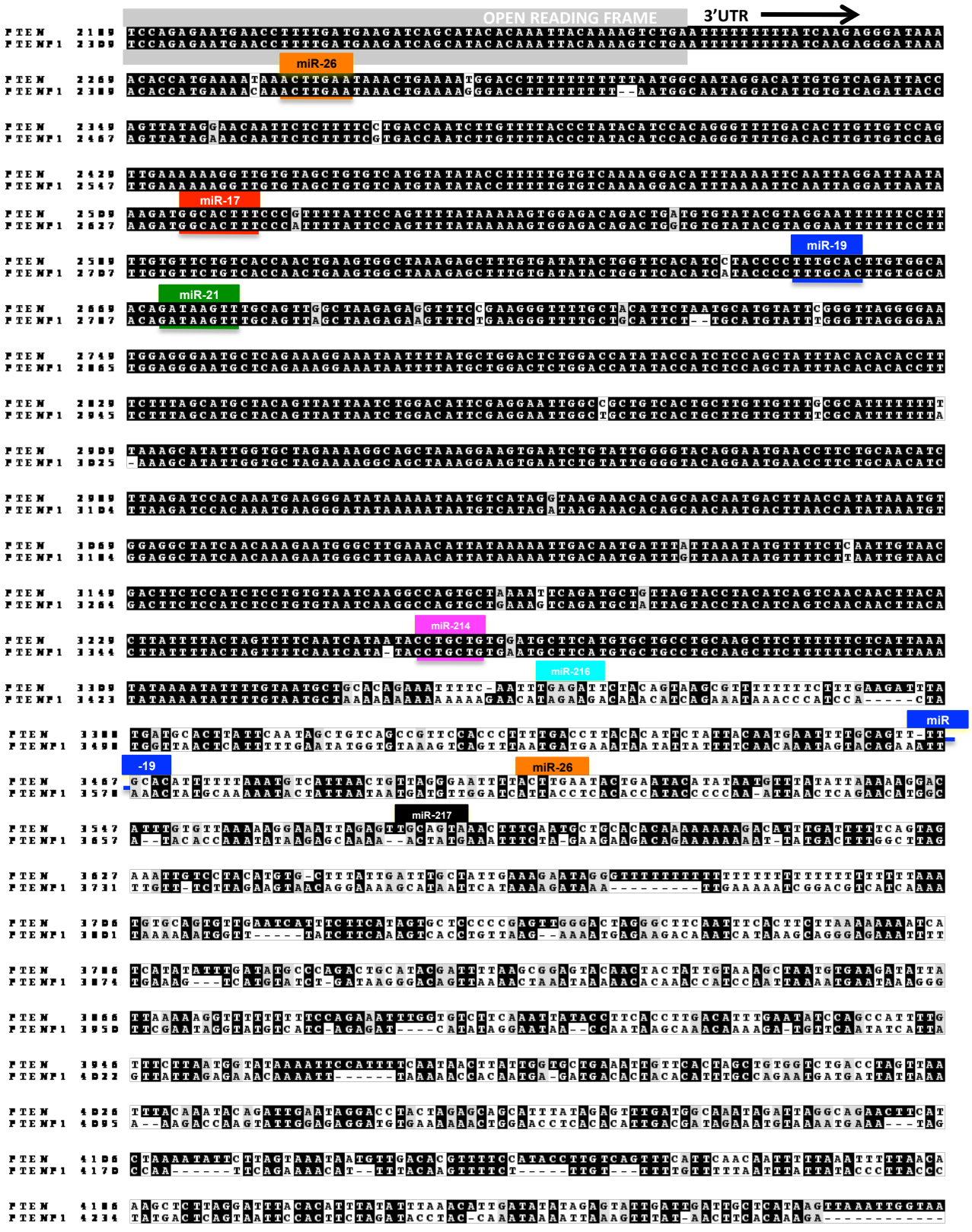
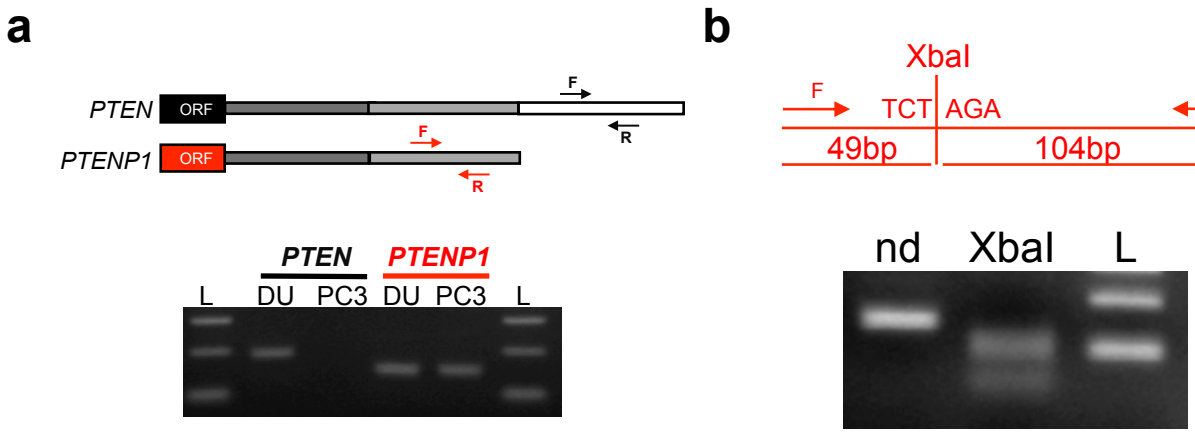


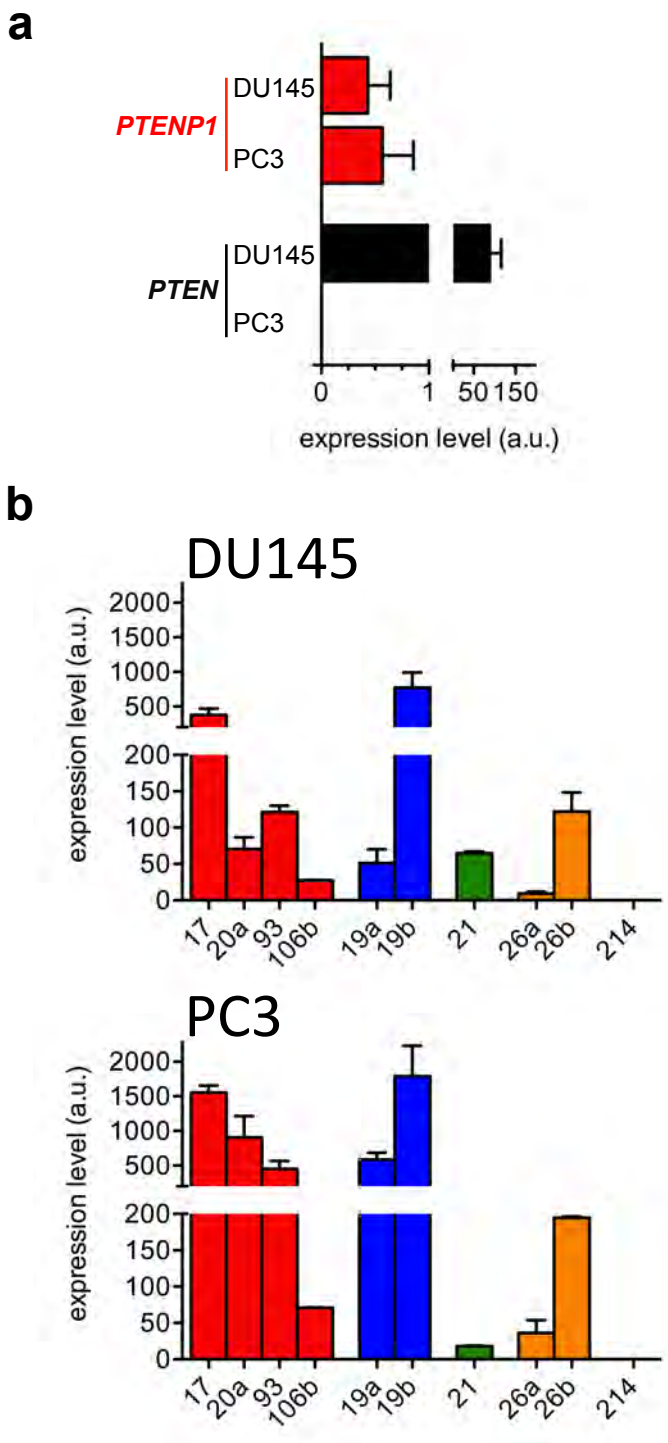
Supplementary Figure 1. Alignment between *PTEN* and *PTENP1* 3'UTR. *PTEN* (NM_000314) and *PTENP1* (NM_023917) 3'UTR are shown. Matched nucleotides are in black, unmatched are in white. The seed matches for the different *PTEN*-targeting microRNA families are shown as colored boxes.



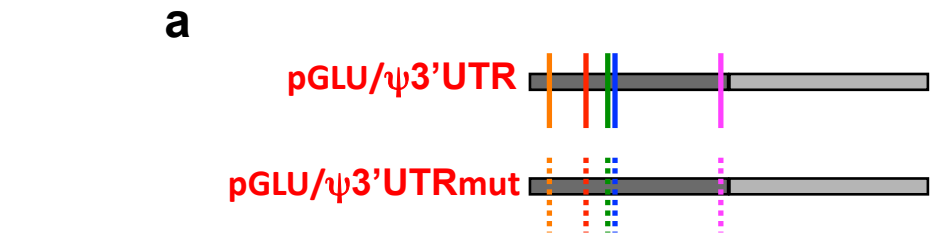
c

***PTEN* forward**	**CCTTCTCCATCTCCTGTGTAATCAA**
***PTEN* reverse**	**GTTGACTGATGTAGGTACTAACAGCAT**
***PTEN* FAM probe**	**CCAGTGCTAAAATTCA**
***PTENP1* forward**	**AGTCACCTGTTAAGAAAATGAGAAGACAAA**
***PTENP1* reverse**	**CTGTCCCTTATCAGATACATGACTTTCAA**
***PTENP1* FAM probe**	**AAGCAGGGAGAAATT**

Supplementary Figure 2. Characterization of *PTEN* and *PTENP1* specific primers. **a-b.** Real time PCR primers. **a.** *(upper)* Localization of *PTEN*-specific (black) and *PTENP1*-specific (red) primers used for real time PCR. *PTEN*-specific primers bind to the 3'UTR region that is not present in *PTENP1* (white rectangle). *PTENP1*-specific primers bind to the 3'UTR region that has low homology with the corresponding *PTEN* region (light grey rectangle). *(lower)* Regular PCR performed in DU145 and PC3 cell lines. While DU145 cells express both *PTEN* and *PTENP1*, PC3 cells, which harbor a homozygous deletion of *PTEN*, express only the pseudogene. **b.** Diagnostic restriction analysis performed on the PCR product obtained with the *PTENP1*-specific primers. The XbaI site is present only in the *PTENP1* sequence and not in the *PTEN* sequence. Therefore, the PCR product obtained using the *PTENP1*-specific primers is indeed derived from *PTENP1*. nd: non digested; L: 100bp ladder. **c.** Taqman probes for *PTEN* (*upper*) and *PTENP1* (*lower*).

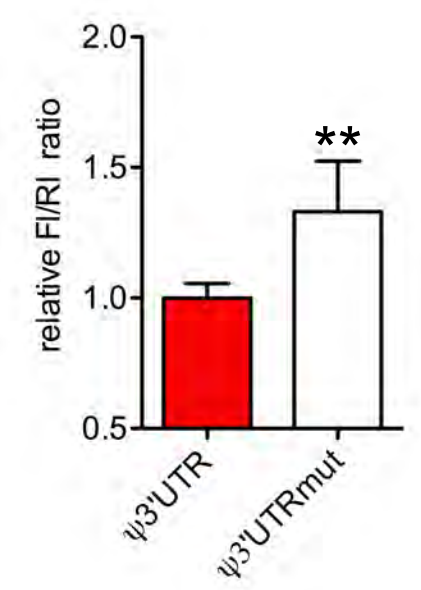


Supplementary Figure 3. Expression level of *PTEN*, *PTENP1* and the *PTEN*-targeting microRNAs in DU145 and PC3 cell lines. a. Real time PCR performed with the isoform-specific primers described in **Supplementary Figure 2a-b** (mean ± s.d, n = 3). In DU145, *PTENP1* is expressed at lower level compared to *PTEN*. This line is therefore suitable for *PTENP1* overexpression experiments. **b.** Real time PCR of the *PTEN*-targeting microRNA family members performed on DU145 (*upper*) and PC3 (*lower*). *miR-17* family: red; *miR-19* family: blue; *miR-21*: green; *miR-26* family: orange; *miR-214*: pink. mean ± s.d, n = 3.

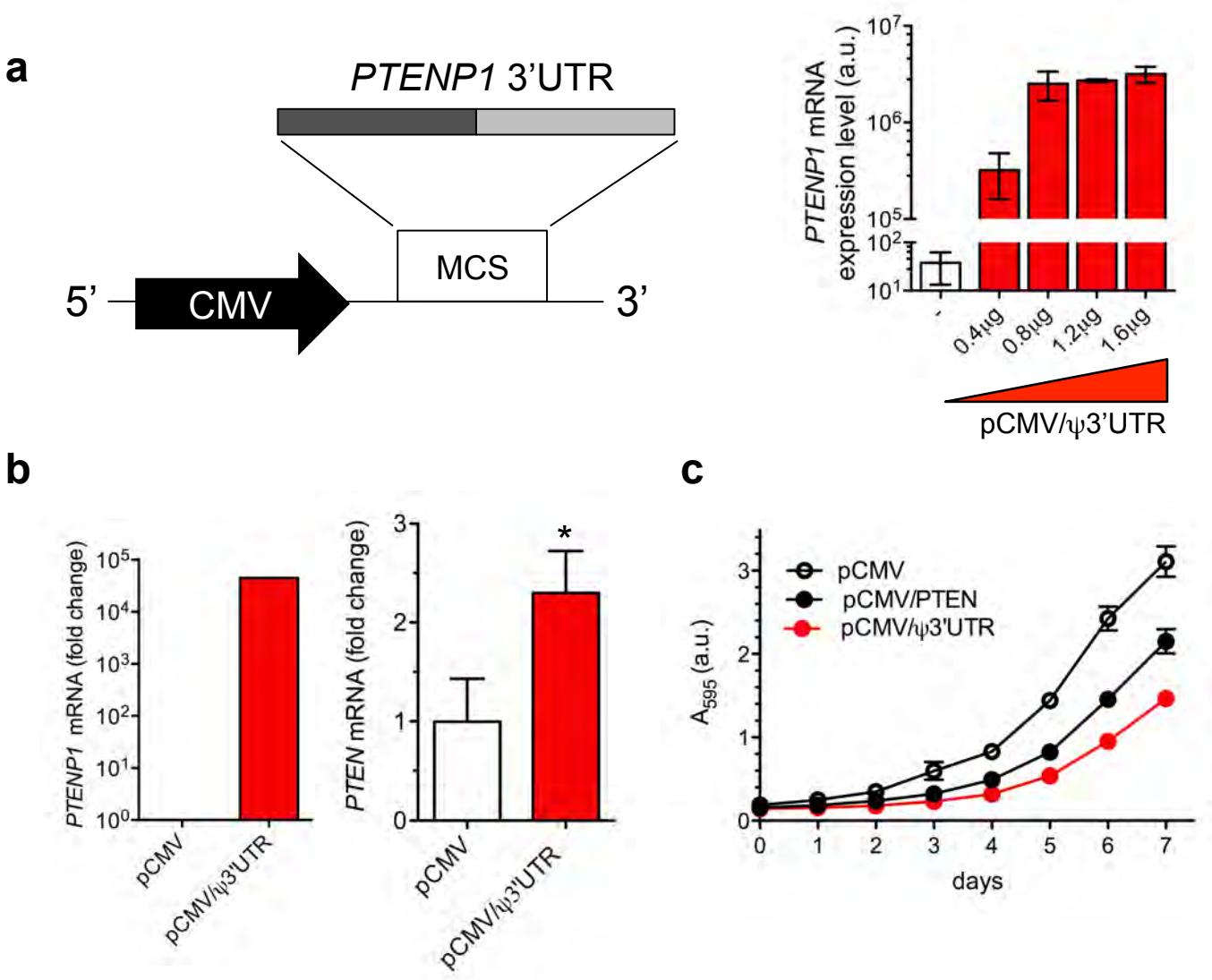


b

miRNA family	*wt* seed match	mutant seed match
17	GGCACTTT	GcCtCaTa
19	TTTGCAC	aTaGgAg
21	GATAAGTT	GtTtAcTt
26	ACTTGAA	tCaTcAt
214	CCTGCTG	gCaGgTc



Supplementary Figure 4. Luciferase assay on *wt* and mutant *PTENP1* 3'UTR. a. Schematic representation of **pGLU** luciferase plasmid expressing the *wt PTENP1* 3'UTR (**pGLU/ψ3'UTR**) or the 3'UTR in which the seed matches of the 5 *PTEN*-targeting microRNAs have been mutagenized (**pGLU/ψ3'UTRmut**). **b.** Sequences of the *wt* and the mutagenized seed matches. **c.** The *wt* and the mutant reporter plasmids were transfected into DU145 cells. 24h later, the luciferase activity of the mutant plasmid was found to be higher than that of the *wt* plasmid. This indicates that the mutations introduced in the seed matches impair the ability of endogenous microRNAs to bind to *PTENP1* 3'UTR, so that the translation of firefly luciferase is increased (mean ± s.d, n > 3).



Supplementary Figure 5. *PTENP1* 3'UTR increases *PTEN* expression level and inhibits cell growth. a. Characterization of **pCMV/ψ3'UTR** plasmid. (*left*) The full ~2kb *PTENP1* 3'UTR was cloned in the multicloning site (MCS) of **pCMV-MCS** expression plasmid. The 5' region that is highly homologous to *PTEN* 3'UTR and the 3' low homology region are depicted as a dark grey and a light grey rectangle, respectively. (*right*) Increasing amounts of **pCMV/ψ3'UTR** plasmid were transiently transfected in 293T cells and 24h later the expression of the insert was measured by real time PCR. **b.** *PTENP1* (*left*) and *PTEN* (*right*) mRNA level 24h after the transient transfection of the empty **pCMV** plasmid or **pCMV/ψ3'UTR** plasmid in DU145 cells. **c.** Growth curve of DU145 prostate cancer cells transiently transfected with equimolar amounts of **pCMV** empty plasmid, **pCMV/PTEN** plasmid (expressing PTEN protein) and **pCMV/ψ3'UTR** plasmid (expressing *PTENP1* 3'UTR). **a**, **b**, and **c**. mean ± s.d, n ≥ 3.

***PTEN*-specific SMARTpool (si-PTEN):**

D-120509-01 GGAAATTAGAGTTGCAGTA
D-120509-02 ACTTATTGGTGCTGAAATT
D-120509-03 GGCAAATAGATTACCCAGA
D-120509-04 GATTCTACAGTAAGCGTTT

***PTENP1*-specific SMARTpool (si-PTENP1):**

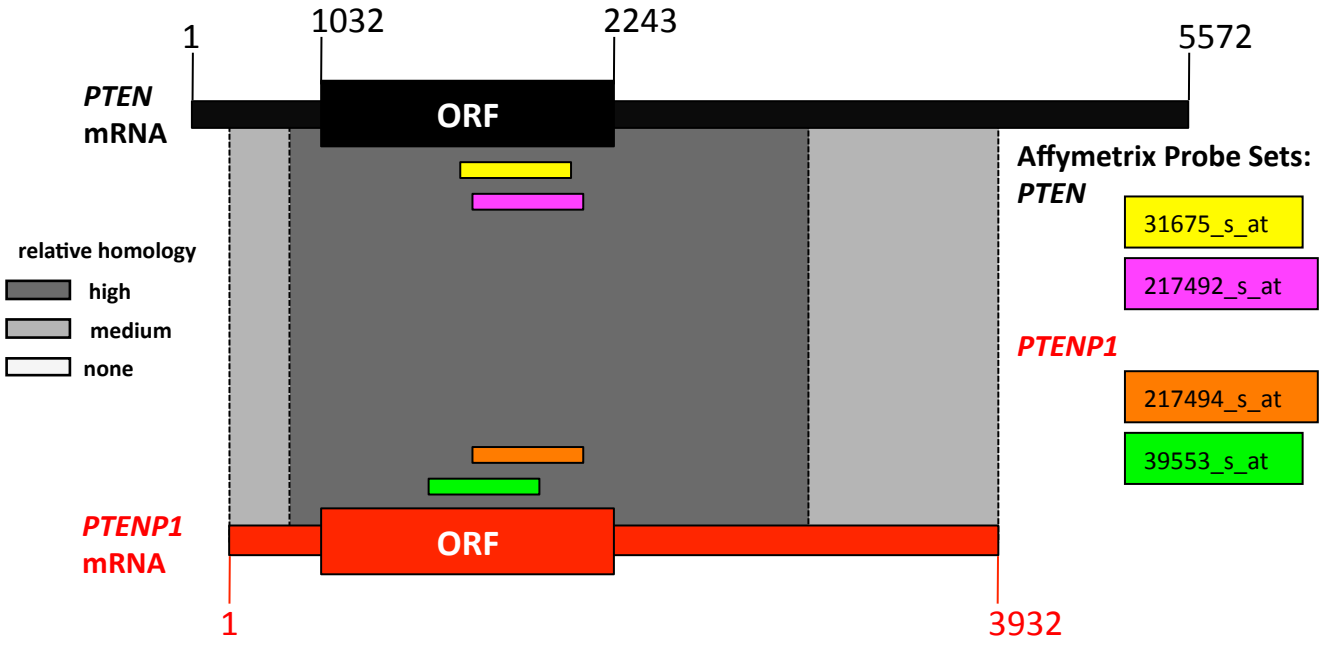
D-120498-01 TGAATAAAGGGTTCGAATA
D-120498-02 GCCAGAATGATGATTATTA
D-120498-03 CATCAGAGATCATATAGGA
D-120498-04 CCTCACACATTGACGATAG

Supplementary Figure 6. si-PTEN and si-PTENP1. The sequences of the *PTEN* and *PTENP1*-specific SMARTpools are reported.

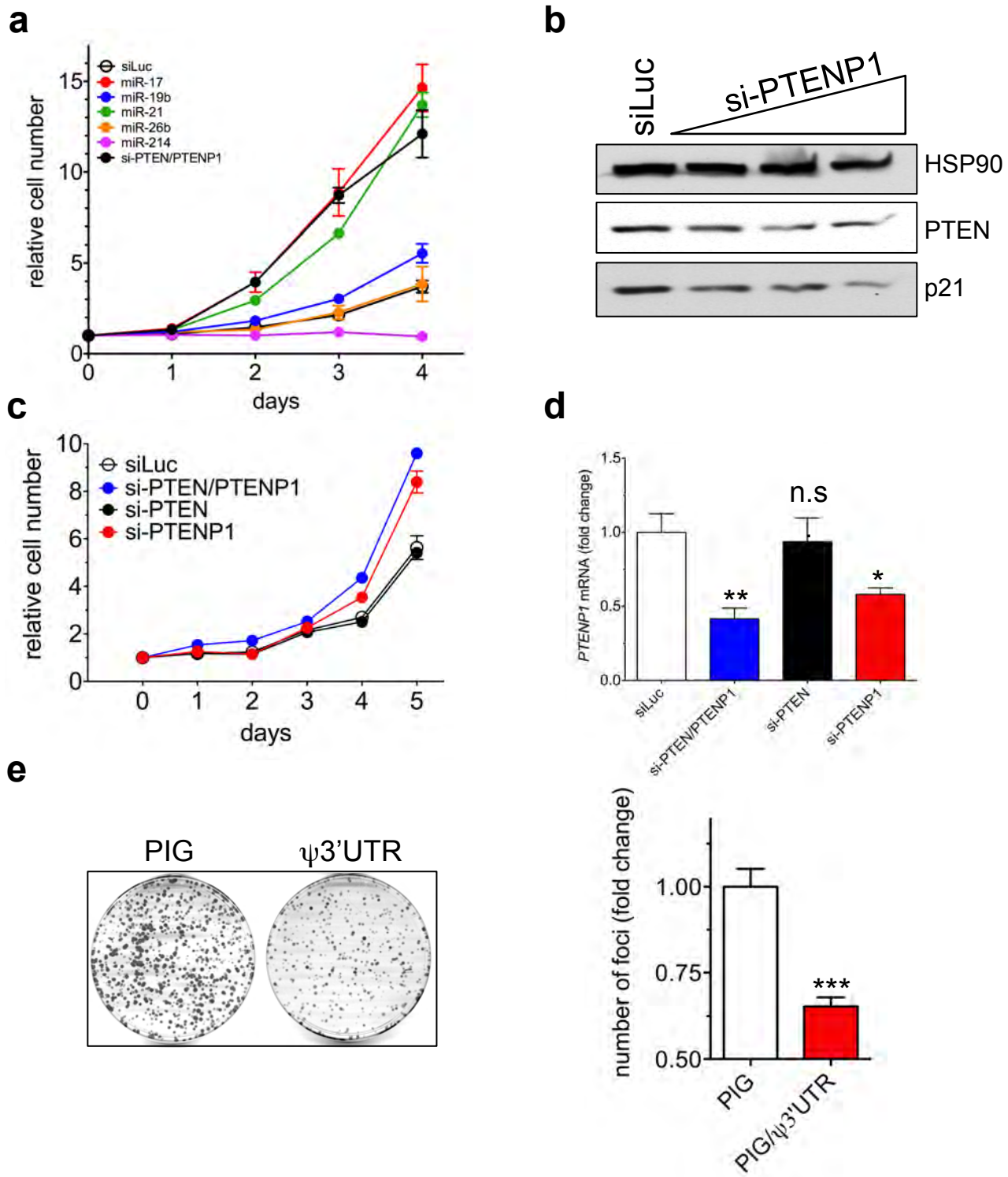
a

```
PTEN        2214-GATCAGCATACACAAATTA-2232
J-003023-09     GAUCAGCAUACACAAAUUA
PTENP1      1952-GATCAGCATACACAAATTA-1970

PTEN        1095-GACTTAGACTTGACCTATA-1113
J-003023-10     GACUUAGACUUGACCUAUA
PTENP1       833-GACTTAGACTTGACCTATA-851

PTEN        1350-GATCTTGACCAATGGCTAA-1368
J-003023-11     GAUCUUGACCAAUGGCUAA
PTENP1      1088-GATCTTGACCAATGGCTAA-1106

PTEN        1931-CGATAGCATTTGCAGTATA-1949
J-003023-12     CGAUAGCAUUUGCAGUAUA
PTENP1      1670-TGATAGCATTTGCAGTATA-1687
```

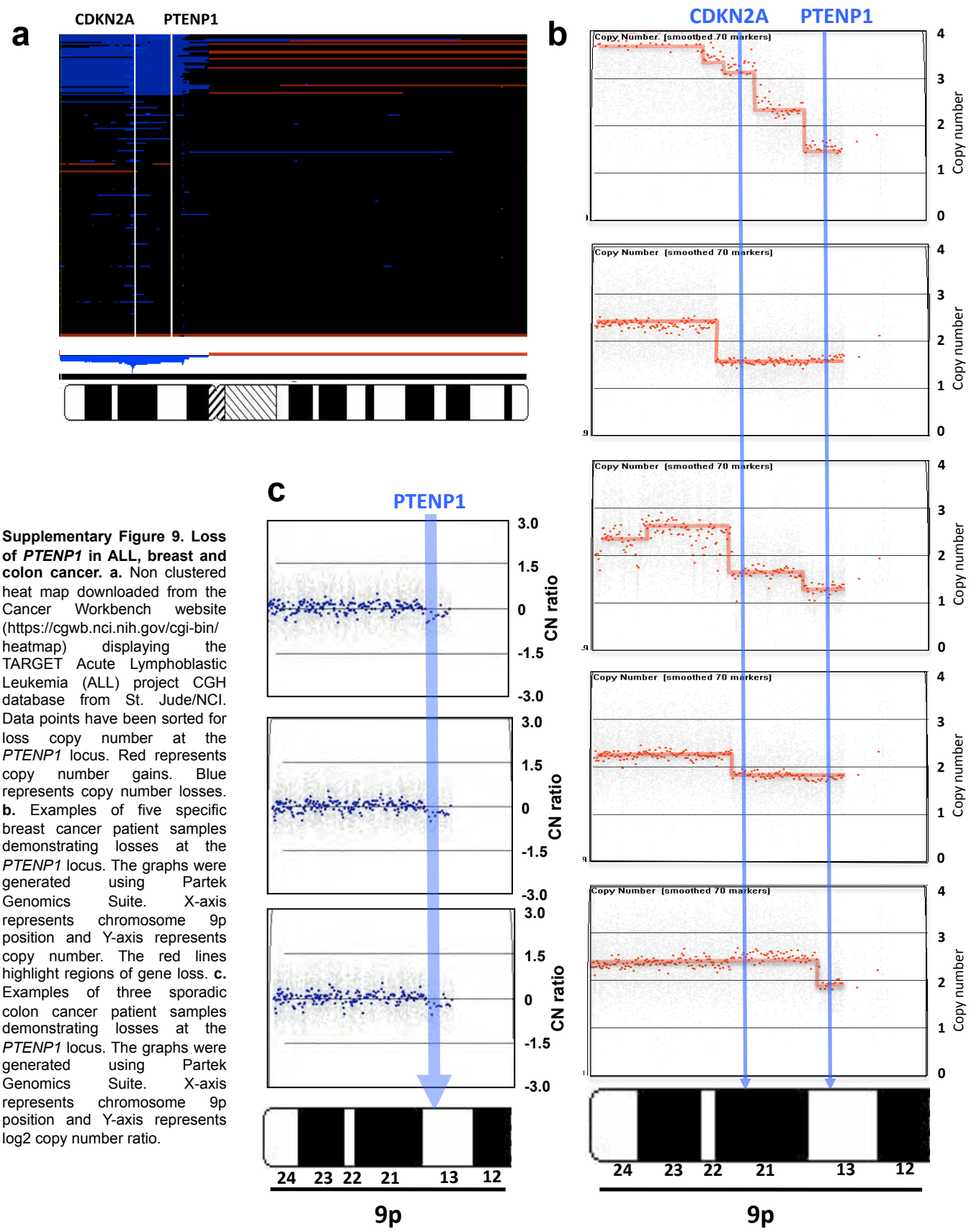
b



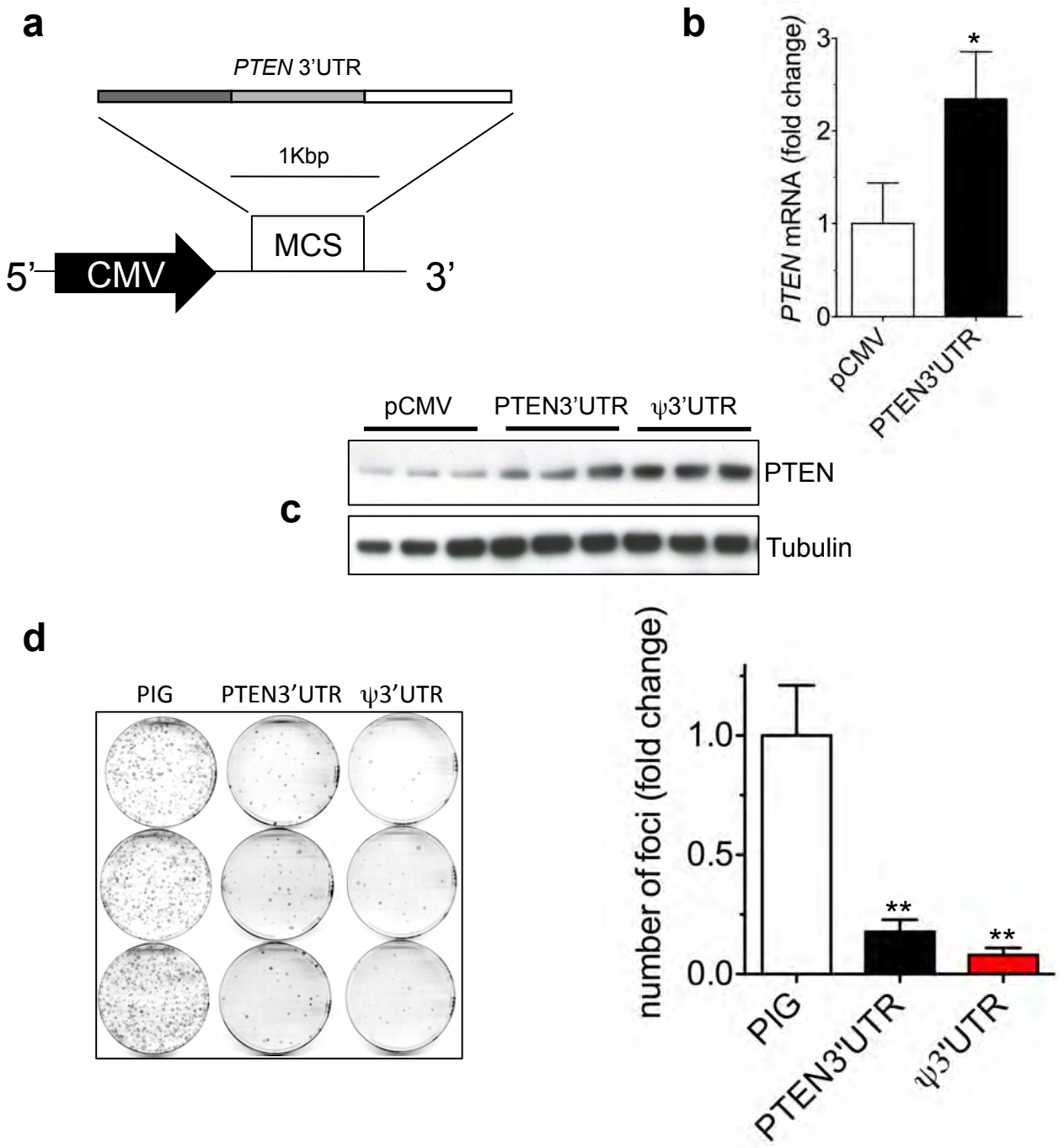
Supplementary Figure 7. Specificity of commercially available siRNAs and Affymetrix probes for *PTEN* and *PTENP1*. a. The four siRNAs that comprise the Dharmacon SMARTpool against *PTEN* are all complementary to the open reading frame, therefore they match *PTENP1* as well. Only one mismatch in the 3'nt of probe J-003023-12 is present (underlined). We call these bi-specific SMARTpool si-PTEN/PTENP1 **b.** The Affymetrix microarray platform contains two probes for *PTEN* (yellow and pink boxes) and two probes for *PTENP1* (orange and green boxes). These two probe sets pair to *PTEN* and *PTENP1* in the open reading frame. Due to the high homology between the two molecules in this region, the probes fail to be specific. Black rectangles: *PTEN* 5'UTR, open reading frame and 3'UTR; red rectangles: *PTENP1* 5'UTR, open reading frame and 3'UTR. The region of high and low conservation between *PTEN* and *PTENP1* are shadowed in dark and light grey, respectively.



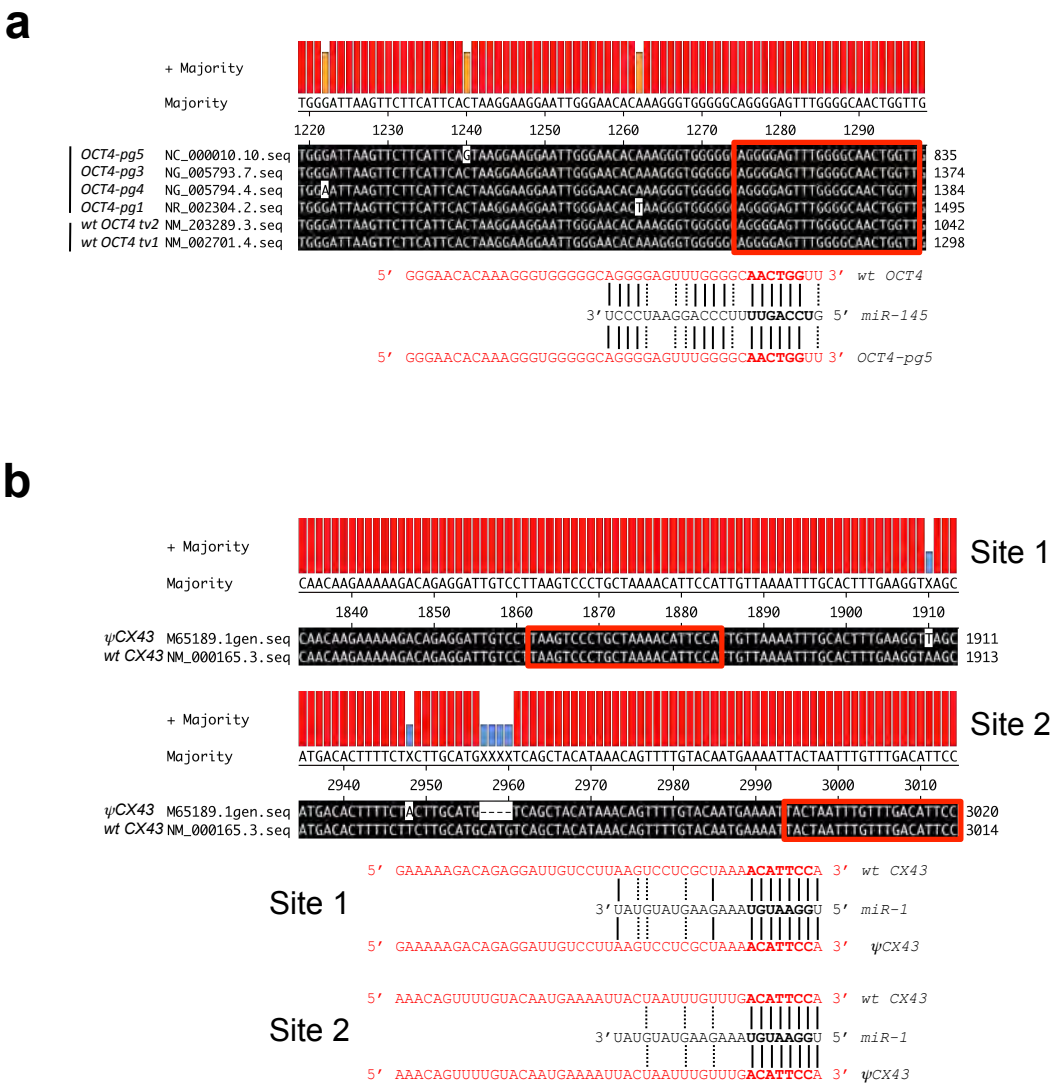
Supplementary Figure 8. *PTENP1* 3'UTR has PTEN-independent functions. a. Growth curve of PC3 cells transiently transfected with a representative member of each of the *PTEN*-targeting microRNA families: *miR-17* (red), *miR-19* (blue), *miR-21* (green), *miR-26* (orange) and *miR-214* (pink). si-PTEN/PTENP1 is included as positive control **b.** Western blot of DU145 cells transiently transfected with control siLuc or increasing doses of si-PTENP1. Two among the targets of miR-17 family, PTEN and p21, are detected. **c.** Growth curve of *PTEN*-null PC3 cells transiently transfected with control siLuc, si-PTEN/PTENP1, si-PTEN and si-PTENP1. **d.** Real time PCR of *PTENP1* performed 24h after the transient transfection of the indicated siRNAs in PC3 cells. **e.** Foci assay of PC3 cells stably infected with **PIG** empty or **PIG/ψ3'UTR** plasmids. A representative of 3 plates (*left*) and the colony counts (*right*) are shown. **a**, **c**, **d** and **e**. mean ± s.d, n ≥ 3.



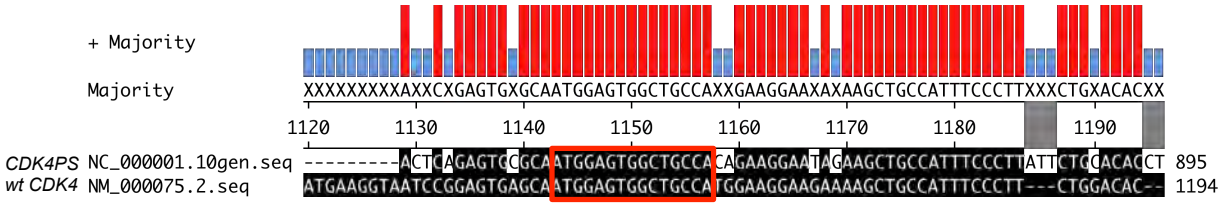
Supplementary Figure 9. Loss of *PTENP1* in ALL, breast and colon cancer. a. Non clustered heat map downloaded from the Cancer Workbench website (https://cgwb.nci.nih.gov/cgi-bin/heatmap) displaying the TARGET Acute Lymphoblastic Leukemia (ALL) project CGH database from St. Jude/NCI. Data points have been sorted for loss copy number at the *PTENP1* locus. Red represents copy number gains. Blue represents copy number losses. **b.** Examples of five specific breast cancer patient samples demonstrating losses at the *PTENP1* locus. The graphs were generated using Partek Genomics Suite. X-axis represents chromosome 9p position and Y-axis represents copy number. The red lines highlight regions of gene loss. **c.** Examples of three sporadic colon cancer patient samples demonstrating losses at the *PTENP1* locus. The graphs were generated using Partek Genomics Suite. X-axis represents chromosome 9p position and Y-axis represents log2 copy number ratio.



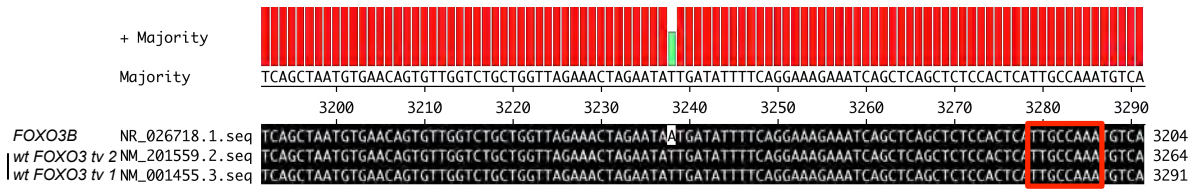
Supplementary Figure 10. *PTEN* 3'UTR increases *PTENP1* expression level and inhibits cell growth. a. Characterization of **pCMV/PTEN3'UTR** plasmid. A ~3kb *PTEN* 3'UTR was cloned in the multicloning site (MCS) of **pCMV-MCS** expression plasmid, so that **pCMV/PTEN3'UTR** was obtained. The 5' region that is highly homologous to *PTENP1* 3'UTR and the middle low homology region are depicted as a dark grey and a light grey rectangle, respectively. The 3' region that is not present in *PTENP1* 3'UTR is depicted as a white rectangle. **b.** *PTEN* mRNA level 24h after the transient transfection of the empty **pCMV** plasmid or **pCMV/PTEN3'UTR** plasmid in DU145 cells. **c.** PTEN level 48h after the transient transfection of the indicated plasmids in DU145. **d.** Foci assay of DU145 cells stably infected with **PIG** empty, **PIG/PTEN3'UTR** and **PIG/ψ3'UTR** plasmids. Representative plates (*left*) and the colony counts (*right*) are shown (mean ± s.d, n ≥ 3).



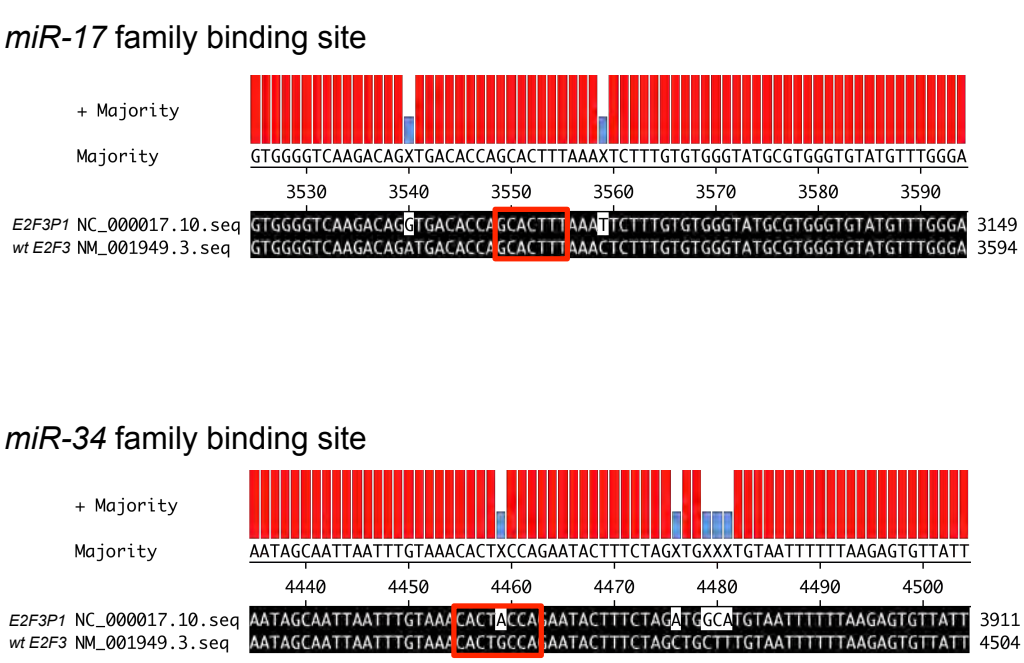
Supplementary Figure 11. Pseudogenes aberrantly expressed in cancer that maintain the binding sites for validated microRNAs. a. *miR-145* binding site is conserved in *OCT4* pseudogenes *OCT4-pg1*, *3*, *4* and *5*. *(upper)* Sequence alignment between the two *OCT4* transcript variants (*tv1* and *tv2*) and 4 out of 6 *OCT4* pseudogenes (*OCT4-pg1*, *3*, *4* and *5*).



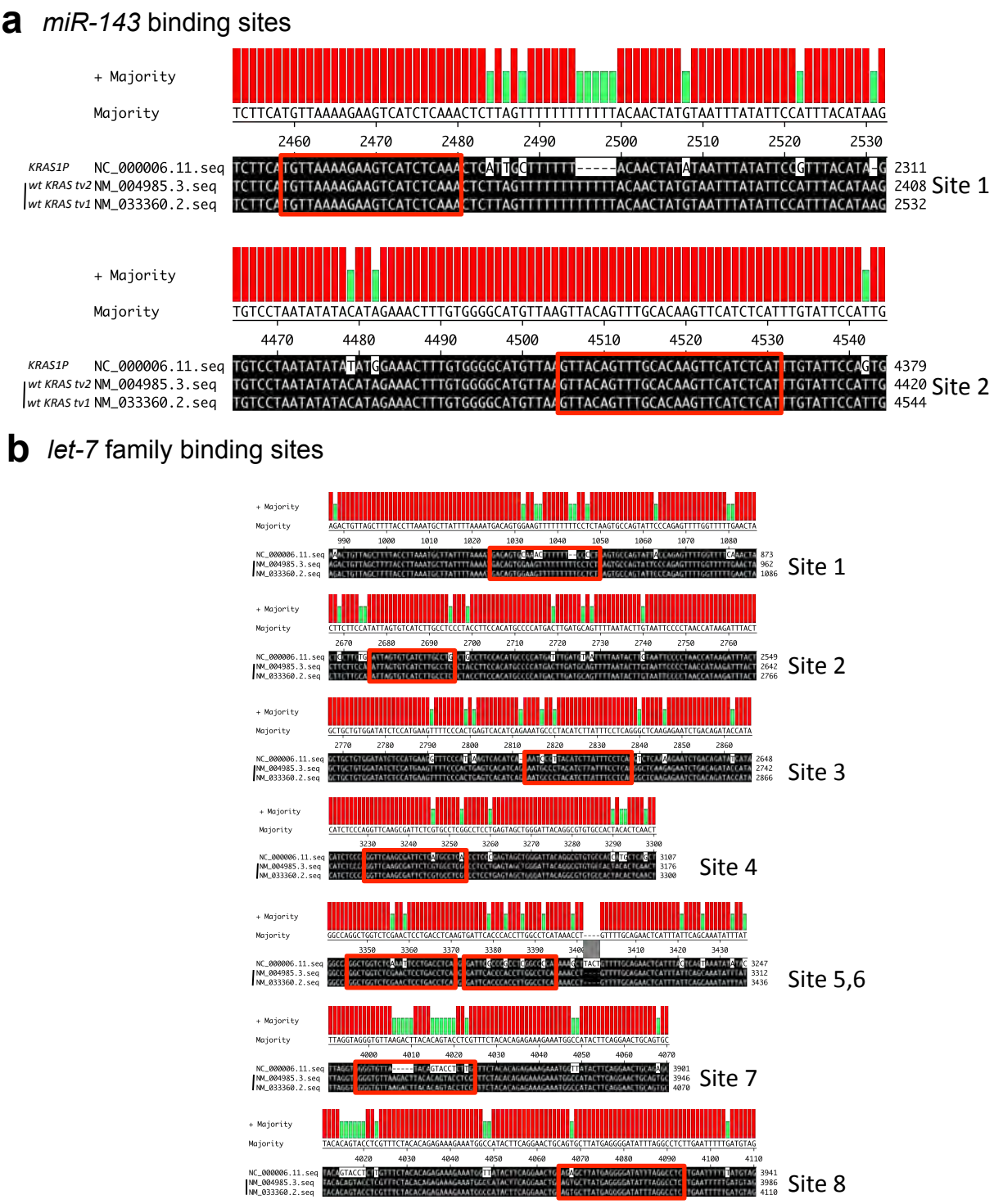
Supplementary Figure 12. *CDK4* pseudogene *CDK4PS* maintains the validated binding site for *miR-34* family. The reported *CDK4PS* sequence has been extended in the 3'UTR region by Blast search.



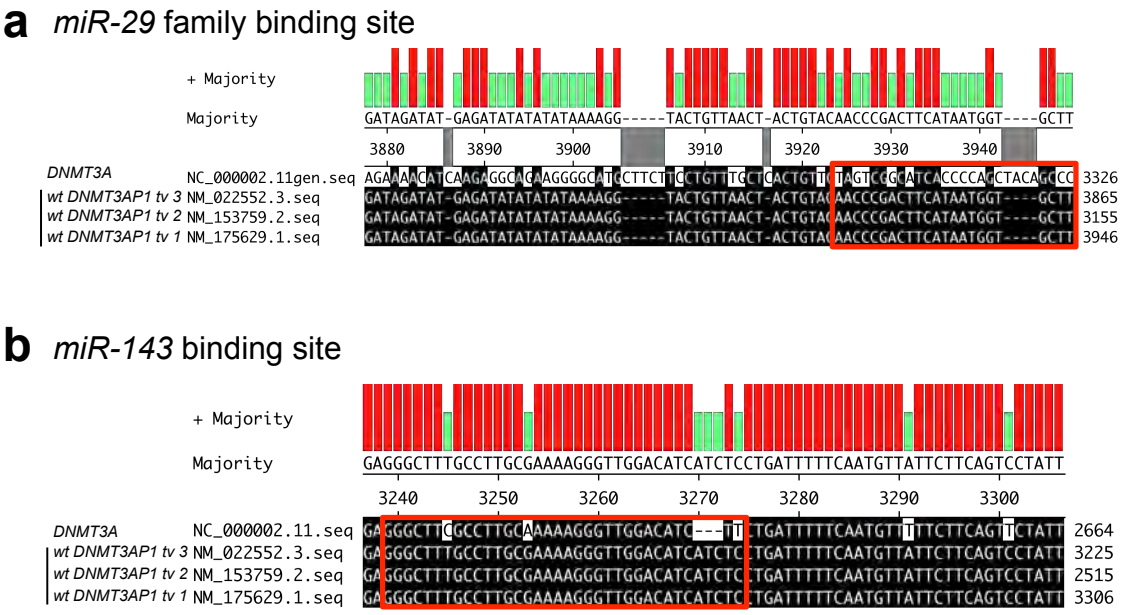
Supplementary Figure 13. *FOXO3* pseudogene *FOXO3B* maintains the validated binding site for *miR-182*. Two transcript variants of *FOXO3* (*tv1* and *tv2*) are reported.



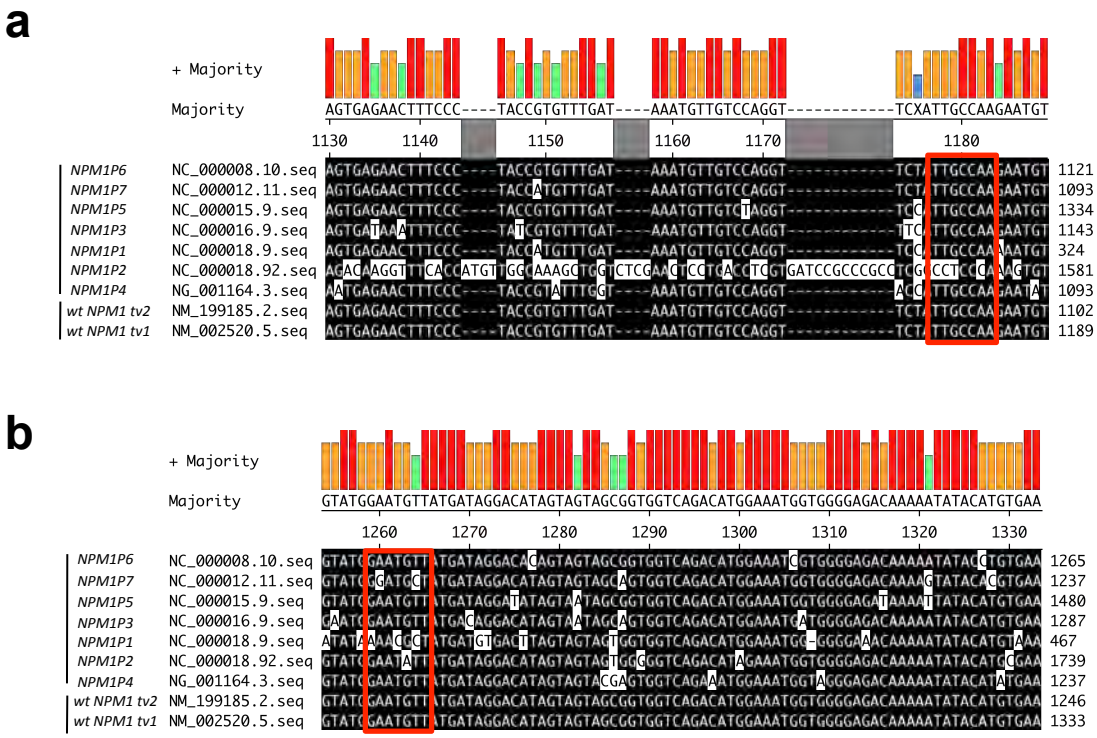
Supplementary Figure 14. *E2F3* pseudogene *E2F3P1* maintains the validated binding site for *miR-17* family, but not for *miR-34* family. The binding site for *miR-17* and *miR-34* families are reported in **a** and **b**, respectively.



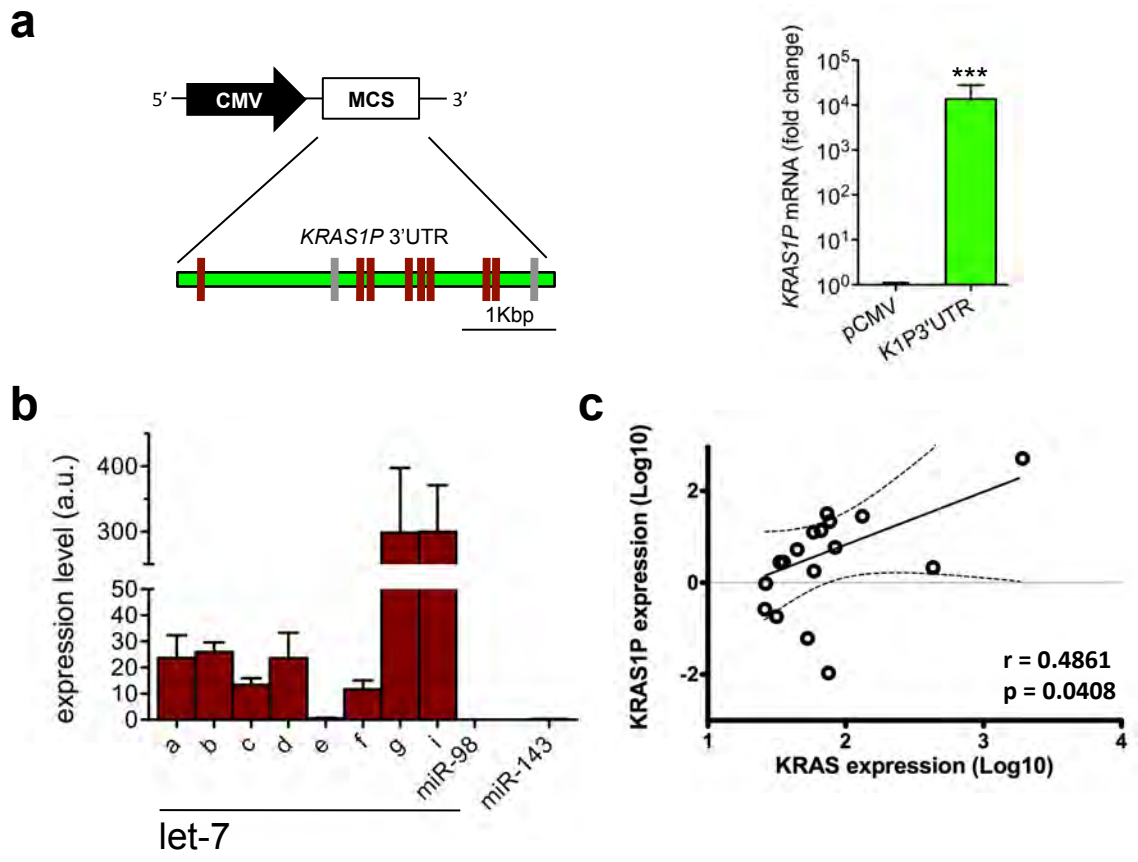
Supplementary Figure 15. *KRAS* pseudogene *KRAS1P* maintains the validated binding sites for *miR-143* and *let-7* family. a. The two binding sites for *miR-143* are both conserved in *KRAS1P*. **b.** *let-7* family has 8 binding sites along *KRAS* 3'UTR. All of them show extensive conservation in *KRAS1P*, especially site 3, 5 and 8 in which the seed match is intact. Two transcript variants of *KRAS* (*tv1* and *tv2*) are reported.



Supplementary Figure 16. *DNMT3A* pseudogene *DNMT3AP1* does not maintain the validated binding site for *miR-29* family and *miR-143*. The binding site for *miR-29* family and *miR-143* are reported in **a** and **b**, respectively. Three transcript variants of *DNMT3A* (*tv1*, *tv2* and *tv3*) are reported.



Supplementary Figure 17. *NPM1* pseudogenes *NPM1P1, 3, 4, 5, 6, 7* maintain the predicted binding sites for *miR-181* and *miR-182*. No microRNAs have yet been reported to target *NPM1*. Nonetheless, PicTar prediction algorithm (http://pictar.mdc-berlin.de/) predicts *miR-181* and *miR-182* to bind *NPM1* 3'UTR. **a.** The predicted *miR-182* seed match is conserved in all NPM1 pseudogenes except for *NPM1P2*. **b.** The predicted *miR-181* seed match is conserved in *NPM1P3*, *4*, *5* and *6*. Two transcript variants of *NPM1* (*tv1* and *tv2*) are reported.



Supplementary Figure 18. *KRAS1P* 3'UTR increases *KRAS* expression level and promotes cell growth. a. (*left*) Characterization of **pCMV/K1P3'UTR** expression plasmid. The full ~4kb 3'UTR was cloned in the multicloning site (MCS) of **pCMV-MCS**, so that **pCMV/K1P3'UTR** was obtained. The seed matches for *miR-143* and *let-7* family are indicated as grey and brown lines, respectively. (*right*) *KRAS1P* mRNA level 24h after the transient transfection of the empty **pCMV** plasmid or **pCMV/K1P3'UTR** plasmid in DU145 cells. **b.** Real time PCR (mean ± s.d, n = 3) of *KRAS*-targeting microRNAs in DU145. *miR-143*: grey. *let-7* family: brown. **c**. Regression analysis of *KRAS* and *KRAS1P* expression in 18 human prostate tumor samples.

miRNA	RT primer (5'-3')	PCR primer F (5'-3')
17	GTCGTATCCAGTGCAGGGTCCGAGGTATTC GCACTGGATACGACctacct	CGGCGGcaaagtgcttacagtgc
20	GTCGTATCCAGTGCAGGGTCCGAGGTATTC GCACTGGATACGACctacct	CGGCGGtaaagtgcttatagtgc
93	GTCGTATCCAGTGCAGGGTCCGAGGTATTC GCACTGGATACGACctacct	CGGCGGcaaagtgctgttcgtgc
106b	GTCGTATCCAGTGCAGGGTCCGAGGTATTC GCACTGGATACGACatctgc	CGGCGGtaaagtgctgacagtgc
19a	GTCGTATCCAGTGCAGGGTCCGAGGTATTC GCACTGGATACGACtcagtt	CGGCGGtgtgcaaatctatgc
19b	GTCGTATCCAGTGCAGGGTCCGAGGTATTC GCACTGGATACGACtcagtt	CGGCGGtgtgcaaatccatgc
21	GTCGTATCCAGTGCAGGGTCCGAGGTATTC GCACTGGATACGACtcaaca	CGGCGGtagcttatcagactgatg
26a	GTCGTATCCAGTGCAGGGTCCGAGGTATTC GCACTGGATACGACagccta	CGGCGGttcaagtaatccagg
26b	GTCGTATCCAGTGCAGGGTCCGAGGTATTC GCACTGGATACGACacctat	CGGCGGttcaagtaattcagg
214	GTCGTATCCAGTGCAGGGTCCGAGGTATTC GCACTGGATACGACactgcc	CGGCGGacagcaggcacagacag
let-7a	GTCGTATCCAGTGCAGGGTCCGAGGTATTC GCACTGGATACGACaacta	GCCGCtgaggtagtaggttgta
let-7b	GTCGTATCCAGTGCAGGGTCCGAGGTATTC GCACTGGATACGACaatcac	GCCGCtgaggtagtaggttgtgt
let-7c	GTCGTATCCAGTGCAGGGTCCGAGGTATTC GCACTGGATACGACaaccat	GCCGCtgaggtagtaggttgta
let-7d	GTCGTATCCAGTGCAGGGTCCGAGGTATTC GCACTGGATACGACactatg	GCCGCagaggtagtaggttgc
let-7e	GTCGTATCCAGTGCAGGGTCCGAGGTATTC GCACTGGATACGACactata	TGCCGGtgaggtaggagg
let-7f	GTCGTATCCAGTGCAGGGTCCGAGGTATTC GCACTGGATACGACaacta	GCCGCtgaggtagtagattgtat
let-7g	GTCGTATCCAGTGCAGGGTCCGAGGTATTC GCACTGGATACGACaactgt	GCCGCtgaggtagtagtttgtac
let-7i	GTCGTATCCAGTGCAGGGTCCGAGGTATTC GCACTGGATACGACaacagc	GCCGCtgaggtagtagtttgtgc
98	GTCGTATCCAGTGCAGGGTCCGAGGTATTC GCACTGGATACGACaacaa	GCCGCtgaggtagtaagttgta
143	GTCGTATCCAGTGCAGGGTCCGAGGTATTC GCACTGGATACGACgagcta	CGGCGGtgagatgaagcactg

Supplementary Figure 19. Sequence of the microRNA-specific primers used for the retrotranscription and the real-time PCR. See Supplementary Methods section for details. The portions of the primers that recognize the microRNAs are in color: red for *miR-17* family, blue for *miR-19* family, green for *miR-21* family, orange for *miR-26* family, pink for *miR-214*, brown for *let-7* family, grey for *miR-143*. In some cases (*miR-17*/*20*/*93*; *miR-19a*/*b*; *let-7a*/*f*), the RT primer is shared by more than one microRNA of the same family.