Supporting Information

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SI Materials and Methods

Plasmids. $PTEN_{1–351}$-CFP was constructed by fusion PCR using the following internal primers: 5′-CTT GCT CAC CAT GCC GCC TAC TGT TTT TGT GAA GTA-3′ and 5′-ACA GTA GGC GGC ATG GTG AGC AAG-3′. The final PCR product was then TOPO cloned into pcDNA3.1D (Invitrogen) following the manufacturer's protocol. $PTEN_{352–403}$-YFP and $PTEN_{352–403(A4)}$-YFP were respectively PCR amplified and TOPO cloned into pcDNA3.1D. $PTEN_{1–351(CBR3)}$-YFP was generated by multi-site mutagenesis of $PTEN_{1–351}$-CFP-FLAG using the following oligonucleotides: 5′-GGT GAT ATC AAA GTA GAG TTC TTC CAC GCA CAG AAC GCG ATG CTA GCA GCG GAC GCA ATG TTT CAC TTT TGG-3′ and 5′-CCA AAA GTG AAA CAT TGC GTC CGC TGC TAG CAT CGC GTT CTG TGC GTG GAA GAA CTC TAC TTT GAT ATC ACC-3′. $PTEN_{10–351}$-CFP was generated by PCR using N-terminal truncation forward oligonucleotide and CFP reverse oligonucleotide and amplified using $PTEN_{1–351}$-CFP cDNA. All GFP variants contain monomeric A206K mutation.

Cell Cultures. HEK293T cells were maintained in DMEM (Invitrogen) supplemented with 10% FBS (Invitrogen). U87MG cells (American Type Culture Collection no. HTB-14) were maintained in Eagle minimal essential medium (American Type Culture Collection) supplemented with 10% FBS. HEK293T cells were transiently transfected on 100-mm dishes with 8 μg of DNA using Polyfect transfection reagent (Qiagen) following the manufacturer's protocol. Cells were then incubated in complete media for 20 h before usage. U87MG cells were transiently transfected on 100-mm dishes with 10 μg of DNA using Fugene HD transfection reagent (Roche) with a ratio of 5 (μL Fugene to 2 μg DNA following the manufacturer's protocol. Cells were then incubated in complete media for 48 h before use.

Microscopy. Cells were seeded on four-welled chambered coverglass (Nunc International) and transfected with appropriate cDNA constructs. Wells were washed and imaged with Hanks' balanced salt solution (Invitrogen) supplemented with 1g/L glucose and 25 mM Hepes. Fluorescent images were acquired using an Olympus IX71 inverted microscope and captured on an EMCCD camera (Photometric). CFP and YFP images were acquired by excitation/emission transmission at 457/470 nm and 514/535 nm, respectively.

Stimulation. U87MG cells were seeded on four-welled chambered cover-glass and transfected with respective constructs for 24 h using the protocol described earlier. Cells were then serum starved for 20 h. Wells were washed and imaged as previously described. The cells were stimulated with 20 ng/mL epidermal growth factor (E9644; Sigma).

Immunoprecipitation. HEK293T cells expressing respective constructs were lysed in buffer containing 10 mM Tris-HCl (pH 7.5), 140 mM NaCl, 5 mM EDTA, and 0.1% Nonidet P-40, and supplemented with 1 mM DTT, as well as phosphatase and protease inhibitor mixture (Sigma). The lysate was then cleared by centrifugation for 10 min 8,200 $\times$ *g* at 4 °C. The supernatant was then added to washed anti-FLAG affinity resin (Sigma) and gently inverted for 2 h. Approximately 10% of cleared whole cell lysate was used for input analysis. The resin containing bound antigen was then washed twice by inversion with lysis buffer for 5 min at 4 °C. The final wash supernatant was discarded and the resin was suspended in sample buffer and boiled.

Western Blotting. Samples were resolved by 4% to 15% Tris-HCl SDS/PAGE gel electrophoresis and transferred onto nitrocellulose (BioRad). GFP was detected using anti-GFP antibody. GFP antibody displayed equal affinity for CFP and YFP variants. Phosphorylation states of $PTEN_{352–403}$ was detected using phospho-PTEN (S380)-specific antibodies (Cell Signaling Technology). All Western blots were resolved by using HRP-conjugated secondary antibody with an ECL detection system (GE Healthcare).

iRap. Inducible PIP_2 depletion using CFP-FKBP-Inp54p, and dual color (i.e., CFP/YFP) confocal imaging of cells have been described previously (34). Briefly, HeLa cells were transfected with dimerization probes, CFP-FKBP-Inp54p and Lyn11-FRB together with YFP-labeled $PTEN_{C124S,A4}$. Cells were treated with iRap, which triggered a rapid translocation of CFP-FKBP-Inp54p to the plasma membrane. Examination of cells co-expressing $PH_{PLC\delta}$, a probe for PIP_2, showed that PIP_2 levels decreased by 84% within 2 min of iRap addition. Fluorescent signals from CFP and YFP in HeLa cells were alternately collected every 30 seconds on a spinning-disk confocal microscope (Yokogawa).

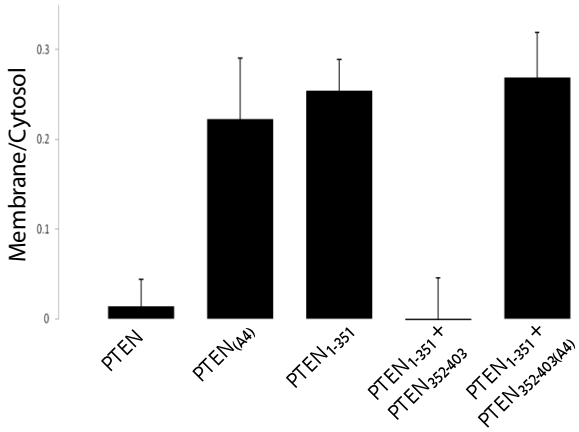


Fig. S1. Membrane localization of $PTEN_{1–351}$-CFP is inhibited by $PTEN_{352–403}$-YFP. Membrane-to-cytosol ratio of respective experiments was calculated using the method referenced by Janetopoulos *et al.* (2005) *Dev Cell* 8:467–477.

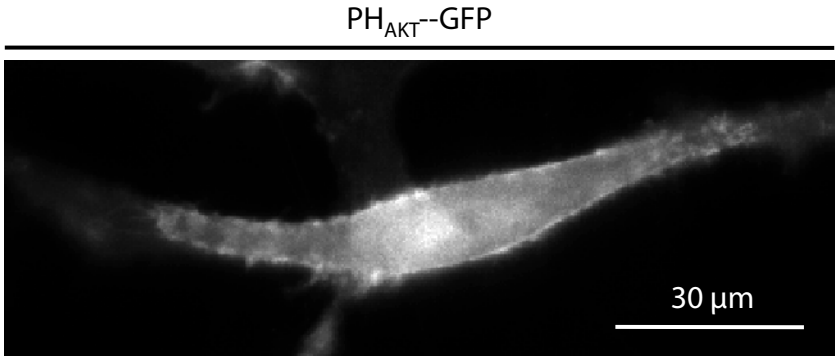


Fig. S2. PH$_{AKT}$-GFP localizes to the U87 plasma membrane. U87 cells were transfected with PH$_{AKT}$-GFP and localization of the probe was assessed.

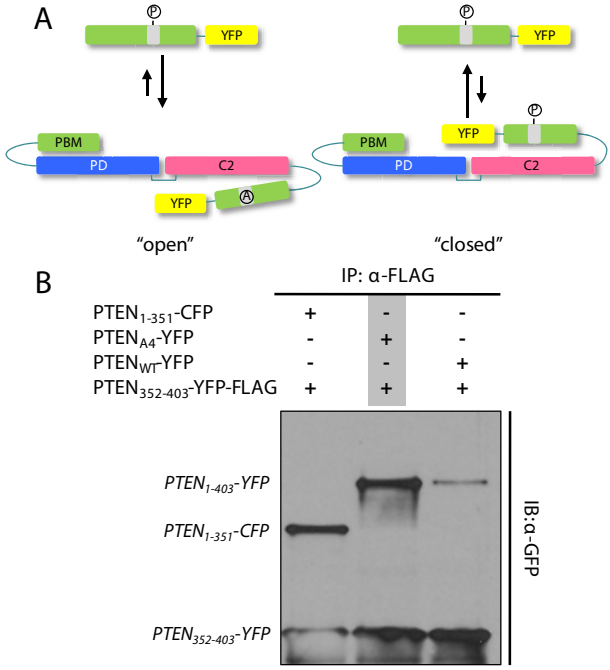


Fig. S3. Exogenous C-terminal tail of PTEN preferentially associates with ''open'' PTEN. (*A*) Schematic representation of the strategy used to assess the relative interaction of exogenous C-terminal tail with $PTEN_{1–351}$-CFP, PTEN $_{A4}$-YFP, and $PTEN_{WT}$-YFP. (*B*) Competition of endogenous carboxy-terminal domain with exogenous co-expressed $PTEN_{352–403}$-YFP-FLAG was assessed by co-transfection with $PTEN_{1–351}$-CFP, $PTEN_{A4}$-YFP, or $PTEN_{WT}$-YFP in HEK293T cells. Cell lysates were immunoprecipitated using anti-FLAG affinity beads, and analyzed by Western blot using anti-GFP antibody.

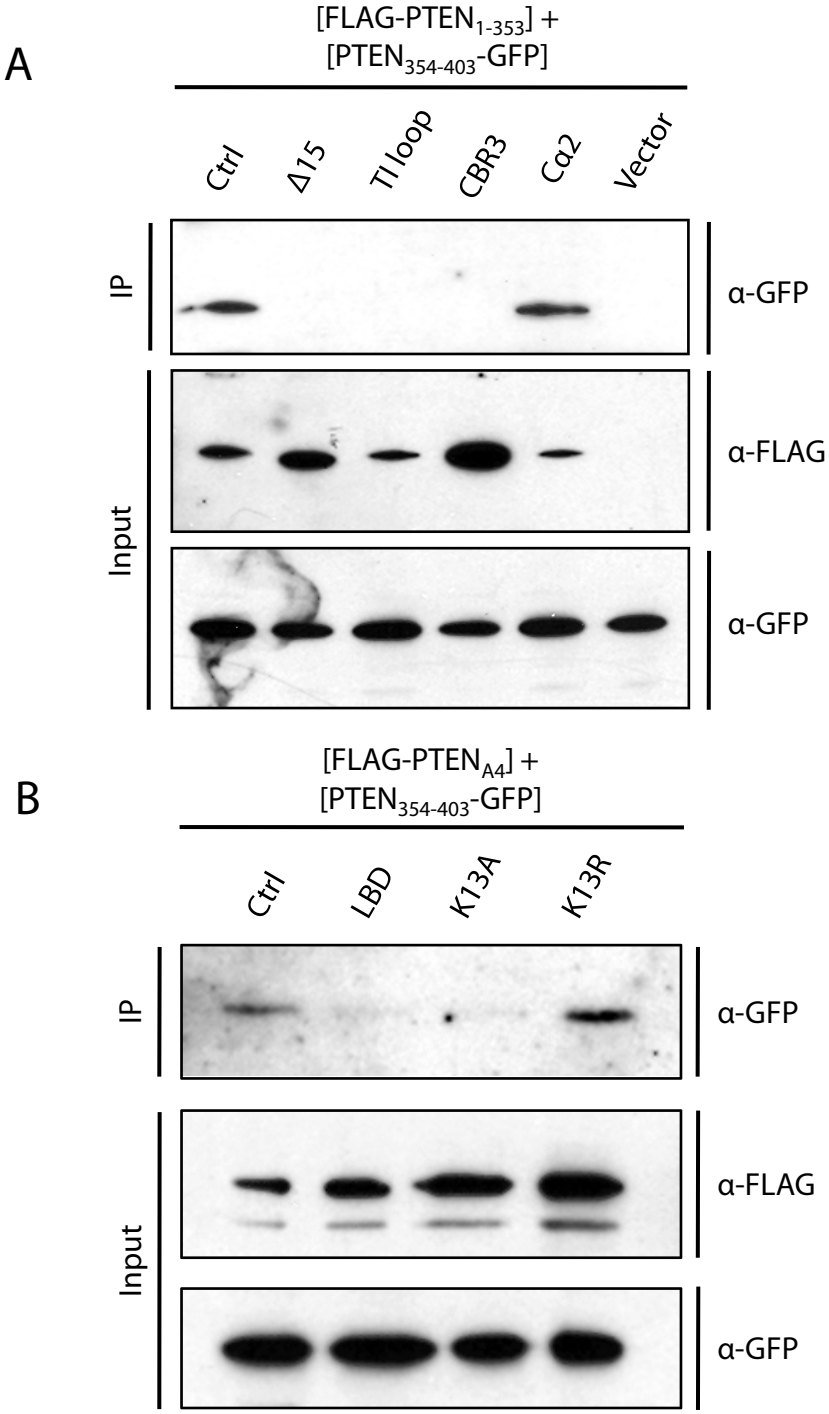
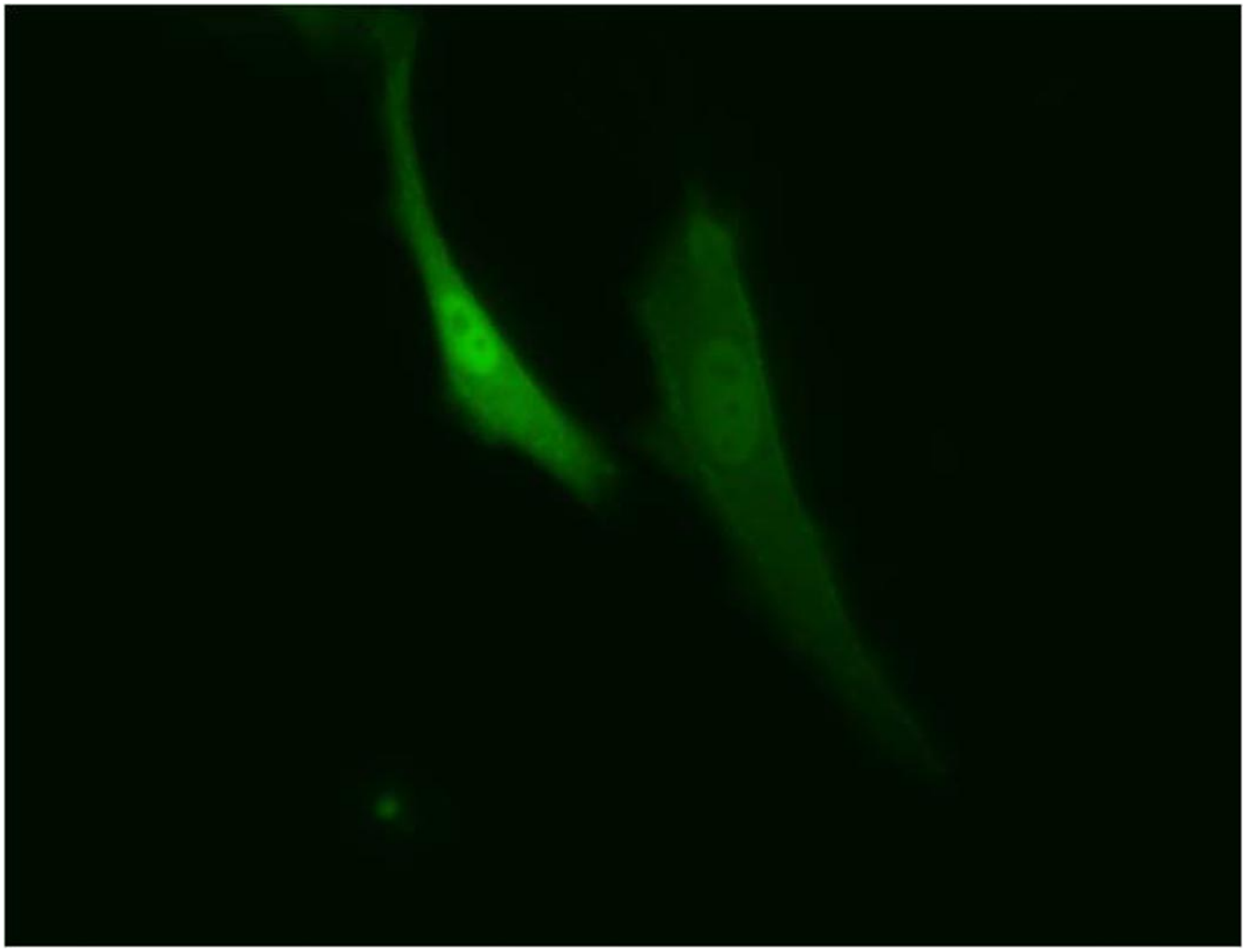
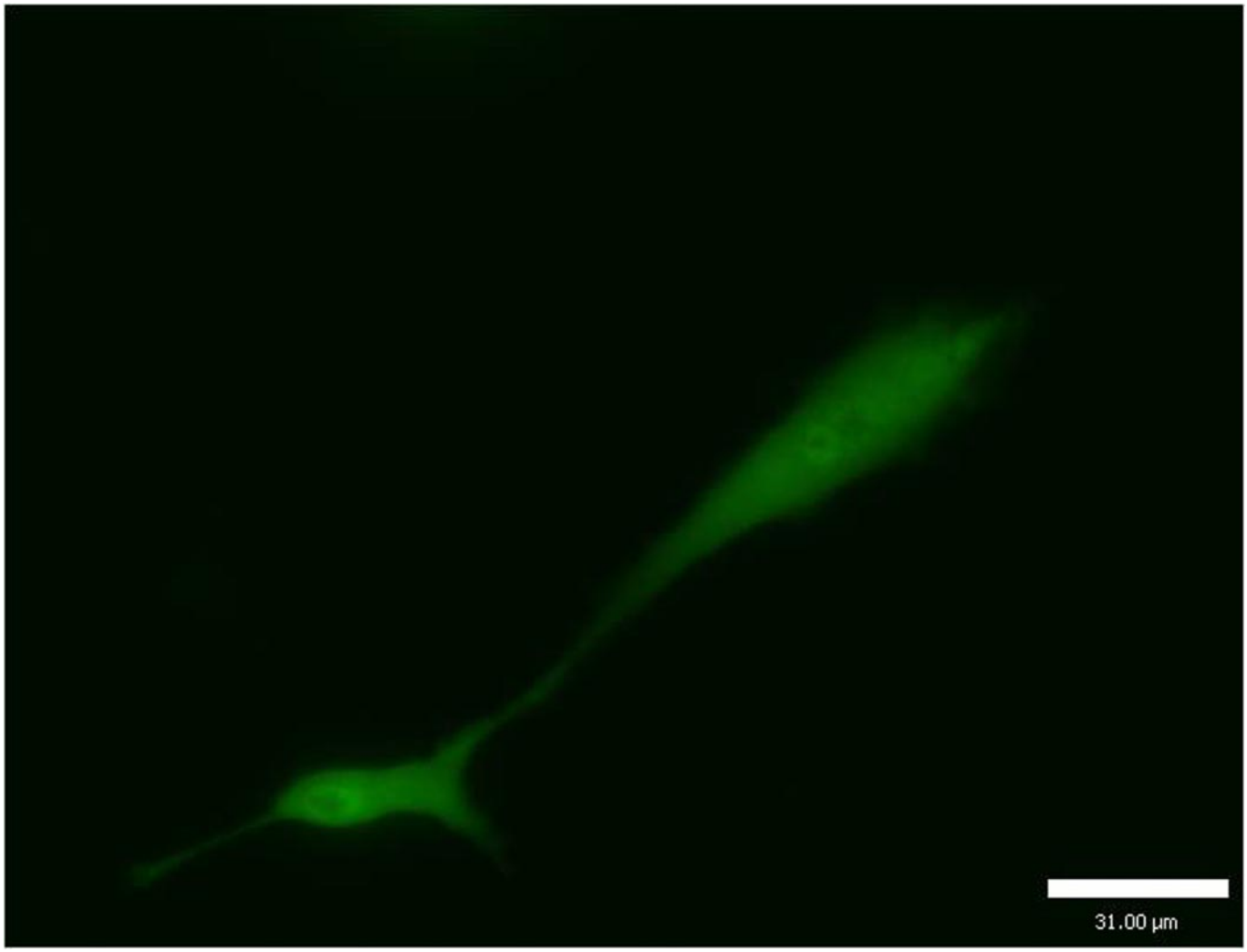


Fig. S4. Perturbations of the lipid binding domain and the TI and CBR3 loops disrupt the intramolecular interaction of $PTEN_{1-353}$ with $PTEN_{354-403}$. Experiments were carried out similarly to previous immunoprecipitations in Figs. 3–5. (*A*) The interaction of FLAG-$PTEN_{1-353}$ and $PTEN_{354-403}$-GFP was assessed (i.e., control). This interaction was also tested after deletion of the first 15 N-terminal residues of FLAG-$PTEN_{1-353}$ (i.e., control), after substitutions of the TI (R161A, K163A, and K164A) and CBR3 (K263A, M264A, L265G, K266A, K267A, and K269A) loops, or perturbation of Cα2 (K327A, N329A, K330A, K332A, and R335A). (*B*) The interaction of FLAG-$PTEN_{A4}$ and $PTEN_{354-403}$-GFP was analyzed after substitutions of K13R, R14A, and R15A in FLAG-$PTEN_{A4}$ (i.e., LBD) or single substitutions K13A and K13R.



Movie S1. PH_{AKT}-GFP translocation in U87 cells expressing $PTEN_{WT}$. U87 cells co-transfected with $PTEN_{WT}$ and PH_{AKT}-GFP. The cells were serum starved 20 h and treated with 20 ng/mL EGF. Localization of the PIP_3 probe PH_{AKT}-GFP was assessed.

Movie S1 (MOV)



Movie S2. PH_{AKT}-GFP translocation in U87 cells expressing $PTEN_{A4}$. U87 cells co-transfected with $PTEN_{A4}$ and PH_{AKT}-GFP. The cells were serum starved 20 h and treated with 20 ng/mL EGF. Localization of the PIP_3 probe PH_{AKT}-GFP was assessed.

Movie S2 (MOV)