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Supplemental Data

Comprehensive Identification of PIP3-Regulated PH Domains from *C. elegans* to *H. sapiens* by Model Prediction and Live Imaging

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Supplemental Experimental Procedures

Lipid Binding Assay

For each of the YFP-PH-domain constructs, 1.6 x 10^6 293F cells were transfected with 1.5 ug of DNA and 9.6 ul Lipofectamine 2000 (Invitrogen) in 1.6 mL Optimem. Transfected cells were incubated at 37^{o}C for 48 hours. Cells were harvested and spun at 450xg for 5min. The supernatant was removed, and the cell pellet was lysed in 500uL lysis buffer (TBT-T: 50mM Tris, pH 8, 10mM EDTA, 100mM NaCl, 0.5% Triton X-100, Complete™ Protease inhibitor cocktail [Roche]). The lysate was vortexed and incubated for 10 min at RT. The lysate was vortexed at 14K rpm for 15 minutes, and the supernatant was transferred directly to 10mL of 3% BSA in TBT-T containing a PIP Strip or PIP Array (Echelon Biosciences, Salt Lake City, Utah). Blots were incubated overnight at 4^{o}C with gentle rocking and then washed 3x 20 minutes with 1% BSA in TBS-T at RT. The blots were then incubated with 1:1000 dilution of rabbit anti-GFP (Molecular Probes cat. no. A-11122) in 1% BSA in TBS-T for one hour, washed, and incubated with 1:1000 anti-rabbit: Alkaline Phosphatase (Molecular Probes cat. no.G-21079) in 1% BSA in TBS-T for one hour at RT. Blots were washed, and the bound PH-

domain constructs were detected by colorimetric reaction (NBT/BCIP) of Alkaline Phosphatase activity.

Lipid blot experiments were carried out in batches. As a control, PSCD3-PH domains were used in each batch. Spot intensities were measured using Metamorph image processing software (Molecular Devices Corporation, Sunnyvale, CA). For each spot, the local background was subtracted, and the value of lipid binding was measured as the average intensity of the whole spot. Lipid binding values on each blot were normalized to the value of the blank spot on the respective blot.

Figure S1. Images of the 29 Mouse PH Domain Constructs that Translocated to the PM in NIH3T3 cells in Response to PDGF Stimulation

Two of the PH-domains are tandem domains.

Figure S2. Basal Subcellular Localization and Lipid Blot Binding Results for the Mouse PH Domains

In the lipid blot bar graphs, to better visualize the relative lipid binding levels and since we were interested mainly in PI(4,5)P2 and PI(3,4,5)P3 binding, we normalized the lipid binding values for each construct by the sum of the respective PI(4,5)P2 and PI(3,4,5)P3 binding values and plotted the values in bar graphs. Standard errors are shown for each of the bars for experiments where two or in some cases more arrays were tested.

Figure S3. Translocation of AKAP13-PH Is Only Indirectly Regulated by PI3K

In agreement with an earlier observation, we found that AKAP13-PH was partially prelocalized to actin cables in unstimulated cells (Olson et al., 1997). While translocation of AKAP13-PH to the PM was reversed by inhibition of PI3K, we found that cytocholasin B, an inhibitor of actin polymerization, could also block translocation of AKAP13-PH but not of AKT1-PH. This implied that the observed PM translocation is an indirect effect of PIP3-induced peripheral actin polymerization and that AKAP13 PH-domain binds to polymerized actin.

(A) CFP-AKT1-PH domain and YFP-AKAP13-PH domain are coexpressed in NIH3T3 cells. Translocation of AKT1-PH domain and AKAP13-PH to the PM coincides in response to 5 nM PDGF and both come off after the addition of 50 μM LY294002.
(B) When the actin cytoskeleton is disrupted by the addition of cytochalasin B (5 μg/ml), only AKAP13-PH can no longer translocate to the PM after PDGF receptor stimulation. Scale bars are 20 μm.

Reference: Olson, M.F., Sterpetti, P., Nagata, K., Toksoz, D., and Hall, A. (1997). Distinct roles for DH and PH domains in the Lbc oncogene. Oncogene *15*, 2827-2831.

Figure S4. Scoring of *S. pombe* and *S. cerevisaie* PH Domains Using the Recursive Functional Classification (RFC) Algorithm

(A) The RFC-scores of the yeast PH domains are overlaid on a plot of the RFC-scores of the tested mouse PH-domains. All the *S. pombe* and most of the *S. cerevisaie* PH-domains (marked in green) scored below the cutoff score, except for *S. cerevisaie* SLM2-PH and OSH3-PH which were in the borderline region (see Table S6 for quantitative scores).
(B) Examples of two *S. cerevisaie* (ySLM2 and yBoi1) and two *S. pombe* (pCSX2 and pKSG1) experiments carried out in NIH3T3 cells.

Figure S5. **Using the RFC Strategy to Predict PIP3-Binding PH Domains in *Dictyostelium Discoideum***

Red bars show PH-domains that have been shown in previous studies to be PIP3-regulated (Sun and Firtel, 2003)

Figure S6. Same as Figure 6D in the Main Text, but with a Control Comparison of the Cumulative S_{RFC} Distribution of Scrambled PH Domains (Dotted Green Line)

31,400 scrambled PH-domain sequences were generated by random selection of amino acids from the 131 tested mouse sequences. The average control S_{RFC} score was 5 +/- 9 (standard deviation).

Figure S7. Basal Images of All Tested PH Domains Used for Validation of RFC Algorithm

The first letter before the gene name specifies which species the gene is from: y=*S.cervesaie*, p=*S.Pombe*, c=*C.elegans*, d=*D.Melanogaster*, and h=human.

Figure S8. Time Course Images of Mammalian Translocating PH Domains

Figure S9.

Inhibition of PI3K using 50 μM LY294002 reverses PDGF receptor-triggered PM translocation of SH3HP2-PH (A) and AKT2-PH (B). (C and D) Four examples showing that pretreatment of cells with 50uM LY294002 for 3 minutes before stimulation prevents PDGF-induced translocation of previously translocating PH-domains.

Figure S10. Constitutive and Receptor-Triggered PM Targeting Partially Correlate with In Vitro Binding to PI(4,5)P2 and PI(3,4)P2, Respectively

PH-domains with observed receptor-triggered PM translocation are shown in red. PH-domains with constitutive, unregulated PM localization are shown in blue while PH-domains that remained constitutively cytosolic are marked in black without labels.