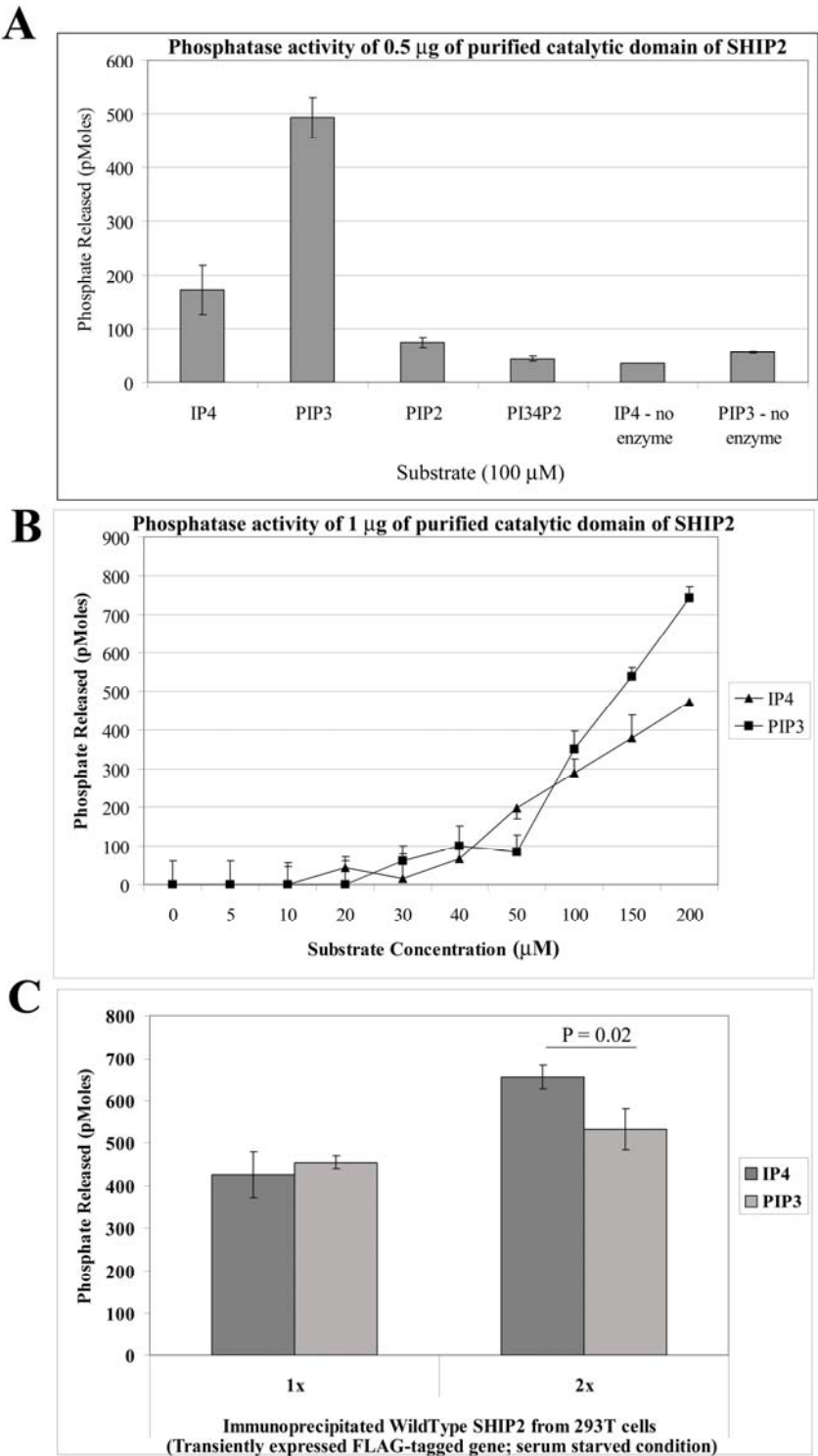
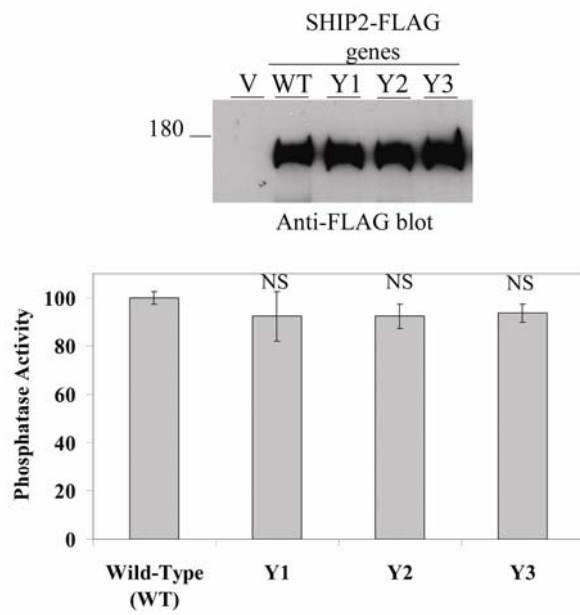


SUPPORTING INFORMATION

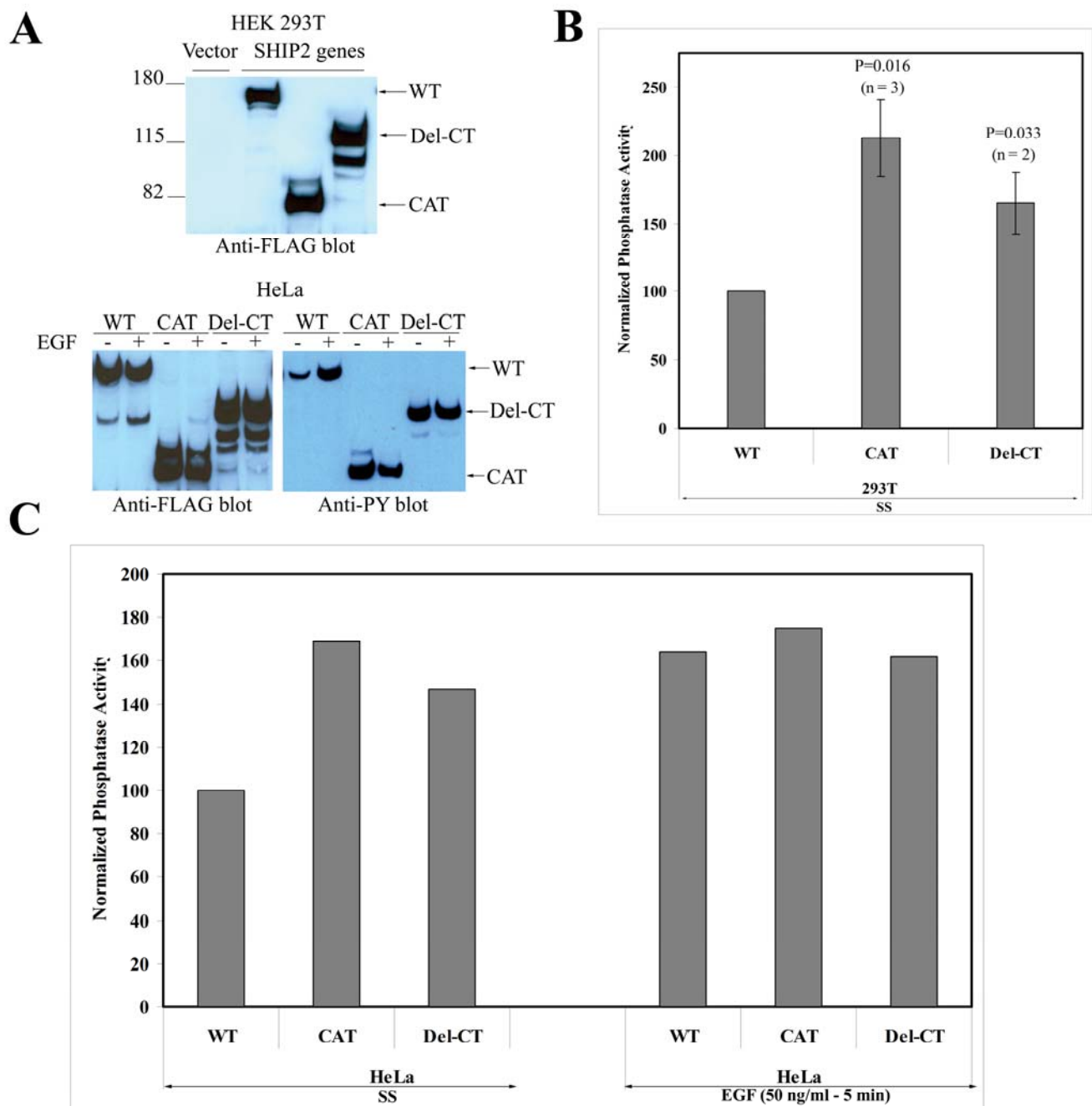


Supplementary Figure 1: **Comparison of Phosphatase activity of SHIP2 on IP4 vs. PIP3.** Bacterially expressed and purified SHIP2 catalytic domain was incubated with the indicated substrates for 30 min at 37°C followed by the addition of Malachite green reagent and quantification of the color development by measuring the absorbance at 650 nm. The amount of phosphates released was estimated by fitting the absorbance values to a standard curve. A – Experiment conducted in triplicates with 0.5 μ g

of enzyme shows higher activity with PIP3 (phosphatidylinositol-3,4,5-trisphosphate) than IP4 (inositol 1,3,4,5 tetrakisphosphate). The activity on PIP2 (phosphatidylinositol 4,5 bisphosphate) was very low and no activity was detected with PI-3,4-P2 (lacks a 5-phosphate) as substrate. B – Experiment conducted in duplicates where increasing concentrations (0 – 200 μ M) of IP4 and PIP3 were interacted with 1 μ g of enzyme. C – Comparison of phosphatase activity of a FLAG-tagged wild-type SHIP2 that was transiently expressed in HEK293/T cells. SHIP2 was immunoprecipitated using anti-FLAG antibody (M2/Sigma) from 15 h serum-starved cells followed by the phosphatase assay using 100 μ M of IP4 or PIP3. 2x sample contains the twice the amount of IP than 1x. Student's *t* test was used to compare the activity and P is shown when significant.



Supplementary Figure 2: **Analysis of the phosphatase activity Y-F mutants of SHIP2.** Wild-type (WT) or three Y-F mutants (Y1-Y497F, Y2-Y747F, and Y3-Y1135F) were expressed in HeLa cells by transient transfection method. pcDNA3 vector (V) transfected cells were used as a negative control. Anti-FLAG immunoprecipitates collected from 15 h serum starved cells were assayed for phosphatase activity. Equal levels of the proteins in the immunoprecipitates were verified using anti-FLAG immunoblot. Data shown are from two separate experiments conducted in duplicates. NS indicates that the differences between samples are not statistically significant (Student's *t* test).



Supplementary Figure 3: **Role of the C-terminus region in the regulation of SHIP2 activity.** A – Two truncated genes of SHIP2 were generated to analyze the role of the C-terminus in the regulation of SHIP2 phosphatase activity. One is deleted in SH2-domain as well as the C-terminus region, thus containing a central portion of SHIP2 with the poyphosphatase domain (aa 282-aa 926; termed *CAT* for catalytic domain). The other is deleted only in the C-terminus region containing a SHIP2 sequence from aa 1 - aa 822 (termed *Del-CT* for *deleted in the C-terminus*). Both *CAT* and *Del-CT* were tagged with FLAG epitope at their C-terminus. Transiently expressed truncated- and the wild-type SHIP2 were

immunoprecipitated using anti-FLAG (M2) from 15 h serum-starved 293/T (upper panel) or HeLa cells (lower panel). HeLa cells were treated with EGF (50 ng/ml for 5 min where indicated). Anti-FLAG immunoblots show the levels of the expressed genes. An anti-PY blot in the lower panel shows that the truncated SHIP2 genes are unexpectedly heavily tyrosine phosphorylated (perhaps indicating the interference with a tyrosine phosphatase interaction/function) in serum-starved conditions (repeated twice) with no further EGF-induced increase in tyrosine phosphorylation. B - Phosphatase activity assays of the wild-type and the truncated SHIP2 genes show significant activation of CAT as well as Del-CT constructs (when compared to the wild-type) in serum starved (SS) 293/T cells. C- A similar experiment conducted in HeLa cells is shown here. EGF-treatment activated the wild-type SHIP2 in HeLa cells but not the CAT or Del-CT genes.

MATERIALS AND METHODS

HeLa and HEK293/Tcells were cultured in DMEM containing 10% FBS (Gibco Life Sciences/Invitrogen) and serum starvation where indicated was done in DMEM plus 0.5% bovine serum albumin. SHIP2 polyclonal antibodies have been previously described (1). EGF was purchased from Collaborative research Inc. or from Invitrogen, H₂O₂ was from Sigma and anti-phosphotyrosine (clone 4G10) was from Upstate/Millipore. Malachite green reagent was from either Upstate/Millipore or from Echelon Biosciences. Inositol (1,3,4,5) tetrakisphosphate (IP4) was from Sigma or Echelon Biosciences. All other phospholipids (PIP3, PIP2 and PI-3,4-P2) were from Echelon Biosciences.

Expression and purification of SHIP2 catalytic domain: The sequence (aa 288-926) containing the inositol polyphosphatase domain of SHIP2 is expressed as a glutathione S-transferase (GST) fusion protein in E-Coli, purified using glutathione beads and eluted with free glutathione.

Site-directed mutagenesis and deletion mutant construction – All SHIP2 mutants used here have been previously reported except D690A which was generated in an identical manner using Stratagene's quick-site mutagenesis protocol (2, 3). SHIP2 sequence encoding amino acids 1 thru 822 (Deleted in the C-terminus region, *Del-CT*) and amino acids 282-926 (lacking the SH2-domain as well as the C-terminus region but harbors the catalytic domain, *CAT*) were generated by PCR amplification and cloned into pcDNA3 vector. All recombinant SHIP2 gene constructs were tagged with a C-terminus FLAG epitope.

Immunoprecipitation-Phosphatase activity assays: Immunoprecipitations were carried out using cell lysates prepared in 1% Triton-X-100 containing HNTG buffer as previously described (2). We used a soluble inositol, IP4 as substrate in our assays. In some experiments (shown in Suppl. Fig. 1), water-soluble phosphatidylinositol 3,4,5 trisphosphate (PIP3) was used. Protein A/G sepharose beads

capturing SHIP2 antibody-antigen complexes were washed twice at 4°C in excess of ice-cold assay buffer (50 mM Tris HCl pH 7.5, 10 mM MgCl₂, 1mM DTT) and resuspended in 1× assay buffer. Immunopurified SHIP2 and 100 µM IP4 (or other substrates at indicated concentrations) was combined in a reaction volume of 20 µl assay buffer. Reagents were mixed briefly (10 seconds) at 1000 rpm on a plate-reader followed by incubation for 30 min at 37°C. Release of free phosphates was measured by malachite green assay. Briefly, 100 µl of assay reagent was added per reaction, incubated for 15 min at room temperature and the absorbance was read at 650 nM using SpectraMax 2 plate reader. Western blot analyses were done as previously described (4).

REFERENCES:

- (1) Habib, T., Hejna, J. A., Moses, R. E., and Decker, S. J. (1998) *J Biol Chem* 273, 18605-18609.
- (2) Prasad, N., Topping, R. S., and Decker, S. J. (2001) *Mol Cell Biol* 21, 1416-1428.
- (3) Prasad, N., Topping, R. S., and Decker, S. J. (2002) *J Cell Sci* 115, 3807-3815.
- (4) Prasad, N., Topping, R. S., Zhou, D., and Decker, S. J. (2000) *Biochemistry* 39, 6929-6935.