Supporting Figure S1. Characterization of kinase-inactive IPK1 transgenic plants.
(a) Positions of amino-acid substitutions (✱) of K168A and D368A in the gene structure of IPK1. Exons are marked by rectangles; shaded areas denote conserved kinase domains shared with ScIPK1 and HsIPK1 (Stevenson-Paulik et al., 2005). T-DNA insertional position of ipk1-1 mutant is indicated. (b) Western analysis of C-terminus YFP-tagged IPK1 or kinase-inactive IPK1 protein level in roots of 14-DAG seedlings grown under Pᵢ-replete conditions. ARF protein is used as an input control. (c) Localization of C-terminus YFP-tagged IPK1 or kinase-inactive IPK1 in roots of 10-DAG transgenic lines grown under Pᵢ-replete conditions. Scale bar, 10 μm. (d) Complementation test of ipk1-1 RSA phenotype of 11-DAG seedlings grown on Pᵢ-replete medium. Scale bar, 1 cm.
Supporting Figure S2. The role of Gle1-InsP₆-Los4 mRNA export machinery in ipk1-mediated Pi-related phenotypes.

Tissue-specific Pi content of 12-DAG seedlings of (a) los4-1, (b) GLE1 RNAi lines (after DEX induction for 6 days), and (d) ipk1-1 mutants expressing GFP-tagged wild-type Gle1 (GFP-Gle1) or Gle1 variants [GFP-Gle1(IS1) and GFP-Gle1(IS2)] grown under Pi-replete (1 mM Pi) conditions. (c) Relative expression of PSR genes in the root tissues of respective lines analyzed in this figure (see Supporting Table S3 for qPCR raw data). Arabidopsis accession C24 was included in (a) as a wild-type control for los4-1. Error bars, S.E. of n=3 independent experiments. Asterisks denote significant difference from the WT (Student t-test, *P < 0.05). No significant differences were observed in (b) between DEX-induction versus ethanol treatments in all genotypes, and (d) between GFP-Gle1(IS1) or GFP-Gle1(IS2) and GFP-Gle1 lines that do not differ from the ipk1-1 mutants (Student t-test; error bars, S.E. of n=3 independent experiments).
Supporting Figure S3. Genetic and phenotypic characterization of mutants of Inositol phosphate biosynthesis enzymes.

(a) Transcript level of target gene in the respective T-DNA insertional line used in this study (Supporting Table S1) by RT-PCR analysis of 32-40 cycles. ACTIN2 was used as an internal control and amplified by 25 cycles. Primer sequences are in Supporting Table 2. (b) T-DNA insertion positions and VIP gene expression of various vip1/vih2 and vip2/vih1 alleles. ATP-grasp kinase domain (shaded in red) and histidine phosphatase domain (shaded in green) are assigned according to The Arabidopsis Information Resource (TAIR) and Laha et al., 2015; primers used for RT-PCR are marked by arrowheads. (c) Root system architecture of 11-DAG seedlings grown on solid medium supplemented with 250 μM P_{i}. Capitalized allele names indicate wild-type isogenic line of respective homozygous mutant alleles. Scale bar, 1 cm. (d) Root system architecture of 11-DAG WT, ipk1-1 and itpk1 seedling grown under P_{i}-replete conditions; scale bar, 1 cm. (e) P_{i} content in the shoot of 22-DAG plant grown in hydroponic medium supplemented with 1 mM P_{i}. Error bars, S.E. of n=4-21 independent experiments. Asterisks indicate significant differences from WT (Student’s t-test; **, P < 0.005).
Supporting Figure S4. Complementation of itpk1 phenotypes.
(a) Complementation of Pi content phenotype of itpk1 mutants by ectopic expression of ITPK1 genomic sequence (spanning 1-kb upstream of start codon and 500 bp downstream of stop codon). Error bar, S.E. of n=3 independent experiments. (b) Complementation of Pi content phenotype of itpk1 mutants by C'-terminus YFP-tagged ITPK1 protein-coding sequence driven by 35S promoter. Pi concentration was analyzed in 14-DAG seedlings grown on medium of 1 mM Pi (a) and 250 μM Pi (b).
Supporting Figure S5. \( P_i \) allocation activities and PSR gene expression in the vip/vih mutants.

(a) Root-to-shoot allocation of \( P_i \) in the 14-DAG vip/vih seedlings under \( P_i \)-replete (250 \( \mu \)M) growth conditions. Seedlings were labeled by \([^{32}P]P_i \) for 3 h, then incubated in medium containing non-radioactive \( P_i \) for the indicated time. Error bar, S.E. of \( n=3-6 \) independent experiments. (b) Relative expression (to WT) of PSR genes in roots of 14-DAG vip/vih seedlings grown under \( P_i \)-replete (1 mM) condition (see Supporting Table S3 for qPCR raw data). (c) Fold change of PSR gene expression in roots of 14-DAG vip/vih seedlings after 3-day \( P_i \) starvation (10 \( \mu \)M) (see Supporting Table S3 for qPCR raw data).
Supporting Figure S6. InsP profile of genotypes labeled with myo-[3H]-inositol.

(a) Comparison of InsP profiles of root tissues of various genotypes. The profiles identify similar peaks to those obtained in [32P]Pi labeling profiles in Fig 5a. Radioactivity was normalized to total count of a HPLC run. (b) Comparison of labeling profiles of shoots and roots of 14-DAG seedlings of WT.
Supporting Figure S7. Characterization of itpk4 mutants and expression pattern of ITPK4.

(a) Complementation of InsP_6 level in seeds of two ITPK4-YFP homozygous lines in the itpk4-1 mutant background. Error bars, S.E. of n = 3. (b) Tissue-specific Pi content of two itpk4 mutant alleles in comparison with their respective WT isogenic lines (ITPK4-1 and ITPK4-2) and ipk1-1. Pi content was measured in 14-DAG seedlings grown under Pi-replete condition (1mM Pi). Error bars, S.D. of n = 3. Asterisks denote significant differences from WT (Student’s t-test; *, P < 0.05; **, P < 0.005). (c) Relative expression of representative PSR genes in the roots of 14-DAG itpk4-1, itpk4-2 and ipk1-1 seedlings to WT grown under Pi-replete Pi condition (1 mM Pi). Error bars, S.E. of n=3-6 independent experiments. (d-l) Promoter activities of ITPK4 at different developmental stages. (d) 3-DAG; scale bar, 0.5 mm. (e) 7-DAG; scale bar, 1 mm. (f) 14-DAG; scale bar, 50 mm. (g) Cross section of 14-DAG root; scale bar, 10 μm. (h) Guard cells of 14-DAG leaf; scale bar, 10 μm. (i) Trichomes of 14-DAG leaves; scale bar, 0.5 mm. (j) 22-DAG floral tissues; scale bar, 1 mm. (k) 22-DAG flowers; scale, 1 mm. (l) Siliques; scale bar, 0.5 mm. (m) Subcellular localization of C-terminus YFP-tagged ITPK4 (ITPK4-YFP) in the roots of 11-DAG itpk4-1 mutants (transgenic line #2). Scale bar, 10 μm.
Supporting Figure S8. Pi starvation responses of WT and various genotypes under different regimes of Pi starvation.

(a) Shoot Pi concentration of WT seedlings after Pi starvation treatments. Error bars, S.D. of n = 6. Asterisks denote significant differences from respective ‘+P’ treatments (Student’s t-test; *, P < 0.05; **, P < 0.005). (b and c) Fold changes of PSR genes (gene expression under Pi-deficient conditions relative to Pi-replete conditions) in roots of WT (b) and various genotypes (c) after Pi starvation treatments. ITPK1 indicates isogenic WT lines of itpkl. qPCR raw data is provided in Supporting Table S3. For Pi starvation treatments, 11-DAG seedlings of WT grown under Pi-replete condition [250 μM Pi for (a and b) and 1 mM Pi for (c)] and transferred to Pi-replete [+P; 250 μM Pi for (a and b) and 1mM Pi for (c)] or Pi-deficient (-P; 10 μM) medium for the indicated time.
Supporting Figure S9. Tissue-specific InsP profiles in response to 1- and 3-day Pi starvation.

(a-d) Scintillation counting of 1-min fractions collected from HPLC analysis of samples in Fig 6. (a) and (b), 1-day Pi-starved shoots and roots, respectively; (c) and (d), 3-day Pi-starved shoots and roots, respectively. InsP_5a, Ins(1,2,4,5,6)P_5 or Ins(2,3,4,5,6)P_5. InsP_4*, Ins(1,4,5,6)P_4 and/or Ins(3,4,5,6)P_4. (e and f) Scintillation counting of 0.5-min fractions collected from isocratic HPLC analysis of samples from the same treatments as Fig 6. [3H]Ins(1,4,5)P_3 was added to extracts before HPLC.
Supporting Figure S10. PPI composition in *itpk1*, *ipk1-1* and *OxITPK1* lines, and P~i~ content in mutants exhibiting elevated PtdIns(4,5)P~2~ levels.

(a and b) Relative content of [32P]-labeled PtdInsP and PtdIns(4,5)P~2~ presented as percentages of total labeled lipids as resolved by TLC analysis (Munnik and Zarza, 2013) of 6-DAG seedlings following 16-h *in vivo* [32P]Pi radiolabeling under P~i~-replete (250 μM) growth conditions. Error bars, S.E. of n=3 independent experiments. (c and d) Tissue-specific P~i~ content of Arabidopsis lines with elevated PtdIns(4,5)P~2~ levels (Ox*PIP5K3*, overexpression of *PIP5K3*). 14-DAG seedlings grown under P~i~-replete conditions (c, 250 μM P~i~), and 12-DAG seedlings grown under P~i~-replete conditions (d, 1 mM P~i~). Error bar, S.E. of n= 6-15 independent experiments (c), n= 3-4 independent experiments (d). Asterisks indicate significant differences from the WT (Student’s t-test; *, P < 0.05; **, P < 0.005).

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