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# Supplementary Materials for

## Identification of the *miR-106b~25* MicroRNA Cluster as a Proto-Oncogenic *PTEN*-Targeting Intron That Cooperates with Its Host Gene *MCM7* in Transformation

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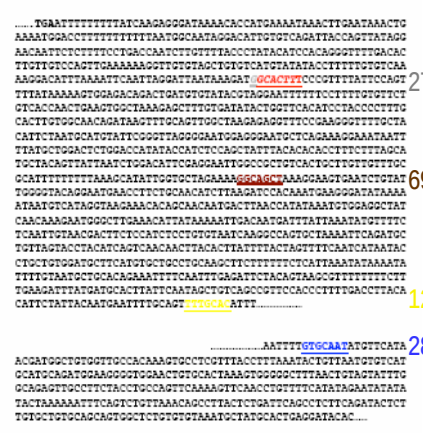
**The PDF file includes:**

Table S8. Primers used to detect human *PTEN*, *MCM7* and intron 13 mRNA by real-time PCR.
Table S9. Primers used to detect mouse *Pten* by real-time PCR.

A




miR-302 family

*hsa/mmu*-miR-302a uaagugcuuccauguuuugguga
*hsa/mmu*-miR-302b uaagugcuuccauguuuuaguag
*hsa/mmu*-miR-302c uaagugcuuccauguuucagugg
*hsa/mmu*-miR-302d uaagugcuuccauguuugagugu
*hsa/mmu*-miR-302e uaagugcuuccaugcuu
*hsa/mmu*-miR-302f uaauugcuuccauguuu
*hsa*-miR-372 aaagugcugcgacauuugagcgu
*hsa*-miR-373 gaagugcuucgauuuuggggugu
*hsa*-miR-518e aaagcgcuucccuucagagug
*hsa*-miR-519a aaagugcaucuuuuagagugu
*hsa*-miR-519b-3p aaagugcaucuuuuagagguu
*hsa*-miR-519c aaagugcaucuuuuuagaggau
*hsa*-miR-520a aaagugcuucccuuuggacugu
*hsa*-miR-520b aaagugcuucuuuuagaggg
*hsa*-miR-520c aaagugcuuccuuuuagagggu
*hsa*-miR-520d aaagugcuucucuuuggugggu
*hsa*-miR-520e aaagugcuuccuuuuugagg

miR-17 family

*hsa/mmu/rno*-miR-17 caaagugcuuacagugcagguagu
*hsa/mmu/rno*-miR-20a uaaagugcuuauagugcagguag
*hsa/mmu/rno*-miR-20b caaagugcucauagugcagguag
*hsa/mmu/rno*-miR-93 caaagugcuguucgugcagguag
*hsa/mmu*-miR-106a caaagugcuaacagugcaggua
*hsa/mmu/rno*-miR-106b uaaagugcugacagugcagau
*hsa*-miR-519d caaagugccucccuuuagagug

miR-19 family

*hsa/mmu/rno*-miR-19a ugugcaaaucuaugcaaaacuga
*hsa/mmu/rno*-miR-19b ugugcaaauccaugcaaaacuga

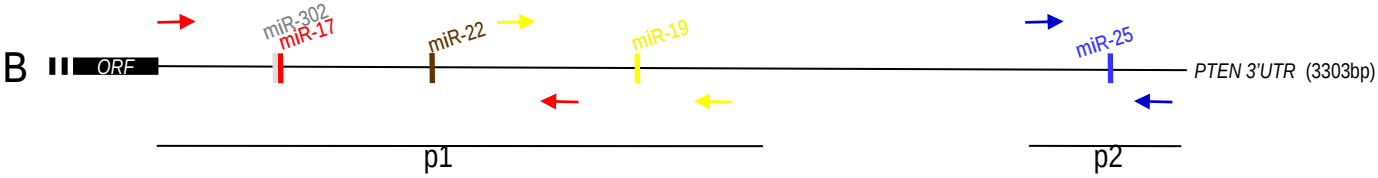
miR-22 family

*hsa/mmu/rno*-miR-22 aagcugccaguugaagaacugu

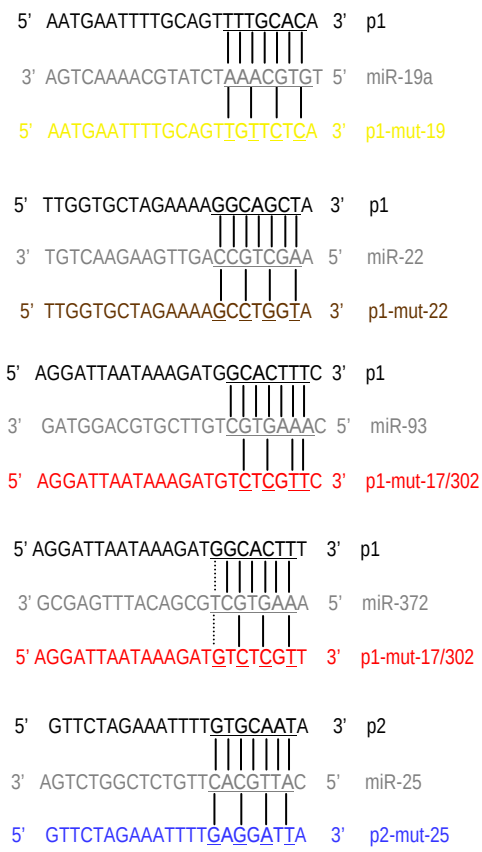
miR-25 family

*hsa/mmu/rno*-miR-25 cauugcacuugucucggucuga
*hsa/mmu/rno*-miR-32 uauugcacauuacuaaguugca
*hsa/mmu/rno*-miR-92a uauugcacuugucccggccugu
*hsa/mmu/rno*-miR-92b uauugcacucgucccggccucc
*hsa/mmu/rno*-miR-363 aauugcacgguauccaucugua
*hsa/mmu/rno*-miR-367 aauugcacuuuagcaaugguga

B




C

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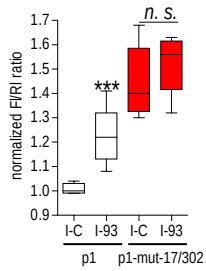



E

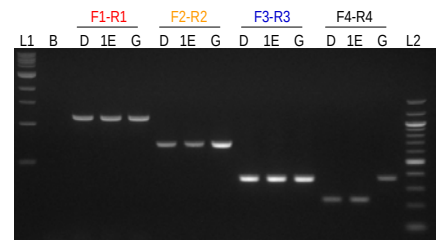



Fig. S1. Direct interaction of miR-17, 19, 22, 25 and 302 with *PTEN* 3'UTR. (A) (*left*) *PTEN* 3' UTR sequence. The seed matches for miR-17 (red), 19 (yellow), 22 (brown), 25 (blue) and 302 (grey) are highlighted. Position is counted starting from the first nucleotide after the PTEN stop codon. miR-17 (GCACTTT) and miR-302 (GGCACTT) seed matches are shifted by 1 nucleotide. (*right*) Sequences of the mature miRNAs belonging to the five families analyzed in this study. The miRNA seed sequence shared by all the members of a family is underlined and in bold. (B) Schematic representation of the seed matches included in p1 and p2 chimeric luciferase reporter plasmids and by the 3 sets of primers used to analyze the length of the *PTEN* 3'UTR in the DU145 and PWR-1E cell lines (see E): F1-R1 (red); F2-R2 (yellow); F3-R3 (blue). (C) Mutant luciferase plasmids with impaired miRNA binding. The miRNA seed sequences and plasmid seed matches are underlined. Canonical and non canonical (GT) pairings are indicated with straight or dotted lines, respectively. Every other base of the seed match was mutated. (D) The *wt* p1 or the mutant p1-mut-17/302 plasmids were transfected into DU145 cells together with the antisense inhibitor of miR-93 (I-93) as a representative inhibitor of the *PTEN*-targeting miRNAs. I-93 increased luciferase activity only of the *wt* plasmid, but not that of the mutant plasmid. This confirms that miR-93 binds to and is released from *PTEN* 3'UTR only when the seed match is *wt*. I-C: control inhibitor. (E) Total RNA was extracted from DU145 (D) and PWR-1E (1E) cells, retrotranscribed and amplified by PCR using 3 sets of primers: F1-R1 encompass the miR-17/302 and the miR-22 seed matches; F2-R2 encompass the miR-19 seed match and F3-R3 encompass the miR-25 seed match. The presence of the PCR product indicates that in these two cell lines PTEN 3'UTR is long enough to contain the seed matches for all the studied miRNA families. PCR amplification from genomic DNA (G) was used as positive control. The F4-R4 set of primers was used to check for the absence of contaminating genomic DNA. These primers recognize two consecutive exons of the ATP1A1 gene (ATPase, Na+/K+ transporting alpha1 polypeptide). If amplification occurs from genomic DNA, a 372bp band is obtained; if amplification occurs from cDNA, a 235bp band is obtained. The presence of the smaller band indicates that the cDNA obtained from DU145 and PWR-1E is free of genomic contamination.

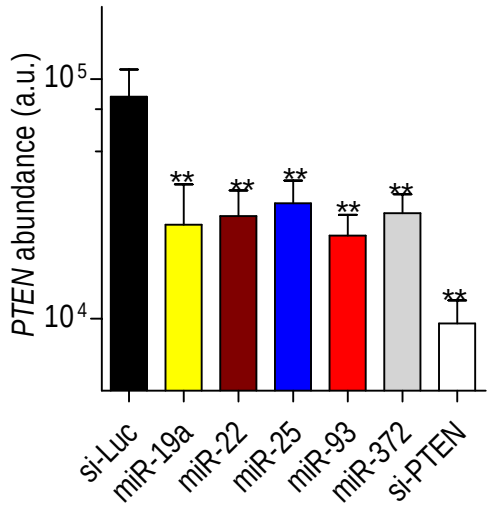



Fig. S2. miR-17, 19, 22, 25 and 302 families cause a decrease in the abundance of *PTEN* mRNA. Real time PCR of *PTEN* 24h after the transfection of control si-Luc, the indicated si-miRNAs, or siRNA directed against human PTEN (si-PTEN) in DU145 prostate cancer cells.

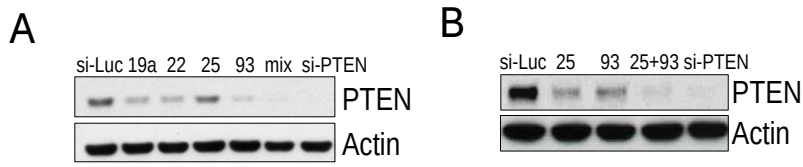
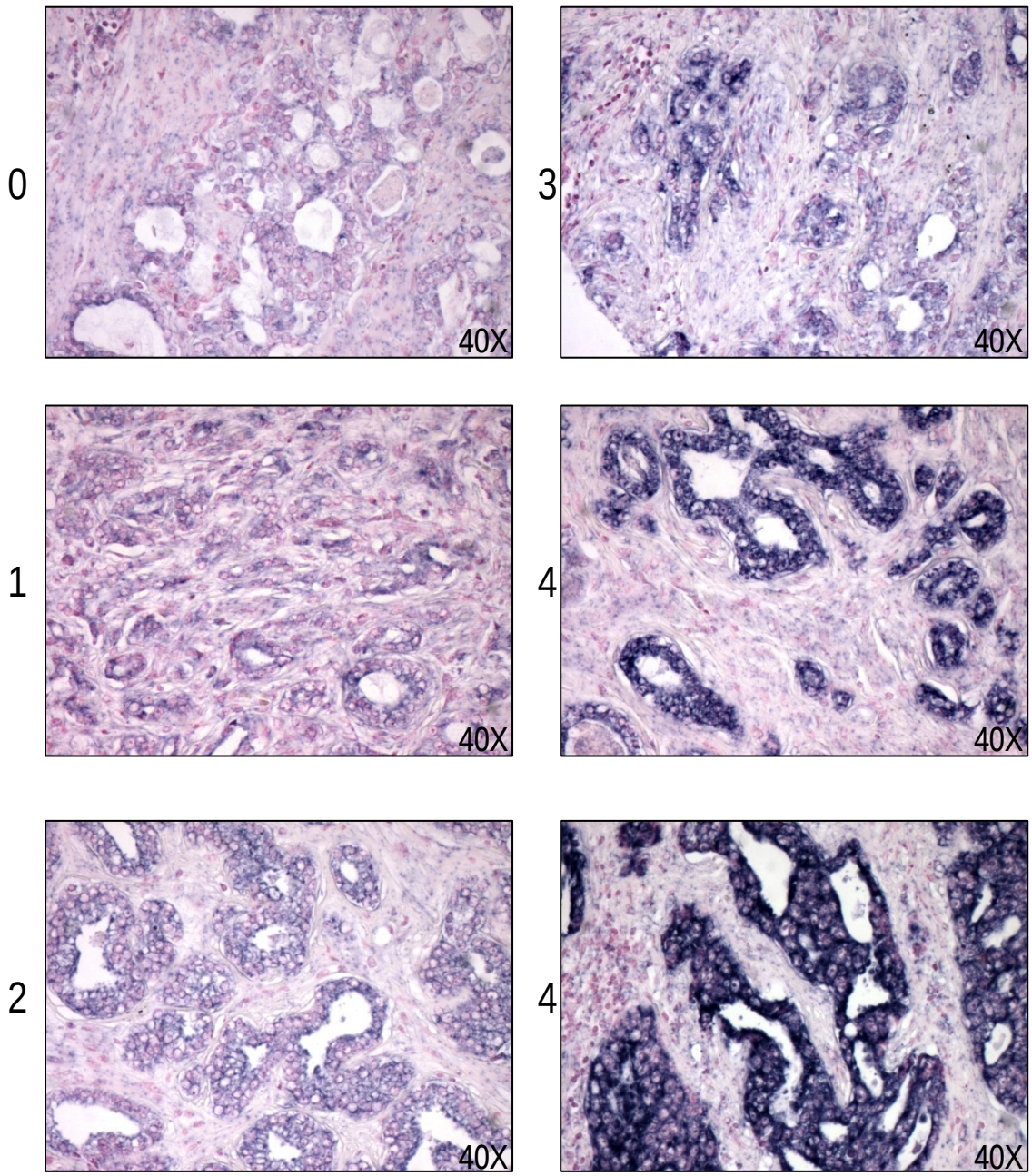



Fig. S3. Cooperation between different miRNAs in decreasing PTEN abundance. (A) The indicated miRNAs were transfected in PWR-1E either alone or in combination (mix). PTEN abundance was analyzed 48h later by Western Blot. (B) miR-25 (miR-25 family) and miR-93 (miR-17 family), which both belong to *miR-106b~25* cluster, cooperate to decrease PTEN abundance.




| SCORE | DESCRIPTION |
|---|---|
| 0 | Negative |
| 1 | Weak, focal |
| 2 | Weak, diffuse |
| 3 | Weak-moderate, diffuse |
| 4 | Moderate-strong, diffuse |

Fig. S4. Score categories for in situ hybridization of miRNAs. TMA samples were considered negative (score 0 to 1) or positive (score 2 to 4) for miRNAs depending on intensity of the staining. A representative sample for each condition (*upper*) and a description of the observed staining (*lower*) are presented.

A

| | pAkt NEG | pAkt POS | |
|---|---|---|---|
| *miR-22*<br>- Neg (0-1)<br>- Pos (2-3-4) | 45 (67.2%)<br>13 (35.1%) | 22 (32.8%)<br>24 (64.9%) | p = 0.004 |
| *miR-106b*<br>- Neg (0-1)<br>- Pos (2-3-4) | 39 (68.4%)<br>13 (31%) | 18 (31.6%)<br>29 (69%) | p = 0.008 |
| *miR-93*<br>- Neg (0-1)<br>- Pos (2-3-4) | 41 (73.2%)<br>19 (37.3%) | 15 (26.8%)<br>32 (62.7%) | p < 0.001 |
| *miR-25*<br>- Neg (0-1)<br>- Pos (2-3-4) | 38 (65.5%)<br>19 (41.3%) | 20 (34.5%)<br>27 (58.7%) | p = 0.018 |

B

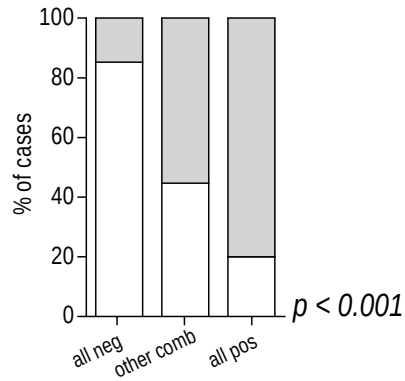



**Fig. S5. The concomitant presence of miR-22, miR-25, miR-93 and miR-106b increases the probability of Akt phosphorylation.** (A) Correlation between the positivity for pAkt and the positivity for miR-22 and miR-106b~25 miRNAs. (B) Percentage of pAkt negative (white) and positive (grey) tumors among those negative for miR-22, 25, 93 and 106b (all neg), positive for at least one of these miRNAs (other comb) or positive for all of them (all pos).

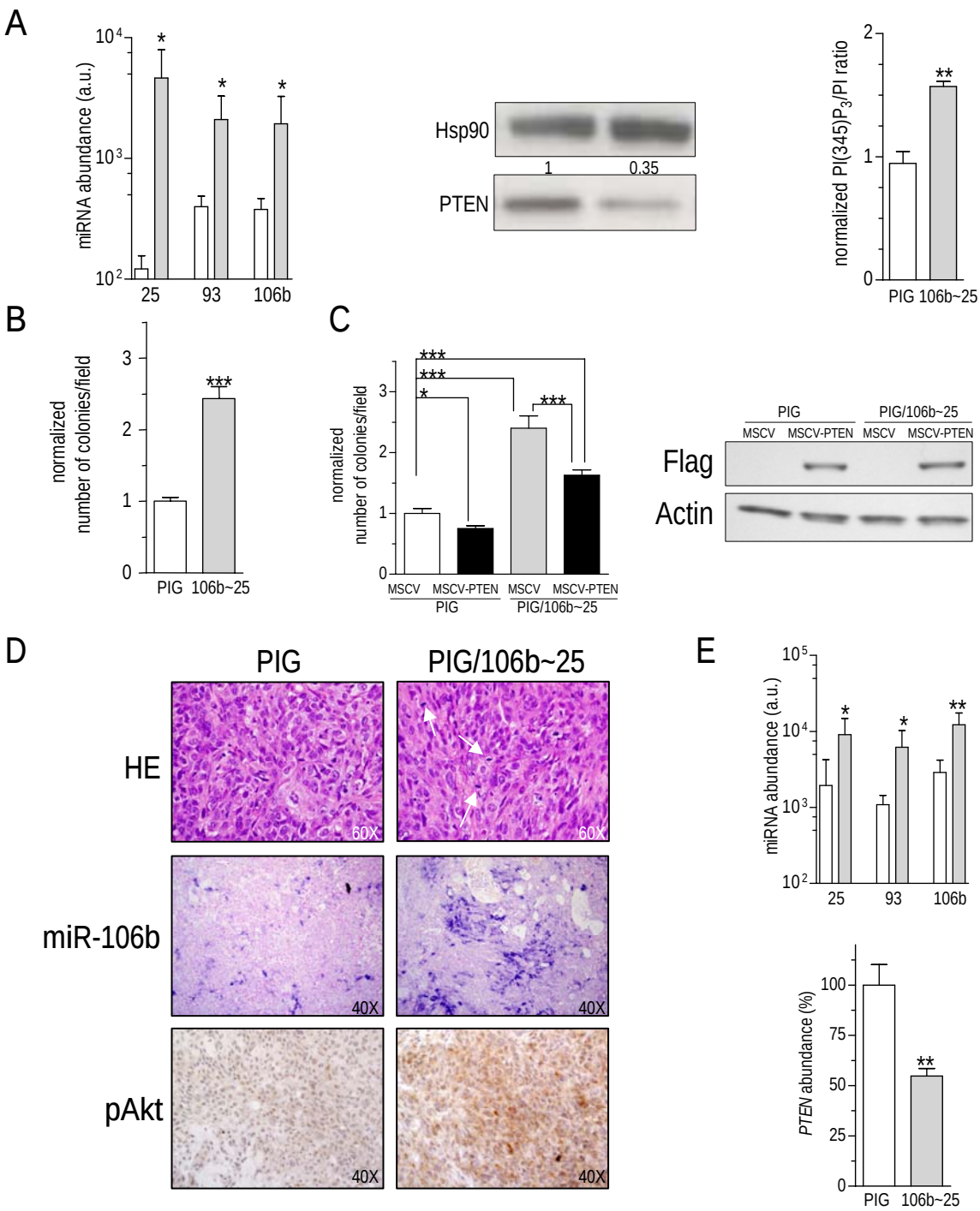



Fig. S6. Analysis of DU145 stably infected with members of the miR-106b~25 cluster. (A) Real time PCR shows that the abundance of the 3 mature miRNAs is higher in PIG/106b~25 infected cells than in PIG control cells (*left*). The increase in miRNA abundance is accompanied by a decrease in PTEN (*middle*, Western Blot analysis) and an increase in PIP3 production (*right*). (B) Overexpression of the miR-106b~25 cluster causes an increase in the number of colonies formed by DU145 in semisolid medium. (C) (*left*) Growth in soft agar of DU145 cells stably infected with PIG/106b~25 and MSCV-neo-Flag-PTEN plasmids. *PTEN* expressed by MSCV-neo-Flag-PTEN contains the entire open reading frame, but lacks the 3'UTR, so that it cannot be targeted by the intronic miRNAs. (*right*) anti-Flag antibody is used to detect exogenous PTEN. (D, E) Analysis of the tumors formed after the injection of PIG and PIG/106b~25 infected cells into the flank of nude mice. (D) H&E staining of the tumors highlights the presence of more mitotic figures (arrows) in PIG/106b~25 tumors than in control PIG tumors (*upper*); in situ hybridization confirms the increased abundance of a representative miRNA of the cluster (miR-106b, *middle*); IHC of pAkt shows that the increased abundance of miR-106b~25 cluster is associated with increased Akt phosphorylation (*lower*). (E) Real time PCR shows that mature miRNA abundance (*upper*) is higher in PIG/106b~25 xenografts compared to PIG empty xenografts. The opposite applies to *PTEN* mRNA (*lower*). White bars: PIG; grey bars: PIG/106b~25.

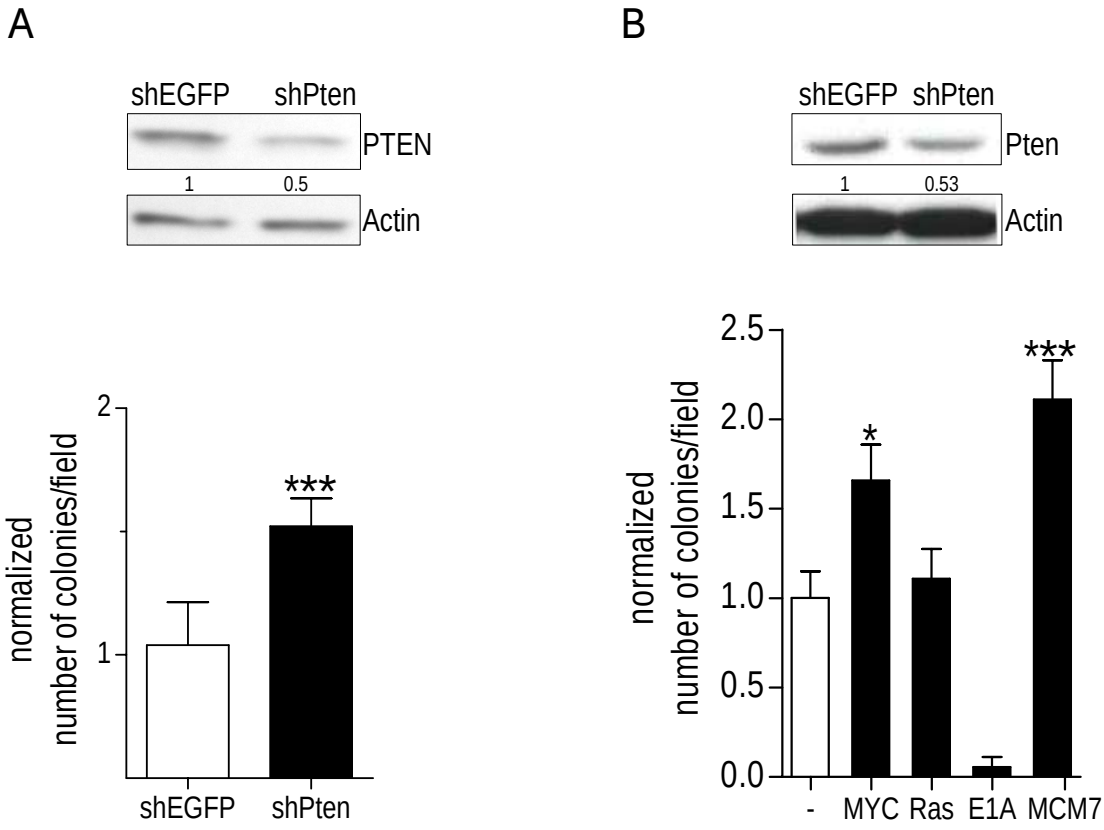



**Fig. S7. shPten partially mimics miR-22 and miR-106b~25 cluster.** (A) DU145 cells were stably infected with a pSUPER-shEGFP control or pSUPER-shPten. shPten induced a <50% reduction in PTEN abundance (*upper*, Western Blot) and a 1.5 fold increase in the number of colonies formed in soft agar (*lower*). (B) Transformation assay performed in *wt* MEF by co-infecting pSUPER-shPten and MSCV empty, MSCV/c-MYC, MSCV/RasV$^{12}$, MSCV/E1A or MSCV/MCM7 plasmids. The <50% decrease in Pten abundance caused by shPten (*upper*, Western Blot), as well as the number of colonies formed in soft agar (*lower*) are shown.

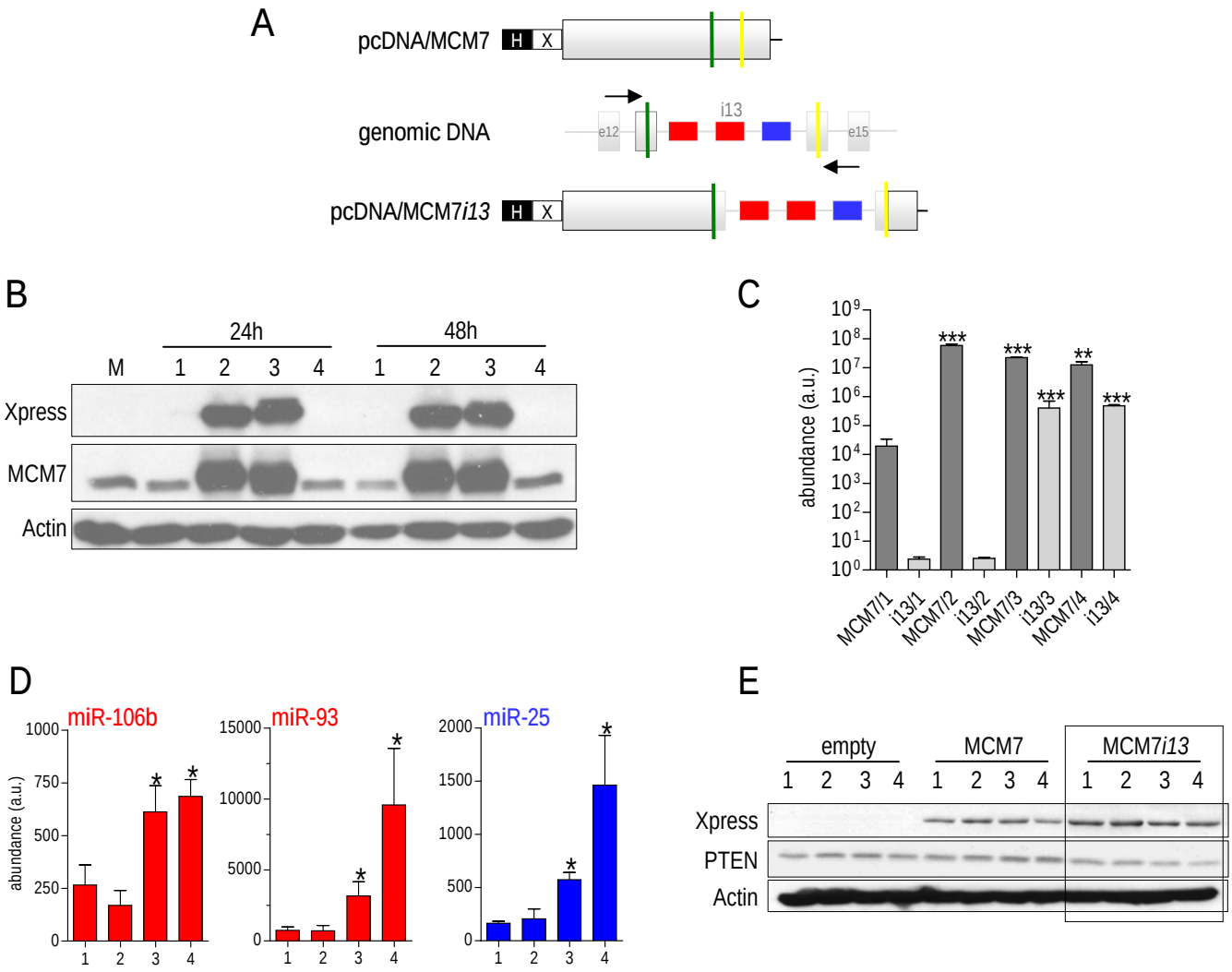



Fig. S8. Constructs for expressing human MCM7 and the intronic miR-106b~25 cluster. (A) Strategy used to insert the miRNA-containing intron 13 into the MCM7 coding sequence. Human MCM7 coding sequence was subcloned into pcDNA3.1/His C expression plasmid, so that pcDNA/MCM7 plasmid was obtained. Then, a fragment encompassing *MCM7* exon 13, intron 13, and exon 14 was amplified by PCR from human genomic DNA. This PCR fragment and pcDNA/MCM7 were both cut with BsaAI (green line) and EarI (yellow line), enzymes with unique restriction sites in exon 13 and exon 14, respectively. In this way, the miRNA-containing intron 13 was inserted between MCM7 exon 13 and exon 14 and the pcDNA/MCM7i13 plasmid was generated. pcDNA/i13 was derived from pcDNA/MCM7i13 by inserting a point mutation that transforms the sixth amino acid of MCM7 (TAC, Tyr) into a stop codon (TAA, STOP). H: HA tag; X: Xpress tag; gray boxes: MCM7 coding sequence; red and blue boxes: *miR-93/106b* and *miR-25* genes. (B) Representative Western Blot of U2OS cells mock-transfected (M) or transiently transfected with the empty pcDNA3.1/His C plasmid (1), pcDNA/MCM7 (2), pcDNA/MCM7i13 (3) or pcDNA/i13 (4). The Xpress tag and MCM7 protein were detected 24 and 48h later. The comparable size and amount of tagged MCM7 protein translated from pcDNA/MCM7 and pcDNA/MCM7i13 plasmids indicate that the intron 13 is correctly spliced out of the MCM7 coding sequence. In contrast, pcDNA/i13 does increase in MCM7 above endogenous amounts because of the early stop codon. (C) Real time PCR performed on the same cells as in B. One set of primers recognizing MCM7 coding sequence (dark gray bars) and one set recognizing intron 13 (pri-miR-106b~25) (light gray bars) were used. (D) Real time PCR for mature miRNA performed on the same cells as in B. Both pcDNA/MCM7i13 and pcDNA/i13, but not pcDNA/MCM7, increased pri-miR-106b~25 and mature miR-106b, miR-93, and miR-25. The increase in precursor abundance was comparable between the 2 constructs, but the processing from pcDNA/i13 appeared to be more efficient, leading to greater amounts of the mature miRNAs. This is probably because precursor mRNA transcribed from pcDNA/i13 is used for miRNA maturation and not for protein translation. (E) U2OS were transfected with pcDNA/MCM7 and pcDNA/MCM7i13 and selected under G418 for 3 weeks. Xpress presence and PTEN abundance were detected in 4 independent clonal populations. Only the pcDNA/MCM7i13 and not the pcDNA/MCM7 construct decreased PTEN abundance, further confirming the intron 13-miRNAs as *PTEN*-targeting.

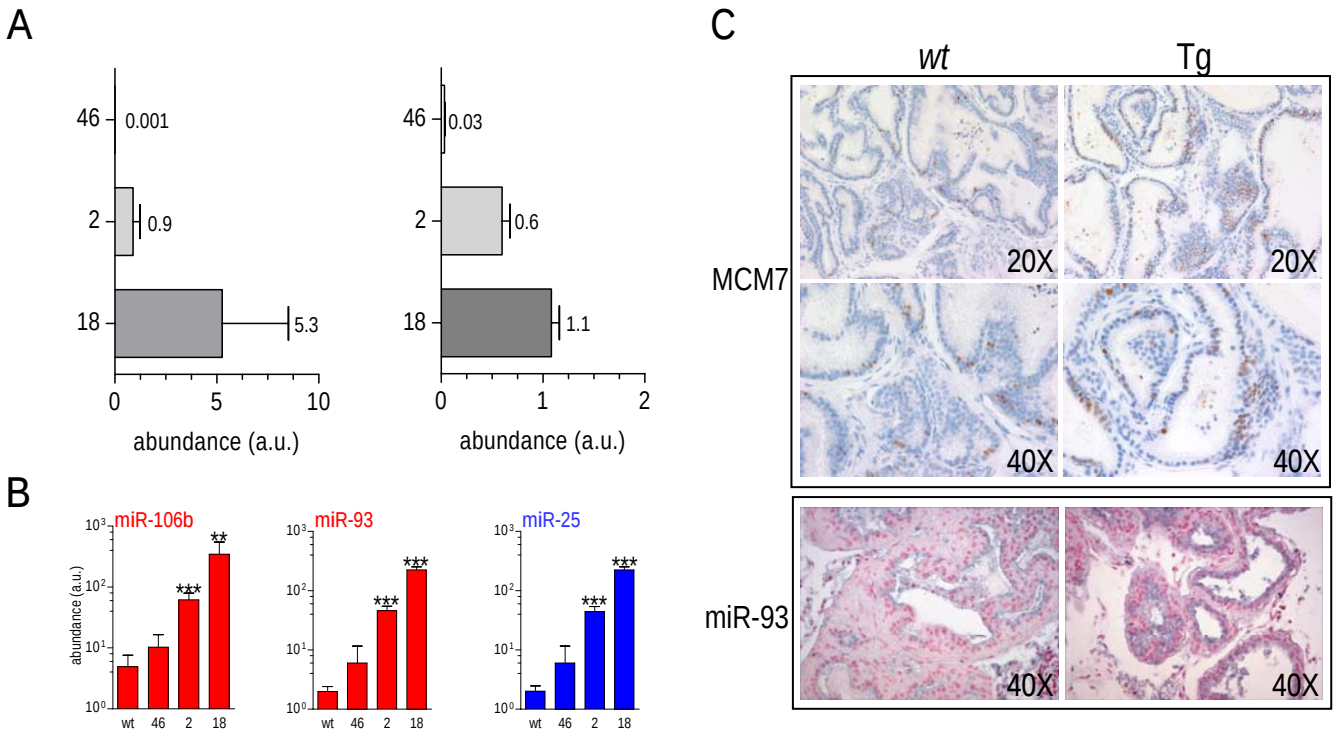
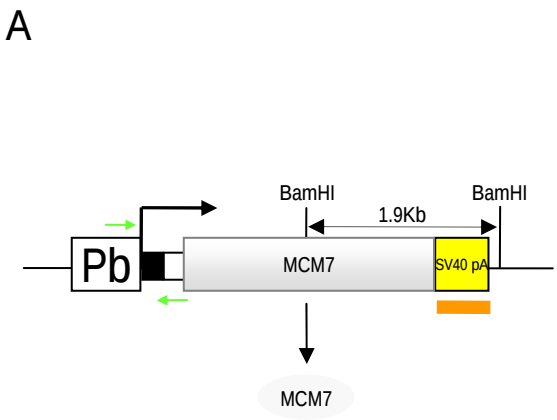
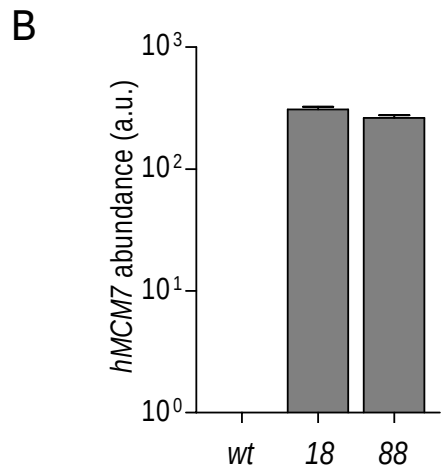
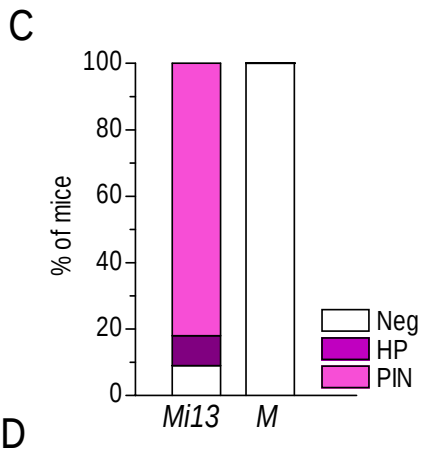



Fig. S9. Transgene expression in the Pb/MCM7i13 lines. (A) The abundance of the transgenic construct was measured by real time PCR using one set of primers recognizing intron 13 (pri-miR-106b~25) (*left*) and another set recognizing the intron 13-exon 14 boundary (*right*). A representative low expressor (46), medium expressor (2) and high expressor (18) line is shown. *Wild type* (*wt*) mice were negative with both sets of primers. (B) Real time PCR of miR-106b, miR-93 and miR-25 in the prostate of transgenic mice belonging to the indicated lines. The 3 miRNAs show a progressive enrichment over endogenous amounts. The mature sequence of the 3 miRNAs is conserved between human and mouse (http://microrna.sanger.ac.uk/sequences/index.shtml). (C) Detection of MCM7 (IHC) and miR-93 (ISH) in the prostate of 3 month old transgenic mice belonging to line 18.




| | *Pb/MCM7i13* HE #18 | *Pb/MCM7* HE #88 |
|---|---|---|
| Neg | 9% (1/11) | 100% (17/17) |
| HP | 9% (1/11) | / |
| PIN | 82% (9/11) | / |

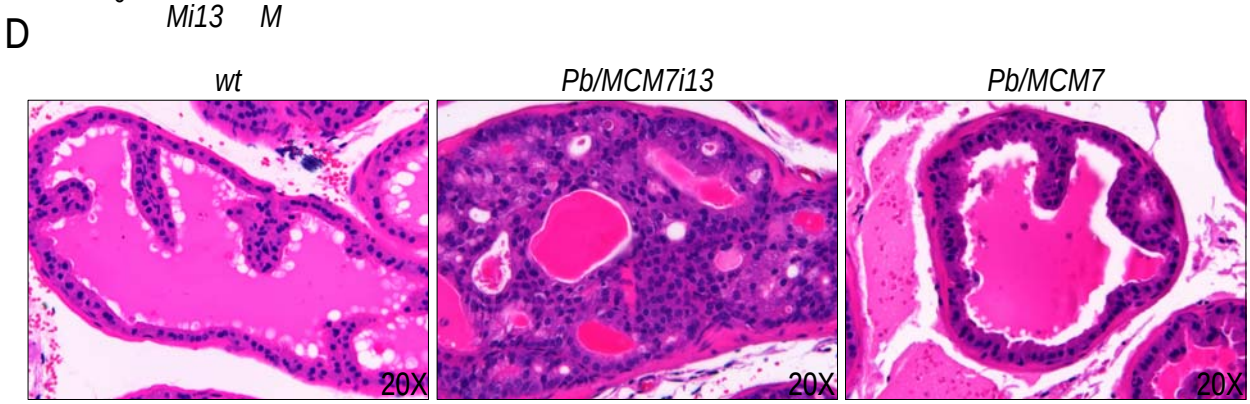
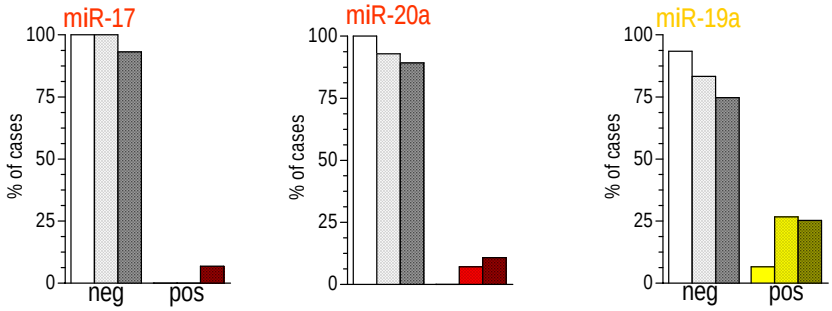



**Fig. S10. MCM7 cannot initiate prostate tumorigenesis.** (A) Schematic representation of the Pb/MCM7 transgenic construct. Pb: prostate-specific rat probasin promoter ARR2PB; black box: HA tag; white box: Xpress tag; gray box: human MCM7 coding sequence; yellow box: SV40 polyA; orange box: probe used for Southern Blot analysis of transgenic founders; green arrows: primers used for PCR analysis of transgenic founders and for genotyping (see **Fig.** 9 for details). (B) Abundance of the transgenic construct measured by real time PCR. A primer pair specific for human MCM7 coding sequence was used. The Pb/MCM7i13 #18 and the Pb/MCM7 #88 lines showed comparable transgene expression. (C) Incidence of hyperplasia (HP) and prostatic intraepithelial neoplasia (PIN) in the dorsolateral prostate of 1-year old transgenic mice belonging to the different lines. (D) Analysis of 1-year old transgenic mice belonging to the high expressor lines 18 (Pb/MCM7i13) and 88 (Pb/MCM7). H&E staining shows PIN in the dorsolateral prostate (DLP) of Pb/MCM7i13 mice, but not of Pb/MCM7 mice

A




B

| | pAkt NEG | pAkt POS | |
|---|---|---|---|
| miR-17<br>-Neg (0-1)<br>- Pos (2-3-4) | 49 (57%)<br>14 (51.9%) | 37 (43%)<br>13 (48.1%) | p = 0.66 |
| miR-19a<br>- Neg (0-1)<br>- Pos (2-3-4) | 47 (61%)<br>8 (32%) | 30 (39%)<br>17 (68%) | p = 0.02 |
| miR-20a<br>- Neg (0-1)<br>- Pos (2-3-4) | 47 (60%)<br>3 (25%) | 31 (40%)<br>9 (75%) | p = 0.2 |

**Fig. S11. Detection of miR-17~92 miRNAs by ISH in prostate tumor samples.** (A) Score of the abundance of miR-17, miR-20a and miR-19a in peritumoral tissues (left bars in each group), PIN (middle bars in each group) and prostate cancer (right bars in each group). 0 to 1: negative, 2 to 4: positive. (B) Correlation between positivity for pAkt and positivity for miR-17, miR-20a, and miR-19a. Only miR-19a is overexpressed in tumor samples and its abundance correlates with that of pAkt. This confirms data from prostate cell lines indicating that miR-19a is the only member of miR-17~92 cluster to be upregulated in prostate cancer (see Fig. 3C).

## *Table S1. Criteria used by four different prediction algorithms.*

| | CoN | S-SM | m-T | ΔG | MIt |
|---|---|---|---|---|---|
| **TargetScanS** | ■ | ■ | | | |
| **miRanda** | ■ | | ■ | ■ | ■ |
| **PicTar** | ■ | | ■ | ■ | ■ |
| **miRBase** | ■ | | ■ | ■ | ■ |

Conservation of the seed match across different species (**CoN**); perfect Watson-Crick complementarity between the miRNA seed and the seed match (**S-SM**); overall complementarity between the miRNA and the target also outside the seed/seed match (**m-T**); stability (low binding energy) of the miRNA-target duplex (**ΔG**); presence of multiple (cooperative) binding sites for the same miRNA (**MLT**).
TargetScanS: http://www.targetscan.org/
miRanda: http://www.microrna.org/microrna/home.do
PicTar: http://pictar.bio.nyu.edu/
miRBase: http://microrna.sanger.ac.uk/targets
The versions of the four algorithms available in 2006 were used.

## *Table S2. Criterion 1 and/or Criterion 2 are fulfilled by 7 miRNA families.*

| | TargetScanS | miRanda | PicTar | miRBASE | target genes (oncosuppressors) | overexpression in prostate cancer (1-4) | CTR 1 | CTR 2 |
|---|---|---|---|---|---|---|---|---|
| 17/20/93/106/519 | * | * | * | | E2Fs (5-9)<br>p21 (9-11)<br>BIM (10)<br>Rb2/p130 (12, 13)<br>PTPRO (14)<br>TP53INP1 (15) | Volinia *et al.* (2006)<br>Ambs *et al.* (2008) | * | * |
| 19 | * | * | | * | TSP1 (16) | | * | * |
| 22 | * | * | * | | | | | * |
| 25/32/92/363/367 | * | * | | | BIM (9)<br>p57 (17) | Volinia *et al.* (2006)<br>Ambs *et al.* (2008) | * | |
| 193 | * | * | * | | | | | * |
| 198 | | * | * | | | Volinia *et al.* (2006)<br>Porkka *et al.* (2007) | * | |
| 302/372/373/519/520 | | * | * | | LATS2 (18)<br>CD44 (19) | Porkka *et al.* (2007) | * | |

The prediction by TargetScanS covers all the members of a given family (20), whereas PicTar, miRanda, and miRBASE predict single miRNAs. The four algorithms use different combinations of criteria for miRNA target selection (listed in **Table S1**) and thus the sets of miRNAs they predict to target human *PTEN* are only partially overlapping. Specifically, TargetScanS predicts a total of 16 miRNA families that target human PTEN, miRANDA predicts more than one hundred miRNAs, PicTar predicts 22 and miRBase predicts only 5 (last update of the target predictions: June 2008). To increase the stringency of our bioinformatic prediction, we filtered the outputs of the 4 individual prediction algorithms using two additional criteria. We assumed that if a miRNA family was predicted to target *PTEN* by a majority of the algorithms, it was more likely to be active. Further, we reasoned that a miRNA family known to have oncogenic features had a greater likelihood to target an oncosuppressor such as PTEN (21). Therefore, Criterion 1 was fulfilled by miRNA families predicted by at least 2 algorithms and reported to have oncogenic features. Oncogenic features were defined as knowledge of oncosuppressor targets or overexpression in prostate cancer according to published miRNA arrays. We considered only those miRNA arrays in which more then 10 benign and malignant samples were analyzed, namely references 1-4. Criterion 2 was fulfilled by miRNA families that were predicted by 3 out of the 4 algorithms, unless reported as oncosuppressors (in which case they were excluded from the analysis.)

The five miRNA families we analyzed for *PTEN*-targeting activity are: miR-17, 19, 22, 25, and 302.

miR-22 is the only member of its family and its gene is located within exon 2 of a non-coding transcriptional unit (22, 23) (see **Fig. 1B**). miR-22 is widely expressed in human tissues (20), but its biologic role is poorly characterized. miR22 blunts tumorigenesis in Abl1-transformed 38B9 pro-B cells (22); it is amplified in breast and ovarian cancer (24) and is abundantly expressed in mammary epithelial progenitor cells (25) and in osteoarthritic chondrocytes, where it contributes to extracellular matrix remodelling (26). This suggests that miR22 might behave as an oncogene or a tumor suppressor depending on the context.

Many members of miR-17, miR-19, miR-25 and miR-302 families are spread among 4 microRNA clusters. miR-372 and miR-373, the miR-302 family members of the *miR-371~373* cluster, are proto-oncogenes with a role in testicular germ cell tumors (18) and breast cancer(19). *miR-17~92*, *miR-106b~25* and *miR-106a~363* are paralogous clusters that arose from gene duplication events (27). The *miR-106a~363* cluster, although a common retroviral integration site (28, 29), is barely detectable in all settings tested so far (30), whereas *miR-17~92* and *miR-106b~25* are abundant in many tissues and cell types (20, 30). The *miR-17~92* cluster is an oncogene that acts through both cell autonomous and non-autonomous mechanisms (see (31) for a review and above for a list of the oncosuppressor target genes). As far as the *miR-106b~25* cluster is concerned, miR-93/106b targets p21 (9, 11), whereas miR-25 targets Bim (9) and p57 (17), suggesting an oncogenic role as well.

The miR-17 and miR-19 families have been associated with PTEN regulation. miR-19 family binds the *PTEN* 3'UTR (32) and an inhibitor of *miR-19a* is able to increase PTEN abundance and decrease the growth of anaplastic thyroid cancer cells (33) Furthermore, miR-17 and miR-19 family members belonging to the *miR-17~92* cluster play a crucial role in development of the lymphoid lineage in mouse, partially through the modulation of PTEN abundance (34, 35).

Four additional *PTEN*-targeting miRNAs have been recently described: miR-21 (36-39), miR-214 (40), miR-26 (41), and miR-216 (42). We did not study these miRNAs because at the time of the analysis they did not fulfil any of our criteria.

miR-21 has a well established oncogenic role (1, 43-45). Nonetheless, it was not predicted by any of the 4 algorithms used in this study.

Analogously, miR-214, known for its role in development (46-48) and overexpression in ovarian cancer, is predicted only by miRanda, and thereby did not fulfil our two criteria.

Despite its prediction by three out of the four algorithms and its consequent fulfilment of criterion 2, miR-26 was not studied because of its prominent oncosuppressor role. The abundance of miR-26 family members in prostate cancer is controversial. Several studies report that it is upregulated in prostate cancer [1,4] whereas others report that it is decreased (2, 3). Furthermore, *miR-26a* is deleted in epithelial cancer (49) *miR-26* is amplified in glioma and exerts an oncogenic role through PTEN downregulation (41).

miR-216 was not studied, despite being predicted by three out of the four algorithms, because like miR-26, it plays a pro-differentiation and oncosuppressive role in pancreatic tissue (50). miR-216 has been added to the list of PTEN-targeting miRNAs (42).

Some miRNAs have already been shown to play a role as oncosuppressors or oncogenes in prostate cancer. Among oncosuppressors, miRNA belonging to the *miR-15a~16* cluster (a genomic region deleted in more than 50% of human prostate cancer samples) simultaneously downregulate Bcl2, Cyclin D, and WNT3A and thereby inhibit survival, proliferation, and invasion (51). Similarly, genomic loss of *miR-101* leads to the overexpression of the polycomb group protein EZH2 (52). miR-23a and miR-23b target the mitochondrial enzyme glutaminase, which catabolizes glutamine to generate ATP. c-Myc represses transcription of the *miR-23a~23b* cluster, promoting energy production and cell proliferation (53). Other examples of oncosuppressor miRNAs in the prostate include: miR-34a/c (induced by p53 upon DNA damage and exert a proapoptotic activity) (54, 55). Their genomic locus often undergoes hypermethylation in prostate cancer (56)); the intronic miR-126* (targets the prostate-specific antigen Prostein and opposes migration (57)); miR-146a (targets Rock1 and in this way impairs the activation of the hyaluronan-induced signal transduction pathway (58)). The oncogenic miRNAs reported so far in the context of prostate tumor are: miR-221 and miR-222 that increase proliferation by targeting the cell cycle inhibitor p27(Kip1) (59, 60), while miR-20a (7) and miR-125b (61) exert an antiapoptotic activity by targeting E2F1/2/3 and Bax, respectively. Among all these miRNAs, only miR-20a (which belongs to miR-17 family) passed our double filter for *PTEN*-targeting miRNAs and was analyzed in this study.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## *Table S3.*
## *Clinical and pathological characteristics of the patients.*

| | |
|---|---|
| **Age at prostatectomy** | |
| Median (range); *n* = 56 | 58.5 (47-72) |
| **PSA at diagnosis** | |
| Median (range); *n* = 97 | 6.4 (1-39.3) |
| **Gleason score** (*n* = 145) | |
| 6 | 33 (22.76%) |
| 7 | 94 (64.83%) |
| 8 | 14 (9.66%) |
| 9 | 4 (2.76%) |
| **Pathological stage** (*n* = 98) | |
| T2A-T2B-T2C (confined to prostate) | 53 (54.08%) |
| T3A (extraprostatic extension) | 27 (27.55%) |
| T3B (seminal vesicle invasion) | 16 (16.32%) |
| N1 | 2 (2.05%) |
| **Follow-up** (months) | |
| Median (range); *n* = 95 | 54 (2-183) |
| **Recurrence** (*n* = 96) | |
| No | 65 (67.71%) |
| yes | 31 (32.29%) |

***Table S4.***
***Correlation between miRNA up-regulation and DICER overexpression in prostate tumor samples.***

| | | DICER | | | |
|---|---|---|---|---|---|
| | | **0** | **1** | **2** | |
| **miR-22** | neg | 11 (14.9%) | 44 (59.5%) | 19 (25.6%) | *p = 0.016* |
| | pos | 3 (5.7%) | 24 (45.3%) | 26 (49%) | |
| **miR-25** | neg | 15 (22.7%) | 38 (57.6%) | 13 (19.7%) | *p < 0.001* |
| | pos | 0 (0%) | 29 (46%) | 34 (54%) | |
| **miR-93** | neg | 12 (19.05%) | 39 (61.9%) | 12 (19.05%) | *p < 0.001* |
| | pos | 4 (5.8%) | 29 (42%) | 36 (52.8%) | |
| **miR-106b** | neg | 10 (14.9%) | 40 (59.7%) | 17 (25.4%) | *p = 0.014* |
| | pos | 4 (6.9%) | 25 (43.1%) | 29 (50%) | |

***Table S5. Primers used to generate chimeric luciferase plasmids.***

| | F | R |
|---|---|---|
| **p1** | ACCACTGACTCTGATCCAG | GTAGTTGTAC TCCGCTTAA |
| **p2** | AGTTGGGCCCTGTACCATCCC | ACACAGAGCCACTGCTGCAC |

***Table S6. Primers used to generate PIG/22 and PIG/106b~25 plasmids.***

| | F | R |
|---|---|---|
| **PIG/22** | ACAGCCACCTCTTGCTGCTC | GGGGAGACAGAGTGTTCTCTC |
| **PIG/106b~25** | GTAAGTGCCCAAATTGCTGG | CTAAGGGGAAGGTAGGGGGG |

***Table S7. Primers used to detect human PTEN 3'UTR by PCR.***

| | F | R |
|---|---|---|
| ***PTEN F1-R1*** | ACCACTGACTCTGATCCAG | CGCTTACTGTAGAATCTCA |
| ***PTEN F2-R2*** | GTAACGACTTCTCCATCTC | GTAGTTGTACTCCGCTTAA |
| ***PTEN F3-R3*** | AGTTGGGCCCTGTACCATCC | CAGGTAGAAGGCAACTCTGCC |
| ***ATP1A1 F4-R4*** | CTCAGATGTGTCCAAGCAAG | GTCAGTGCCCAAGTCAATG |

***Table S8. Primers used to detect human PTEN, MCM7 and intron 13 mRNA by real-time PCR.***

| | F | R |
|---|---|---|
| ***PTEN*** | GTTTACCGGCAGCATCAAAT | CCCCCACTTTAGTGCACAGT |
| ***MCM7*** | CCTCGCAGCCAGTACACAA | GCCCCACCCTCTAAGGTCA |
| ***intron 13 (pri-miR-106b~25)*** | TAGTGGTCCTCTCCGTGCTAC | GGATCTAGGACACATGGAGTG |
| ***intron13-exon14 boundary*** | CCTCACAGGACAGCTGAACTCC | CCCCTTGTCTCCTAGAAGAGAG |
| ***ACTIN*** | CATGTACGTTGCTATCCAGGC | CTCCTTAATGTCACGCACGAT |

***Table S9. Primers used to detect mouse Pten by real-time PCR.***

| | F | R |
|---|---|---|
| ***Pten*** | TGGATTCGACTTAGACTTGACCT | GCGGTGTCATAATGTCTCTCAG |
| ***GAPDH*** | TGACCACAGTCCATGCCATC | GACGGACACATTGGGGGTAG |