Supplemental Data

Figure S1. Identification of *SPL2* as a suppressor of *pho84*Δ strain. The *pho84*Δ strain was transformed with PCR products that replaced the indicated ORFs with the *KlLEU2* gene (Wykoff and O'Shea, 2001). Transformants were screened for correct integration by PCR and then assayed for growth as described in Figure 2.

Figure S2. Spl2 down-regulates the low-affinity phosphate transporters Pho87 and Pho90. The indicated strains (see Table S1) were assayed for growth in a variety of conditions. Ten-fold dilutions of strains grown in YEPGal were grown for different amounts of time so that differences are most clear (2-7 days at 30ºC). To identify the transporter(s) targeted for Spl2-mediated inactivation, we exploited the observation that deletion of *SPL2* alters the arsenate resistance and low phosphate growth phenotypes of strains containing low-affinity transporters (Figure 2A). Inactivation of *PHO4* or *SPL2* in a strain lacking the five known phosphate transporters does not influence growth in different phosphate levels or in the presence of arsenate, suggesting that Spl2 down-regulates one of these previously identified phosphate transporters (Figure S2; the strain lacking five phosphate transporters and Pho4 does not grow on SD or YEPD medium because it cannot express *GIT1*, a Pho4-induced transporter for glycerophosphoinositol which can also uptake phosphate at a low rate). When Pho87, Pho90, or Pho91 is the only phosphate transporter present, growth on high phosphate and partially limiting phosphate medium (SD and YEPD, respectively) is restored, but cells do not have sufficient transport activity to generate arsenate sensitivity or the ability to grow on low phosphate medium. When *SPL2* or *PHO4* is deleted in strains that contain only Pho87 or

Pho90, we observe increased growth in low phosphate conditions and the reciprocal loss of growth in the presence of arsenate, consistent with the model that deletion of *PHO4* or *SPL2* results in increased phosphate uptake. In contrast, no such effects of *PHO4* or *SPL2* deletion are observed in a strain that expresses only Pho91. We conclude that Pho87 and Pho90 are down-regulated by Spl2 and Pho4. Given the weak growth of the Pho91-only strain on standard medium (YEPD and SD), we cannot draw any conclusions regarding its regulation.

Figure S3. Deletion of *SPL2* rescues the *pho84*Δ phenotype. Strains EY1745 (wild-type), EY1746 (*pho84*Δ), EY2097 (*pho84*Δ*pho4*Δ), EY1759 (*pho84*Δ*spl2*Δ), and EY2098 (*pho80*Δ*pho4*Δ*)* all contain Pho4-YFP and were grown to mid-log phase, transferred to SD medium or SD medium lacking phosphate and grown for 3 hours at 30ºC, concentrated 100-fold, transferred into a 96-well microscope plate, and visualized by fluorescence microscopy. These strains contained a nuclear marker (Hrp1-mRFP) ((Huh et al., 2003)) and punctuate fluorescence corresponds to nuclear localization. In a *pho84*Δ strain Pho4-YFP is localized to the nucleus in high phosphate conditions, presumably due to low internal phosphate levels (Ausukaree et al., 2004). Conversely, in the p*ho84*Δ*spl2*Δ strain Pho4-YFP is localized to the cytoplasm, suggesting that the loss of Spl2 results in an increase in intracellular phosphate in high phosphate conditions. Conducting the same experiment in a *pho84*Δ*pho4*Δ strain using a Pho4-YFP fusion lacking the Pho4 DNA binding domain (which prevents transcription and dimerization but not phosphate-dependent localization (Kaffman et al., 1998b)), reveals that, like the *SPL2* deletion, loss of Pho4 function in the *pho84*Δ strain corrects the localization defect.

Figure S4. Spl2 protein expression. The following strains were grown to mid-logarithmic phase and then transferred to either high phosphate or no phosphate conditions for four hours: wild-type (EY57), *pho87*Δ*pho90*Δ*pho91*Δ (EY1982), *PGK1*pr-*SPL2* (EY1959), and *spl2*Δ (EY1872). Cells were harvested and extracts were analyzed as described in experimental procedures utilizing a 15% Criterion gel (Bio-Rad). Immunoblot analysis was performed with polyclonal rabbit antibodies against Pho4 and Spl2.

Figure S5. Immunoblot analysis of extracts derived from cells expressing GFP under the control of the *PHO84* promoter grown in intermediate phosphate. Cells expressing high levels of *PHO84* (GFP) and those expressing low levels were isolated by flow cytometry, cell extracts were generated, and Spl2 and Pho4 expression was monitored by Western blotting using polyclonal rabbit antibodies against Pho4 and Spl2. Spl2 is abundant in the population expressing *PHO84*, whereas it is not detectable in the uninduced population. Pho4 levels remain unchanged in both populations. The asterisk denotes a degradation product commonly observed with the Pho4 antibody (O'Neill et al., 1996).

Table S1. Genotypes of yeast strains used in this study.

Methods:

Strain Construction:

Gene disruptions were performed to precisely delete the open reading frame utilizing a PCR-based inactivation protocol (Kitada et al., 1995; Longtine et al., 1998). The deletion of *PHO84*, *PHO80*, *PHO81*, *PHO4* and phosphate transporters is from previously published work (Bun-Ya et al., 1991; Carroll et al., 2001; Madden et al., 1988; Schneider et al., 1994). pRS316-*GAL1*pr-*PHO84* is EB1280 and has been previously described (Wykoff and O'Shea, 2001). C-terminal tagging of *HRP1* is described in (Huh et al., 2003) and C-terminal tagging of Pho4 with YFP utilized identical primers as reported in (Springer et al., 2003), but with a plasmid (EB1648) containing eYFP (Longtine et al., 1998; Raser and O'Shea, 2004). Truncation of Pho4 to remove the DNA binding domain was performed so that eYFP begins at residue 251. To replace the *PHO5* ORF with eGFP the following primers were utilized to amplify a product from EB1632 (pFA6a-yEGFP-KANMX6): agaacaacaacaaatagagcaagcaaattcgagattaccaaccatgtctaaaggtgaaga and tattcgtatttagtttccaatattatttagttatacaaaagaattcgagctcgtttaaac. To integrate the *PHO84*pr reporter at an ectopic locusplasmid EB1632 was used as a template to precisely replace the ORF of *PHO84*. Genomic DNA from this strain was isolated and oligonucleotides cg**ggatcc**ccacattgaacttttcacttcg and cc**gcggccgc**ttatttgtacaattcatccatacc were utilized to amplify this genomic region and clone it into pRS306 (Sikorski and Hieter, 1989). This plasmid (EB1656) was then linearized with *Stu*I, transformed into yeast strains that had a wild-type *PHO84* locus, and selected for by uracil prototrophy. Initial experiments with Spl2 demonstrated that the start codon is incorrectly annotated on the Saccharomyces genome database (www.yeastgenome.org); thus we generated the p*PGK1-SPL2* strain with the following primers and EB1654 (pFA6a-HIS3MX6-PGK1): cttgtgaaactgctgtttccattttgccgcgtggagacattatagttttttctccttgac and

tacaacgccgtaaagttccaaacgcagcggcctgagataggaattcgagctcgtttaaac. Overexpressed Spl2 migrated at the same size as native Spl2 in this strain.

Microscopy:

Fluorescence imaging was conducted using a Zeiss Axiovert inverted microscope. Excitation light was provided by a Sutter Lambda DG-4 utilizing excitation filters placed within the light path. Emission filters were placed in a 5 position Ludl filter wheel controlled by a MAC5000 shutter controller (Ludl, Hawthorne, NY). Images were taken using a Cascade 512B camera operated at 5 MHz. Cells were imaged in glass bottom 96 well plates (BD Falcon) using an oil-immersion Zeiss 63x achromatic objective. Images were analyzed using Metamorph software (Molecular Devices, Downingtown, PA), and were normalized utilizing Adobe Photoshop 6.0 (Adobe Systems, San Jose, CA).

Immunoblot analysis and antibody generation:

Extracts were prepared in 8M urea, as described previously (Wykoff and O'Shea, 2005). Thirty micrograms of extract were subjected to electrophoresis and blotted on nitrocellulose. Pho4 was detected utilizing a previously described rabbit antibody at a 1:5000 dilution and Spl2 was detected by a polyclonal rabbit antibody at a 1:2500 dilution raised against the peptide CENDCVIDEDIFEDSSDEEQS (Bethyl Laboratories, TX). Immunoblots were processed and detected as previously reported, except that 0.3% Triton X-100 was added to TBST in all steps to minimize Spl2 antibody cross reactivity (Wykoff and O'Shea, 2005).

Supplemental References

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