Table of contents

Supplementary Table S1. Patient demographics. Additional information about radical prostatectomy samples recollected between 1997 and 2000.

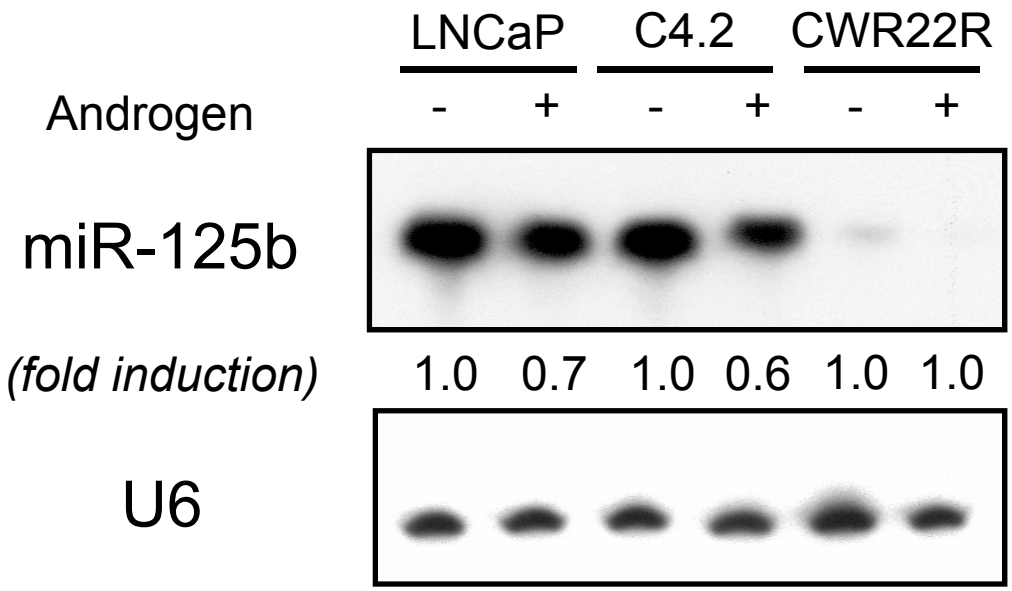
Number of Patients	10
Caucasian	7
African-American	1
Other	1
Unknown	1

	Median (Range)
Age	55 (45 - 71)
Weight (lbs.)	192.2 (156.4 - 273.5)
Height (inchs)	70.3 (67.0 - 72.0)
PSA (Pre surgery) (ng/ml)	7.5 (4.69 - 15.19)
Gleason Score (Post Surgery)	7 (6 - 9)
Weight of Prostate	51.00 (42.50 - 90.09)

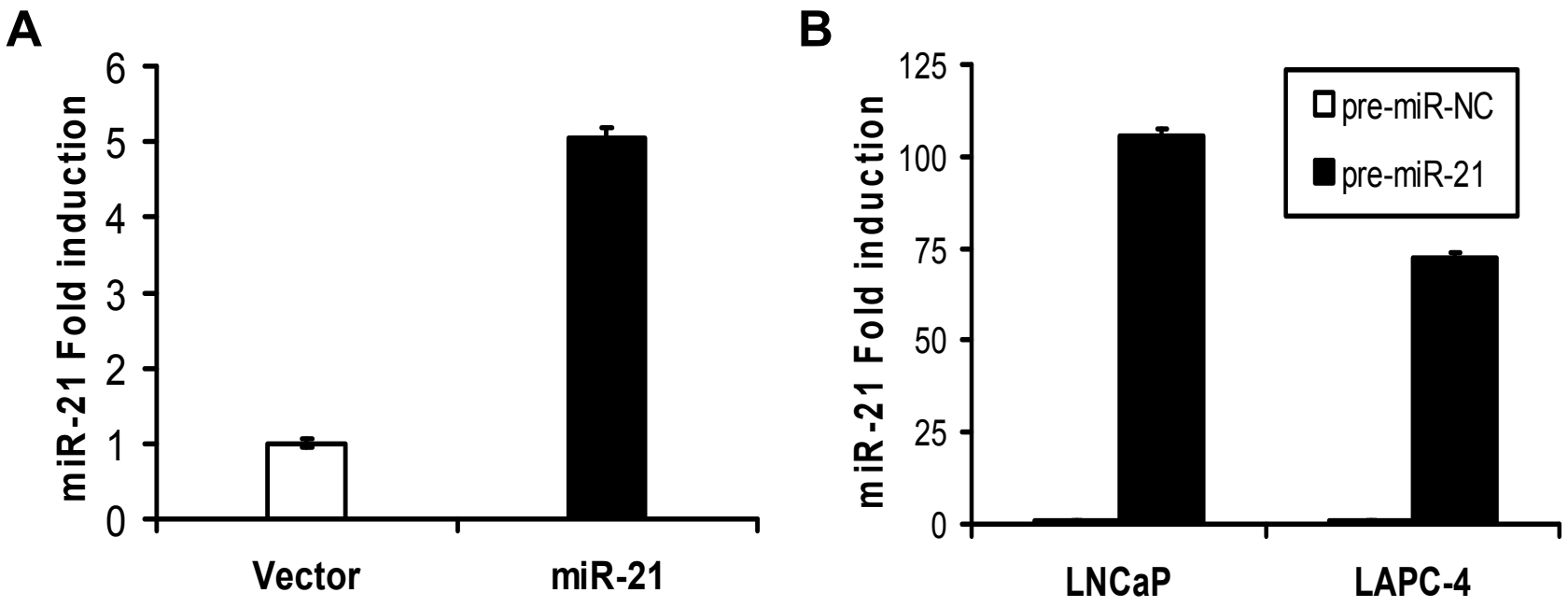
Grade	
T1c	10 % (1/10)
T2a	50 % (5/10)
T2b	20 % (2/10)
T2c	20 % (2/10)

Supplementary Table S2. Primers used for PCR

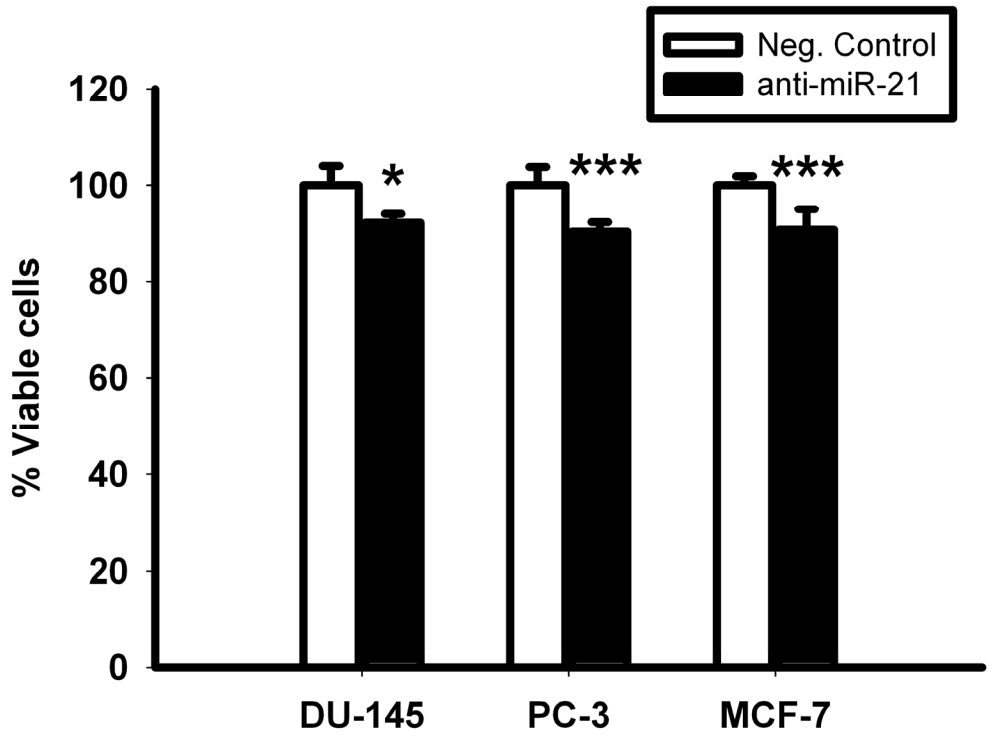
Primers used in CHIP:		
Name	**Sense**	**Antisense**
Neg	ATTGGCTATCTTTGTGTGCCTTG	TGCTCAATAAAACACATTGTTCTTCAT
miPPR-21	TCCCAATCATCTCAGAACAAGCT	TGCACAGAAACTCCAGTACATTAGTAAC
PSA Enhancer	TTTTTCTGGATTGTTGTTTCAAGG	TTGCAAGGATGCCTGCTTT
Unrelated seq	AAACCACCCATCCAGAAGGG	CGTGGCAGCACTCGTAAGACT
Primers used for construction of MSCV-miR-21 retrovirus:		
miR-21	CCGAGATCTTTGAACTTGTTCATTTTGTTTTGCTT	CCGCTCGAGCAGCTTAGTTTTCCTTTATTTATTTG



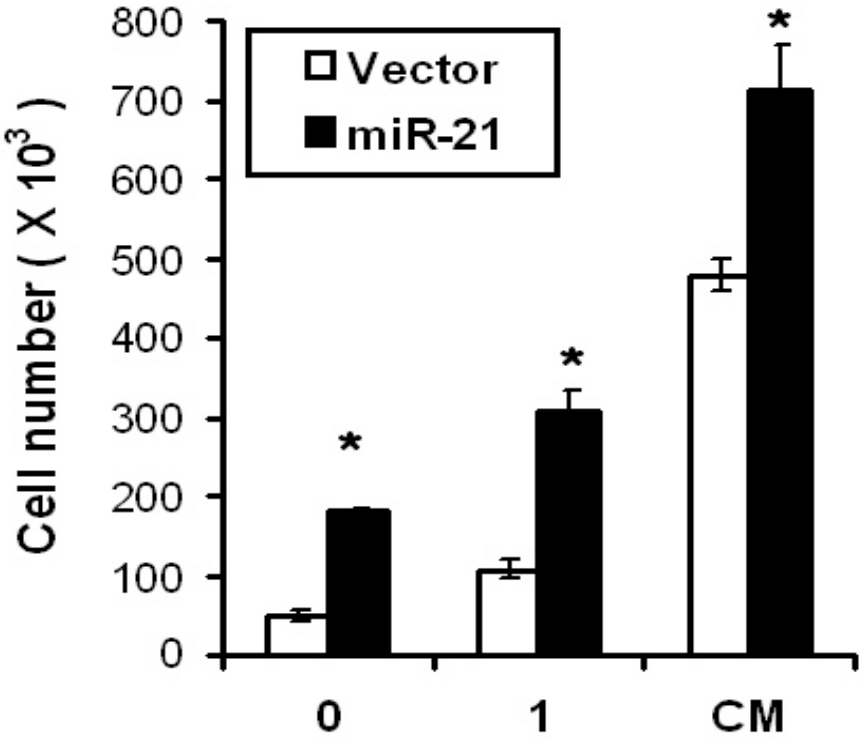
Supplementary Fig. S1. miR-125b Northern blotting. miR-125b induction in AR-positive CaP cell lines, LNCaP, C4-2 and CWR22Rv1. Cells were treated for 72 hours with Androgens (+, 10nM R1881) or vehicle (-) prior to RNA extraction.



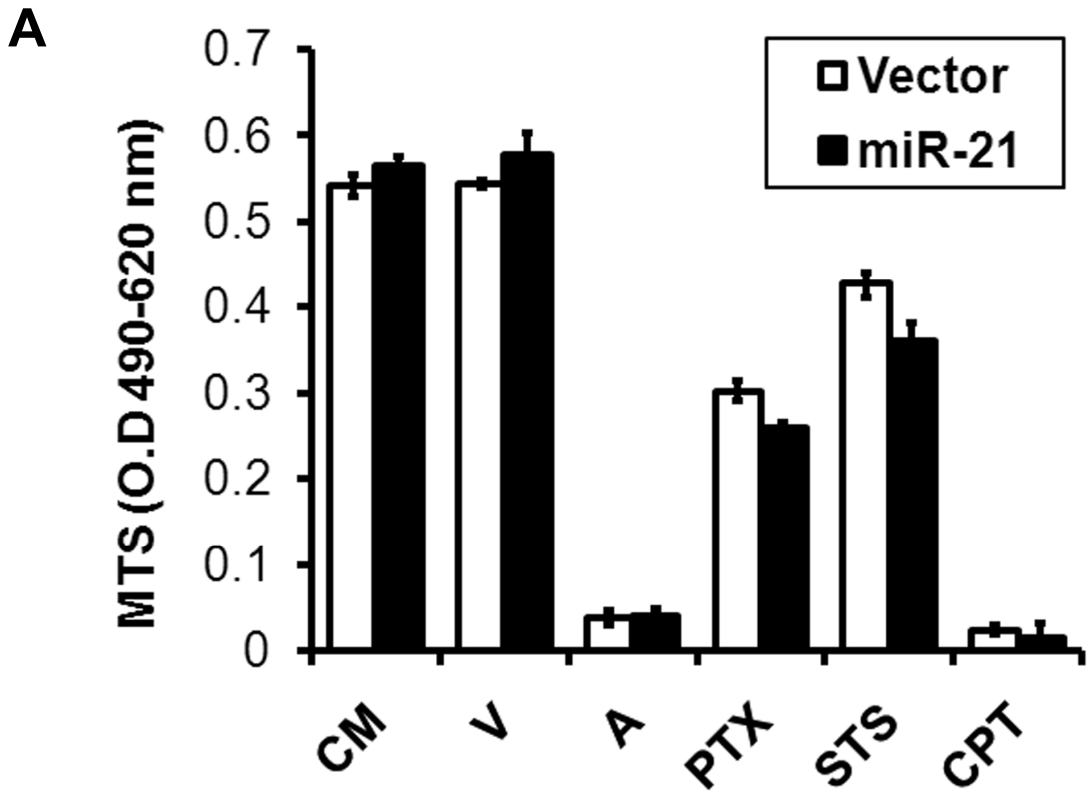
Supplementary Fig. S2. miR-21 transgene levels. A, Levels of miR-21 in retrovirally transduced LNCaP stably expressing miR-21 (black bar) or an empty vector (white bar). B, Levels of miR-21 in LNCaP and LAPC-4 transfected with the synthetic pre-miR-21 (black bars) or a FAM-labeled negative control (white bars). miRNAs were quantified by RT-PCR after 48 hours and normalized to U6 RNA. Bars are M± S.E. (n=3 different wells).



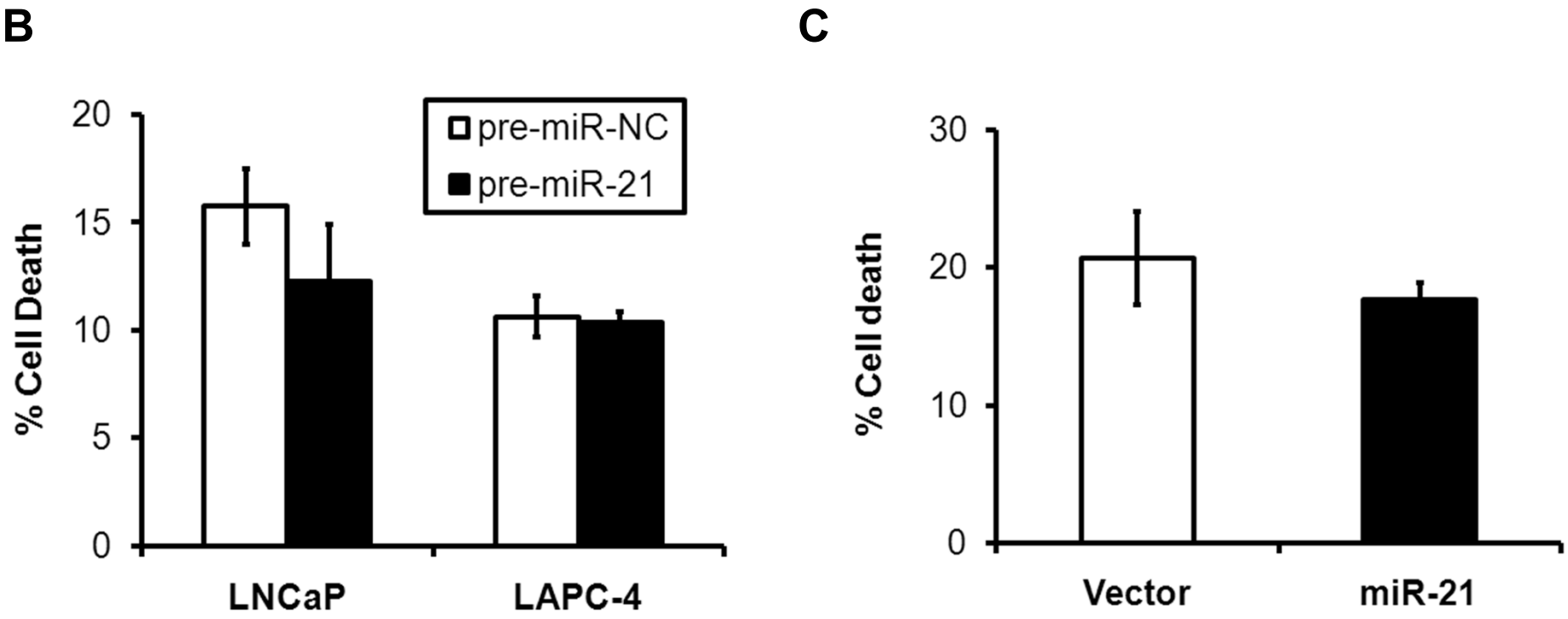
Supplementary Fig. S3. Effects of miR-21 inhibition on AR Negative CaP cell proliferation. miR-21 levels were inhibited either by a synthetic miR-21 inhibitor (MCF-7 and DU-145) or the Ad-Sponge-miR-21 (PC-3). MCF-7 Breast Cancer cells served as a control and DU-145 and PC-3 represent AR negative CaP cell lines. miR-21 inhibited samples are represented by Black bars and referred to their corresponding negative control (White bars). Proliferation was quantified by MTS following incubation for 3 days in complete media. Data are Mean (columns) $\pm$ STD (bars) from 6 independent measurements, * $P< 0.05$, *** $P< 0.001$ (t-student test).



Supplementary Fig. S4. Effects of miR-21 on cell proliferation. A, Retrovirally transduced LNCaP populations stably expressing miR-21 (black bars) or a control vector (white bars) were maintained for 6 days in charcoal stripped media supplemented with vehicle (0), 1 nM R1881 (1) or in complete media (CM). Viable cells were counted by trypan blue exclusion. Bars are M$\pm$ S.E. (n=3 different wells). * P$<$ 0.05 (t-student test).



Supplementary Fig. S5. Effects of miR-21 on cell death. A, Retrovirally transduced LNCaP expressing miR-21 (black bars) or control vector (white bars) populations were challenged with 0.5 μg/ml Adriamycin (A), 100 nM Paclitaxel (PTX), 0.5 μM Staurosporine (STS), 2.5 μM Campthotecine (CPT) or a maximal amount of vehicle (V, 0.25% DMSO) in complete medium (CM). Thereafter, cell viability was determined by MTS assay. Values obtained by subtracting the absorbance readings at 490 and 620 nanometers were plotted. Bars are the Mean ± S.E of 6 independent measurements.



Supplementary Fig. S5. Effects of miR-21 on cell death. B, LNCaP and LAPC-4 transfected with the synthetic pre-miR-21 (black bars) or a FAM-labeled negative control (white bars) were maintained for 6 days in androgen deprived (charcoal stripped) media. The number of dead cells was quantified by direct staining with Trypan blue. Figures show the result of one representative experiment. Bars are M± S.E. (n=3 different wells). C, Cell populations of retrovirally transduced LNCaP stably expressing miR-21 (black bar) or an empty vector (white bar) were maintained for 6 days in androgen deprived media and the degree of cell death was determined by Trypan blue staining.

Supplemental Materials and Methods

Cell lines culture. PC-3 and DU-145 were grown in RPMI 1640 (Cellgro) containing 10% heat-inactivated FBS. MCF-7 were passaged in MEM (InVitrogen) plus 10% FBS. All cell media contained 10 μg/ml Ciprofloxacin Hydrochloride (US Biological).

miR-21 Sponge vector construction. To inhibit the mature miR-21, we applied a previously reported microRNA Sponge vector containing 7 tandemly arrayed miR-21 binding sites in the 3' UTR of destabilized GFP driven by the CMV promoter (1). By removing the miR-21 seed sequences, we constructed a negative control vector. The microRNA sponge expression cassettes were subcloned into the adenoviral E1 expression cassette pAdTrack-CMV. Replication-defective virus expressing GFP plus the 3' UTR containing the miR-21 binding sites (Ad-Sponge-miR-21) or alternatively, GFP without these binding sites (Ad-Sponge) were generated by homologous recombination with AdEasy1 and propagation in 293 cells as previously described (2). After determining the viral titer with the Adeno-X^{TM} Rapid titer kit (Clontech), we infected the cell lines with 10 MOI (multiplicity of infection) of either Ad-Sponge-miR-21 or Ad-Sponge.

miR-21 suppression. DU-145 and MCF-7 were Dharmafect 3 (Dharmacon) transfected with 20 nM of meridian inhibitors against miR-21 or a negative control (Dharmacon). PC-3 cells were infected with 25 MOIs of Ad-Sponge-miR-21 or

Ad-Sponge-control in the presence of human Factor X (Innovative Research). After 24 hours of infection or transfection in complete media cells were harvested and seeded at a confluence of 5 x 10^3 cells per 96 multiwell plate. MTS (Promega) was performed at 3 days according to manufacturer's instructions.

Retroviral expression of miR-21. HeLa genomic DNA was used to PCR a fragment containing the human miR-21 (primers miR-21 FW & miR-21 RV [Supplementary Table 2]). The resulting PCR product was cloned into BglII and XhoI sites of pMSCV-PIG (3). Empty vector served as a negative control. Viruses were packaged in Phoenix cells (G. Nolan, Stanford University, Stanford, CA). LNCaP cells were infected in the presence of 8 μg/mL polybrene. Two days after the initial infection, cultures were selected with 1 μg/mL puromycin.

RT-PCR. Reverse transcribed miR-21 was quantified by real-time PCR with TaqMan® MicroRNA Assay Kit (Applied Biosystems) and Taqman 2X Universal PCR master mix, No AmpErase® UNGb (Applied Biosystems). RNU6B was used as normalization control.

miRNA Microarrays.. Custom microarrays (Combimatrix) containing probes against 474 human mature miRNAs plus mismatch probes for each miRNA were applied as described previously (4). Briefly, arrays were pre-hybridized at 37 ^oC for 1 hour in 3X SSC, 0.1% SDS, 0.2% BSA. 10 μg of total RNA Cy3-labeled was hybridized to arrays at 37^oC overnight in 400mM Na_2HPO_4 (pH 7.0), 0.8% BSA, 5% SDS, and 12% formamide. Arrays were washed once at room temperature in 2X SSC, 0.25% SDS, 3 times at room temperature in 1.6X SSC,

and twice in ice-cold 0.8X SSC. Hybridized arrays were then scanned using a GenePix 4000B microarray scanner (Axon) and signal intensities were extracted using the Combimatrix Microarray Imager software. The background value was determined by calculating the median signal from the mismatch probes and this value was subtracted from all perfect match probes. Signals that were less than 1.5 times background were removed and datasets were median centered prior to calculating fold-change values. All array data were submitted using GEO database from NCBI (Accession number is GSE16225).

References

1. Ebert, M. S., Neilson, J. R., and Sharp, P. A. MicroRNA sponges: competitive inhibitors of small RNAs in mammalian cells. Nat Methods, *4:* 721-726, 2007.
2. He, T. C., Zhou, S., da Costa, L. T., Yu, J., Kinzler, K. W., and Vogelstein, B. A simplified system for generating recombinant adenoviruses. Proc Natl Acad Sci U S A, *95:* 2509-2514, 1998.
3. Hemann, M. T., Fridman, J. S., Zilfou, J. T., Hernando, E., Paddison, P. J., Cordon-Cardo, C., Hannon, G. J., and Lowe, S. W. An epi-allelic series of p53 hypomorphs created by stable RNAi produces distinct tumor phenotypes in vivo. Nat Genet, *33:* 396-400, 2003.
4. Chang, T. C., Wentzel, E. A., Kent, O. A., Ramachandran, K., Mullendore, M., Lee, K. H., Feldmann, G., Yamakuchi, M., Ferlito, M., Lowenstein, C. J., Arking, D. E., Beer, M. A., Maitra, A., and Mendell, J. T. Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. Mol Cell, *26:* 745-752, 2007.