
Figures and figure supplements

Replication Study: A coding-independent function of gene and pseudogene mRNAs regulates tumour biology

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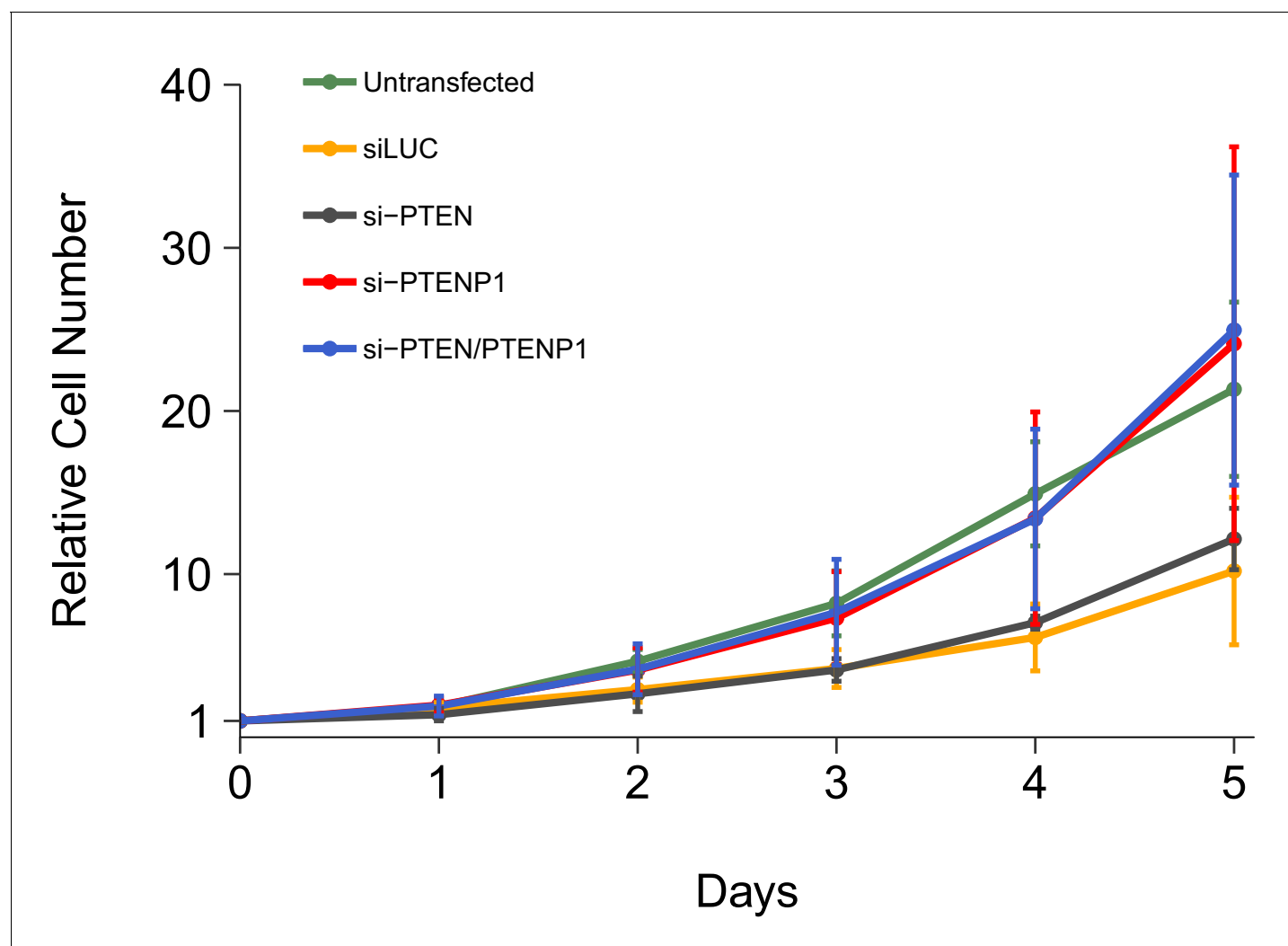


Figure 1. Cell growth of DU145 cells depleted of *PTEN* and/or *PTENP1*. DU145 cells were transfected with either a non-targeting siRNA (siLUC), si-*PTEN*, si-*PTENP1*, or an siRNA pool targeting *PTEN* and *PTENP1* (si-*PTEN/PTENP1*), or not transfected. Crystal violet proliferation assays were performed each day as indicated starting the day after transfection. Relative cell number was calculated relative to the average Day 0 values for each condition. Means reported and error bars represent SD from five independent biological repeats. Two-way ANOVA interaction between *PTEN* (targeted or not-targeted) and *PTENP1* (targeted or not-targeted) on Day 5 relative cell numbers: $F(1,16) = 0.02$, $p=0.878$; main effect of *PTEN*: $F(1,16) = 0.15$, $p=0.703$; main effect of *PTENP1*: $F(1,16) = 13.8$, $p=0.0019$. Planned contrasts between siLUC and si-*PTEN*: $t(16) = 0.38$, uncorrected $p=0.705$ with a *priori* Bonferroni adjusted significance threshold of 0.01, Bonferroni corrected $p>0.99$; siLUC and si-*PTENP1*: $t(16) = 2.73$, uncorrected $p=0.015$, Bonferroni corrected $p=0.074$; siLUC and si-*PTEN/PTENP1*: $t(16) = 2.90$, uncorrected $p=0.011$, Bonferroni corrected $p=0.053$; si-*PTEN/PTENP1* and si-*PTEN*: $t(16) = 2.51$, uncorrected $p=0.023$, Bonferroni corrected $p=0.115$; si-*PTEN/PTENP1* and si-*PTENP1*: $t(16) = 0.16$, uncorrected $p=0.872$, Bonferroni corrected $p>0.99$. Additional details for this experiment can be found at <https://osf.io/kjmxj/>.

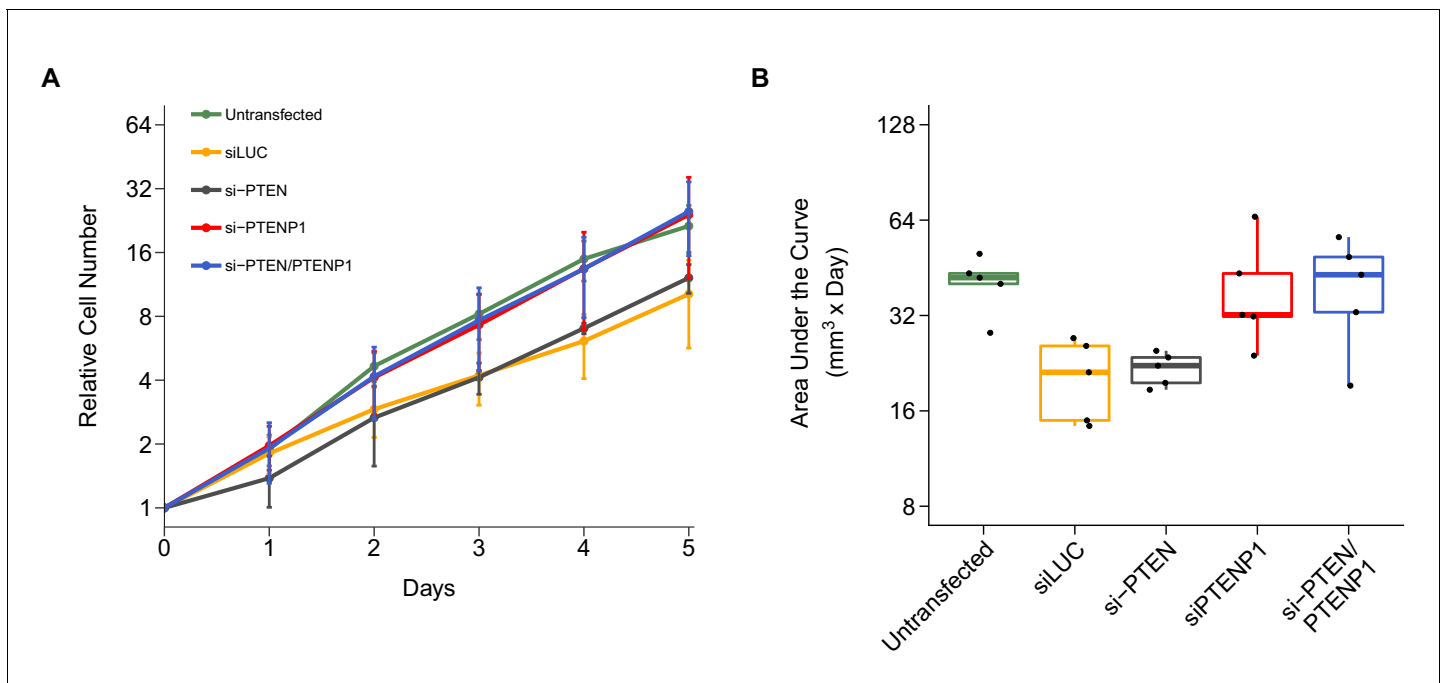


Figure 1—figure supplement 1. Alternative visualization of cell growth. This is the same experiment as **Figure 1**. (A) Relative cell numbers were natural log transformed with means reported and error bars represent SD. (B) The area under the curve (AUC) was calculated for each condition of each biological repeat ($n = 5$). Box and whisker plot with median represented as the line through the box, individual AUC values represented as dots, and whiskers representing values within 1.5 IQR of the first and third quartile (y-axis is natural log scale). Two-way ANOVA interaction between *PTEN* (targeted or not-targeted) and *PTENP1* (targeted or not-targeted) on AUC values: $F(1,16) = 0.0015$, $p=0.970$; main effect of *PTEN*: $F(1,16) = 0.033$, $p=0.858$; main effect of *PTENP1*: $F(1,16) = 13.3$, $p=0.0022$. Planned contrasts between siLUC and si-PTEN: $t(16) = 0.16$, uncorrected $p=0.878$ with *a priori* Bonferroni adjusted significance threshold of 0.01, Bonferroni corrected $p>0.99$; siLUC and si-PTENP1: $t(16) = 2.60$, uncorrected $p=0.019$, Bonferroni corrected $p=0.115$; siLUC and si-PTEN/PTENP1: $t(16) = 2.71$, uncorrected $p=0.016$, Bonferroni corrected $p=0.093$; si-PTEN/PTENP1 and si-PTEN: $t(16) = 2.55$, uncorrected $p=0.021$, Bonferroni corrected $p=0.128$; si-PTEN/PTENP1 and si-PTENP1: $t(16) = 0.10$, uncorrected $p=0.920$, Bonferroni corrected $p>0.99$. Additional details for this experiment can be found at <https://osf.io/kjmxj/>.

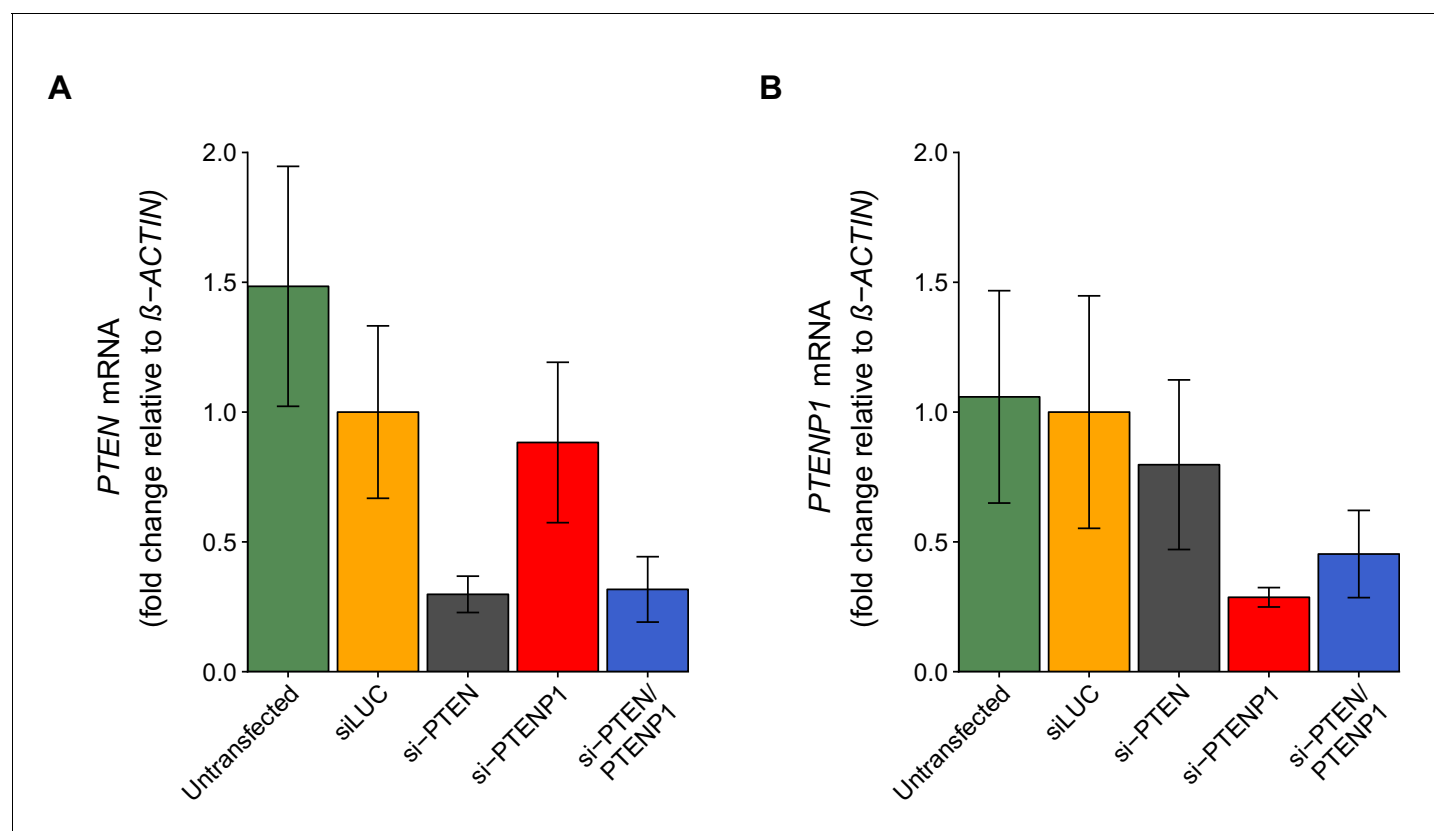


Figure 2. *PTEN* and *PTENP1* abundance in DU145 cells depleted of *PTEN* and/or *PTENP1*. DU145 cells were transfected with either a non-targeting siRNA (siLUC), si-*PTEN*, si-*PTENP1*, or an siRNA pool targeting *PTEN* and *PTENP1* (si-*PTEN/PTENP1*), or not transfected. Total RNA was isolated 24 hr later and qRT-PCR analysis was performed to detect *PTEN*, *PTENP1*, and β -*ACTIN* levels. (A) Fold change in *PTEN* expression (*PTEN*/ β -*ACTIN*) is presented for each condition relative to siLUC cells. Means reported and error bars represent SD from five independent biological repeats. Two-way ANOVA interaction between *PTEN* (targeted or not-targeted) and *PTENP1* (targeted or not-targeted) on *PTEN* expression: $F(1,16) = 0.41$, $p=0.532$; main effect of *PTEN*: $F(1,16) = 35.5$, $p=2.01 \times 10^{-5}$; main effect of *PTENP1*: $F(1,16) = 0.21$, $p=0.651$. Planned contrasts between siLUC and si-*PTEN* for *PTEN* expression: $t(16) = 4.66$, uncorrected $p=0.00026$ with *a priori* Bonferroni adjusted significance threshold of 0.0083, Bonferroni corrected $p=0.0016$; siLUC and si-*PTENP1*: $t(16) = 0.78$, uncorrected $p=0.448$, Bonferroni corrected $p>0.99$; siLUC and si-*PTEN/PTENP1*: $t(16) = 4.54$, uncorrected $p=0.00034$, Bonferroni corrected $p=0.0020$. (B) Fold change in *PTENP1* expression (*PTENP1*/ β -*ACTIN*) is presented for each condition relative to siLUC cells. Means reported and error bars represent SD from five independent biological repeats. Two-way ANOVA interaction on *PTENP1* expression: $F(1,16) = 2.03$, $p=0.174$; main effect of *PTEN*: $F(1,16) = 0.019$, $p=0.891$; main effect of *PTENP1*: $F(1,16) = 16.6$, $p=0.00088$. Planned contrasts between siLUC and si-*PTEN* for *PTENP1* expression: $t(16) = 1.10$, uncorrected $p=0.286$ with *a priori* Bonferroni adjusted significance threshold of 0.0083, Bonferroni corrected $p>0.99$; siLUC and si-*PTENP1*: $t(16) = 3.89$, uncorrected $p=0.0013$, Bonferroni corrected $p=0.0079$; siLUC and si-*PTEN/PTENP1*: $t(16) = 2.98$, uncorrected $p=0.0089$, Bonferroni corrected $p=0.053$. Additional details for this experiment can be found at <https://osf.io/4uard/>.

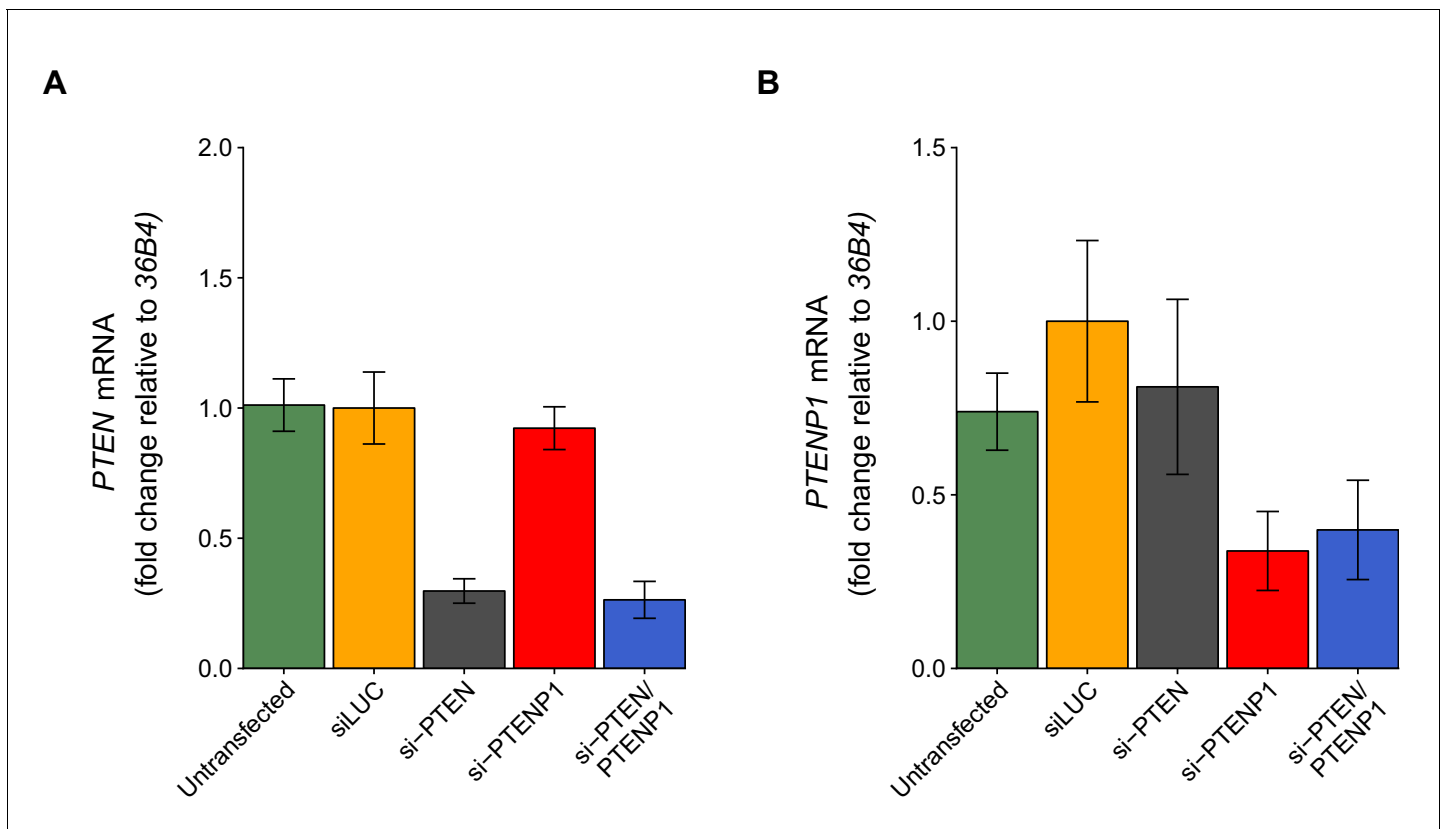


Figure 2—figure supplement 1. *PTEN* and *PTENP1* abundance using *36B4* to normalize expression. This is the same experiment as **Figure 2**, but with *PTEN* and *PTENP1* expression normalized to *36B4* instead of β -*ACTIN*. (A) Fold change in *PTEN* expression (*PTEN/36B4*) is presented for each condition relative to siLUC cells. Means reported and error bars represent SD from five independent biological repeats. Exploratory analysis: Two-way ANOVA interaction between *PTEN* (targeted or not-targeted) and *PTENP1* (targeted or not-targeted) on *PTEN* expression: $F(1,16) = 0.29$, $p=0.600$; main effect of *PTEN*: $F(1,16) = 280.5$, $p=1.45 \times 10^{-11}$; main effect of *PTENP1*: $F(1,16) = 1.88$, $p=0.190$. Planned contrasts between siLUC and si-PTEN for *PTEN* expression: $t(16) = 12.2$, uncorrected $p=1.58 \times 10^{-9}$ with a priori Bonferroni adjusted significance threshold of 0.0083, Bonferroni corrected $p=9.48 \times 10^{-9}$; siLUC and si-PTENP1: $t(16) = 1.35$, uncorrected $p=0.197$, Bonferroni corrected $p>0.99$; siLUC and si-PTEN/PTENP1: $t(16) = 12.8$, uncorrected $p=7.93 \times 10^{-10}$, Bonferroni corrected $p=4.76 \times 10^{-9}$. (B) Fold change in *PTENP1* expression (*PTENP1/36B4*) is presented for each condition relative to siLUC cells. Means reported and error bars represent SD from five independent biological repeats. Exploratory analysis: Two-way ANOVA interaction on *PTENP1* expression: $F(1,16) = 2.07$, $p=0.170$; main effect of *PTEN*: $F(1,16) = 0.55$, $p=0.471$; main effect of *PTENP1*: $F(1,16) = 38.2$, $p=1.32 \times 10^{-5}$. Planned contrasts between siLUC and si-PTEN for *PTENP1* expression: $t(16) = 1.54$, uncorrected $p=0.143$ with a priori Bonferroni adjusted significance threshold of 0.0083, Bonferroni corrected $p=0.860$; siLUC and si-PTENP1: $t(16) = 5.39$, uncorrected $p=6.06 \times 10^{-5}$, Bonferroni corrected $p=0.00036$; siLUC and si-PTEN/PTENP1: $t(16) = 4.89$, uncorrected $p=0.00016$, Bonferroni corrected $p=0.00098$. Additional details for this experiment can be found at <https://osf.io/4uard/>.

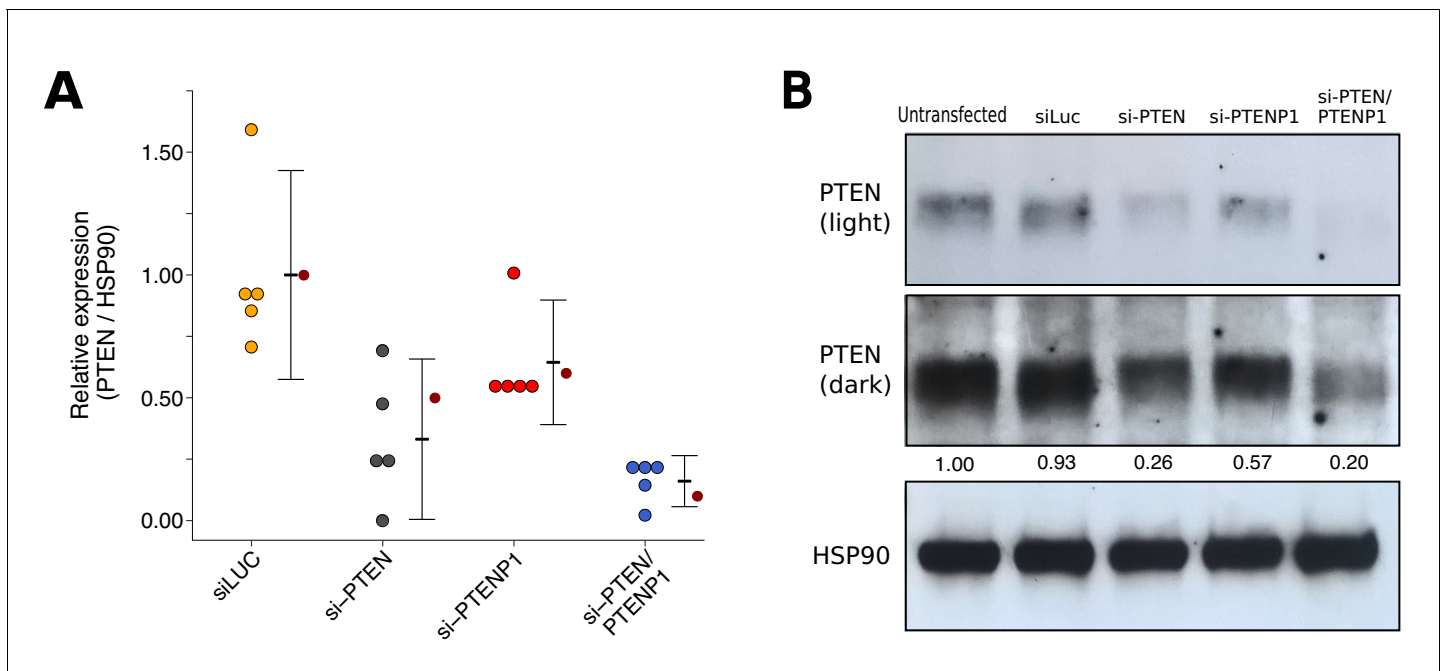


Figure 3. *PTENP1* abundance in DU145 cells expressing *PTEN* 3'UTR. DU145 cells were transfected with either a vector control plasmid (pCMV) or a plasmid to express *PTEN* 3'UTR (pCMV-*PTEN*), or not transfected. Total RNA was isolated 24 hr later and qRT-PCR analysis was performed to detect *PTENP1* and β -*ACTIN* levels. Fold change in *PTENP1* expression (*PTENP1*/ β -*ACTIN*) is presented for each condition relative to pCMV transfected cells. Means reported and error bars represent *SD* from three independent biological repeats. Unpaired two-tailed Student's *t* test between pCMV and pCMV-*PTEN*: $t(4) = 0.46$, $p=0.671$. Additional details for this experiment can be found at <https://osf.io/rkuxh/>.

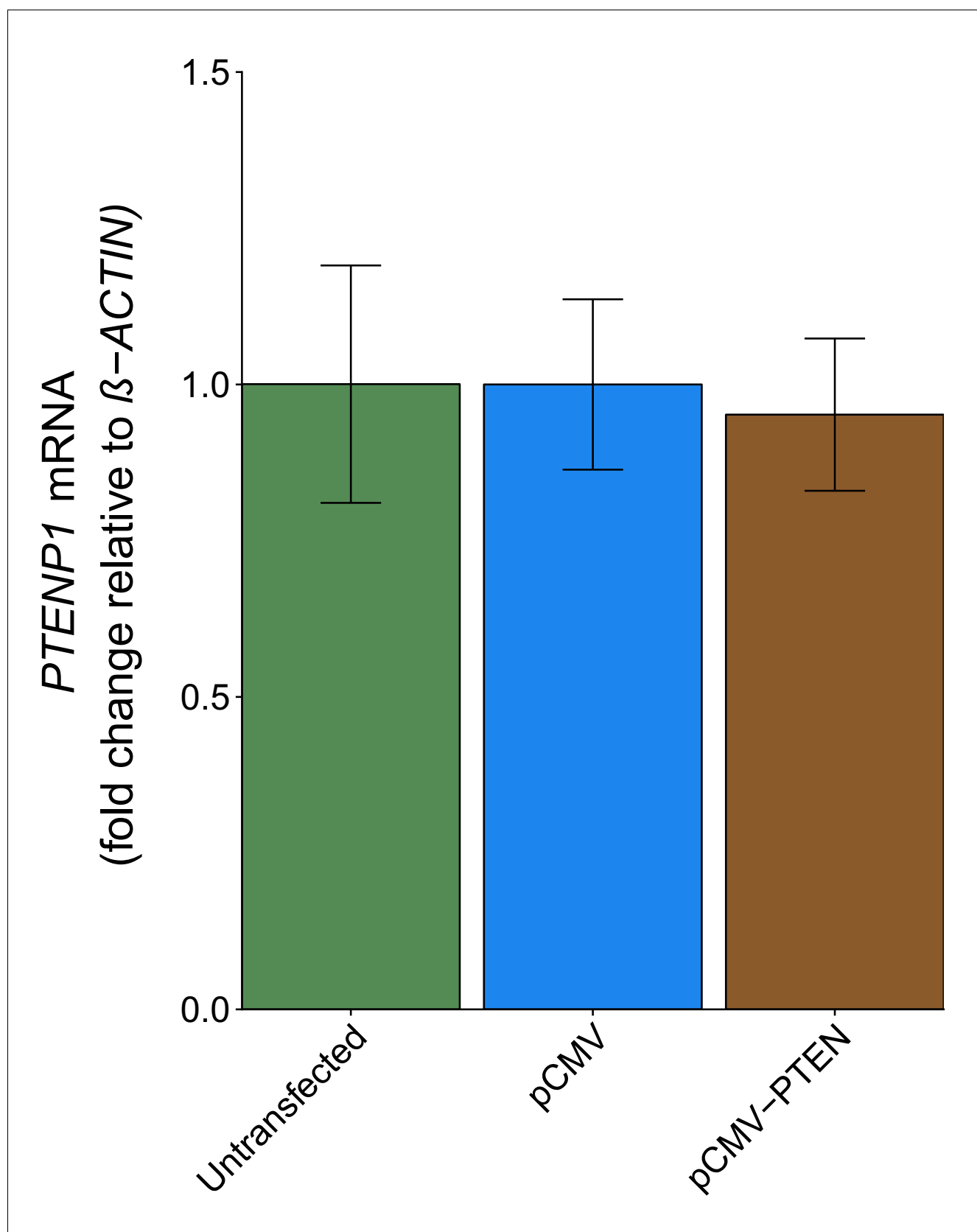


Figure 4. PTEN expression in DU145 cells depleted of *PTEN* and/or *PTENP1*. DU145 cells were transfected with either a non-targeting siRNA (siLUC), si-PTEN, si-PTENP1, or an siRNA pool targeting *PTEN* and *PTENP1* (si-PTEN/PTENP1), or not transfected. Cells were harvested 48 hr later for Western
Figure 4 continued on next page

Figure 4 continued

blot analysis. (A) Relative protein expression (PTEN/HSP90) are presented for each condition. Western blot bands were quantified, PTEN levels were normalized to HSP90, then for each biological repeat values were normalized to the untransfected condition with protein expression presented relative to siLuc. Dot plot of independent biological repeats ($n = 5$), means reported as crossbars and error bars represent 95% CI. Data reported in Figure 2H of **Poliseno et al. (2010)** is displayed as a single point (small dark red circle) for comparison. Planned comparisons (two-tailed Wilcoxon-Mann-Whitney tests): siLUC and si-PTEN: $U = 25$, uncorrected $p=0.0079$ with *a priori* Bonferroni adjusted significance threshold of 0.01, Bonferroni corrected $p=0.040$; siLUC and si-PTENP1: $U = 21$, uncorrected $p=0.095$, Bonferroni corrected $p=0.476$; siLUC and si-PTEN/PTENP1: $U = 25$, uncorrected $p=0.0079$, Bonferroni corrected $p=0.040$; si-PTEN/PTENP1 and si-PTEN: $U = 6$, uncorrected $p=0.222$, Bonferroni corrected $p>0.99$; si-PTEN/PTENP1 and si-PTENP1: $U = 0$, uncorrected $p=0.0079$, Bonferroni corrected $p=0.040$. (B) Representative Western blots probed with an anti-PTEN antibody (two exposures presented to facilitate detection) and anti-HSP90 antibody. Relative PTEN/HSP90 expressions are reported below PTEN images. Additional details for this experiment can be found at <https://osf.io/re87y/>.

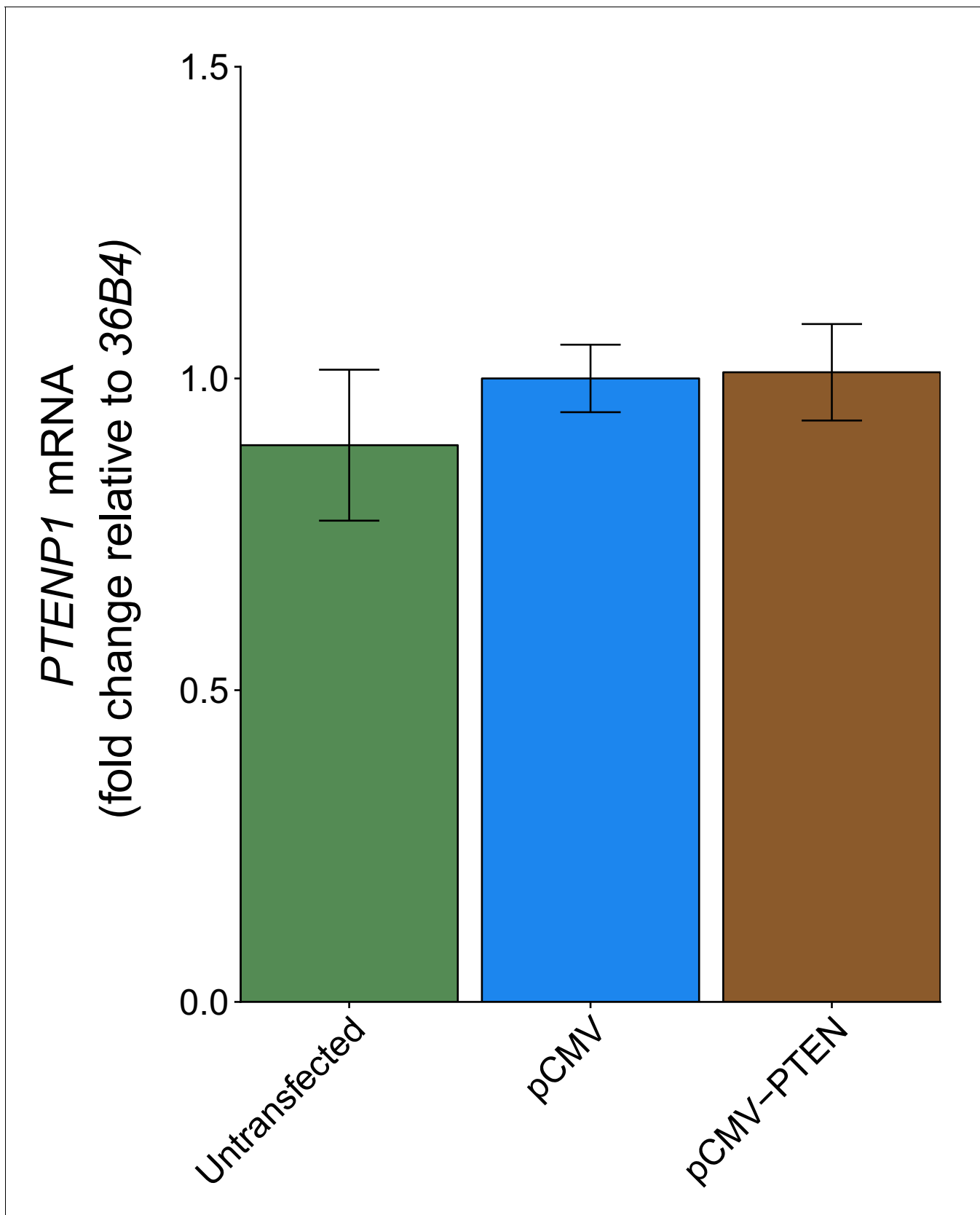


Figure 4—figure supplement 1. *PTENP1* abundance using *36B4* to normalize expression. This is the same experiment as **Figure 4**, but with *PTENP1* expression normalized to *36B4* instead of β -ACTIN. Fold change in *PTENP1* expression (*PTENP1*/*36B4*) is presented for each condition relative to **Figure 4—figure supplement 1** continued on next page

Figure 4—figure supplement 1 continued

pCMV transfected cells. Means reported and error bars represent *SD* from three independent biological repeats. Exploratory analysis: Unpaired two-tailed Student's *t* test between pCMV and pCMV-*PTEN*: $t(4) = 0.18$, $p=0.865$. Additional details for this experiment can be found at <https://osf.io/rkuxh/>.

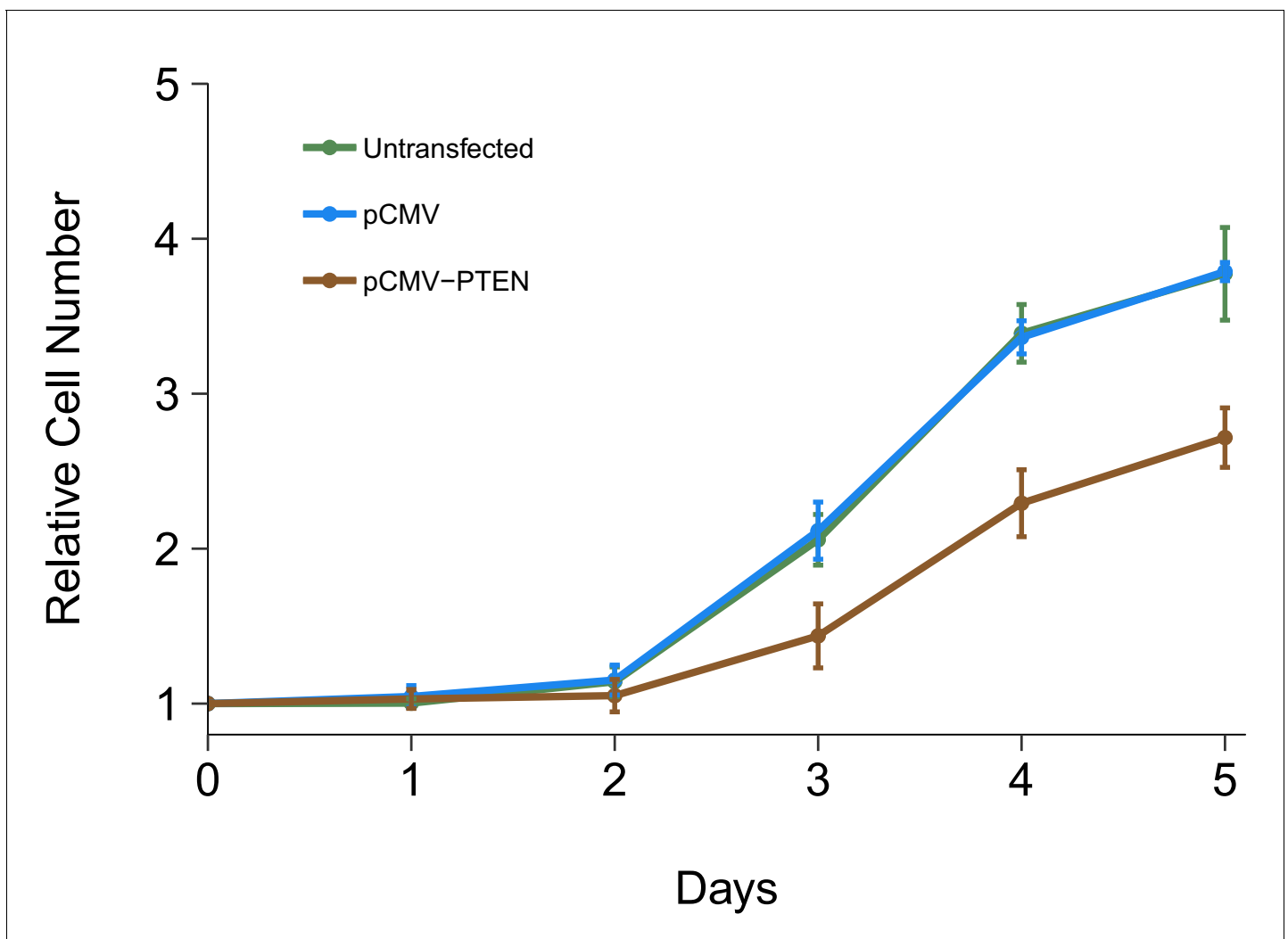


Figure 5. Cell growth of DU145 cells expressing *PTEN* 3'UTR. DU145 cells were transfected with either vector control plasmid (pCMV) or a plasmid to express *PTEN* 3'UTR (pCMV-*PTEN*), or not transfected. Crystal violet proliferation assays were performed each day as indicated starting the day after transfection. Relative cell number was calculated relative to the average Day 0 values for each condition. Means reported and error bars represent *SD* from three independent biological repeats. Unpaired two-tailed Student's *t* test between pCMV and pCMV-*PTEN* on Day five relative cell numbers: $t(4) = 9.25$, $p=0.00076$. Additional details for this experiment can be found at <https://osf.io/jgp6n/>.

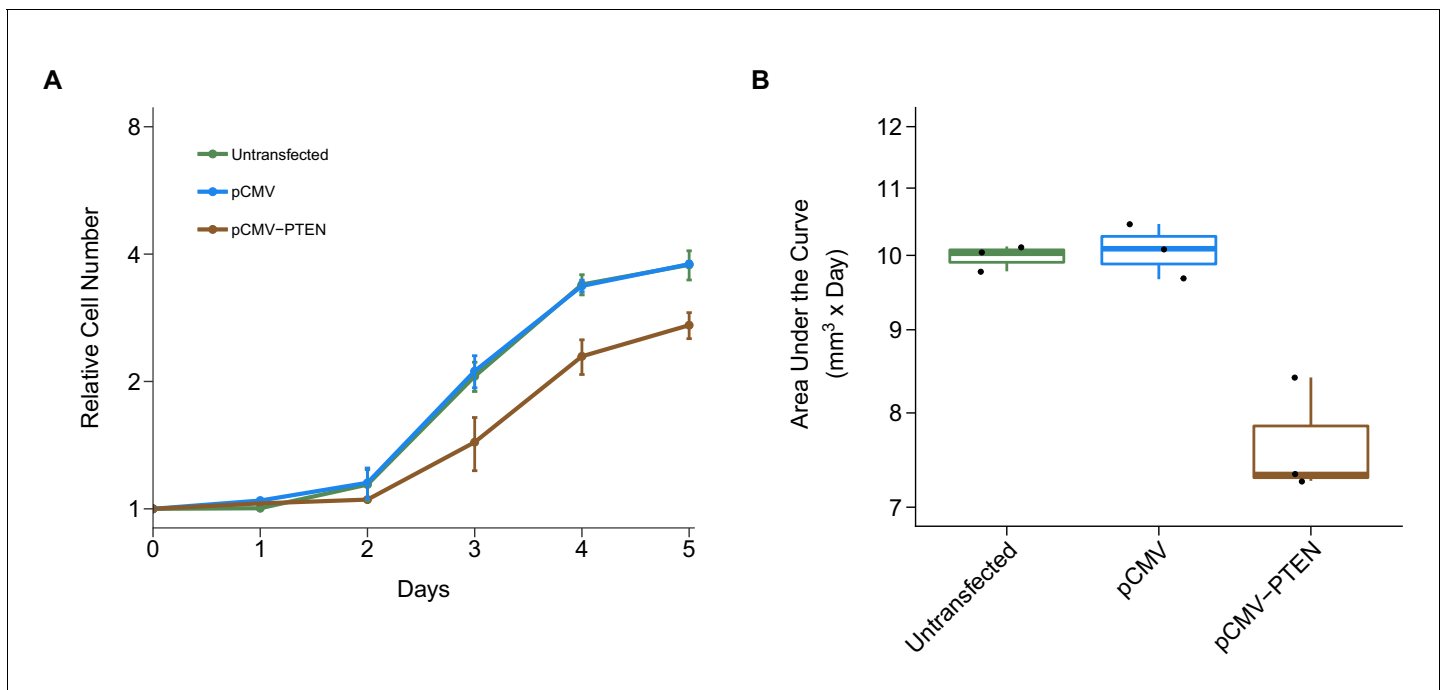


Figure 5—figure supplement 1. Alternative visualization of cell growth. This is the same experiment as **Figure 5**. (A) Relative cell numbers were natural log transformed with means reported and error bars represent SD. (B) AUC was calculated for each condition of each biological repeat ($n = 3$). Box and whisker plot with median represented as the line through the box, individual AUC values represented as dots, and whiskers representing values within 1.5 IQR of the first and third quartile (y-axis is natural log scale). Unpaired two-tailed Student's t test between pCMV and pCMV-PTEN on AUC values: $t(4) = 5.52$, $p = 0.00528$. Additional details for this experiment can be found at <https://osf.io/jgp6n/>.

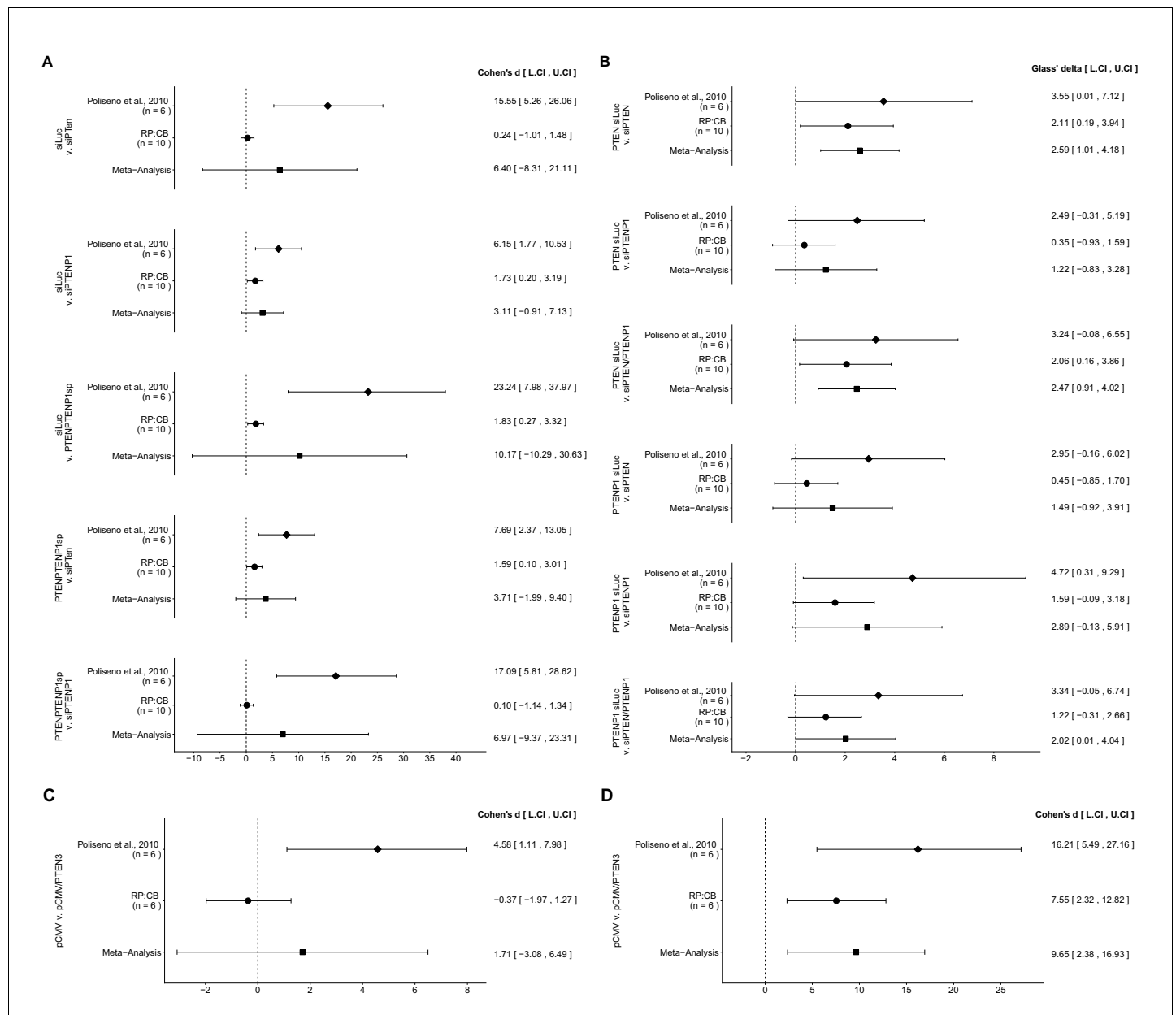


Figure 6. Meta-analyses of each effect. Effect size and 95% confidence interval are presented for *Poliseno et al. (2010)*, this replication study (RP:CB), and a random effects meta-analysis of those two effects. Cohen's *d* and Glass' delta are standardized differences between the two indicated measurements with the calculated effects for the original study effects reported as positive values. Sample sizes used in *Poliseno et al. (2010)* and RP:CB are reported under the study name. (A) These effects are related to the change in Day 5 relative cell numbers between the conditions reported in *Figure 1* of this study and *Figure 2F* of *Poliseno et al. (2010)*. Meta-analysis *p* values: siLUC and si-PTEN ($p=0.394$); siLUC and si-PTENP1 ($p=0.129$); siLUC and si-PTEN/PTENP1 ($p=0.330$); si-PTEN/PTENP1 and si-PTEN ($p=0.202$); si-PTEN/PTENP1 and si-PTENP1 ($p=0.403$). (B) These effects are related to the fold change differences in *PTEN* and *PTENP1* expression between the conditions reported in *Figure 2* of this study and *Figure 2G* of *Poliseno et al. (2010)*. Meta-analysis *p* values: *PTEN* expression between siLUC and si-PTEN ($p=0.0013$); *PTEN* expression between siLUC and si-PTENP1 ($p=0.244$); *PTEN* expression between siLUC and si-PTEN/PTENP1 ($p=0.0019$); *PTENP1* expression between siLUC and si-PTEN ($p=0.225$); *PTENP1* expression between siLUC and si-PTENP1 ($p=0.060$); *PTENP1* expression between siLUC and si-PTEN/PTENP1 ($p=0.049$). (C) These effects are related to the fold change differences in *PTENP1* expression between pCMV and pCMV-*PTEN*-3'UTR reported in *Figure 4* of this study and *Figure 4A* of *Poliseno et al. (2010)* (meta-analysis $p=0.485$). (D) These effects are related to the change in Day 5 relative cell numbers between pCMV and pCMV-*PTEN*-3'UTR reported in *Figure 5* of this study and *Figure 4A* of *Poliseno et al. (2010)* (meta-analysis $p=0.0093$). Additional details for these meta-analyses can be found at <https://osf.io/9yh6p/>.