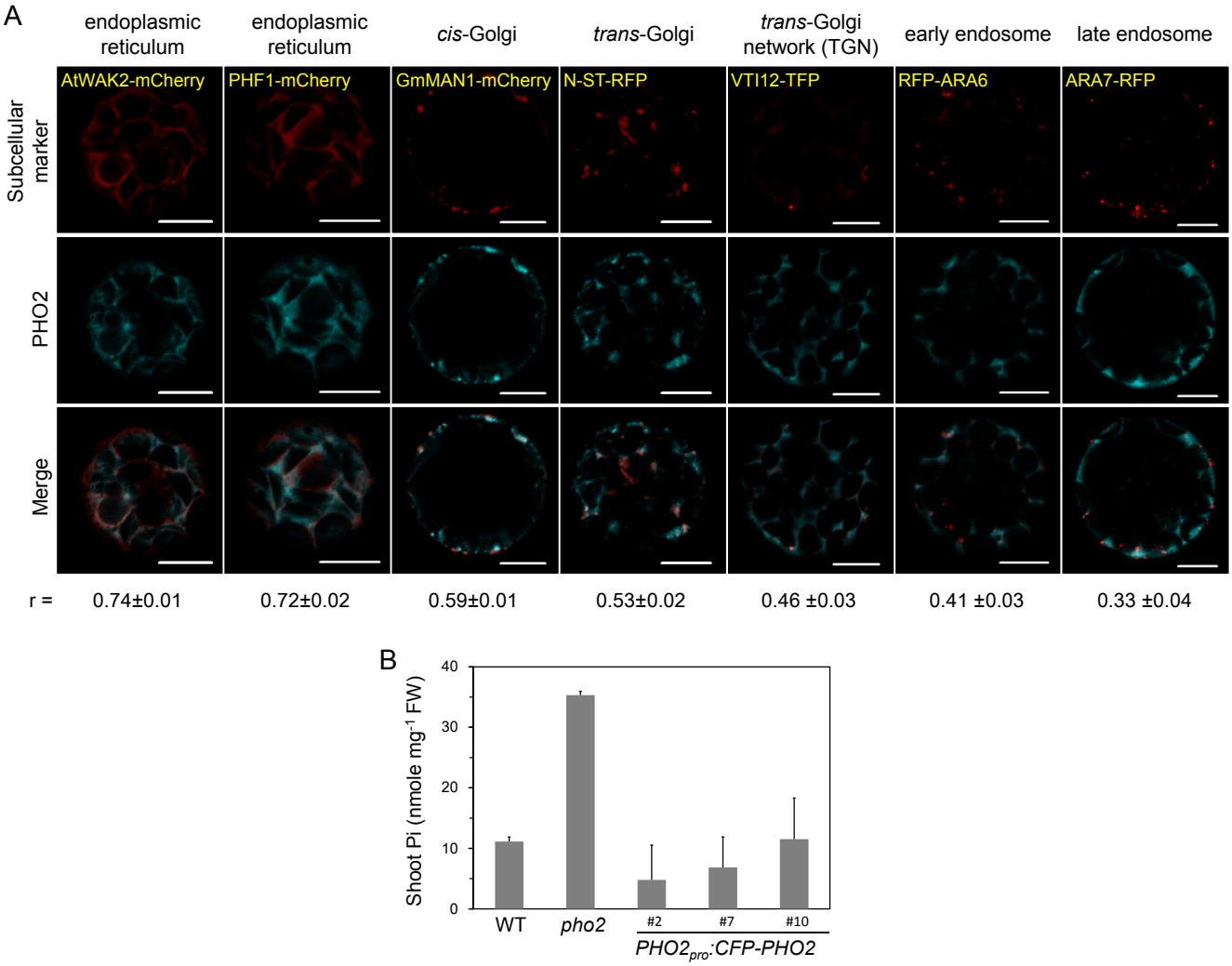
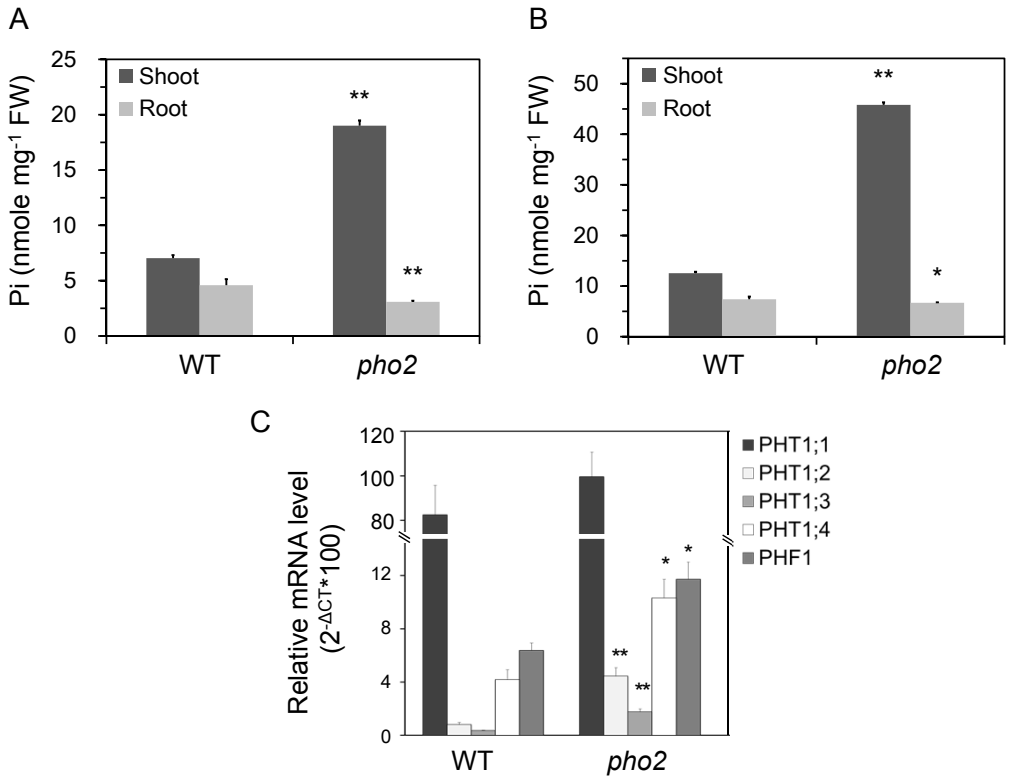


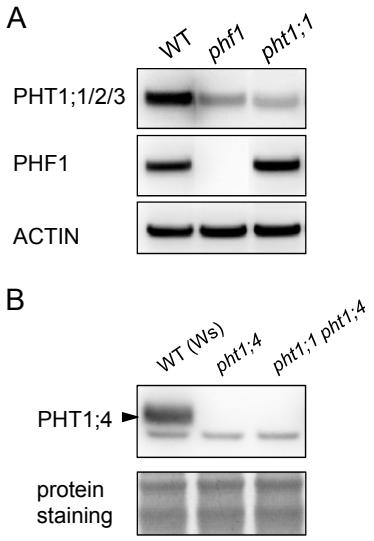
Supplemental Figure 1. PHO2 resides at the endomembrane.

(A) Subcellular localization of CFP-PHO2 or RFP-PHO2 in *Arabidopsis* protoplasts. The protein markers representing the specific subcellular compartments (showed on the top panel) are tagged with the fluorescent protein as indicated. Bar = 10 μm. Co-localization of the signal with different markers was analyzed by Pearson's correlation coefficiency (r) which is indicated below the image (± standard error, n=11 to 24 cells).
(B) The shoot P_i concentrations of *pho2* mutants complemented by the expression of *CFP-PHO2* driven by *PHO2* native promoter. Error bars represent SD (n = 3).



Supplemental Figure 2. Analyses of WT and *pho2* seedlings subjected to membrane proteomic analysis.

The shoot and root P_i concentrations of 12-day-old seedlings grown under solid agar medium (A) or 17-day-old seedlings grown under hydroponic medium (B) containing 250 μM KH_2PO_4. (C) Quantitative RT-PCR analysis of *PHT1;1*, *PHT1;2*, *PHT1;3*, *PHT1;4* and *PHF1* mRNA in the roots of 12-day-old seedlings grown under solid agar medium. Error bars represent SD (n = 4). Data significantly different from the controls are indicated (* $p < 0.05$, ** $p < 0.01$; Student's *t* test). FW, fresh weight.

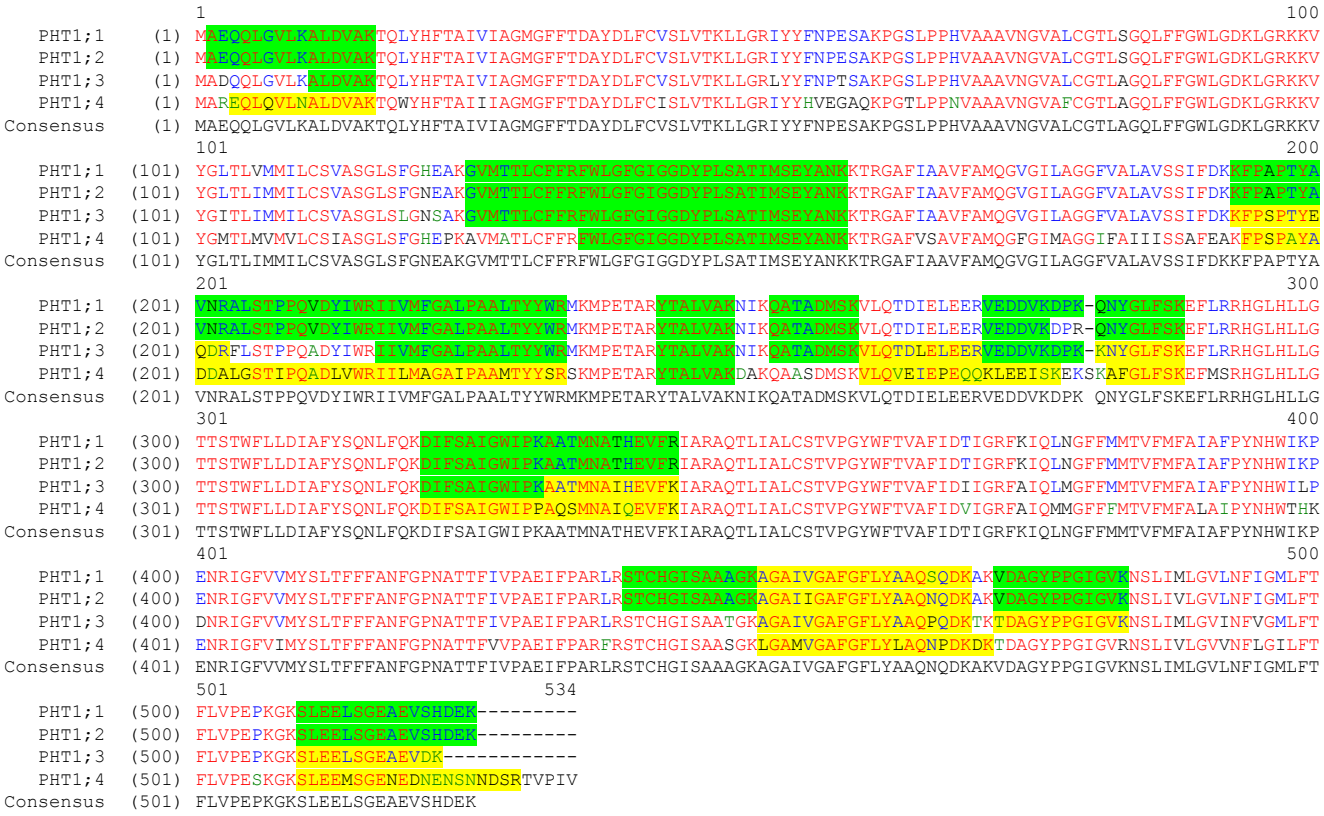


Supplemental Figure 3. Evaluation of the specificity of antibodies.

Immunoblot analyses of PHT1;1/2/3 (A), PHT1;4 (B) and PHF1 (C) proteins in the WT and corresponding mutants.
(A) The signal for PHT1;1/2/3 proteins was significantly reduced in 12-day-old *pht1;1* seedlings when grown under P_i-sufficient conditions. PHF1 proteins were detected only in the WT but not the *phf1* mutant.
(B) A specific signal corresponding to PHT1;4 protein was detected only in the WT (Ws, indicated by arrowhead) but was absent in the *pht1;4* (Misson et al., 2004) and *pht1;1 pht1;4* (Shin et al., 2004) seedlings grown under P_i deficient conditions.

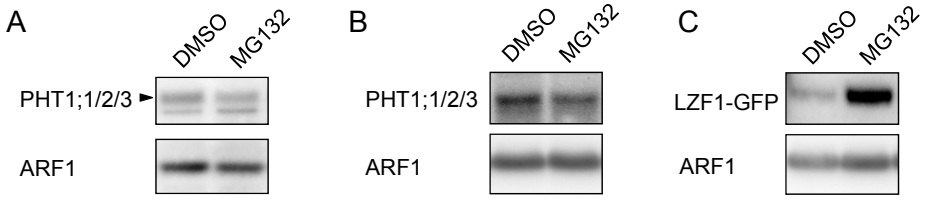
Misson, J., Thibaud, M.-C., Bechtold, N., Raghothama, K., and Nussaume, L. (2004). Transcriptional regulation and functional properties of *Arabidopsis* Pht1;4, a high affinity transporter contributing greatly to phosphate uptake in phosphate deprived plants Plant Mol. Biol. **55,** 727-741.

Shin, H., Shin, H.S., Dewbre, G.R., and Harrison, M.J. (2004). Phosphate transport in *Arabidopsis*: Pht1;1 and Pht1;4 play a major role in phosphate acquisition from both low- and high-phosphate environments. Plant J. **39,** 629-642.



Supplemental Figure 4. The identified peptides corresponding to PHT1;1, PHT1;2, PHT1;3 or PHT1;4 proteins.

Identical amino acids are marked in red. The identified peptides shared with PHT1 proteins are highlighted in green. The unique peptides mapped specifically to individual PHT1 are highlighted in yellow.



Supplemental Figure 5. Effect of MG132 on the accumulation of PHT1;1/2/3 proteins.

Immunoblot analyses of PHT1;1/2/3 proteins in the 11-day-old WT roots (A) or 5-day-old WT seedlings (B) subjected to treatment with MG132 (25 μM) for 24 h. The LZF1-GFP protein detected from 5-day-old *LZF1-GFP* transgenic plants (Chang et al. 2011) was used as a positive control for MG132 treatment. DMSO treated plants were used as negative controls.

Chang C-SJ, Maloof J, Wu S-H (2011) COP1-mediated degradation of BBX22/LZF1 optimizes seedling development in Arabidopsis. Plant Physiol 156: 228-239

Supplemental Table 1. Sequences of primers used in this study

Construct	Primer	Sequence (5' to 3')
35S:CFP-PHO2	Forward	ATGGAAATGTCCCTTACTGACTC
UBQ_{pro}:RFP-PHO2	Reverse	TTATGATTCTGGTCCAATCTCTTGGA
UBQ_{pro}:$PHO2^{C748A}$-nYFP	Forward	GTTGCCTTGAGTCTGCTGAATACAT
UBQ_{pro}:$PHO2^{C748A}$-cYFP	Reverse	TCTTCCTGACTCATACAGGTTCGG
$PHO2_{pro}$:CFP-PHO2	Forward	ATGGTGAGCAAGGGCGAGGA
	Reverse	TTATGATTCTGGTCCAATCTCTTGGA
35S:PHF1	Forward	ATGGAGATTGAAGAAGCGAGTC
UBQ_{pro}:nYFP-PHF1	Reverse	CTATAGGTCCAAGTTCCACCTACTATG
35S:PHF1-mCherry	Forward_1	ATGGAGATTGAAGAAGCGAGTC
	Reverse_1	TAGGTCCAAGTTCCACCTACTATG
	Forward_2	GGCCGGCCTGGAGGTGGAGGTGGAGCTGTGAGCA
	Reverse_2	TTAAGCACCAGCAGGATCCTTGTACAGCTCGTC
UBQ_{pro}:PHT1;1-cYFP	Forward	ATGGCCGAACAACAACTAGGAGT
	Reverse	TTTCTCGTCATGGCTAACCTCA
UBQ_{pro}:PHT1;4-cYFP	Forward	ATGGCAAGGGAACAATTACAAGTG
UBQ_{pro}:PHT1;4-nYFP	Reverse	AACTATTGGGACCGTTCTACTATCATT
Nub-PHO2	Forward	ACAGGATCCATGGAAATGTCCCTTACT
	Reverse	TTATGATTCTGGTCCAATCTCTTGGA
Nub-PHF1	Forward	ACACGGATCCATGGAGATTGAAGAAGCGAG
	Reverse	ACACGAATTCCTATAGGTCCAAGTTCCACC
PHF1-Cub	Forward	ACATCTAGAATGGAGATTGAAGAAGCGAG
	Reverse	GTCTATAGGTCCAAGTTCCACCT
Nub-PHT1;1	Forward	TCATGGCCGAACAACAACTAGG
	Reverse	AGCTTGAGACCAGCTTCGAAGT
PHT1;4-Cub	Forward	ACATCTAGAATGGCAAGGGAACAATTAC
	Reverse	TATGTCTAGAAACTATTGGGACCGTTCTAC
pPHO2proMDC32	Forward	ACAGTCGAGTTCAAGGAAGTCACA
	Reverse	ACGTGGTACCACACACACAACTCTACA

Supplemental Methods

iTRAQ labeling and LC-MS/MS analysis

The iTRAQ reagent (Applied Biosystems) was thawed and reconstituted in 70 µl of ethanol by vortexing for 1 min. The peptide solutions (140 µg) derived from different samples were subjected to two labeling-reactions with iTRAQ reagents that were allowed to proceed at room temperature for 1 hour. $iTRAQ_{114}$ and $iTRAQ_{115}$ were used to label the peptides from agar-grown WT and *pho2* and $iTRAQ_{116}$ and $iTRAQ_{117}$ were used to label the peptides from hydroponic-grown WT and *pho2* plants, respectively. The labeled peptides from the 4 different samples were pooled and acidified in strong cation exchange (SCX) buffer A (5 mM KH_2PO_4 solution in 25% [v/v] ACN, pH adjusted to 3.0 with phosphoric acid) to a final volume of 1 ml and subsequently subjected to fractionation by SCX chromatography. The iTRAQ-labeled peptides in SCX buffer A were loaded onto a 2.1 mm × 200 mm polysulfoethyl A column containing 5-µm particles with a pore size of 30 nm (PolyLC). The elution was monitored by absorbance at 214 nm, and fractions were collected every minute. A total of 38 fractions were vacuum-dried and resuspended in 0.1% (v/v) Trifluoroacetic acid (TFA) for further desalting and concentration using C18 ZipTip. Each SCX fraction was analyzed in duplicate by LC-MS/MS.

For LC-MS/MS analysis, the first set of replicate samples was analyzed by mass spectrometer (Waters Q-TOF Premier, Waters Corporation). Samples were injected into a 2 cm × 180 µm capillary trap column and separated by 25 cm × 75 µm Waters ACQUITY 1.7 µm BEH C18 column using a nanoACQUITY Ultra Performance LC system (Waters). The mass spectrometer was operated in ESI positive V mode with a resolving power of 9,000. A NanoLockSpray source was used for accurate mass measurement and the lock mass channel was sampled every 30 sec. The mass spectrometer was calibrated with a

synthetic human [Glu1]-Fibrinopeptide B solution (500 pmol/µl, Sigma Aldrich) delivered through the NanoLockSpray source. Data acquisition was operated in data directed analysis. The method included a full MS scan (*m/z* 400 – 1,600, 0.6 sec) and 3 MS/MS scans (*m/z* 100 – 1,990, 1.2 sec each scan) sequentially on the three most intense ions present in the full scan mass spectrum. The other 2 sets of replicate samples were analyzed using an AB SCIEX TripleTOF 5600 instrument in information dependent acquisition mode. Samples were separated on a 15 cm × 100 µm self-packed C_{18} reversed-phase column (ReproSil-Pur C18-AQ, 3 µm; Dr. Maisch-GmbH, Ammerbuch, Germany) using a nanoACQUITY Ultra Performance LC system (Waters). The mass spectrometer was operated in high sensitivity mode with rolling collision energy on. Precursor ions were selected across the *m/z* range of 300-1500 using 250 ms accumulation time per spectrum. A maximum of 15 precursors per cycle from each MS spectrum were selected for MS/MS analyses with 150 ms minimum accumulation time for each precursor and dynamic exclusion for 6s.

Spectra Processing

The MS/MS raw spectra generated from the Waters Q-TOF Premier (the first replicate) and the AB SCIEX TripleTOF 5600 (the second and third replicates) instruments were processed to detect MS/MS peaks using UniQua (Chang et al., 2013). For the UniQua processing, the spectra were converted into mzXML format using massWolf (version 4.3.1) and ProteoWizard (version 3.0.4623) for the raw data generated from the Waters and AB SCIEX instruments, respectively. The UniQua parameters for Waters Q-TOF Premier were: smoothing = 9, centroiding high = 80%, maximum resolution = 16,000, baseline cutoff = 1.5 counts, reporter m/z range = 114-117, and reporter dynamic range = 5-1,000.

The UniQua parameters for AB SCIEX TripleTOF 5600 were: smoothing = 11, centroiding high = 80%, maximum resolution = 25,000, baseline cutoff = 4 counts, reporter m/z range= 114-117 and reporter dynamic range = 5-100,000. The processed MS/MS spectra were then converted into Mascot generic format (.mgf) using mzXML2Search in Trans Proteomics Pipeline (TPP)[1] (Deutsch et al., 2010) version 4.4 rev. 1. To obtain better quantification results, the MS/MS spectra with peak number < 10, average intensity of the top 10 abundant peaks < 15 counts and iTRAQ average intensity < 30 counts were further removed from the peaklist file.

Protein Qualification and Quantitation

The processed spectra were searched against the TAIR10 database (December 2011, 27,416 sequences) using the Mascot version 2.3 (Matrix Science, London, UK). For the MASCOT search, the mass tolerance for peptide precursor and MS/MS fragments was 0.1 Da. Only one missed cleavage was allowed for tryptic peptides. The methylthiolation (C), iTRAQ4plex (N-term) and iTRAQ4plex (K) were set as fixed modification. The Oxidation (M) and iTRAQ4plex (Y) were set as variable modification. The quantitation option was enabled and set to the iTRAQ4-plex approach. The peptide was considered to be identified if the MASCOT ion score > 27 ($P < 0.05$). In addition, the protein was considered to be identified if the MASCOT protein score was ≥ 27 with at least one unique and two different peptides identified. For the protein quantitation, only unique peptide was quantified in MASCOT and used to determine the protein ratio in MASCOT. To minimize the systematic ratio error due to sample preparation, the protein ratios were normalized by the mode of the overall protein ratios.

For the data acquired by the Q-TOF Premier, 72,817 peptides and 3107 proteins were quantified. For the data acquired by the TripleTOF 5600, 41,885 peptides and 2893 proteins were quantified in the first replicate; 82,397 peptides and 4939 proteins were quantified in the second replicate.

Chang, W.-H., Lee, C.-Y., Lin, C.-Y., Chen, W.-Y., Chen, M.-C., Tzou, W.-S., and Chen, Y.-R. (2013). UniQua: A Universal Signal Processor for MS-Based Qualitative and Quantitative Proteomics Applications. Anal. Chem. **85,** 890-897.

Deutsch, E.W., Mendoza, L., Shteynberg, D., Farrah, T., Lam, H., Tasman, N., Sun, Z., Nilsson, E., Pratt, B., Prazen, B., Eng, J.K., Martin, D.B., Nesvizhskii, A.I., and Aebersold, R. (2010). A guided tour of the Trans-Proteomic Pipeline. Proteomics **10,** 1150-1159.