

Supporting Information

Identification of microRNA signature and potential pathway targets in prostate cancer

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Supporting Figures

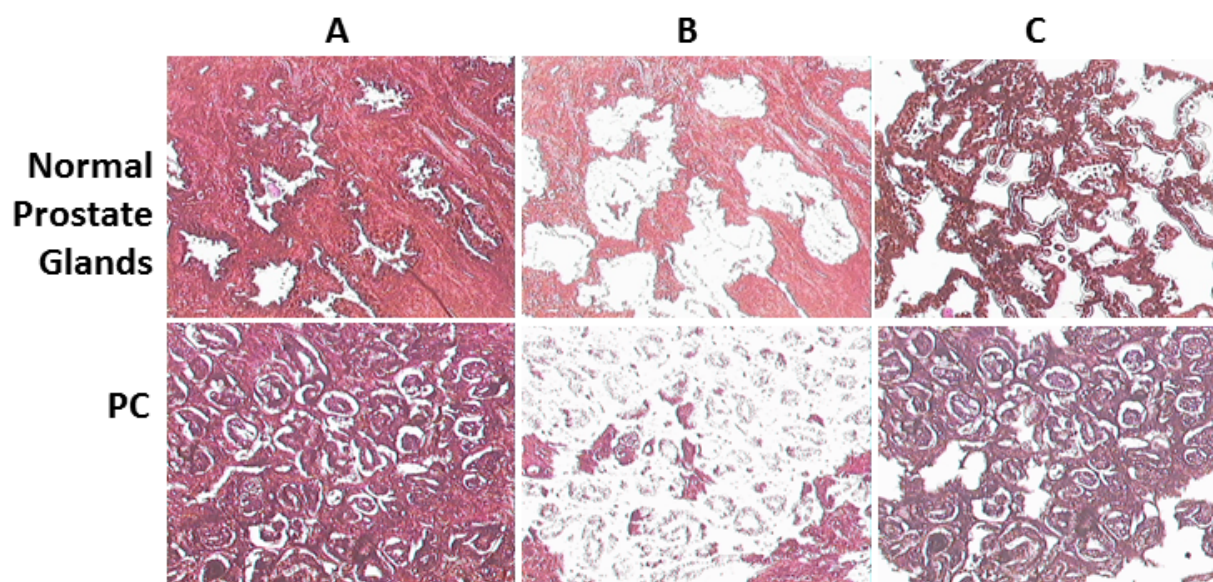


Figure 1 Microdissected normal and prostate tumor cells. Formalin-fixed paraffin-embedded (FFPE) PC tissue sections mounted onto uncoated glass slides were dewaxed (Materials and Methods), stained with Arcturus® Paradise® solution, and dehydrated before microdissection. Top, a representative case depicting non-neoplastic normal prostate glands before (A) after microdissection (B) and the captured cells on the cap (C). Bottom, representative section of organ-confined prostate cancer (Gleason score 6) before and after microdissection and the captured cells (magnification, 40×).

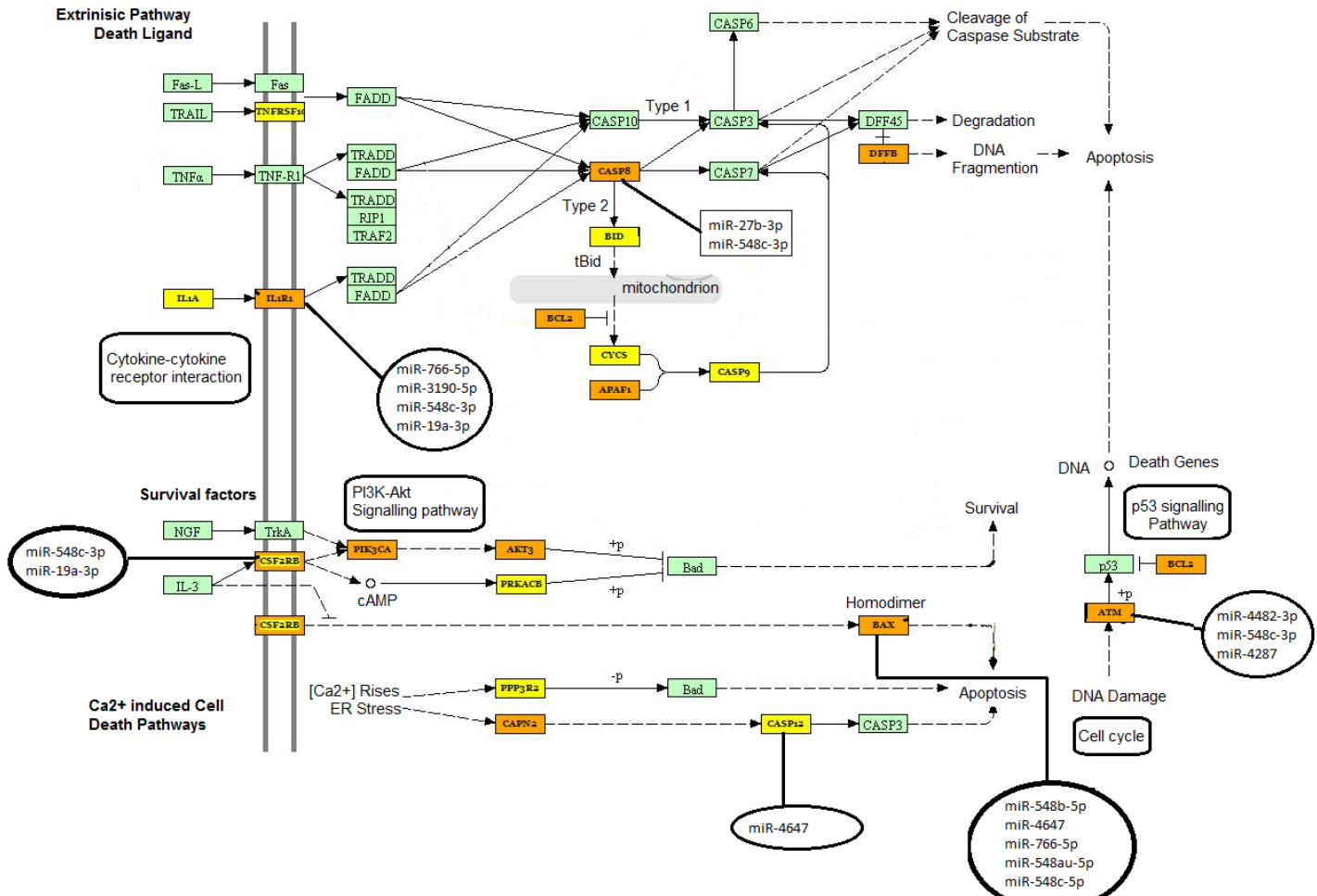


Figure 2 Diagrammatic scheme depicting targeted apoptosis-related genes by upregulated miRNAs in prostate tumor cells. An integrated *in silico* and computational prediction databases (DIANA-micro T-CDS, miRBase, and TargetScan) were employed as described in “Materials and methods” to predict potential targets of a subset of highly upregulated miRNAs (>2 fold) in prostate tumors. DIANA-miRPath “miRPath v2.0” was used to construct potential miRNA interactions in modulating apoptosis-related genes.

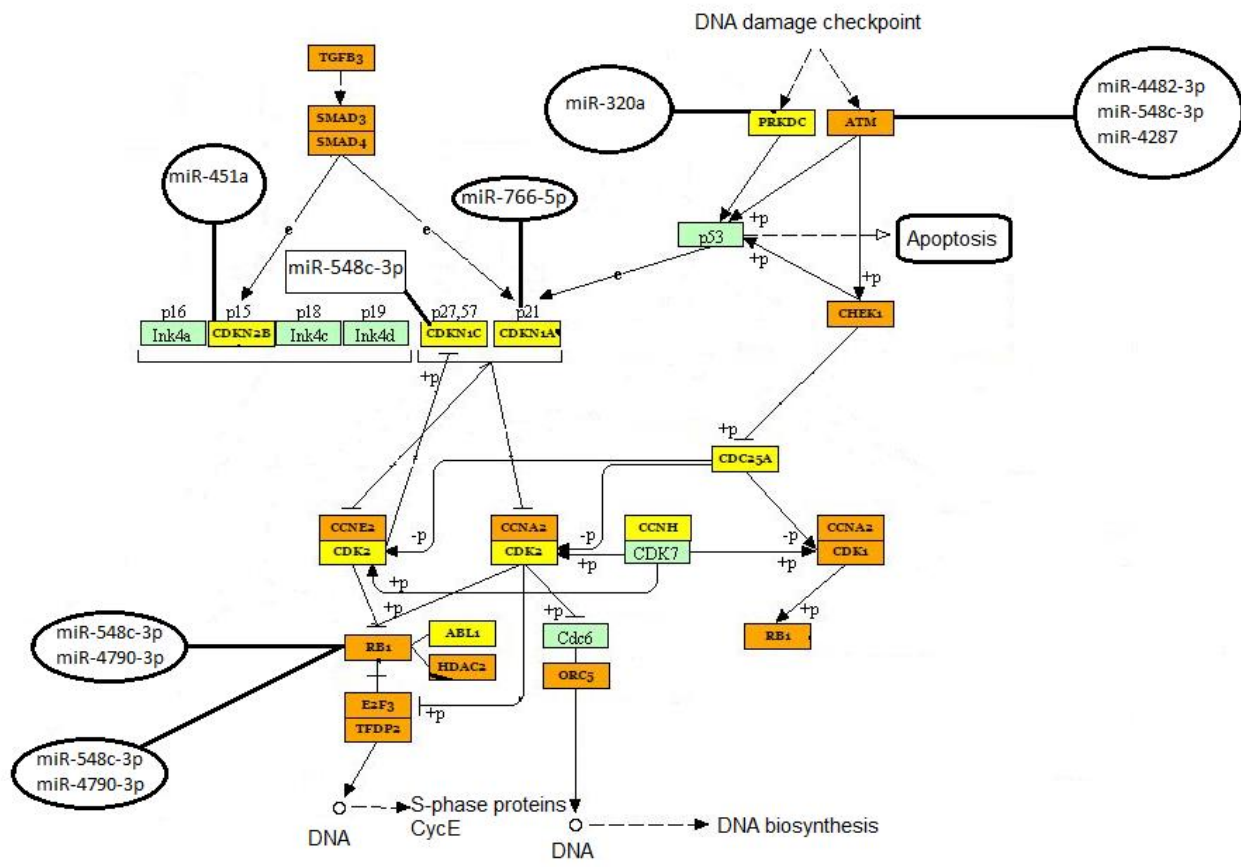


Figure 3 Diagrammatic scheme depicting targeted cell cycle-related genes by upregulated miRNAs in prostate tumor cells. An integrated *in silico* and computational prediction databases (DIANA-micro T-CDS, miRBase, and TargetScan) were employed as described in “Materials and methods” to predict potential targets of a subset of highly upregulated miRNAs (>2 fold) in prostate tumors. DIANA-miRPath “miRPath v2.0” was used to construct potential miRNA interactions in modulating cell cycle-related genes.

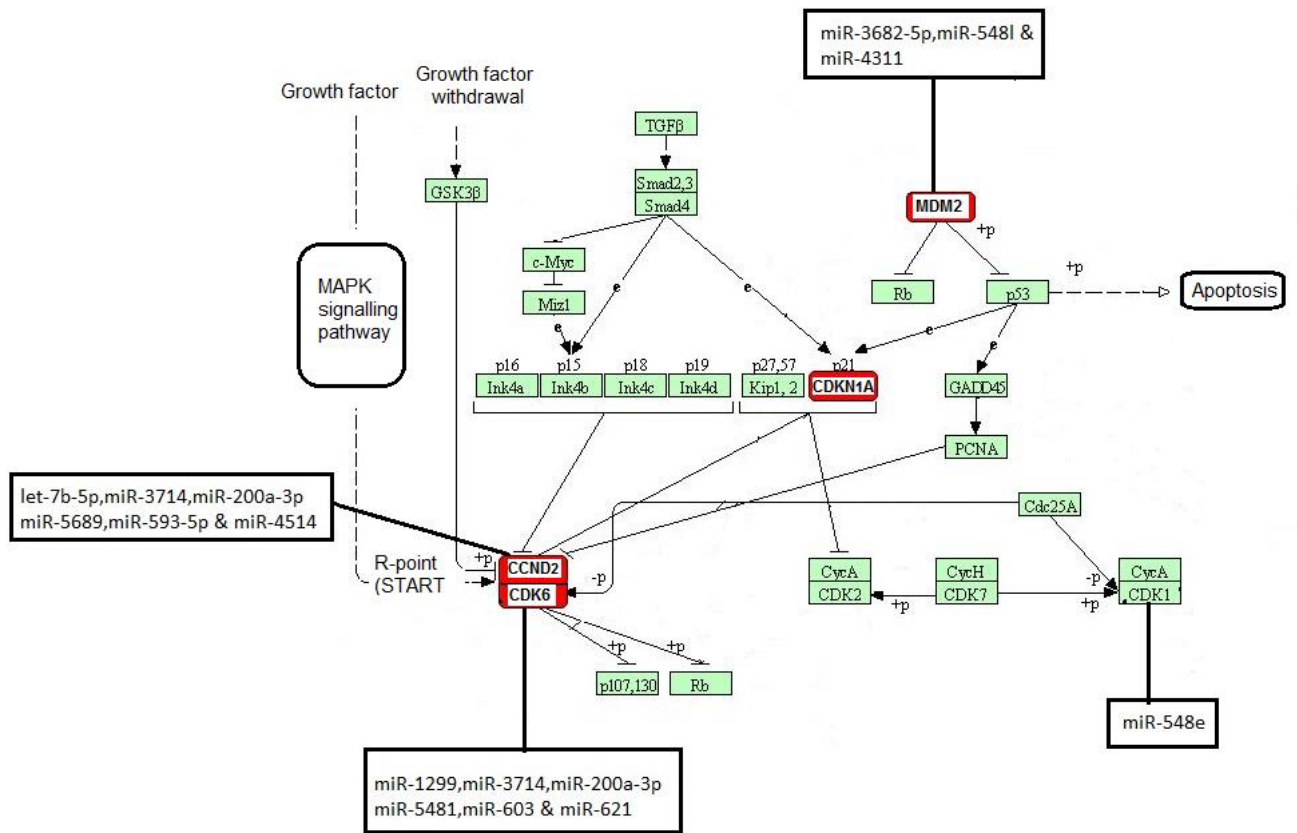


Figure 4 Diagrammatic scheme depicting potential cell cycle gene targets of the down-regulated microRNAs and their signaling pathways in prostate cancer cells. An integrated *in silico* and computational prediction databases (DIANA-micro T-CDS, miRBase, and TargetScan) were employed to predict potential targets of a subset of highly down-regulated miRNAs (>2 fold) in prostate tumors as described in “Materials and methods”. DIANA-miRPath “miRPath v2.0” was used to construct potential key targets in cell cycle.

Supporting Tables

Table 1 List of miRNA primer sequences used for qRT-PCR validation analysis in cell lines

microRNA	sequence
miR-30d-5p	5' - GCGTG TGT AAA CAT CCC CGA C- 3'
miR-603	5' - GGGG CAC ACA CTG CAA TTA C- 3'
miR-363-3p	5' - GGGAATTGCACGGTATCCA - 3'
miR-3120-5p	5' - TTGCCTGTCTGTGCCTGC - 3'
miR-1299	5' - GTTTGGTTCTGGAATTCTGTGT - 3'
let-7b-5p	5' - GGGTGAGGTAGTAGGTTGT - 3'
miR-200a-3p	5' - GTTTGGTAACACTGTCTGGTAA - 3'
miR-4790-3p	5' - GGGTT TGG TGA ATG GTA AAG CGA T- 3'
miR-1305	5' - GCGTT GGT GTG CAA ATC TAT GCA A- 3'
miR-19a-3p	5'-GTTGGTGTGCAAATCTATGCAA - 3'
miR-548c-3p	5'-CGCGTGGGGCAAAAATCTCAATTAC- 3'

Table 2 List of primer sequences of miRNA targets analyzed by qRT-PCR cell lines

Primer	Forward	Reverse	Reference
H-ras	GGGGCAGTCGCGCCTGTGAA	CCGGCGCCCACCACCACCAG	(1)
K-ras	GGGGAGGGCTTTCTTTGTGTA	GTCCTGAGCCTGTTTTGTGTC	(1)
Akt	GCACAAACGAGGGGAGTACAT	CCTCACGTTGGTCCACATC	(2)
Bcl2	GGAGGCTGGGATGCCTTT	GCCAAACTGAGCAGAGTC	(3)
c-Fos	GGGCAAGGTGGAACAGTTAT	AGTTGGTCTGTCTCCGCTTG	(4)
c-Jun	CCCCAAGATCCTGAAACAGA	CCGTTGCTGGACTGGATTAT	(4)
ATM	GAGACAGGGTTGCCATTGTA	CTCCAGCAGTGTATTGGGATAG	Designed
GAPDH	TCC CTG AGC TGA ACG GGA AG	GGA GGA GTG GGT GTC GCT GT	(5)

Table 3 Fold change of upregulated miRNAs in microdissected prostate tumors relative to the matched adjacent normal epithelium.

miRNA	Fold	miRNA	Fold	miRNA	Fold
hsa-miR-4469	11.6	hsa-miR-548c-3p	4.4	hsa-miR-520f	2.7
hsa-miR-548b-5p	11.6	hsa-miR-5687	4.4	hsa-miR-548al	2.7
hsa-miR-410	10.7	hsa-miR-652-5p	4.4	hsa-miR-548au-3p	2.7
hsa-miR-1179	9.8	hsa-miR-95	3.6	hsa-miR-5591-5p	2.7
hsa-miR-155-3p	9.8	hsa-miR-182-3p	3.6	hsa-miR-591	2.7
hsa-miR-3189-3p	8.9	hsa-miR-186-3p	3.6	hsa-miRPlus-C1114	2.7
hsa-miR-3622b-3p	8.0	hsa-miR-192-5p	3.6	hsa-miR-490-5p	2.7
hsa-miR-639	7.1	hsa-miR-4287	3.6	hsa-miR-5701	2.4
hsa-miR-18a-3p	7.1	hsa-miR-4486	3.6	hsa-miR-3064-5p	2.4
hsa-miR-92b-5p	7.1	hsa-miR-449a	3.6	hsa-miR-376a-5p	2.4
hsa-miRPlus-A1083	7.1	hsa-miR-4783-3p	3.6	hsa-miR-4477b	2.4
hsa-miR-3193	6.2	hsa-miR-548am-	3.6	hsa-miR-4669	2.4
hsa-miR-3661	6.2	hsa-miR-1269b	3.3	hsa-miR-4719	2.4
hsa-miR-378i	6.2	hsa-miR-136-3p	3.1	hsa-miR-211-3p	2.3
hsa-miR-4647	6.2	hsa-miR-19a-3p	3.1	hsa-miR-4438	2.3
hsa-miR-505-3p	6.2	hsa-miR-3614-5p	3.1	hsa-miR-654-5p	2.3
hsa-miR-511	6.2	hsa-miR-518d-3p	3.1	hsa-miR-10a-5p	2.2
hsa-miR-604	6.2	hsa-miR-5195-3p	3.1	hsa-miR-2682-5p	2.2
hsa-miR-4317	5.8	hsa-miR-548ao-3p	3.1	hsa-miR-376a-3p	2.2
hsa-miR-4430	5.8	hsa-miR-616-3p	3.1	hsa-miR-3943	2.2
hsa-miR-296-3p	5.3	hsa-miR-3972	2.9	hsa-miR-4433-3p	2.2
hsa-miR-3195	5.3	hsa-miR-221-3p	2.9	hsa-miR-4492	2.2
hsa-miR-34a-5p	5.3	hsa-miR-3689a-3p	2.9	hsa-miR-561-5p	2.2
hsa-miR-3650	5.3	hsa-miR-4254	2.9	hsa-miR-34b-3p	2.1
hsa-miR-451a	5.3	hsa-miR-5047	2.9	hsa-miR-1185-1-3p	2.1
hsa-miR-586	5.3	hsa-miR-1207-5p	2.7	hsa-miR-3165	2.1
hsa-miR-650	5.3	hsa-miR-215	2.7	hsa-miR-5686	2.1
hsa-miR-744-5p	5.3	hsa-miR-30b-3p	2.7	hsa-miR-611	2.1
hsa-miR-766-5p	5.3	hsa-miR-3123	2.7	hsa-miR-759	2.1
hsa-miR-27b-3p	4.4	hsa-miR-3151	2.7	hsa-miR-1247-3p	2.0
hsa-miR-3138	4.4	hsa-miR-3192	2.7	hsa-miR-4445-5p	2.0
hsa-miR-3190-5p	4.4	hsa-miR-376b-3p	2.7	hsa-miR-1302	2.0
hsa-miR-320a	4.4	hsa-miR-452-5p	2.7	hsa-miR-1305	2.0
hsa-miR-378e	4.4	hsa-miR-4709-5p	2.7	hsa-miR-3613-5p	2.0
hsa-miR-4482-3p	4.4	hsa-miR-4746-3p	2.7	hsa-miR-3692-5p	2.0
hsa-miR-4661-5p	4.4	hsa-miR-4766-5p	2.7	hsa-miR-5583-3p	2.0
hsa-miR-4680-5p	4.4	hsa-miR-4999-5p	2.7	hsa-miR-578	2.0
hsa-miR-4790-3p	4.4	hsa-miR-5194	2.7		

Table 4 Fold change of down-regulated miRNAs in microdissected prostate tumors relative to the matched adjacent normal epithelium.

miRNA	Fold	miRNA	Fold	miRNA	Fold
hsa-miR-3149	-113.3	hsa-miR-466	-7.3	hsa-miR-4701-3p	-5.6
hsa-miR-3689d	-76.5	hsa-miR-520g/hsa-miR-520h	-7.3	hsa-miR-10b-3p	-5.4
hsa-miR-3682-5p	-25.3	hsa-miR-499a-3p	-7.2	hsa-miR-4288	-5.2
hsa-miR-200a-5p	-20.0	hsa-miR-5689	-7.0	hsa-miR-4264	-5.1
hsa-miR-4518	-16.9	hsa-miR-4423-3p	-6.8	hsa-miR-4801	-5.1
hsa-miR-3121-5p	-16.3	hsa-miR-140-5p	-6.8	hsa-miR-4311	-4.9
hsa-miR-5189	-15.8	hsa-miR-203a	-6.8	hsa-miR-139-5p	-4.5
hsa-let-7b-5p	-14.6	hsa-miR-3130-3p	-6.8	hsa-miR-363-3p	-4.5
hsa-miR-3677-3	-14.6	hsa-miR-431-3p	-6.8	hsa-miR-654-3p	-4.5
hsa-miR-21-5p	-13.5	hsa-miR-4321	-6.8	hsa-miR-1251	-4.5
hsa-miR-1299	-13.0	hsa-miR-4325	-6.8	hsa-miR-2277-3p	-4.5
hsa-miR-3120-5p	-12.4	hsa-miR-4711-3p	-6.8	hsa-miR-521	-4.5
hsa-miR-4490	-12.4	hsa-miR-4734	-6.8	hsa-miR-621	-4.5
hsa-miR-595	-12.4	hsa-miR-4755-3p	-6.8	hsa-miR-5699	-4.3
hsa-miR-4449	-11.3	hsa-miR-4763-3p	-6.8	hsa-miR-10b-3p	-5.4
hsa-miR-30d-5p	-10.7	hsa-miR-593-5p	-6.8	hsa-miR-4288	-5.2
hsa-miR-3714	-10.7	hsa-miR-603	-6.8	hsa-miR-4264	-5.1
hsa-miR-3679-5p	-10.1	hsa-miR-892a	-6.5	hsa-miR-4801	-5.1
hsa-miR-3132	-9.9	hsa-miR-3689b-3p	-6.2	hsa-miR-4311	-4.9
hsa-miR-200a-3p	-9.0	hsa-miR-4665-3p	-6.2	hsa-miR-139-5p	-4.5
hsa-miR-3664-3p	-9.0	hsa-miR-4727-3p	-6.2	hsa-miR-363-3p	-4.5
hsa-miR-139-3p	-7.9	hsa-miR-483-5p	-6.2	hsa-miR-654-3p	-4.5
hsa-miR-2277-5p	-7.9	hsa-miR-3157-3p	-6.1	hsa-miR-1251	-4.5
hsa-miR-3122	-7.9	hsa-miR-1285-5p	-5.9	hsa-miR-2277-3p	-4.5
hsa-miR-4776-5p	-7.9	hsa-miR-4638-5p	-5.9	hsa-miR-521	-4.5
hsa-miR-5481	-7.9	hsa-miR-1272	-5.6	hsa-miR-621	-4.5
hsa-miR-589-5p	-7.9	hsa-miR-194-5p	-5.6	hsa-miR-5699	-4.3
hsa-miR-624-5p	-7.5	hsa-miR-377-3p	-5.6		
hsa-miR-337-3p	-7.3	hsa-miR-4517	-5.6		

Table 5 List of upregulated miRNA targets and their associated cellular pathways

Target	miRNA	Pathway
RB1	miR-548c-3p, miR-4790-3p	Cell Cycle
CDKN2B	miR-451a	
CDKN1B	miR-548c-3p	
CDKN1A	miR-766-5p	
TGFB1	miR-548c-3p	
ATM	miR-4482-3p, miR-548c-3p, miR-4287	
PRKDC	miR-320a	
BAX	miR-548b-5p, miR-4647, miR-766-5p, miR-548au-5p, miR-548c-5p	Apoptosis
ATM	miR-4482-3p, miR-548c-3p, miR-4287	
IL1R1	miR-766-5p, miR-3190-5p, miR-548c-3p, miR-19a-3p	
DFFB	miR-548c-3p	
CSF2RB	miR-548c-3p, miR-19a-3p	
CASP8	miR-27b-3p, miR-548c-3p	
CAPN1	miR-548b-5p, miR-4482-3p, miR-548au-5p, miR-548c-5p	
CASP12	miR-4647	
P53/ TP53	miR-1305	
TNFRSF10B	miR-548c-3p	
CASP8	miR-27b-3p, miR-548c-3p	
PMAIP1	miR-548b-5p	
BBC3	miR-548c-3p, miR-1305	
EI24	miR-27b-3p, miR-548c-3p	
ZMAT3	miR-449a, miR-548au-3p	
SIAH1	miR-27b-3p, miR-320a,	
CYCS	miR-548c-3p, miR-1305	
APAF1	miR-548c-3p, miR-1305	
CASP9	miR-548c-3p	
SERPINE1	miR-548c-3p	
CYCS	miR-5194	
APAF1	miR-449a	
SESN3	miR-548b-5p, miR-27b-3p, miR-320a, miR-4482-3p, miR-548c-3p, hsa-miR-182-3p, miR-19a-3p, miR-1305	
PTEN	hsa-miR-548b-5p, miR-27b-3p, miR-320a, miR-4482-3p, miR-548c-3p, miR-182-3p, miR-19a-3p, miR-1305	
NKX3-1	miR-378e, miR-378i	Prostate Cancer
FOXO1	miR-4680-5p, miR-548c-3p, miR-4719, miR-1185-1-3p, miR-4445-5p, miR-578	
RB1	miR-4790-3p, miR-548c-3p	
CASP9	miR-766-5p	

Table 6 List of down-regulated miRNA targets and their associated cellular pathways.

Target	miRNA	Pathway	
CCND1	let-7b-5p, miR-3714, miR-200a-3p, miR-5689, miR-593-5p, miR-4517	Cell cycle & P53	
CDK4	miR-1299, miR-3714, miR-200a-3p, miR-548l, miR-603, miR-621		
CDK2	miR-1299, miR-3714, miR-200a-3p, miR-548l, miR-603, miR-621		
CCNE1	miR-30d-5p, miR-200a-3p		
CCNB3	miR-4763-3p		
MDM2	miR-3682-5p, miR-548l, miR-4311		
MDM4	miR-3682-5p, let-7b-5p, miR-3120-5p, miR-548l, miR-4755-3p, miR-4311		
JUN	miR-1299		
JUND	miR-4763-3p, miR-603, miR-4801		
RRAS2	miR-548l, miR-4801, miR-4311		
PRKCA	miR-3689d, miR-1299, miR-200a-3p, miR-466		
RAF1	miR-3714, miR-589-5p		
BRAF	miR-4755-3p, miR-2277-3p, miR-5699		
GRB2	miR-3149, miR-3664-3p, miR-466, miR-603		
FOS	miR-603, miR-4311		
MCL1	miR-3679-5p, miR-3664-3p, miR-548l		Apoptosis
MYB	let-7b-5p, miR-3714, miR-194-5p		
BIRC8	miR-4801		
BCL2L1	let-7b-5p, miR-140-5p, miR-4755-3p, miR-377-3p		
BCL2	miR-548l		
PIK3R5	miR-3682-5p, miR-1299, miR-548l, miR-4325, miR-363-3p		
CSF2RB	miR-548l		
NGF	let-7b-5p		
AKT3	miR-3682-5p, miR-3120-5p, miR-139-5p		
PRKACA	miR-3679-5p, miR-200a-3p, miR-4321, miR-4517, miR-4801		
STAT5A	miR-603, miR-194-5p		
STAT3	miR-21-5p, miR-337-3p		
CFLAR	miR-3122		
CHUK	miR-4311		
NFKB1	miR-4311	Prostate Cancer	
HRAS	miR-3682-5p, miR-4490, miR-30d-5p, hsa-miR-548l, miR-337-3p, miR-4517		
EGF	miR-200a-5p, miR-30d-5p, miR-3664-3p, miR-603		
EGFR	miR-4763-3p, miR-3120-5p, miR-3664-3p, miR-4311		
GRB2	miR-3149, miR-3664-3p, miR-466, miR-603		
SOS1	miR-30d-5p, miR-3714, miR-548l, miR-466, miR-4311		
ARAF	miR-3714, miR-589-5p		
HSP90AA1	miR-3664-3p		
MAPK1	miR-593-5p		

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