#### SUPPLEMENTARY DATA FOR KROISS ET AL.

Supplementary Data 1-2

Supplementary Tables 1-3

Supplementary Data 1. Global microRNA expression analysis in androgen-stimulated LNCaP cells. A reverse transcription was performed using TaqMan® microRNA Reverse Transcription Kit and Megaplex RT Primers Human Pool A v2.1 (Applied Biosystems), with 500 ng total RNA. A high-throughput qPCR of 377 microRNAs and 3 endogenous controls was performed with TaqMan® Array Human MicroRNA A Cards v2.0 (Applied Biosystems), according to manufacturer's instructions, on an AB7900HT system (Applied Biosystems). Ct values were determined using RQManager v1.2 software (Applied Biosystems). Values were corrected by normalization to the median of endogenous controls expression (RNU6B, RNU44 and RNU48). Fold induction of microRNA expression after R1881 stimulation was determined, using the  $2^{-\Delta\Delta Ct}$  equation. Selection of deregulated microRNAs was based on statistical analyses performed using the RealTimeStatMiner® software (Integromics). The putative androgen-regulated microRNA quantification assays, as described in Material and Methods.

# Supplementary Data 2. Screening of miR-135a regulated genes using microarray technology.

- <u>Probe labelling and array hybridization</u>. RNA profiling in LNCaP cells were performed using a Human Whole Genome GE 4x44k V1 oligonucleotides microarray kit (Agilent). An antisens RNA (aRNA) amplification procedure was performed (*AminoAllyl MessageAmp II aRNA amplification kit*, Ambion), using 2 µg total RNA isolated from cells transfected with miR-135a or miR-NC. The aRNA was chemical coupling to the Cy-monoreactive dye ester (Amersham). One  $\mu$ g of combined Cy3/Cy5 dye-labeled aRNA were hybridized competitively (miR-135a versus miR-NC transfected cells). Each experiment was repeated with a switch in fluorescent Cy3/Cy5 labels to follow a dye-swap design and account for dye effects. Four data points per hybridization spot were produced, using samples from 24 and 48 h post-transfection experiments.

- <u>Computational analysis of microarray data.</u> Microarrays were scanned on an Agilent DNA Microarray Scanner, using two color scan setting (for both Cy3 and Cy5 channels) for 4x44k array slides. Probes quantification was performed using Feature Extraction Software 10.5 (Agilent) and data files (txt format) were generated. Background subtraction (half with an offset of 100)) and loess normalisation were performed using limma package (Smyth GK. Linear models and empirical bayes methods for assessing differential expression in microarray experiments. *Stat Appl Genet Mol Biol*, 2004;3:Article3) in R environment. Quantile normalization was added for between array normalization. Controls and low intensity/flagged probes in both channels were filtered out. Only probes present in the four experiments were analyzed (21 257) and used for performing statistical analysis.

- <u>Data deposition</u>. The datasets are posted at NCBI's Gene Expression Omnibus and are available through the GEO Series accession number GSE45903, with the following link:

#### http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE45903

- <u>Shift expression profile analysis</u>. AMEN suite of tools was used (46), as previously described (47).

## **Supplementary Table 1. Primer sequences.**

#### Sequence of primers for qPCR

	Forward	Reverse
Pri-miR-135a1	GTTGGGGTGGAAGAAGTG	GGCTCCAATCCCTATATGA
Pri-miR-135a2	GGTTAGCTTTGAAATGGTTG	TCCATCCCTACATGAGACTT
ROCK1	TACCAGCTGCAAGCTATATTAGA	TTCGAGATTATGTTTGAGATGC
ROCK2	ATCAAAGAGATGATGGCTAGAC	GCAAGATTGGCAACATCAC
Histone H4 promoter	AAATGGTGGGATCACAGACG	CGAGCTTCTTGTTTCCGTGT
TBP promoter	GTGGCGGTCCACATAAAAAC	GTCCTCTCATGCCCTGTGTT
PSA AREIII	ACAGACCTACTCTGGAGGAAC	AAGACAGCAACACCTTTTT
miR-135a2 ARE-2	GGCCAGGTTGTTAAAGCACT	CATCGGCCAGAACTCTTAGC

### Sequence of primers for plasmid constructions

	Forward	Reverse
-1402/-22 miR-	GGTACCAAGGAGAGATGTGGTAGA	GCTAGCTGGTCGGATGCAAAACTT
135a2	GG	AT
-6762/-5522 miR-	GGTACCAGCTGCTATTTCATCATTT	GCTAGCTAAGCCATCGGCCAGAAC
135a2	G	ТС
-8822/-7622 miR-	GGTACCTCTACAGCATATGGCATTG	GCTAGCCAGAATGAAGATTACTGA
135a2	С	GGC
Construct with	GGGTCTTGGTCAACAAGTAAAGCTT	CCATGACGAAGGAGAAAAAGCTTT
ARE-2 mutant	TTTCTCCTTCGTCATGG	ACTTGTTGACCAAGACCC
3'UTR ROCK1	CCATGTGACTGAGTGCCCTG	AATGCAACCCCCATTGAAAG
3'UTR ROCK2	CTGCCTTCTATGAAAGCAGT	TCCTAAGAGCTGGTAAACGC

Sequence of gene specific primers for 5'RACE assay

GSP1 for RT: 5'-TCCATCCCTACATGAGACTTT-3' GSP2 for first PCR: 5'-ATCTTGGTCGGATGCAAAAC-3'

GSP3 for nested PCR: 5'-CTTCACATGACTTCACAACC-3'

# Supplementary Table 2. Clinicopathological features of samples from patients with prostate cancer.

N°	Age	Gleason	Stage	рN	Margin	PSA
						[ng/ml]
1.	67	3+3	pT2c	N0	R1	15
2.	72	3+3	pT2c	N0	R0	8.16
3.	66	3+4	pT2a	N0	R0	5.64
4.	60	3+4	pT3a	N0	R0	12.3
5.	60	3+4	pT3a	N0	RI	9.8
6. -	51	3+4	pT3a	N0	RI	11
7.	66	3+4	pT3a	N0	R0	22
8.	62	3+4	p12c	N0	R0	4.8
<b>9.</b>	49	3+4	p12c	N0	RI	5
10.	63	3+4	p12c	N0	R0	6.8
11.	03 70	3+4	p12c	NU NO	KI DO	5.55
12.	70 72	3+4	p13a	NU N	R0	9.33
13.	13	3+4	p12b	INX NO	KI D1	0.1
14.	50 52	3+4	p13a	NU NO	KI D1	5.85
15.	55 54	3+4	p13a	NU NO		5.5
10.	54	3+4	p13a	NU NO	R0	0.0
17.	00	3+4 2+4	p13a	NU Nu	R1 D0	13.43
18.	08 60	3+4	p13a mT2h	INX NO	R0 D0	3.2 7.22
19. 20	00 61	5 <del>+</del> 4	p120	NO NO	R0 R0	1.25
20.	01 67	4+3	p12c	NO NO	R0 D1	0.0
21. 22	65	4+3	p130 pT2b	NO NO	R1 R0	10
22.	03 57	4+3	p130	NO	R0 R0	5.6
25. 24	57	4+3	p120	NO	R0 R0	5.0 1.9
24. 25	04 60	4+3	pT2c	NU N1	R0 P0	4.0 17
25. 26	66	4+3	p130	NO	R0 D1	7.83
20. 27	60	4+3	p120	NO	R1 R0	83
27.	60	4+3	p13a pT3b	NU N1	R0 R1	10.13
20. 20	61	4+3	p130 pT3a	Nr	R1	8 1
29. 30	61	4+3	pT3a	NO	RO	4.04
31	59	4+3	pT2e	NO	R0 R1	13.1
32	65	4+3	pT3a	Ny	R1	4 96
33	69	4+3	pT2e	N1	R1	6 48
34	75	4+3	pT3b	Nx	RO	3 23
35	62	4+3	pT3b	N1	RO	197
36.	62	4+3	pT3a	NO	RO	13.3
37.	57	4+3	pT3a	NO	RO	26
38.	60	4+3	pT3b	NO	RO	10
39.	70	4+3	pT2c	NO	RO	5.42
40.	60	4+3	pT3b	NO	R1	7.7
41.	60	4+3	pT3b	NO	R1	12.7
42.	72	4+5	pT3a	N1	R0	6
43.	50	5+4	pT3a	N1	R1	8.96
44.	64	4+5	pT3a	N1	<b>R</b> 1	13
45.	70	4+4	pT3a	NO	R0	6.33
46.	71	5+4	pT3a	N1	<b>R</b> 1	8.9
47.	65	4+4	pT3a	N1	<b>R</b> 1	3.36
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N°	Age	Gleason	Stage	pN	Margin	PSA [ng/ml]
48.	44	4+5	pT3b	N0	R1	44
49.	58	4+4	pT3a	N1	R1	14
50.	74	4+5	pT3b	N0	R0	nd
51.	66	4+5	pT3b	N1	R1	16.8
52.	69	4+4	pT3a	N0	R1	10.6
53.	51	4+4	pT3b	N0	R0	10.51
54.	70	4+4	pT3b	N1	R1	4.35
55.	63	4+5	pT3a	N0	R1	4.6
56.	65	4+5	pT3b	N0	R1	13.3

The cases were all selected from Centre Hospitalier Lyon-Sud. Prostate tissue samples were obtained from 56 patients who underwent radical prostatectomy, between 2003 and 2012. A non-interventional biomedical research protocol for tissue samples conservation has been set-up with the approval of the Ethics Committee in Lyon (CPP Sud-Est 2; medical faculty and the state medical board) to these investigations. An informed consent was obtained for all patients included in these studies, prior to any tissue sample conservation and for research use.

Immediately after prostate removal (delay inferior to 10 min), small pieces of tissue (at least 6 tissue samples of 0.5 to 1 cc) were gross dissected by the pathologist (M.D.-P.), in the left and right peripheral zones, the transitional area and the seminal vesicles. All fragments were snapped frozen and stored in liquid nitrogen until analysis. To ensure the tissue was cancerous or non-cancerous and to confirm the Gleason score, histological analysis of a frozen section was performed for each sample by the same pathologist before any extraction. The fragments fully composed of tumor glands were selected and named "tumor tissue".

pT2 pathological stages: primary tumor confined to prostate. pT2a, unilateral disease involving one-half of one lobe or less; pT2b, unilateral disease involving more than one-half

of lobe but not both lobes; pT2c, bilateral disease; pT3 pathological stages: extra-prostatic extension of the disease. pT3a, extra-prostatic extension; pT3b, seminal vesicle invasion. N0, no distant metastasis in regional lymph nodes; N1, presence of metastasis in regional lymph nodes; Nx, regional lymph nodes not assessed.

R0, negative surgical resection margins; R1, positive surgical resection margins.

nd, PSA concentration not determined.

Supplementary Table 3. Pathological characteristics of samples from patients with prostate cancer.

		<b>Overall</b> (n=56)	<b>Low</b> <sup>1</sup> (n=0)	Intermediate <sup>2</sup> (n=16)	<b>High<sup>3</sup></b> (n = 40)
Gleason score	2				
	6	3.57 % (2)	0 % (0)	12.5 % (2)	0 % (0)
	7 (3+4)	30.36 % (17)	0 % (0)	43.75 % (7)	25 % (10)
	7 (4+3)	39.28 % (22)	0 % (0)	43.75 % (7)	37.5 % (15)
	8-9	26.78 % (15)	0 % (0)	0 % (0)	37.5 % (15)
Pathological s	stage				
	≤pT2a	1.79 % (1)	0 % (0)	6.25 % (1)	0 % (0)
	pT2b	3.57 % (2)	0 % (0)	12.5 % (2)	0 % (0)
	pT2c	23.21 % (13)	0 % (0)	81.25 % (13)	0 % (0)
	pT3a	44.64 % (25)	0 % (0)	0 % (0)	62.5 % (25)
	pT3b	26.79 % (15)	0 % (0)	0 % (0)	37.5 % (15)
Lymph node					
	N0	69.64 % (39)	0 % (0)	87.5 % (14)	62.5 % (25)
	N1	21.43 % (12)	0 % (0)	0 % (0)	30 % (12)
	Nx	8.93 % (5)	0 % (0)	12.5 % (2)	7.5 % (3)
Surgical merg	ges				
	R0	48.21 % (27)	0 % (0)	62.5 % (10)	42.5 % (17)
	<b>R</b> 1	51.79 % (29)	0 % (0)	37.5 (6)	57.5 % (23)

() are frequencies for parameters.

Risk classification:

- <sup>1</sup> Low Risk: stage pT1 to pT2a; Gleason score  $\leq$  6; PSA < 10 ng/ml.
- <sup>2</sup> Intermediate Risk: stage pT2b to pT2c; Gleason score of 7; 10 ng/ml < PSA < 20 ng/ml.

<sup>3</sup> High Risk: stage  $\geq$  pT3a; Gleason score  $\geq$  8; PSA > 20 ng/ml.