SUPPLEMENTARY INFORMATION

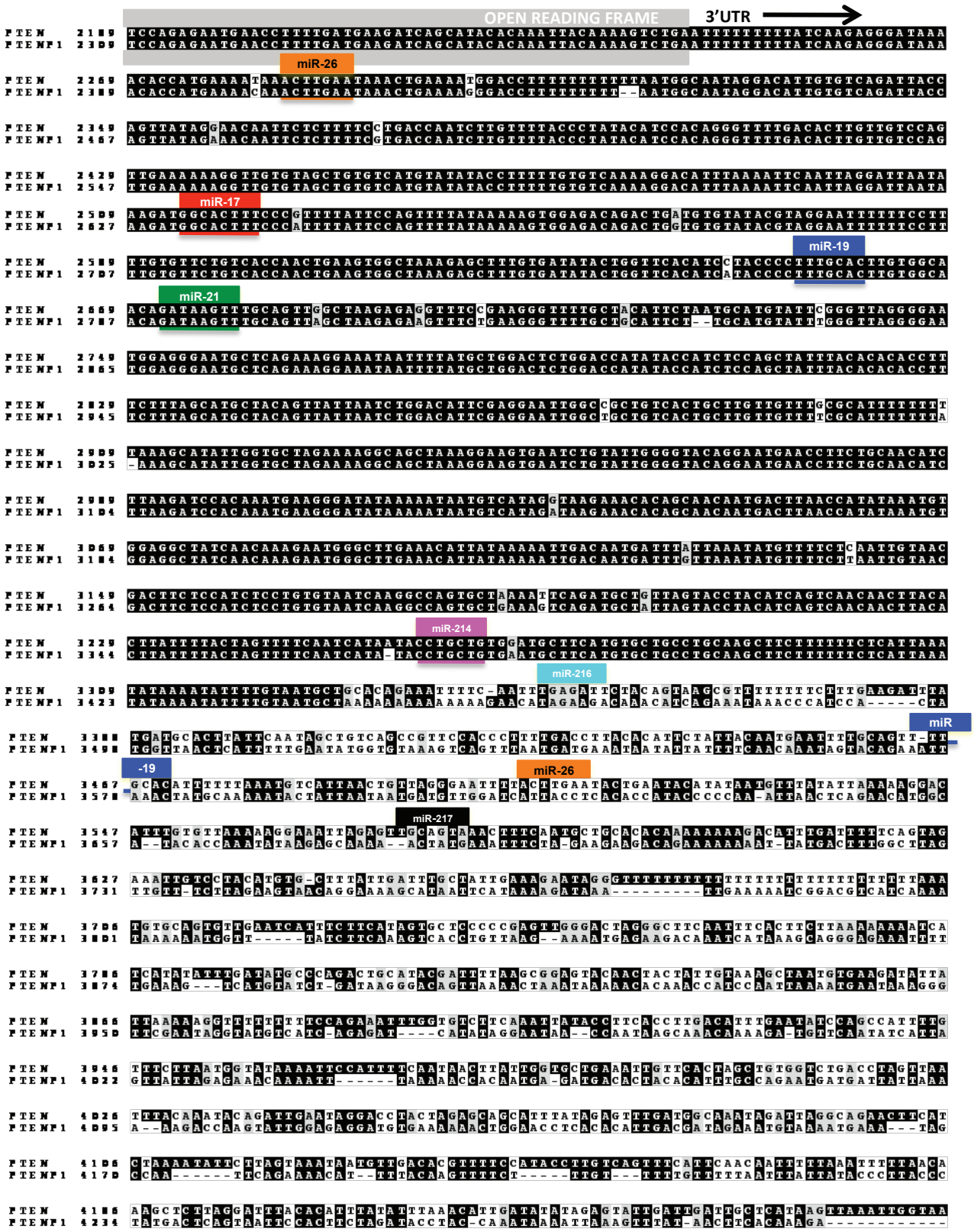
## SUPPLEMENTARY DATA ANALYSIS

### Analysis of *PTENP1* genomic status

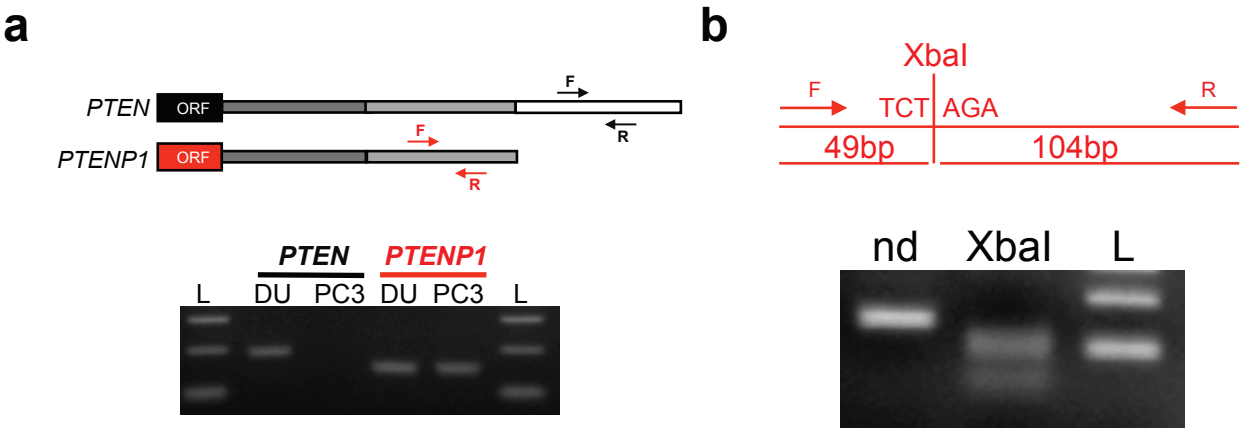
We examined alterations of the *PTENP1* genomic loc*us*. Array-based comparative genomic hybridization (aCGH) databases from *The Cancer Workbench* (https://cgwb.nci.nih.gov/cgi-bin/heatmap) indicated that the *PTENP1* locus undergoes copy number (CN) losses in a subset of tumors. For instance, in the TARGET Acute Lymphoblastic Leukemia (ALL) project (St. Jude/NCI), *PTENP1* is lost in approximately 20% of ALL patient samples **(Supplementary Fig. 9a)**. In these tumors *PTENP1* loss is commonly, but not always part of larger losses of the 9p arm. This observation corroborates previous reports in different tumor types, *PTENP1* has been shown to undergo LOH (detected as loss of the microsatellite marker D9S1878)[1-3]. Importantly, concomitant loss of *CDKN2A* with *PTENP1,* as observed in large losses of 9p*,* may provide an additional advantage over specific loss of only *CDKN2A*, because PTEN expression would be consequently decreased.

Furthermore, we mined various databases available through NCBI GEO (http://www.ncbi.nlm.nih.gov/geo/) for changes in *PTENP1* genomic status. In a study of 118 breast cancer and 44 normal samples, 11/118 (9.3%) demonstrated significant deletion of a region overlapping with *PTENP1* (chr 9:33592959-33692166) and 11 independent breast cancer samples had significant deletion on chromosome 10 region overlapping with *PTEN* (chr10:89499031-89747774; **Table 1**). The magnitude of deletion was similar for *PTEN* and *PTENP1* with 1.48 and 1.4 copies, respectively compared to normal copy number 2. Upon closer analysis of 9p in the 11 cases of *PTENP1* CN losses, it is apparent that *PTENP1* loses can occur independently of *CDKN2A* loss **(Supplementary Fig. 9b)**. However, only 1/11 cases demonstrated a statistically significant loss of the *PTENP1* region only **(Supplementary Fig. 9b, bottom panel)**. These findings indicate that both *PTEN* and *PTENP1* copy number losses occur in breast cancer.

1. Herbst, R.A., et al., *PTEN and MXI1 allelic loss on chromosome 10q is rare in melanoma in vivo.* Arch Dermatol Res, 1999. **291**(10): p. 567-9.
2. Perinchery, G., et al., *High frequency of deletion on chromosome 9p21 may harbor several tumor-suppressor genes in human prostate cancer.* Int J Cancer, 1999. **83**(5): p. 610-4.
3. Marsit, C.J., et al., *Alterations of 9p in squamous cell carcinoma and adenocarcinoma of the lung: association with smoking, TP53, and survival.* Cancer Genet Cytogenet, 2005. **162**(2): p. 115-21.



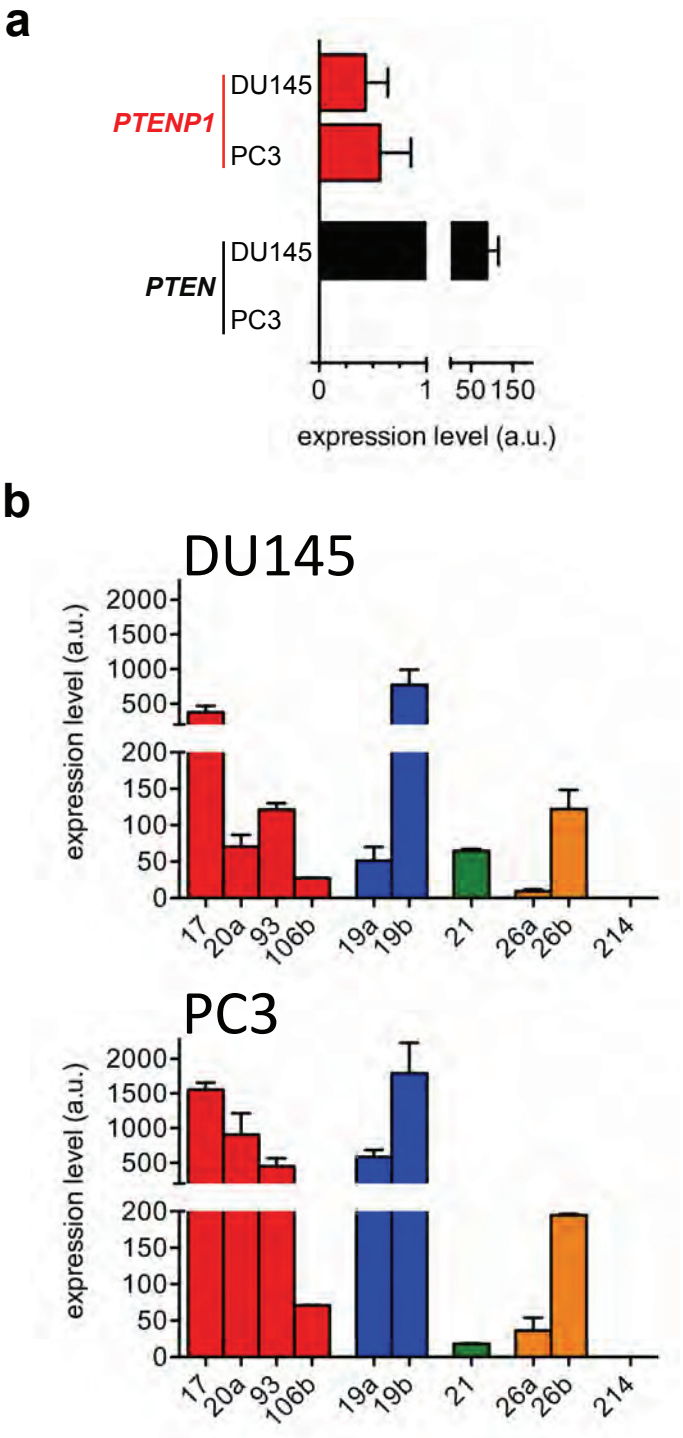

**Supplementary Figure 1. Alignment between *PTEN* and *PTENP1* 3'UTR.** *PTEN* (NM_000314) and *PTENP1* (NM_023917) 3'UTR are shown. Matched nucleotides are in black, unmatched are in white. The seed matches for the different *PTEN*-targeting microRNA families are shown as colored boxes.




**c**

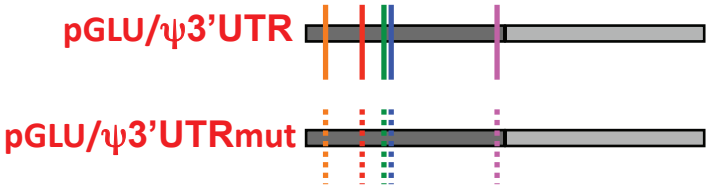
| | |
|---|---|
| ***PTEN* forward** | **CCTTCTCCATCTCCTGTGTAATCAA** |
| ***PTEN* reverse** | **GTTGACTGATGTAGGTACTAACAGCAT** |
| ***PTEN* FAM probe** | **CCAGTGCTAAAATTCA** |
| | |
| ***PTENP1* forward** | **AGTCACCTGTTAAGAAAATGAGAAGACAAA** |
| ***PTENP1* reverse** | **CTGTCCCTTATCAGATACATGACTTTCAA** |
| ***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.

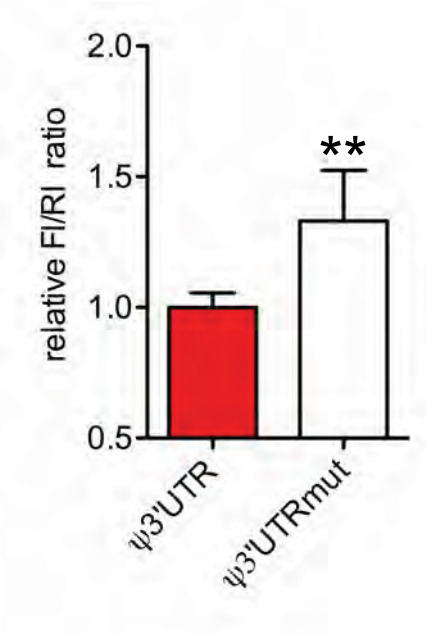
**a**



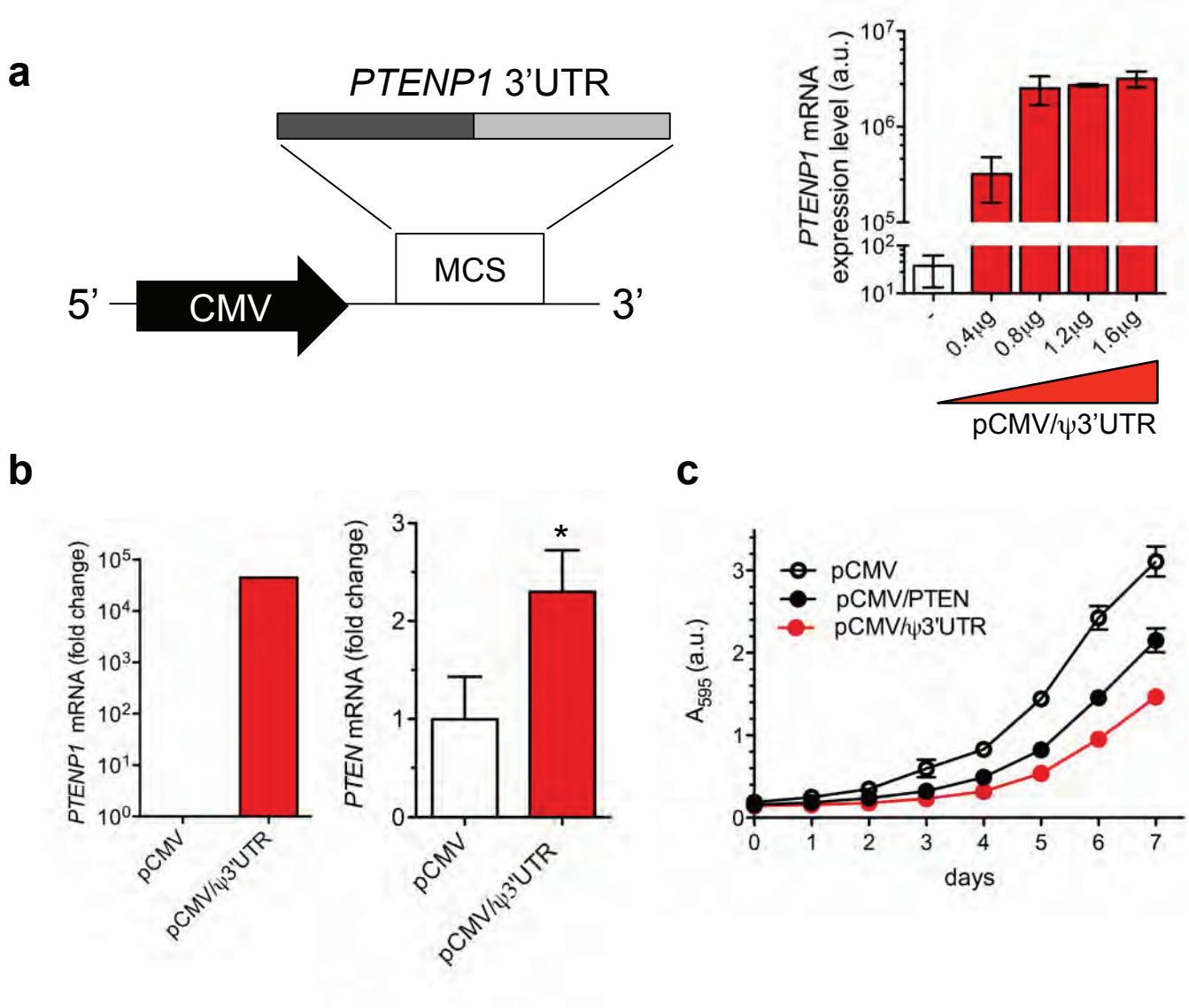

**b**

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

**c**




**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):**

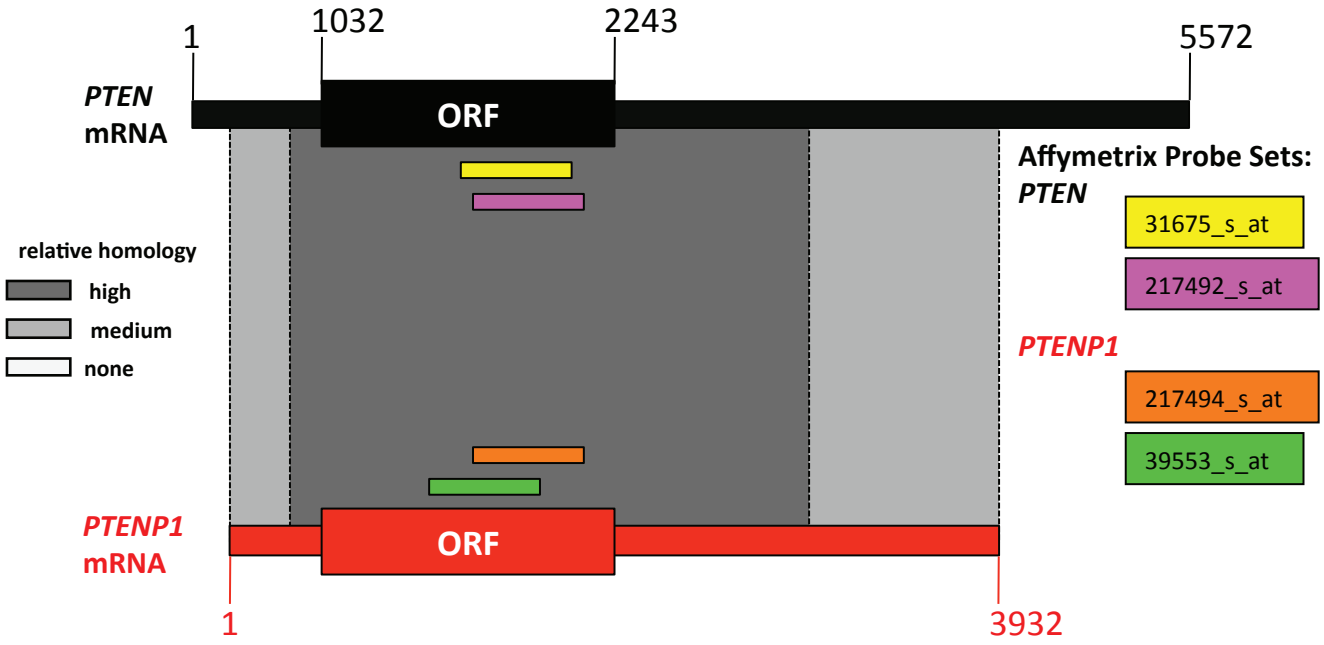
**D-120498-01 TGAATAAAGGGTTCGAATA**
**D-120498-02 GCCAGAATGATGATTATTA**
**D-120498-03 CATCAGAGATCATATAGGA**
**D-120498-04 CCTCACACATTGACGATAG**

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

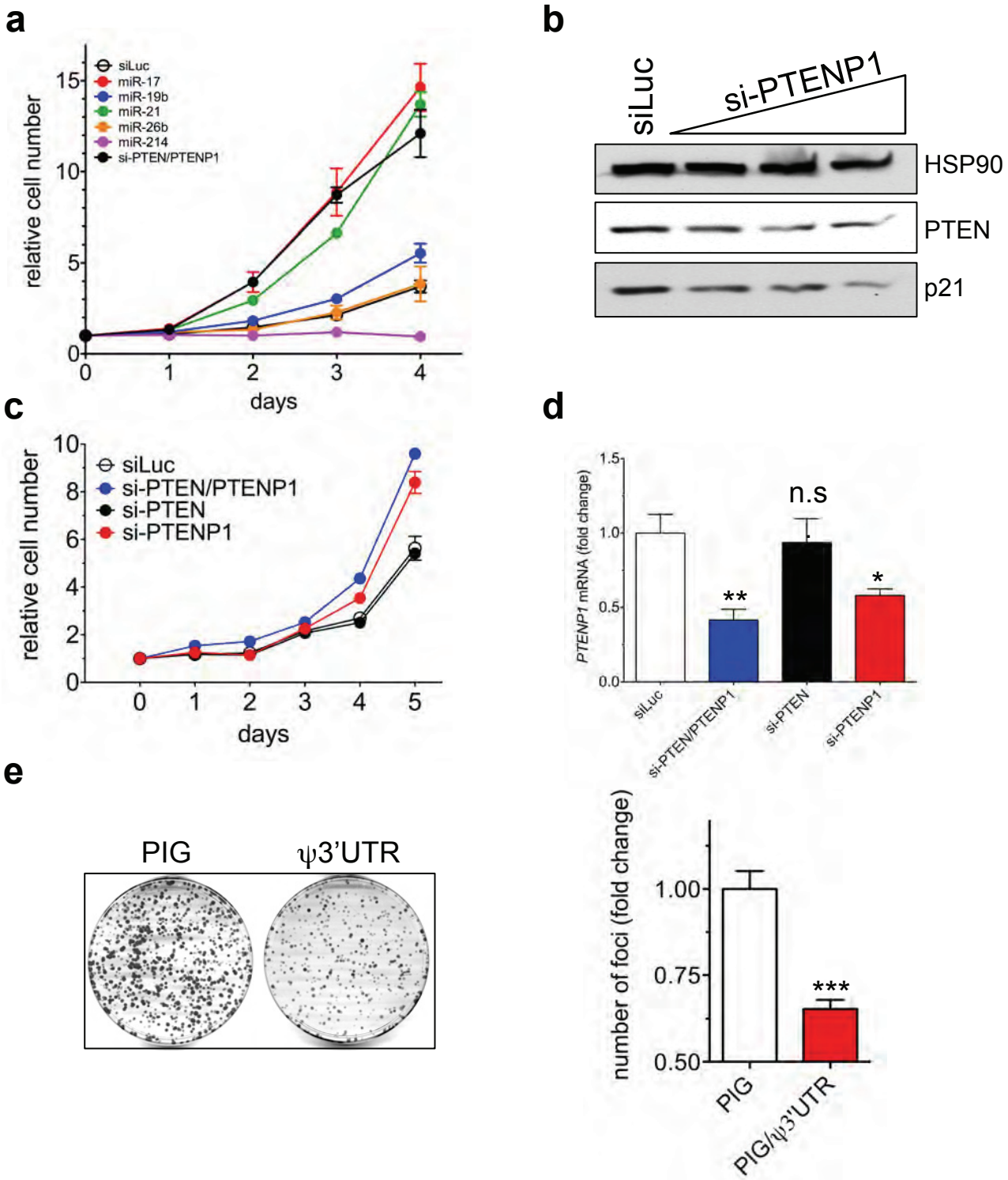
**a**

```
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
```

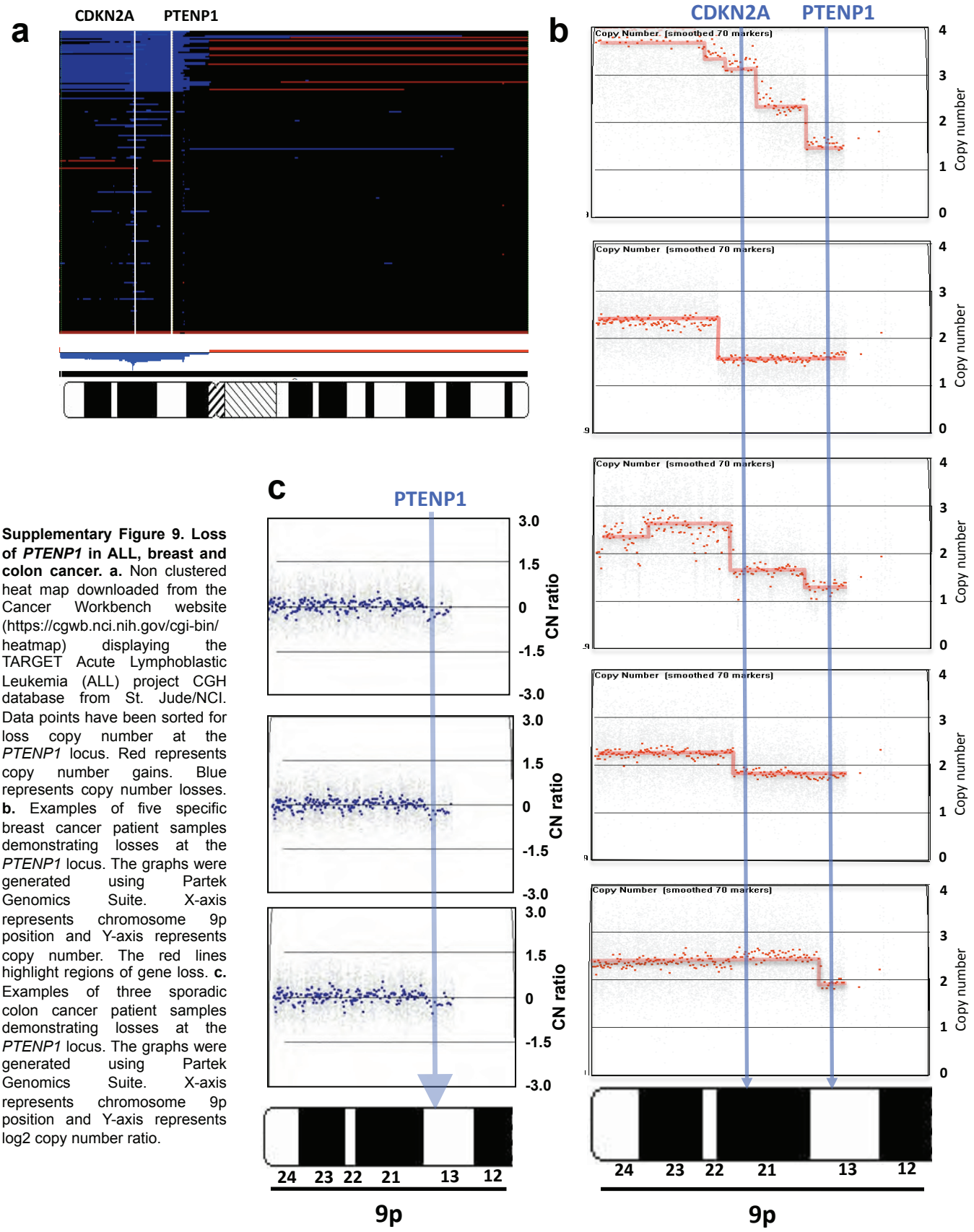
**b**



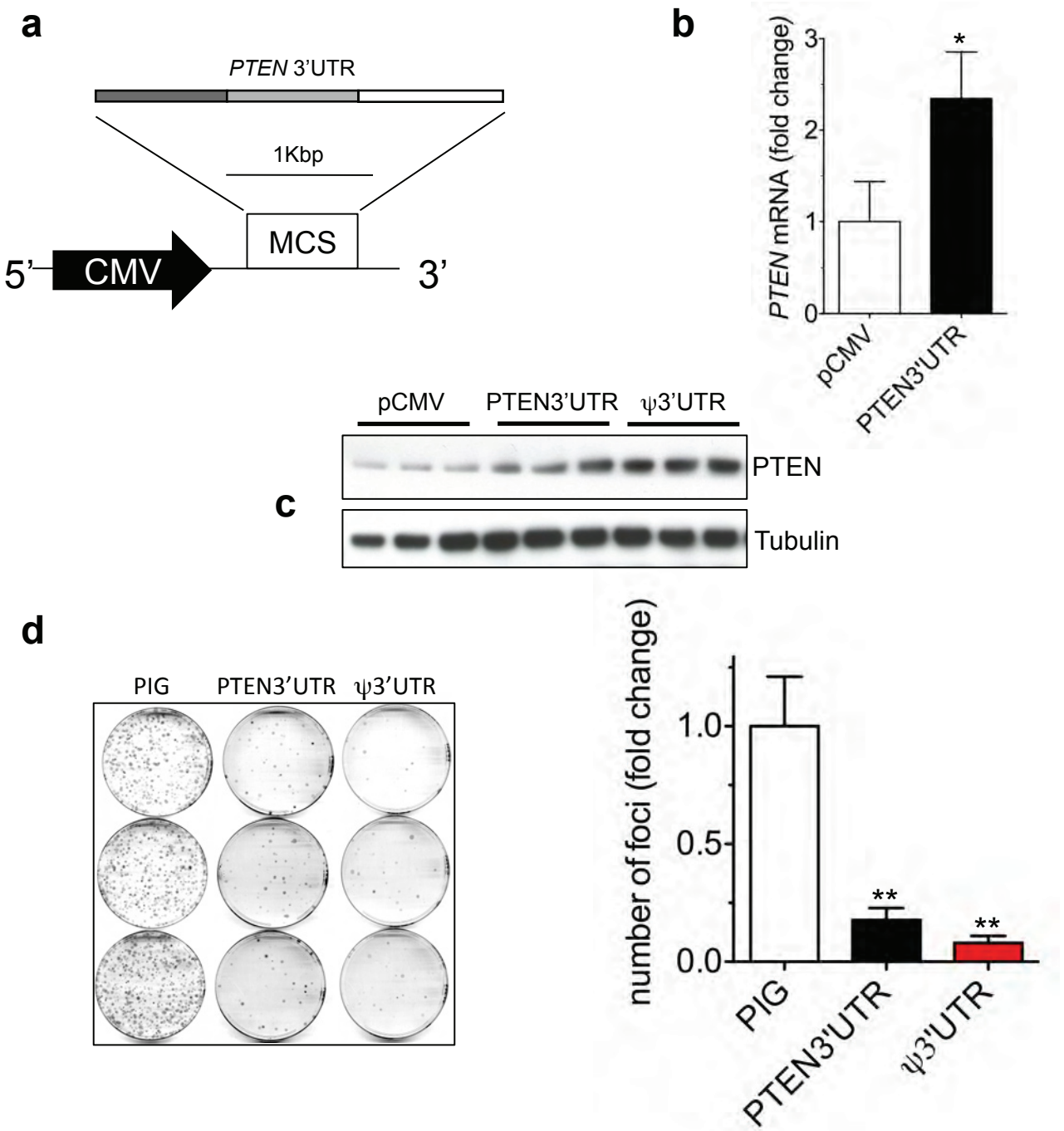

**Supplementary Figure 7. Specificity of commercially available siRNAs and Affymetrix probes for *PTEN* and *PTENP1*. a**. The four siRNAs that comprise the Dharmacon SMARTpool against *PTEN* are all complementary to the open reading frame, therefore they match *PTENP1* as well. Only one mismatch in 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.



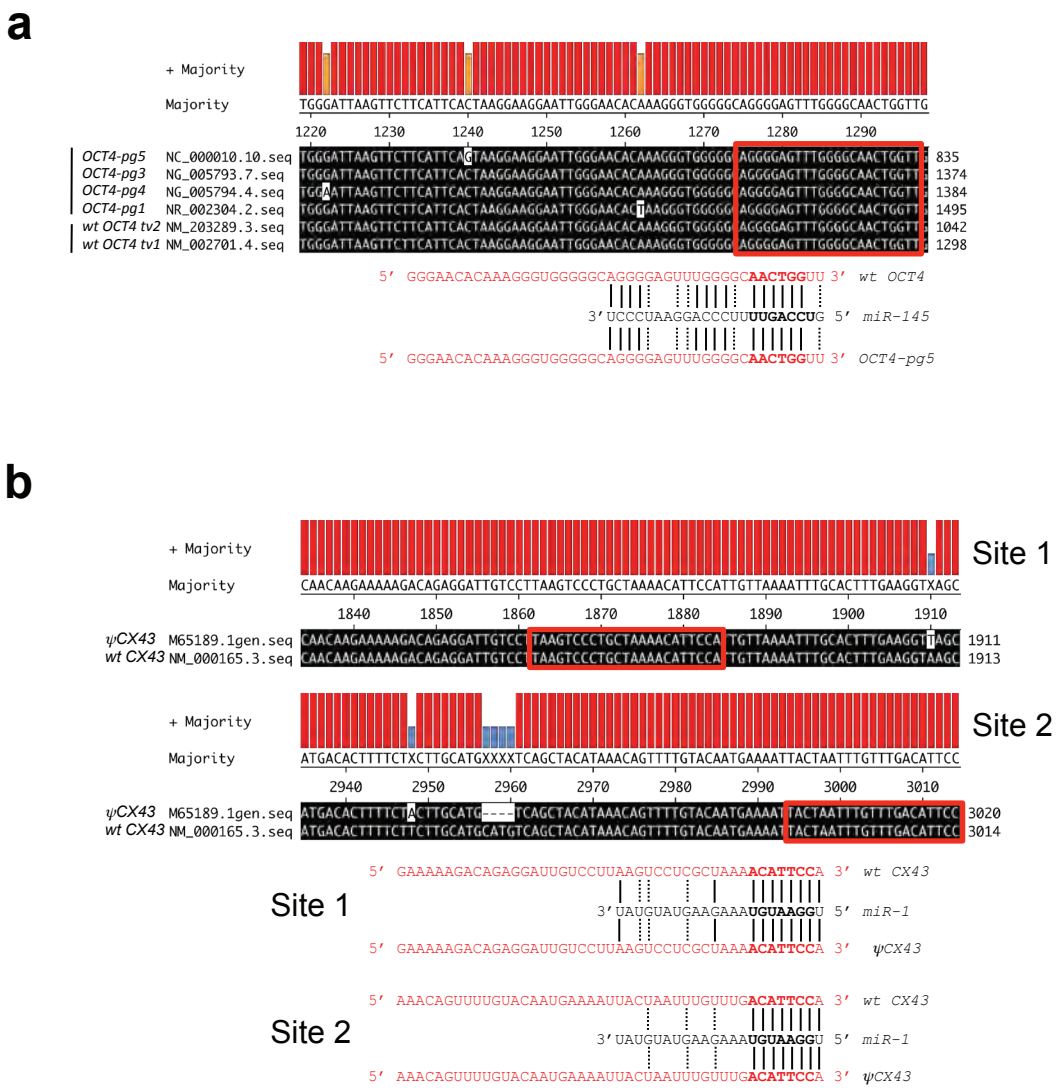

**Supplementary Figure 8. *PTENP1* 3'UTR has PTEN-independent functions. a.** Growth curve of PC3 cells transiently transfected with a representative member of each of the *PTEN*-targeting microRNA families: *miR-17* (red), *miR-19* (blue), *miR-21* (green), *miR-26* (orange) and *miR-214* (pink). si-PTEN/PTENP1 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.



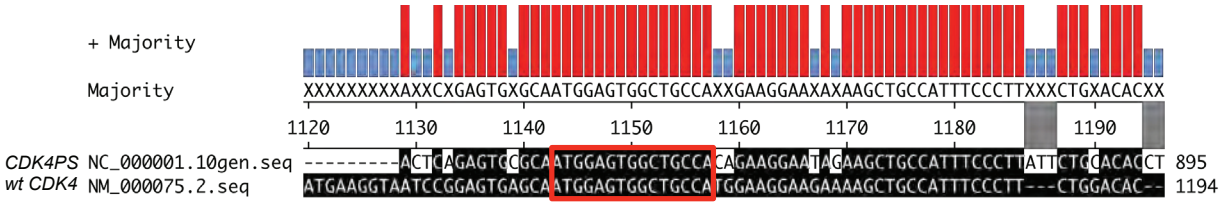

**Supplementary Figure 9. Loss of *PTENP1* in ALL, breast and colon cancer. a.** Non clustered heat map downloaded from the Cancer Workbench website (https://cgwb.nci.nih.gov/cgi-bin/heatmap) displaying the TARGET Acute Lymphoblastic Leukemia (ALL) project CGH database from St. Jude/NCI. Data points 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.



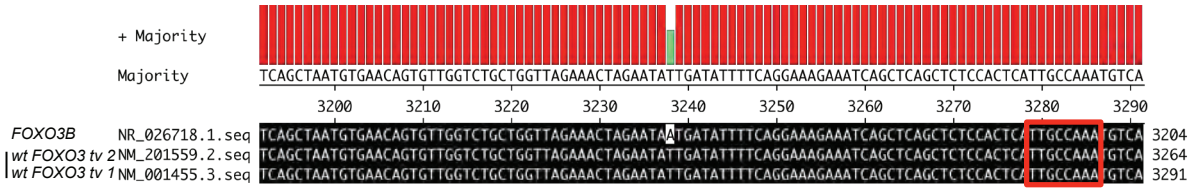

**Supplementary Figure 10. *PTEN* 3'UTR increases *PTENP1* expression level and inhibits cell growth. a.** Characterization of **pCMV/PTEN3'UTR** plasmid. A ~3kb *PTEN* 3'UTR was cloned in the multicloning site (MCS) of **pCMV-MCS** expression plasmid, so that **pCMV/PTEN3'UTR** was obtained. The 5' 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*).

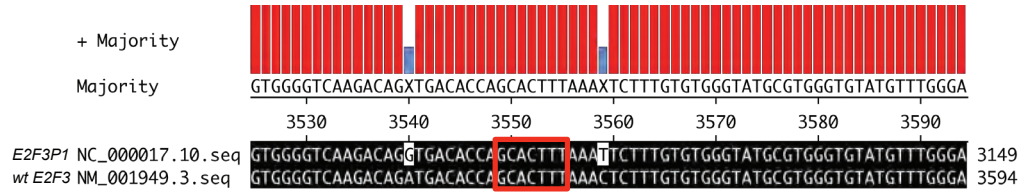



**Supplementary Figure 12. *CDK4* pseudogene *CDK4PS* maintains the validated binding site for *miR-34* family.** The reported *CDK4PS* sequence has been extended in the 3'UTR region by Blast search.

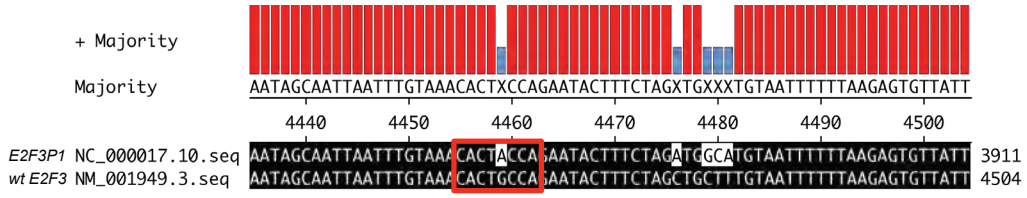



**Supplementary Figure 13. *FOXO3* pseudogene *FOXO3B* maintains the validated binding site for *miR-182*.** Two transcript variants of *FOXO3* (*tv1* and *tv2*) are reported.

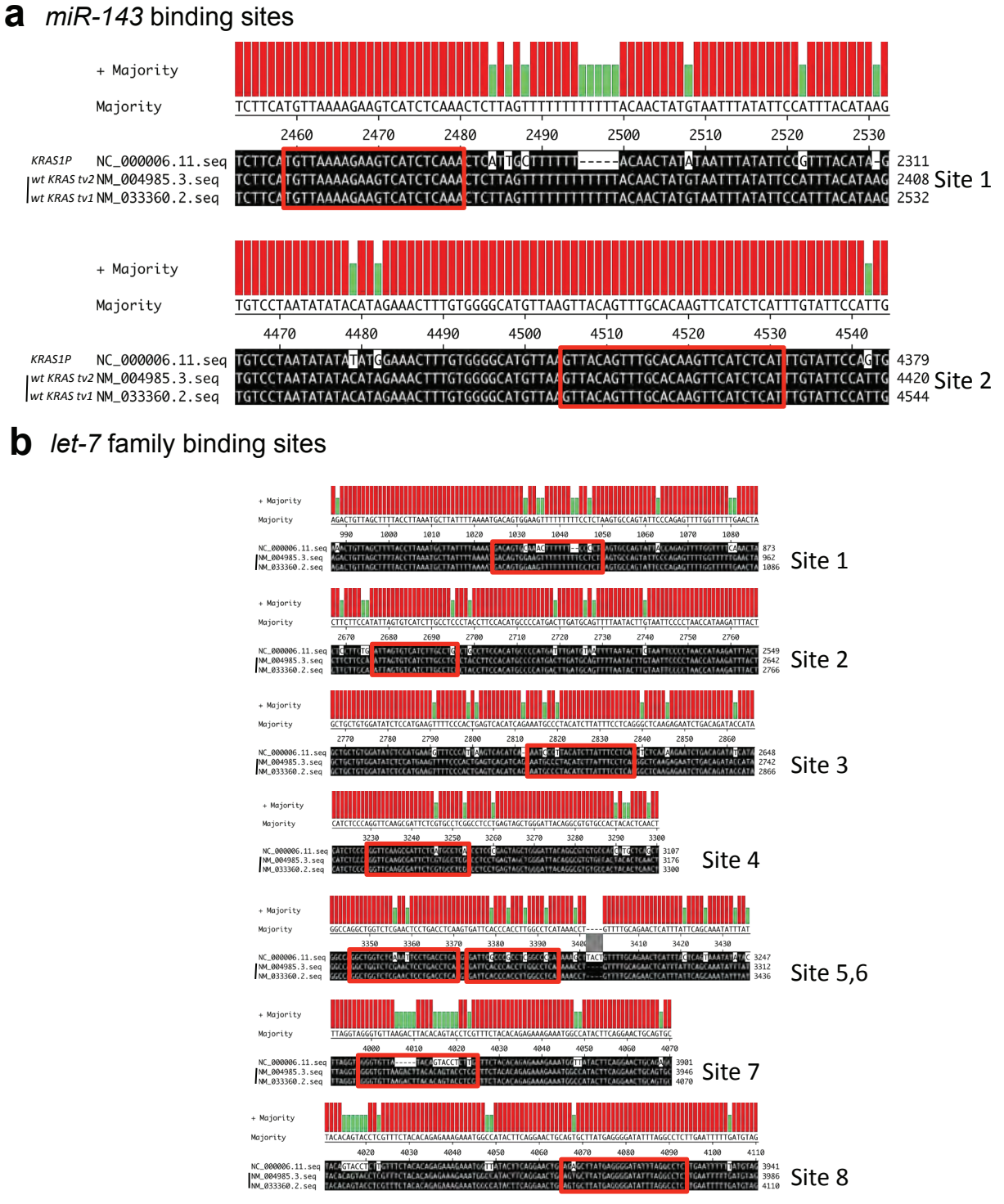
**a** *miR-17* family binding site




**b** *miR-34* family binding site

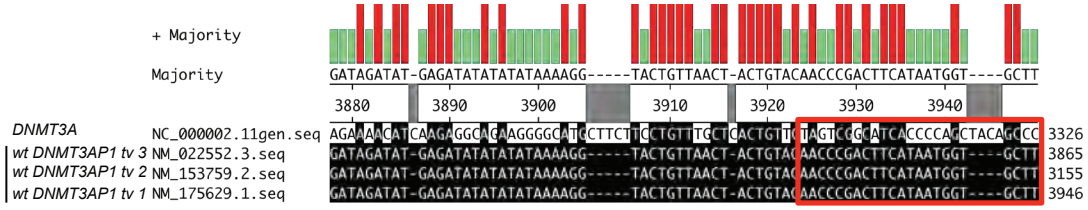



**Supplementary Figure 14. *E2F3* pseudogene *E2F3P1* maintains the validated binding site for *miR-17* family, but not for *miR-34* family.** The binding site for *miR-17* and *miR-34* families are reported in **a** and **b**, respectively.

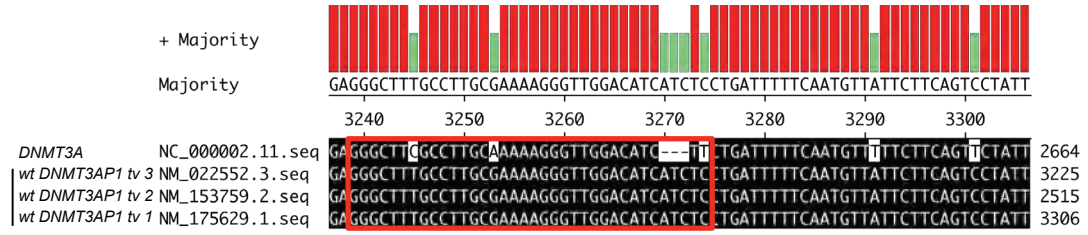



**Supplementary Figure 15. *KRAS* pseudogene *KRAS1P* maintains the validated binding sites for *miR-143* and *let-7* family. a.** The two binding sites for *miR-143* are both conserved in *KRAS1P*. **b.** *let-7* family has 8 binding sites along *KRAS* 3'UTR. All of them show extensive conservation 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.

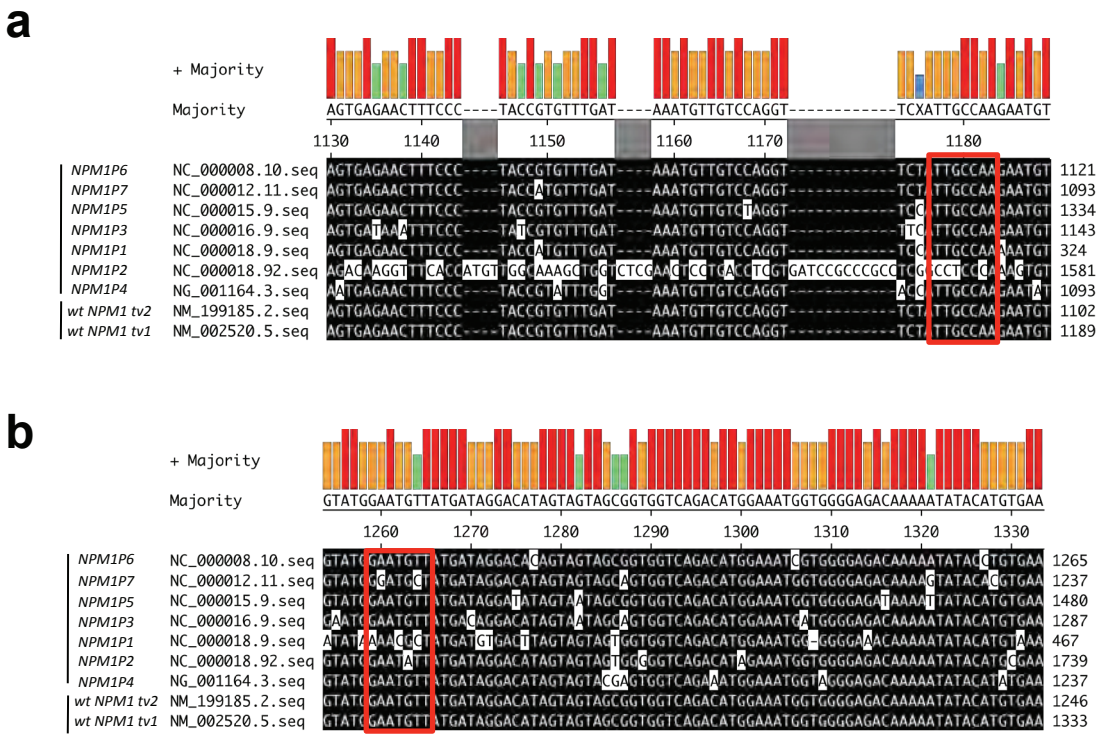
**a** *miR-29* family binding site



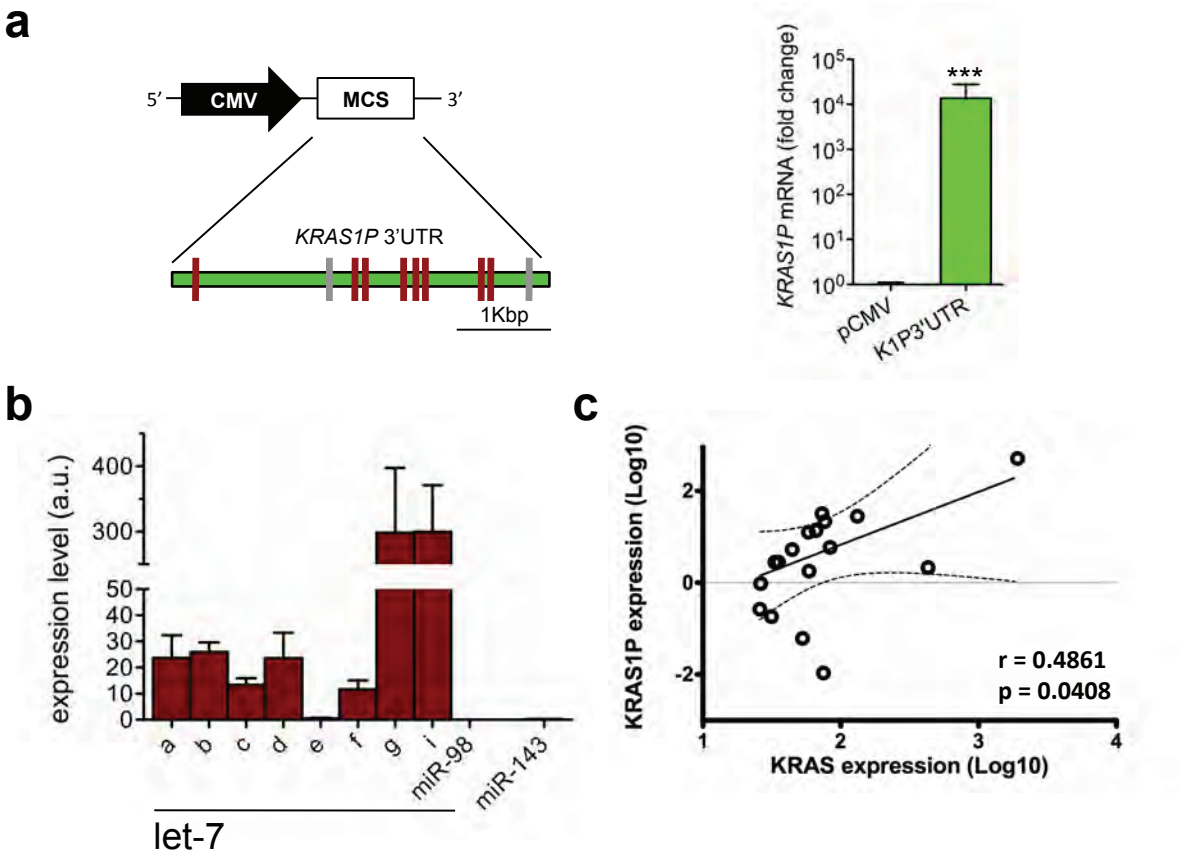

**b** *miR-143* binding site




**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.

**Supplementary Table 1**: Genomic status of *PTEN* and *PTENP1* genomic loci in human breast cancer samples

| chromoso | start | end | length(bp | Sample_Nun | Sample_ID | type_of_alter | Copy Number | |
|---|---|---|---|---|---|---|---|---|
| 9 | 32078636 | 32253053 | 174418 | 5 | GSM417221_BC93.CEL GSM417251_BC123.CEL GSM417254_BC126.CEL GSM417363_BC138.CEL GSM417385_BC160.CEL | Amplification | 2.697738 | |
| 9 | 32127295 | 32276746 | 149452 | 10 | GSM417193_BC65.CEL GSM417196_BC68.CEL GSM417205_BC77.CEL GSM417206_BC78.CEL GSM417220_BC92.CEL GSM417234_BC106.CEL GSM417243_BC115.CEL GSM417249_BC121.CEL GSM417256_BC12 | Deletion | 1.531107 | |
| 9 | 32253053 | 32765029 | 511977 | 5 | GSM417221_BC93.CEL GSM417251_BC123.CEL GSM417254_BC126.CEL GSM417363_BC138.CEL GSM417385_BC160.CEL | Amplification | 2.598098 | |
| 9 | 32276746 | 32429424 | 152679 | 9 | GSM417196_BC68.CEL GSM417205_BC77.CEL GSM417206_BC78.CEL GSM417220_BC92.CEL GSM417234_BC106.CEL GSM417243_BC115.CEL GSM417249_BC121.CEL GSM417256_BC128.CEL GSM417366_BC1 | Deletion | 1.574833 | |
| 9 | 32429424 | 32765029 | 335606 | 9 | GSM417196_BC68.CEL GSM417205_BC77.CEL GSM417206_BC78.CEL GSM417220_BC92.CEL GSM417234_BC106.CEL GSM417243_BC115.CEL GSM417249_BC121.CEL GSM417256_BC128.CEL GSM417366_BC1 | Deletion | 1.537774 | |
| 9 | 32765029 | 33202543 | 437515 | 4 | GSM417251_BC123.CEL GSM417254_BC126.CEL GSM417363_BC138.CEL GSM417385_BC160.CEL | Amplification | 2.66008 | |
| 9 | 32765029 | 32885697 | 120669 | 10 | GSM417196_BC68.CEL GSM417205_BC77.CEL GSM417206_BC78.CEL GSM417220_BC92.CEL GSM417221_BC93.CEL GSM417234_BC106.CEL GSM417243_BC115.CEL GSM417249_BC121.CEL GSM417256_BC12 | Deletion | 1.53958 | |
| 9 | 32885697 | 32900858 | 15162 | 11 | GSM417196_BC68.CEL GSM417205_BC77.CEL GSM417206_BC78.CEL GSM417220_BC92.CEL GSM417221_BC93.CEL GSM417234_BC106.CEL GSM417243_BC115.CEL GSM417249_BC121.CEL GSM417256_BC12 | Deletion | 1.515865 | |
| 9 | 32900858 | 33202543 | 301686 | 10 | GSM417196_BC68.CEL GSM417205_BC77.CEL GSM417206_BC78.CEL GSM417220_BC92.CEL GSM417221_BC93.CEL GSM417234_BC106.CEL GSM417243_BC115.CEL GSM417249_BC121.CEL GSM417256_BC12 | Deletion | 1.53958 | |
| 9 | 33202543 | 33228068 | 25526 | 4 | GSM417251_BC123.CEL GSM417254_BC126.CEL GSM417363_BC138.CEL GSM417385_BC160.CEL | Amplification | 2.947445 | |
| 9 | 33202543 | 33232916 | 30374 | 11 | GSM417196_BC68.CEL GSM417205_BC77.CEL GSM417206_BC78.CEL GSM417220_BC92.CEL GSM417221_BC93.CEL GSM417234_BC106.CEL GSM417243_BC115.CEL GSM417249_BC121.CEL GSM417256_BC12 | Deletion | 1.511565 | |
| 9 | 33228068 | 33905339 | 677272 | 4 | GSM417251_BC123.CEL GSM417254_BC126.CEL GSM417363_BC138.CEL GSM417385_BC160.CEL | Amplification | 2.664718 | |
| 9 | 33232916 | 33303397 | 70482 | 10 | GSM417196_BC68.CEL GSM417205_BC77.CEL GSM417206_BC78.CEL GSM417220_BC92.CEL GSM417221_BC93.CEL GSM417234_BC106.CEL GSM417243_BC115.CEL GSM417249_BC121.CEL GSM417256_BC12 | Deletion | 1.53958 | |
| 9 | 33303397 | 33389254 | 85858 | 10 | GSM417196_BC68.CEL GSM417205_BC77.CEL GSM417206_BC78.CEL GSM417220_BC92.CEL GSM417221_BC93.CEL GSM417234_BC106.CEL GSM417243_BC115.CEL GSM417249_BC121.CEL GSM417256_BC12 | Deletion | 1.51332 | |
| 9 | 33389254 | 33431670 | 42417 | 11 | GSM417196_BC68.CEL GSM417205_BC77.CEL GSM417206_BC78.CEL GSM417220_BC92.CEL GSM417221_BC93.CEL GSM417234_BC106.CEL GSM417243_BC115.CEL GSM417249_BC121.CEL GSM417256_BC12 | Deletion | 1.493437 | |
| 9 | 33431670 | 33466451 | 34782 | 10 | GSM417196_BC68.CEL GSM417205_BC77.CEL GSM417206_BC78.CEL GSM417220_BC92.CEL GSM417221_BC93.CEL GSM417234_BC106.CEL GSM417243_BC115.CEL GSM417249_BC121.CEL GSM417256_BC12 | Deletion | 1.51332 | |
| 9 | 33466451 | 33592959 | 126509 | 10 | GSM417196_BC68.CEL GSM417205_BC77.CEL GSM417206_BC78.CEL GSM417220_BC92.CEL GSM417221_BC93.CEL GSM417234_BC106.CEL GSM417243_BC115.CEL GSM417249_BC121.CEL GSM417256_BC12 | Deletion | 1.549747 | |
| 9 | 33592959 | 33692166 | 99208 | 11 | GSM417196_BC68.CEL GSM417205_BC77.CEL GSM417206_BC78.CEL GSM417220_BC92.CEL GSM417221_BC93.CEL GSM417234_BC106.CEL GSM417243_BC115.CEL GSM417249_BC121.CEL GSM417256_BC12 | Deletion | 1.48509 | PTENP1 |
| 9 | 33692166 | 33795191 | 103026 | 10 | GSM417196_BC68.CEL GSM417205_BC77.CEL GSM417206_BC78.CEL GSM417220_BC92.CEL GSM417221_BC93.CEL GSM417234_BC106.CEL GSM417243_BC115.CEL GSM417249_BC121.CEL GSM417256_BC12 | Deletion | 1.549747 | |
| 9 | 33795191 | 33995061 | 199871 | 10 | GSM417196_BC68.CEL GSM417205_BC77.CEL GSM417206_BC78.CEL GSM417220_BC92.CEL GSM417221_BC93.CEL GSM417234_BC106.CEL GSM417243_BC115.CEL GSM417249_BC121.CEL GSM417256_BC12 | Deletion | 1.517871 | |
| 9 | 33905339 | 34142613 | 237275 | 4 | GSM417251_BC123.CEL GSM417254_BC126.CEL GSM417363_BC138.CEL GSM417385_BC160.CEL | Amplification | 2.89649 | |
| 9 | 33995061 | 34084816 | 89756 | 12 | GSM417196_BC68.CEL GSM417205_BC77.CEL GSM417206_BC78.CEL GSM417210_BC82.CEL GSM417220_BC92.CEL GSM417221_BC93.CEL GSM417234_BC106.CEL GSM417247_BC119.CEL GSM417249_BC12 | Deletion | 1.501715 | |
| 9 | 34084816 | 34295732 | 210917 | 11 | GSM417196_BC68.CEL GSM417205_BC77.CEL GSM417206_BC78.CEL GSM417220_BC92.CEL GSM417221_BC93.CEL GSM417234_BC106.CEL GSM417243_BC115.CEL GSM417247_BC119.CEL GSM417249_BC12 | Deletion | 1.514609 | |
| 9 | 34142613 | 34340254 | 197642 | 4 | GSM417251_BC123.CEL GSM417254_BC126.CEL GSM417363_BC138.CEL GSM417385_BC160.CEL | Amplification | 2.636783 | |
| 9 | 34295732 | 34399591 | 103860 | 10 | GSM417196_BC68.CEL GSM417205_BC77.CEL GSM417206_BC78.CEL GSM417220_BC92.CEL GSM417221_BC93.CEL GSM417234_BC106.CEL GSM417243_BC115.CEL GSM417249_BC121.CEL GSM417256_BC12 | Deletion | 1.517871 | |
| 9 | 34340254 | 34924481 | 584228 | 3 | GSM417251_BC123.CEL GSM417254_BC126.CEL GSM417385_BC160.CEL | Amplification | 2.582387 | |
| 9 | 34399591 | 34990034 | 590444 | 10 | GSM417196_BC68.CEL GSM417205_BC77.CEL GSM417206_BC78.CEL GSM417220_BC92.CEL GSM417221_BC93.CEL GSM417234_BC106.CEL GSM417243_BC115.CEL GSM417249_BC121.CEL GSM417256_BC12 | Deletion | 1.541888 | |
| 9 | 34924481 | 34990034 | 65554 | 4 | GSM417203_BC75.CEL GSM417251_BC123.CEL GSM417254_BC126.CEL GSM417385_BC160.CEL | Amplification | 2.647237 | |
| 9 | 34990034 | 35174234 | 184201 | 3 | GSM417251_BC123.CEL GSM417254_BC126.CEL GSM417385_BC160.CEL | Amplification | 2.582387 | |
| 9 | 34990034 | 36060199 | 1070166 | 11 | GSM417196_BC68.CEL GSM417203_BC75.CEL GSM417205_BC77.CEL GSM417206_BC78.CEL GSM417220_BC92.CEL GSM417221_BC93.CEL GSM417234_BC106.CEL GSM417243_BC115.CEL GSM417249_BC121 | Deletion | 1.54138 | |
| 9 | 35174234 | 35885245 | 711012 | 2 | GSM417251_BC123.CEL GSM417385_BC160.CEL | Amplification | 2.63522 | |
| 9 | 35885245 | 35952590 | 67346 | 3 | GSM417204_BC76.CEL GSM417251_BC123.CEL GSM417385_BC160.CEL | Amplification | 2.868233 | |
| 9 | 35952590 | 36109866 | 157277 | 2 | GSM417251_BC123.CEL GSM417385_BC160.CEL | Amplification | 2.63522 | |
| 9 | 36060199 | 36078512 | 18314 | 12 | GSM417196_BC68.CEL GSM417203_BC75.CEL GSM417205_BC77.CEL GSM417206_BC78.CEL GSM417220_BC92.CEL GSM417221_BC93.CEL GSM417234_BC106.CEL GSM417243_BC115.CEL GSM417249_BC121 | Deletion | 1.540713 | |
| 9 | 36078512 | 36123410 | 44899 | 12 | GSM417196_BC68.CEL GSM417203_BC75.CEL GSM417205_BC77.CEL GSM417206_BC78.CEL GSM417220_BC92.CEL GSM417221_BC93.CEL GSM417234_BC106.CEL GSM417243_BC115.CEL GSM417249_BC121 | Deletion | 1.522727 | |
| 9 | 36109866 | 36127416 | 17551 | 3 | GSM417251_BC123.CEL GSM417385_BC160.CEL NA10831_Op1_011206_VnV_B06_r1.CEL | Amplification | 2.708793 | |
| | | | | | | | | |
| 10 | 88124601 | 88180506 | 55906 | 10 | GSM417185_BC57.CEL GSM417199_BC71.CEL GSM417203_BC75.CEL GSM417204_BC76.CEL GSM417206_BC78.CEL GSM417221_BC93.CEL GSM417247_BC119.CEL GSM417252_BC124.CEL GSM417359_BC134 | Deletion | 1.513198 | |
| 10 | 88180506 | 88183213 | 2708 | 10 | GSM417185_BC57.CEL GSM417199_BC71.CEL GSM417203_BC75.CEL GSM417204_BC76.CEL GSM417206_BC78.CEL GSM417221_BC93.CEL GSM417247_BC119.CEL GSM417252_BC124.CEL GSM417359_BC134 | Deletion | 1.483394 | |
| 10 | 88183213 | 88902159 | 718947 | 11 | GSM417185_BC57.CEL GSM417199_BC71.CEL GSM417201_BC73.CEL GSM417203_BC75.CEL GSM417204_BC76.CEL GSM417206_BC78.CEL GSM417221_BC93.CEL GSM417247_BC119.CEL GSM417252_BC124.( | Deletion | 1.490272 | |
| 10 | 88902159 | 88946942 | 44784 | 10 | GSM417185_BC57.CEL GSM417199_BC71.CEL GSM417201_BC73.CEL GSM417203_BC75.CEL GSM417204_BC76.CEL GSM417206_BC78.CEL GSM417221_BC93.CEL GSM417252_BC124.CEL GSM417359_BC134.( | Deletion | 1.476881 | |
| 10 | 88946942 | 89123572 | 176631 | 10 | GSM417185_BC57.CEL GSM417199_BC71.CEL GSM417201_BC73.CEL GSM417203_BC75.CEL GSM417204_BC76.CEL GSM417206_BC78.CEL GSM417221_BC93.CEL GSM417252_BC124.CEL GSM417359_BC134.( | Deletion | 1.508145 | |
| 10 | 89123572 | 89175140 | 51569 | 10 | GSM417185_BC57.CEL GSM417199_BC71.CEL GSM417201_BC73.CEL GSM417203_BC75.CEL GSM417204_BC76.CEL GSM417206_BC78.CEL GSM417221_BC93.CEL GSM417252_BC124.CEL GSM417359_BC134.( | Deletion | 1.46746 | |
| 10 | 89175140 | 89259656 | 84517 | 10 | GSM417185_BC57.CEL GSM417199_BC71.CEL GSM417201_BC73.CEL GSM417203_BC75.CEL GSM417204_BC76.CEL GSM417206_BC78.CEL GSM417221_BC93.CEL GSM417252_BC124.CEL GSM417359_BC134.( | Deletion | 1.511312 | |
| 10 | 89259656 | 89267197 | 7542 | 10 | GSM417185_BC57.CEL GSM417199_BC71.CEL GSM417201_BC73.CEL GSM417203_BC75.CEL GSM417204_BC76.CEL GSM417206_BC78.CEL GSM417221_BC93.CEL GSM417252_BC124.CEL GSM417359_BC134.( | Deletion | 1.471873 | |
| 10 | 89267197 | 89309487 | 42291 | 11 | GSM417185_BC57.CEL GSM417199_BC71.CEL GSM417201_BC73.CEL GSM417203_BC75.CEL GSM417204_BC76.CEL GSM417206_BC78.CEL GSM417207_BC79.CEL GSM417221_BC93.CEL GSM417252_BC124.C | Deletion | 1.437335 | |
| 10 | 89309487 | 89499031 | 189545 | 11 | GSM417185_BC57.CEL GSM417199_BC71.CEL GSM417201_BC73.CEL GSM417203_BC75.CEL GSM417204_BC76.CEL GSM417206_BC78.CEL GSM417207_BC79.CEL GSM417221_BC93.CEL GSM417252_BC124.C | Deletion | 1.472769 | |
| 10 | 89499031 | 89637504 | 138474 | 11 | GSM417185_BC57.CEL GSM417199_BC71.CEL GSM417201_BC73.CEL GSM417203_BC75.CEL GSM417204_BC76.CEL GSM417206_BC78.CEL GSM417207_BC79.CEL GSM417221_BC93.CEL GSM417252_BC124.C | Deletion | 1.407877 | PTEN |
| 10 | 89637504 | 89642275 | 4772 | 11 | GSM417185_BC57.CEL GSM417199_BC71.CEL GSM417201_BC73.CEL GSM417203_BC75.CEL GSM417204_BC76.CEL GSM417206_BC78.CEL GSM417207_BC79.CEL GSM417221_BC93.CEL GSM417252_BC124.C | Deletion | 1.342655 | PTEN |
| 10 | 89642275 | 89686146 | 43872 | 12 | GSM417185_BC57.CEL GSM417199_BC71.CEL GSM417201_BC73.CEL GSM417203_BC75.CEL GSM417204_BC76.CEL GSM417206_BC78.CEL GSM417207_BC79.CEL GSM417220_BC92.CEL GSM417221_BC93.CE | Deletion | 1.290048 | PTEN |
| 10 | 89686146 | 89689789 | 3644 | 11 | GSM417185_BC57.CEL GSM417199_BC71.CEL GSM417201_BC73.CEL GSM417203_BC75.CEL GSM417204_BC76.CEL GSM417206_BC78.CEL GSM417207_BC79.CEL GSM417221_BC93.CEL GSM417252_BC124.C | Deletion | 1.342655 | PTEN |
| 10 | 89689789 | 89702343 | 12555 | 11 | GSM417185_BC57.CEL GSM417199_BC71.CEL GSM417201_BC73.CEL GSM417203_BC75.CEL GSM417204_BC76.CEL GSM417206_BC78.CEL GSM417207_BC79.CEL GSM417221_BC93.CEL GSM417252_BC124.C | Deletion | 1.410367 | PTEN |
| 10 | 89702343 | 89747774 | 45432 | 12 | GSM417185_BC57.CEL GSM417193_BC65.CEL GSM417199_BC71.CEL GSM417201_BC73.CEL GSM417203_BC75.CEL GSM417204_BC76.CEL GSM417206_BC78.CEL GSM417207_BC79.CEL GSM417221_BC93.CE | Deletion | 1.432106 | PTEN |
| 10 | 89747774 | 89763256 | 15483 | 13 | GSM417185_BC57.CEL GSM417189_BC61.CEL GSM417193_BC65.CEL GSM417199_BC71.CEL GSM417201_BC73.CEL GSM417203_BC75.CEL GSM417204_BC76.CEL GSM417206_BC78.CEL GSM417207_BC79.CE | Deletion | 1.446913 | |
| 10 | 89763256 | 90676196 | 912941 | 13 | GSM417185_BC57.CEL GSM417189_BC61.CEL GSM417193_BC65.CEL GSM417199_BC71.CEL GSM417201_BC73.CEL GSM417203_BC75.CEL GSM417204_BC76.CEL GSM417206_BC78.CEL GSM417207_BC79.CE | Deletion | 1.414049 | |
| 10 | 90676196 | 91009592 | 333397 | 12 | GSM417185_BC57.CEL GSM417189_BC61.CEL GSM417193_BC65.CEL GSM417199_BC71.CEL GSM417203_BC75.CEL GSM417204_BC76.CEL GSM417206_BC78.CEL GSM417207_BC79.CEL GSM417221_BC93.CE | Deletion | 1.437568 | |
| 10 | 91009592 | 91159152 | 149561 | 11 | GSM417185_BC57.CEL GSM417189_BC61.CEL GSM417193_BC65.CEL GSM417199_BC71.CEL GSM417203_BC75.CEL GSM417204_BC76.CEL GSM417206_BC78.CEL GSM417221_BC93.CEL GSM417252_BC124.C | Deletion | 1.468987 | |
| 10 | 91159152 | 91239000 | 79849 | 11 | GSM417185_BC57.CEL GSM417189_BC61.CEL GSM417193_BC65.CEL GSM417199_BC71.CEL GSM417203_BC75.CEL GSM417204_BC76.CEL GSM417206_BC78.CEL GSM417221_BC93.CEL GSM417252_BC124.C | Deletion | 1.529715 | |
| 10 | 91239000 | 91646139 | 407140 | 12 | GSM417185_BC57.CEL GSM417189_BC61.CEL GSM417193_BC65.CEL GSM417199_BC71.CEL GSM417203_BC75.CEL GSM417204_BC76.CEL GSM417206_BC78.CEL GSM417221_BC93.CEL GSM417249_BC121.C | Deletion | 1.536131 | |
| 10 | 91418007 | 91485537 | 67531 | 2 | GSM417253_BC125.CEL NA07357_Op1_011206_VnV_A07_r1.CEL | Amplification | 2.68051 | |
| 10 | 91646139 | 92214056 | 567918 | 11 | GSM417185_BC57.CEL GSM417193_BC65.CEL GSM417199_BC71.CEL GSM417203_BC75.CEL GSM417204_BC76.CEL GSM417206_BC78.CEL GSM417221_BC93.CEL GSM417249_BC121.CEL GSM417252_BC124.( | Deletion | 1.528092 | |

To gain a more detailed understanding of the specific losses at the *PTENP1* locus, Affymetrix GeneChip® Human Mapping 500K Array datasets GSE7545 and GSE16619 were downloaded from NCBI GEO and analyzed with the Partek Genomic Suite (Partek Inc) for detection of genomic regions with alterations and data visualization. 118 breast cancer and 44 normal samples were included in the study. In 11/118 (9.3%) of breast cancer samples significant deletion on region chr9:33592959-33692166 (overlapping with *PTENP1*) was found. In 11 breast cancer samples significant deletion on chromosome 10 region overlapping with *PTEN* (chr10:89499031-89747774) was also found. The magnitude of deletion is similar, with 1.48 copy for *PTENP1* and 1.4 copy for *PTEN* with respect to normal copy number as 2. No amplification was detected in these regions. Rows highlighted in green represent the genomic loci of *PTENP1* and *PTEN*.

**Table 2. Pseudogenes aberrantly expressed in human cancer and validated miRNAs binding to their cognate *wt* genes.**

| Pseudogene | Role in human cancer | *wt* gene | Validated miRNA families | Conservation of the binding site between *wt* and pseudo |
|---|---|---|---|---|
| *PTENP1* | *see text* | *PTEN*: *see text* | ***miR-17*** | **yes** |
| | | | ***miR-19*** | **yes** |
| | | | ***miR-21*** | **yes** |
| | | | ***miR-26*** | **yes** |
| | | | ***miR-214*** | **yes** |
| | | | *miR-216* | no |
| | | | *miR-217* | no |
| *ψCX43* | specifically expressed in breast cell lines (not in normal mammary epithelium)[1] | *CONNEXIN 43 (CX43)*: one of the monomers that compose gap junctions. CX43 expression is aberrantly lost in cancer. | ***miR-1***[2] | **yes** |
| *NA88-A* | specifically expressed in melanoma cell lines (not in normal melanocytes)[3] | *HPX42B* | - | |
| *OCT4-pg1* *OCT4-pg5* | specifically expressed in cancer cell lines and tissues (not in normal tissues)[4] | *OCT4*: transcription factor expressed in embryonic stem cells where it plays a critical role in mantaining the pluripotent and self-renewing state. Oct4 is aberrantly expressed in cancer cells. | ***miR-145***[5] | **yes** |
| | | | *miR-470*[6] | *miR-470* is mouse-spec |
| *NANOGP8* | specifically expressed in cancer cell lines and tissues (not in normal fibroblasts and fetal liver)[7] | *NANOG*: transcription factor expressed in embryonic stem cells where it plays a critical role in mantaining the pluripotent and self-renewing state. Oct4 is aberrantly expressed in cancer cells | *miR-134*[8] | no* |
| | | | *miR-296*[6] | *miR-296* binding sites are not conserved between human and mouse |
| *ψBRAF* | specifically expressed in thyroid tumor samples (especially if they don't carry BRAF mutations), and not in normal thyroid[9] | BRAF: Ser/Thr kinase that serves as downstream effector of RAS in the MAPK signaling cascade. Mutations that render BRAF constitutively active are common in cancer. | - | - |

The conservation of *miR-17*, *19*, *21*, *26* and *214* binding sites in *PTENP1* has been discussed elsewhere **(Fig. 1)**. The asterisk indicates those *wt*/pseudogene pairs that show an overall low sequence conservation (<60%).

**References 1-9**


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**Table 3. Conservation of validated miRNA binding sites in cancer-related target genes.**

| *wt* genes | corresponding pseudogene(s) | validated miRNA families | conservation of the binding site between *wt* and pseudo |
|---|---|---|---|
| *CCND3* | *CCND3P* | *miR-16* [1] | no* |
| *CDK4* | *CDK4PS* | *miR-34* [2] | yes |
| *DNMT3A* | *DNMT3AP1* | *miR-29* [3] | no |
| | | *miR-143* [4] | no |
| *E2F3* | *E2F3P1* | *miR-17* [5] | yes |
| | | *miR-34* [6] | no |
| *c-MYC* | *MYCL3* | *let-7* [7] | no* |
| | | *miR-145* [8] | no* |
| *OCT4* | *OCT4-pg1,2,3,4,5,6* | *miR-145* [9] | yes |
| *KRAS* | *KRAS1P* | *let-7* [10] | yes |
| | | *miR-143* [11] | yes |
| | | | |
| *PTEN* | *PTENP1* | *miR-17* [12] | yes |
| | | *miR-19* [13,14] | yes |
| | | *miR-21* [15] | yes |
| | | *miR-26* [16] | yes |
| | | *miR-214* [17] | yes |
| | | *miR-216* [18] | no |
| | | *miR-217* [18] | no |
| *FOXO3* | *FOXO3B* | *miR-182* [19] | yes |

A list of miRNA families with a well recognized oncogenic or oncosuppressor role was obtained merging the most recent reviews about microRNAs and cancer [20-24].
The validated targets of these miRNAs that have at least 1 pseudogene (http://www.genecards.org) are listed above. The conservation of the binding sites of the validated miRNAs in the pseudogene(s) is also reported. The asterisk indicates those wt/pseudogene pairs that show an overall low sequence conservation (<60%).
The conservation of *miR-17*, *19*, *21*, *26* and *214* binding sites in *PTENP1* has been discussed elsewhere **(Fig. 1)**. Analogously, the conservation of *miR-145* binding sites in *OCT4* pseudogenes has been described in **Table 2**.

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