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SUPPLEMENTARY INFORMATION

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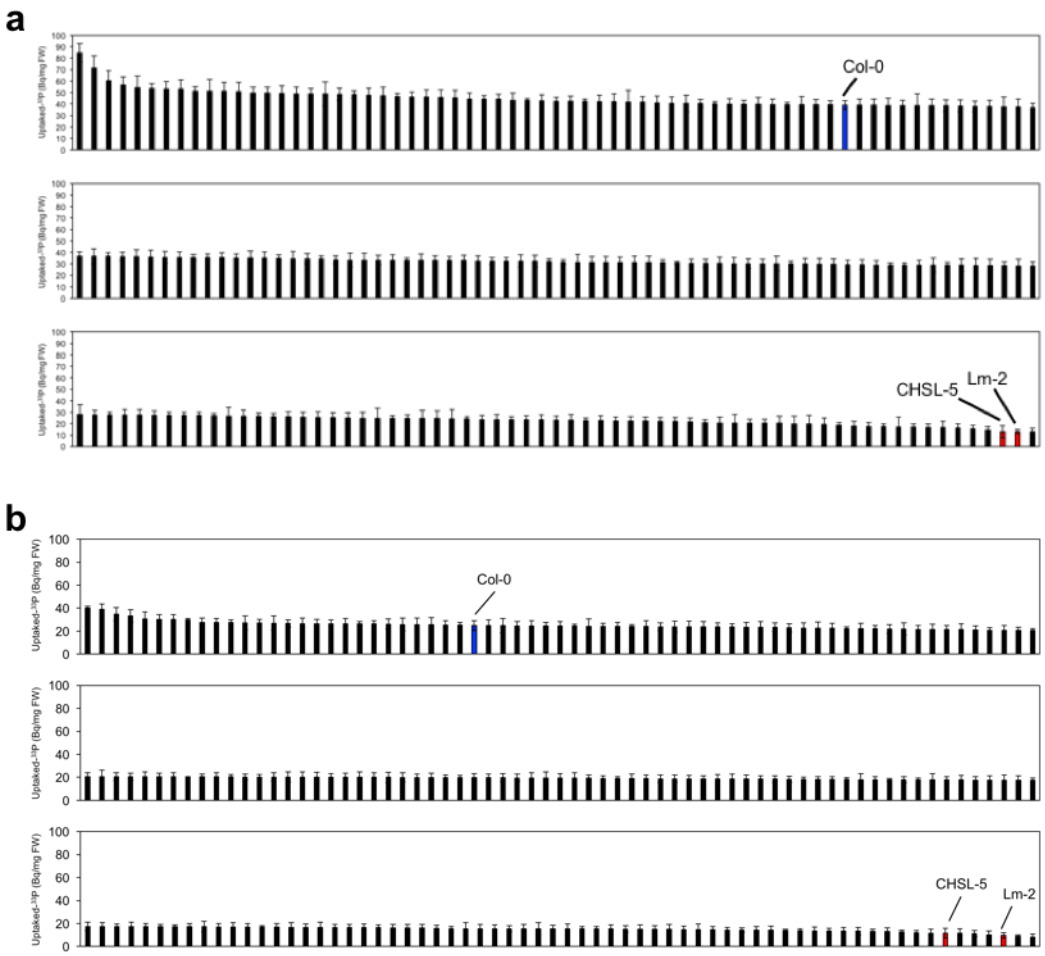
A phytochrome-B-mediated regulatory mechanism of phosphorus acquisition

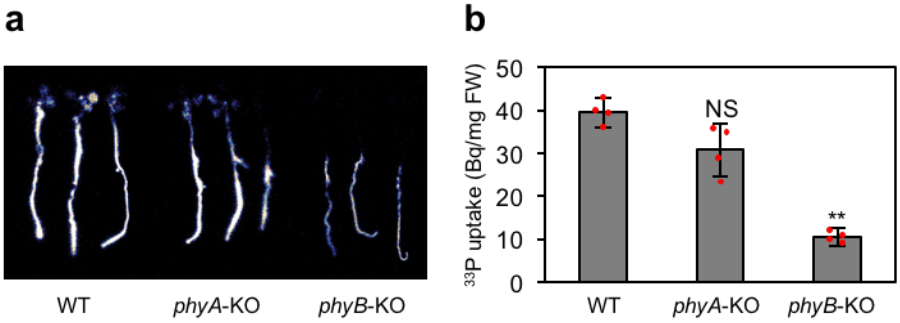
Yasuhito Sakuraba[1], Satomi Kanno[1], Atsushi Mabuchi[2], Keina Monda[2], Koh Iba[2] and Shuichi Yanagisawa[1]*

[1]Plant Functional Biotechnology, Biotechnology Research Center, The University of Tokyo, Tokyo, Japan. [2]Department of Biology, Faculty of Science, Kyushu University, Fukuoka, Japan. *e-mail: asyanagi@mail.ecc.u-tokyo.ac.jp

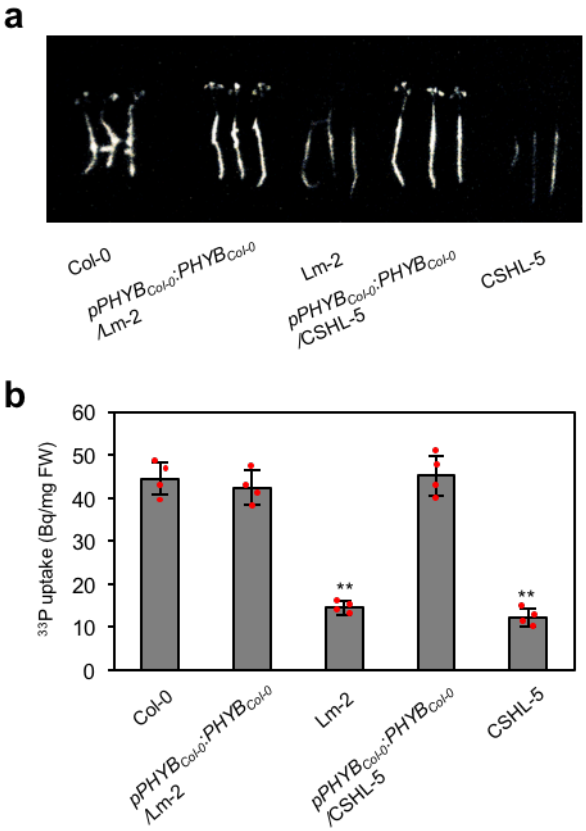
Supplementary Information – Sakuraba *et al*.



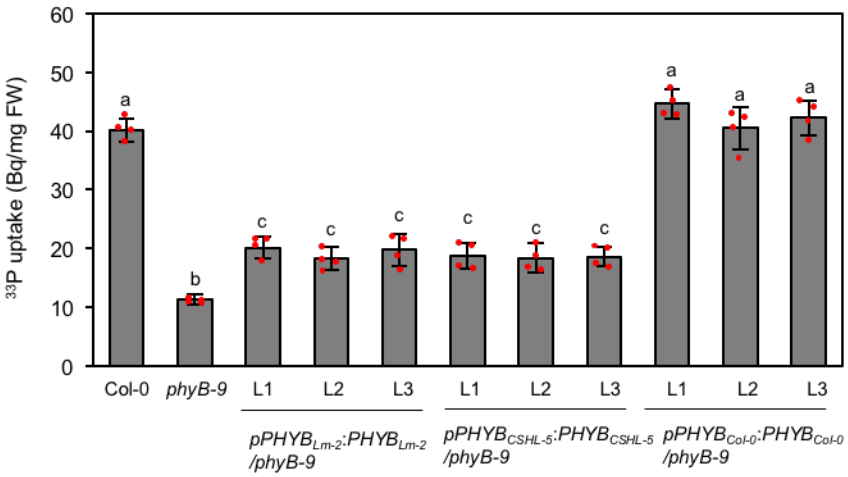
Supplementary Fig. S1 | Pi uptake activities in 10-day-old seedlings from 200 natural accessions of Arabidopsis. Seedlings used in these assays were grown under Pi-sufficient conditions for 5 days and then under Pi-deficient conditions for 5 days (a), or continuously grown under Pi-sufficient conditions for 10 days (b). The blue bar represents the activity of Col-0, whereas red bars indicate activity of CSHL-5 and Lm-2. n=4 biologically independent samples.



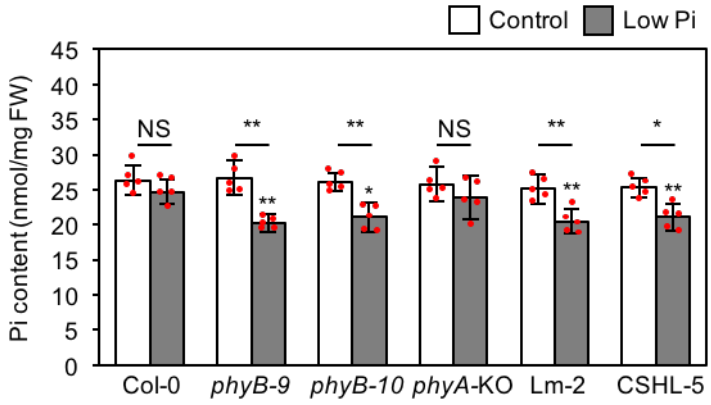
Supplementary Fig. S2 | Pi uptake is similar in a *phyA*-KO mutant and Col-0 (WT). Visualization (**a**) and quantification (**b**) of ^{33}P-labeled phosphate uptake by Col-0 (WT), *phyA*-KO and *phyB*-KO (*phyB-9*) seedlings grown under Pi-sufficient conditions for 5 days and then under Pi-deficient conditions for 5 days. n=4 biologically independent samples. ** $P < 0.01$ by two-tailed Student's *t*-test compared with the value obtained from Col-0 (WT) seedlings; NS: not significant.



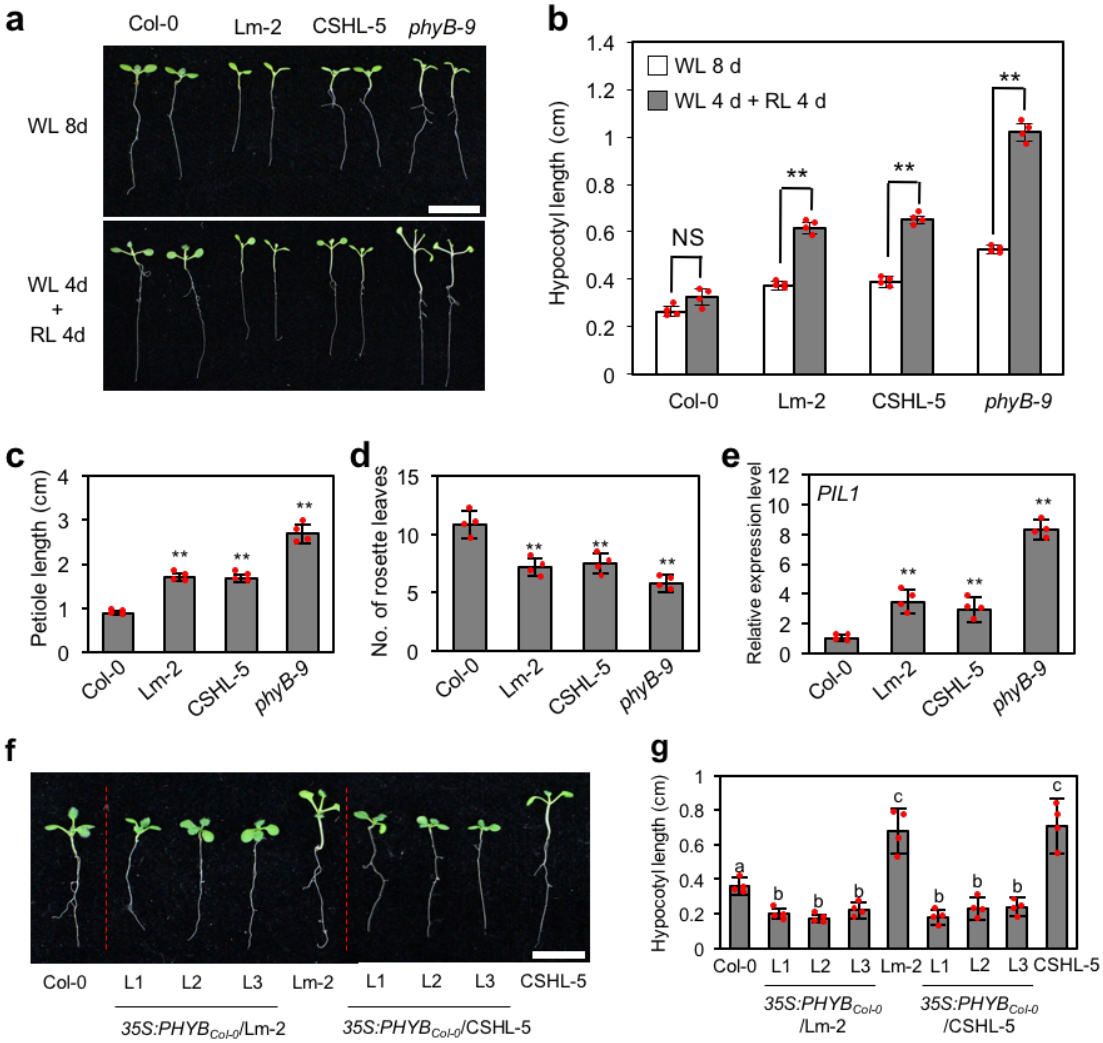
Supplementary Fig. S3 | Low Pi uptake in Lm-2 and CSHL-5 was rescued by expressing Col-0-type *PHYB* using its native promoter. Visualization (**a**) and quantification (**b**) of ^{33}P-labeled phosphate uptake by Col-0 (WT), *pPHYB*$_{Col\text{-}0}$*:PHYB*$_{Col\text{-}0}$/Lm-2, Lm-2, *pPHYB*$_{Col\text{-}0}$*:PHYB*$_{Col\text{-}0}$/CSHL-5, and CSHL-5 seedlings grown under Pi-sufficient conditions for 5 days and then under Pi-deficient conditions for 5 days. n=4 biologically independent samples. ** $P < 0.01$ by two-tailed Student's *t*-test compared with the value obtained from Col-0 (WT) seedlings.



Supplementary Fig. S4 | Low Pi uptake in *phyB-9* was not completely recovered by expressing Lm-2- and CSHL-5-type PHYB with their native promoter. **Q**uantification of ^{33}P-labeled phosphate uptake by seedlings of Col-0 (WT), *phyB-9*, and three independent lines of $pPHYB_{Lm\text{-}2}$:$PHYB_{Lm\text{-}2}$/*phyB-9*, $pPHYB_{CSHL\text{-}5}$:$PHYB_{CSHL\text{-}5}$/*phyB-9*, and $pPHYB_{Col\text{-}0}$:$PHYB_{Col\text{-}0}$/*phyB-9* that were grown under Pi-sufficient conditions for 5 days and then under Pi-deficient conditions for 5 days. n=4 biologically independent samples. The same letters above each bar indicate that means did not differ significantly at the 0.05 level in Tukey's multiple comparison test.

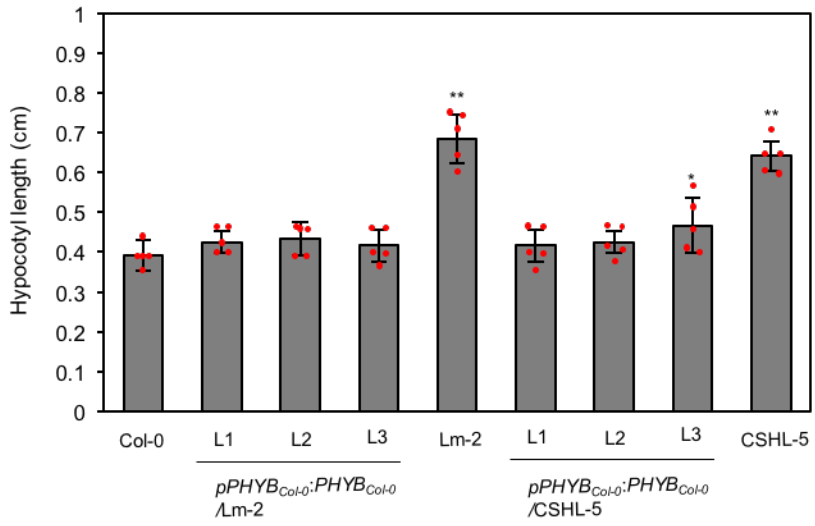


Supplementary Fig. S5 | Pi content of Col-0 (WT), *phyB-9*, *phyB-10*, *phyA*-KO, Lm-2 and CSHL-5 seedlings. Seedlings were grown for 5 days under Pi-sufficient conditions and for an additional 5 days under Pi-deficient conditions. n=5 biologically independent samples. ** ($P < 0.01$) and * ($P < 0.05$) above white bars indicate comparisons with the value obtained from Col-0 (WT) seedlings in two-tailed Student's *t*-tests. ** ($P < 0.01$), * ($P < 0.05$) and NS (not significant) marked above horizontal bars indicate whether the values obtained from seedlings before Pi-starvation treatment (control) differed significantly from those obtained after Pi starvation (Low Pi) in a Student's *t*-test.

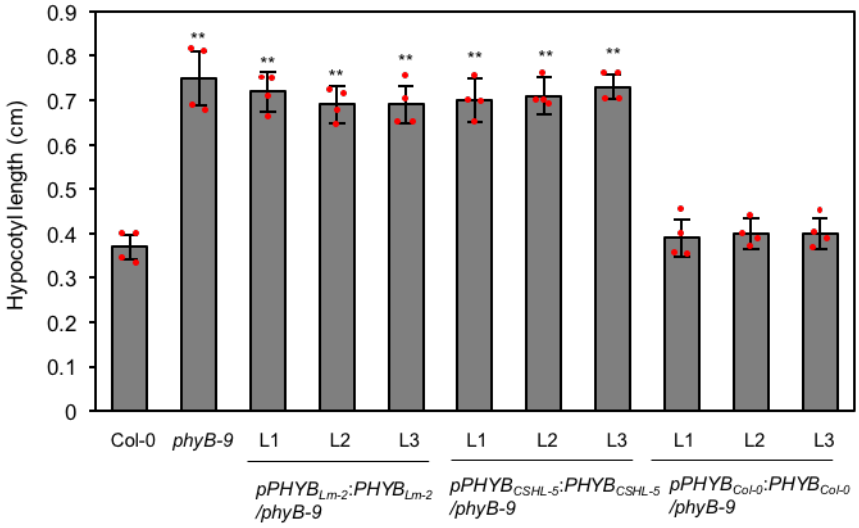


Supplementary Fig. S6 | Phenotypic characterization of Lm-2 and CSHL-5. (**a-d**) Growth of Col-0, Lm-2, CSHL-5 and *phyB*-KO mutant (*phyB-9*) seedlings. Images (**a**) and hypocotyl lengths (**b**) of seedlings grown under white light (WL) for 8 days (WL 8d) or under WL for 4 days and subsequently under red light (RL) for 4 days (WL 4d + RL 4d). n=4 biologically independent samples. Petiole lengths (**c**) of 3-week-old Lm-2, CSHL-5 and *phyB-9* plants and the number of rosette leaves at the bolting phase (**d**) of Lm-2, CSHL-5 and *phyB-9* plants were compared with those of WT Col-0 plants. n=4 biologically independent samples. (**e**) Relative expression levels of *PIL1* in 10-day-old Col-0, Lm-2, CSHL-5 and *phyB-9* seedlings. The expression levels of *PIL1* were normalized against the transcript level of *GAPDH*. n=4 biologically independent samples. In **b**, ** ($P < 0.01$) and NS indicate significance and no significance of RL induction of hypocotyl elongation in a two-tailed Student's *t*-test, respectively. In **c-e,**

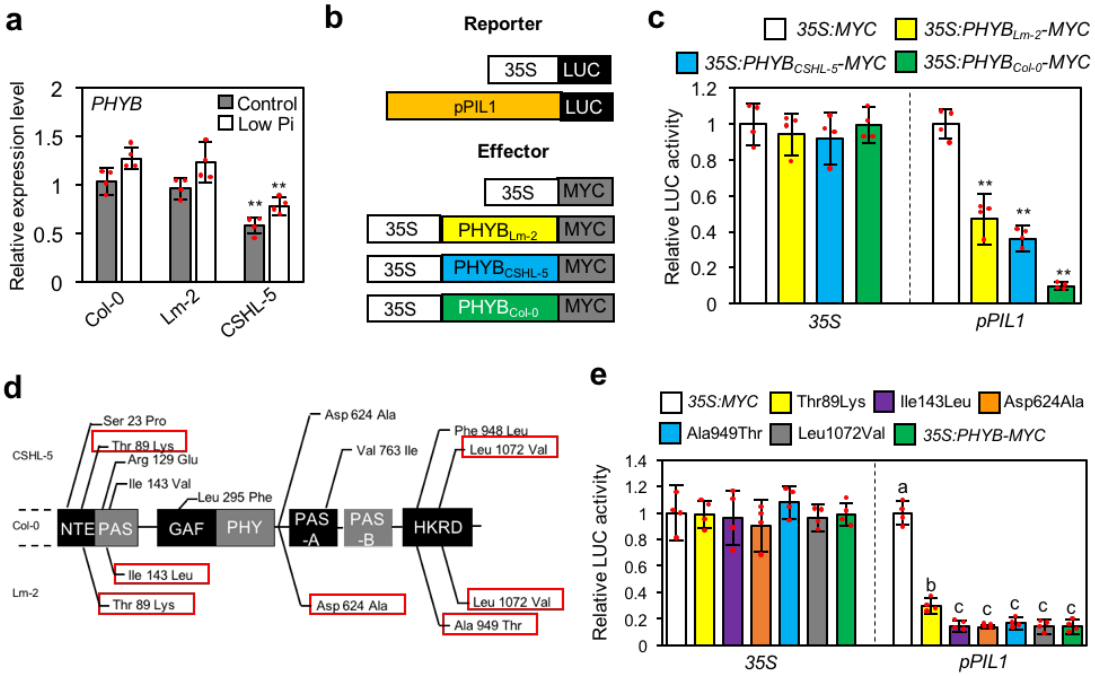
** ($P < 0.01$) indicates whether values significantly differed from those of Col-0 plants in a two-tailed Student's *t*-test. (**f, g**) Growth of Lm-2 and CSHL-5 seedlings expressing Col-0-type *PHYB* (*35S:PHYB*$_{Col\text{-}0}$/Lm-2 or *35S:PHYB*$_{Col\text{-}0}$/CSHL-5). Images (**f**) and hypocotyl lengths (**g**) of 10-day-old seedlings from three independent transgenic lines (L1–3). n=4 biologically independent samples. The same letters above each bar indicate that means did not differ significantly at the 0.05 level in Tukey's multiple range test. **a** and **f** scale bars: 1 cm.



Supplementary Fig. S7 | Hypocotyl lengths of 10-day-old seedlings of Col-0 (WT), *pPHYB$_{Col-0}$:PHYB$_{Col-0}$*/Lm-2, Lm-2, *pPHYB$_{Col-0}$:PHYB$_{Col-0}$*/CSHL-5, and CSHL-5. Three independent transgenic lines were used for the analysis of *pPHYB$_{Col-0}$:PHYB$_{Col-0}$*/Lm-2 and *pPHYB$_{Col-0}$:PHYB$_{Col-0}$*/CSHL-5. n=5 biologically independent samples. ** $P < 0.01$ and * $P < 0.05$ by two-tailed Student's *t*-test compared with the value of Col-0 (WT) seedlings.

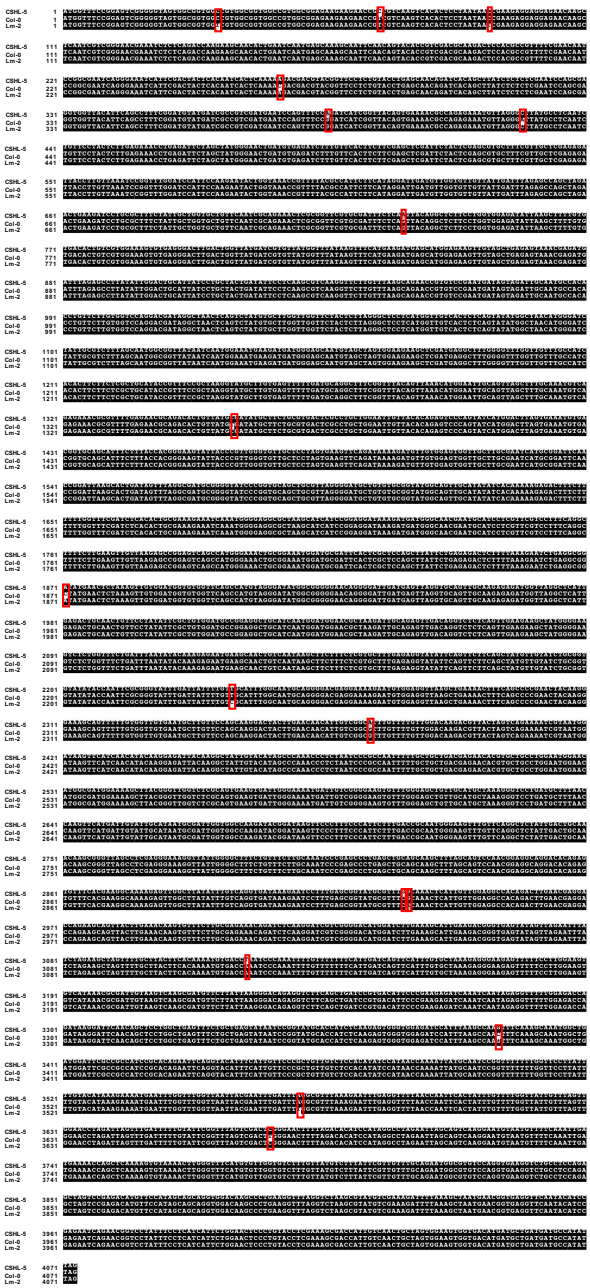


Supplementary Fig. S8 | Hypocotyl lengths of 10-day-old seedlings of Col-0 (WT), *phyB-9*, *pPHYB$_{Lm-2}$:PHYB$_{Lm-2}$/phyB-9*, *pPHYB$_{CSHL-5}$:PHYB$_{CSHL-5}$/phyB-9*, and *pPHYB$_{Col-0}$:PHYB$_{Col-0}$/phyB-9*. Three independent transgenic lines were used for the analysis of *pPHYB$_{Lm-2}$:PHYB$_{Lm-2}$/phyB-9*, *pPHYB$_{CSHL-5}$:PHYB$_{CSHL-5}$/phyB-9*, and *pPHYB$_{Col-0}$:PHYB$_{Col-0}$/phyB-9*. n=4 biologically independent samples. ** $P < 0.01$ by two-tailed Student's *t*-test compared with the value of Col-0 (WT) seedlings.

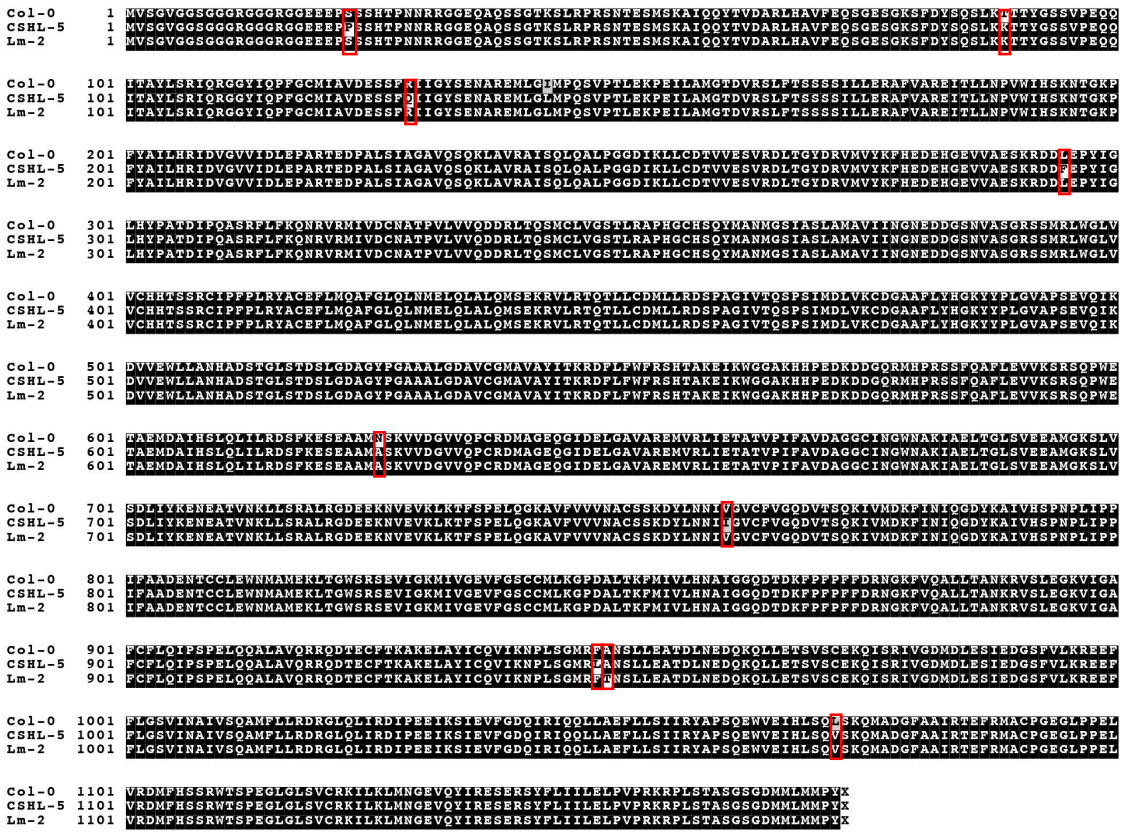


Supplementary Fig. S9 | Amino acid substitutions in Lm-2-type and CSHL-5-type phyB proteins decrease their RL-sensing capacity. (**a**) Relative expression levels of *PHYB* in 10-day-old Col-0, Lm-2, and CSHL-5 seedlings grown under Pi-sufficient (control) or -deficient (low Pi) conditions. The expression levels of *PHYB* were normalized against the transcript level of *GAPDH*. n=4 biologically independent samples. (**b**) Reporter and effector constructs used in trans-repression assay in **c**. In reporter constructs, *LUC* was under the control of the *PIL1* or *35S* (control) promoter. Each construct also contained the NOS terminator (not shown). (**c**) Repression of the *PIL1* promoter by Lm2-type ($PHYB_{Lm\text{-}2}$-Myc), CSHL-5-type ($PHYB_{CSHL\text{-}5}$-Myc), and Col-0-type ($PHYB_{Col\text{-}0}$-Myc) phyB in protoplasts that were incubated under the light. The *35S* promoter and an empty expression vector (*35S:MYC*) were served as negative controls. n=4 biologically independent samples. (**d**) Differences in amino acid sequences of phyB proteins from three Arabidopsis accessions. Amino acid sequences of phyB proteins from Col-0, CSHL-5, and Lm-2 accessions were deduced by DNA sequencing of cDNA clones. Amino acid substitutions examined in **e** are marked (**e**) Repression of the *PIL1* promoter by $PHYB_{Col\text{-}0}$-Myc with or without an amino acid substitution (Thr89Lys, Ile143Leu, Asp624Ala, Ala949Thr or Leu1072Val). The *35S* promoter and an empty expression vector (*35S:MYC*) were served as negative controls.

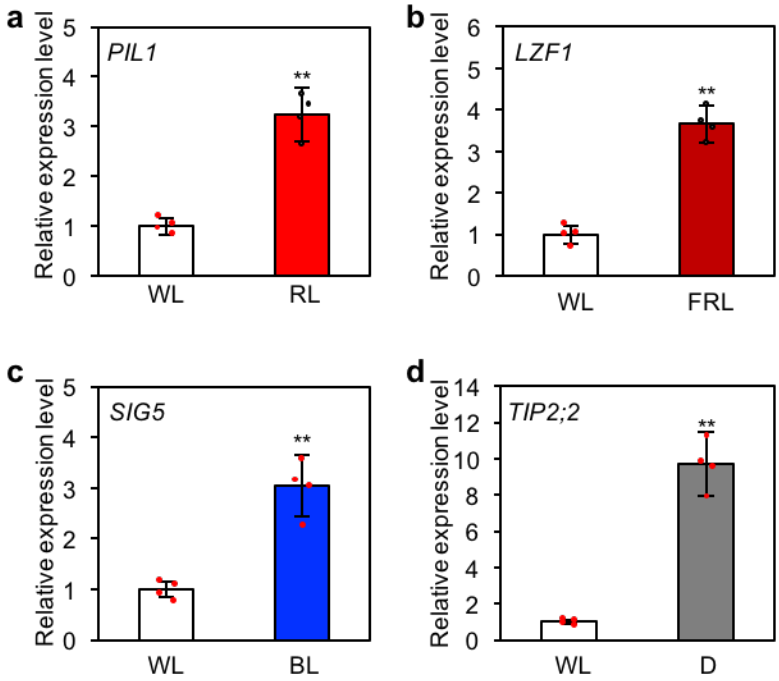
n=4 biologically independent samples. Significant differences by two-tailed Student's *t*-test (** $P < 0.01$, * $P < 0.05$), compared to values of Col-0 seedlings grown under the same conditions (**a**) or values obtained with an empty vector (**c**). The same letters above each bar in **e** indicate that means did not differ significantly at the 0.05 level in Tukey's multiple comparison test.



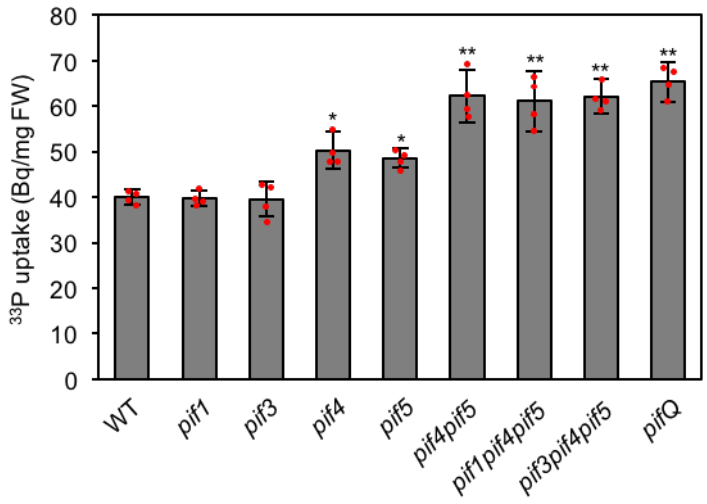
Supplementary Fig. S10 | Alignment of nucleotide sequences of Col-0-, Lm-2-, and CSHL-5-type *PHYB*. The alignment was obtained using Clustal Omega program (http://www.ebi.ac.uk/Tools/clustali/). Differences among these nucleotide sequences are indicated with red boxes.



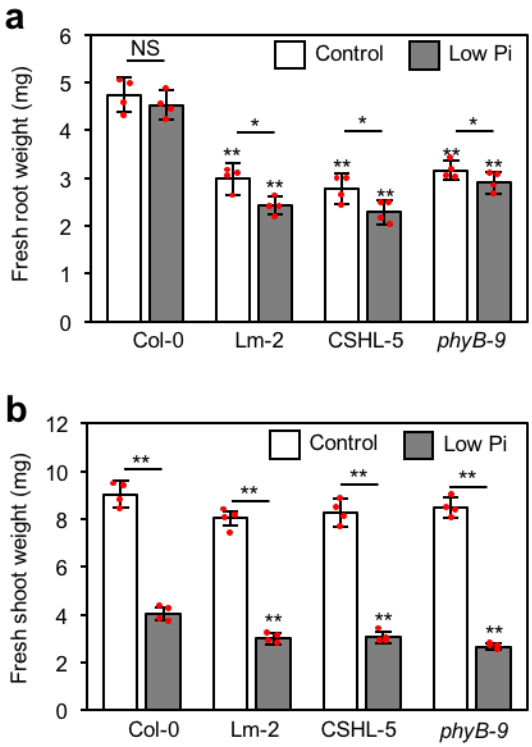
Supplementary Fig. S11 | Alignment of amino acid sequences of Col-0-, Lm-2-, and CSHL-5-type phyB. The alignment was obtained using Clustal Omega program (http://www.ebi.ac.uk/Tools/clustali/). Differences among these amino acid sequences are indicated with red boxes.



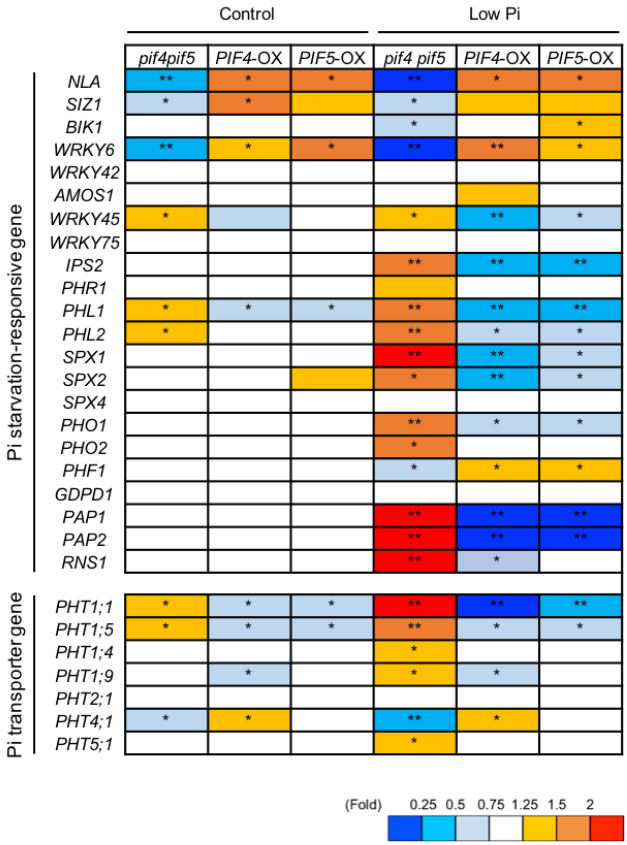
Supplementary Fig. S12 | Gene expression in red, far-red or blue light, or in the dark. Col-0 seedlings were grown under constant white light (WL) for 5 days under Pi-sufficient conditions and then for an additional 5 days under Pi-deficient conditions, and subsequently exposed to red light (RL), far-red light (FRL), blue light (BL) or WL (control) or placed in the dark for 1 h. Total RNA was extracted from the seedlings, and gene expression was analyzed using qRT-PCR. (**a**) RL-responsive *PIL1* expression, (**b**) FRL-responsive *LZF1* expression, (**c**) BL-responsive *SIG5* expression, and (**d**) dark-inducible *TIP2;2* expression. Expression levels of each gene of interest were normalized against expression of *GAPDH*. n=4 biologically independent samples. ** $P < 0.01$ by two-tailed Student's *t*-test, compared with values obtained from control seedlings exposed to WL.



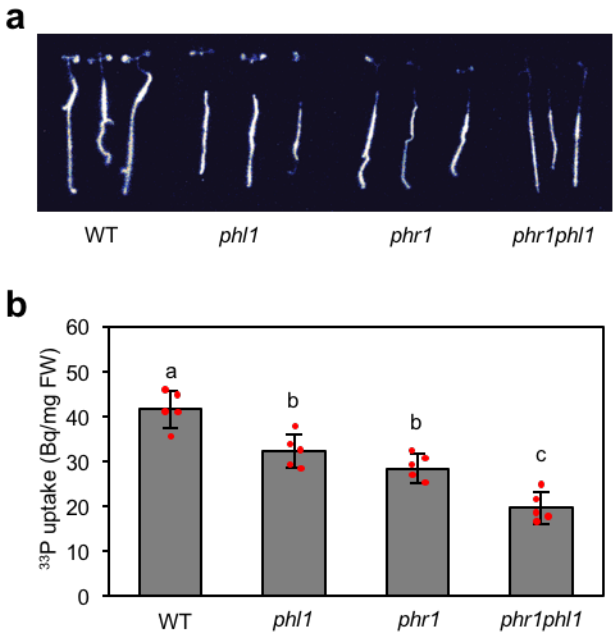
Supplementary Fig. S13 | Pi uptake by *pif* double and triple mutants. ^{33}P-labeled Pi uptake was measured in Col-0 (WT), *pif1*, *pif3* and *pif4* single mutant, *pif4 pif5* double mutant, *pif1 pif 4pif5* and *pif3 pif4 pif5* triple mutant and *pif1 pif3 pif4 pif5* quadruple (*pifQ*) mutant seedlings. Seedlings were grown for 5 days under Pi-sufficient conditions and for an additional 5 days under Pi-deficient conditions. n=4 biologically independent samples. ** $P < 0.01$ and * $P < 0.05$ by two-tailed Student's *t*-test, compared with the value obtained from Col-0 (WT) seedlings.



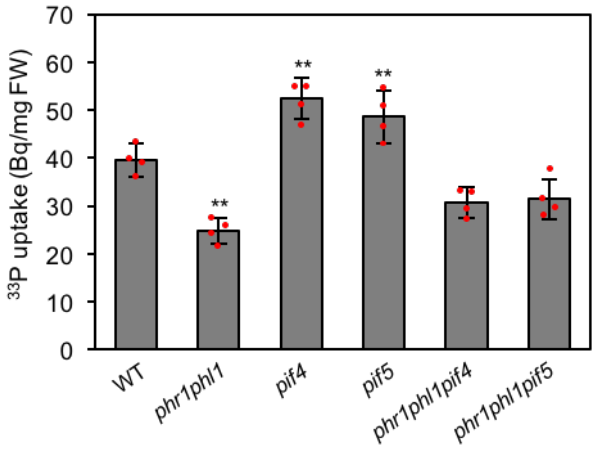
Supplementary Fig. S14 | Phenotypes of Col-0, Lm-2, CSHL-5 and *phyB-9* seedlings under Pi-deficient conditions. Col-0, Lm-2, CSHL-5 and *phyB-9* seedlings were grown for 5 days under Pi-sufficient conditions and then for an additional 5 days under either Pi-sufficient (black bars) or Pi-deficient (white bars) conditions. n=4 biologically independent samples. ** $P < 0.01$ indicates comparisons of values with those obtained from Col-0 (WT) seedlings in two-tailed Student's *t*-tests. ** $P < 0.01$, * $P < 0.05$ and NS (not significant) marked above horizontal bars indicate whether the values obtained from seedlings before Pi-starvation treatment (control) differed significantly from those obtained after Pi starvation (Low Pi) in a Student's *t*-test.



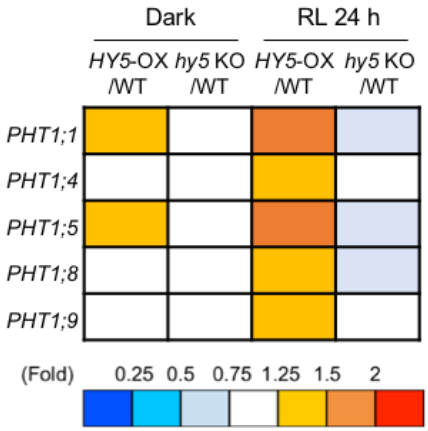
Supplementary Fig. S15 | Expression of Pi uptake-related genes is modified in *pif4 pif5* and *PIF*-over-expression lines. Col-0 (WT), *pif4 pif5*, *PIF4*-OX and *PIF5*-OX seedlings were grown under Pi-sufficient conditions for 5 days and for an additional 5 days under either Pi-sufficient (control) or Pi-deficient (Low Pi) conditions. Expression levels of each gene of interest were normalized first against expression of *TUB2*, and then against expression in Col-0 (WT) seedlings. n=4 biologically independent samples. ** ($P < 0.01$) and * ($P < 0.05$) indicate that values differed significantly from those of Col-0 seedlings in a two-tailed Student's *t*-test.



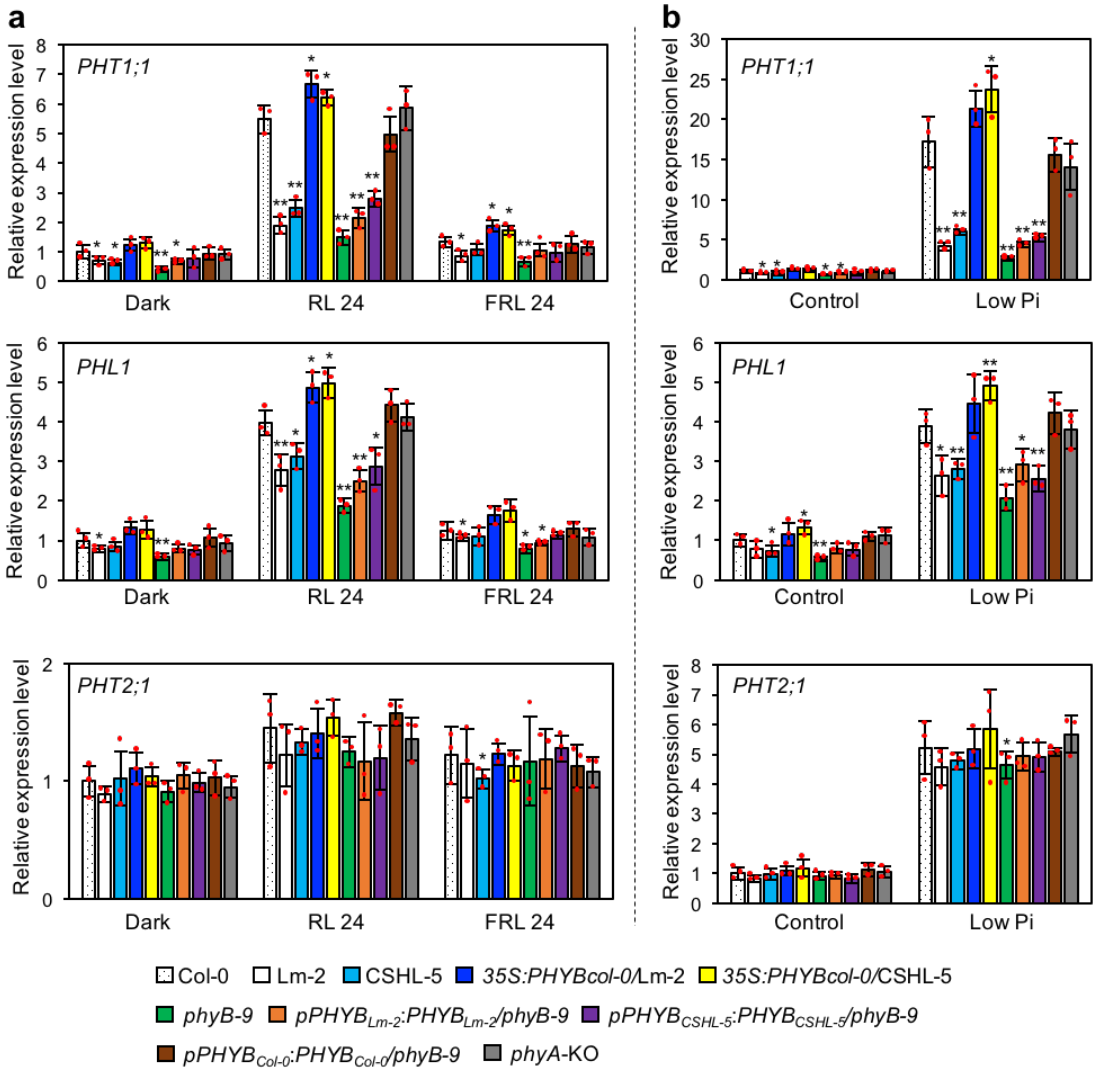
Supplementary Fig. S16 | Pi uptake activity of *phl1*, *phr1*, and *phr1 phl1* seedlings. Visualization (**a**) and quantification (**b**) of ^{33}P-labeled phosphate uptake by Col-0 (WT), *phl1*, *phr1*, and *phr1 phl1* seedlings grown under Pi-sufficient conditions for 5 days and then under Pi-deficient conditions for 5 days. n=4 biologically independent samples. The same letters above each bar indicate that means did not differ significantly at the 0.05 level in Tukey's multiple range test.



Supplementary Fig. S17 | Pi uptake by *phr1 phl1 pif4* and *phr1 phl1 pif5* triple mutants. Uptake of ^{33}P-labeled Pi was measured in seedlings of Col-0 (WT), *phr1 phl1*, *pif4*, *pif5*, *phr1 phl1 pif4* and *phr1 phl1 pif5* seedlings. Seedlings were grown for 5 days under Pi-sufficient conditions and for an additional 5 days under Pi-deficient conditions. n=4 biologically independent samples. ** $P < 0.01$ by two-tailed Student's *t*-test, compared with the value obtained from Col-0 (WT) seedlings.

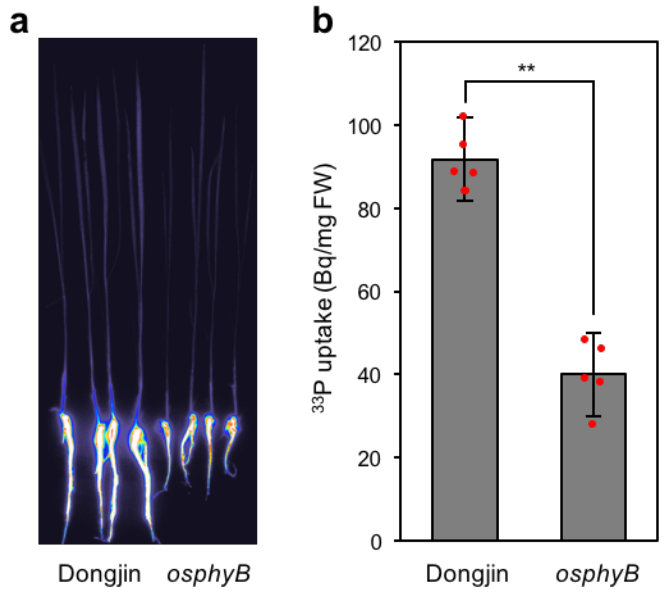


Supplementary Fig. S18 | *PHT1* genes are differentially expressed in *HY5*-OX and *hy5*-KO seedlings. Total RNA was extracted from 4-day-old seedlings grown in the dark and then exposed to red light (RL) for 24 h. Expression of *PHT1;1*, *PHT1;4*, *PHT1;5*, *PHT1;8* and *PHT1;9* was analyzed using qRT-PCR. The expression level of each *PHT1* gene was normalized against the transcript level of *TUB2*. The mean expression levels in *HY5*-OX and *hy5*-KO seedlings (n = 4) are shown, relative to expression levels in Col-0 (WT).

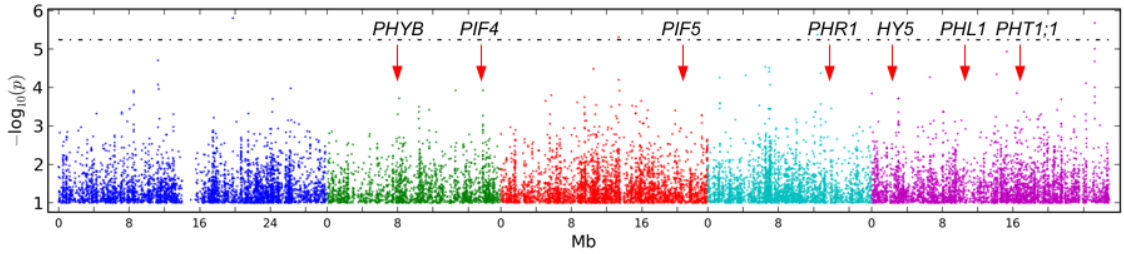


Supplementary Fig. S19 | The effect of Lm-2- and CSHL-5-type phyB on RL- and Pi starvation-responsive activation of *PHT1;1* and *PHL1*. (**a**) RL-induced expression of *PHT1;1* and *PHL1* in Col-0, Lm-2, CSHL-5, *35S:PHYB*$_{Col\text{-}0}$/Lm-2, *35S:PHYB*$_{Col\text{-}0}$/CSHL-5, *phyB-9*, *pPHYB*$_{Lm\text{-}2}$*:PHYB*$_{Lm\text{-}2}$*/phyB-9*, *pPHYB*$_{CSHL\text{-}5}$*:PHYB*$_{CSHL\text{-}5}$*/phyB-9*, *pPHYB*$_{Col\text{-}0}$*:PHYB*$_{Col\text{-}0}$*/phyB-9,* and *phyA*-KO seedlings. *PHT2;1* expression, which is not under the control of phyB, was also analyzed as a control. Total RNA was extracted from seedlings grown for 4 days in the dark and then exposed to red light (RL) or far-red light (FRL) for 24 h and used for qRT-PCR. (**b**) Pi starvation-induced expression of *PHT1;1*, *PHL1*, and *PHT2;1* in the seedlings listed above. Total RNA

was extracted from seedlings grown under Pi-sufficient conditions for 5 days and then for an additional 5 days under Pi-sufficient (control) or Pi-deficient (Low Pi) conditions and used for qRT-PCR. Relative expression levels were obtained by normalizing first against transcript levels of *TUB2*, and then against the values obtained from 4-day-old, dark-grown Col-0 seedlings (**a**) or Col-0 seedlings grown under Pi-sufficient conditions (**b**). n=3 biologically independent samples. ** ($P < 0.01$) and * ($P < 0.05$) indicate significant differences compared with Col-0 seedlings grown under the same growth conditions in a two-tailed Student's *t*-test.



Supplementary Fig. S20 | Pi uptake is lower in a rice *phyB* mutant. Visualization (**a**) and quantification (**b**) of ^{32}P-labeled Pi uptake by *phyB* mutant (*osphyB*) and Dongjin (WT) rice seedlings. Rice seedlings were grown in a culture solution containing 100 μM Pi for 18 days and then for an additional 3 days in a culture solution containing 5 μM Pi. n=4 biologically independent samples. ** $P < 0.01$ by two-tailed Student's *t*-test.



Supplementary Fig. S21 | The Manhattan plot for GWAS for Pi uptake in 135 Arabidopsis accessions. The Manhattan plot was generated with Pi uptake activity in Supplementary Table S1 and Supplementary Fig. S1a, using the GWAPP web interface (http://gwapp.gmi.oeaw.ac.at) and the accelerated mixed model (AMM). Arabidopsis accessions used for GWAS are listed in Supplementary Table S6. Each color represents different chromosomes. Positions of *PHYB*, *PIF4*, *PIF5*, *HY5*, and the Pi uptake-related genes (*PHR1*, *PHL1* and *PHT1;1*) are indicated.