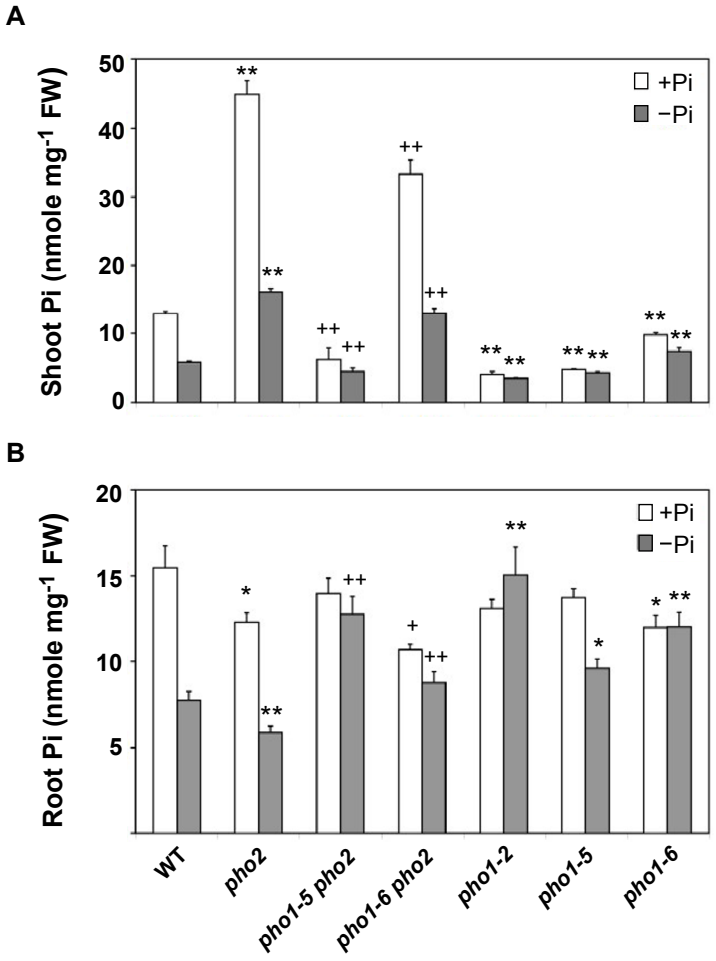


Supplemental Figure 1. The P_i concentrations of 20-day-old *pho2* suppressors.

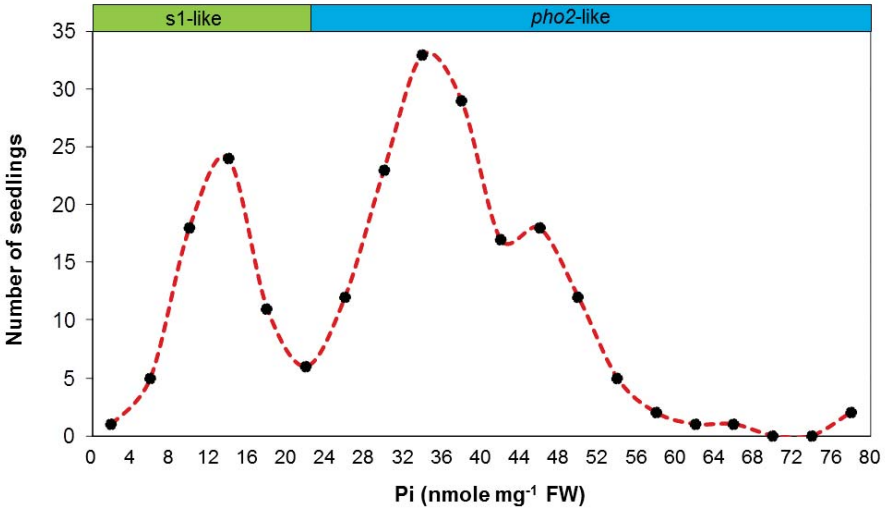
The shoot (A) and root (B) P_i concentrations of WT, *pho2*, *pho1-5 pho2*, *pho1-6 pho2*, *pho1-2*, *pho1-5*, and *pho1-6* plants hydroponically grown under $+P_i$ and $-P_i$ conditions (5 days of P_i deficiency). Error bars represent SE (n = 4 - 6). Data significantly different from the corresponding controls are indicated (mutant versus the WT, * $P < 0.05$, ** $P < 0.01$; mutant versus the *pho2* mutant, $^{+}$ $P < 0.05$, $^{++}$ $P < 0.01$; Student's *t*- test). FW, fresh weight.

A

Genotype Crosses and Generation Screened	Phenotype of Progeny[a]		Ratio Tested	χ^2	P^b
	s1-like	*pho2*-like			
s1 x *pho2* F2	62	158	s1:*pho2* = 1:3	1.188	0.2- 0.3

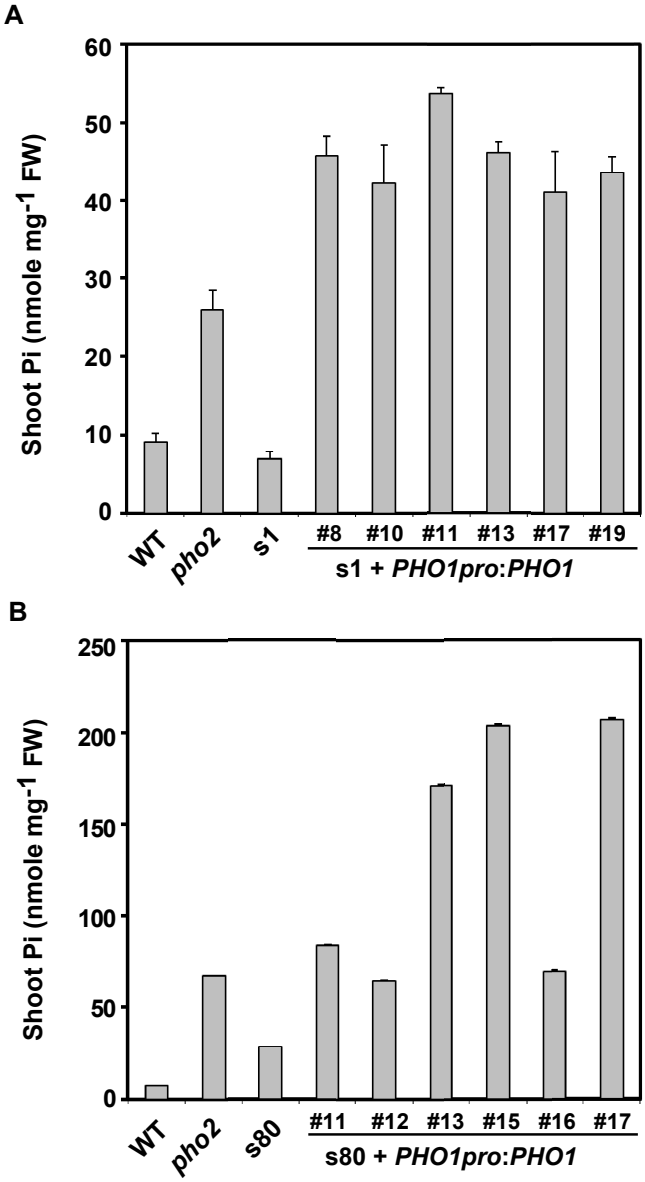
[a] Twelve-day-old seedlings were grown on agar plates containing 1 mM KH_2PO_4. Seedlings with P_i concentrations greater than 22 nmole mg^{-1} FW were classified as *pho2*-like and those with less than 22 nmole mg^{-1} FW as s1-like, as indicated in the graph below.
[b] Probabilities of departure from the stated ratios are due to sampling error.

B



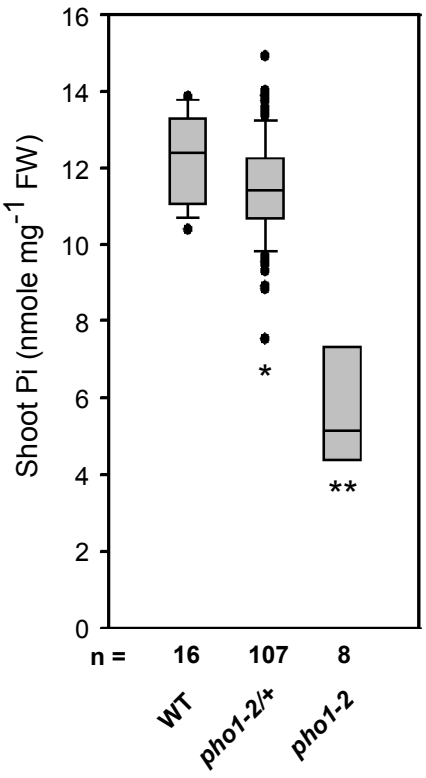
Supplemental Figure 2. Segregation analysis of F2 progeny resulting from genetic crosses between s1 and *pho2.*

(A) Segregation ratios of F2 progeny.
(B) Distribution and range of shoot P_i concentrations among the F2 progeny.



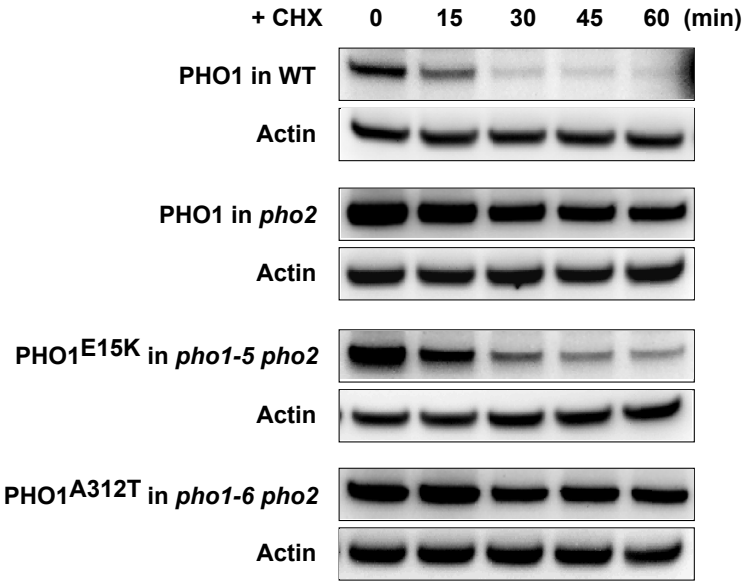
Supplemental Figure 3. The shoot P_i concentrations of PHO1-complemented *pho2* suppressors.

(A) PHO1-complemented s1 T2 lines were 11-day-old seedlings grown on agar plates containing 1 mM KH_2PO_4. Error bars represent SD (n = 3). FW, fresh weight.
(B) PHO1-complemented s80 T1 lines were 6-week-old plants grown in soil. Error bars represent SD of two technical replicates. FW, fresh weight.



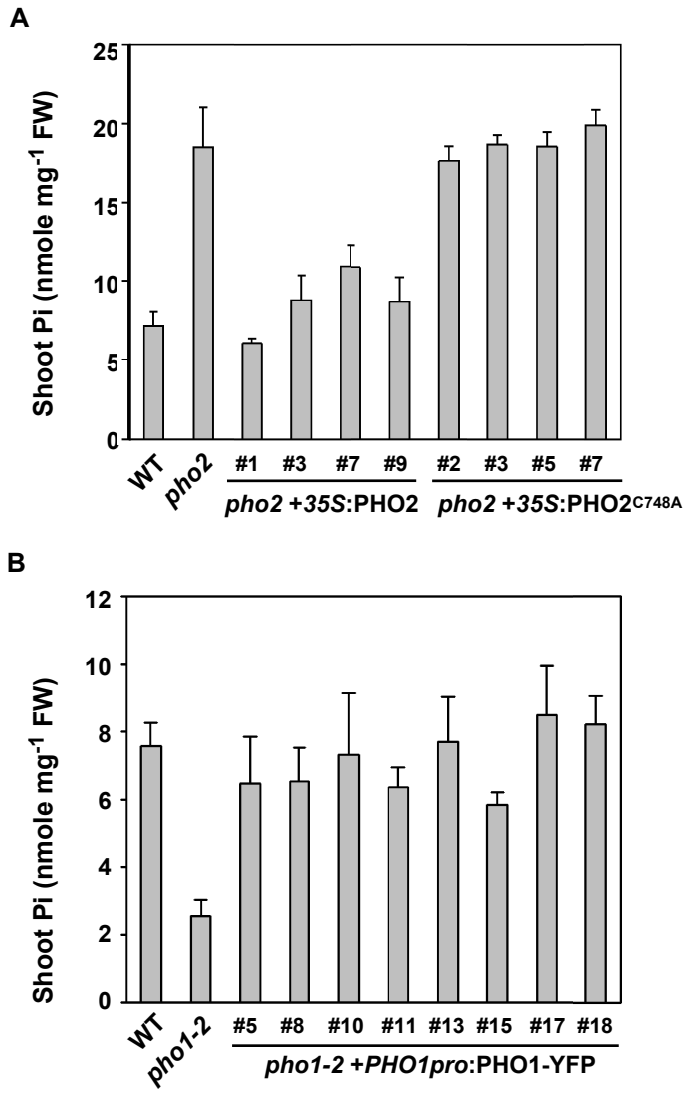
Supplemental Figure 4. The shoot P_i concentrations of heterozygous *pho1-2/+* relative to WT and *pho1-2* plants.

The growth conditions used are the same as described in the figure legend for Figure 3. Data significantly different from the WT controls are indicated (* $P < 0.05$, ** $P < 0.01$; Student's *t*-test). FW, fresh weight. n, the number of plants.



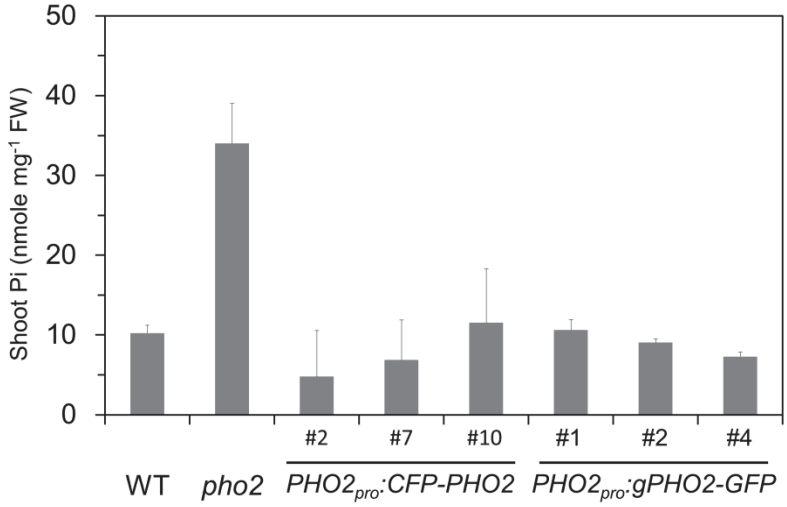
Supplemental Figure 5. Protein stability of PHO1 variants.

The expression level of the PHO1 mutant variants in the roots of 14-day-old *pho1-5 pho2* and *pho1-6 pho2* over a 60-minute period of cycloheximide treatment (CHX, 200 μM) under +P_i conditions. Actin was used as a loading control.



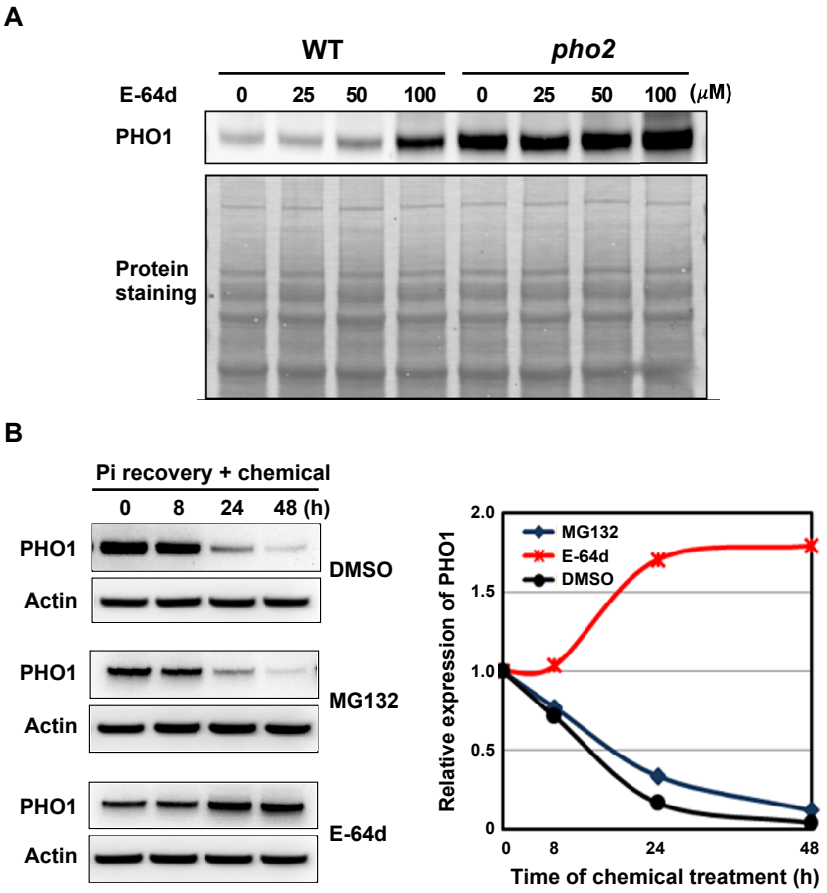
Supplemental Figure 6. Complementation tests of *pho2* and *pho1-2* mutants by mutated PHO2 and PHO1-YFP, respectively.

(A) The shoot P_i concentrations of 12-day-old PHO2- and $PHO2^{C748A}$-expressing *pho2* T2 lines grown under $+P_i$ conditions. Error bars represent SD (n = 3). FW, fresh weight. Four independent transgenic lines are shown for each construct.
(B) The shoot P_i concentrations of 14-day-old PHO1-YFP-expressing *pho1-2* T2 lines grown under $+P_i$ conditions. Eight independent transgenic lines are shown.



Supplemental Figure 7. Complementation of *pho2* by fluorescent protein-tagged PHO2.

The shoot P_i concentrations of 20-day-old plants grown hydroponically under $+P_i$ conditions were measured. A 3.1-kb genomic fragment of *PHO2* promoter was cloned into pMDC32 by replacing the 35S promoter (p$PHO2_{pro}$MDC32). The DNA fragment containing the coding sequence of the N-terminal CFP fusion of PHO2 was then recombined into p$PHO2_{pro}$MDC32, designated as $PHO2_{pro}$*:CFP-PHO2*. The genomic 10.7-kb fragment encompassing the promoter and the complete coding region of *PHO2* was cloned and recombined into pMDC107 to obtain the construct $PHO2_{pro}$*:gPHO2-GFP*. Three independent transgenic lines are shown for each construct. Error bars represent SD (n = 3).



Supplemental Figure 8. The effect of E-64d on the expression of PHO1 under P_i-sufficient conditions.

(A) The expression level of PHO1 in the root of 14-day-old WT and *pho2* seedlings over 24 hours of E-64d treatment (25, 50 and, 100 μM) under +P_i conditions. The bottom panel shows the protein staining on the membrane.
(B) The expression level of PHO1 in the root of WT seedlings over 48 hours of P_i recovery (250 μM KH_2PO_4) and MG132 (50 μM) or E-64d (50 μM) treatment following 8 days of P_i deficiency. The relative expression level of PHO1 following different chemical treatments is plotted on curves after normalization with the corresponding expression level of actin shown in the left panel.

Supplemental Table 1.

Oligonucleotides used for plasmid constructs

Construct	Primer name	Sequence (5' to 3')
PHO1$_{pro}$:PHO1	PHO1 –3566.F	GGACAAGCTGTGGCTCGTGGAAGAT
	PHO1 +5921.R	CGGAACCCTAGAAAGCACCTCCTCC
PHO1$_{pro}$*:gPHO1insYFP*	PHO1 –2108.F	TTCAGAATTCTTTCAGTTTTAGCC
	PHO1YFP +5391.R	CACAGCTCCACCTCCACCTCCAGGCCGGCCC GGTACGGTCTTCACTGCCCTAAAT
	PHO1YFP +5392.F	TGCTGGTGCTGCTGCGGCCGCTGGGGCCTTA CCGTTCCTTGACAGGGACTCAG
	PHO1 P4.R	TTGGACATCTTCGTATTCAACG
	YFP.F	GGCCGGCCTGGAGGTGGAGGTGGAGCTGTGA GCA
	YFP.R	GGCCCCAGCGGCCGCAGCAGCACCAGCAGG ATC
35S:gPHO1insYFP	PHO1.F	ATGGTGAAGTTCTCGAAGGAGCTA
	PHO1 P4.R	TTGGACATCTTCGTATTCAACG
UBQ10: gPHO1-nYFP *UBQ10: gPHO1*-cYFP	PHO1.F	ATGGTGAAGTTCTCGAAGGAGCTA
	PHO1 –2331.R1	ACCGTCTGAGTCCCTGTCA
35S:gPHO1	PHO1.F	ATGGTGAAGTTCTCGAAGGAGCTA
	PHO1 –2331.R2	TTAACCGTCTGAGTCCCTG
35S:PHO1N381-HA	PHO1.F	ATGGTGAAGTTCTCGAAGGAGCTA
	PHO1N381_HA.R	TTAAGCGTAATCTGGAACATCGTATGGGTCTT TGGTCTGGTGGGGTTTCA
35S:PHO1C399-HA	PHO1C399.F	ATGGTCACTTTCTTTGTTGGGTTA
	PHO1_HA.R	TTAAGCGTAATCTGGAACATCGTATGGGTAA CCGTCTGAGTCCCTGTCA
35S:PHO2 *35S*:CFP-PHO2	PHO2.F	ATGGAAATGTCCCTTACTGACTC
	PHO2.R1	TTATGATTCTGGTCCAATCTCTTGGA
35S:PHO2-CFP	PHO2.F	ATGGAAATGTCCCTTACTGACTC
	PHO2.R2	TGATTCTGGTCCAATCTCTTG
PHO2$_{pro}$*:PHO2-GFP*	attB1-pho2(-6973)	GGGGACAAGTTTGTACAAAAAAGCAGGCTCC TTGTGAAAGGAGGGAGAAATAG
	attB2-pho2(+2718)	GGGGACCACTTTGTACAAGAAAGCTGGGTCT GATTCTGGTCCAATCTCTTGGAC
pPHO2$_{pro}$*MDC32*	E2 promoter forward	ACAGTCGAGTTCAAGGAAGTCACA
	E2 promoter reverse	ACGTGGTACCACACACACAACTCTACA
PHO2$_{pro}$*:CFP-PHO2*	FP-START	ATGGTGAGCAAGGGCGAGGA
	At2g33770 3'2724	TTATGATTCTGGTCCAATCTCTTGGA
35S:PHO2^{C748A} *UBQ10:*cYFP-PHO2^{C748A} *UBQ10*:PHO2^{C748A}-nYFP	PHO2 C748A.F	GTTGCCTTGAGTCTGCTGAATACAT
	PHO2 C748A.R	TCTTCCTGACTCATACAGGTTCGG
PHO2.DL-Nx	PHO2 BamHI.F	ACAGGATCCATGGAAATGTCCCTTACT
	PHO2.R	TTATGATTCTGGTCCAATCTCTTGGA
PHO1N472.AMBV4	PHO1 XbaI.F	ACATCTAGAATGGTGAAGTTCTCGAAG
	PHO1N472.R	GTACCGTCTGAGTCCCTGTCA

Oligonucleotides used for qRT-PCR analysis

Gene	Primer name	Sequence (5' to 3')
UBQ10	UBQ10.for	GGCCTTGTATAATCCCTGATGAATAAG
	UBQ10.rev	AAAGAGATAACAGGAACGGAAACATAGT
PHO1	PHO1.for	GGGTTTAAGGATCGAACCAA
	PHO1.rev	GGGAGTTTCCCAAAGGTTTT