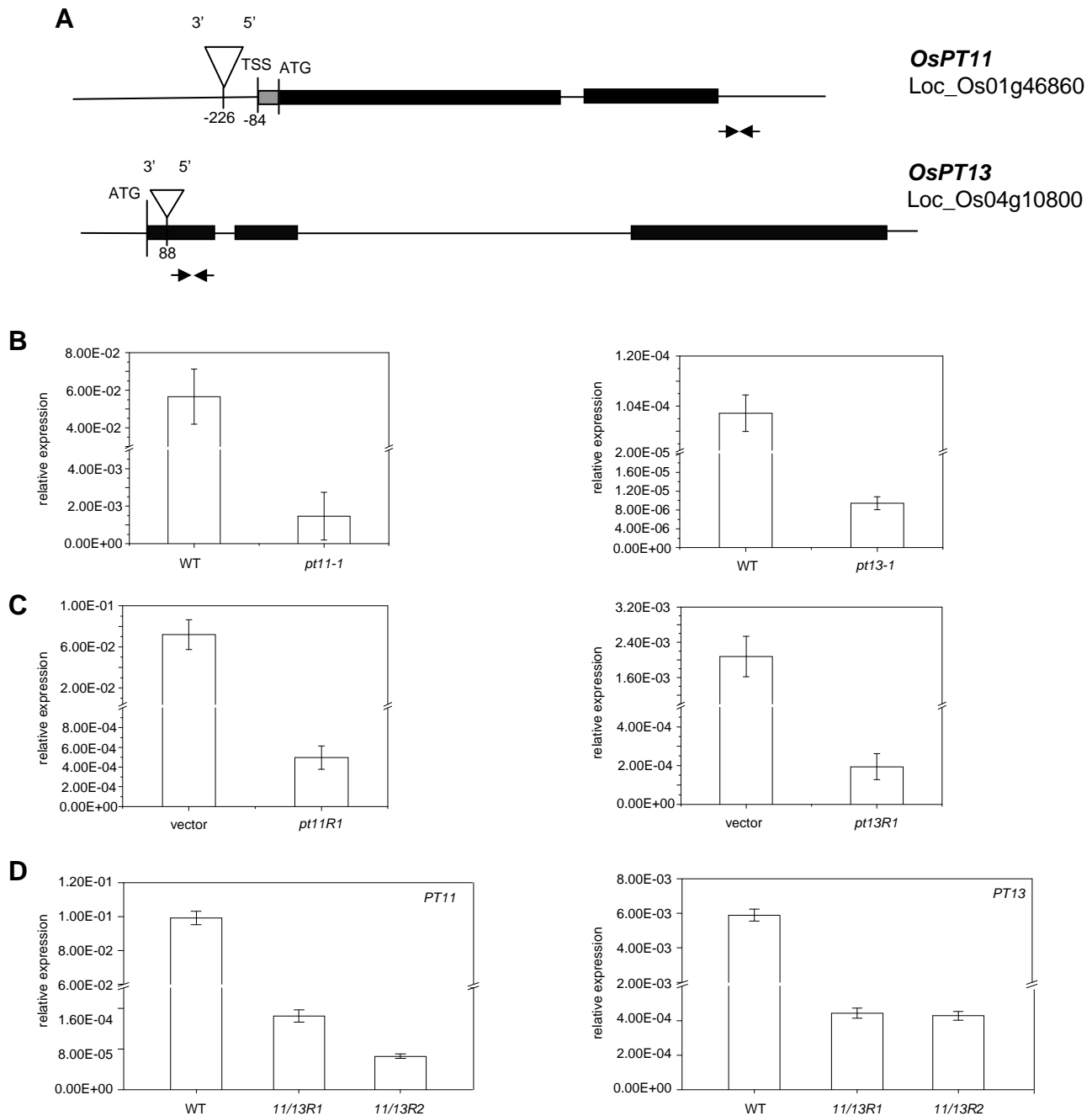


## Supplemental Figure 1

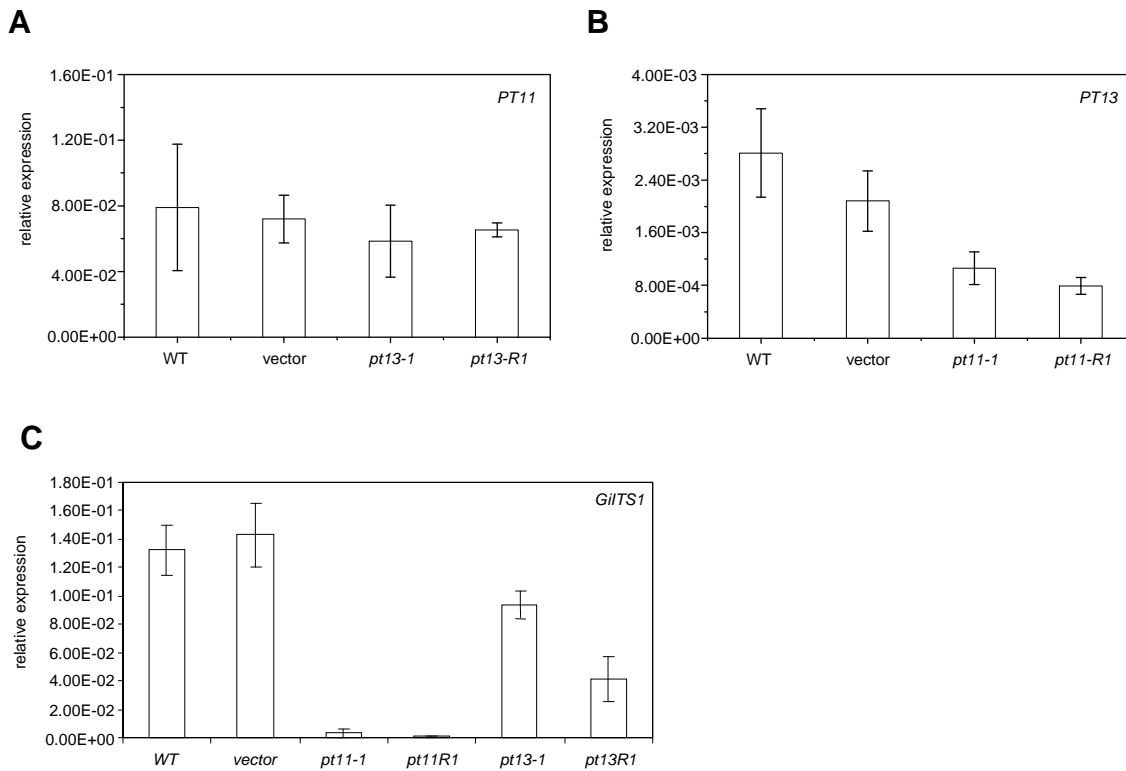


**Supplemental Figure 1. Characterization of *PT11* and *PT13* insertion mutants and corresponding RNAi lines.**

(A) The structure of *PT11* and *PT13* drawn to scale with positions of repetitive insertions. The A of each ATG designates nucleotide 1. Black boxes represent exons separated by introns (solid lines). The *pt11* mutant carries a *Tos17* retrotransposon insertion within the promoter region of *PT11*. The *pt13* mutant contains a *dSpm* element in the first exon of *PT13*. TSS: transcription start site; Arrows indicate primers used for expression analysis.

(B-D) Analysis of *PT11* and *PT13* transcript levels in *Glomus intraradices* colonized wild-type, empty vector controls, homozygous mutant (*pt11-1* or *pt13-1*), and RNAi lines (*pt11R1*, *pt13R1*, *pt11/13R1* and *pt11/13R2*) by real-time RT-PCR using primer pairs as indicated. Gene expression levels have been normalized to the constitutive rice *Cyclophilin2* gene. Error bars refer to the standard error of two to five biological replicates.

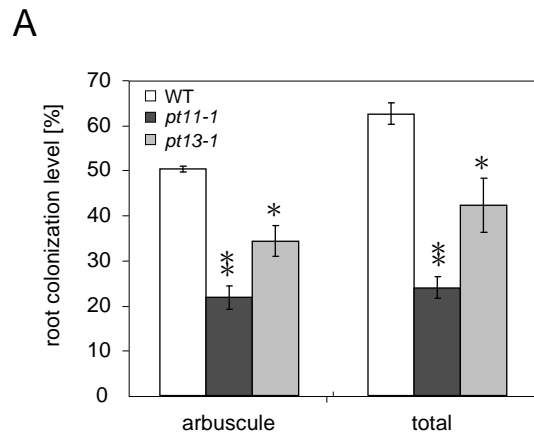
## Supplemental Figure 2



### Supplemental Figure 2. Absence of off-target silencing as monitored by real-time RT-PCR.

Transcript levels were reciprocally determined for *PT11* in the *PT13* mutant (A) and for *PT13* in the *PT11* mutant lines (B). The *Glomus intraradices internal transcribed spacer 1* (*GilTS1*) was used to quantitatively reflect root colonization (C). Gene expression levels have been normalized to the constitutive rice *Cyclophilin2* gene. The graph includes mRNA data from three biological replicates of each genotype.

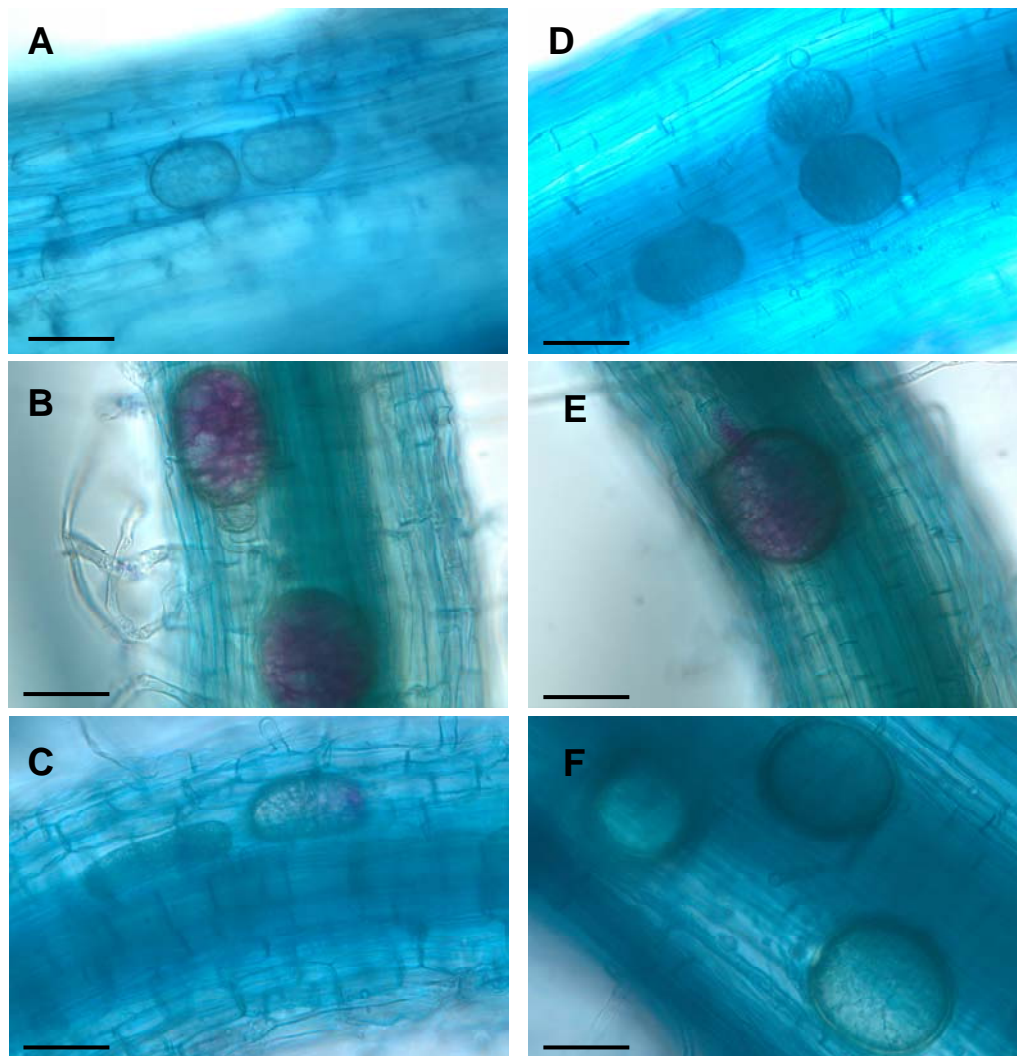
## Supplemental Figure 3



### Supplemental Figure 3. Colonization of the *PT11* and *PT13* mutant lines by *Gigaspora rosea*.

(A) Percentage of root length colonized by arbuscules and total fungal structures of *Gigaspora rosea* in control and mutant lines as indicated at 10 wpi. Mean values of three biological replicates are shown, each consisting of two pooled plants. Statistically significant differences between mutant and control alleles is indicated by a single asterisks  $p < 0.05$ ; double asterisks  $p < 0.01$ .

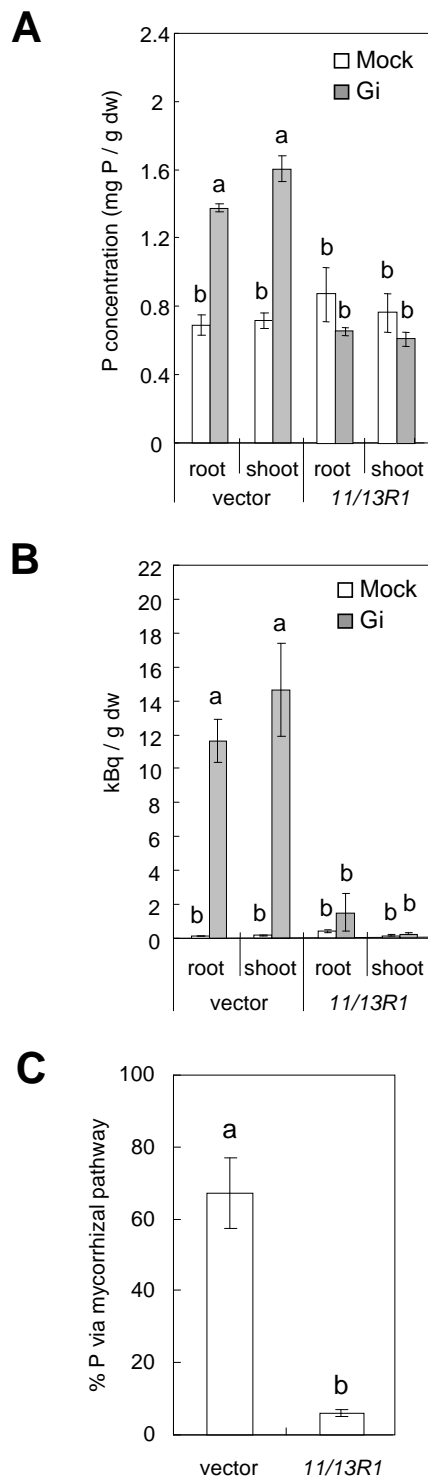
## Supplemental Figure 4



**Supplemental Figure 4: Polyphosphate (polyP) stain of mycorrhizal control, *PT11* and *PT13* mutant roots.**

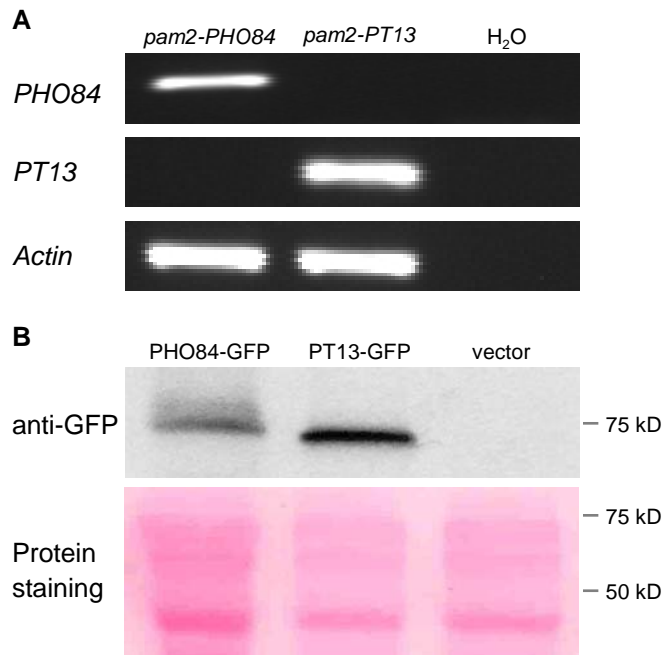
In *pt11-1* (B) and *pt11R1* (E) mutants purple polyP granules accumulated in vesicles of *Glomus intraradices* which was neither observed in wild-type (A) or empty vector (D) controls nor in *pt13-1* (C) or *pt13R1* (F) mutants. Bar = 50  $\mu$  m.

## Supplemental Figure 5

**Supplemental Figure 5. Quantitative symbiotic Pi-transfer in double mutant of *PT11* and *PT13*.**

(A and B) Tissue concentration at 8wpi of <sup>31</sup>P and <sup>33</sup>P in roots and shoots of *Glomus intraradices* or mock-inoculated control plants. (C) Percentage contribution of the mycorrhizal pathway to P uptake at 8wpi in the control and mutant RNAi lines colonized by *Glomus intraradices*. The standard error refers to three to five biological replicates and each biological replicate consists of a pool of two plants. Boxes with dissimilar letters are significantly different (Tukey test after one-way ANOVA) at  $p < 0.01$  in (A) and at  $p < 0.001$  in (B) and (C).

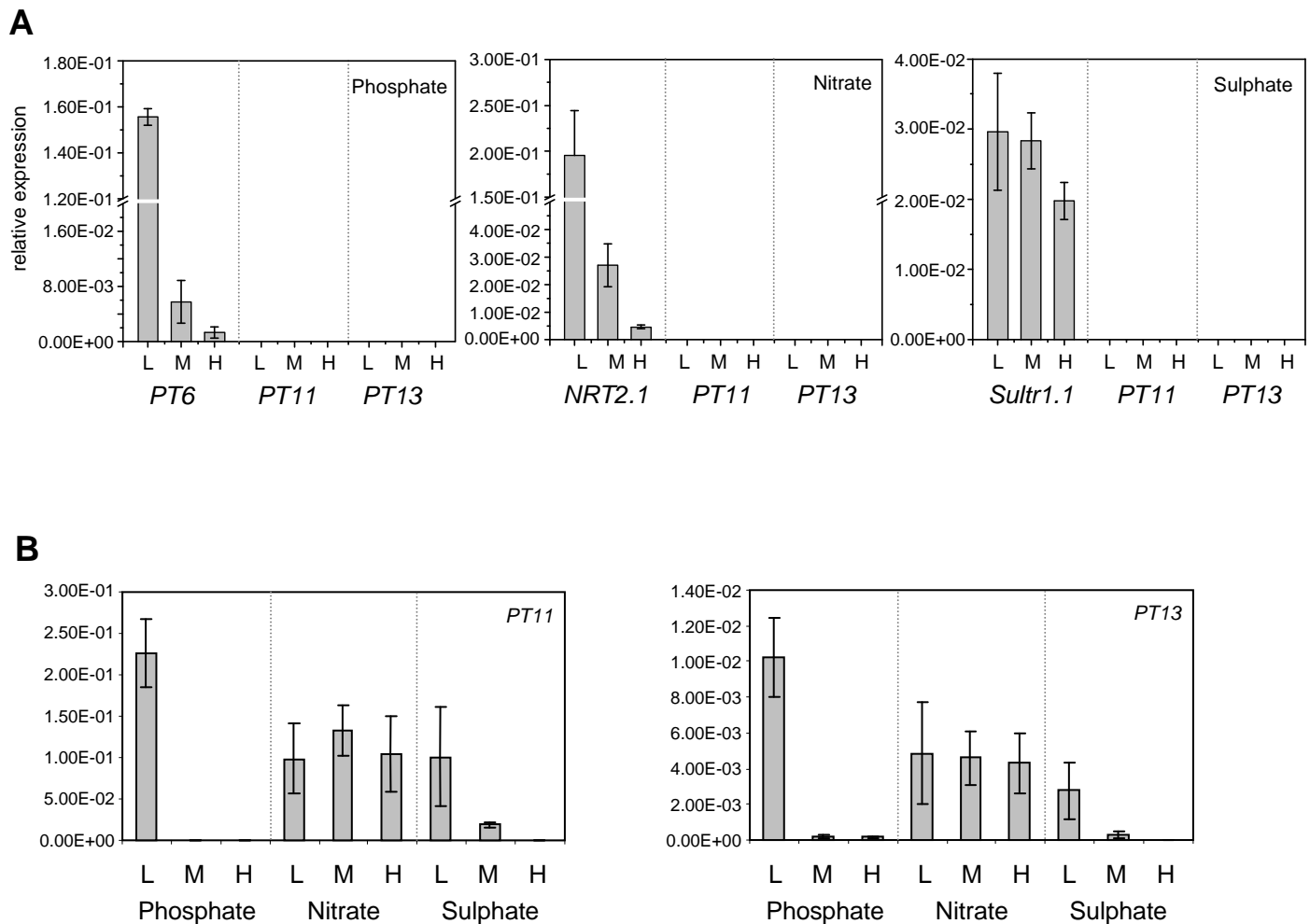
## Supplemental Figure 6



**Supplemental Figure 6. Transcript and protein levels of ectopically expressed yeast *PHO84* and rice *PT13* in yeast.**

(A) RT-PCR based analysis of mRNA levels of the yeast *PHO84* and the rice *PT13* under the control of the constitutive *Plasma Membrane ATPase1 (PMA1)* promoter in the yeast *pam2* mutant. *Actin* was used as a constitutively expressed endogenous control gene. (B) Protein levels of PHO84-GFP and PT13-GFP detected by anti-GFP antibodies. Ponceau S staining shows amount of protein loaded.

## Supplemental Figure 7



**Supplemental Figure 7: Expression analysis of *PT11* and *PT13* in roots in response to increasing regimes of phosphate, nitrate or sulphate fertilization.**

Real-time RT-PCR based gene expression analysis of 7 weeks old rice roots after 5 weeks of fertilization with the respective nutrient regime. (A) mRNA levels of the nutrient marker genes *PT6* (Pi transporter), *NRT2.1* (nitrate transporter), *Sultr1.1* (sulfate transporter) and the AM-associated *PT11* and *PT13* in roots of mock-inoculated plants grown under increasing supply of the indicated mineral nutrient. (B) Transcript levels of *PT11* and *PT13* in mycorrhizal roots of plants growing under increasing regimes of the indicated mineral nutrient. Mean and standard error values of three biological replicates are shown. Gene expression levels have been normalized to the constitutive rice *Cyclophilin2* gene. Fertilization conditions correspond to L-low, M-medium and H-high levels of phosphate, nitrate and sulfate as indicated.

Supplemental Table 1  
Concentration (mM) of compounds in one strength Hoagland solution

|  | P-L     | P-M     | P-H     | N-L     | N-M     | N-H     | S-L     | S-M     | S-H     |
|--|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| KNO <sub>3</sub>   | 5       | 5       | 5       | 0       | 2       | 7       | 5       | 5       | 5       |
| Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O                               | 0.5     | 0.5     | 0.5     | 0.5     | 0.5     | 0.5     | 0.5     | 0.5     | 0.5     |
| MgSO <sub>4</sub> ·7H <sub>2</sub> O   | 2       | 2       | 2       | 2       | 2       | 2       | 0.1     | 1       | 4       |
| KH <sub>2</sub> PO <sub>4</sub>  | 0.05    | 0.5     | 2       | 0.05    | 0.05    | 0.05    | 0.05    | 0.05    | 0.05    |
| KCl  | 0.95    | 0.5     | 0       | 5.95    | 3.95    | 0       | 0.95    | 0.95    | 0.95    |
| MnSO <sub>4</sub> ·H <sub>2</sub> O  | 8.0E-04 | 8.0E-04 | 8.0E-04 | 8.0E-04 | 8.0E-04 | 8.0E-04 | 8.0E-04 | 8.0E-04 | 8.0E-04 |
| ZnSO <sub>4</sub> ·7H <sub>2</sub> O   | 7.0E-05 | 7.0E-05 | 7.0E-05 | 7.0E-05 | 7.0E-05 | 7.0E-05 | 7.0E-05 | 7.0E-05 | 7.0E-05 |
| CuSO <sub>4</sub> ·5H <sub>2</sub> O   | 3.2E-05 | 3.2E-05 | 3.2E-05 | 3.2E-05 | 3.2E-05 | 3.2E-05 | 3.2E-05 | 3.2E-05 | 3.2E-05 |
| Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O                  | 5.0E-04 | 5.0E-04 | 5.0E-04 | 5.0E-04 | 5.0E-04 | 5.0E-04 | 5.0E-04 | 5.0E-04 | 5.0E-04 |
| (NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O | 8.0E-06 | 8.0E-06 | 8.0E-06 | 8.0E-06 | 8.0E-06 | 8.0E-06 | 8.0E-06 | 8.0E-06 | 8.0E-06 |

Supplemental Table 2

Primer sequences used for genotyping of wild type and insertion mutant alleles of *PT11* and *PT13*

| Gene        | Primer sequence  |
|-------------|--|
| <i>PT11</i> | Forward: AAGGGACAAAACACACAGGC<br>Reverse: TCTGATCACCAAAGTCTGCTCG |
| <i>pt11</i> | Forward: AAGGGACAAAACACACAGGC<br>Reverse: AGGTTGCAAGTTAGTTAAGA   |
| <i>PT13</i> | Forward: AGCAAGGACAGGATCGAAGA<br>Reverse: GGCGTACTCTGACATGATGG   |
| <i>pt13</i> | Forward: GCGTCGGTTTCATCGGGACC<br>Reverse: GGCGTACTCTGACATGATGG   |



## Supplemental Table 3

Primer sequences for constructing RNAi and reporter lines of *PT11* and *PT13*

| Gene        | Vector   | Goal              | primer sequence  |
|-------------|----------|-------------------|--|
| <i>PT11</i> | pANDA    | RNAi line         | F: CACC TAC GCT CAT CTG GGA<br>R: GTTGCAATTGTCCCTCTCATTC |
|             | pHGWS7.0 | promoter activity | F: CACCGTCACGTCCTCTTCC<br>R: CTCCGATGATGCCGTCGAT         |
| <i>PT13</i> | pANDA    | RNAi line         | F: CACCCCAACAATACGATGAC<br>R: TGTATCCGATCCGGTACTAG       |
|             | pHGWS7.0 | promoter activity | F: CACCGCACATAATGATTTAATTT<br>R: TGTTGCTTTCCGATCCGACT    |

## Supplemental Table 4

Primer sequences used for genotyping of RNAi segregating populations

| RNAi lines       | Primer sequence   |
|------------------|---|
| <i>pt11</i>      | Forward: ACTGTTTCTTTTGTCGATGCTCACC<br>Reverse: CACCTAC GCTCATCTGGGA |
| <i>pt13</i>      | Forward: ACTGTTTCTTTTGTCGATGCTCACC<br>Reverse: CACCCCAACAATACGATGAC |
| <i>pt11/pt13</i> | Forward: ACTGTTTCTTTTGTCGATGCTCACC<br>Reverse: CACCTAC GCTCATCTGGGA |

Supplemental Table 5

Real-time RT-PCR primers for gene expression analysis of PHT1 genes, Pi starvation induced genes, mycorrhizal marker genes, nitrate and sulfate transporters, and constitutive genes.

| Gene                | Primer sequence               |                               |
|---------------------|-------------------------------|-------------------------------|
|                     | Forward primer                | Reverse primer                |
| <i>PT2</i>          | GACGAGACCGCCCAAGAAG           | TTTTCAGTCACTCACGTCGAGAC       |
| <i>PT6</i>          | CCGCCCCTGCAAAGTGTGTA          | CAACTGGCGGTTTCTTCGAT          |
| <i>PT11</i>         | CATATCCCAGATGAGCGTATCATG      | GAGAAGTTCCTGCTTCAAGCA         |
| <i>PT13</i>         | CTTCTCCATCTCCCTTGTCG          | TTTTGTGCGCTAGCCAGCC           |
| <i>IPS1</i>         | TTGGCAATTATTCGGTGGAT          | ACCATTTCACCATCCTCTTTATG       |
| <i>miR399j</i>      | GGAGCATGTAAGTCTTTTTGTAGC      | GGCAACTCTCCTTTGGCAGA          |
| <i>AM1</i>          | TTTGCTTGCCACACGTTTTAA         | ACCTCGCCAAAATATATGTATGCTATT   |
| <i>AM3</i>          | CTGTTGTTACATCTACGAATAAGGAGAAG | CAACTCTGGCCGGCAAGT            |
| <i>AM14</i>         | CCAACACCGTTGCAAGTACAATAC      | GCACTTTGAAATTGGACTGTAAGAAA    |
| <i>GiITS1</i>       | GAGACCATGATCAGAGGTCAGGT       | GGTCATTTAGAGGAAGTAAAAGTCGTAAC |
| <i>NRT2.1</i>       | GCGACCGAGACCAGCAATAC          | CCTCCGTTTGTATCGGAGAAA         |
| <i>Sultr1.1</i>     | GCTCCATGTTTGCAGGTGTG          | CCCAAAGTGCTTGCCAACAT          |
| <i>Cyclophilin2</i> | GTGGTGTTAGTCTTTTTATGAGTTCGT   | ACCAAACCATGGGCGATCT           |
| <i>Ubiquitin</i>    | CATGGAGCTGCTGCTGTTCTAG        | CAGACAACCATAGCTCCATTGG        |

## Supplemental Table 6

RT-PCR primer sequences used for gene expression analysis of *PHO84* and *PT13* in the yeast *pam2* mutant.

| Gene            | Primer sequence      |                       |
|-----------------|----------------------|-----------------------|
|                 | Forward primer       | Reverse primer        |
| <i>ScPHO84</i>  | TGCCGTCGAATCTCTTGACA | CGTTTTTGGAAACCGGCATAA |
| <i>ScActin1</i> | CCTACGTTGGTGATGAAGCT | GTCAGTCAAATCTCTACCGG  |
| <i>PT13</i>     | TGGACATTGCAAGGACACAG | TTTTGTCGCCTAGCCAGCC   |