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# Supplementary Materials for

## PTEN inhibits PREX2-catalyzed activation of RAC1 to restrain tumor cell invasion

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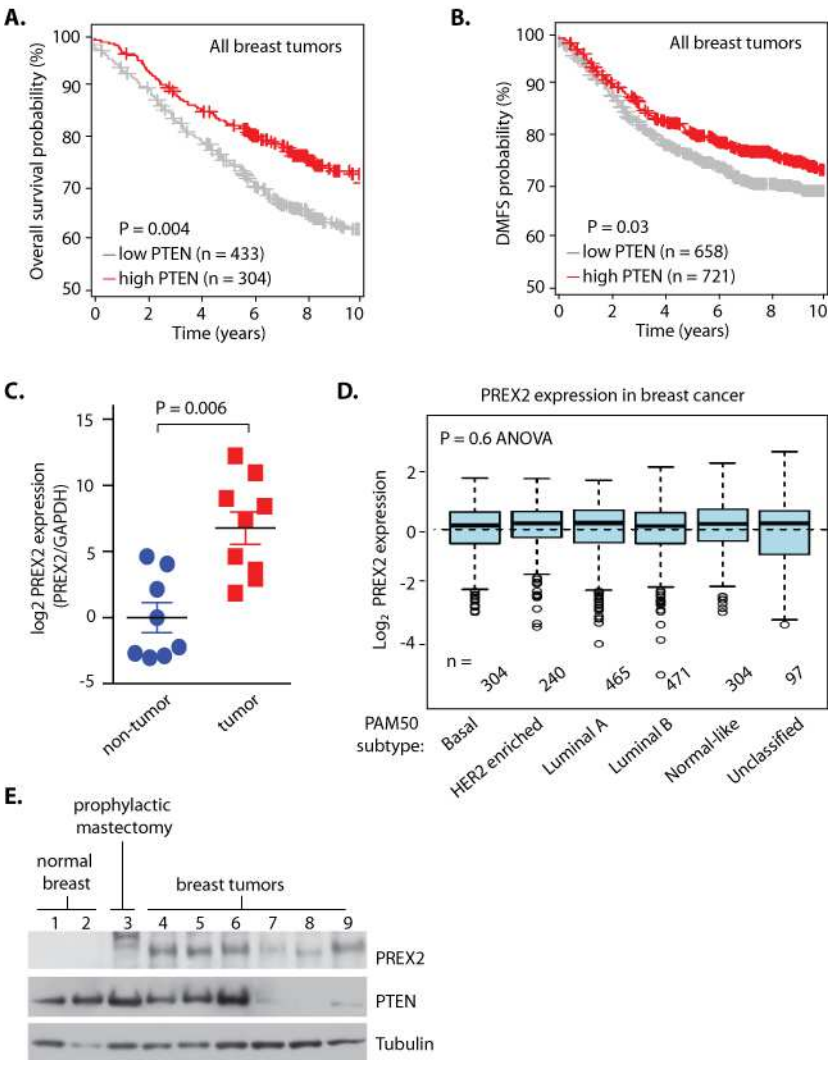
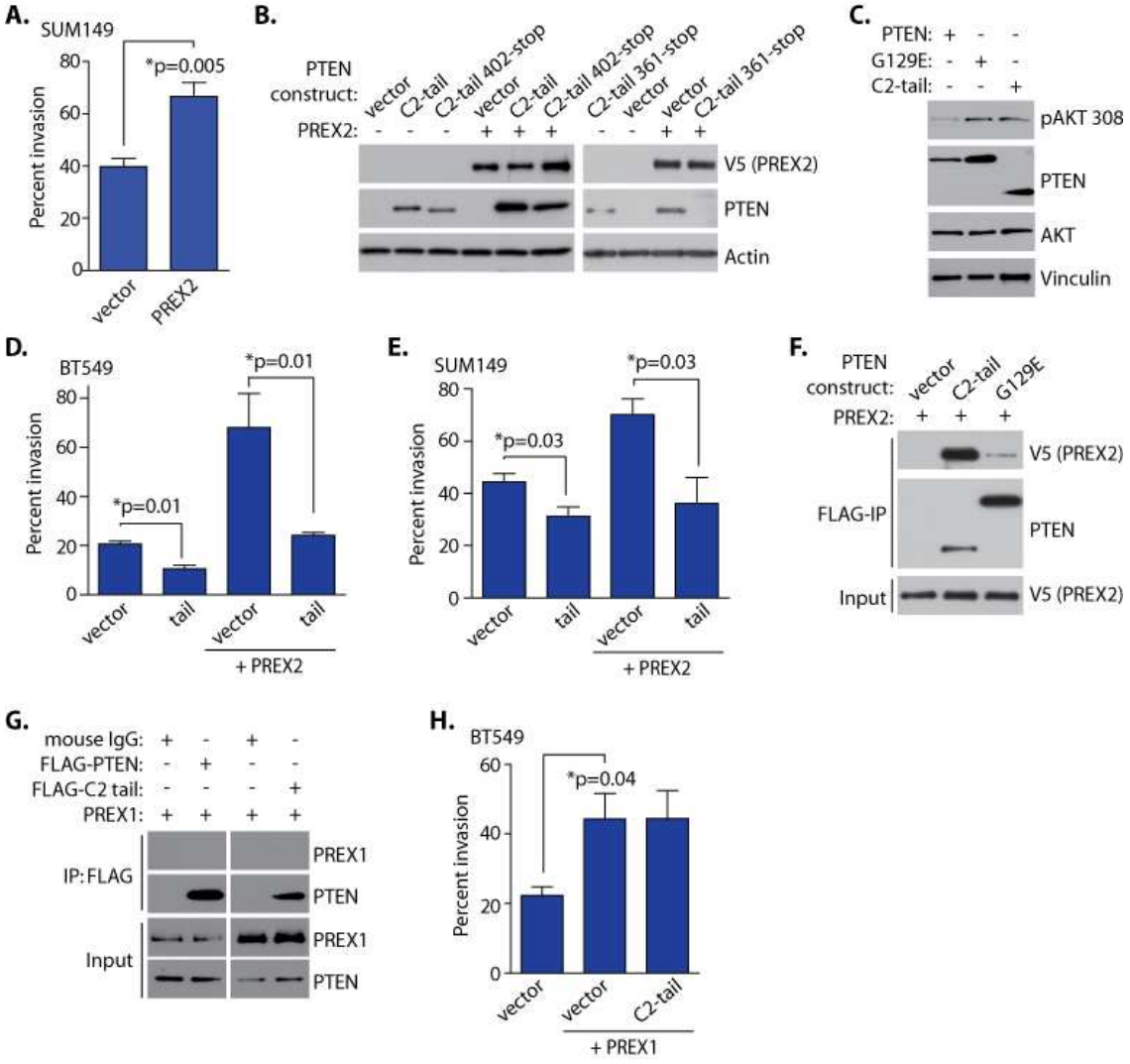
**The PDF file includes:**



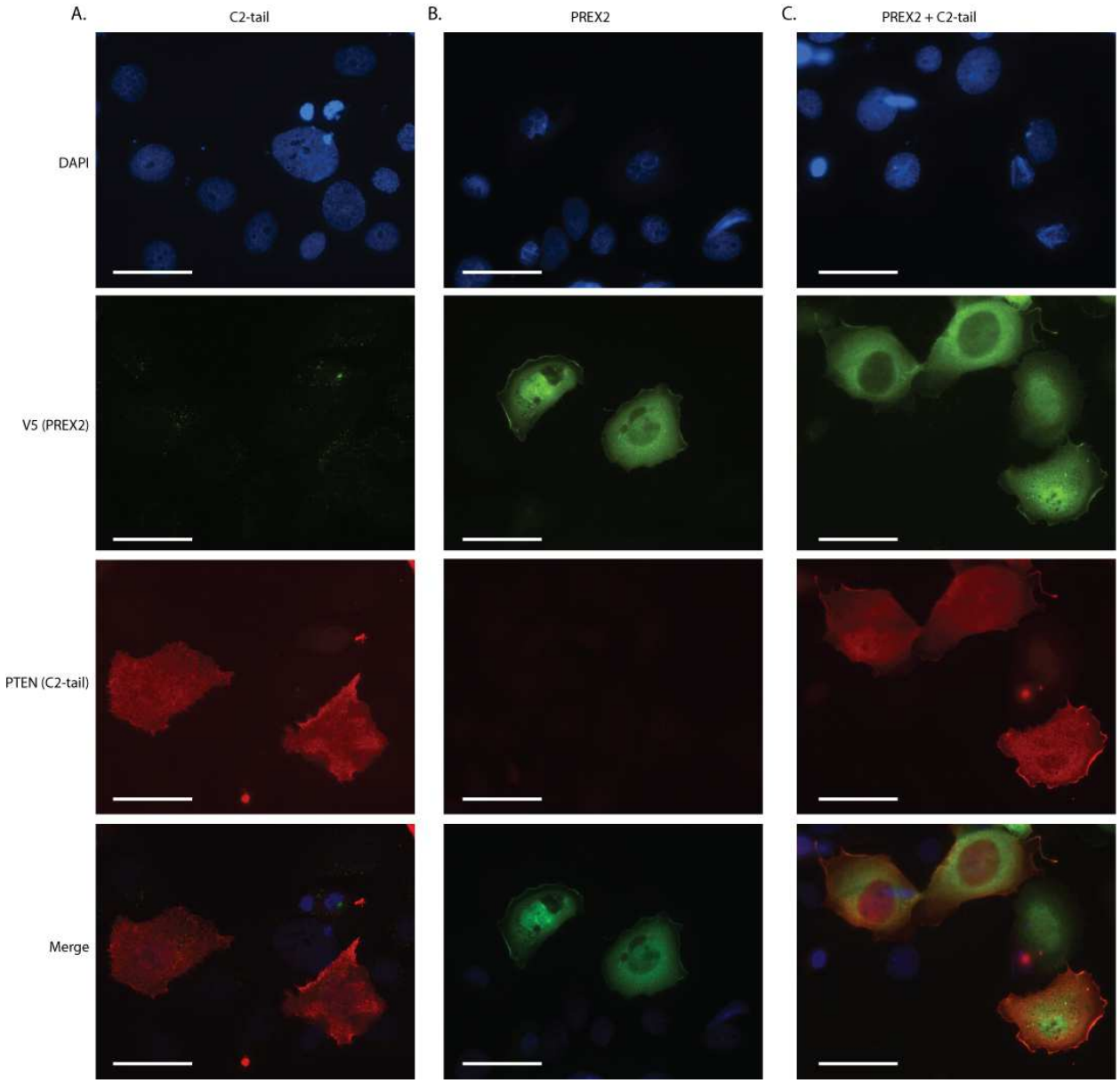

**Supplemental Figure 1. *PTEN* loss is associated with poor prognosis in breast cancer; *PREX2* is expressed in breast cancer.** (A and B) We used GOBO (Gene Expression-Based Outcome for Breast Cancer Online), a publicly-available database that has expression data for 1881 breast tumor samples, to examine the relationship between PTEN and survival *(24)*. Samples were divided into PTEN low (log2 expression -2.8 to -0.01) and high (log2 expression -0.01 to 2.4) groups. (A) Low *PTEN* expression is associated with decreased survival and (B) decreased distant metastasis free survival (DMFS) in breast cancer overall. (C) *PREX2* mRNA expression was measured by using quantitative PCR in breast tumors relative to non-tumor

breast tissue. $Log_2$ *PREX2* mRNA expression is shown. P values were calculated using two-tailed t tests. (D) *PREX2* is widely expressed is breast cancer. *PREX2* expression was compared among the PAM50 breast cancer subtypes by using the GOBO database *(24).* (E) PREX2 protein abundance was compared across the indicated tissue samples from normal breast, prophylactic mastectomy and breast tumors. Immunoblots are representative of two experiments.

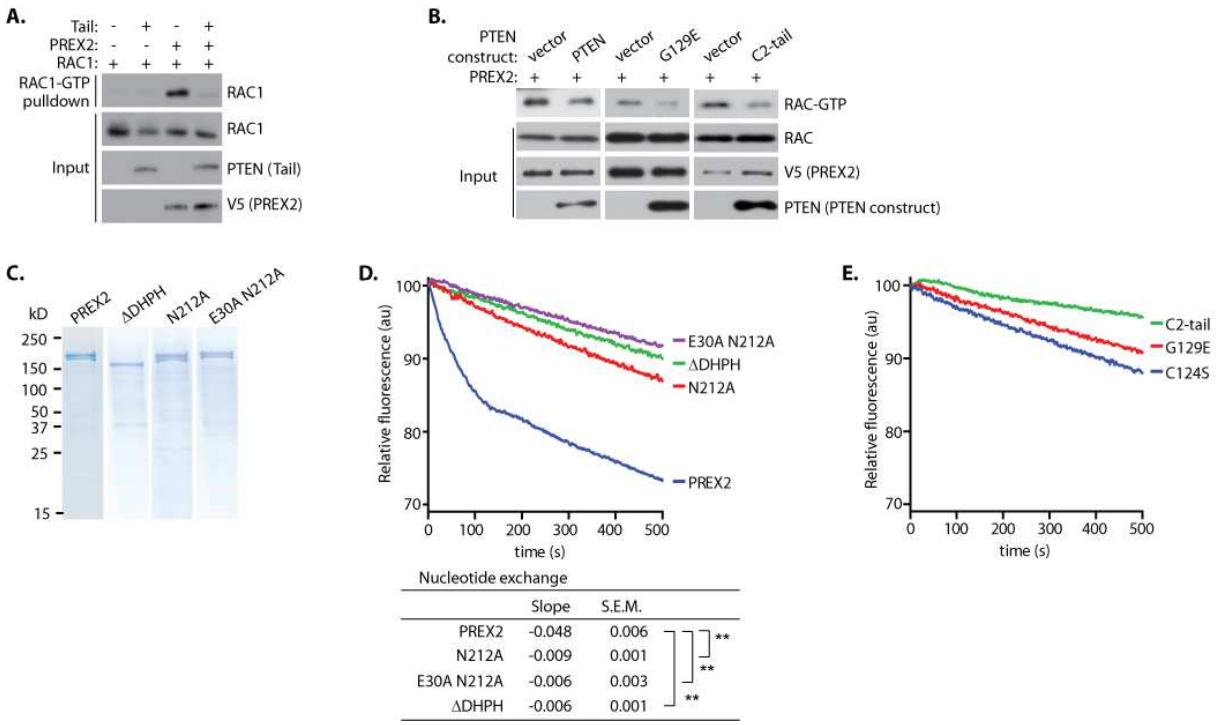



**Supplemental Figure 2. PTEN C2-tail suppresses invasion in BT549 and SUM149 cells; C2-tail does not antagonize PREX1-driven invasion.** (A) PREX2 drives invasion in SUM149 cells. (B) Representative blots from cells transfected with FLAG-PTEN deletion constructs with and without PREX2-V5 and used in invasion studies. (C) PTEN C2-tail does not decrease the amount of phosphorylated AKT (pAKT). U87-MG cells were transfected as indicated, starved one hour and then stimulated with 10 µg/ml insulin. Immunoblots were probed as indicated. (D)

PTEN GFP-tail inhibits invasion in BT549 cells. (E) PTEN GFP-tail inhibits invasion in SUM149 cells. (F) Co-immunoprecipitation of PREX2 with PTEN G129E and PTEN C2-tail. (G) Neither full-length PTEN nor C2-tail interacts with PREX1. BT549 cells were transfected with PREX1 plus FLAG-PTEN or FLAG-C2-tail, and FLAG constructs were immunoprecipitated. (H) C2-tail does not suppress PREX1-driven invasion. BT549 cells were transfected as indicated and invasion experiments were performed using an FBS gradient. “Percent invasion” was calculated by dividing the number of invading cells by the total number of cells plated. Error bars for invasion data in (A), (D) (E), and (H) represent mean ± s.e.m. for three independent experiments. P values were calculated using two-tailed t tests. Immunoblots in (B), (C), (F), and (G) are representative of two experiments.

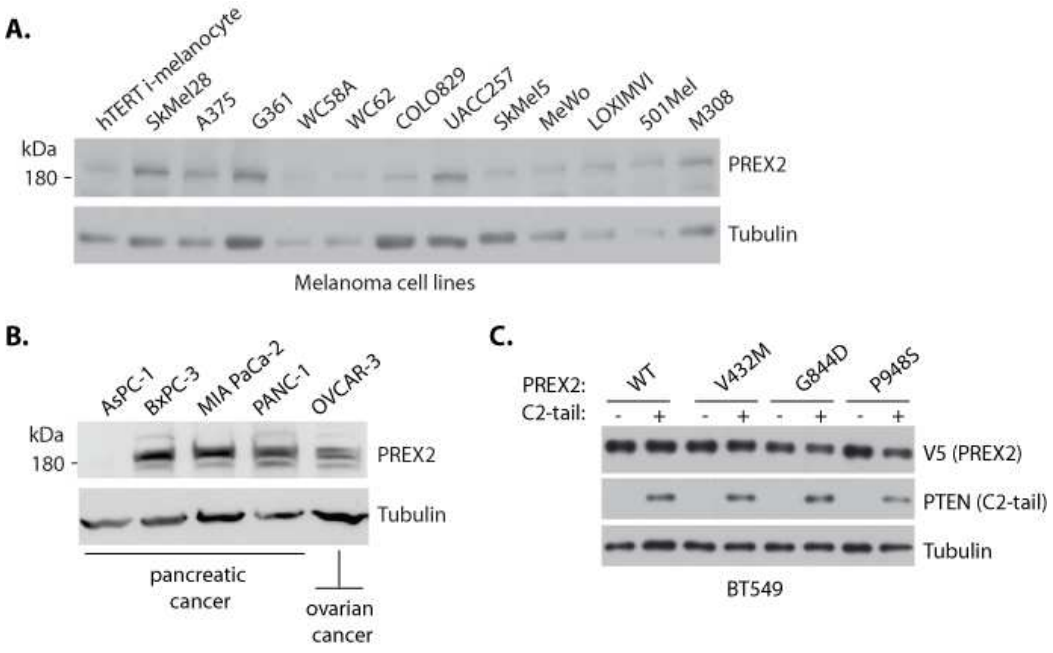



**Supplemental Figure 3. PTEN C2-tail does not alter PREX2 localization.** HCC1937 cells were transfected with either (A) C2-tail, (B) PREX2, or (C) co-transfected with both C2-tail and PREX2. All images were captured at 400x. Images are representative of 2 sets of cells. Scale bar, 50 µm.



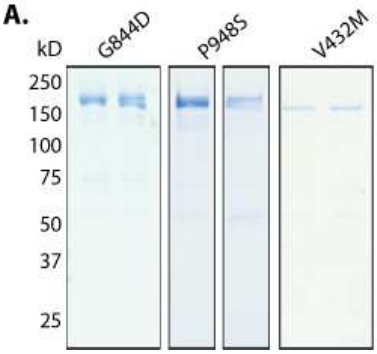

**Supplemental Figure 4. PTEN inhibits PREX2-catalyzed RAC activation.** (A) PTEN GFP-tail decreases RAC activation. Cells were starved overnight and then RAC activation was measured by pulldown of RAC-GTP. Immunoblots are representative of two experiments. (B) Wild-type PTEN, G129E, and C2-tail decrease the activation of endogenous RAC1. BT549 cells were co-transfected with PREX2 and either wild-type PTEN, G129E, or C2-tail. Cells were stimulated with media containing 10% FBS, and RAC activation was measured by pulldown of RAC-GTP normalized to total RAC. Immunoblots are representative of at least two experiments. (C) Coomassie stains of purified PREX2 proteins. (D) Effect of PREX2 GEF-dead mutants (N212A single mutant and N212A, E30A double mutant) and PREX2 ΔDHPH on the kinetics of mant-GDP dissociation from RAC as measured by a fluorimeter. (E) Effect of PTEN constructs (C2-tail, G129E, and C124S) on the kinetics of mant-GDP dissociation from RAC as measured by a fluorimeter. *PREX2 was not added to any of these reactions. For (D) and (E) RAC, PTEN

and PREX2 were present in exchange reactions at concentrations of approximately 20 nM, 10 nM and 5 nM, respectively. 1000-fold molar excess of GTP was added to the reaction to initiate dissociation of mant-GDP from RAC. Lines represent the average of at least three independent runs, and the slopes of the linear phases of nucleotide exchange were calculated. Two-tailed Mann-Whitney non-parametric tests were used to compare slopes of nucleotide exchange experiments.




**Supplemental Figure 5. PREX2 abundance in melanoma and pancreatic cancer cells and expression of PREX2 cancer mutants used in invasion studies.** (A) Melanoma cell lines were probed for PREX2 and tubulin. (B) Pancreatic and ovarian cancer cell lines were probed for PREX2 and tubulin. (C) Representative blots of BT549 cells transfected with the indicated constructs and used in invasion assays. Immunoblots in (A), (B), and (C) are representative of at least two experiments.

A.




B.

Nucleotide exchange

| | Slope | S.E.M. |
|---|---|---|
| C2-tail | -0.001 | 0.005 |
| V432M | -0.019 | 0.004 |
| V432M+C2-tail | -0.017 | 0.004 |
| P948S | -0.014 | 0.001 |
| P948S+C2-tail | -0.013 | 0.003 |
| G844D | -0.018 | 0.005 |
| G844D+C2-tail | -0.009 | 0.002 |

**Supplemental Figure 6. Purification of PREX2 mutants for use in nucleotide exchange assays.** (A) Coomassie stains of purified PREX2 mutants. Purified proteins were quantified by loading onto gels along with BSA standards. (B) Slopes of the linear phases of nucleotide exchange were calculated and the mean slope ± s.e.m of at least three independent experiments is shown.