## Supplementary Figure legends

**Figure S1.** *Schematic diagram of the genomic locus of miR-128 (Homo sapiens-miR-128)*

The pre-miR-128a (i.e., miR-128-1) gene is embedded in the intron 18 of the R3HDM1 gene on chromosome 2q21.3. The pre-miR-128b (i.e., miR-128-2) gene is embedded in the intron 17 of the ARPP21 gene (also called R3HDM3) on chromosome 3p22.3. The pre-miR-128a and pre-miR-128b genes encode the same mature miR-128 (sequence shown at the bottom).

**Figure S2.** *miR-128 mRNA was down-regulated in human PCa cell lines*.

qRT-PCR analysis of miR-128 expression levels in eight human PCa lines and one immortalized normal human prostatic (NHP) epithelial cell line NHP9. The expression levels in each cell line were normalized to RNU48 and data (log10 scale) presented as relative to the miR-128 level in NHP9 cells.

**Figure S3.** *Validation of transfection efficiencies and tumor suppression by miR-128*

**A**. miR-128 levels in PC3, PPC-1, Du145 and LNCaP cells transfected with miR-NC or miR-128 mimics (30 nM) were analyzed at 48 h by TaqMan qRT-PCR. Shown are the miR-128 levels (mean ± SEM) relative to miR-NC transfected cells. *P<0.05; **P<0.01, ***P<0.001.

**B**. Independent tumor regeneration assays in Du145 cells. Cells were transfected with 30 nM of NC or miR-128 mimics and 48 h later, injected (at 100,000 or 10,000 cells) subcutaneously (s.c) into NOD/SCID mice. The endpoint tumor images were shown on the left and tumor

incidence, harvest time, tumor weight (mean ± S.D) and the P values (for tumor weight comparisons; Student *t*-test) were indicated on the right.

**Figure S4.** *Characterizations of the miR-128 sensor*

**A**. Schematic of miR-128 sensor construct bearing four copies of sequences, at the 3'-UTR of GFP, complementary to the miR-128 seed sequence.

**B**. Quantification of $GFP^+$ % in PC3 and Du145 cells 48 h after transfection with the miR-128 sensor construct or the parent control pEGFP-N1.

**C**. Exogenously introduced miR-128 mimic, dose-dependently, reduces % $GFP^+$ cells. PC3 and Du145 cells were co-transfected with pEGFP-N1 or miR-128-sensor vectors and miR-128 mimic at different concentrations (1, 10, and 30 nM) and images taken 72 h after transfections. See Fig. 4A for representative images of cells transfected with the vectors without the miR-128 mimic. Original magnifications, x 400.

**Figure S5.** *miR-128 'decoy' promoted clonal expansion and sphere-forming capacity in PC3 and Du145 cells*.

**A-B**. Clonal assays in PC3 (A) and Du145 (B) cells transfected with pEGFP N1 or miR-128 sensor and plated in 6-well plates (100 cells per well) on Swiss 3T3 fibroblast feeder layer. Clones were counted 14 days after plating. Presented are the mean ± S.D from triplicate wells. *P<0.05 when compared with the corresponding NC controls.

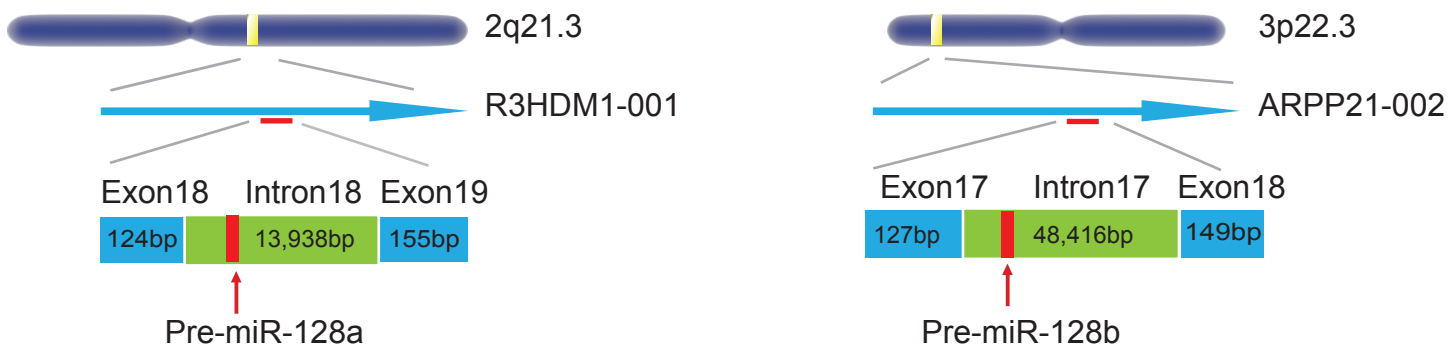
**C-D**. Sphere assays in PC3 (C) and Du145 (D) cells. The experiments were conducted similarly to the clonal assays except that the transfected cells were plated in ULA plates. Spheres were enumerated 12 d after plating. Data presented are the mean ± S.D from triplicate wells.

**Figure S6.** *BMI-1 effects on proliferation of PCa cells as measured by MTT assays*

**A**. *BMI-1* knockdown decreases proliferation of $CD44^+$ Du145 cells, as well as bulk PC3 and PPC-1 cells. Cells were infected with shBMI-1 or shCtrl for 72 h followed by MTT assays. Values presented are mean ± S.D from triplicate wells.

**B**. *BMI-1* overexpression increases proliferation of $CD44^-$ Du145 cells as well as bulk PC3 and PPC-1 cells. Cells were infected with pBABE-BMI-1 or pBABE control vector for 72 h followed by MTT assays. Data presented are mean ± S.D from triplicate wells.

**Fig. S1 The genomic locus of hsa-miR-128**




hsa-pre-miR128a [MI0000447]

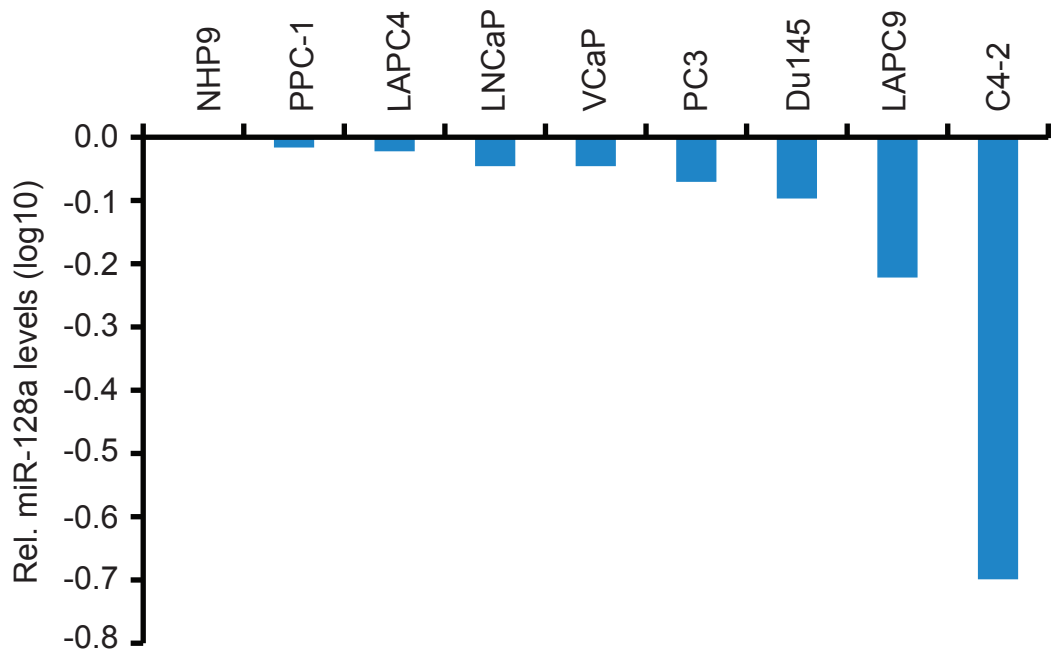
UGAGCUGUUGGAUUCGGGGCCGUAGCACUGUCUGAGAGGUUUACAUUUCUCACAGUGAACCGGUCUCUUUUUCAGCUGCUUC

UGUGCAGUGGGAAGGGGGGCCGAUACACUGUACGAGAGUGAGUAGCAGGUCUCACAGUGAACCGGUCUCUUUCCCUACUGUGUC

hsa-pre-miR128b [MI0000727]

hsa-mature-miR128: UCACAGUGAACCGGUCUCUUUU (22)

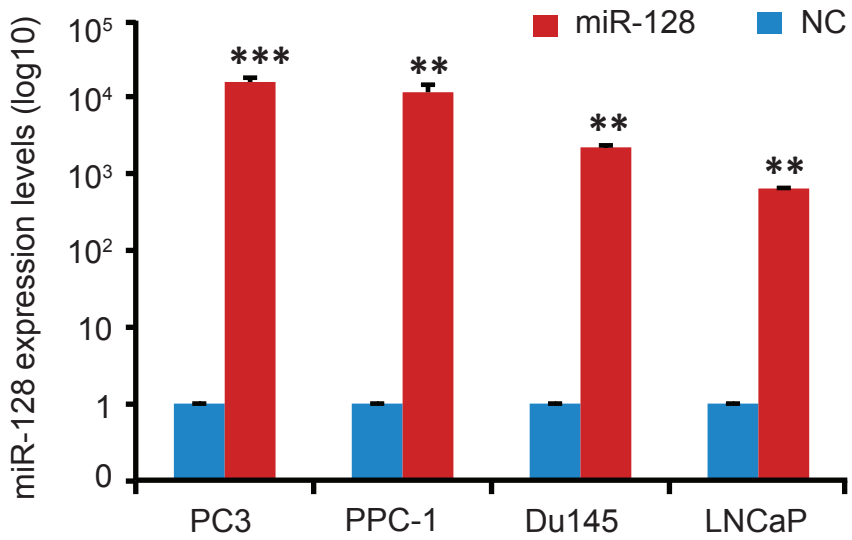
**Fig. S2. Relative expression levels of miR-128 in PCa cell lines**



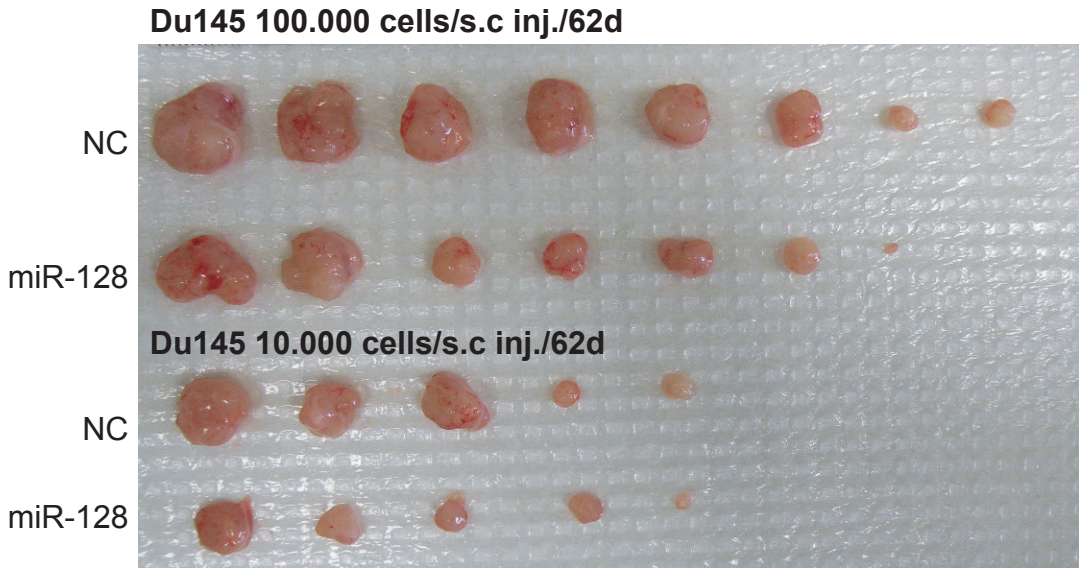

Jin et al. Supplementary Fig. S2

**Fig. S3. Transfection efficiency and tumor-suppressive effect of miR-128**

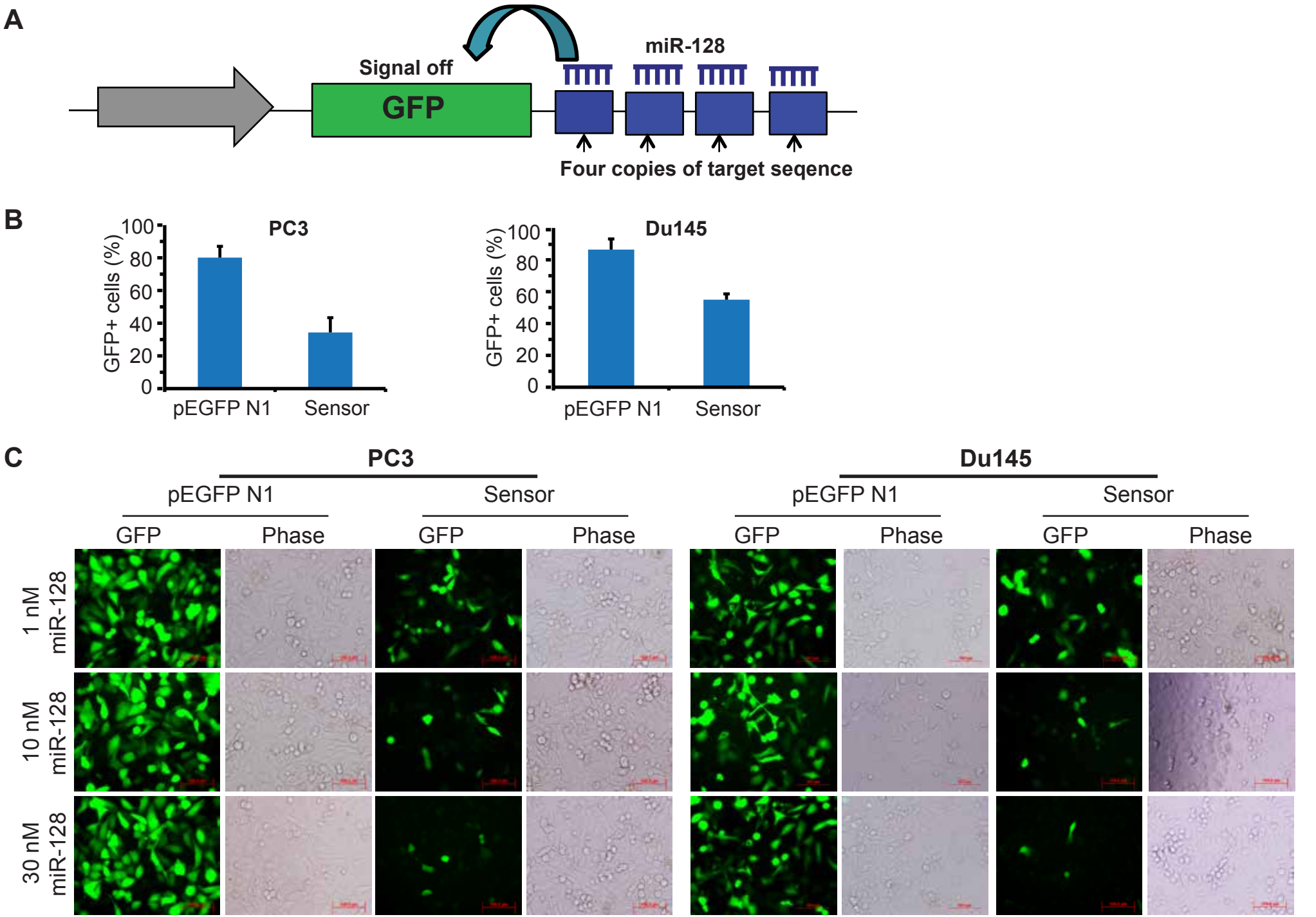
**A**



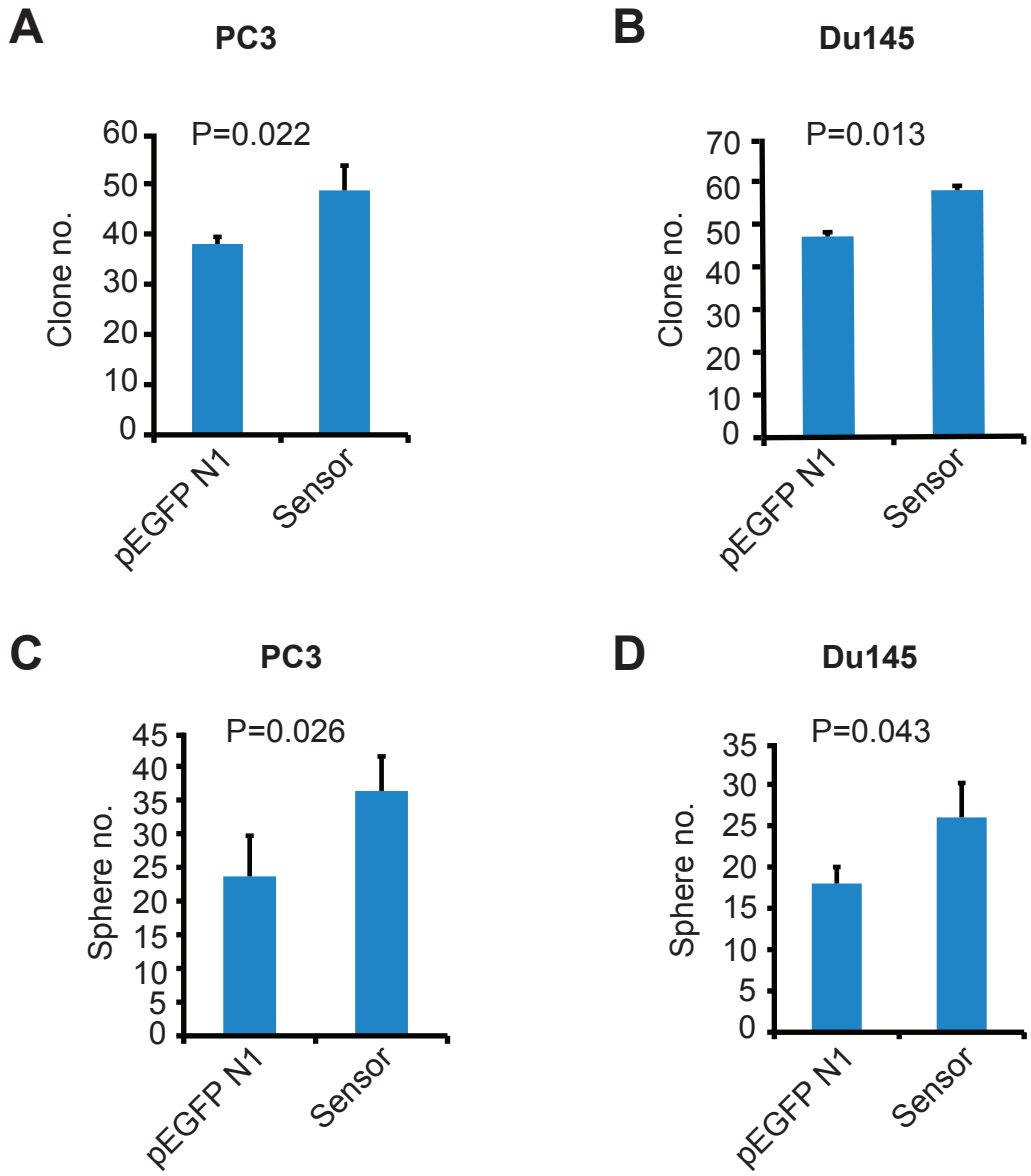

**B**




| | Incidence | Mean weight | P value |
|---|---|---|---|
| NC | 8/10 (80%) | 0.331 ± 0.234 | |
| miR-128 | 7/10 (70%) | 0.165 ± 0.212 | 0.035 |
| NC | 5/10 (50%) | 0.244 ± 0.136 | |
| miR-128 | 5/10 (50%) | 0.088 ± 0.083 | 0.029 |

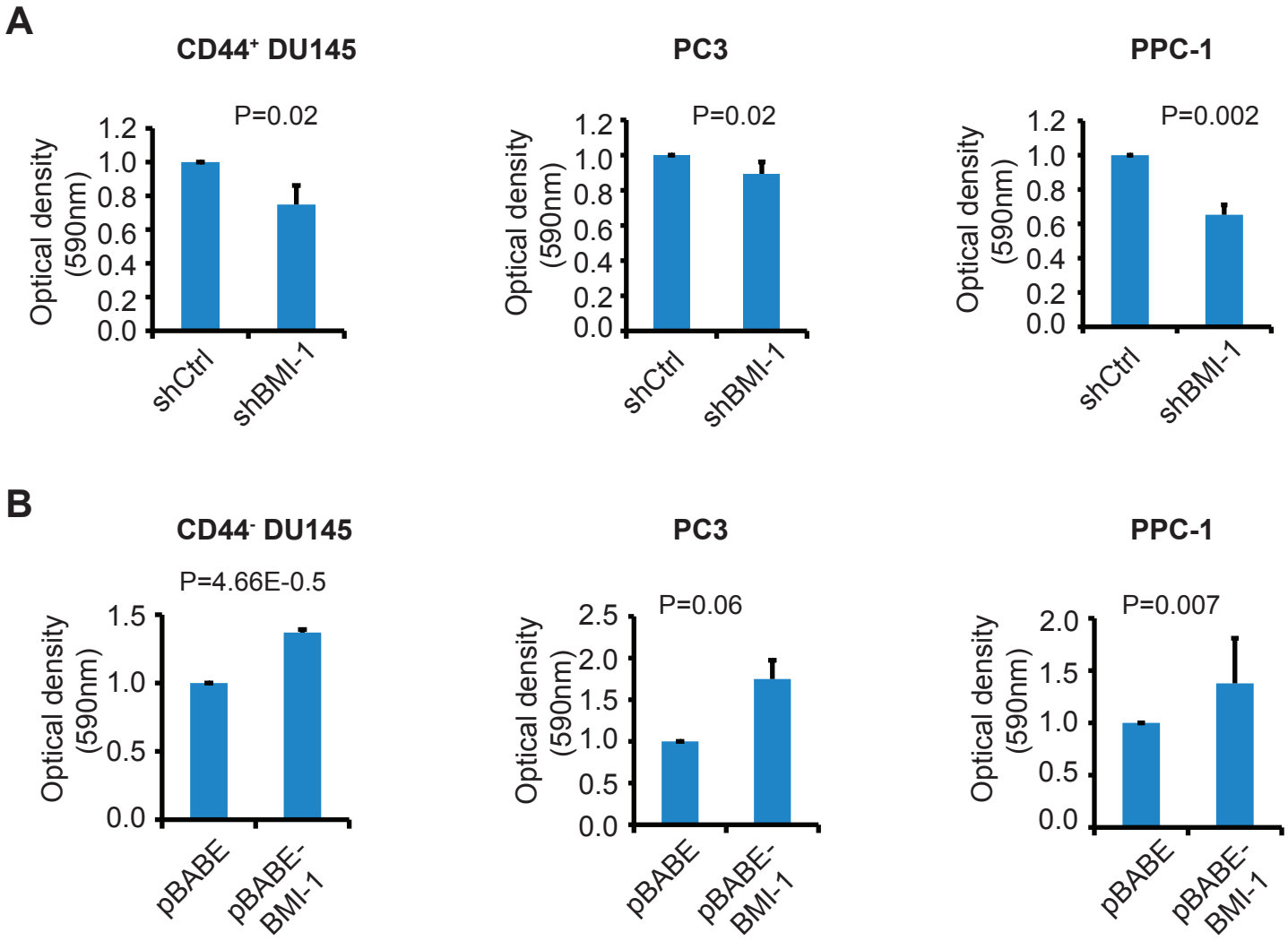



Jin et al. Supplementary Fig. S4




Jin et al, Supplementary Fig. S5

**Fig. S6. Effects of *BMI-1* knockdown and overexpression on PCa cell proliferation**



**Supplementary Table S1. Primers used in qPCR analysis miR-128 target quantification, BMI-1 and NANOG 3'-UTR cloning, and sequencing**

| Primers | Sequence (5'- 3') |
|---|---|
| BMI-1 F | TGGAGAACTGGAAAGTGACTCTGG |
| BMI-1 R | AAGAAGATTGGTGGTTACCGCTG |
| NANOG F | TAGCAATGGTGTGACGCAGAAG |
| NANOG R | TCTGGTTGCTCCACATTGGAAGG |
| TGFBR1 F | CAACTCAGTCAACAGGAAGGCATC |
| TGFBR1 R | TGGGAAAGAAGCGTTCATAGTGC |
| E2F3 F | AGAAAGACATCCCCATTGTGTGAG |
| E2F3 R | ACCCTGGCATTGTTTTGCTTC |
| EGFR F | CCTATCAAGTGGATGGCATTGG |
| EGFR R | TTTGGGCGACTATCTGCGTC |
| GAPDH F | AATCCCATCACCATCTTCCA |
| GAPDH R | TGGACTCCACGACGTACTCA |
| AR F | GAGAAGCCTTAGAATGGGTGG |
| AR R | TGGCTTATGGGATAGGACAAC |
| PSA F | GGGAGGGTCTTCCTTTGGCA |
| PSA R | ATCTGAGGGTTGTCTGGAGGA |
| BMI-1 3'UTR F | ATGAGCTCTACCTGAGACTGTTAAGGA |
| BMI-1 3'UTR R | GCAAGCTTGGCAAACATTGGTAACTTT |
| NANOG 3'UTR F | GAGCTCATATTACTCAATTTCAGTCTGGA |
| NANOG 3'UTR R | CAAGCTTATATTTACTCATTAGAACACTCG |
| BMI-1 3'UTR mutagenic F | TAAATATGCAAGAAATCGTTATTCACAACAACATGGCTACATAGAATGCATTA |
| BMI-1 3'UTR mutagenic R | TAATGCATTCTATGTAGCCATGTTGTTGTGAATAACGATTTCTTGCATATTTAG |
| NANOG 3'UTR mutagenic F | TTAGTAGAGACGGGGTTTCGAGTTGTTAGCCAGGATGGT |
| NANOG 3'UTR mutagenic R | ACCATCCTGGCTAACAACTCGAAACCCCGTCTCTACTAA |
| BMI-1 3'UTR sequencing F1 | CGCCTACATTACATTCTCCTTG |
| BMI-1 3'UTR sequencing F2 | ATTCTCCCAACTGGTTCG |
| BMI-1 3'UTR sequencing F3 | TGCCTTCTCTGCTATGTCTG |