300	GGGAGGACAAACAGGATTGA	AGGATTGGGGTTGTTCATCA
CREB1	TGCCACATTAGCCCAGGTAT	AGAGTTACGGTGGGAGCAGA
MKNK2	GATCACCAGCCAGGAGTACG	CATCTCCACCTCCCTGAAAA
FHL2	CTGCGAGGAGTGTGGGAAG	GGCTTGTCCACCAGTGAGTT
HNRNPA1	GGAATGACAACTTCGGTCGT	ATTATAGCCATCCCCACTGC
MED26	ACCCCCAGAGCAACATCC	AGTGCCTCTTTGGTAATAGGG
MED29	TGACAAGTGCCTGGAAGAGTT	ACGTTGGAGAGTGCTTGG
CDK8	GGAACTGGGATCTCTATGTCG	AGCCACACCTTCCTATCAGC
MED1	CCTTCTGACCTACTGGATGACA	ATTCATGCCCAAAGATCGAG
MED14	CGTTAATAGCCTTCACCAAGC	CATTCCATTTTAGCTGTGTTGC
MED17	TAGACTTCAGCCAGGGTTCG	TACCACTCCTTCCTCGTCGT
MED12	TTGCTATCCACATCGACTGC	GAAGACTGGCGCATGGTC
MED23	GCTGATTTTCTCCCTGTGATG	GGGTTGACTGGGGTTTGTTA
MED15	CCAGCAAGGATATGGAGAGC	GGCCACGAGAGAAAGGTATTC
MED4	GCAGCTACAAAAACAGCTAAAGG	TCCTTCGCTTGGTAAACAGC
MED6	TGGTCAAAATGCAGAGGCTA	TCCGAATGATGAAAAGAATGG
MED16	CCCAAGATCGACCACCTG	CTGCTCCCACTGCTTCACC
CCNC	TGCTCCAGTATGTGCAGGAC	CAGTAGGCAAAGATCCGTTC

Supplementary Methods

Statistical Protocol for Genome Wide Screen Analysis

The screen was performed in duplicate and data was analyzed using six distinct statistical protocols to identify significant modulators of AR activity, (aka "hits"). The six different statistical parameters allowed us to be inclusive and not miss any potential hits. Genes were considered a hit in the genome-wide RNAi screen, only if it occurred in duplicate plates and satisfy at least 2 of the parameters below. A hit was considered weak if condition 6 (Nexp-median/IQR) and only one other parameter was met or if the 2 parameters were Z score and robust Z score. A strong hit would be one where 2 parameters were met without the use of condition 6. We decided to follow all hits, strong and weak in order to be inclusive.

1) Nexp=FF/RL
Data from firefly luciferase readings were normalized to Renilla luciferase (Nexp=FF/RL).
To establish cut-off values for inhibition or activation of AR transcriptional activity, the GFP average (4 controls included on each plate) was used. A gene was considered to inhibit or activate AR transcription if there was at least a 1.5-fold reduction or 3-fold increase with respect to the GFP average.

2) Log (Nexp)
Logarithmic transformation of Nexp was used to produce a linear progression of values. A gene was considered to inhibit or activate AR transcription if there was at least a 1.5 or 3-fold, respectively, reduction or increase with respect to the GFP average.

3) Log ($Nexp/N_{GFP}$)
Log ($Nexp/N_{GFP}$) represents a fold change of Nexp with respect to the N_{GFP} (normalized GFP average). A 1.5 fold decrease or 3 fold increase was considered significant.

4) Z score [$(Nexp-N_{GFP\ AVG})/ N_{GFP\ SD}$]
Z score measures in SD's, the deviation of Nexp from the $N_{GFP\ AVG}$. A Z score of zero equals the $N_{GFP\ AVG}$. Z scores less than -1.5 or greater than 5 were considered significant.

5) Robust Z score [(Nexp-median)/ Median Absolute Deviation]
Robust Z score measures the deviation of Nexp from the median. The use of robust z scores minimizes the presence of outliers. Robust Z scores less than -1.5 or greater than 5 were considered significant.

6) (Nexp-median)/IQR
(Nexp-median) / Interquartile Range measures the deviation from the median using the interquartile range (quartile 3 – quartile 1). Similar to the robust Z score, it is less sensitive to outliers. A less than 50% increase or a 2 fold increase with respect to the AR siRNA average (positive control) was considered significant. No value was less than AR siRNA.

Statistical Protocol for Kinome Screen Analysis

The screen was performed in duplicate and data was analyzed using six distinct statistical protocols to identify significant modulators of AR activity (aka "hits"). Genes were considered a hit in the Kinome RNAi screen only if it occurred in duplicate plates and satisfy at least 2 of the parameters below. Genes were considered strong hits if they met at least 2 conditions from the first three parameters and weak if only conditions 5 and 6 were met or if only conditions 4, 5 and 6 were met.

1) Nexp=FF/RL
Data from firefly luciferase readings were normalized to Renilla luciferase (Nexp=FF/RL).
To establish cut-off values for inhibition of AR transcriptional activity, the siAR positive controls average was used. A gene was considered to inhibit AR transcription if the levels of activation were equal or lowered to 3 folds the siAR average levels. To establish cut-off values for activation of AR transcriptional activity, the LacZ negative controls average was used. A gene was considered to activate AR transcription if there was 2 fold increase with respect to the LacZ average.

2) Log (Nexp)
Logarithmic transformation of Nexp was used to produce a linear progression of values. A gene was considered to inhibit AR transcription if there was at least a 3 fold reduction with respect to the siAR average or to activate if there was 2 fold increase with respect to the LacZ average.

3) Log ($Nexp/N_{LacZ}$)
Log ($Nexp/N_{LacZ}$) represents a fold change of Nexp with respect to the N_{LacZ} (normalized LacZ average). A 2 fold decrease or increase was considered significant.

4) Z score [$(Nexp-N_{siAR\ AVG})/ N_{siAR\ SD}$]
Z score measures in SD's, the deviation of Nexp from the $N_{siAR\ AVG}$. A Z score of zero equals the $N_{siAR\ AVG}$. A gene was considered to inhibit AR transcription if it was less than 3 SDs away from the siAR z score average. A gene was considered to activate AR transcription if it was greater than 2 SDs away from the LacZ z score average.

5) Robust Z score [(Nexp-median)/ Median Absolute Deviation]
Robust Z score measures the deviation of Nexp from the median. The use of robust z scores minimizes the presence of outliers. Robust Z scores less than -2 or greater than 2 were considered significant.

6) (Nexp-median)/IQR
(Nexp-median) / Interquartile Range measures the deviation from the median using the interquartile range (quartile 3 – quartile 1). Similar to the robust Z score, it is less sensitive to outliers. A less than 50% increase or a 2 fold increase with respect to the AR siRNA average (positive control) was considered significant. No value was less than AR siRNA.