

**Supplemental information**

**ITPK1 is an InsP<sub>6</sub>/ADP phosphotransferase that controls phosphate signaling in *Arabidopsis***

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**Suppl. Table 1. Expression of P<sub>i</sub> starvation-induced genes in response to changes in P<sub>i</sub> availability.** Wild-type plants (Col-0) were cultivated in hydroponics under sufficient P<sub>i</sub> (+P), deficient P<sub>i</sub> for 7 days (-P) or resupplied with P<sub>i</sub> for 6 h. Values represent expression levels normalized to +P. Shown are means  $\pm$  SE ( $n = 3$  biological replicates).

Gene	+P	-P	Pi RS 6h
<i>SPX1</i>	1.0 $\pm$ 0.17	267 $\pm$ 10.53	6.93 $\pm$ 1.59
<i>IPS1</i>	1.0 $\pm$ 0.27	1601 $\pm$ 154	90 $\pm$ 13.8
<i>PHR1</i>	1.0 $\pm$ 0.06	1.04 $\pm$ 0.05	0.86 $\pm$ 0.03
<i>PHT1;1</i>	1.0 $\pm$ 0.22	16.4 $\pm$ 1.93	2.42 $\pm$ 0.52
<i>PHT1;2</i>	1.0 $\pm$ 0.19	126.67 $\pm$ 10.88	10.14 $\pm$ 0.79
<i>PHT1;8</i>	1.0 $\pm$ 0.23	50.39 $\pm$ 5.75	8.11 $\pm$ 0.57
<i>PHT1;9</i>	1.0 $\pm$ 0.07	27.06 $\pm$ 4.21	3.08 $\pm$ 0.58

**Suppl. Table 2. List of primers used in this study.**

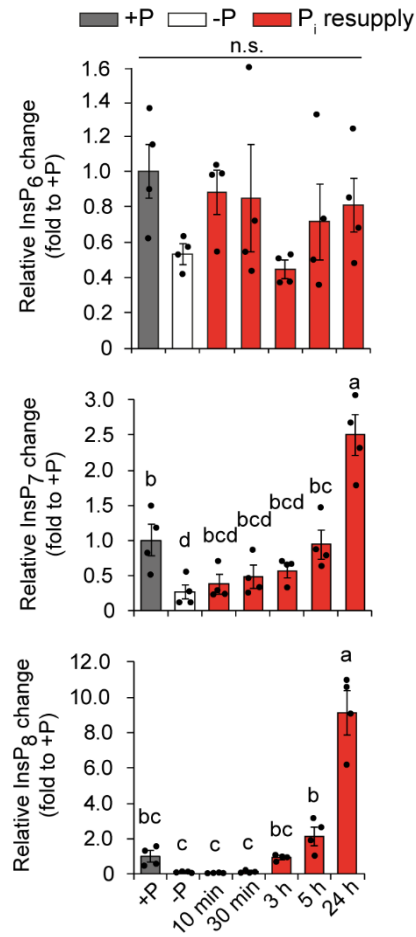
Primers used for qPCR analysis		
AGI ID	Gene name	Primer sequence
AT2G36170	<i>UBQ2</i>	F: 5'-CCAAGATCCAGGACAAAGAAGGA-3' R: 5'-TGGAGACGAGCATAACACTTG-3'
AT5G43350	<i>PHT1;1</i>	F: 5'-AGGCGATCACGTTGCTTACA-3' R: 5'-TCTCTGGAGAGTTGAGGAGAGAC-3'
AT5G43370	<i>PHT1;2</i>	F: 5'-CCATTAGCGCACAAACGGAAAG-3' R: 5'-GAAACCCATACCGGCGATGA-3'
AT5G43360	<i>PHT1;3</i>	F: 5'-GCTTTCATCGCGGCAGTGTT-3' R: 5'-TGAGGAGGCGTTGATAGAAACC-3'
AT2G38940	<i>PHT1;4</i>	F: 5'-AGCCTTTGTCTCTGCGGTTT-3' R: 5'-CGTGGATCCCAAGGCATCAT-3'
AT1G20860	<i>PHT1;8</i>	F: 5'-AGAGAAGTGGCGGTGGTTTG-3' R: 5'-TCTTGCGGTTTCAGGCATCA-3'
AT1G76430	<i>PHT1;9</i>	F: 5'-TTCGGAGAAGACGAACGTGG-3' R: 5'-GTATCTGGCGGTTTCAGGCA-3'
AT3G23430	<i>PHO1</i>	F: 5'-GACTTACAGCTCGTTGAATATGATAGC-3' R: 5'-CGATCTCTTTACGACTTTGAGATACG-3'
AT4G28610	<i>PHR1</i>	F: 5'-TGTGGAATTGCGACCTGTTA-3' R: 5'-GCTCTTTCACCTACCGCCAAG-3'
AT5G20150	<i>SPX1</i>	F: 5'-GTTGATTTCCATGGAGAAATGG-3' R: 5'-GGTAAACGCATGAGATCACCAG-3'
AT3G09922	<i>IPS1</i>	F: 5'-TCCCTCTAGAAATTGGGCAAC-3' R: 5'-GGGAGTGGGTACAACCCAAA-3'
AT2G33770	<i>PHO2</i>	F: 5'-TTGCACCATGTGAAATTTGG-3' R: 5'-AGACCCGTTTCTGATGGTT-3'
AT5G16760	<i>ITPK1</i>	F: 5'-ATTGGGACGTCGAAAGGGTC-3' R: 5'-CTCAGTCAACACAGGCTCGT-3'
Primers used for genotyping in F1, F2 and F3		
itpk1_LP	5'-ACCAATATTCGATTCCACACG-3'	
itpk1_RP	5'-CCATGTCCCAGAAGAACTCAG-3'	
itpk2-2_LP	5'-TCGCTTGTAAGTTTCAAGTTGC-3'	

itpk2-2_RP	5'-TAAGGACAAAAACATGGCAGG-3'
SAIL_LB3	5'-TAGCATCTGAATTTTCATAACCAATCTCGATACAC-3'
phr1_LP	5'-GAGAGACCTCACACGCACTTC-3'
phr1_RP	5'-CTTTCTGGCGAACCTGTAGTG-3'
phl1_LP	5'-GTGGAGACGTTTCTGCACTTC-3'
phl1_RP	5'-TCCCACAATCCAAATTCAGAG-3'
LBb1.3	5'-ATTTTGCCGATTCGGAAC-3'
vih2-4_GK_LP	5'-AACAACAGCAATGACACAACG-3'
vih2-4_GK_RP	5'-CATTCCCATCTTTTGGACAAC-3'
GABI_LB1	5'-ATAATAACGCTGCGGACATCTACATTTT-3'

**Suppl. Table 3. Mass spectrometer parameters used for the analysis of inositol (pyro)phosphates.**

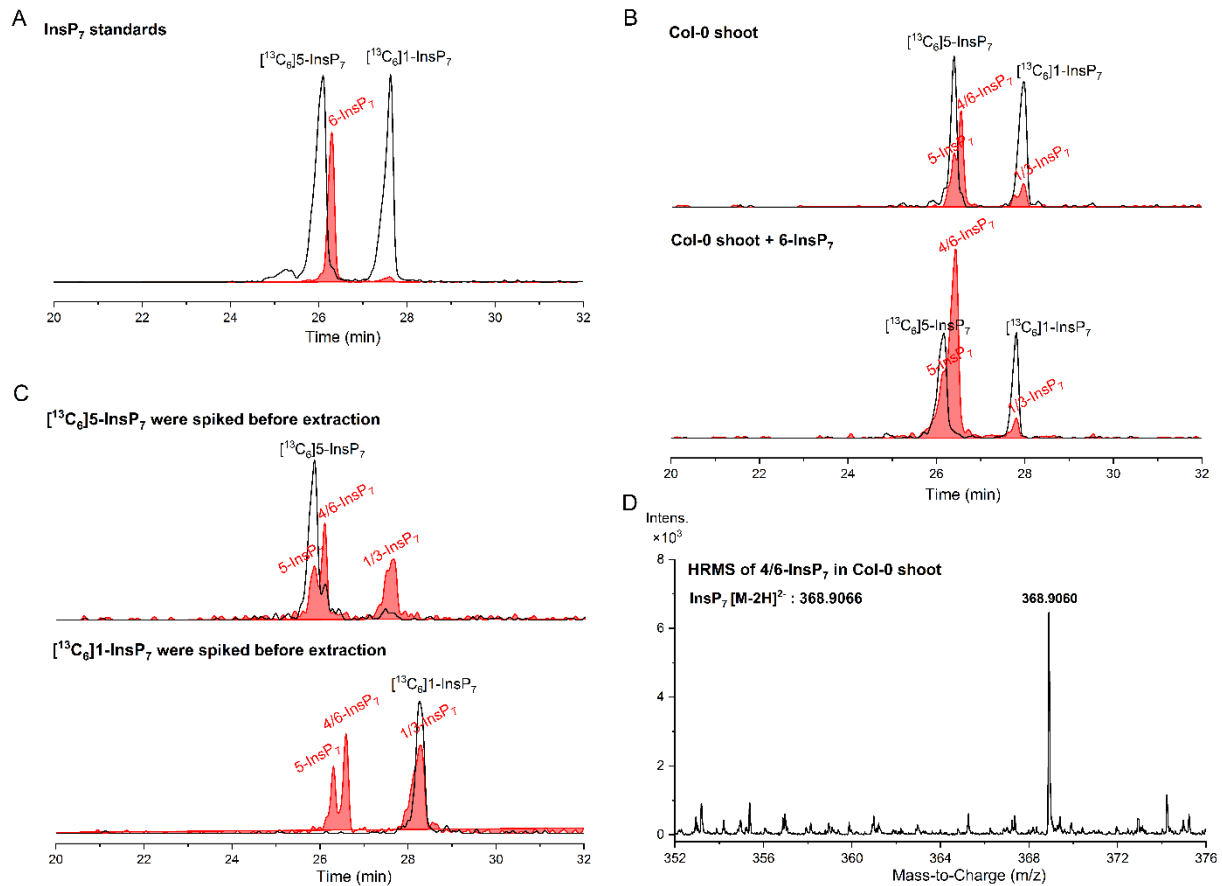
Compound	MRM transitions	Type of transition	Collision Energy (V)	Cell Acc (V)
InsP <sub>8</sub>	408.9 → 359.6 <sup>a</sup>	[M-2H] <sup>2-</sup> → [M-2H-H <sub>3</sub> PO <sub>4</sub> ] <sup>2-</sup>	10	1
	408.9 → 78.9	[M-2H] <sup>2-</sup> → [PO <sub>3</sub> ] <sup>-</sup>	42	4
[ <sup>13</sup> C <sub>6</sub> ]InsP <sub>8</sub>	411.9 → 362.6 <sup>a</sup>	[M-2H] <sup>2-</sup> → [M-2H-H <sub>3</sub> PO <sub>4</sub> ] <sup>2-</sup>	10	1
	411.9 → 78.9	[M-2H] <sup>2-</sup> → [PO <sub>3</sub> ] <sup>-</sup>	42	4
InsP <sub>7</sub>	368.9 → 319.6 <sup>a</sup>	[M-2H] <sup>2-</sup> → [M-2H-H <sub>3</sub> PO <sub>4</sub> ] <sup>2-</sup>	10	3
	368.9 → 78.9	[M-2H] <sup>2-</sup> → [PO <sub>3</sub> ] <sup>-</sup>	38	3
[ <sup>13</sup> C <sub>6</sub> ]InsP <sub>7</sub>	371.9 → 322.6 <sup>a</sup>	[M-2H] <sup>2-</sup> → [M-2H-H <sub>3</sub> PO <sub>4</sub> ] <sup>2-</sup>	10	3
	371.9 → 78.9	[M-2H] <sup>2-</sup> → [PO <sub>3</sub> ] <sup>-</sup>	38	3
InsP <sub>6</sub>	328.9 → 78.9 <sup>a</sup>	[M-2H] <sup>2-</sup> → [PO <sub>3</sub> ] <sup>-</sup>	46	1
	328.9 → 481	[M-2H] <sup>2-</sup> → [M-H-HPO <sub>3</sub> -H <sub>3</sub> PO <sub>4</sub> ] <sup>-</sup>	10	3
[ <sup>13</sup> C <sub>6</sub> ]InsP <sub>6</sub>	331.9 → 78.9 <sup>a</sup>	[M-2H] <sup>2-</sup> → [PO <sub>3</sub> ] <sup>-</sup>	46	1
	331.9 → 487	[M-2H] <sup>2-</sup> → [M-H-HPO <sub>3</sub> -H <sub>3</sub> PO <sub>4</sub> ] <sup>-</sup>	10	3
InsP <sub>5</sub>	288.9 → 498.7 <sup>a</sup>	[M-2H] <sup>2-</sup> → [M-H-HPO <sub>3</sub> ] <sup>-</sup>	10	1
	288.9 → 78.9	[M-2H] <sup>2-</sup> → [PO <sub>3</sub> ] <sup>-</sup>	14	1
[ <sup>13</sup> C <sub>6</sub> ]InsP <sub>5</sub>	291.0 → 504.7 <sup>a</sup>	[M-2H] <sup>2-</sup> → [M-H-HPO <sub>3</sub> ] <sup>-</sup>	10	1
	291.0 → 78.9	[M-2H] <sup>2-</sup> → [PO <sub>3</sub> ] <sup>-</sup>	14	1
InsP <sub>4</sub>	249.0 → 418.6 <sup>a</sup>	[M-2H] <sup>2-</sup> → [M-H-HPO <sub>3</sub> ] <sup>-</sup>	10	1
	249.0 → 320.6	[M-2H] <sup>2-</sup> → [M-H-HPO <sub>3</sub> -H <sub>3</sub> PO <sub>4</sub> ] <sup>-</sup>	14	1
InsP <sub>3</sub>	419.0 → 320.6 <sup>a</sup>	[M-H] <sup>-</sup> → [M-H-H <sub>3</sub> PO <sub>4</sub> ] <sup>-</sup>	18	4
	419.0 → 78.9	[M-H] <sup>-</sup> → [PO <sub>3</sub> ] <sup>-</sup>	50	1

<sup>a</sup> MRM transition with the highest response for each compound (used for quantification).



**Supplemental Figure 1. InsP<sub>6</sub> and InsP<sub>7</sub> levels respond strongly to P<sub>i</sub> deficiency and P<sub>i</sub> resupply in rice plants.**

Shown are fold changes of quantified signal intensities of independent PAGE gels. Data are means  $\pm$  SE ( $n = 3$  gels loaded with independent biological samples). Plants were cultivated in hydroponics under sufficient P<sub>i</sub> (+P), deficient P<sub>i</sub> (-P) for 10 days, or -P resupplied with P<sub>i</sub> for the indicated times. Different letters indicate significant differences according to Tukey's test ( $P < 0.05$ ). n.s., not significant according to one-way ANOVA ( $P < 0.05$ ).



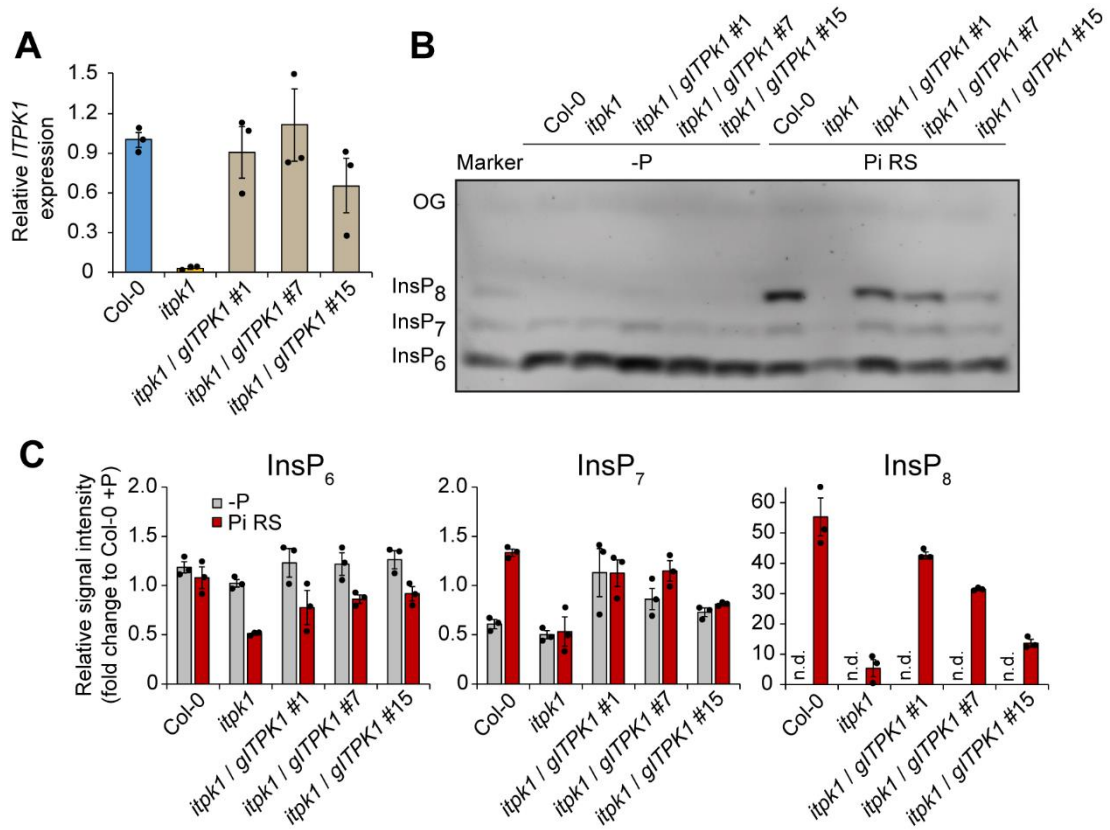
**Supplemental Figure 2. Determination of 4/6-InsP<sub>7</sub> as a novel PP-InsP found in plants via CE-ESI-qTOF analysis.**

(A) 6-InsP<sub>7</sub> could be readily distinguished with the aid of isotopic standards  $[^{13}\text{C}_6]5\text{-InsP}_7$  and  $[^{13}\text{C}_6]1\text{-InsP}_7$  with CE-ESI-MS.

(B) An undefined InsP<sub>7</sub> isomer found in *Arabidopsis* (Col-0) showed same migration time as 6-InsP<sub>7</sub> standard.

(C)  $[^{13}\text{C}_6]5\text{-InsP}_7$  and  $[^{13}\text{C}_6]1\text{-InsP}_7$  were spiked before InsP extractions, ruling out the possibility that 4/6-InsP<sub>7</sub> were generated during sample preparation.

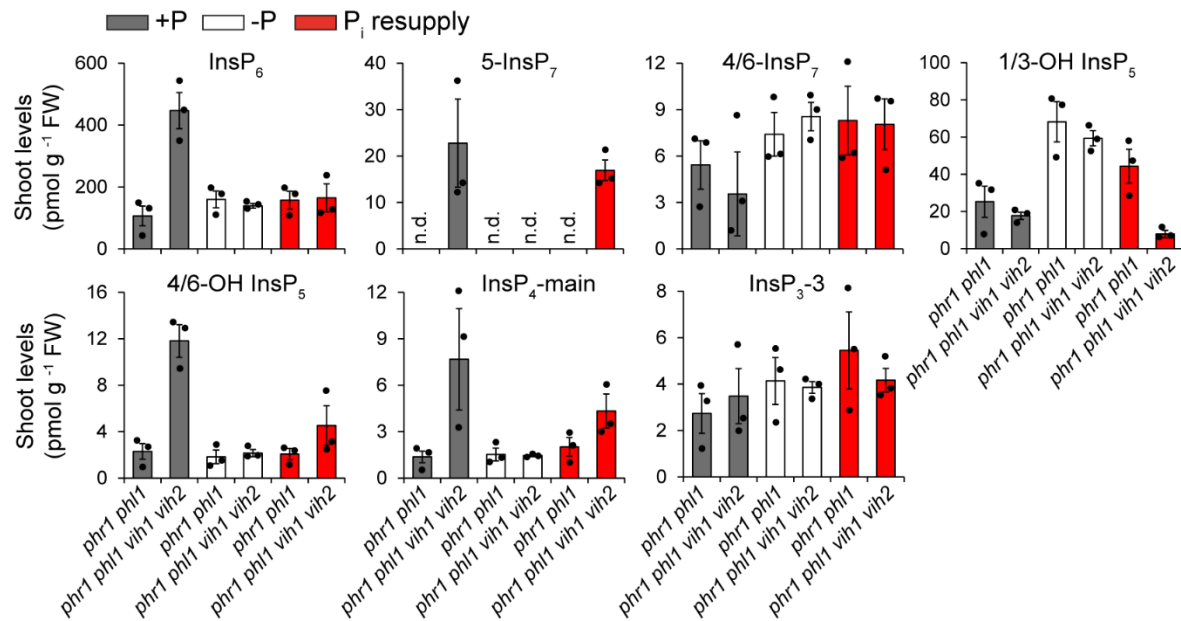
(D) A representative high-resolution mass spectrum (HRMS) of 4/6-InsP<sub>7</sub> in *Arabidopsis*.



### Supplemental Figure 3. Complementation of *itpk1* mutant.

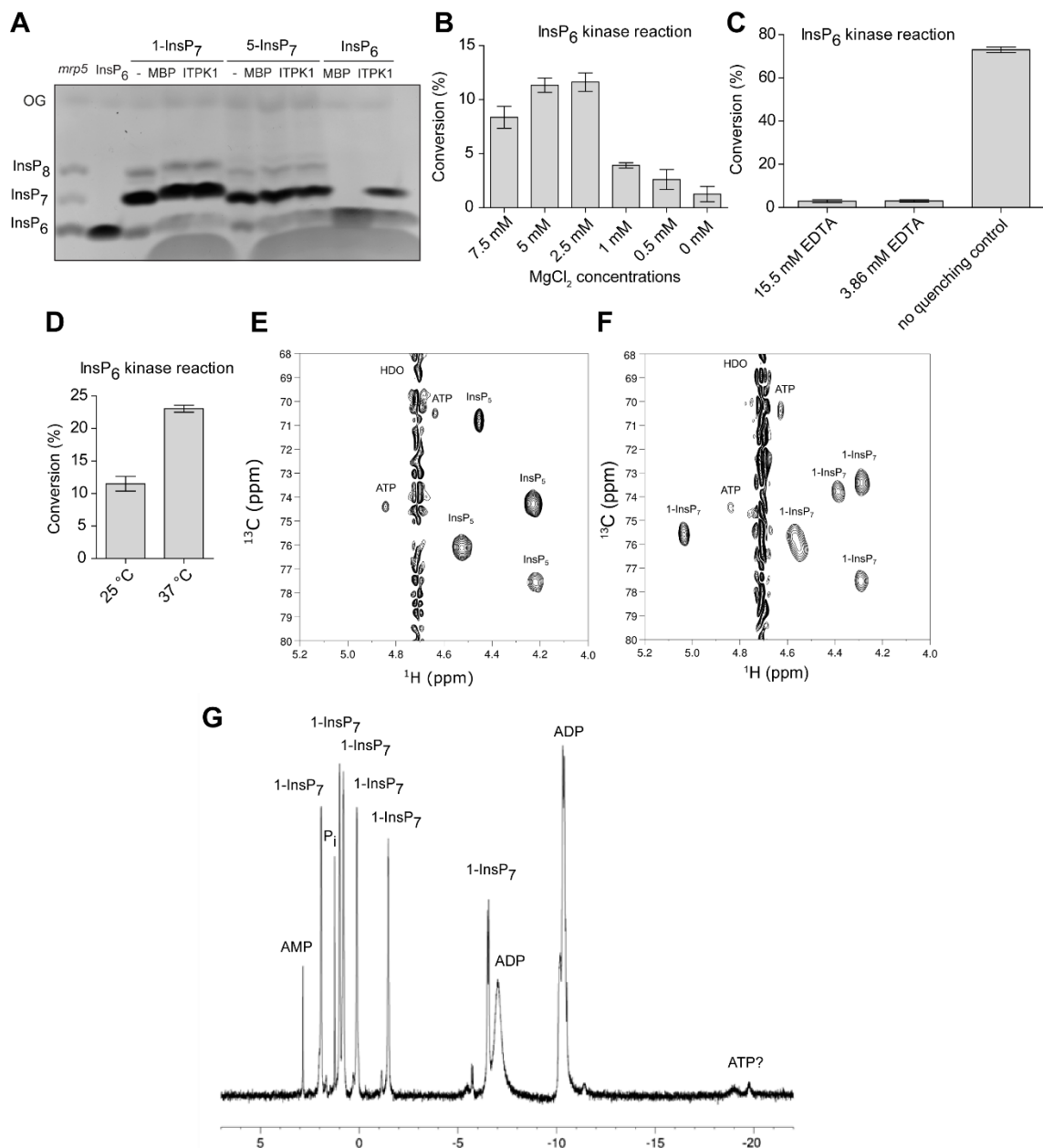
(A) *ITPK1* expression in 3-week-old WT (Col-0), *itpk1* and three independent *itpk1* lines transformed with *ITPK1* genomic DNA. Data represent means  $\pm$  SD ( $n = 3$  biological replicates).

(B and C) PAGE detection (B) and fold change of quantified signal intensities (C) of inositol (pyro)phosphates in shoots of WT (Col-0), *itpk1* and three independent *itpk1* lines transformed with *ITPK1* genomic DNA. Plants were cultivated in hydroponics under deficient P<sub>i</sub> for 7 days (-P) or -P resupplied with P<sub>i</sub> for 12 h (Pi RS). Data represent means  $\pm$  SE of values normalized to Col-0 plants continuously grown under sufficient P<sub>i</sub> ( $n = 3$  biological replicates). n.d., not detected. OG, orange G.



#### Supplemental Figure 4. VIH1- and VIH2-dependent inositol (pyro)phosphate metabolism.

CE-ESI-MS analysis of *VIH1* and *VIH2* mutations in the *phr1 phl1* background. Plants were cultivated in hydroponics under sufficient P<sub>i</sub> (+P), deficient P<sub>i</sub> (-P) or -P resupplied with P<sub>i</sub> for the indicated times. Data represent means ± SE (*n* = 3 biological replicates). n.d., could not be detected in any of these samples. Note that InsP<sub>8</sub>, 1/3-InsP<sub>7</sub>, InsP<sub>3</sub>-1 and 2-OH InsP<sub>5</sub> could not be detected in none of the analyzed samples.



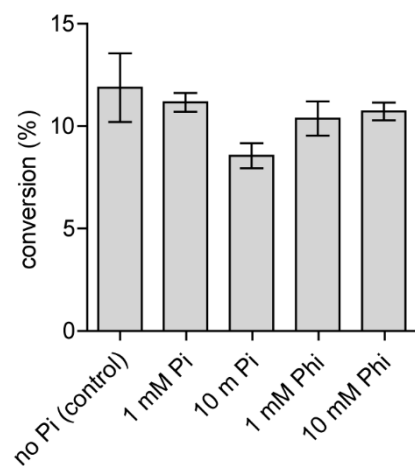
**Supplemental Figure 5. Activity of *Arabidopsis* ITPK1 on InsP<sub>5</sub> [2-OH] and InsP<sub>7</sub> isomers and control experiments for kinase assays.**

(A) ITPK1 has no kinase activity on 1-InsP<sub>7</sub> and 5-InsP<sub>7</sub>. InsP<sub>7</sub> isomers or InsP<sub>6</sub> were incubated with recombinant *Arabidopsis* ITPK1 as indicated in presence of 12.5 mM ATP. Inositol (pyro)phosphates were separated via PAGE and visualized by toluidine blue staining. The identity of bands was determined by migration compared to the substrates in absence of enzyme (-) and TiO<sub>2</sub>-purified *mrp5* seed extract. Purified His<sub>8</sub>-MBP tag (MBP) served as negative control for ITPK1. OG, orange G.

(B-D) Control experiments for NMR analyses. InsP<sub>6</sub> was incubated with recombinant *Arabidopsis* ITPK1 at 25°C in the presence of 2.5 mM ATP. Enzymatic activity was determined after 24 h in the presence of varying EDTA concentrations (B), after 1.5 h at changing Mg<sup>2+</sup> concentrations (C) and temperature (D). The conversion was determined by NMR spectroscopy after quenching with an excess of EDTA.

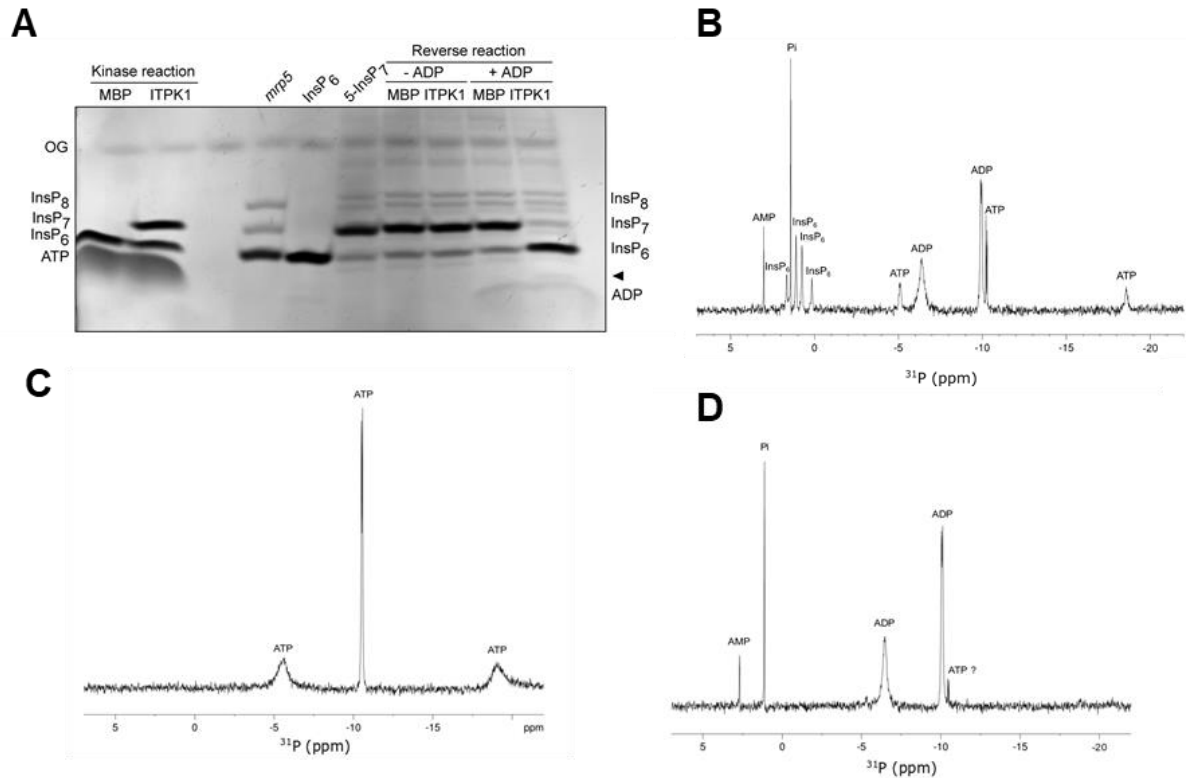
(E-G) 2D <sup>1</sup>H-<sup>13</sup>C-HMBC spectra. Recombinant *Arabidopsis* ITPK1 was incubated with InsP<sub>5</sub> (E) or 1-InsP<sub>7</sub> (F) at 25°C in the presence of an ATP recycling system for 24 h. The reaction mixture was analyzed by HSQC NMR spectroscopy. (G) Overview of the reaction shown in (F) as analyzed by <sup>31</sup>P NMR spectroscopy after 24 h. A small, unidentified signal potentially reflecting ATP is marked with a question mark.





**Supplemental Figure 6. Dependency of *Arabidopsis* ITPK1 kinase activity on P<sub>i</sub>.**

InsP<sub>6</sub> was incubated with recombinant *Arabidopsis* ITPK1 at 25°C in the presence of 2.5 mM ATP and the indicated concentrations of P<sub>i</sub> or its non-metabolizable analog phosphite (Phi). The conversion was determined by NMR spectroscopy after quenching with an excess of EDTA. The experiment was repeated three times.



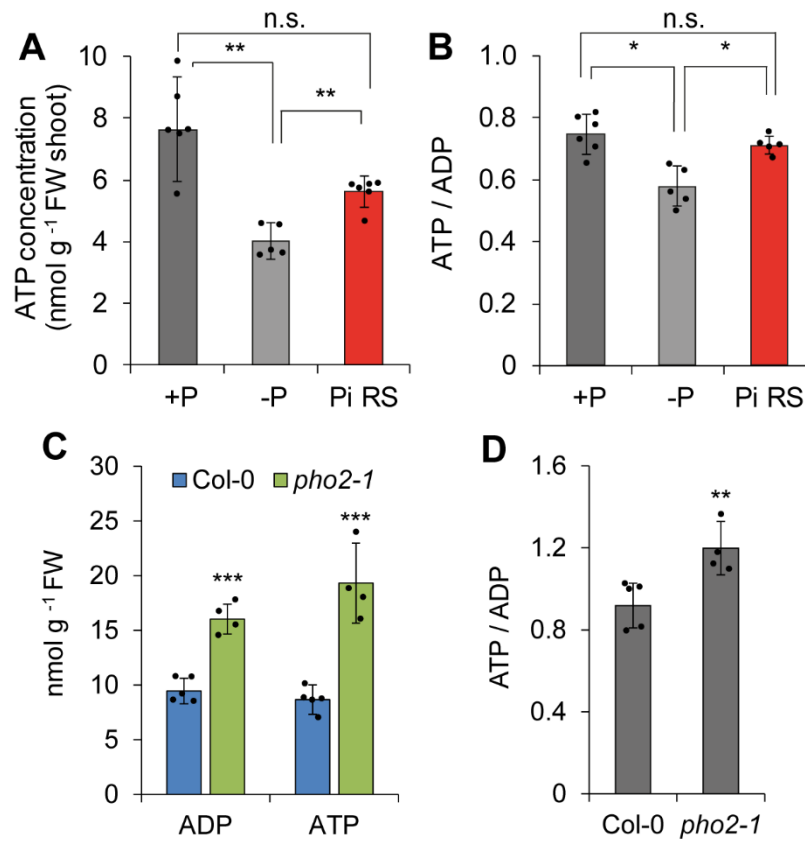
**Supplemental Figure 7. Recombinant *Arabidopsis* ITPK1 can dephosphorylate 5-InsP<sub>7</sub> in the presence of ADP.**

(A) ADP-dependent dephosphorylation of 5-InsP<sub>7</sub> by recombinant *Arabidopsis* ITPK1. InsPs were separated via PAGE and visualized by toluidine blue staining. The identity of bands was determined by migration compared to InsP<sub>6</sub> and 5-InsP<sub>7</sub> standards and TiO<sub>2</sub>-purified *mrp5* seed extract. InsP<sub>6</sub> kinase reaction served as positive control for the reverse reactions. Purified His<sub>8</sub>-MBP tag (MBP) served as negative control for ITPK1. Arrowhead indicates the presence of a small ATP band just above ADP. OG, orange G.

(B) <sup>31</sup>P NMR spectroscopy analysis of recombinant *Arabidopsis* ITPK1 incubated with [<sup>13</sup>C<sub>6</sub>]-labelled 5-InsP<sub>7</sub> at 25°C in the presence of ADP. After 24 h the mixture was analyzed by NMR.

(C) <sup>31</sup>P NMR analysis of ATP in ATP synthase reaction buffer.

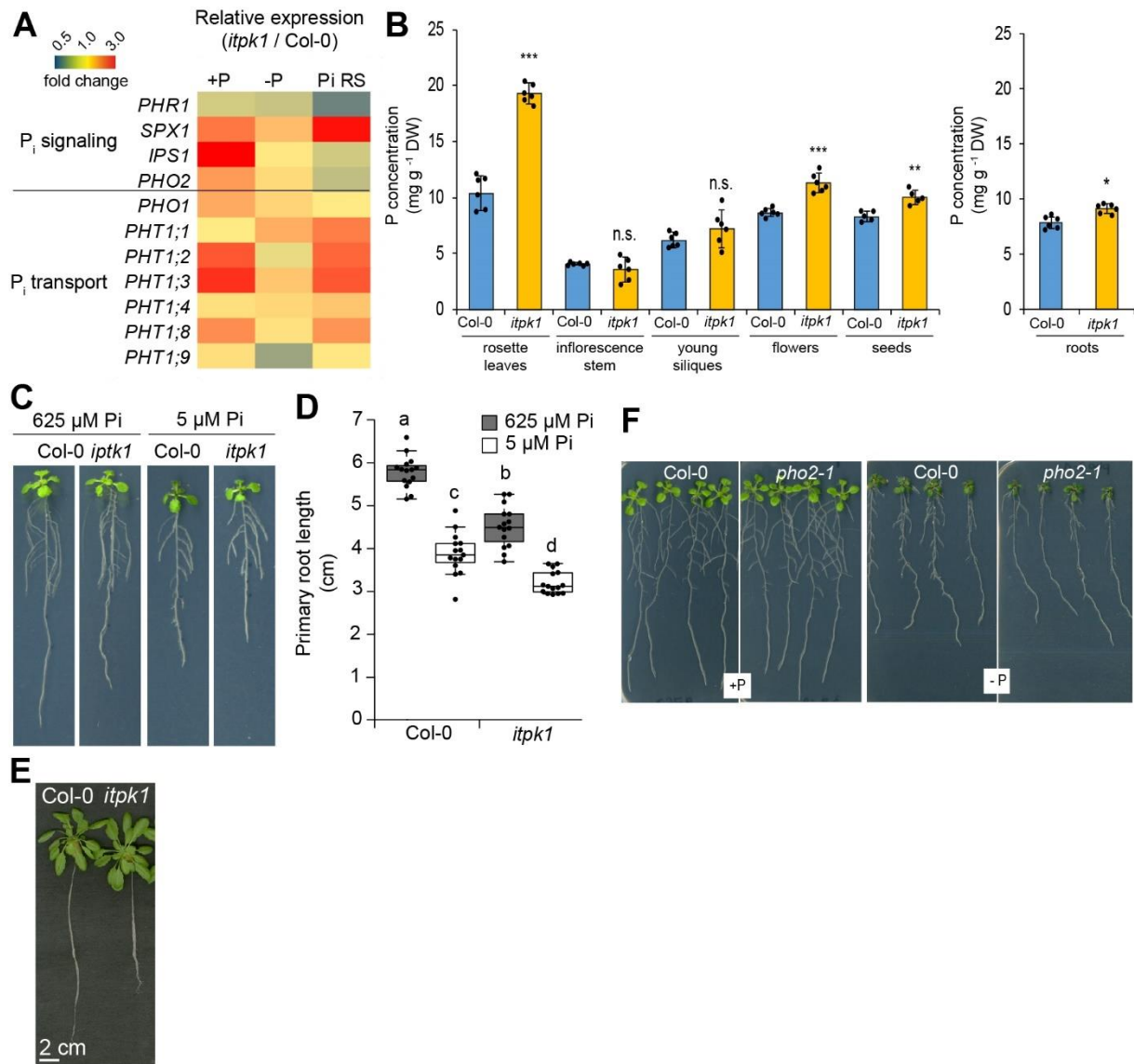
(D) <sup>31</sup>P NMR spectroscopy analysis of recombinant *Arabidopsis* ITPK1 incubated with ADP without 5-InsP<sub>7</sub> at 25°C and analyzed after 24 h. A small, unidentified signal potentially reflecting ATP is marked with a question mark.



**Supplemental Figure 8. Effect of P<sub>i</sub> availability and resupply on shoot ATP levels.**

**(A and B)** Concentration of ATP (**A**) and ATP/ADP ratios (**B**) in shoots of Col-0 plants grown in hydroponics with P<sub>i</sub>-sufficient solution (+P), exposed to 4 days of P<sub>i</sub> starvation (-P) or resupplied with P<sub>i</sub> for 12 h (Pi RS). Data represent means ± SD (*n* = 5-6 biological replicates). n.s., not significant.

**(C and D)** Concentration of ATP and ADP (**C**) and ATP/ADP ratios (**D**) in shoots of Col-0 and the P<sub>i</sub>-overaccumulating mutant *pho2-1* grown in hydroponics with sufficient P<sub>i</sub>. Data represent means ± SD (*n* = 4-5 biological replicates). \**P* < 0.05, \*\**P* < 0.01 and \*\*\**P* < 0.001 denote significant difference to wild type (Col-0) according to Student's *t*-test.



**Supplemental Figure 9. ITPK1-dependent P overaccumulation in different plant organs and root phenotypes.**

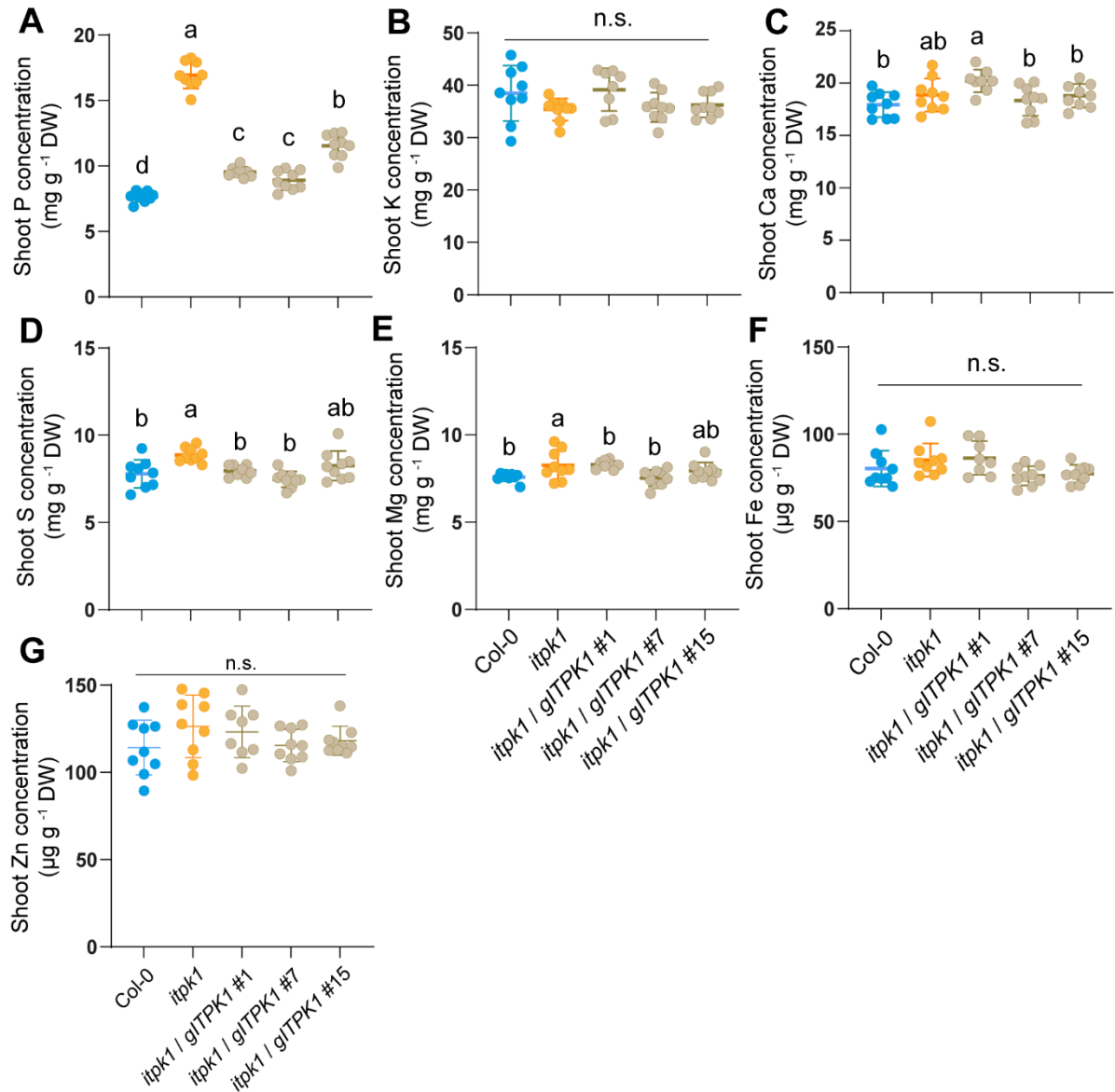
(A) Disruption of *ITPK1* results in mis-regulated expression of P<sub>i</sub> starvation-induced genes in roots.

(B) Total P levels in different parts of wild-type (Col-0) and *itpk1* plants. Data represent means ± SD ( $n$  = samples from 5 independent plants). Samples of above-ground tissues were collected from 5-week-old plants grown on peat-based substrate. Roots were collected from plants grown under sufficient P<sub>i</sub> in hydroponics. \* $P$  < 0.05, \*\* $P$  < 0.01 and \*\*\* $P$  < 0.001 denote significant difference to wild type (Col-0) according to Student's  $t$ -test. n.s., not significant. Young siliques = green siliques with a length of 0.8 cm to 1.5 cm.

(C and D) P<sub>i</sub>-independent root growth repression in *itpk1* plants. Plant phenotype (C) and primary root length (D) after 7 days of growth under sufficient (625 μM P<sub>i</sub>) or deficient P<sub>i</sub> supply (5 μM P<sub>i</sub>). Horizontal lines show medians; box limits indicate the 25th and 75th percentiles; whiskers extend to 1.5 times the interquartile range from the 25th and 75th percentiles ( $n$  = 15 independent plants). Different letters indicate significant differences according to Tukey's test ( $P$  < 0.05).

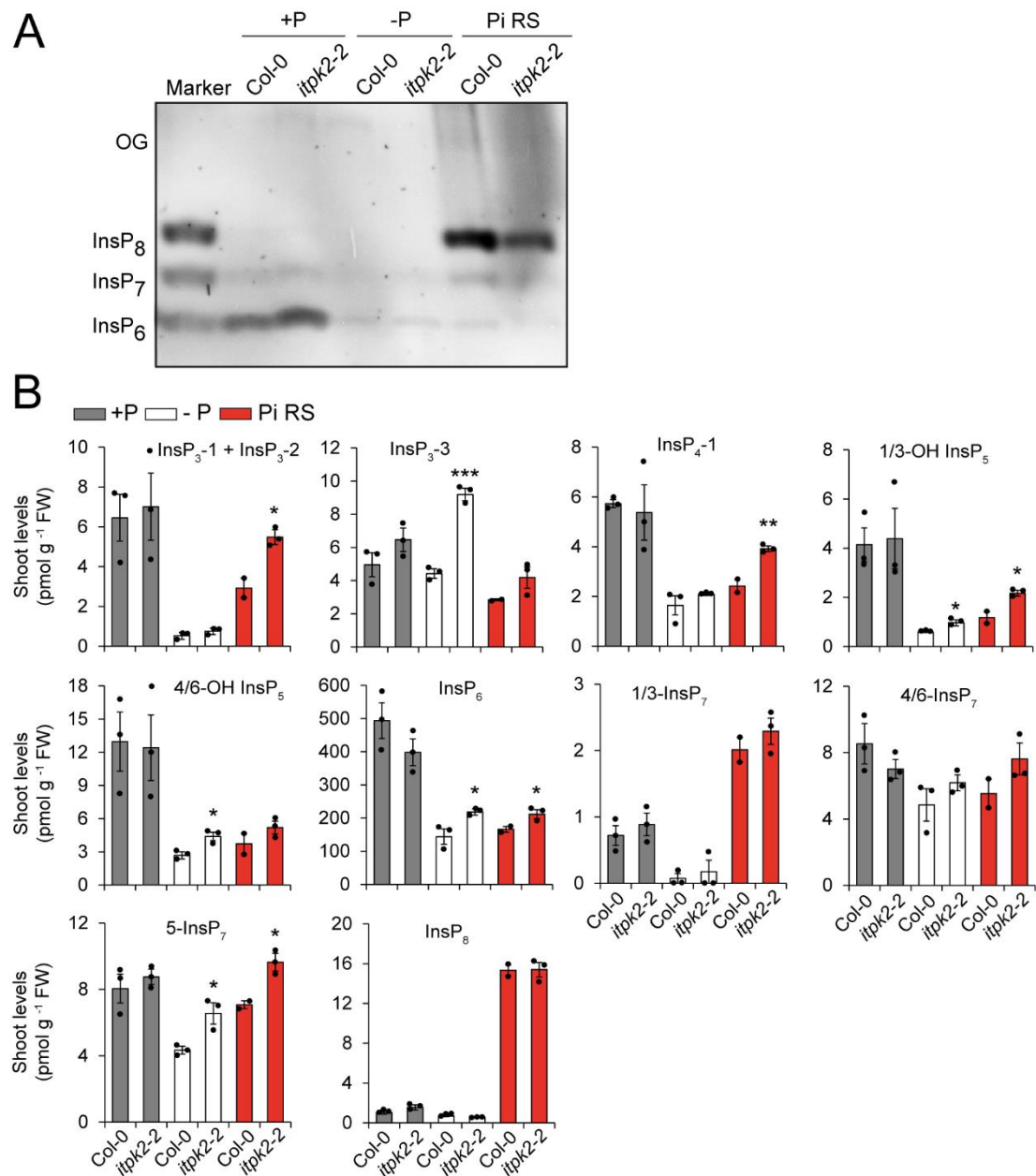
(E) Short-root phenotype of 5-week-old *itpk1* plants grown in hydroponics with sufficient P<sub>i</sub>. Representative plants are shown.

(F) Phenotype of wild-type (Col-0) and *pho2-1* plants grown in agar plates. Seven-day-old seedlings germinated on half-strength solid Murashige and Skoog agar media containing 625 μM P<sub>i</sub> were transferred to +P (625 μM P<sub>i</sub>) or -P (5 μM P<sub>i</sub>) and grown for additional 7 days until imaging.



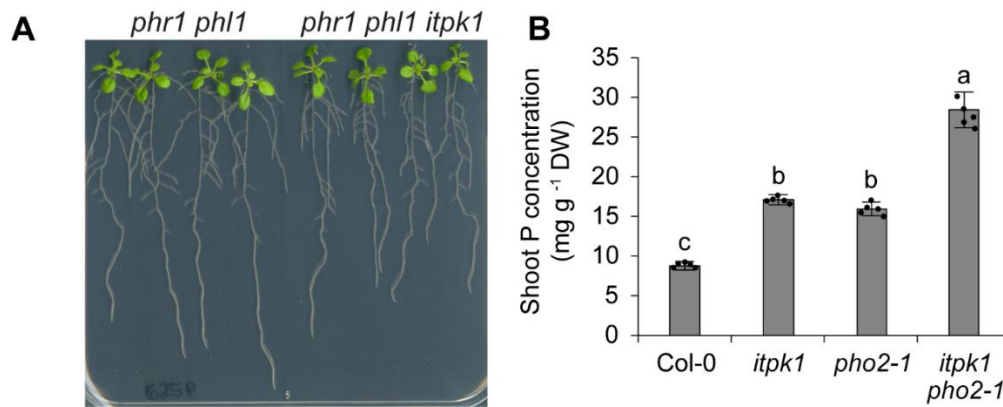
**Supplemental Figure 10. The *itpk1* mutant shows a specific overaccumulation of P that can be complemented with genomic *ITPK1*.**

Shoot concentrations of the macronutrients phosphorus (A), potassium (B), calcium (C), sulfur (D) and magnesium (E), and the micronutrients iron (F) and zinc (G) of 3-week-old plants grown on peat-based substrate. Graphs depict the means  $\pm$  SD ( $n = 8-9$  plants) and raw data points. Different letters indicate significant differences according to Tukey's test ( $P < 0.05$ ). n.s., not significant according to one-way ANOVA.



**Supplemental Figure 11. P<sub>i</sub>-dependent InsP<sub>7</sub> and InsP<sub>8</sub> synthesis is not substantially altered in shoots of the *itpk2-2* mutant.**

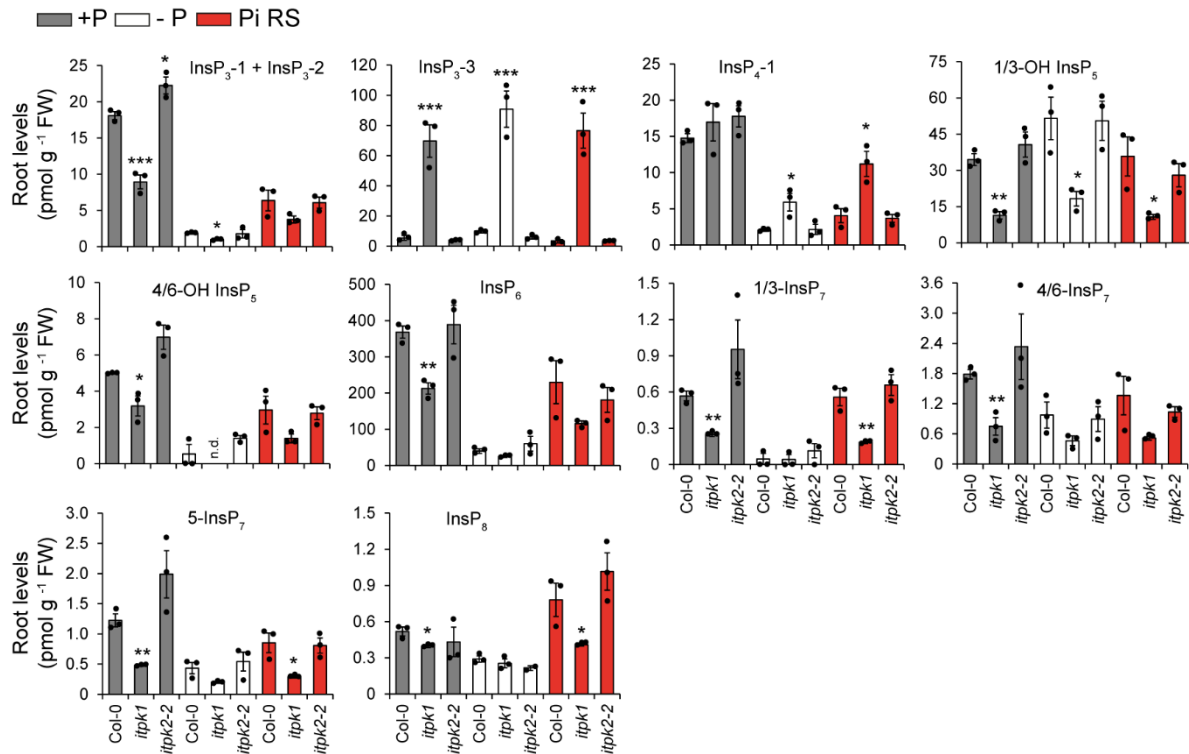
(A and B) PAGE detection (A) and CE-ESI-MS analysis (B) of inositol (pyro)phosphates in shoots of WT and *itpk2-2* plants. Plants were grown in hydroponics under sufficient P<sub>i</sub> (+P), after 7 days of P<sub>i</sub> deficiency (-P) or after resupply of P<sub>i</sub> to P<sub>i</sub>-deficient plants for 12 h (P<sub>i</sub> RS). Data represent means ± SE (*n* = 2-3 biological replicates). OG, orange G. \**P* < 0.05, \*\**P* < 0.01 and \*\*\**P* < 0.001 denote significant difference to wild type (Col-0) according to Student's *t*-test.



**Supplemental Figure 12. The short-root phenotype of *itpk1* plants is independent of PHR1/PHL1 and evidence for the additive role of ITPK1 and PHO2 in the regulation of P<sub>i</sub> homeostasis.**

**(A)** Root phenotypes of *phr1 phl1* double mutant and *phr1 phl1 itpk1* triple mutant. Seven-day-old seedlings germinated on half-strength solid Murashige and Skoog agar media containing 625  $\mu$ M P<sub>i</sub> were transferred to +P (625  $\mu$ M P<sub>i</sub>) and grown for additional 7 days. Shown are representative images of the indicated mutants grown side-by-side on the same agar plate.

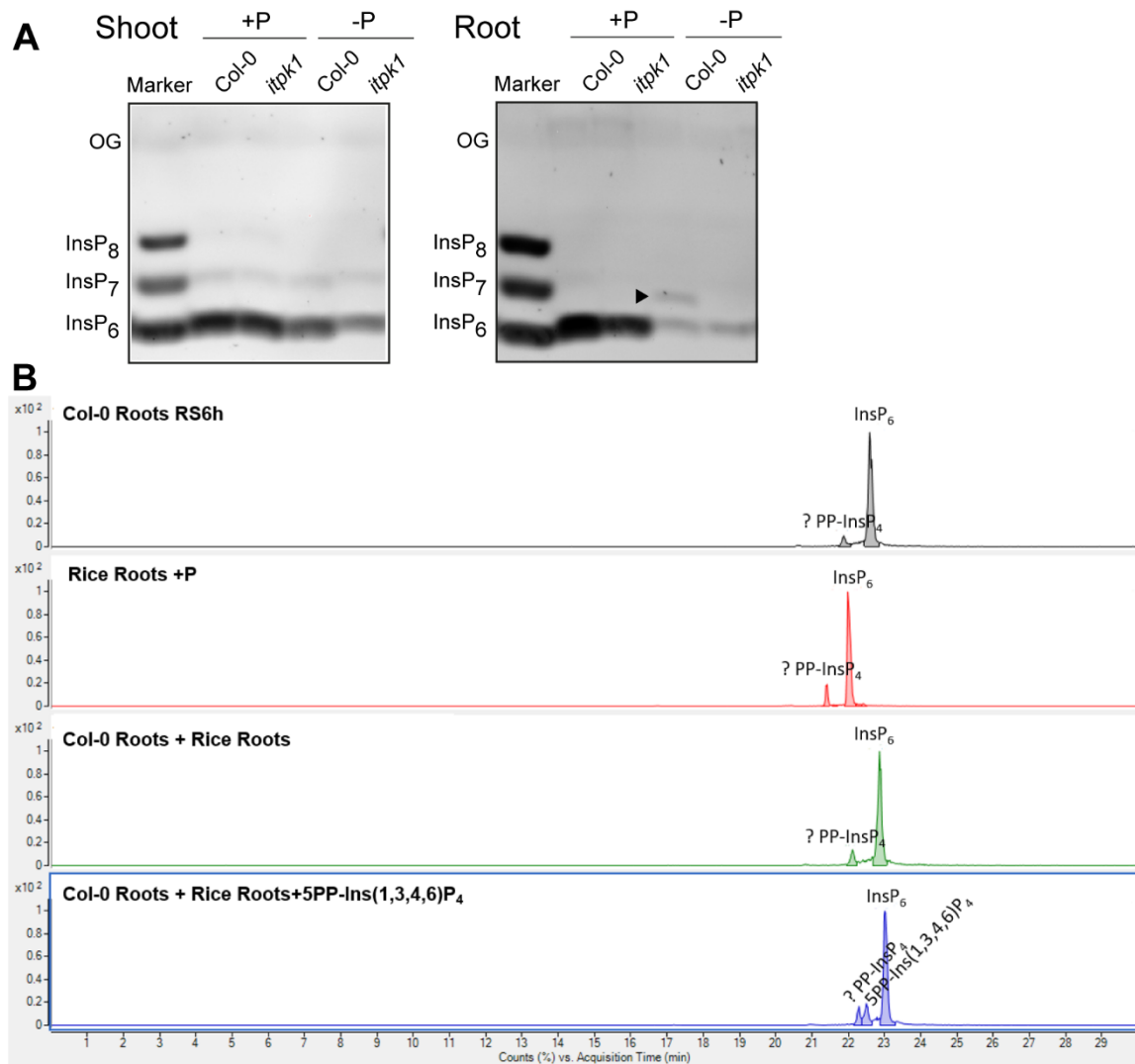
**(B)** Total P levels were assessed in shoots of 4-week-old wild-type (Col-0) and the indicated single and double mutant. Samples of above-ground tissues were collected from 5-week-old plants grown on peat-based substrate. Data represent means  $\pm$  SD ( $n = 5$  plants). Different letters indicate significant differences according to Tukey's test ( $P < 0.05$ ).



**Supplemental Figure 13. ITPK1- and ITPK2-dependent inositol (pyro)phosphate metabolism in roots.**

CE-ESI-MS analysis of inositol (pyro)phosphates in roots of WT (Col-0) and *itpk1* plants. Plants were cultivated in hydroponics under sufficient P<sub>i</sub> (+P), deficient P<sub>i</sub> for 7 days (-P) or -P resupplied with P<sub>i</sub> for 12 h (Pi RS). Data represent means ± SE (*n* = 3 biological replicates). n.d., not detected. \**P* < 0.05, \*\**P* < 0.01 and \*\*\**P* < 0.001 denote significant difference to wild type (Col-0) according to Student's *t*-test.

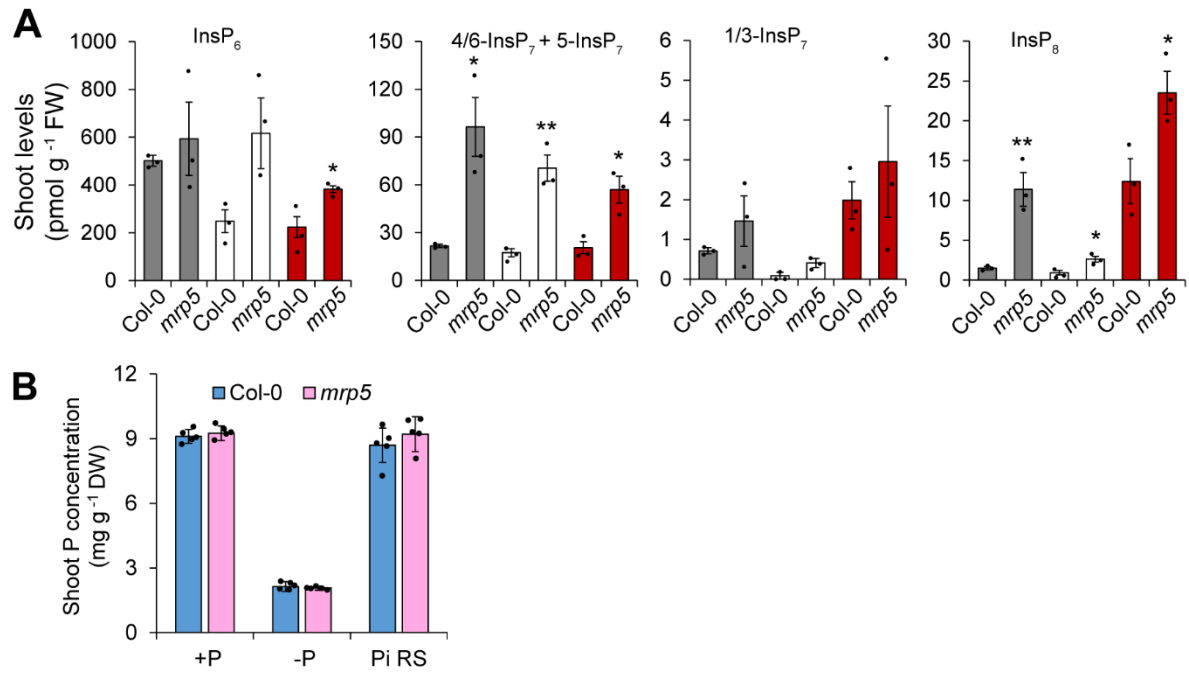




**Supplemental Figure 14. Roots of *Arabidopsis* and rice plants produce a PP-InsP<sub>4</sub> isomer not previously identified in plants.**

(A) PAGE detection of InsPs and PP-InsPs in shoots and roots of WT (Col-0) and *itpk1* mutant plants grown in hydroponics in P<sub>i</sub>-sufficient solution (+P) or exposed for 7 days to P<sub>i</sub> starvation (-P). Shown are PAGE results of shoots and roots of the same plants. The PAGE of roots is also shown in Fig. 7C and is displayed here for a direct comparison with shoot. The arrowhead indicates the appearance of a band between InsP<sub>6</sub> and InsP<sub>7</sub> specifically in WT root samples. OG, orange G.

(B) CE-ESI-MS identification of a new PP-InsP<sub>4</sub> isomer in roots of *Arabidopsis* and rice plants. The identified isomer generated in the roots of both species was indistinguishable and did not co-migrate with a 5PP-Ins(1,3,4,6)P<sub>4</sub> standard.



**Supplemental Figure 15. Disruption of *MRP5* increases PP-InsP synthesis in shoots but does not alter P<sub>i</sub>-dependent regulation of InsP<sub>8</sub> synthesis.**

(A) CE-ESI-MS analysis of InsP<sub>6</sub> and PP-InsPs in shoots of wild-type (Col-0) and *mrp5* plants. Plants were grown in hydroponics in P<sub>i</sub>-sufficient solution (+P), exposed for 7 days to P<sub>i</sub> starvation (-P) or -P resupplied with P<sub>i</sub> for 12 h (Pi RS). In this run, a baseline separation of 5-InsP<sub>7</sub> and 4/6-InsP<sub>7</sub> was not possible and combined results are shown. Data represent means ± SE (*n* = 3 biological replicates). \**P* < 0.05 and \*\**P* < 0.01 denote significant difference to wild type (Col-0) according to Student's *t*-test.

(B) Total P concentration in shoots of WT (Col-0) and *mrp5* mutant plants grown as described in (A). Data represent means ± SD (*n* = 5 plants).

## Supplemental Methods

### Hydroponics culture of *Arabidopsis* and rice

For hydroponic culture, *Arabidopsis* seeds were pre-cultured on rock wool moistened with tap water. After 1 week, the tap water was replaced by half-strength nutrient solution containing 2 mM  $\text{NH}_4\text{NO}_3$ , 1 mM  $\text{KH}_2\text{PO}_4$ , 1 mM  $\text{MgSO}_4$ , 1 mM  $\text{KCl}$ , 250  $\mu\text{M}$   $\text{K}_2\text{SO}_4$ , 250  $\mu\text{M}$   $\text{CaCl}_2$ , 100  $\mu\text{M}$   $\text{Na-Fe-EDTA}$ , 30  $\mu\text{M}$   $\text{H}_3\text{BO}_3$ , 5  $\mu\text{M}$   $\text{MnSO}_4$ , 1  $\mu\text{M}$   $\text{ZnSO}_4$ , 1  $\mu\text{M}$   $\text{CuSO}_4$  and 0.7  $\mu\text{M}$   $\text{NaMoO}_4$  (pH adjusted to 5.8 by  $\text{KOH}$ ). After 7 days, the nutrient solution was changed to full-strength and replaced once a week during 2 weeks, then twice a week during 1 week, and every 2 days once the treatments were imposed. Aeration was provided to roots from the third week onwards. To induce  $\text{P}_i$  deficiency,  $\text{KH}_2\text{PO}_4$  was replaced by  $\text{KCl}$  and  $\text{P}_i$  resupply was performed by refeeding  $\text{P}_i$ -starved plants with 1 mM  $\text{KH}_2\text{PO}_4$  for 12 h. Plants were grown hydroponically in a growth chamber under the above-mentioned conditions except that the light intensity was 200  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  and supplied by halogen lamps.

Rice plants (cv. Nipponbare) were cultivated in hydroponics inside a glasshouse with natural light supplemented with high pressure sodium vapor lamps to ensure a minimum light intensity of 300  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , and 30°C/25°C day (16 h)/night (8 h) temperature. Seeds were germinated in darkness at 30°C for 3 days and then transferred to meshes floating on a solution containing 0.5 mM  $\text{CaCl}_2$  and 10  $\mu\text{M}$   $\text{Na-Fe-EDTA}$ , which was exchanged every third day. After 10 days, homogenous seedlings were transplanted into 60-L tanks filled with a modified Yoshida nutrient solution (Shrestha et al., 2018). Ten days later, the nutrient solution was changed to full-strength and exchanged every 10 days. During the whole growing period, the pH value was adjusted to 5.5 every second day.  $\text{P}_i$  starvation was imposed for 10 days before starting  $\text{P}_i$  resupply.

### Agar plate culture of *Arabidopsis*

Cultivation of *Arabidopsis* plants under sufficient  $\text{P}_i$  (625  $\mu\text{M}$   $\text{P}_i$ ) or low  $\text{P}_i$  (5  $\mu\text{M}$   $\text{P}_i$ ) in sterile solid medium was performed exactly as described in Gruber et al. (2013).

### Analysis of ATP and ADP

Adenosine nucleotides were specifically determined according to Haink and Deussen (2003) with some modifications. Frozen leaf material (100 mg) was homogenized in liquid  $\text{N}_2$  and extracted with methanol/chloroform according to Ghaffari et al. (2016). An aliquot of extracted samples was used for derivatization. Twenty  $\mu\text{L}$  of extract was added to 205  $\mu\text{L}$  of a buffer

containing 62 mM sodium citrate and 76 mM  $\text{KH}_2\text{PO}_4$  for which pH was adjusted to 5.2 with KOH. To this mixture, 25  $\mu\text{L}$  chloroacetaldehyde (Sigma-Aldrich, Germany) was added and the whole solution was incubated for 40 min at 80°C followed by cooling and centrifugation for 1 min at 14000 rpm. Two blanks containing all reagents except plant extract were used as control. For quantification, external ATP and ADP standards were established with different concentrations. Separation of adenosine nucleotides was performed on a newly developed UPLC-based method using ultra pressure reversed phase chromatography (Acquity H-Class, Waters GmbH, Eschborn, Germany). The UPLC system consisted of a quaternary solvent manager, a sample manager-FTN, a column manager and a fluorescent detector (PDA eλ Detector). The separation was carried out on a C18 reversed phase column (YMC Triart, 1.9  $\mu\text{m}$ , 2.0x100 mm ID, YMC Chromatography, Germany) with a flow rate of 0.6 ml per min and duration of 7 min. The column was heated at 37°C during the whole run. The detection wavelengths were 280 nm for excitation and 410 nm as emission. The gradient was accomplished with two solutions. Eluent A was 5.7 mM tetrabutylammonium bisulfate (TBAS) and 30.5 mM potassium  $\text{KH}_2\text{PO}_4$ , pH adjusted to 5.8. Eluent B was a mixture of pure acetonitrile and TBAS in a ratio of 2:1. The column was equilibrated with eluent A (90%) and eluent B (10 %) for at least 30 minutes. The gradient was produced as follow: 90% A and 10% B for 2 min, changed to 40% A and 60% B and kept for 2.3 min, changed to 10% A and 90% B for 1.1 min and reversed to 90% A and 10% B for another 1.6 min.

## Supplemental References

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