Supplementary information

Supplementary methods

INF-α induction

PC3 cells were transfected with 100 nM siRNAs or left untreated. Cell culture supernatant was collected 72 h after transfection. INF-α in the supernatant was measured using the Flow Cytomix Human INF-α Simplex Kit (Bender MedSystems, Wien, Austria) according to the manufacturer's instructions. Human recombinant INF-α at concentrations ranging from 20,000 to 32 pg/ ml was used as control. INF-α concentrations were calculated using by FlowCytomix Pro 2.2 Software (Bender MedSystems GmbH, Wien, Austria).

Supplementary Figure Legends

Supplementary Figure S1. Activity of promoter-targeted siRNA in prostate cancer cells. DU145 and LNCaP cells were transfected with 100nM of siRNAs and harvested after 72 h to evaluate *c-myc* mRNA by RT-PCR.

Supplementary Figure S2. Lack of INF-α induction upon transfection of siRNAs. PC3 cells were transfected with 100 nM of the indicated siRNAs or left untreated. Cell culture supernatant was collected 72 h and INF-α was measured using the Flow Cytomix Human INF-α Simplex Kit. Samples containing human recombinant INF-α were used as control. INF-α was undetectable in siRNA-treated cells.

Supplementary Figure S3. Positive controls for chromatin pull-down with antibodies against K9 dimethylated and K27 trimethylated histone H3. DNA immunoprecipitated with H3K9me2 and H3K27me3 antibodies was assessed by SYBR Green q-PCR with primers for the *c-myc*, p16 (*upper panel*) and RARB2 (*lower panel*) gene promoters. Data are normalized to the amount of input DNA.

Supplementary Figure S4. Negative control reactions to exclude contamination with genomic DNA in the detection of promoter-associated transcripts. RNA samples from PC3, DU145 and LNCaP cells were amplified by PCR (without RT step) in order to exclude DNA contamination. Genomic DNA and a DNA-free RNA sample are included as positive and negative control, respectively. NT, no template reaction.

Supplementary Figure S5. Identification of promoter-associated transcripts in the *c-myc* promoter. 5'RACE products were cloned and sequenced. The position of the 5' ends of the five cloned transcripts relative to the major *c-myc* TSS (P2) is indicated in the upper panel and their sequences are shown in the lower panel.

Supplementary Figure S6. Selective knock-down of Ago1 and Ago2 in PC3 cells. Cells were transfected with 100 nM of siRNAs and harvested after 48 h to isolate total RNA. Levels of Ago 1 and Ago2 were measured by RT-PCR.

Supplementary Figure S7. Limited induction of apoptosis in siRNA transfected prostate cancer cells. PC3 cells were harvested 4 days after transfection with siRNAs. Active caspase-3 and c-Myc levels were measured by FACS using PE-labeled anti-active caspase 3 and anti-c-Myc mouse monoclonal antibody followed by a FITC-labeled anti-mouse secondary antibody.

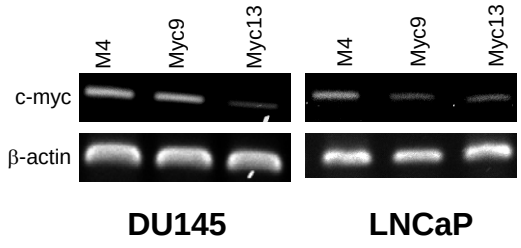
Table S1. Sequenses of small interfering RNAs and antisense oligonucleotides.

Small interfering RNAs	**Sequence**
Myc13	5′-CGGGGCUUUAUCUAACUCGtt-3′ 3′-ttGCCCCGAAAUAGAUUGAGC-5′
Myc13cm	5′-CGGGGCUUGCGCUAACUCGtt-3′ 3′-ttGCCCCGAACGCGAUUGAGC-5′
Myc9	5′-GCUUUAUCUAACUCGCUGUtt-3′ 3′-ttCGAAAUAGAUUGAGCGACA-5′
Myc24	5′-AAGCCGGUUUUCGGGGCUUtt-3′ 3′-ttUUCGGCCAAAAGCCCCGAA-5′
Myc59	5′-AAAAAGAACGGAGGGAGGGtt-3′ 3′-ttUUUUUCUUGCCUCCCUCCC-5′
GL3	5′-CUUACGCUGAGUACUUCGAtt-3′ 3′-ttGAAUGCGACUCAUGAAGCU-5′
M3	5′-CUGAGUAAGCGCGAUCUAAtt-3′ 3′-ttGACUCAUUCGCGCUAGAUU-5′
M4	5′-CGGGUCUCUAUCUGACUAGtt-3′ 3′-ttGCCCAGAGAUAGACUGAUC-5′
M5	5′-CGCGGAUUUAUCGAAGUCGtt-3′ 3′-ttGCGCCUAAAUAGCUUCAGC-5′
Ago1	5′-GAGAAGAGGUGCUCAAGAAuu-3′ 3′-uuCUCUUCUCCACGAGUUCUU-5′
Ago2	5′-GCACGGAAGUCCAUCUGAAUU-3′ 3′-uuCGUGCCUUCAGGUAGACUU-5′
Antisense Oligonucleotides	
ASO	5′-TTATACTCAGCGCGATCCCT-3′
Scrambled ASO	5′-TCCCTCGCGACTCAGTTATA-3′

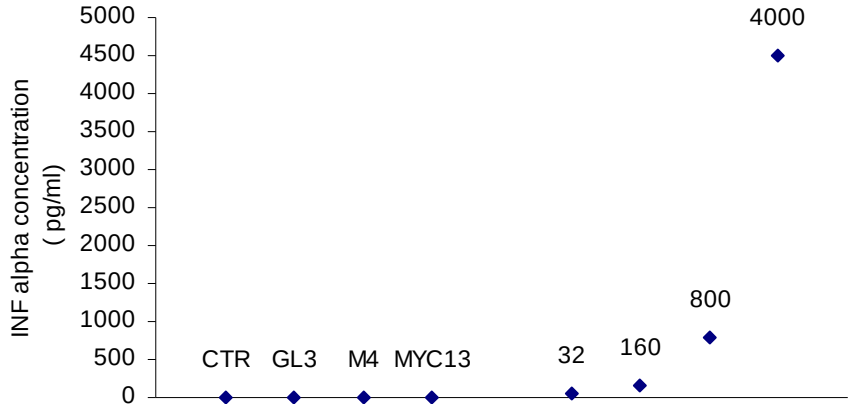
Table S2. Primers sets used for RT-PCR, nuclear run-on, ChIP, biotin pull-down, qPCR and 5'RACE.

Primer set	Primers	Sequence	Applications
Myc/exon2/3	Forward Reverse	5′-ggtggtcttcccctaccctctcaa-3′ 5′-ggcagcaggatagtccttccg-3′	RT-PCR
β-Actin	Forward Reverse	5′-aagagaggcatcctcaccct-3′ 5′-tacatggctggggtgttgaa-3′	RT-PCR
hTERT	Forward Reverse	5′-tctggatttgcaggtgaacagcc-3′ 5′-gggtggccatcagtccaggatgg-3′	RT-PCR
Ago1	Forward reverse	5′-gcactgcccattggcaacgaa-3′ 5′-cattcgccagctcacaatggct-3′	RT-PCR
Ago2	Forward Reverse	5′-cgcgtccgaaggctgctcta-3′ 5′-tggctgtgccttgtaaaacgct-3′	RT-PCR
GAPDH	Forward Reverse	5′-ggctgtgggcaaggtcatccc-3′ 5′-tccaccaccctgttgctgta-3′	Nuclear run on
Myc/exon1	Forward Reverse	5′-ggcactttgcactggaactt-3′ 5′-gcaaggagagcctttcagag-3′	Nuclear run-on
Myc/-83/+124	Forward Reverse	5′-agggcttctcagaggcttg-3′ 5′-cctattcgctccggatctc-3′	ChIP, RT-PCR Biotin pull-down
Myc/-217/-75	Forward Reverse	5′-aagatcctctctcgctaatctcc-3′ 5′-agaagccctgcccttctc-3′	ChIP, RT-PCR
Myc/+220/+403	Forward Reverse	5′-ggcactttgcactggaactt-3′ 5′-ggtgcttacctggttttcca-3′	ChIP
Myc/-44/+68	Forward Reverse	5′-agggatcgcggctgagtataa-3′ 5′-ggctcttccaccctagcc-3′	Biotin pull-down
Myc/-217/+124	Forward Reverse	5′-aagatcctctctcgctaatctcc-3′ 5′-cctattcgctccggatctc-3′	RT-PCR
Myc/-374/-75	Forward Reverse	5′-catgcggctctcttactctg-3′ 5′-agaagccctgcccttctc-3′	RT-PCR
Myc/mRNA	Forward Reverse	5′-ggtgctccatgaggagaca-3′ 5′-cctgcctcttttccacagaa-3′	Real time PCR
Myc/paRNA -226/-158	Forward Reverse	5′-aagatcctctctcgctaatc-3′ 5′-ggtcctcagcctgtccaga-3′	Real time PCR
Myc/P2	Forward Reverse	5′-agggatcgcgctgagtataa-3′ 5′-tgcctctcgctggaattact-3′	Real time RT-PCR
β-Actin	Forward Reverse	5′-aactggtctcaagtcagtgtacagg-3′ 5′-tcccccaacttgagatgtatg-3′	Real time PCR
RARß2	Forward Reverse	5′-GCACGTAGGCTGTTGGTCTTT-3′ 5′-GCTGGCTTGTCTGTCATAATTCA-3′	Real time PCR
p16	Forward Reverse	5′- TCCTGAAAATCAAGGGTTGAG-3′ 5′-GCAAAACTATTCTTTCCTAGTTGTGA -3′	Real time PCR
GSP1 -202	Reverse	5′-CGGAGATTAGCGAGAGAGGA-3′	5′RACE
GSP2 -297	Reverse	5′-GGAAGGTGGGGAGGAGACT-3′	5′RACE
GSP3 -333	Reverse	5′-CAGCCGAGCACTCTAGCTCT-3′	5′RACE

Supplementary Figure S1

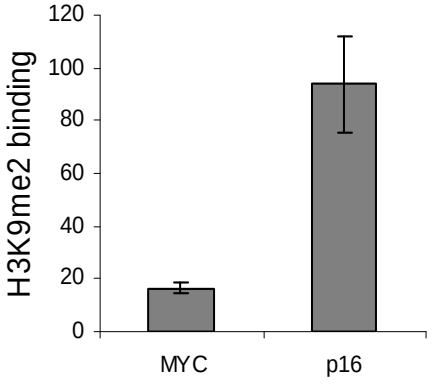


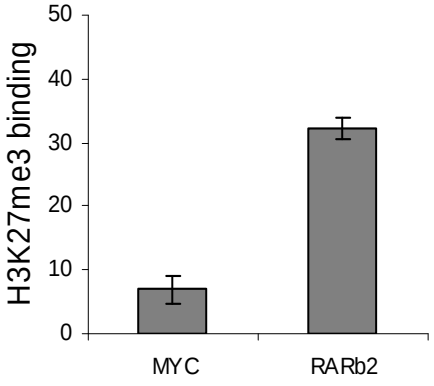
Supplementary Figure S2



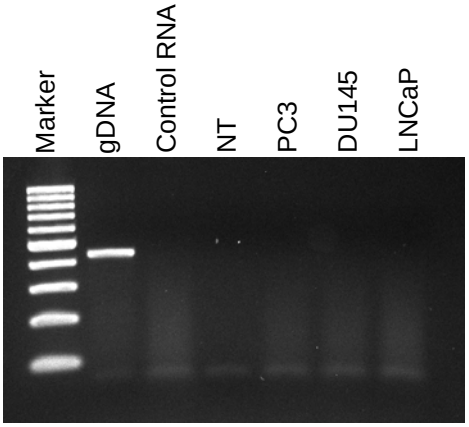
SAMPLE NAME	NOMINAL VALUE pg/ml	MEASURED VALUE pg/ml
ST.1	32	64.65
ST.2	160	150.71
ST.3	800	793.49
ST.4	4000	4487.92
ST.5	20000	16391.89
CTR	unknown	not detectable
GL3	unknown	not detectable
M4	unknown	not detectable
MYC13	unknown	not detectable

Supplementary Figure S3

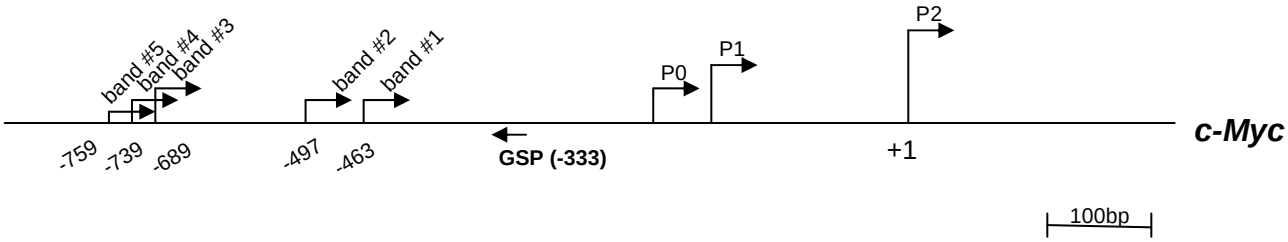




Supplementary Figure S4

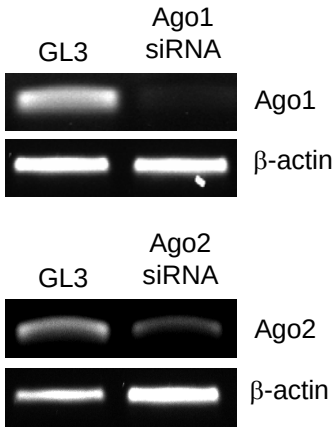


Supplementary Figure S5



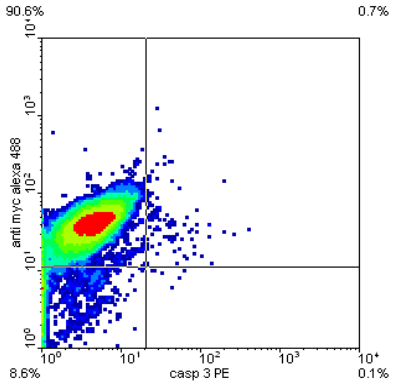
Fragment	Sequence of cloned fragment	Position relative to P2 site
Band #1	GTCAAACAGTACTGCTACGGAGGAGCAGCAGAGAAAGGGAGAGGGTTTGAGAGGGAG CAAAAGAAAATGGTAGGCGCGCGTAGTTAATTCATGCGGCTCTCTTACTCTGTTTACAT CCTAGAGCTAGAGTGCTCGGCTG	-463
Band #2	GTGATGATTTATACTCACAGGACAAGGATGCGGTTTGTCAAACAGTACTGCTACGGAG GAGCAGCAGAGAAAGGGAGAGGGTTTGAGAGGGAGCAAAAGAAAATGGTAGGCGCGC GTAGTTAATTCATGCGGCTCTCTTACTCTGTTTACATCCTAGAGCTAGAGTGCTCGGCTG A	-497
Band #3	ATCTACACTAACATCCCACGCTCTGAACGCGCGCCCATTAATACCCTTCTTTCCTCCAC TCTCCCTGGGACTCTTGATCAAAGCGCGGCCCTTTCCCCAGCCTTAGCGAGGCGCCCTG CAGCCTGGTACGCGCGTGGCGTGGCGGTGGGCGCGCAGTGCGTTCTCGGTGTGGAGGG CAGCTGTTCCGCCTGCGATGATTTATACTCACAGGACAAGGATGCGGTTTGTCAAACAG TACTGCTACGGAGGAGCAGCAGAGAAAGGGAGAGGGTTTGAGAGGGAGCAAAAGAAA ATGGTAGGCGCGCGTNGTTAATTCATGCGGCTCTCTNNCNCNNNTACNNCCTAGAGCT AGAGTGNTCGGCTG	-689
Band #4	ATCATTCCTCCCTATCTACACTAACATCCCACGCTCTGAACGCGCGCCCATTAATACCC TTCTTTCCTCCACTCTCCCTGGGACTCTTGATCAAAGCGCGGCCCTTTCCCCAGCCTTAG CGAGGCGCCCTGCAGCCTGGTACGCGCGTGGCGTGGCGGTGGGCGCGCAGTGCGTTCT CGGTGTGGAGGGCAGCTGTTCCGCCTGCGATGATTTATACTCACAGGACAAGGATGCG GTTTGTCAAACAGTACTGCTACGGAGGAGCAGCAGAGAAAGGGAGAGGGTTTGAGAG GGAGCAAAAGAAAATGGTAGGCGCGCGTAGTTAATTCATGCGGCTCTCTTACTCTGTTT ACATCCTAGAGCTAGAGTGCTCGGCTG	-739
Band #5	TGAATGCGTTGCTGGGTTATTTTAATCATTCTAGGCATCGTTTTCCTCCTTATGCCTCTA TCATTCCTCCCTATCTACACTAACATCCCACGCTCTGAACGCGCGCCCATTAATACCCT TCTTTCCTCCACTCTCCCTGGGACTCTTGATCAAAGCGCGGCCCTTTCCCCAGCCTTAGC GAGGCGCCCTGCAGCCTGGTACGCGCGTGGCGTGGCGGTGGGCGCGCAGTGCGTTCTC GGTGTGGAGGGCAGCTGTTCCGCCTGCGATGATTTATACTCACAGGACAAGGATGCGG TTTGTCAAACAGTACTGCTACGGAGGAGCAGCAGAGAAAGGGAGAGGGTTTGAGAGG GAGCAAAAGAAAATGGTAGGCGCGCGTAGTTAATTCATGCGGCTCTCTTACTCTGTTTA CATCCTAGAGCTAGAGTGCTCGGCTG	-759

Supplementary Figure S6



Supplementary Figure S7

GL3



Myc13