## SUPPLEMENTARY METHODS

### Construction of recombinant proteins and expression

The different GFP-tagged versions of PTEN were made by in-frame ligation of the EGFP coding sequence (Clontech) with the amino terminus of rat PTEN. The truncated form of PTEN (PTEN-ΔPDZ), lacking the C-terminal residues $Ile^{400}$-$Thr^{401}$-$Lys^{402}$-$Val^{403}$, the point mutants C124S and G129E, and the double mutant C124S-ΔPDZ were made by site-directed PCR mutagenesis. The catalytically inactive PTEN mutants (C124S and G129E) have been characterized previously (Myers et al., *Proc Natl Acad Sci USA*, **95**, 13513-13518, 1998). All constructs were prepared in pSinRep5 for expression using Sindbis virus. Recombinant proteins were expressed in hippocampal CA1 pyramidal neurons from organotypic slice cultures. Slices were prepared from postnatal day 5-7 rats and cultured from 5 to 10 days. Expression of the recombinant proteins was for 15 to 20 hours. All biosafety procedures and animal care protocols were approved by the University of Michigan Committee on Use and Care of Animals and the bioethics committee of the Consejo Superior de Investigaciones Científicas (CSIC).

### Antibodies

Antibodies used in this study were: PTEN (Neomarkers -mouse, Cell Signalling -rabbit); PSD-95 (Neuromab -mouse, Abcam -rabbit); MAGI-2 and αCaMKII (Sigma); Akt, phospho-Akt (Ser473) and phospho-Tyrosine (Cell Signalling); GluA1, phospho-GluA1 (Ser831 and Ser845), GluA2, phospho-GluA2 (Ser880) and GluN1 (Millipore); GFP (Roche).

**Synaptosomal/PSD fractionations and *in vitro* activation of NMDA receptors**

Biochemical isolation of synaptosomes and PSD fractions were carried out essentially as previously described (Carlin et al., *J Cell Biol*, **86**, 831-845, 1980). Briefly, hippocampi from 3-4 week rats were homogenize in a Dounce glass homogenizer with buffer A (0.32 M sucrose, 1 mM $MgCl_2$, 0.5 mM $CaCl_2$, 10 mM HEPES, 1 mM EGTA, 1 mM dithiothreitol and a cocktail of protease inhibitors from Roche –"Complete Mini EDTA-free"). This homogenate was spun down at 1400g for 10 min at 4°C. The supernatant was kept and the pellet was resuspended in buffer A and spun again at 710g for 10 min at 4°C. Both supernatants were mixed and spun down at 11,600g for 12 min at 4°C. The pellet (crude synaptosomal fraction) was resupended in buffer B (10 mM HEPES, 1 mM dithiothreitol, protease inhibitor cocktail) plus 0.32 M sucrose, and overlaid on a discontinuous density gradient containing two layers of buffer B plus 1.0 M and 1.4 M sucrose. After centrifugation at 82,500g for 1 hour at 4°C, the interphase between 1.0 M and 1.4 M was collected (synaptosomal fraction).

The NMDA receptor activation was induced on the purified synaptosomes by adding 1.2 mM $MgCl_2$, 2.5 mM $CaCl_2$, 20 μM of NMDA and 10 μM glycine, and incubating for 5 min at room temperature. The reaction was stopped by addition of 100 μM DL-2-Amino-5-phosphonovaleric acid (AP-5) and 1 mM EDTA. After 10 min incubation at room temperature, the fractionation was continued by adding 0.5% Triton X-100 and stirring for 15 min at 4 °C. Then, samples were spun down at 165,000g for 2h at 4°C to obtain the Triton insoluble pellet (PSD fraction). This pellet was resuspended in 5% SDS and analyzed by 7% PAGE-SDS and western blot.

**Electrophysiology methods**

Solutions: The recording chamber was perfused with ACSF: 119 mM NaCl, 2.5 mM KCl, 4 mM $CaCl_2$, 4 mM $MgCl_2$, 26 mM NaHCO3, 1 mM $NaH_2PO_4$, 11 mM glucose, 0.1 mM picrotoxin, and 4 μM 2-chloroadenosine (pH 7.4), gassed with 5% $CO_2$ / 95% $O_2$. Patch recording pipettes (3-6 MΩ) were filled with 115 mM cesium methanesulfonate, 20 mM CsCl, 10 mM HEPES, 2.5 mM $MgCl_2$, 4 mM $Na_2ATP$, 0.4 mM $Na_3GTP$, 10 mM sodium phosphocreatine, 0.6 mM EGTA (pH 7.25). In experiments with intracellular perfusion of peptide pep2m/G10 (Tocris; 0.5 mM final concentration), the patch recording pipette solution was supplemented with protease inhibitors leupeptin and pepstatin (100 mM each).

Synaptic plasticity methods: NMDAR-dependent LTD was induced by pairing low-frequency presynaptic stimulation (500 pulses at 1 Hz) with moderate postsynaptic depolarization (–40 mV). LTP was induced by pairing 3 Hz presynaptic stimulation (300 pulses) with postsynaptic depolarization at 0 mV. In LTD and LTP experiments, one pathway did not receive stimulation during depolarization, and therefore was used as a control pathway. mGluR-dependent LTD was induced by bath application of 50 μM (RS)-3,5-dihydroxyphenylglycine (DHPG) for 5 min. Cells were held under current clamp without synaptic stimulation during DHPG application and 5 minutes afterwards (Huber et al., *J Neurophysiol*, **86**, 321-325, 2001). These recordings were carried out in the presence of 100 μM (2R)-amino-5-phosphonovaleric acid (APV), to prevent any contribution to synaptic plasticity from NMDARs. When blocking PTEN activity, slices were incubated with 15 nM bpV(HO)pic for 15-20 hours before LTD or LTP induction. bpV(HO)pic was also present during the recordings.

## SUPPLEMENTARY FIGURE LEGENDS

**Supplementary Figure 1. Specificity of the NMDA receptor-dependent association between PTEN and PSD-95**. **A**. Total protein extracts from hippocampal slices were immunoprecipitated with anti-PSD95 antibody. Some slices were treated with 20 μM NMDA for 5 min before the immunoprecipitation and transferred to regular ACSF for 15, 25 or 60 min (+15', +25' or +60', as indicated). For the two right-most lanes, slices were preincubated with the NMDA receptor antagonist AP5 (100 μM) before and during the NMDA treatment (+AP5). Input lanes (left) contain 10% of the input from the baseline condition (without NMDA treatment). Coimmunoprecipitations were analyzed by western blot using PTEN and GluN1 antibodies, as indicated. **B**. Similar to A, with slices pretreated for 5 min with 20 μM NMDA, and transferred to regular ACSF for 15 min (+15') or 25 min (+25') after the NMDA treatment. Differences in protein migration between panels A and B can be explained by the different types of gels used (4–12% gradient Bis-Tris gels from Invitrogen –A–, *versus* custom-made gradient polyacrylamide gels –B–). Appropriate protein markers were used to estimate the molecular weight in each case. Star (*) indicates the position of the IgG used for immunoprecipitation in both experiments.

**Supplementary Figure 2. Expression of recombinant PTEN variants and effect on the PI3K pathway. A.** Western blot analysis of the phosphorylation of Akt at Ser473 from hippocampal slices expressing GFP, wild-type GFP-PTEN or the catalytically dead mutant GFP-PTEN-C124S. **B.** Quantification of Akt phosphorylation from three experiments as the one shown in A. Akt phosphorylation relative to total Akt levels was normalized to the values obtained from

GFP-expressing slices. **C.** Western blot analysis of the expression of recombinant GFP-PTEN, the point mutants C124S and G129E, the truncated mutant ΔPDZ and the double mutant C124S-ΔPDZ. Recombinant (GFP-fused) and endogenous PTEN were detected with an anti-PTEN antibody.

**Supplementary Figure 3. FRAP fluorescence recovery rates and PTEN distribution between spines and dendrites are not altered by NMDA receptor activation. A.** Fluorescence recovery time constants were estimated by exponential fits to the time courses shown in Fig. 2C-E. Values are plotted for untreated slices (0 min) or at different times after the 5 min NMDA treatment (black bar). Average values ± s.e.m. are plotted for GFP-PTEN (black symbols), GFP-PTEN-ΔPDZ (grey symbols) and GFP (white symbols). There was no significant change in FRAP time constant before and after NMDA treatment. **B.** Net GFP signal from GFP-PTEN (black symbols), GFP-PTEN-ΔPDZ (grey symbols) and GFP (white symbols) was quantified at spines and the adjacent dendritic shafts at different times, as described in A. The partition of these proteins between spines and dendrites (spine/dendrite ratio) was not significantly altered along the course of the experiment.

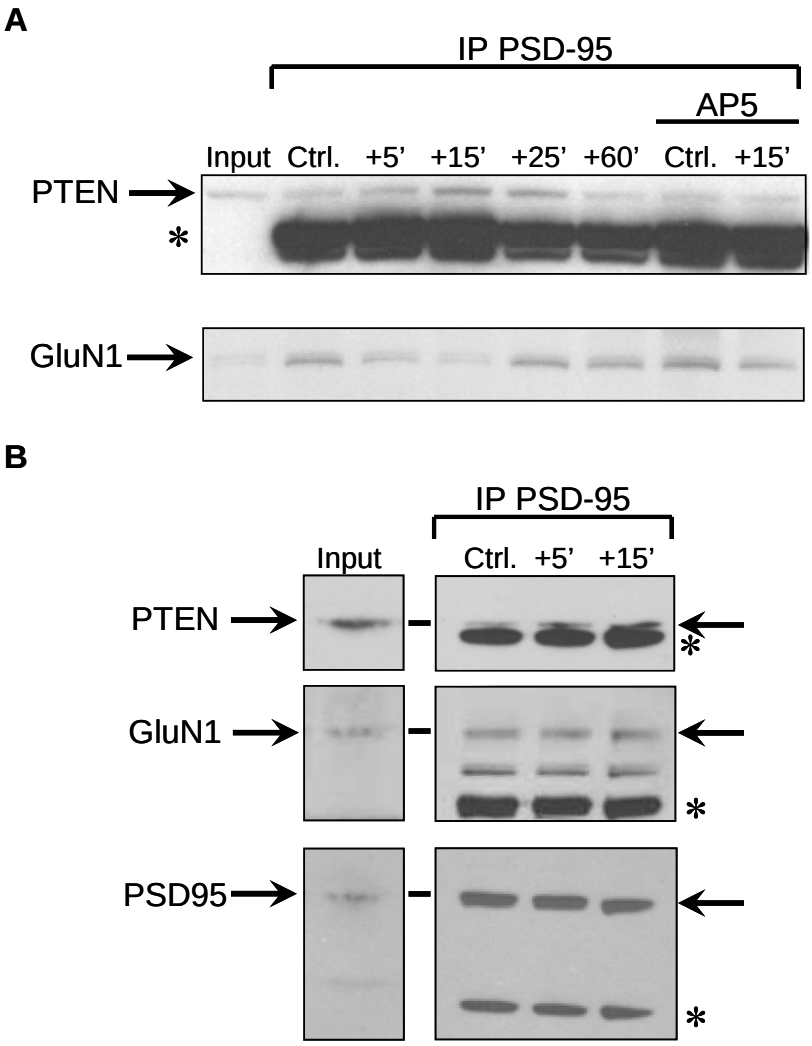
**Supplementary Figure 4. Effects of recombinant PTEN expression on passive membrane properties of CA1 hippocampal neurons. A-C.** The expression of GFP-PTEN, the point mutant C124S, the truncated mutant ΔPDZ and the double mutant C124S-ΔPDZ in CA1 hippocampal neurons did not alter input resistance (A), holding current (B) or whole-cell capacitance (C) when compared with uninfected control neurons. “n” represents number of cells.

**Supplementary Figure 5. Overexpression of wild-type or dominant negative (C124S) PTEN does not alter GluA1 or GluA2 phosphorylation. A.** Western blot analysis of GluA1, GluA2 and their phosphorylated forms (p-S880 for GluA2, p-S831 and p-S845 for GluA1) from hippocampal slices expressing GFP, GFP-PTEN or GFP-PTEN-C124S. **B.** Quantification of total and phosphorylated GluA1 and GluA2 from three experiments as the one shown in A. Values are normalized to GFP-infected slices (dashed line).

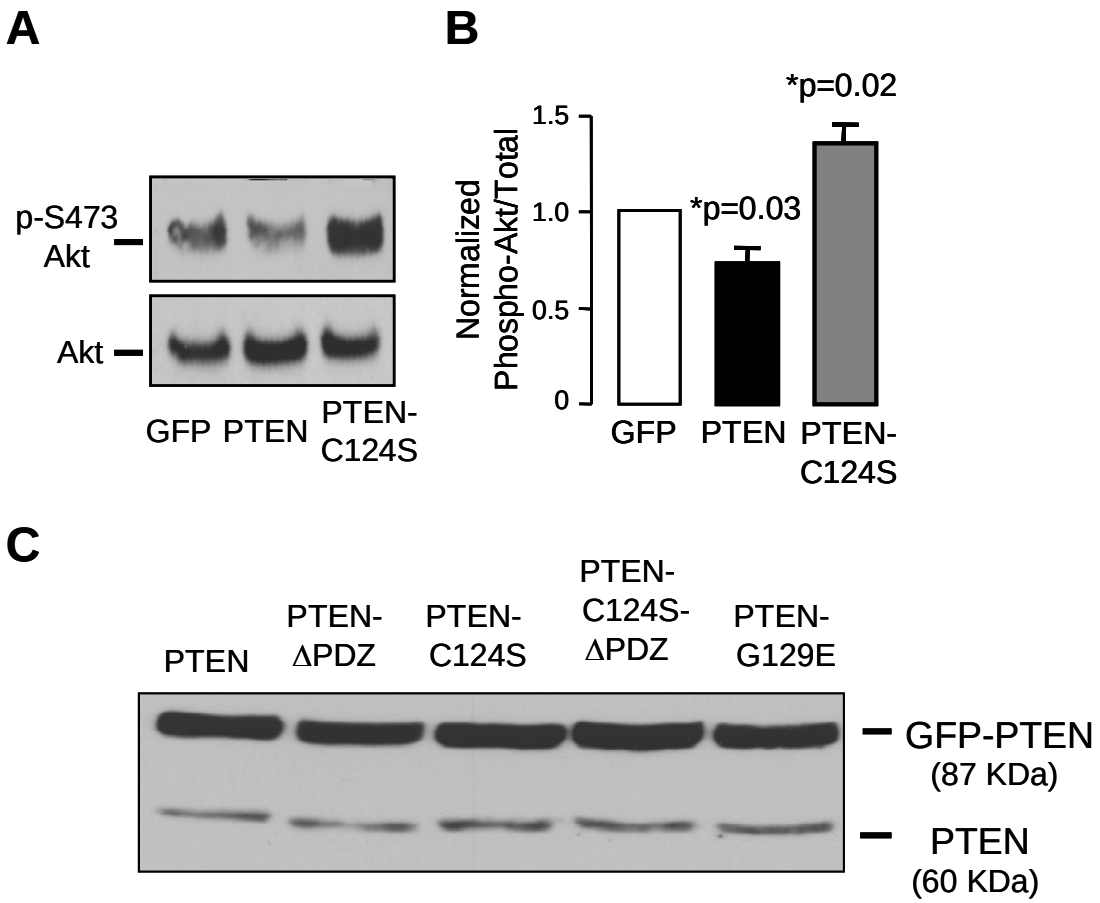
**Supplementary Figure 6. Characterization of bpV(HO)pic as a specific PTEN inhibitor. A.** Hippocampal slices expressing GFP-PTEN or uninfected were treated with different concentrations of bisperoxo(5-hydroxypyridine-2-carboxyl)oxovanadate (bpV(HO)pic) (Schmid et al., FEBS Lett. 566, 35-38, 2004), as indicated. After 16 hours, total protein extracts were prepared and analyzed by western blot with antibodies for phospho-Akt (Ser473), total Akt and phospho-tyrosine. Note the decrease in phospho-Akt in GFP-PTEN-expressing neurons, which is recovered with low (15 nM) concentrations of bpV(HO)pic. Phospho-tyrosine signal is only apparent at much higher (500 nM) concentrations. **B.** Quantification of phospho-Akt and phospho-tyrosine signal from three experiments as the one shown in A. The decrease in P-Akt signal in GFP-PTEN overexpressing slices is indicated with a star (*). P-Akt signal is recovered with 15 nM bpV(HO)pic, without affecting phospho-Tyr signal, indicating that low nanomolar concentrations of this compound selectively inhibit PTEN over phospho-Tyr phosphatases. These results essentially replicate previously published ones (Schmid et al., FEBS Lett. 566, 35-38, 2004).

**Supplementary Figure 7. PTEN activity during LTD induction is necessary for LTD expression.** LTD was induced in CA1 hippocampal neurons by pairing presynaptic stimulation (1 Hz, 500 pulses) with moderate postsynaptic depolarization (-40 mV) (black bar below graph). On some slices, the PTEN inhibitor bpV(HO)pic was applied 5 min before LTD induction and was washed out 5 min after the induction (black bar above graph). Treated slices are represented with white symbols, and untreated controls with black symbols. Amplitude of the synaptic response is normalized to a 5 min baseline. "n" represents number of cells. Statistical significance was calculated according to the Mann-Whitney test.

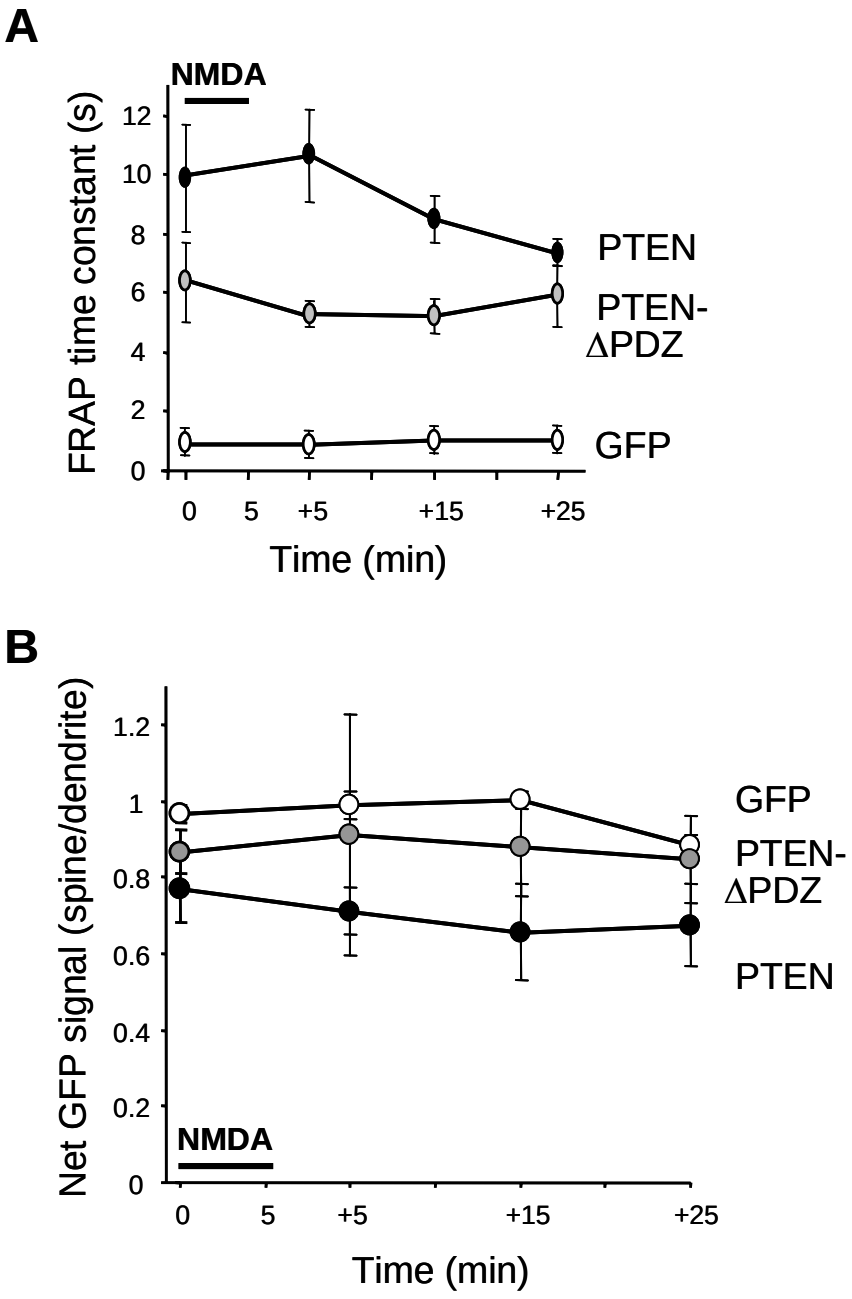
**Supplementary Figure 8. PTEN overexpression does not affect LTD. A.** LTD was induced in CA1 hippocampal neurons overexpressing wild-type PTEN (black symbols) or control, uninfected neurons (white symbols). Amplitude of the synaptic responses is normalized to a 10 min baseline. Insets: sample traces averaged from the baseline (thin lines) or from the last 10 min of the recording (thick lines). **B.** Average of AMPAR-mediated responses collected from the last 10 min of the recording and normalized to the baseline. Left columns (Paired, LTD) correspond to the stimulation pathway in which postsynaptic depolarization (-40 mV) was paired to low-frequency stimulation (1 Hz). Right columns (Control, unpaired) correspond to the pathway that was not stimulated during depolarization. "n" represents number of cells.



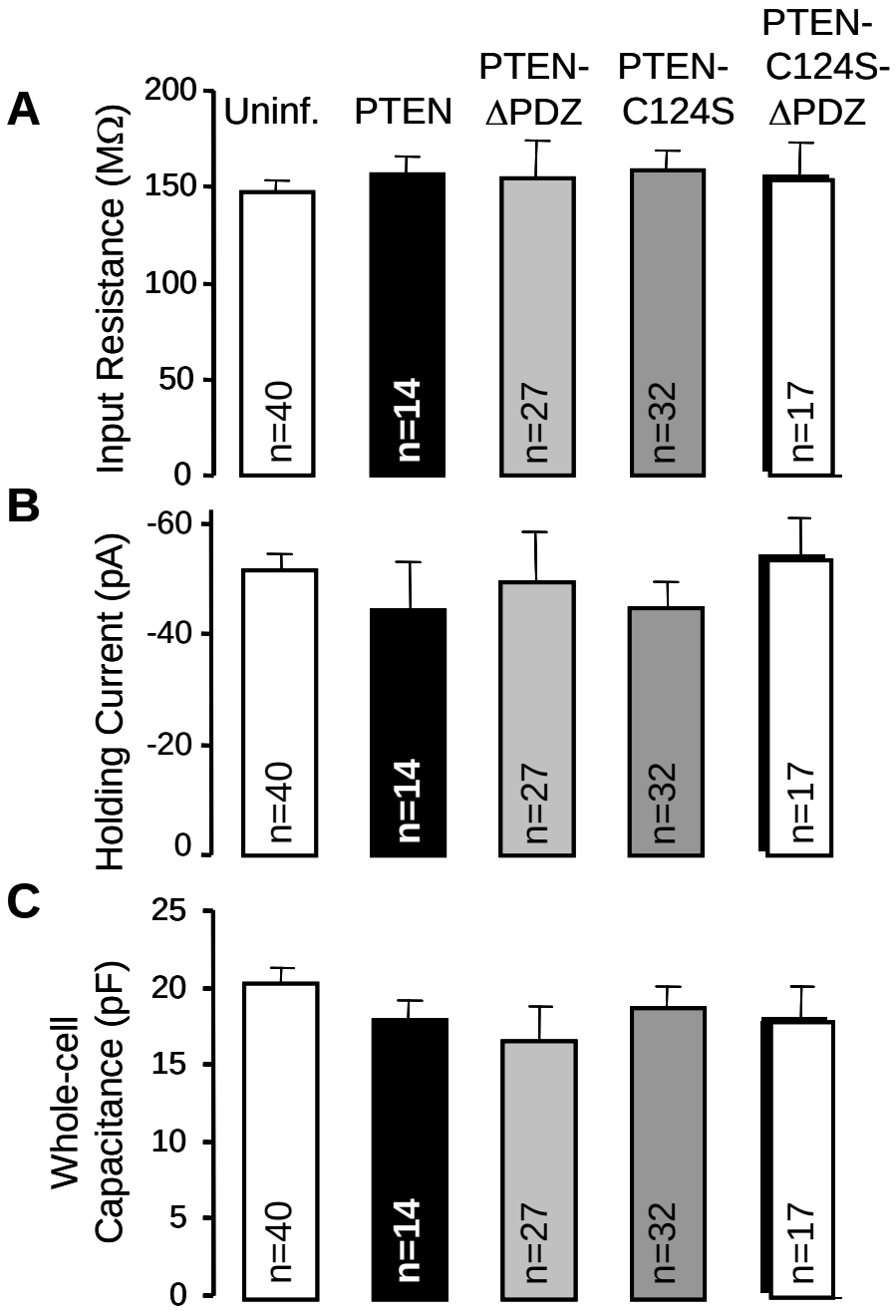

Supplementary Figure 1



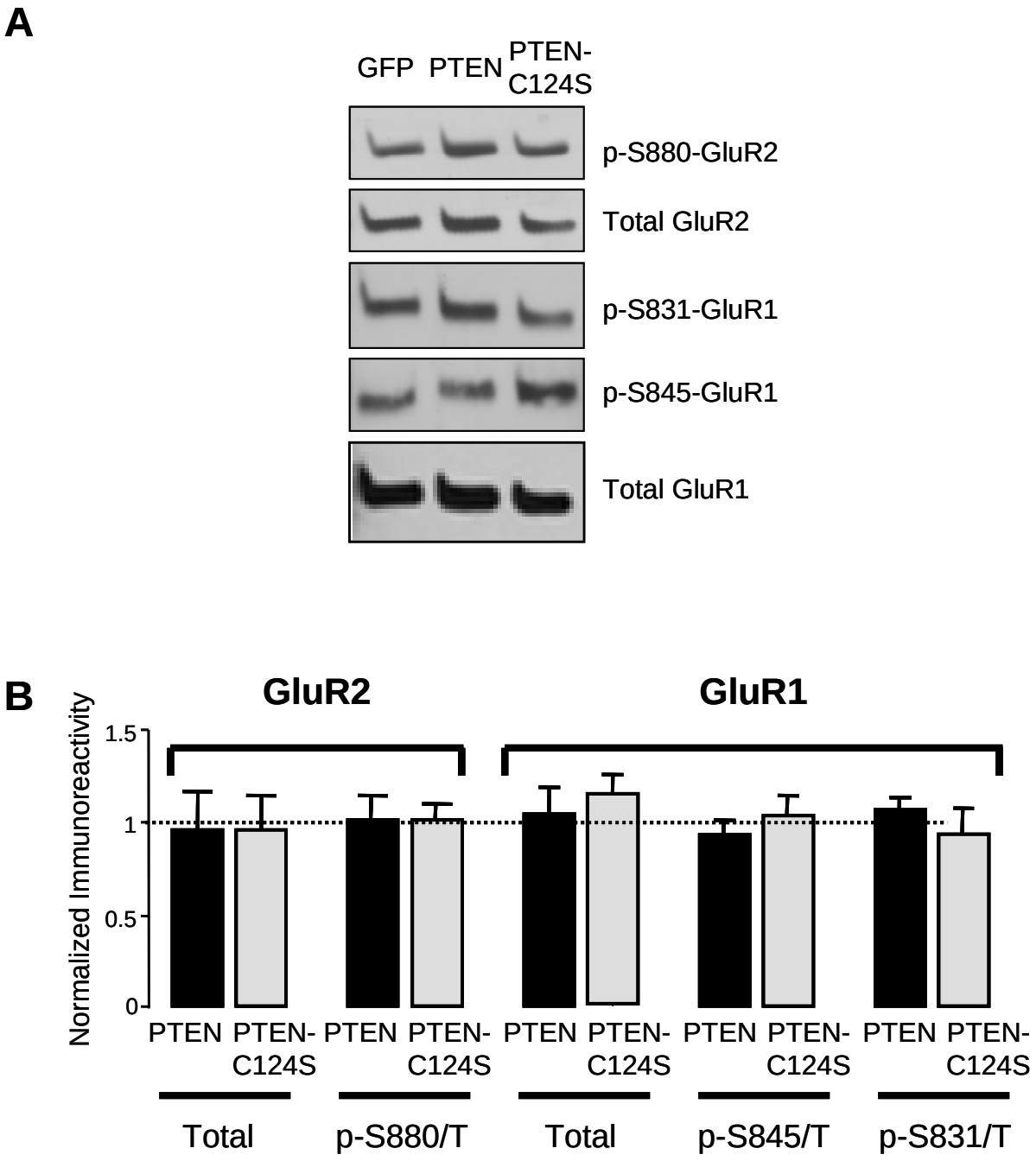

Supplementary Figure 2



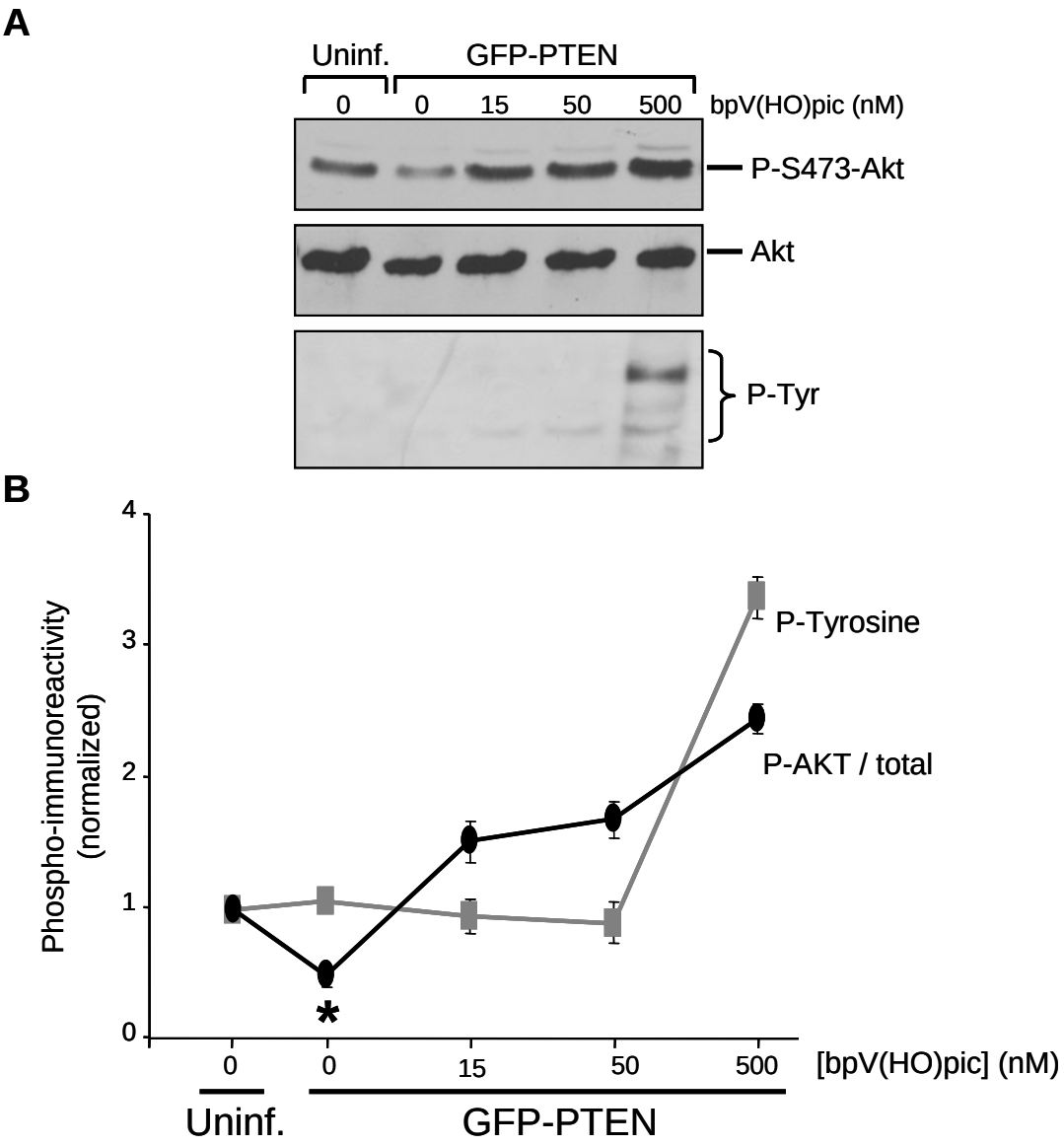

Supplementary Figure 3



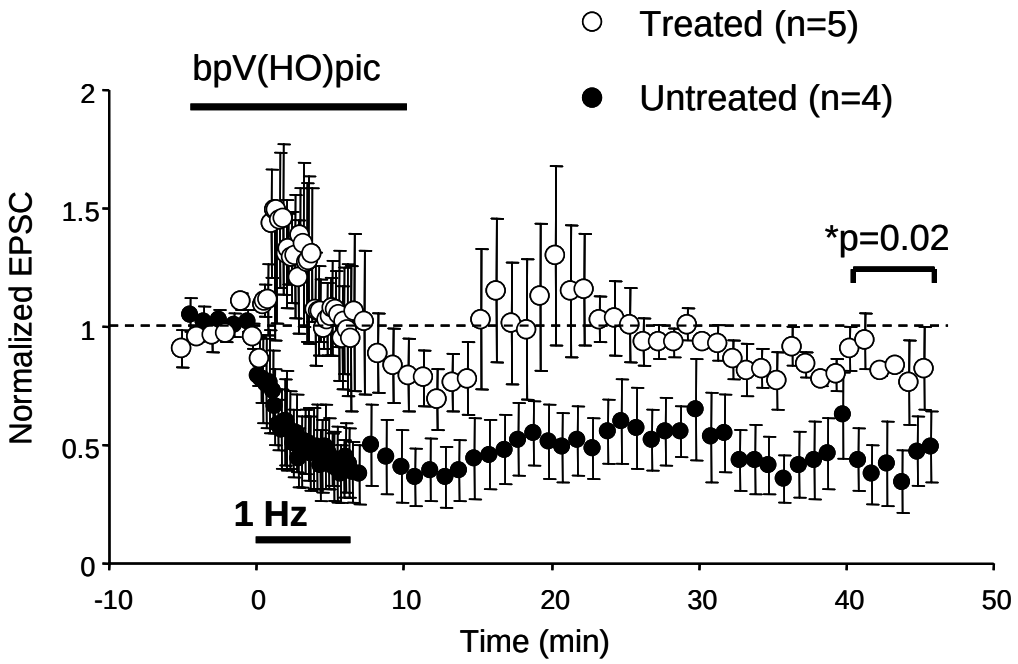

Supplementary Figure 4



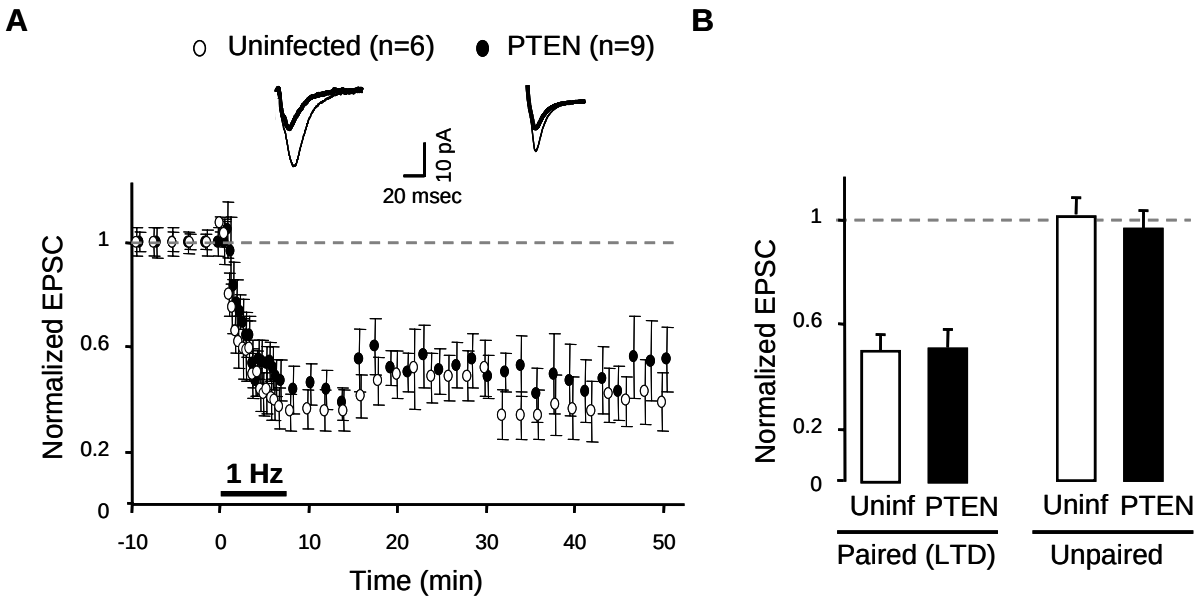

Supplementary Figure 5




Supplementary Figure 6




Supplementary Figure 7




Supplementary Figure 8