Supplementary Material

Materials and Methods

DNA constructs: The HA-tagged cDNAs of wtPTEN, PTEN(G129E) and PTEN(C124S) were a kind gift from N. K. Tonks (Cold Spring Harbor, NY, USA). cDNAs and deletion constructs were subcloned into pRK5-myc or pRK5-flag. The C2A domain of synaptotagmin was cloned into pRK5-myc using PCR primers on a synaptotagmin cDNA [A. D. Blagoveshchenskaya, E. W. Hewitt, D.F. Cutler, Mol Biol Cell 10, 3979-3990 (1999)] that was a kind gift from D. F. Cutler (University College London, London, UK). The alanine point mutants were generated using PCR primers on a PTEN(C124S) template and cloned into pRK5-myc. The aspartic acid mutants were generated using PCR primers on a wtPTEN or PTEN(C124S) template and cloned into pRK5-myc.

Migration assays: Human glioma cells were grown in DMEM, supplemented with 10% heat-inactivated fetal calf serum (PAA laboratories Ltd), 5% non-essential amino acids (GIBCO) and 5% penicillin / streptomycin (GIBCO-BRL), plated on acid-washed glass coverslips in four-well plates and allowed to reach confluency. The monolayer was scratched (wounded) with a sterile micropipette and 1h later constructs (0.1mg/ml in PBS) were microinjected into the nucleus of the cells in the first row at the wound edge. Cells were returned to a 5%CO2 incubator and left to migrate for 16h. Cells were fixed in 4% paraformaldehyde for 15 min and processed for immunofluorescence as described (15). Rat 3F10 anti-HA IgG (ROCHE) or anti-Myc mAb 9E10 was used to visualize HA and MYC tagged expressed constructs,
followed by FITC-goat anti-rat or anti-mouse IgG (Jackson ImmunoResearch Laboratories), respectively. Mouse anti-flag IgG (SIGMA) was used for detection of the flag-tagged constructs, followed by FITC-goat anti-mouse IgG (Jackson ImmunoResearch Laboratories). Rhodamine-labeled phalloidin (SIGMA) was used to visualize actin. Images were captured with a CCD camera and processed using Openlab software. Cells were scored as being positive for migration, if they were still found at the leading edge of the wound after fixation.

Immunoprecipitation and western blot analysis: COS-7 cells cultured in DMEM supplemented with 10% fetal calf serum (PAA laboratories Ltd) and 5% penicillin/streptomycin (GIBCO BRL) were plated onto six-well plates at a density of 1x 10⁵ cells per well and transfected 16h later by the GeneJuice method (NOVAGEN) according to the manufacturer’s specifications. Cells were rinsed on ice with cold PBS-A containing 1mM orthovanadate (SIGMA) and lysed on ice with Nonidet P-40 buffer (10 mM Tris/HCl [pH 7.5], 140 mM NaCl, 1 mM orthovanadate and 1% Nonidet P-40, 2 mM PMSF, 5 mM EDTA, 20 µg/ml aprotinin, 20 µg/ml leupeptin) 48h post-transfection. The nuclei were discarded following centrifugation at 13000 rpm for 2 min and the supernatant lysates were incubated at 4°C for 2h with specific antibodies and G-sepharose beads. The beads with the immunoprecipitates were centrifuged at 13000 rpm for 30 sec and extensively washed in Nonidet P-40 buffer. The immunoprecipitated proteins were eluted with SDS-sample buffer and resolved by 12% SDS-PAGE. The proteins were transferred to nitrocellulose membranes for western blot analysis. Mouse Anti-Myc mAb 9E10 or mouse anti-flag IgG (SIGMA) was used for detection of the MYC or FLAG tagged constructs, followed by peroxidase-conjugated goat anti-mouse IgG (PIERCE). Immunoblotting with rabbit
anti-PTEN (Upstate Biotechnology), followed by peroxidase-conjugated goat anti-rabbit IgG (PIERCE) was used for detection of endogenous PTEN.

**Phosphorylation assay:** COS-7 cells were plated onto six-well plates at a density of 2 x 10^5 cells per well and transfected 16h later by the GeneJuice method (NOVAGEN) according to the manufacturer's specifications. 20h post-transfection cells were washed twice with sodium phosphate-free MEM (SIGMA) and incubated with 32P-solution [PBS43, 5mCi (185MBq), Amersham Biosciences, 200µCi in 1ml MEM / well] for 4h at 37°C and 5% CO2. Cells were rinsed once with 5ml of cold PBS-A and lysed for 5min on ice with 150µl/ well of cold lysis buffer (40mM TrisHCl pH7.6, 150mM NaCl, 1mM EDTA, 1% Triton X-100), supplemented with one complete inhibitor tablet/ 50ml of buffer. Lysates were cleared by centrifugation at 13000rpm for 3 min and the Myc epitope-tagged PTEN constructs were precipitated by incubating the samples with 40µl of 50% Glutathione-sepharose beads + 5µl of anti-Myc 9E10 antibody for 2h at 4°C on a rotating wheel. The immunocomplexes were washed twice with cold lysis buffer, twice with wash buffer (40mM TrisHCl pH7.6, 150mM NaCl, 1mM EDTA) and finally twice with cold phosphatase buffer (25mM HEPES pH7.2, 50mM NaCl, 2.5mM EDTA, 10mM DTT). The proteins were eluted with sample buffer, resolved by 10%-SDS-PAGE and transferred onto nitrocellulose membranes for western blot analysis. The radioactive proteins were visualized by exposing the membrane to the Biorad Imaging screen K and the data were analysed using a phosphorimager (Biorad Molecular Imager FX) and Quantity One software (Biorad).