Supplementary information

Identification of PTEN at the ER and MAMs and its regulation of Ca^{2+} signaling and apoptosis in a protein phosphatase-dependent manner

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Supplementary Figure Legends

Supplementary Figure 1. Intracellular distributions of PTEN in MEFs. Protein components of subcellular fractions prepared from mouse embryonic fibroblast (MEF) cells were loaded on 10% SDS-polyacrylamide gels and revealed by western blot. PTEN was detected with a specific monoclonal antibody. Marker proteins indicate ER (IP3R3), MAMs (FACL4), cytosol (β-tubulin), nucleus (PCNA) and mitochondria (SOD-2). H: homogenate; Mc: crude mitochondrial fraction; Mp: pure mitochondrial fraction; ER; MAMs; C: cytosol.

Supplementary Figure 2. Densitometric analysis and plotting of Akt phosphorylation status presented as the ratio between phosphorylated and total protein. Quantification of WB analysis on HEK-293 lysates from mock- (control) or siRNAs-PTEN transfected cells (**a**), and form cells transfected with either control, PTEN, GFP-PTEN[NLS], GFP-PTEN[NES], snap25-PTEN, AKAP-PTEN or ER-PTEN encoding plasmids (**b**). Densitometry of band intensity is expressed relative to control (100%). Data are mean ± SEM from 3 to 5 independent experiments. (**c**) Quantification of Akt phosphorylation in subcellular fractions prepared from HEK-293 cells transfected with recombinant wild type PTEN or ER-PTEN encoding plasmids. Densitometric

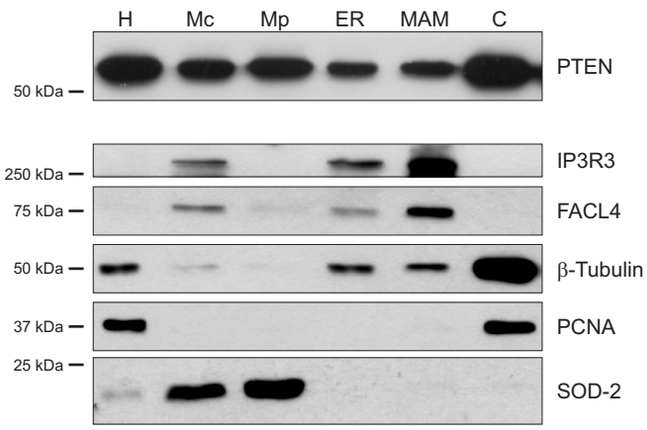
analysis of $pAkt^{Ser473}$/Akt bands intensity in homogenate (H), crude mitochondria (Mc), ER, MAM and cytosol (C), is expressed relative to PTEN overexpressing cells (100%). Data are mean ± SEM for 3 independent experiments. * indicates $P < 0.05$.

Supplementary Figure 3. Effects of ArA on intracellular Ca^{2+} mobilization, apoptotic responses and PTEN interaction with IP3R3, Related to Figures 4 and 5. (**a**) Fura-2/AM measurements in HEK-293 cells preincubated with 2 μM xestospongin C (XeC) or vehicle for 16 hours. XeC is a membrane-permeable inhibitor of the IP3-mediated Ca^{2+} release when used at low micromolar concentrations. In the presence of XeC, the increase of $[Ca^{2+}]_c$ in response to 80 μM ArA was significantly depressed, suggesting that the latter involved Ca^{2+} release via IP3R. (**i**) Typical tracing of the $[Ca^{2+}]_c$ response is presented as a 340/380 nm ratio. Each of the traces shown is representative of at least three independent experiments. (**ii**) Bars graphs (mean ± SEM) show the change in percentage of cytosolic Ca^{2+} increases normalized as $\Sigma(F_{340}/F_{380})$ over time, in comparison to cells incubated with vehicle. (**b**) Fura-2/AM measurements in Ca^{2+}-free medium. HEK-293 cells overexpressing PTEN, ER-PTEN, or mock-transfected, were loaded with Fura-2/AM. Where indicated, Ca^{2+}-free conditions were obtained as described in Materials and Methods. The basal fluorescence signal was monitored prior to treatment: 1 mM EGTA was added to Ca^{2+}-free medium at 15 s to chelate residual Ca^{2+}, and 80 μM ArA was added at 75 s. Under these conditions, the addition of ArA still stimulated an initial increase in intracellular free Ca^{2+}, however, the slower subsequent rise in intracellular Ca^{2+} which was detected in the presence of extracellular Ca^{2+} (Figures 4a-i and 4b-i) was not observed. (**i**) The kinetic behavior of the $[Ca^{2+}]_c$ response is presented as a 340/380 nm ratio. Each of the traces shown is representative of at least three independent experiments. (**ii**) Bars graphs (mean ± SEM) show the change in percentage of cytosolic Ca^{2+} increases normalized as $\Sigma(F_{340}/F_{380})$ over time, in comparison to untransfected cells. (**c**) Densitometric analysis of normalized cleaved caspase-3 protein levels in HEK-293 cells treated with 80 μM ArA for 120 min. In the bar graphs mean ± SEM is expressed relative to control mock-tranfected cells (100%), for 4 independent experiments. (**d**) Quantification of CoIP data presented in Figure 5c. Protein levels were normalized to the IgG light chain bands. Densitometry of bands intensity is expressed relative to untreated control (100%). Data are mean ± SEM for 3 independent experiments.

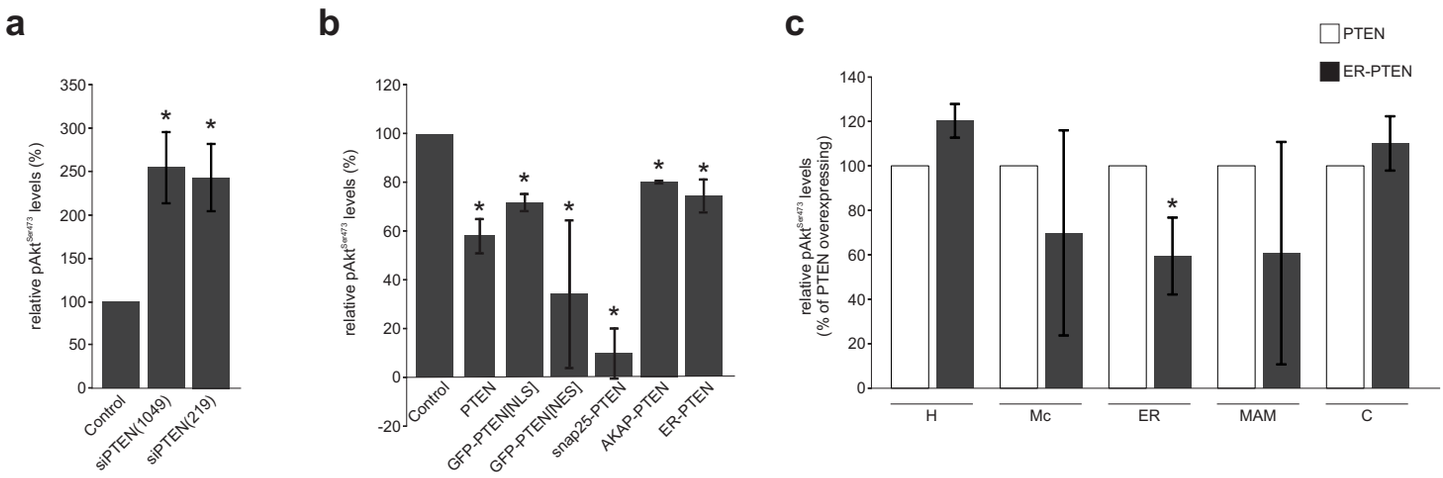
Supplementary Figure 4. Regulation of ER Ca^{2+} mobilization requires PTEN protein, but not lipid, phosphatase activity, Related to Figure 6. (**a**) Densitometric quantification of WB and plotting of the $pAkt^{Ser473}$/Akt ratios is expressed relative to ER-PTEN transfected cells (100%).

Normalization between phosphorylated Akt and Akt indicates an increased of phosphorylation in HEK-293 cells lysate from ER-PTEN(C124S) transfected HEK-293 cells in comparison to ER-PTEN or ER-PTEN(G129E) transfected cells. Data are mean ± SEM for 5 independent experiments. (**b**) HEK-293 cells overexpressing ER-PTEN, ER-PTEN(C124S), ER-PTEN(G129E) or mock-transfected, were loaded with Fura-2/AM to measure changes in intracellular Ca^{2+} response induced by ArA in Ca^{2+}-free conditions, as in Supplementary Figure 3a. (**i**) The kinetic behavior of the $[Ca^{2+}]_c$ response is presented as a 340/380 nm ratio. Each of the traces shown is representative of at least three independent experiments. (**ii**) Bars graphs (mean ± SEM) show the change in percentage of cytosolic Ca^{2+} increases normalized as $\Sigma(F_{340}/F_{380})$ over time, in comparison to untransfected cells. (**c**) Quantification of CoIP data presented in Figure 6g. Protein levels were normalized to the IgG light chain bands. Densitometry of bands intensity is expressed relative to ER-PTEN(C124S) transfected cells (100%). Data are mean ± SEM for 3 independent experiments. * indicates $P < 0.05$.

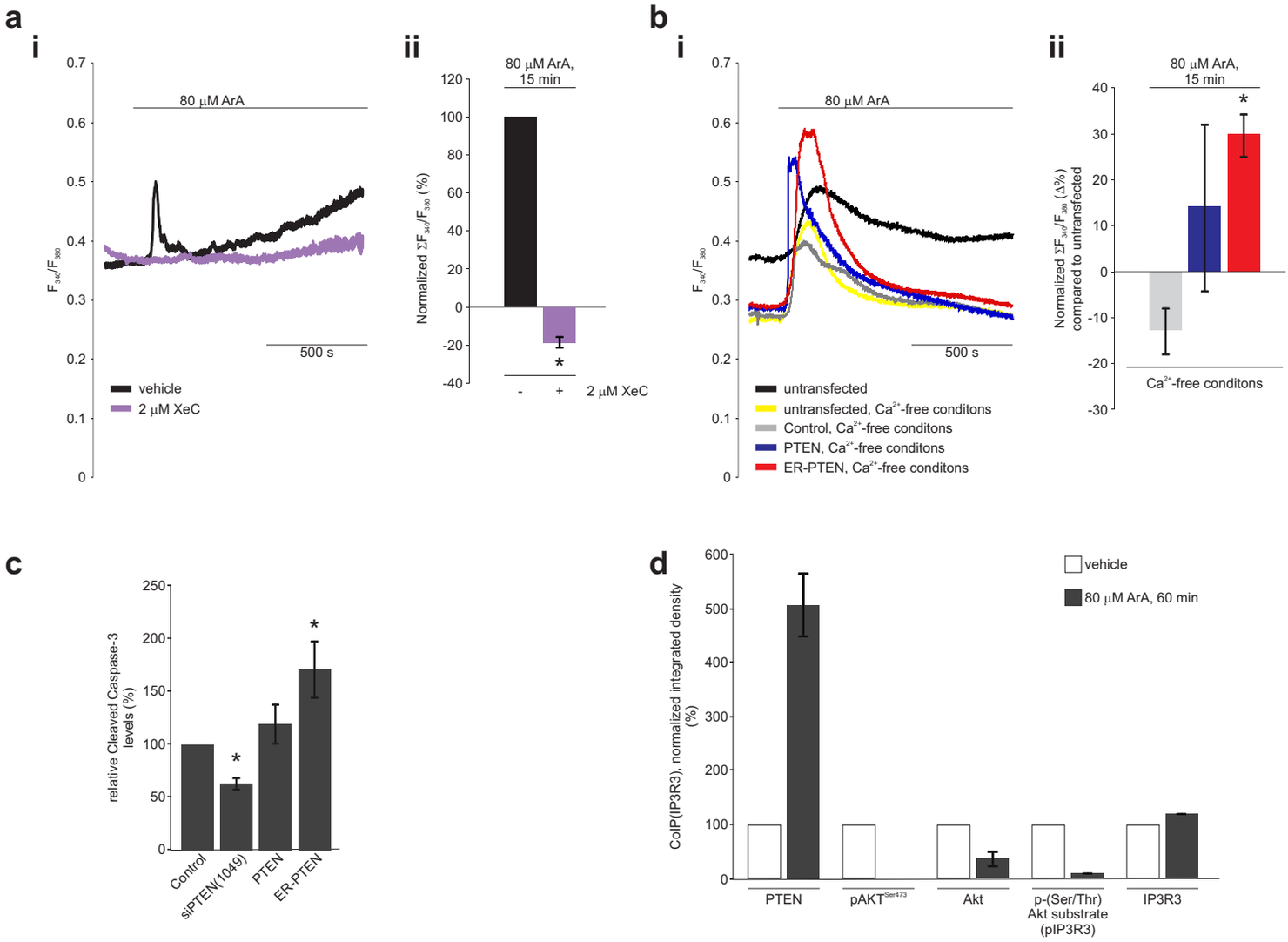
Supplementary Video data. Increased localization of PTEN at the ER induced by ArA treatment, Related to Figure 5. Cells were transfected with GFP tagged PTEN (GFP-PTEN) or GFP. At 48 hours after transfection, cells were labeled with ER-tracker RED (Life Technologies), according to the manufacturer's protocol. Time-lapse recording during 80 μM ArA treatment was performed as in Figure 5a. (**Video 1** and **Video 2**) Time-lapse recording of GFP-PTEN dynamics during ArA treatment; GFP-PTEN (left), ER-Tracker (middle) and merged (right). (**Video 3**) Time-lapse recording of GFP dynamics during ArA treatment; GFP (left), ER-Tracker (middle) and merged (right). Times are shown in the bottom left corner of the frame.



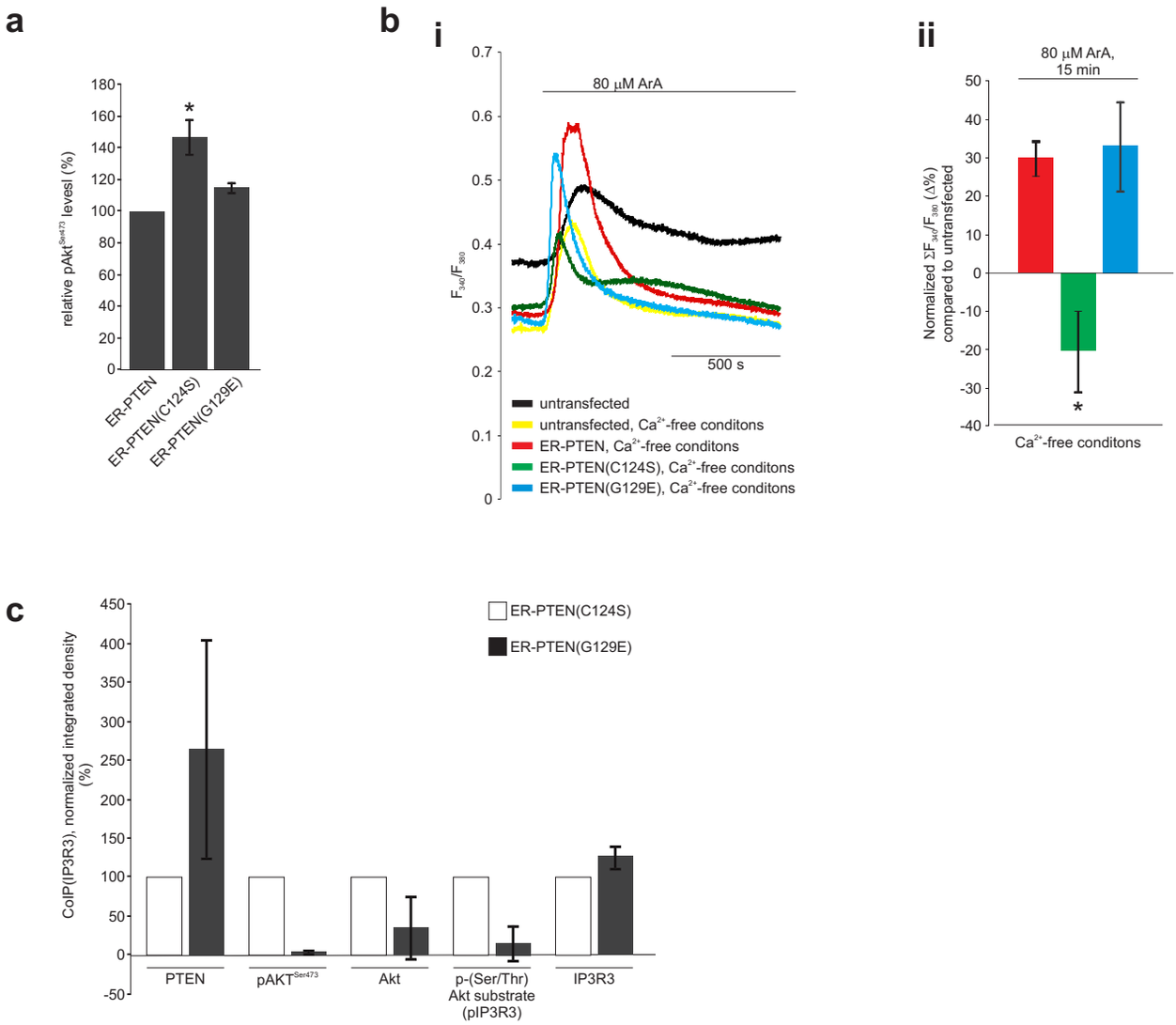
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4