Supplemental methods:

Analysis of the Pi concentration effect on *PPsPase1* and *PECP1* expression

Prom:GUS lines used in **Sup Fig. 2 A-D** were generated by PCR-amplifying a 1611-bp fragment from the PPsPase1 promoter and a 1605-bp fragment from the PECP1 promoter (upstream of the translation start site) from Arabidopsis genomic DNA using the SP_PromPPsPase1_V2/ ASP_PromPPsPase1_V2 and SP_PromPECP1_V2 / ASP_PromPECP1_V2 primers, respectively. The primers contained additional restriction enzyme sites (*Kpn*I or *Xho*I) for direct cloning into a pGreen plant transformation vector, containing GUS as a reporter gene (see Sup Table 3). The resulting binary vectors were transferred into *Agrobacterium* (pGV3101). Arabidopsis plants (Columbia) were transformed using the floral dip method. The transformed T1 seeds were selected on 0.7% agar plates with standard half-strength MS medium containing 15 g/L sucrose and 50 mg/L kanamycin. The presence of the transgene was confirmed by PCR, and homozygous plants with strong GUS expression were selected through successive seed amplification and kanamycin resistance selections.

For GUS quantification in different Pi conditions, seven-day-old seedlings were grown in half-strength standard MS liquid medium, rinsed with sterile distilled water followed by a single rinse with MS medium without Pi, and then transferred to 20 ml of full-strength MS medium supplemented with different Pi concentrations (0, 5, 10, 50, 100, 250, 500 or 1,250 µM). Seedlings were grown under a 16 h-light/8 h-dark cycle at 22°C with 75 µmol photons m^{-2} s^{-1}. Plants were then harvested after 5 days for GUS staining or quantification. All experiments were repeated twice with at least four biological replicates.

Histochemical staining for GUS activity was done in GUS reaction mix (25 mg of 5-bromo-4-chloro-3-indolyl-β-D-glucuronide in 50 ml of 100 mm sodium Pi buffer with 0.1% [v/v] Triton X-100). The stained plants were transferred to 70% (v/v) alcohol to remove chlorophyll. Images of GUS stained plants were taken under the stereo microscope equipped with a SPOT RT-slider digital camera (Diagnostic Instruments Inc.).

For fluorometric quantification of GUS activity, ~ 200 mg of plant tissue powder were thoroughly mixed with 500 µl of extraction buffer (50 mm $NaHPO_4$, pH 7.0; 10 mm β-mercaptoethanol; 10 mm EDTA; 0.1% [v/v] Triton X-100 and 0.1% [w/v] sodium lauryl sarcosyl). After centrifugation (15,000 rpm; 5 min; 4°C), 50 µl of the supernatant was mixed with 450 µl prewarmed (37°C) GUS assay buffer (2 mM 4-Methylumbelliferyl-β-D GlcUA in extraction buffer) and incubated at 37°C for 30 min. The reaction was terminated by transferring 100 µl of the reaction mix to 900 µl of 2% (w/v) Na_2CO_3. The amount of fluorescent product produced in the reaction was determined using a Versa FluorTM fluorometer (BIO-RAD). The GUS activity was expressed as pmoles of 4-methylumbelliferyl formed mg total $protein^{-1}$ min^{-1}.

Plants used in **Sup Fig. 2 E-H** were grown *in vitro* for 12 days on plates containing 50ml of MS/10 medium (2µM $FeCl_2$) with distinct concentrations of Pi (10, 50, 100, 150, 200, 250, 500 or 1,250 µM including Pi contained in the agar). Pi content and transcript quantification analysis were performed as described in the method section.

Confirmation of transcript knockdown in T-DNA insertion lines

In order to verify transcript levels in mutant and WT plants, mRNA was first isolated from frozen plant tissues using the RNeasy extraction kit (Qiagen), in combination with the RNase-free DNase Set (Qiagen). Reverse transcription of poly(dT) cDNA was performed starting from 400 ng of total RNA using the SuperScript™ III Reverse Transcriptase kit (Invitrogen), following the manufacturer's instructions. cDNA was then PCR amplified (25 to 28 cycles) using the primers indicated in **Sup Fig. 4** (and provided in Sup Table 3). Amplification with the ACT2 primers (**Sup Table 3**) was performed to verify that a similar amount of cDNA was used as a template.