The miR-130 family promotes cell migration and invasion in bladder cancer through FAK and Akt phosphorylation by regulating PTEN

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Supporting Figure legend

Supplementary Figure 1 Functional verification of miR-130 family hairpin inhibitors and establishment of miR-130 family stable expression cell lines. (a) MiR-130 family reporter vectors containing perfectly matched target sites of miR-130b, miR-301a or miR-301b (50 ng) were cotransfected with 50 nM miRIDIAN hairpin inhibitors or a negative control inhibitor into 5637 cells. Luciferase activity was determined using a dual reporter assay system. Data are presented as mean ± S.D. of more than three independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p< 0.001$. (b) Relative miR-130 family expression in established UM-UC-2 stable cell lines. The expression levels of each miRNAs were determined with qRT-PCR by a ΔΔCT method, and compared with a mock-transfected cell line.

Supplementary Figure 2 Functional analysis of another clone of UM-UC-2 cells stably expressing miR-130 family. (a) Relative miR-130 family expression in another clone of UM-UC-2 cells stably expressing miR-130 family (Clone No. 2). The expression level of each miRNA was determined with qRT-PCR by ΔΔCT method, and compared with a mock-transfected cell line. (b) The established UM-UC-2 cells (clone No. 2) were seeded into a 96-well plate dish, incubated for the indicated times and relative cell proliferation was measured by a WST-1 assay. Data are mean ± S.D. of five independent experiments. (c) Relative cell motility was measured 12 h after a scratch wound was formed in the cell layer. Representative results of cell motility in the scratch wound- healing assay are shown in the lower panels. Data are mean ± S.D. of three independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

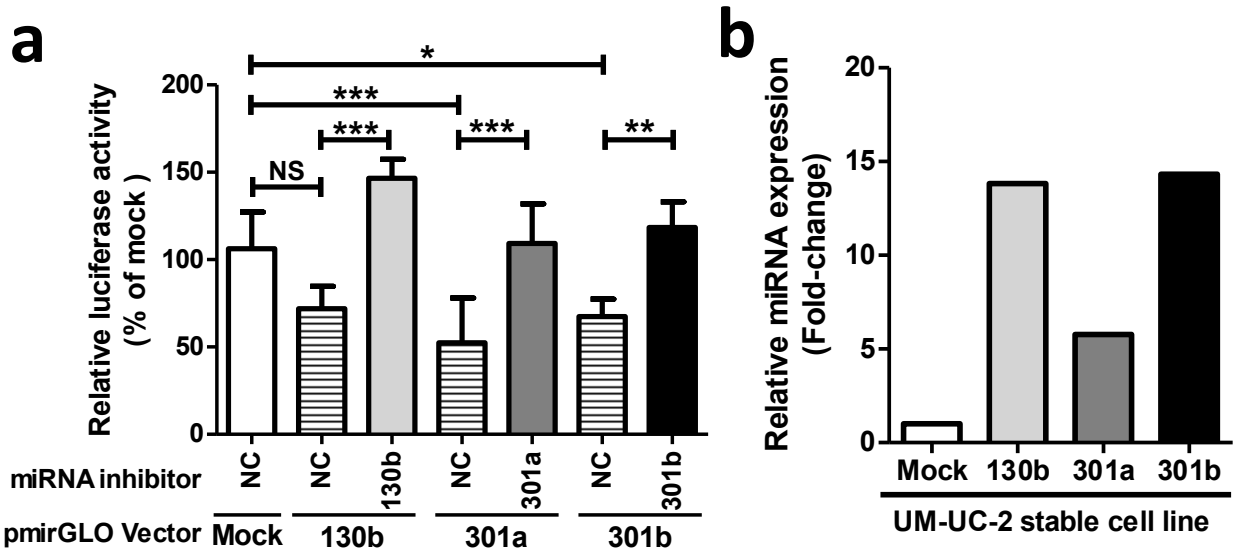
Supplementary Figure 3 Transient expression of PTEN suppresses 5637 cell migration.

One μg pcDNA 3.0 (Empty) or pcDNA 3.0-PTEN (PTEN) vectors were transfected into 5637 cells. The following experiments were performed 72 h after transfection: (a) Western blot analysis of PTEN, Akt, FAK and their phosphorylated forms using lysates of the transfected 5637 cells. (b) A wound healing assay was performed and relative cell migration was shown as mean ± S.D. of triplicate experiments. *p <0 .05. (c) Representative images of cell membrane localization of PTEN in transfected 5637 cells. The cells were stained with anti-PTEN antibody (PTEN) and 4',6-Diamidino-2- phenylindole dihydrochloride (DAPI, Nucleus). (d) The number of cells in which cell membrane localization of PTEN was detectable (indicated by arrowheads in (c)), was counted. Data are mean ± S.D. of triplicate experiments (> 27 cells /view). **p < 0.01.

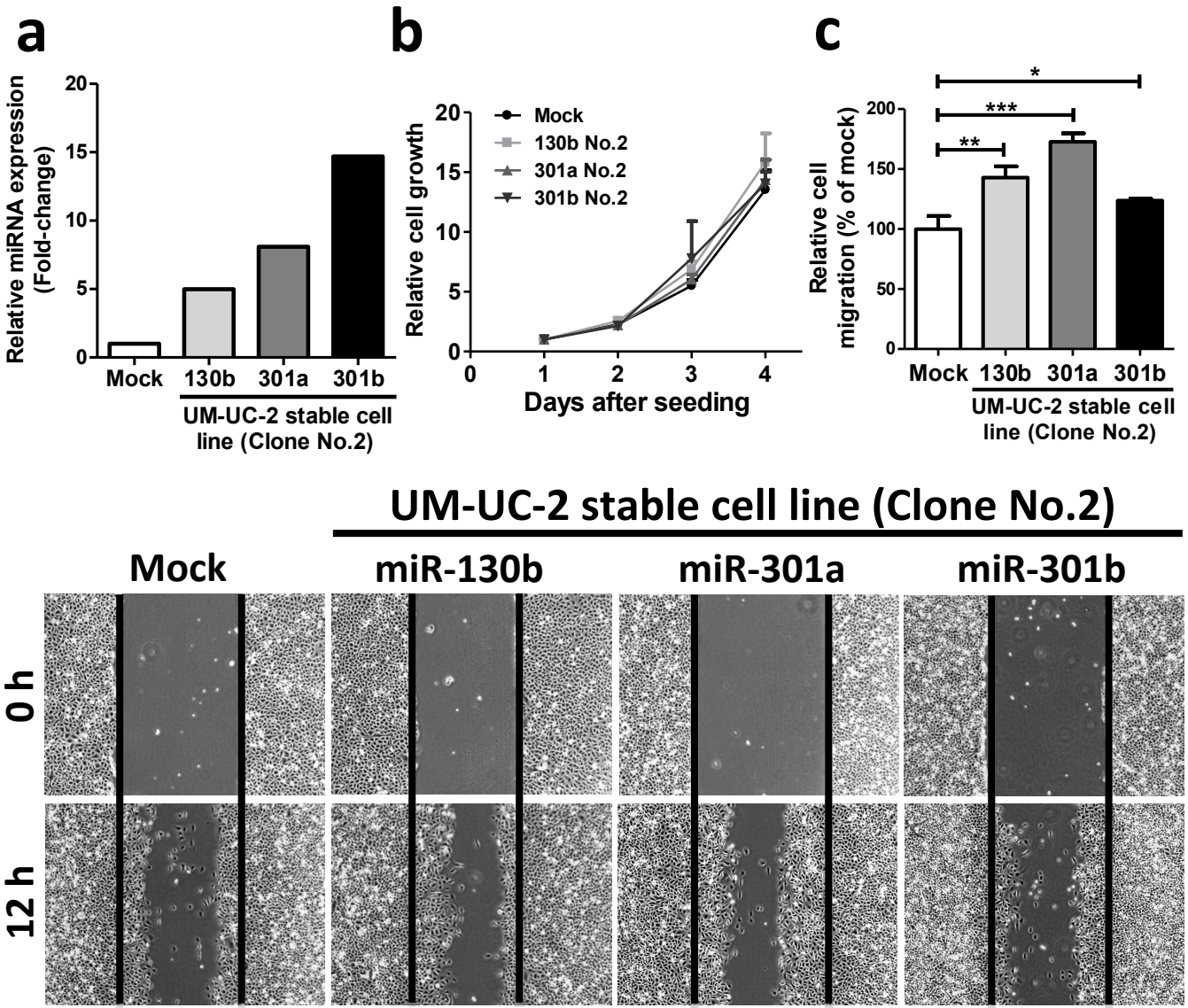
Supplementary Figure 4 The miR-130 family regulates PTEN localization via its phosphorylation. (a) UM-UC-2 cells stably expressing MiR-130 family were stained with anti-PTEN antibody and DAPI (Nucleus). (b) Representative images of sub-cellular localization of PTEN in miR-130 family-stably expressing UM-UC-2 cells. Cell membrane localization of PTEN is indicated by arrowhead. The number of cells in which cell membrane localization of PTEN was detectable were counted and are shown as mean ± S.D. of triplicate experiments (>34 cells/view). *** p < 0.001. (c) The cell lysates were immunoblotted with anti-PTEN and anti-Ser^{380} antibodies, and relative expression of phosphorylated PTEN (P-PTEN) is shown as mean±S.D. of three independent experiments.

Supplementary Figure 5 Identification and validation of an additional miR-130 family target gene in bladder cancer. (a) A schematic model of predicted miR-130 family-binding sites within the 3'-UTR of human MAGI2 gene and the result of dual-luciferase reporter assay for UM-UC-2 cells stably expressing miR-130 family. (b) A schematic model of predicted miR-130 family-binding sites within the 3'-UTR of human PTPN11 gene. A dual-luciferase reporter assay was performed, with pmirGLO-PTPN11 wild-type (WT) or -PTPN11 mutant (Mut) vector, for UM-UC-2 cells stably expressing miR-130 family. (c) The effect of miR-130 family overexpression or inhibition on PTPN11 protein expression was evaluated by western blot analysis. Data shown are mean ± S.D. of five independent experiments (*$p < 0.05$).

Supplementary figure 1



Supplementary figure 2



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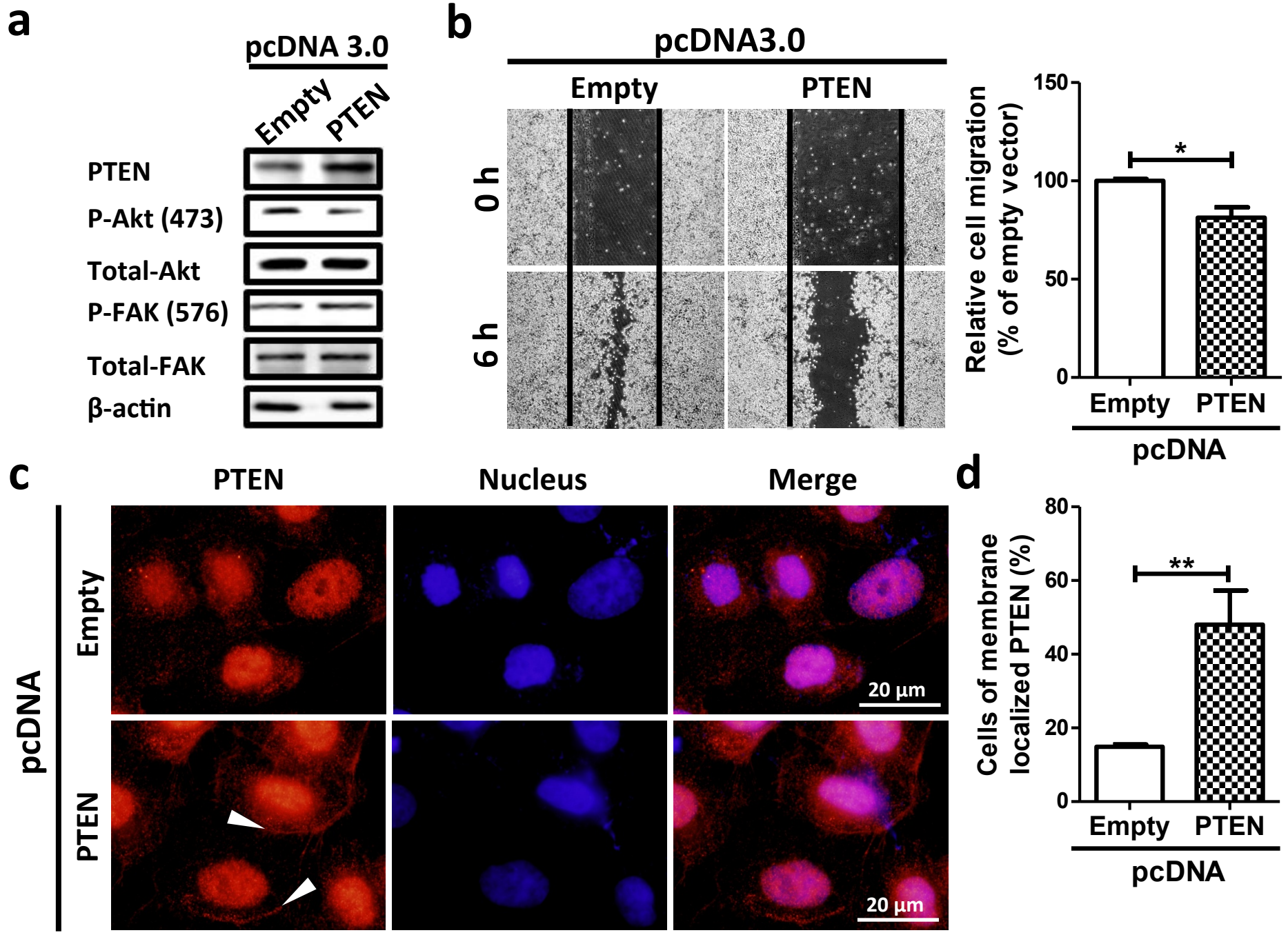
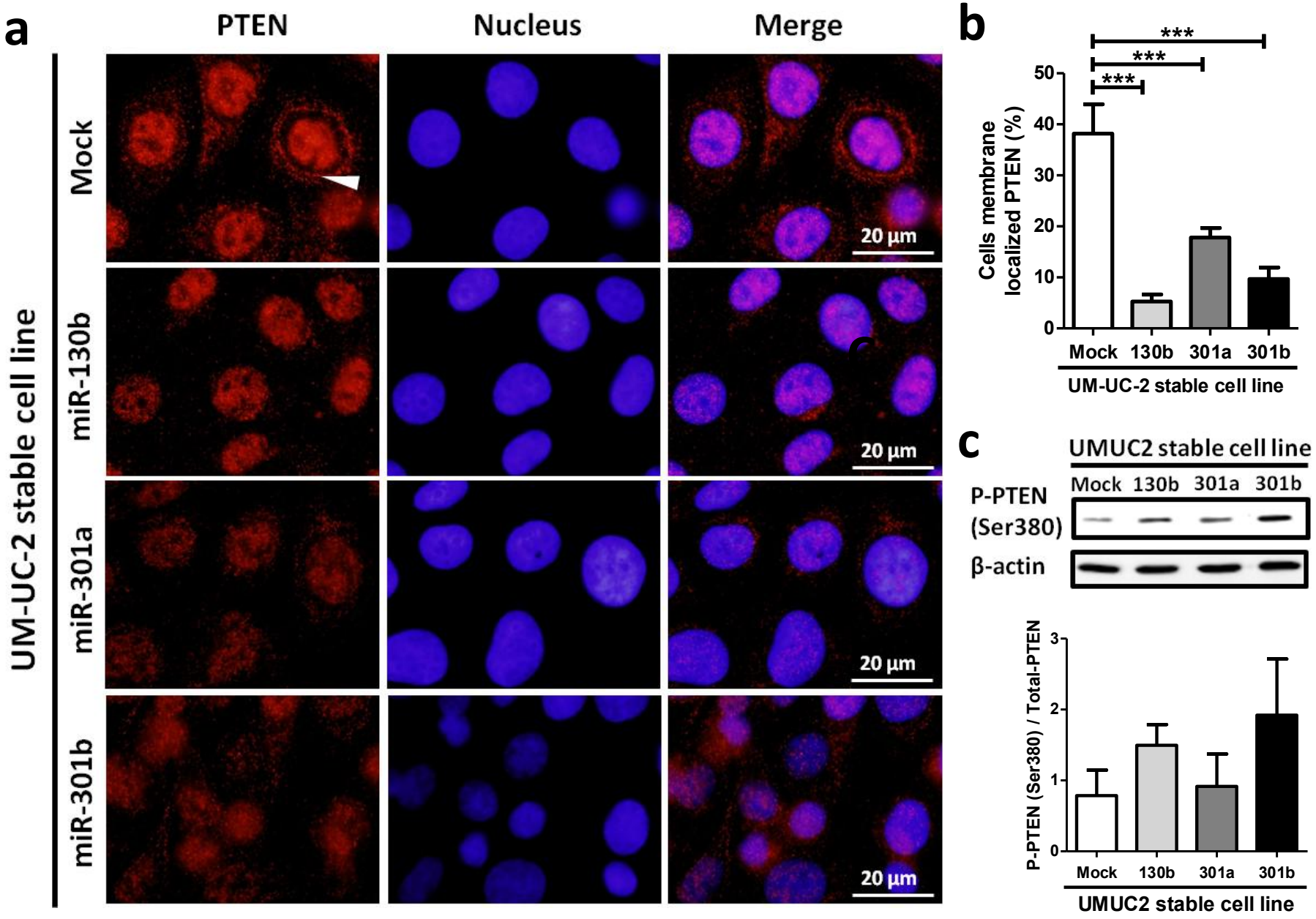


Supplementary figure 4



Supplementary figure 5