

Supplementary Figure Legends

Fig. S1 β -arr enhances PTEN lipid phosphatase activity. **a.** Schematic representation of the bait chimera used to screen a human thymus cDNA library. An adaptor coding for five glycine residues was inserted between the cDNAs for hSos and β -arr2 Δ C1 encoding amino acids 1-337 of β -arr2, to reduce structural interference. **b.** Myc immunoprecipitates from HEK cells expressing vector control, Myc-PTEN, Myc-PTEN and FLAG- β -arr1 or Myc-PTEN and FLAG- β -arr2 were analyzed for the capacity to dephosphorylate water-soluble diC8-PIP3 (100 μ M).

Fig. S2 Active RhoAV14 increases β -arr-PTEN association. **a.** Co-immunoprecipitation analysis of FLAG- β -arr1 with PTEN in the presence or absence of active RhoA-V14 from lysates of cotransfected COS cells. Experiment was conducted as in Fig. 4a. **b.** Western blot showing siRNA-mediated reduction in β -arr levels using either a pan- β -arr1/2 rabbit polyclonal antibody (A1CT) or a β -arr2 specific rabbit monoclonal antibody (Cell Signalling).

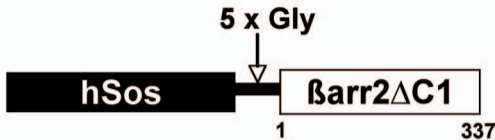
Fig. S3 β -arr1 and β -arr2 cooperate with PTEN to inhibit Akt activation. **a.** PTEN-null prostate cancer PC-3 cells were transfected with control vector, PTEN alone or PTEN in combination with β -arr1 or β -arr2, and pAkt levels were monitored by Western blot (lanes 1-4). Both β -arr1 and β -arr2 enhanced PTEN-mediated inhibition of Akt activation. When expressed alone (lanes 8-9) or with PTEN-G129E (lanes 6-7), β -arrs had no effect on pAkt levels versus relative control transfected cells. **b.** Active Akt levels (pThr308 and pSer473) in wild-type and β -arr1/2 DKO MEFs before and after stimulation with LPA. **c.** Transfection of β -arr1/2 DKO MEFs with β -arr1 inhibits Akt activation following LPA stimulation (0-60 mins).

Fig. S4 U373-PTEN cells express physiological levels of PTEN. **a.** 50 μ g of cell lysates from parental U373 cells, U373-PTEN replete cells and various cell lines expressing endogenous PTEN were monitored by Western blot for PTEN expression using an anti-PTEN mAb Santa Cruz PTEN (A2B1): sc-7974. The higher molecular mass observed with U373-PTEN cells is due to the presence of N-terminal His and Xpress tags (PTEN subcloned in pcDNA3HisC). **b.** Western blot of endogenous PTEN found in endogenous β -arr immunoprecipitates from wounded primary rat astrocytes. A1CT is a polyclonal antibody against β -arrs.

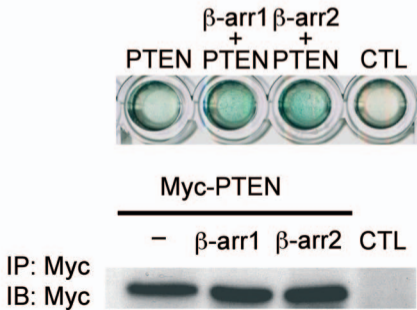
Fig. S5 Summary of PTEN constructs used for migration studies. Schematic representation of PTEN constructs used. The capacity of PTEN constructs to inhibit migration when injected alone is shown. In addition, the ability of β -arr to rescue migration of the corresponding PTEN mutants is shown.

Lima-Fernandes Fig. S1

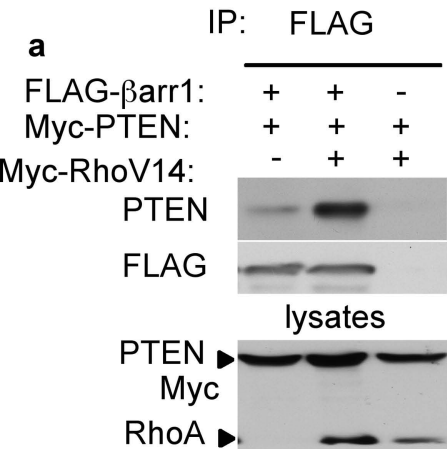
a



b

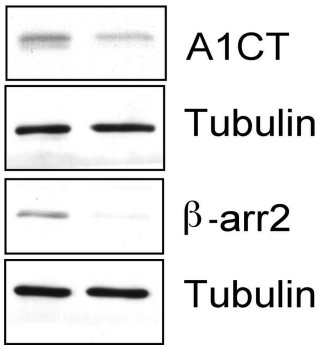


Lima-Fernandes Fig. S2



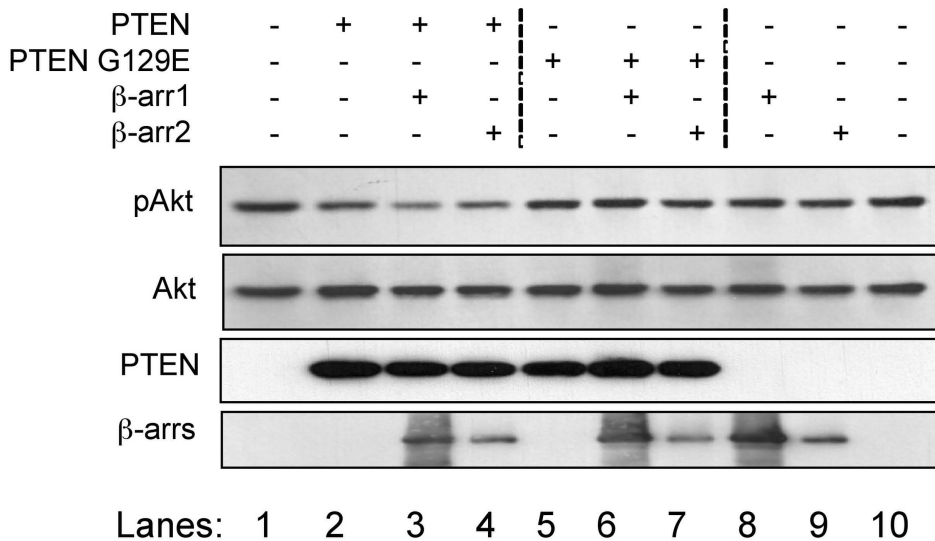
b

siRNA: CTL β -arr1/2

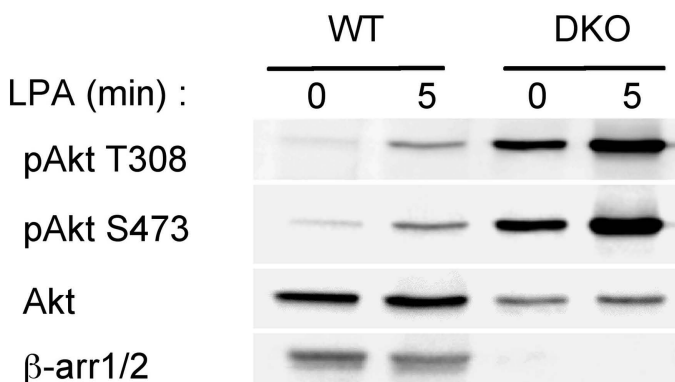


Lima-Fernandes Fig. S3

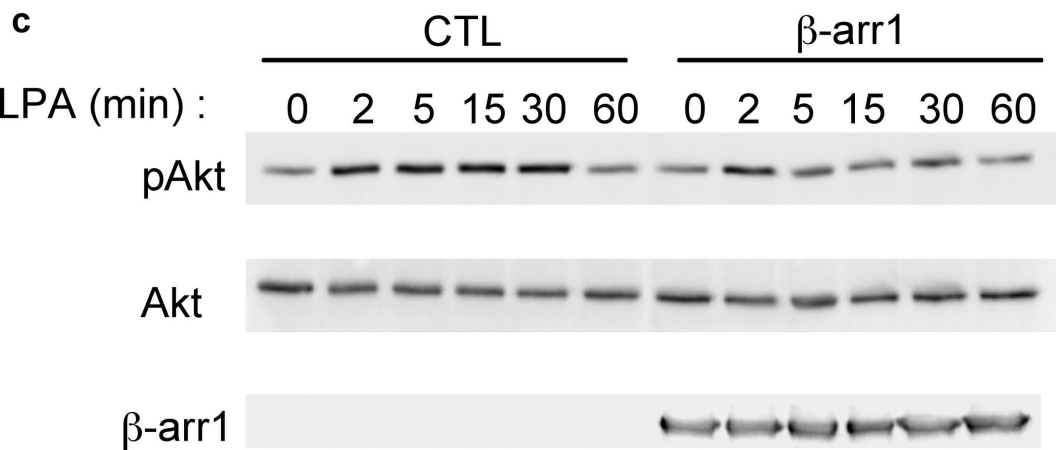
a



b

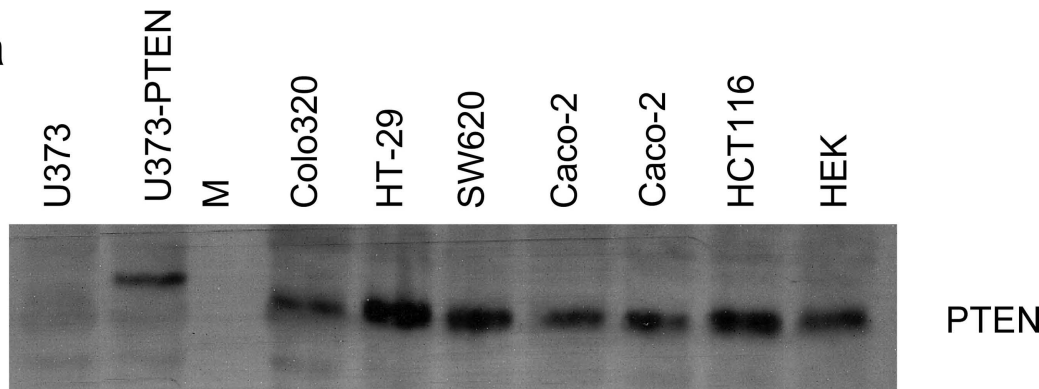


c

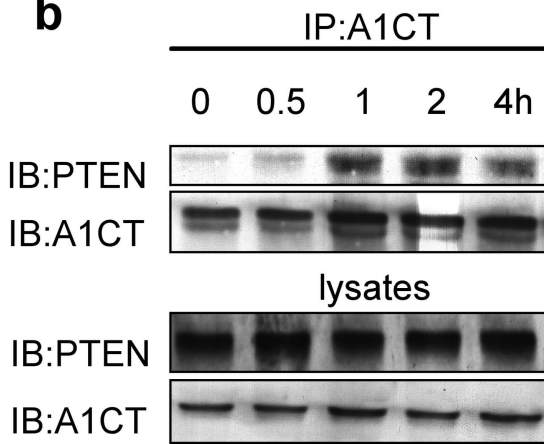


Lima-Fernandes Fig. S4

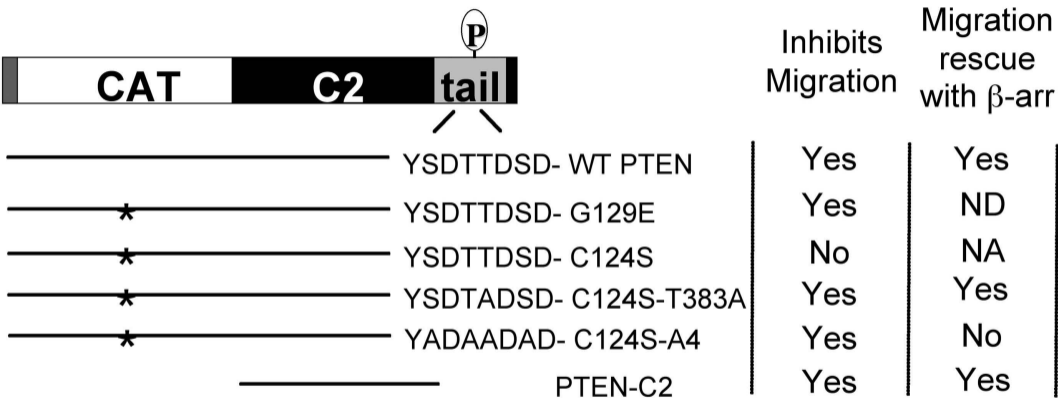
a



b



Lima-Fernandes Fig. S5



ND: not determined

NA: non-applicable