

MicroR-449a enhances radiosensitivity by downregulation of c-Myc in prostate cancer cells

Aihong Mao^{1, 2, 3, 6}, Qiuyue Zhao^{1, 4, 5, 6}, Xin Zhou^{1, 4, 5}, Chao Sun^{1, 4, 5},

Jing Si^{1, 4, 5}, Rong Zhou^{1, 4, 5}, Lu Gan^{1, 4, 5}, Hong Zhang^{1, 4, 5*}

Supplementary tables

Table S1 Primer sequences of miR-449a, b, c and RNU6

Gene	Sequence	
miR-449a	5'-TGGCAGTGTATTGTTAGCTGGT-3'	
miR-449b	5'-AGGCAGTGTATTGTTAGCTGGC-3'	
MiR-449c	5'-TAGGCAGTGTATTGCTAGCGGCTGT-3'	
RNU6	F	5'-CTCGCTTCGGCAG CACA-3'
	R	5'-AACGCTTCACGAATTG-3'

Table S2 Primer sequences for c-Myc and GADPH

Gene	Sequence	
c-Myc	F	5'-GCAGCTGCTTAGACGCTGGA-3'
	R	5'-CGCAGTAGAAATACGGCTGCAC-3'
GADPH	F	5'-TCGCTCTCTGCTCCTCCTGTTC-3'
	R	5'-CGCCCAATACGACCAAATCC-3'

Table S3 miRNA/Oligonucleotide sequences for expression miR-449a inserted GV214 vector

Gene	Sequence
miR-449a	5'- <u>CGGATCCATTGTTAGCTGGCTGCTTGGGTC</u> AAGTCAGCAGCCACA ACTACCCTGCCACTTGCTTCTGGATAAATTCTTCTTGTC AATGAAGTG CTCTGGATACCTGTGTGTGATGAGC <u>TGGCAGTGTATTGTTAGCTGGTT</u> GAATATGTGAATGGCATCGGCTAACATGCAACTGCTGTCTTATTGCATA TACAATGAACATCAGAGTGTA ACTGAATCTGTAATTAGTGTGTGTTTA TGTGTACTTTCTGCTATGAAG <u>CAAGCT</u> -3'
miR-con	5'-TTCTCCGAACGTGTCACGT-3'

Table S4 SiRNA sequences for targeting c-Myc

miRNA/siRNA	Sequence
Anti-miR-449a	5'-AUCGGCUAACAUGCAACUGCUG-3'
Anti-NC	5'-UCUACUGUCACUCAGUAGUUU-3'
si-c-Myc1(S1)	5'- GCUUCACCAACAGGAACUATT-3' 3'-TTCGAAGUGGUUGUCCUUGAU-5'
si-c-Myc2(S2)	5'- GGAAACGACGAGAACAGUUTT-3' 3'-TTCCUUUGCUGCUCUUGUCA-5'
si-c-Myc3(S3)	5'- CCACACAUCAGCACAACUATT-3' 3'-TTGGUGUGUAGUCGUGUUGAU-5'
NC	5'- UUCUCCGAACGUGUCACGUTT-3' 3'-TTAAGAGGCUUGCACAGUGCA-5'

Table S5 Oligonucleotide sequences for c-Myc-3'UTR and c-Myc-3'UTR mutant

Oligonucleotide	Sequence
c-Myc-3'UTR	<u>TCTAGA</u> AATGTCCTGAGCAATCACCTATGAACTTGTTTCAAATGCA TGATCAAATGCAACCTCACAACCTTGGCTGAGTCTTGAGACTGA AAGATTTAGCCATAATGTAAACTGCCTCAAATTGGACTTTGGGCA TAAAAGAACTTTTTTATGCTTACCATCTTTTTTTTTTCTTTAACAG ATTTGTATTTAAGAATTGTCTAGA
c-Myc-3'UTR MUT	<u>TCTAGA</u> AATGTCCTGAGCAATCACCTATGAACTTGTTTCAAATGCA TGATCAAATGCAACCTCACAACCTTGGCTGAGTCTTGAGACTGA AAGATTTAGCCATAATGATGTGACGGTCAAATTGGACTTTGGGCA TAAAAGAACTTTTTTATGCTTACCATCTTTTTTTTTTCTTTAACAG ATTTGTATTTAAGAATTGTCTAGA

Supplementary figures and figure legends

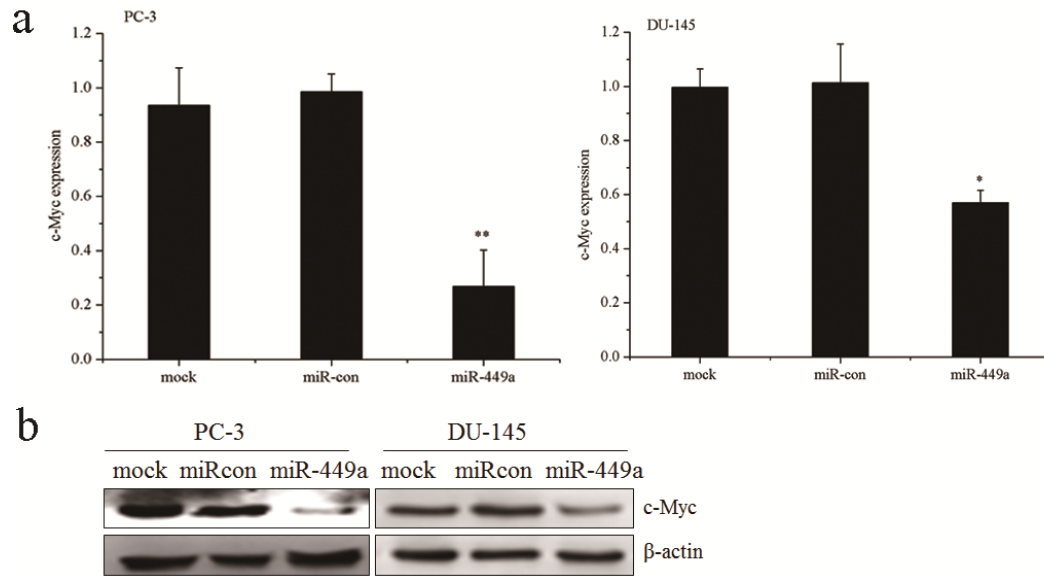


Figure S1. c-Myc was downregulated by miR-449a in prostate cancer cells. (a) The effect of miR-449a on c-Myc mRNA expression in PC-3 and DU-145 cells, versus control. **(b)** The effect of miR-449a on c-Myc protein expression in PC-3 and DU-145 cells. * $P < 0.05$ and ** $P < 0.01$.

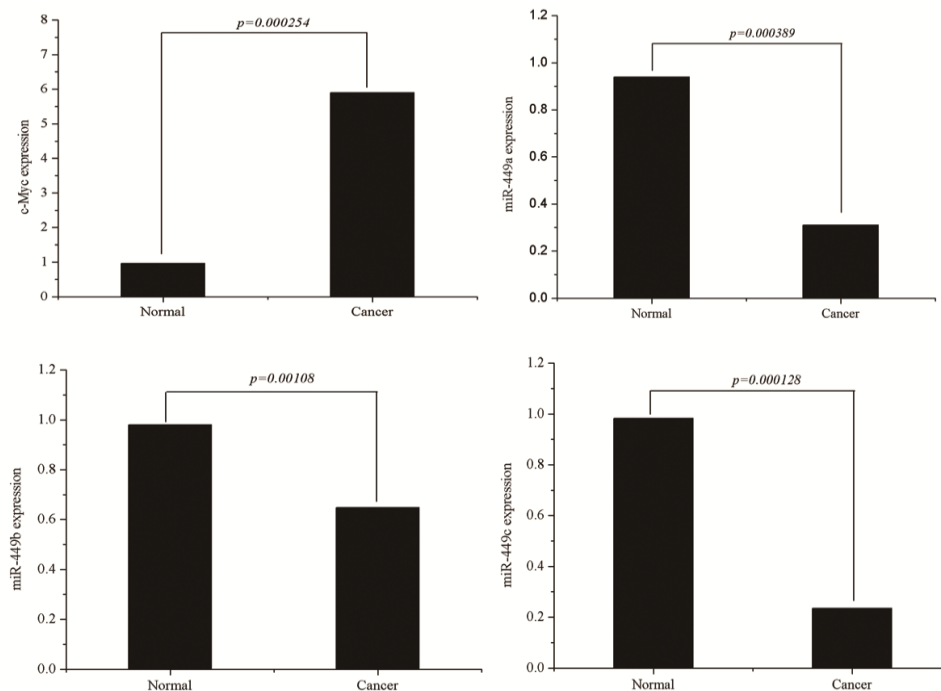


Figure S2. c-Myc and miR-449a/b/c were expressed in prostate cancer tissues.
 Real-time RT-PCR analysis of mature miR-449a/b/c and c-Myc expression in prostate cancer and normal prostatic tissues prepared from the same patients.

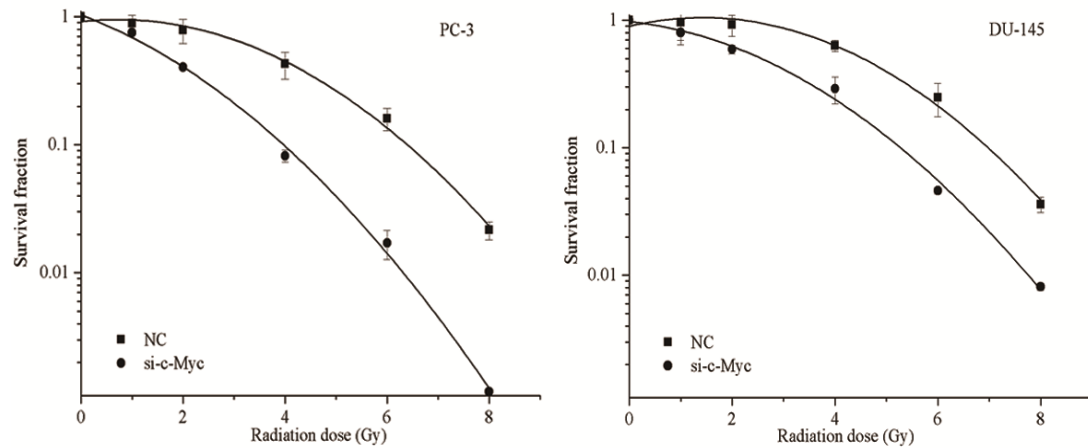


Figure S3. Knockdown of c-Myc enhanced the radiosensitivity of PC-3 and DU-145 cells to IR.

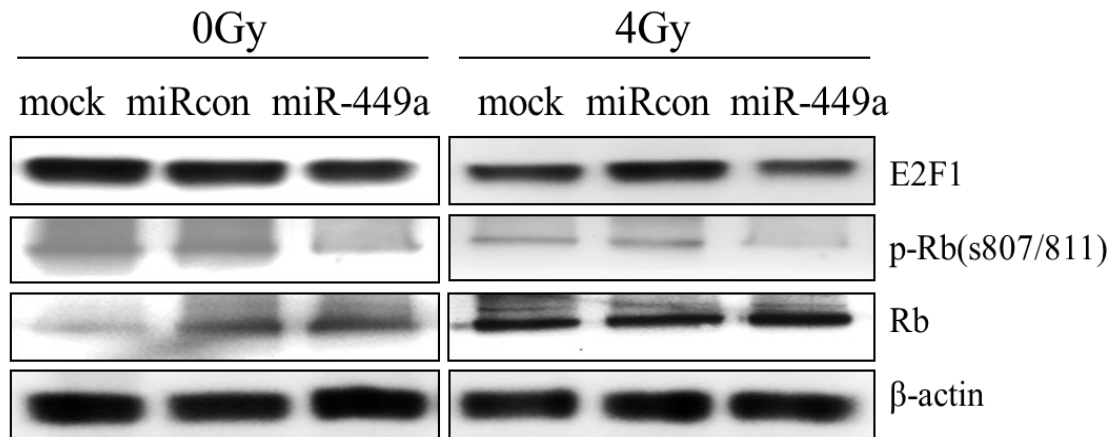


Figure S4. MiR-449a regulated Rb/E2F1 and Rb phosphorylation in response to IR.