Supporting Information

Materials and Methods

Phylogenetic Analysis and Transmembrane Domain Prediction. Full-length amino acid sequences of the SPX-domain containing proteins from *Arabidopsis* and the vacuolar phosphate transport proteins from *Saccharomyces cerevisiae* were aligned using ClustalW (v. 2.0.12) (1). A phylogenetic tree was constructed using the neighbor-joining method (2) implemented in MEGA5 software with the following parameters: bootstrap method (1000 replicates), Poisson model, uniform rates, and complete deletion. For trans-membrane prediction of SPX-MFS proteins, the protein sequences were analyzed by the TMHMM Server v. 2.0 (http://www.cbs.dtu.dk/services/TMHMM/). Motif and topology prediction were carried out using the blast tool on NCBI website (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and the SOSUI program (http://bp.nuap.nagoya-u.ac.jp/sosui/), respectively.

Pi Concentration in Nutrient Soil Sap. The nutrient soil (PINDSTRUP, Denmark) was watered once every 5 days with the same volume of de-ionized water. The soil samples were collected on the first, third, and fifth day after watering and filtered with 80 mesh nylon net. After a centrifugation at 14,000 rpm for 5 min, the supernatant was used for Pi measurement by ascorbate-molybdate-antimony method as described previously (3).

Death Rate Analysis. The wild-type (Col-0) and *vpt1* mutant plants were planted in a hydroponic system containing medium reported by Tocquin et al (4) except that $NH_4H_2PO_4$ was replaced with NaH_2PO_4 as Pi source. Some seedlings turned albino on the third day after germination in the medium containing mM level of Pi, and then stopped growing in the next culture phase. 2-week-old seedlings showing albino phenotype were considered as “dead”. The death rate was calculated as % of albino seedlings to all seedlings. Plants were grown under long-day conditions (16-h illumination of 150 μmol/m^2/s, and 8-h dark cycle) at 22°C.

Quantitative Real-Time RT-PCR (qRT-PCR) Analysis of Pi Response Genes. Total RNA was extracted using TRIzol reagent (Invitrogen) from two-week-old plants grown in the hydroponic system containing 1.3 mM Pi or after transfer from 1.3 mM to 1.3 μM Pi condition for 48 h . The first-strand cDNA was synthesized by M-MLV Reverse Transcriptase (Promega) with oligo(dT) primers. Quantitative PCR was performed using QuantiFast™ SYBR Green PCR Kit (QIAGEN) on a CFX Connect™ Real-Time System (BIO-RAD). Target quantifications were performed with specific primer pairs designed using Primer 5 software (Table S1). *NLA* is induced by high Pi levels, and *WRKY45*, *miR399*, *RNS1*, *PHT1;1* and *PHT1;4* are genes induced by low Pi as shown in previous studies (5-9).

Construction of Dexamethasone-Inducible Transgenic *Arabidopsis* Lines. For the inducible expression of *VPT1*, the full-length *VPT1* CDS was amplified by polymerase chain reaction (PCR) from a wild-type cDNA pool using specific primers (Supplemental Table 1), and the 2.1-kb amplicon was cloned into the binary vector pTA7002 with ClonExpress® MultiS One Step

Cloning Kit (Vazyme^{TM}, China). The generated construct was further sequenced for confirmation and introduced into *Agrobacterium tumefaciens* (strain GV3101) cells for transformation into the *vpt1* mutant plants. More than 70 independent lines were selected for hygromycin resistance. Homozygous transformants (T3) were confirmed by both segregation and PCR analysis. Three representative lines were used for phenotypic analysis and molecular characterization. The 3-week-old transgenic *Arabidopsis* seedlings were splayed with 10 μM dexamethasone (DEX) and Pi contents and expression of *VPT1* and *ACTIN2* were measured after 12 hours of treatment.

Measurement of Anion Content. Rosettes of 3-week-old seedlings grown in the soil were used for measuring contents of various anions. . To measure Pi content, 100 mg of fresh plant sample was grounded in 1 ml ice-cold 5% perchloric acid solution. After centrifugation, the supernatant was used for the determination of Pi content using the ascorbate-molybdate-antimony method as described previously (3). For measuring sulfate content, 100 mg of fresh plant sample was incubated in 1 ml of 0.1 M HCl for 2 h at room temperature. After brief mixing and centrifugation, the supernatant was used for the analysis of sulfate content using the turbidimetric method (10). For analyzing nitrate content, 100 mg of fresh leaves was cut into small pieces and vacuum-infiltrated in 5 ml deionized water. Nitrate was extracted by boiling for 30 min and the supernatant was used for nitrate measurement by the salicylic acid nitration procedure (11). To measure the chloride content, 100 mg of fresh leaves was grounded in liquid nitrogen, and extracted with 5 ml 1 M HNO_3 at 95 ^{o}C for 30 min. After centrifugation, the chloride concentration of the supernatant was determined by the silver titration method (12). For determination of malate content, 100 mg of fresh leaves were ground in liquid nitrogen and extracted with 1 ml of boiling 80% ethanol for 5 min. After centrifugation, the supernatant was filtered through a 0.45-μm filter. The malate content was analyzed by HPLC using an RP-18 column (13).

References

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Supplementary Figures

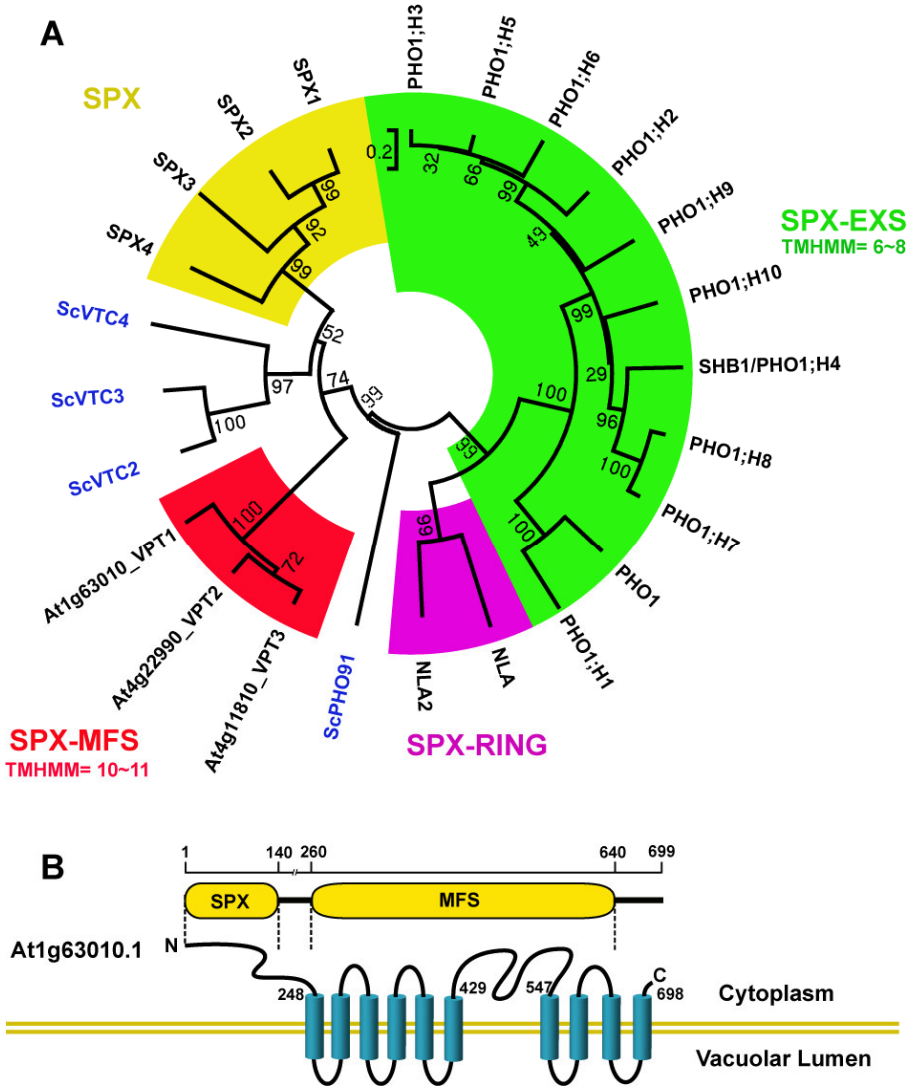

Fig. S1. **Phylogenetic analysis of SPX-domain proteins and the predicted ten transmembrane domains in VPT1**. (***A***) Phylogenetic tree of SPX domain-containing proteins in *Arabidopsis thaliana* (ecotype *Columbia*-0) and *Saccharomyces cerevisiae*. *Arabidopsis* has four classes of SPX domain-containing proteins, SPX, SPX-RING, SPX-EXS, and SPX-MFS. The amino acid sequences were aligned with Clustal W and the phylogenetic tree was made using the Neighbor-Joining method of MEGA5.2 software. The trans-membrane information was obtained by TMHMM Server v. 2.0. (http://www.cbs.dtu.dk/services/TMHMM/). (***B***) Predicted topology for AtVPT1 encoded by At1g63010.1 with SOSUI program (http://bp.nuap.nagoya-u.ac.jp/sosui/). The protein domains were drawn according to the result of protein blast on the NCBI website (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

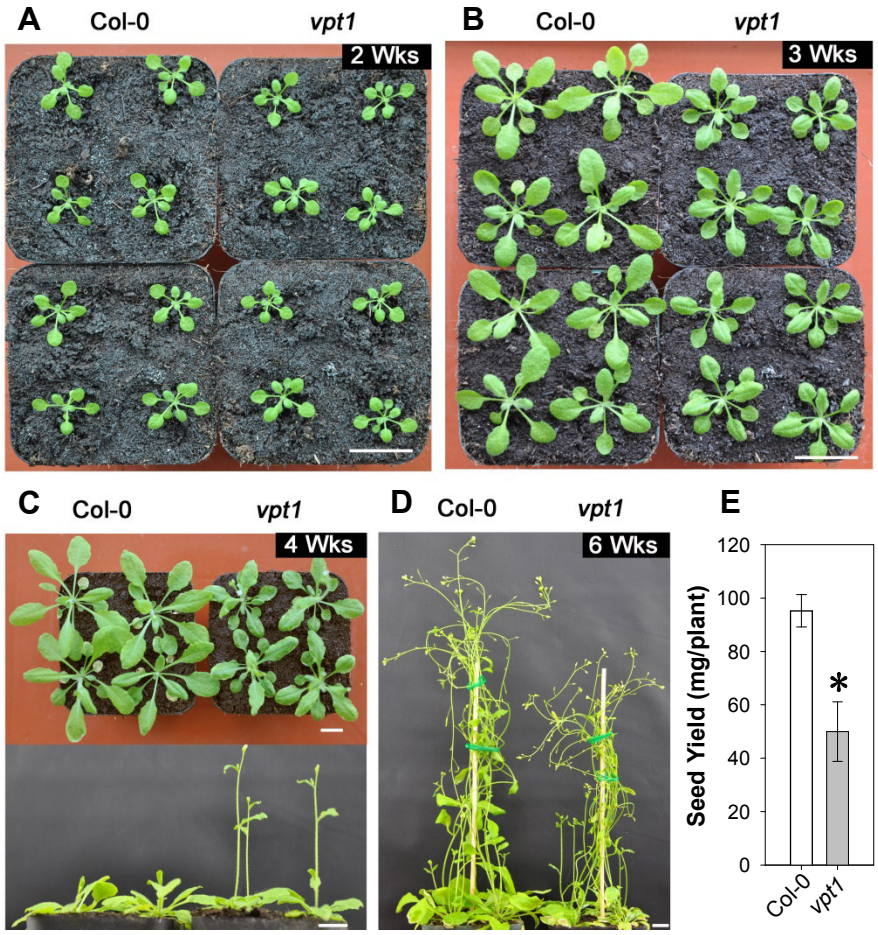

Fig. S2. Growth phenotype of the *vpt1* mutants in soil. Wild-type (Col-0) and the *vpt1* mutant seeds were plated on 1/2 MS medium with 1% agar and grown for 3 days, and then were transplanted to the soil. The images were taken after 2 weeks (***A***), 3 weeks (***B***), 4 weeks (***C***), and 6 weeks (***D***) after transfer to soil. The images in (***C***) were taken either from the top (upper) or rom the side (lower) to show both stunted growth and early flowering of *vpt1* mutant. Bars = 1 mm. (***E***) Reduced seed production in *vpt1* mutants. Dried seeds were weighted and compared. Error bars indicate ± SD (n = 8 plants). Statistical analysis was performed between wild-type (Col-0) and *vpt1* mutant plants using Student's t test (*P < 0.01).

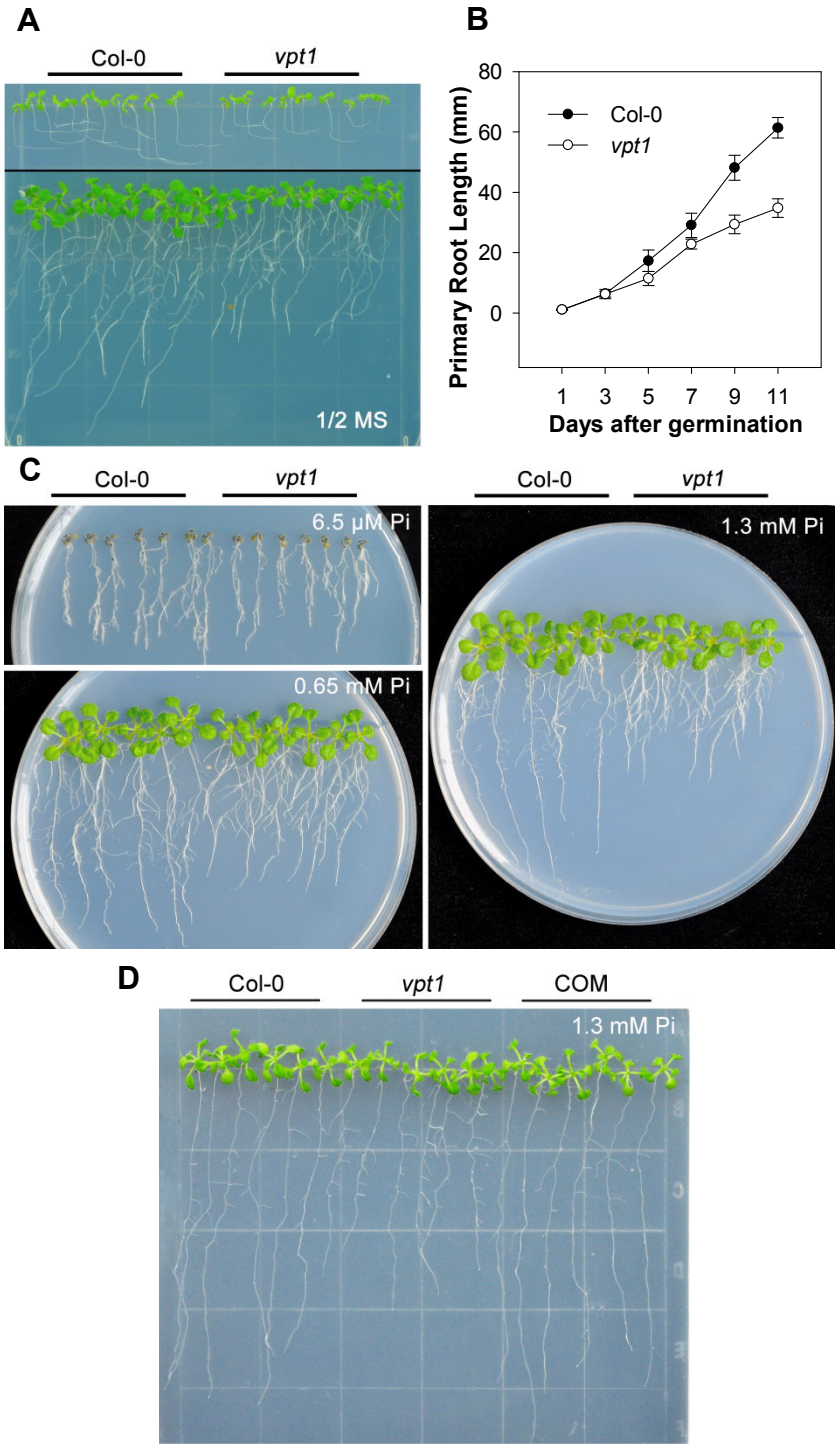

Fig.S3. The *vpt1* mutant plants are sensitive to Pi in the 1/2 MS agar medium. (***A,B***) Root growth of wild-type (Col-0) and *vpt1* mutants. (***A***) Upper panel: three-day-old seedlings were rotated 90^{o} and cultured for another 3 days. Lower panel: vertically cultured for 9 days 1/2 MS medium containing 0.65 mM Pi. (***B***) Root lengths measured (using Image J) on the indicated days after germination (n=10). The data present mean±SD. (***C***) Growth phenotype of two-week-old Col-0 and *vpt1* mutant seedlings on the 1/2 MS agar medium containing 6.5 µM, 0.65 mM, or 1.3 mM mM KH_2PO_4. (***D***) Comparing growth of WT, *vpt1*, and complementation line (COM) under high Picondition (1.3 mM KH_2PO_4).

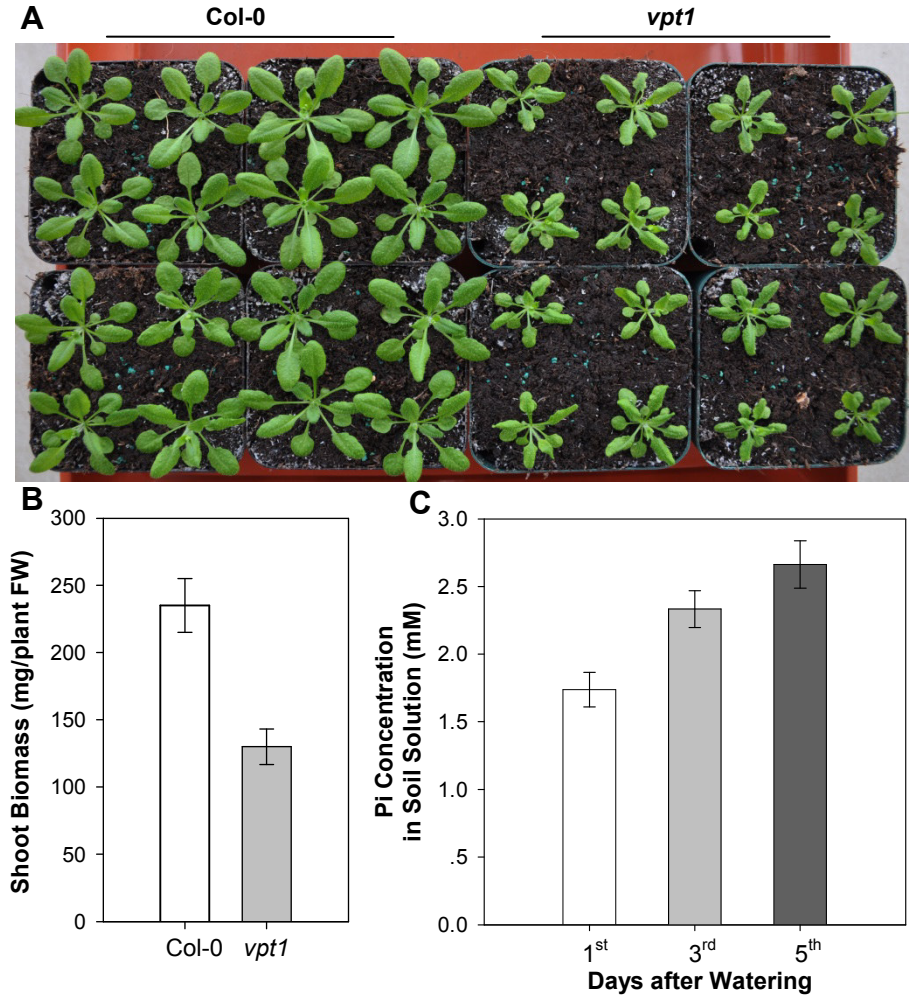

Fig. S4. Pi content in the nutrient-rich soil. The growth phenotype (***A***) and shoot biomass (***B***) of 3-week-old wild-type (Col-0) and *vpt1* mutants grown in the soil on the first day of watering cycle. Shoot biomass was summarized from 16 Col-0 and *vpt1* mutant seedlings. (***C***) The Pi concentration in the soil. The data presented in (***B***) and (***C***) are mean±SD.

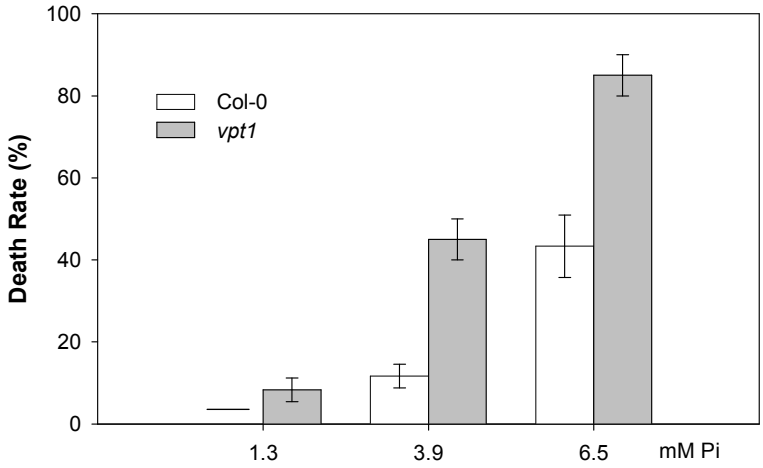

Figure S5. The death rate of *vpt1* is higher than wild-type (Col) in hydroponic medium containing high concentration of Pi. Col-0 and *vpt1* mutants were germinated and grown in the hydroponic medium containing 1.3, 3.9, or 6.5 mM Pi. The 2-week-old severely arrested seedlings with bleached leaves were counted as "dead" and death rate is the % of such seedlings versus all seedlings. The data represent mean ± SD of four biological replicates.

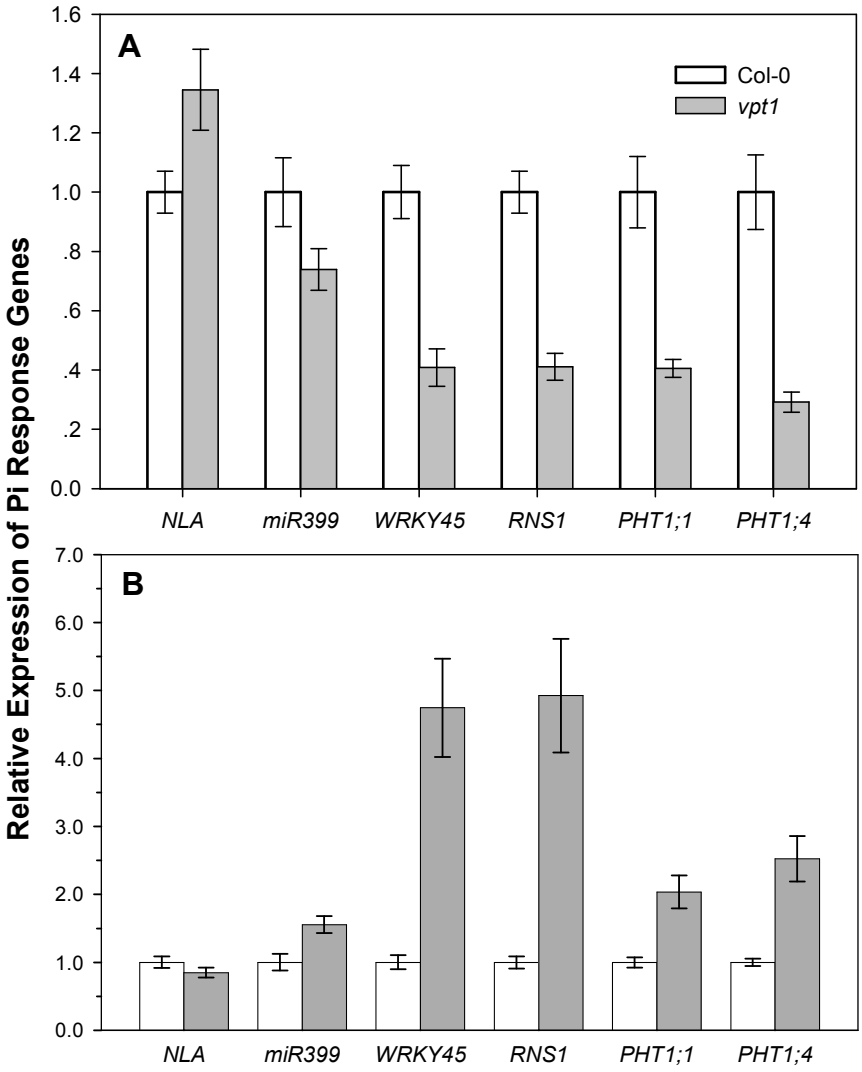

Fig. S6. qRT-PCR analysis of Pi response genes under different Pi conditions. (***A***) Expression levels of Pi response genes under Pi sufficient condition. Total RNA was extracted from two-week-old seedlings of Col-0 and *vpt1* grown in hydroponic culture containing 1.3 mM Pi, and the transcripts of the indicated genes were analyzed. (***B***) Expressions of Pi response genes in plants transferred from Pi sufficient to Pi deficient condition. Two-week-old seedlings grown under 1.3 mM Pi condition were transferred to 1.3 μM Pi condition. Total RNA was extracted from Col-0 and *vpt1* at 48 h after the transfer. Expression of *NLA* is induced by high Pi levels, and *miR399*, *WRKY45*, *RNS1*, *PHT1;1* and *PHT1;4* are induced by low Pi levels according to previous studies (5-9). Values from qRT-PCR analysis were normalized to *ACTIN2* as an internal control. The relative expression levels of the indicated genes were compared against level of mRNA in the wild-type sample (as 1.0). The data points represent mean ± SD of four biological replicates.

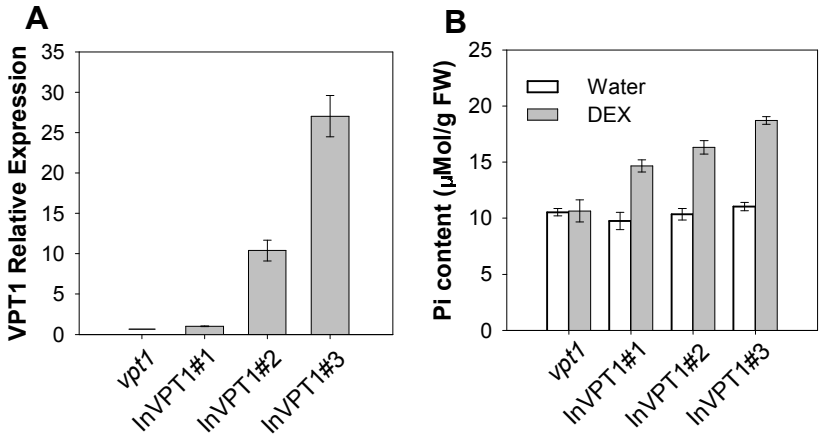

Fig. S7. Dexamethasone (DEX)-induced transient *VPT1* expression caused an increase in Pi accumulation in the *vpt1* mutant plants. Spraying 10 μM DEX induced *VPT1* expression (***A***) and promoted Pi accumulation (***B***) in *vpt1* mutant plants transformed with *VPT1* expression system (InVPT1). 3-week-old seedlings cultured in the hydroponic solution containing 0.13 mM Pi were sprayed with 10 μM DEX. Rosette leaves were obtained at 12h after treatment for mRNA measurement and Pi content determination. Values were normalized to *ACTIN2* and the relative expression of *VPT1* was calculated as the ratio of *VPT1* to the InVPT1#1 in (***A***). The data presented in (***A***) and (***B***) are mean ± SD of four biological replicates.

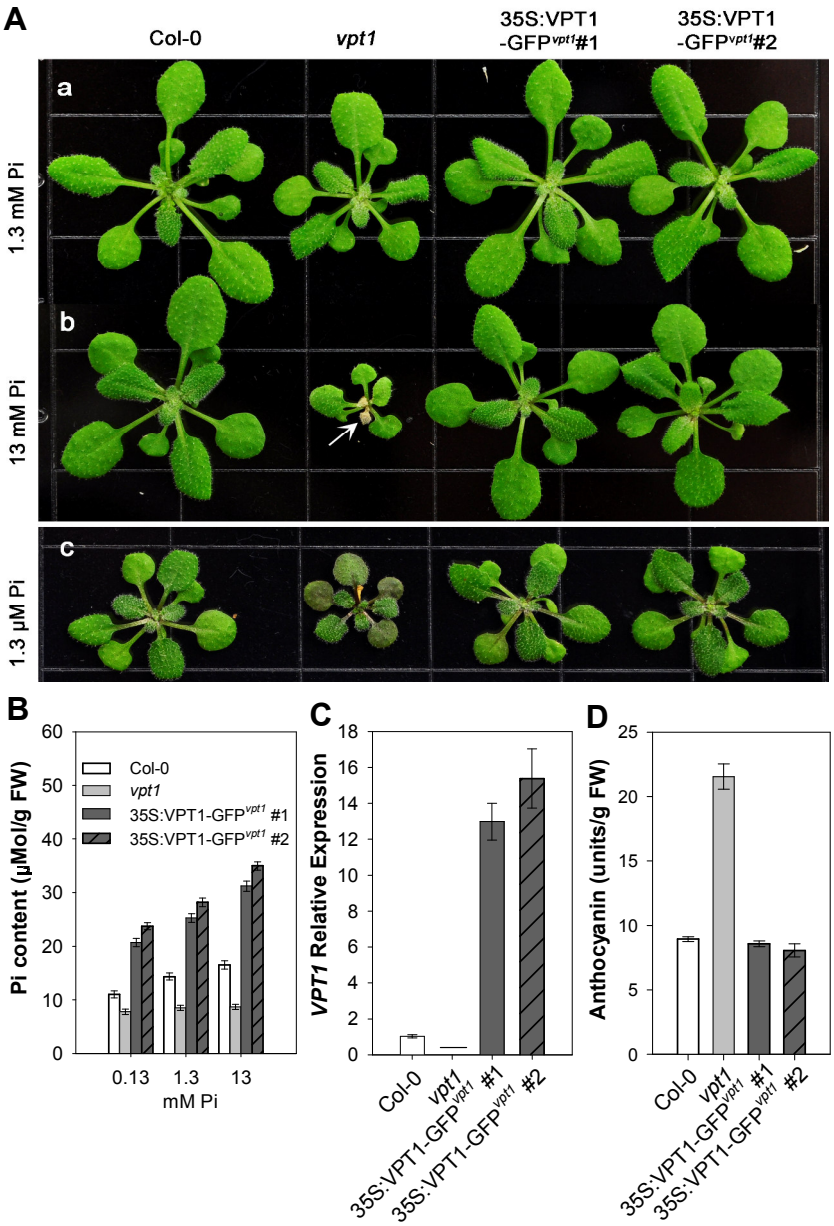

Fig. S8. The VPT1-GFP fusion protein complements growth phenotype and Pi accumulation deficiency of *vpt1* mutant. (***A***) The growth phenotype of wild-type (Col-0), *vpt1* mutant and 35S:VPT1-GFP^{vpt1} line 1 (35S:VPT1-GFP^{vpt1} #1) and line 2 (35S:VPT1-GFP^{vpt1} #2) in hydroponic medium containing 1.3 mM Pi (a), 13 mM Pi (b), or 1.3 µM Pi (c). The arrow in (b) indicates the albino and "dead" young leaves. (***B***) The Pi content of Col-0, *vpt1* mutant, 35S:VPT1-GFP^{vpt1} #1, and 35S:VPT1-GFP^{vpt1} #2 in hydroponic medium containing 0.13, 1.3, or 13 mM Pi. (***C***) Relative expression of *VPT1* in Col-0, *vpt1* mutant, 35S:VPT1-GFP^{vpt1} #1, and 35S:VPT1-GFP^{vpt1} #2 in hydroponic medium containing 0.13 mM Pi. (***D***) Anthocyanin accumulation in Col-0, *vpt1* mutant, 35S:VPT1-GFP^{vpt1} #1, and 35S:VPT1-GFP^{vpt1} #2 treated with 1.3 µM Pi. Two-week-old seedlings cultured in hydroponic medium containing 0.13 mM Pi were transferred into the medium containing 1.3 mM (a) or 13 mM (b) Pi, or two-week-old seedlings cultured under 1.3 mM were transferred into the medium containing 1.3 µM Pi (c), and were grown for 7 days before photographs were taken (***A***), Pi content was measured (***B***), expression of *VPT1* and *ACTIN2* (***C***) were analyzed, and anthocyanin was determined (***D***). The data presented in (**B**), (***C***) and (***D***) indicates mean ± SD of four biological replicates.

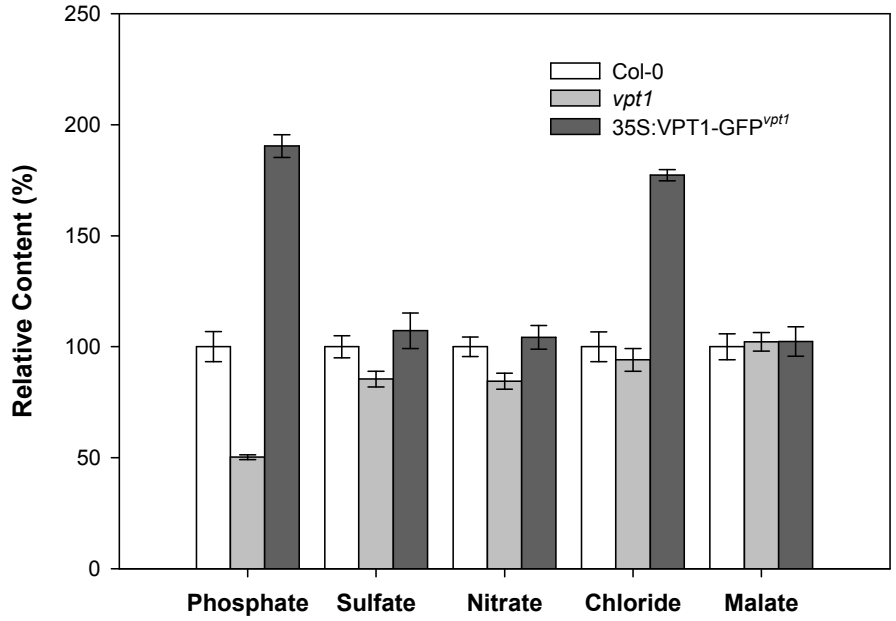

Fig. S9. Relative contents of various anions in the rosette of Col-0, *vpt1*, and 35S:VPT1-GFP^{vpt1} line. The rosettes were collected from three-week-old seedlings grown in nutrient soil for determining the contents of phosphate, sulfate, nitrate, chloride, and malate. The data were normalized to the contents in Col-0, and presented as percentage ±SD of four biological replicates.

Supplementary Tables

Table S1. List of PCR Primers

Names	Primer sequences	Purposes
VPT1-RT-PCR-F	5'-CGTTGCTGATCACTTCAGGA-3'	RT-PCR analysis of
VPT1-RT-PCR-R	5'-GCAATCCTCCGTTGTACGTT-3'	*VPT1* mRNA
g*VPT1*-Com-F	5'-GCTATGACCATGATTACGAATTCATACATAATCATATAACTGCAGATAA AATATCG-3'	complementation with genomic DNA
g*VPT1*-Com-R	5'-GCAGGTCGACTCTAGAGGATCCTGATCCTTTCACTGAAGTTATAGG-3'	
pEZS-*VPT1*-F	5'-TCGAGCTCAAGCTTCGAATTCATGGTGGCTTTTGGGAAATA-3'	Subcellular localization
pEZS-*VPT1*-R	5'-GTACCGTCGACTGCAGAATTCTATAGAGTGAGTTATAAGTACAACAAG TAG-3'	analysis of VPT1 in transgenic plants
35s: VPT1-GFP -F	5'-GCTATGACCATGATTACGAATTCCCTCTAATACGACTCACTATAGG-3'	
35s: VPT1-GFP -R	5'-GCAGGTCGACTCTAGAGGATCCGTGACACTATAGAATATGCATCAC-3'	
GUS-F	5'-GCATGCCTGCAGGTCGACTCTAGAATACATAATCATATAACTGCAGATA AAATATCG-3'	Tissue-specific expression analysis of
GUS-R	5'-GGACTGACCACCCGGGGATCCCTTTTAATCGCAGAAAGCAGAG-3'	*VPT1*
p7002-*VPT1*-F	5'-TCGAGGCTAGAGGATCGACTAGTATGGTGGCTTTTGGGAAATAC-3'	construction of
p7002-*VPT1*-R	5'-TACGGACGAAAGCTGGGAGGCCTTCAATAGAGTGAGTTATAAGTACA ACAAG-3'	DEX-inducible expression system of *VPT1*
VPT1-qPCR-F	5'-GGTTTATTTCAGCGCATGGT-3'	qRT-PCR analysis of
VPT1-qPCR-R	5'-TCCGGTTAACAGCTCTTGCT-3'	*VPT1* expression
ACTIN2-qPCR-F	5'-GCCATCCAAGCTGTTCTCTC-3'	qRT-PCR analysis of
ACTIN2-qPCR-R	5'-GCTCGT AGTCAACAGCAACAA-3'	*ACTIN2*
NLA-F	5'-GCCATTCGAAAAATCCTCAA-3'	qRT-PCR analysis of Pi
NLA-R	5'- TGAACAGGAGGAGGAGGAGA-3'	response genes
WRKY45-F	5'-GTTTCATGGGGTCGACAACT-3'	
WRKY45-R	5'-CTGCTTTTTGGCCGTACTTC-3'	
miR399-F	5'-ATATGCATTACAGGGCAAGATC-3'	
miR399-R	5'-AAGAGAATTACCGGGCAAATC-3'	
RNS1-F	5'-ATCCAAATTCAGGCAAACCA-3'	
RNS1-R	5'-GTGTTGGCCAGCTCTTCTTC-3'	
PHT1;1-F	5'-GGTTCCTATATGCGGCTCAA-3'	
PHT1;1-R	5'-GCTAACCTCAGCCTCACCAG-3'	
PHT1;4-F	5'-TGATAAGCTCGGGAGGAAGA-3'	
PHT1;4-R	5'-TGGTTGCGGATAAAGGGTAG-3'	