

Niclosamide for prostate cancer

Table S1. Sequence of siRNA and primer for experiments

Sequence of siRNA		
Name	Sequence (5' to 3')	
siFOX1-1	CAACAGGAGUCUAAUCAAG	
siFOX1-2	GGACCACUUUCCCUACUUU	
siFOX1-3	CUCUUCUCCUCAGAUUA	
siEXO1	GCACGUAAUUAAGUGAUG	
Primer for RT-qPCR		
Gene	Forward sequence (5' to 3')	Reverse sequence (5' to 3')
FOX1	TTCAGAACCCTTAGACCTCATC	GCTGAGGCTGTCATTATTGTG
BARD1	TCTGTAGCCAACCATCTGTATCTC	ACTTCATTCTGCTCTTAGTGTCTG
EXO1	CCTGCCATTCAAGAAGTCATAG	TAATCACTCGTTCCACTCCCAC
BLM	CAGACTCCGAAGGAAGTTGTATG	GAAGTCTCAGAAGTATCAAAGTCATCC
BRCA2	CTTGCCCTTTTCGTCTATTTG	GTCGCCACTGGAGGTTGC
RAD51	TTGTAGACAGTGCCACCGCC	AACATCGTGTCCATCCAC
β -actin	GAGACCTTCAACACCCAGC	ATGAGGTAGTCAGTCAGGTCCC
Primer for ChIP assay		
Name	Forward sequence (5' to 3')	Reverse sequence (5' to 3')
EXO1 P1	GCTAAATCTGGCAACCCTACC	AGGCATAAAGAGATGTCCTGTGTC
EXO1 P2	AGGTAAATGGTAGGGGCAGAT	CTCGGAAGTTGGGAGTGTTC
Primer for promoter cloning		
Name	Forward sequence (5' to 3')	Reverse sequence (5' to 3')
pGL3-EXO1 P1	TAAGTGGCCGTACCTACCTCAAAGGTTTTCAAGTCTATTGA	CGGATTGCCAAGCTTCAAAGGTTTCAAAGTCTATTCTTGG
pGL3-EXO1 P2	TAAGTGGCCGTACCTACTACTGCAATGGGGAAAAGAACC	CGGATTGCCAAGCTTAACACGGGTAAGTTCCTACACAGCGC
pGL3-BLM P	TAAGTGGCCGTACCAAGGAATTGTCAGTCTTTTCATTC	CGGATTGCCAAGCTTAAATCTGCCTGTACACAGTAAGTCC

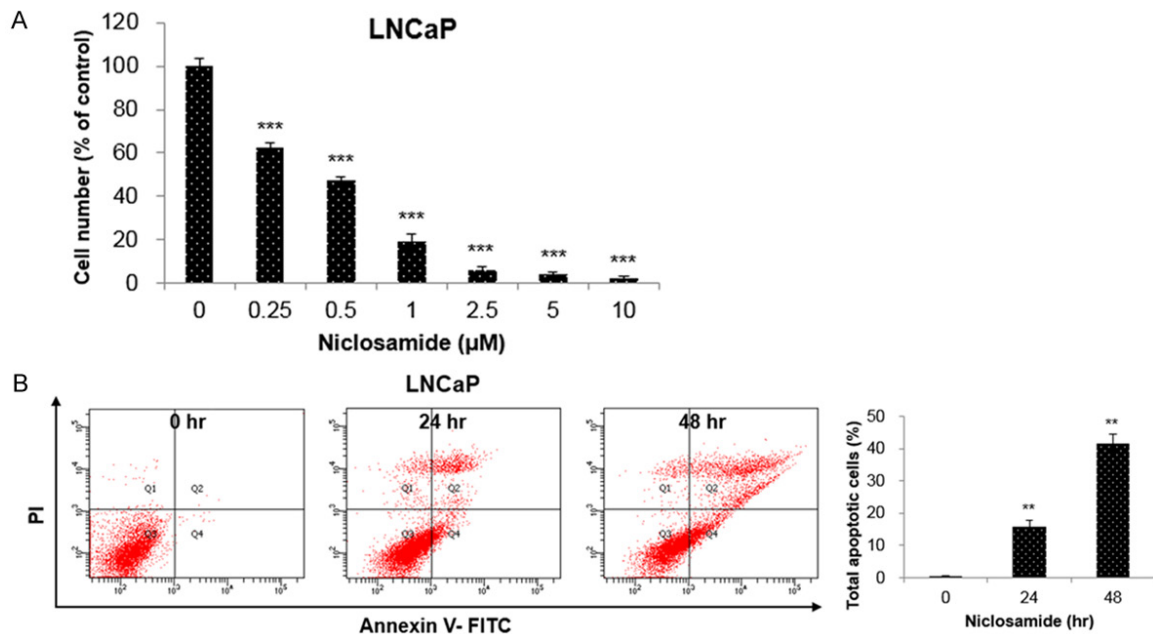


Figure S1. Niclosamide inhibits cell proliferation and induces apoptosis in LNCaP cells. (A) LNCaP cells were incubated with the indicated concentrations of niclosamide for 48 hr and cell viability was measured using the WST assay. (B) LNCaP cells were incubated with 0.5 μM niclosamide for 24 and 48 hr. Apoptosis was determined by analyzing FITC Annexin V-PI staining. All data are presented as the mean \pm SD of two experiments performed in triplicate. ** $P < 0.01$, *** $P < 0.001$, two-tailed Student's t test.

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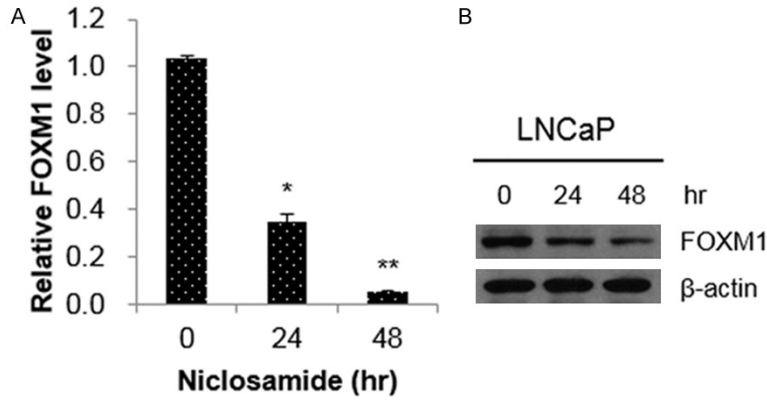
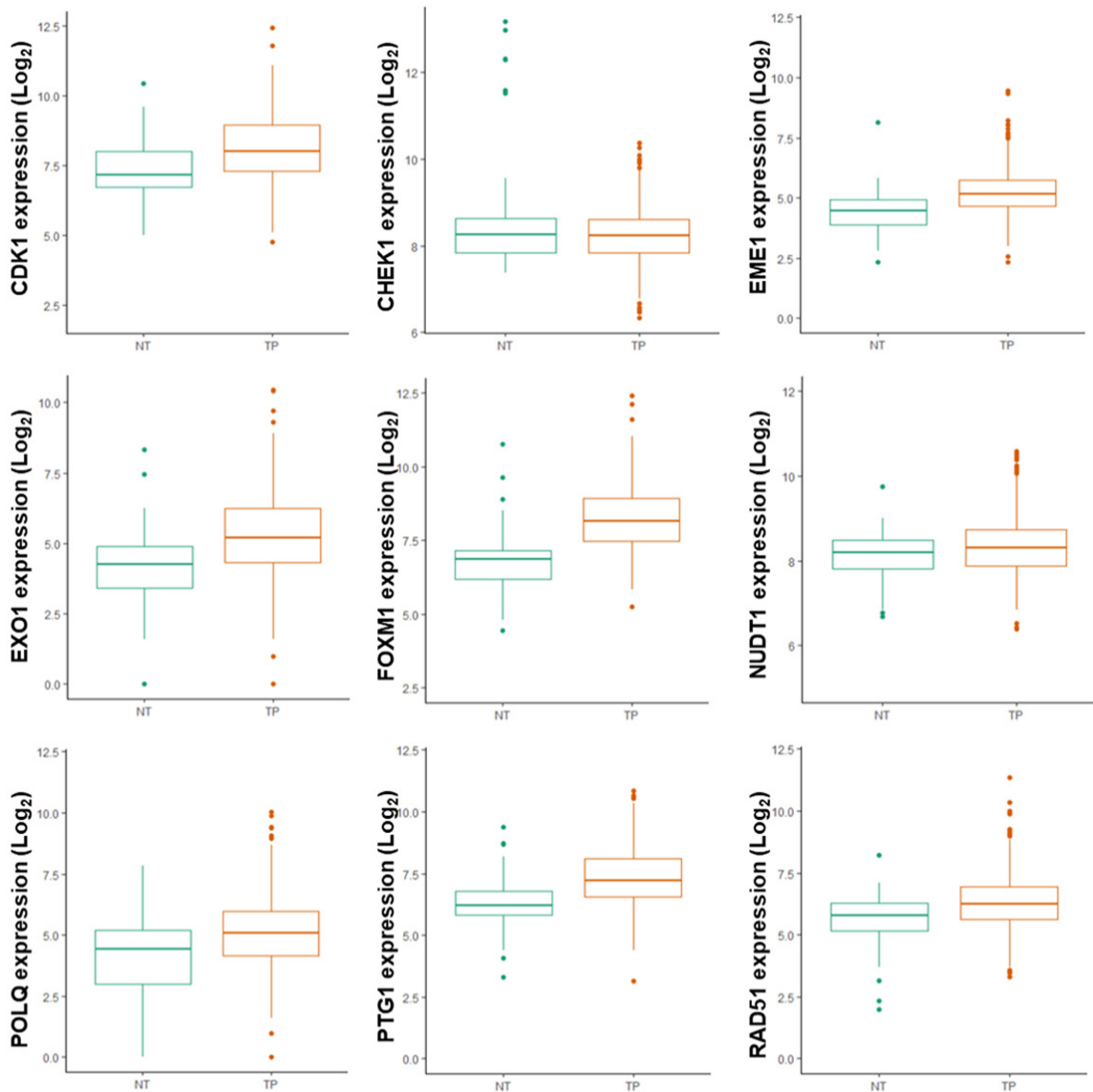


Figure S2. Niclosamide reduces the expression of FOXM1 in LNCaP cells. (A and B) LNCaP cells were incubated with 0.5 μ M niclosamide for 24 or 48 hr. FOXM1 mRNA (A) and protein (B) expression was determined using qRT-PCR and western blot analyses, respectively. β -actin was used as an internal control. Data are presented as the mean \pm SD of two experiments performed in triplicate. * $P < 0.05$, ** $P < 0.01$, two-tailed Student's t test.



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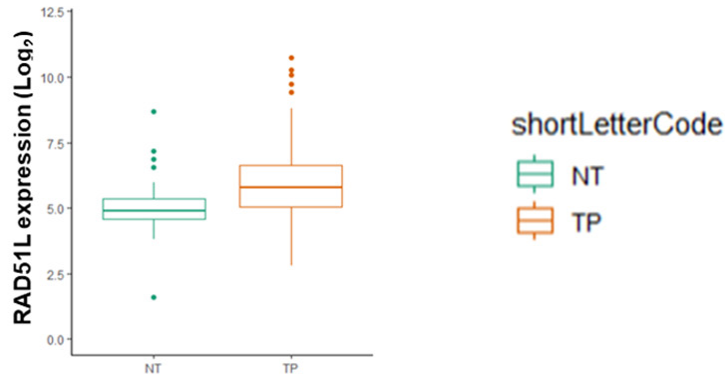


Figure S3. Analysis of mRNA expression for 10 clinically important genes in tumor and adjacent normal tissues using the TCGA data set.

Table S2. Clinical significance of the 10 candidate target genes

Gene Symbol	FC (PC3-Niclosamide/PC3-DMSO)	Log2 FC (Tumor/Normal, GDC portal, TCGA-PRAD)	Overall survival <i>p</i> value (TCGA, Firehose Legacy)
CDK1	0.078	1.123259	0.0276
EXO1	0.168	1.405179	<0.0001
RAD54L	0.200	1.281174	0.38
PTTG1	0.218	1.356720	0.357
RAD51	0.270	1.140352	0.179
NUDT1	0.271	-1.16502	0.791
POLQ	0.281	1.252423	0.646
FOXM1	0.322	1.627793	0.0113
CHEK1	0.381	-1.25142	0.678
EME1	0.394	1.048552	0.12

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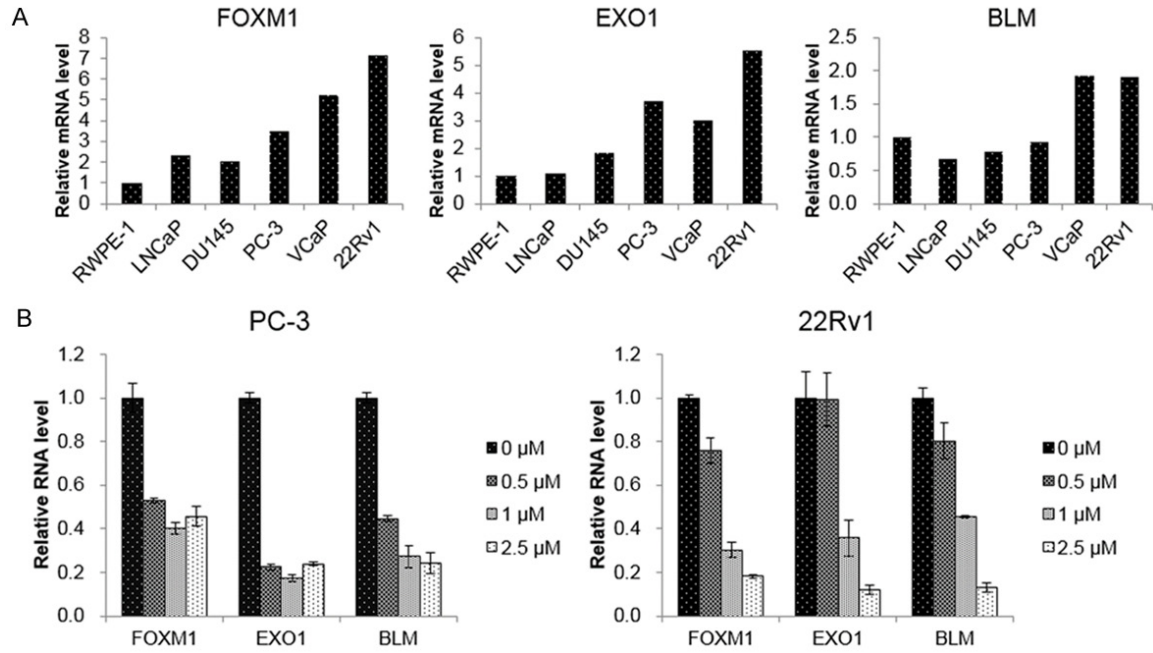


Figure S4. FOXM1, EXO1, and BLM mRNA expression in the different cell lines. (A) qRT-PCR was used to evaluate the basal expression of FOXM1, EXO1, and BLM in the indicated cell lines. The control cell line was the non-tumorigenic human prostate epithelial cell line RWPE-1. (B) PC-3 and 22Rv1 cells were incubated with the indicated concentrations of niclosamide for 48 hr. mRNA expression was quantified by qRT-PCR. β -actin mRNA was used as an internal control to normalize the data.

Table S3. Correlation coefficient and overall and disease-free survival from the TCGA database

	co-expression with FOXM1		Overall Survival (<i>p</i> -value)	Disease Free Survival (<i>p</i> -value)
	Pearson's correlation	Spearman's correlation		
RAD54B	0.63	0.46	0.126	0.0831
BLM	0.72	0.70	0.237	0.00488
RAD51	0.49	0.69	0.182	0.0579
SHFM1	0.14	0.14	0.199	0.342
BRCA2	0.54	0.43	0.086	0.200
XRCC2	0.59	0.56	0.482	0.00123
FANCM	0.26	0.24	0.0935	0.535
EXO1	0.88	0.80	0.0001701	0.0138
POLH	0.26	0.15	0.00114	0.746
EME1	0.69	0.61	0.198	0.164
BARD1	0.25	0.19	0.502	0.709