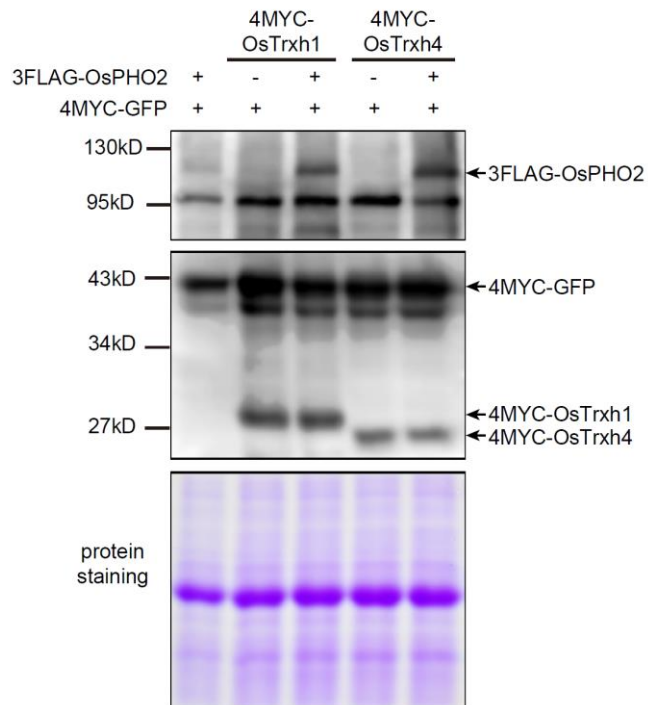
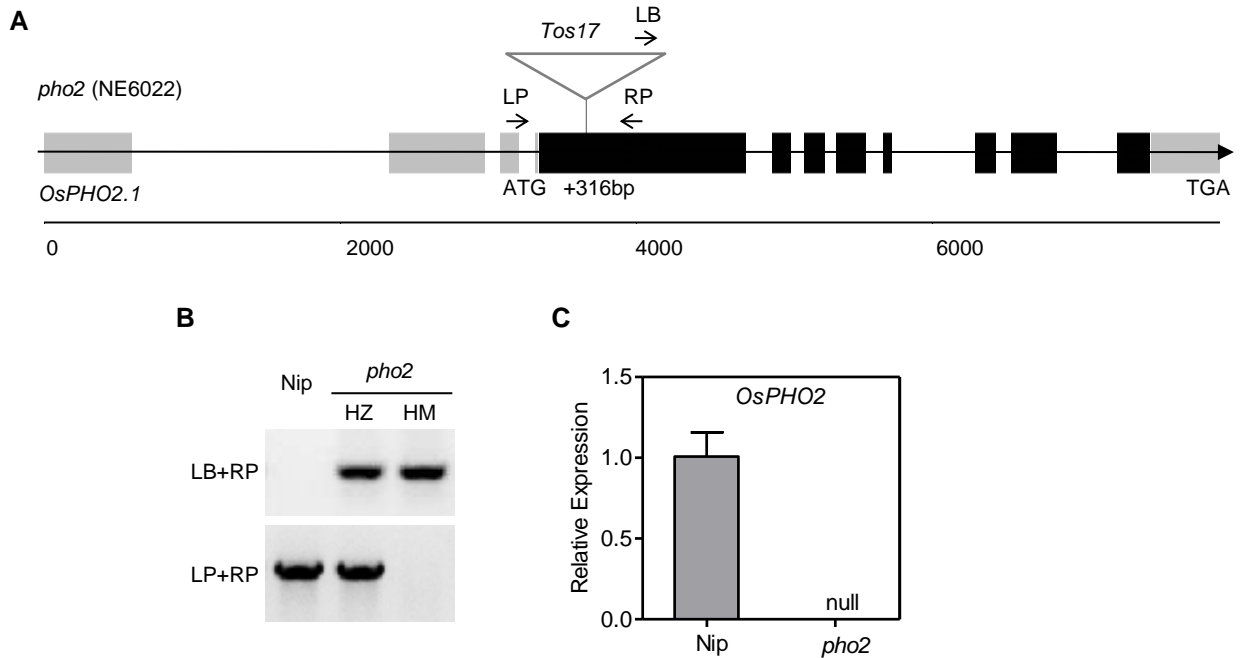


**Supplemental Figure S2.** Subcellular localization of OsPHO2, OsTrxh1 and OsTrxh4-FP fusion proteins in rice protoplasts. The plasmid pSAT6-EYFP-N1 (YFP control) was served as a positive control. Bars = 10 µm.



**Supplemental Figure S3.** OsPHO2 could not target OsTrxh1 and OsTrxh4 to degradation in tobacco leaves. Co-expression of 3FLAG-tagged OsPHO2 with 4MYC-tagged OsTrxh1 or OsTrxh4. The immunoblots were conducted using the tag specific antibodies. 4MYC-GFP was used as the control for the infiltration event. The bottom panel showed protein staining.



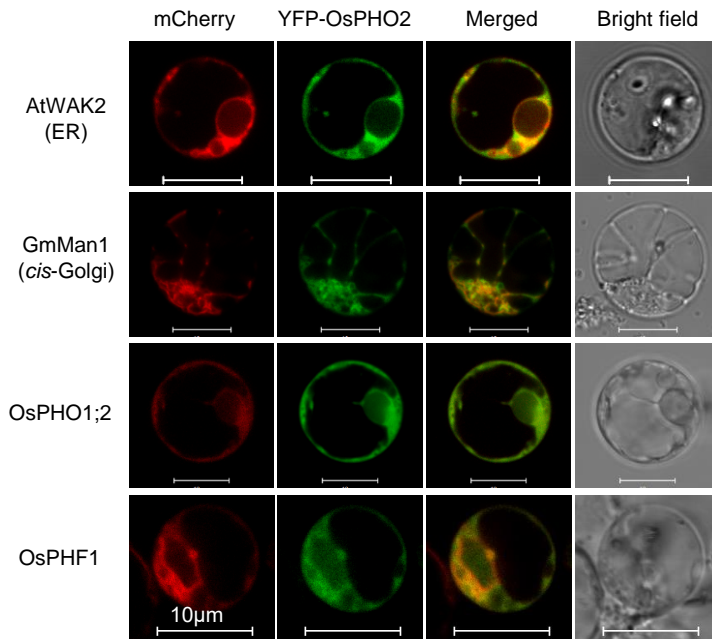
**Supplemental Figure S4.** Identification of rice *Tos17* insertion mutant *pho2* (NE6022).

**A**, *OsPHO2* gene structure and the *Tos17* insertion site. The exon region of *OsPHO2.1* is represented with boxes (black, coding region; grey, untranslated region). The *Tos17* insertion site is at +316bp after the ATG code. LB, LP, and RP are primers used in B to identify *pho2* mutant.

**B**, PCR analysis of the *Tos17* insertion at the first coding exon of *OsPHO2*. HM, homozygous *pho2* mutant; HZ, heterozygous; Nip, wild type *Nipponbare*.

**C**, qRT-PCR analysis of the *OsPHO2* expression in the wild type (Nip) and *pho2* mutant seedlings.

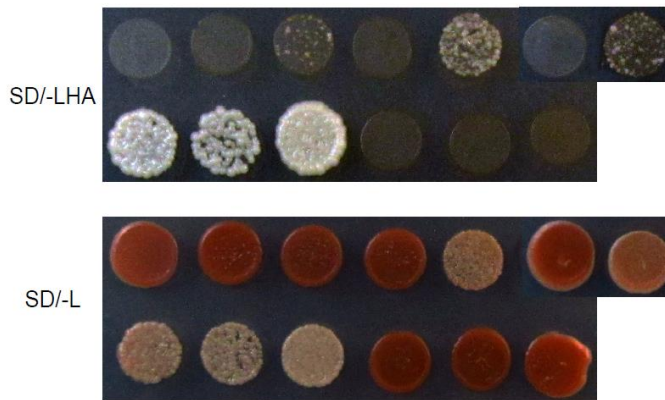
The primers used for PCR and qRT-PCR analysis are listed in Table S2 and S3.



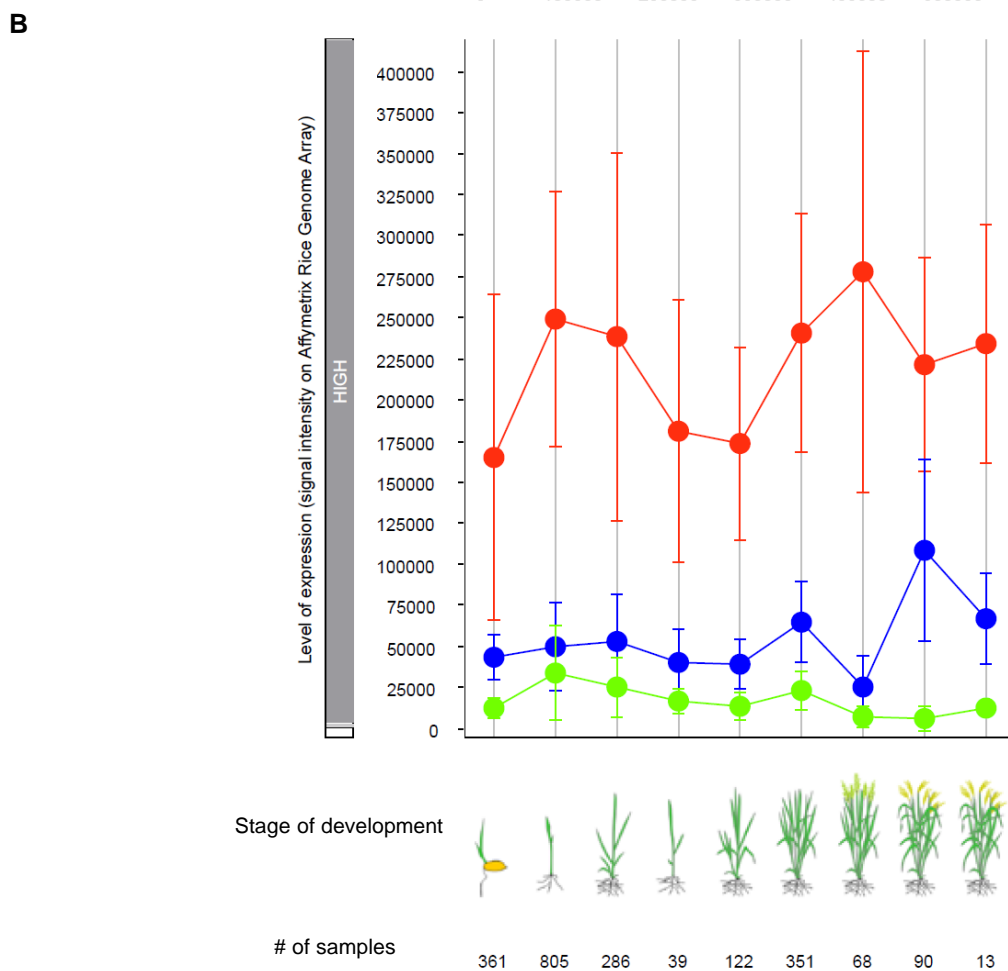
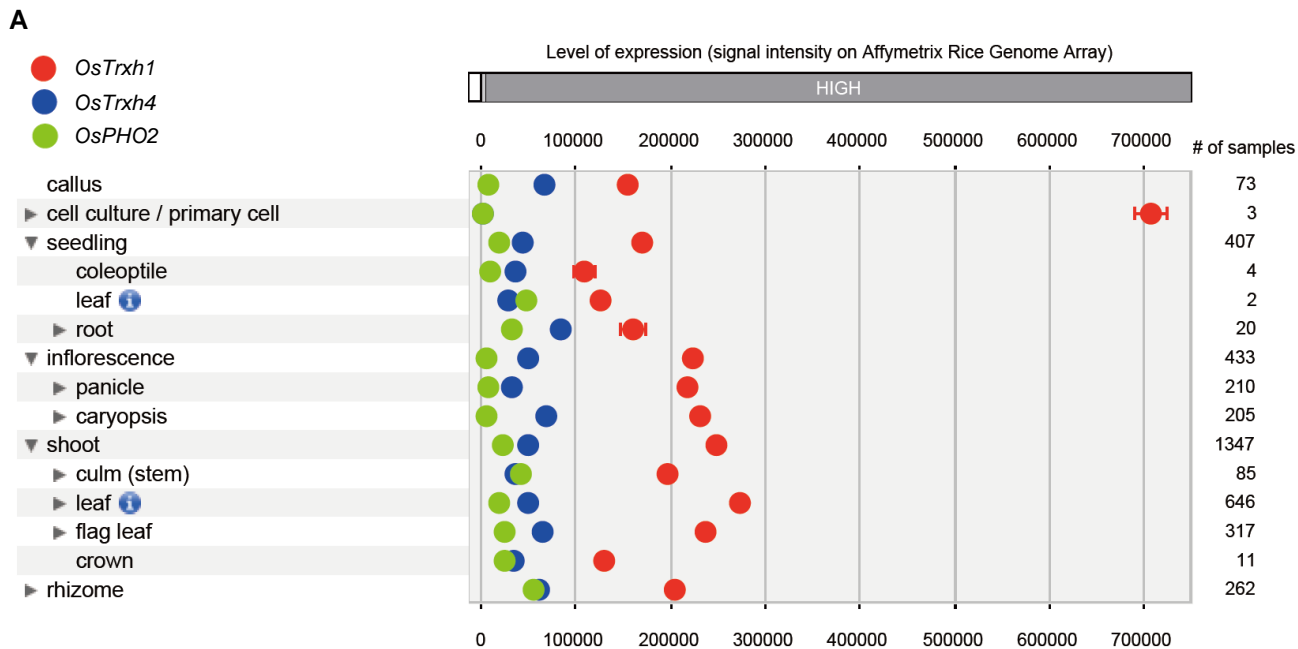
**Supplemental Figure S5.** Co-subcellular localization of YFP-OsPHO2 with ER/Golgi markers and OsPHF1/OsPHO1;2-mCherry fusion proteins in rice protoplasts.

The ER/Golgi markers were AtWAK2/GmMan1-mCherry, respectively. Bars = 10 µm.

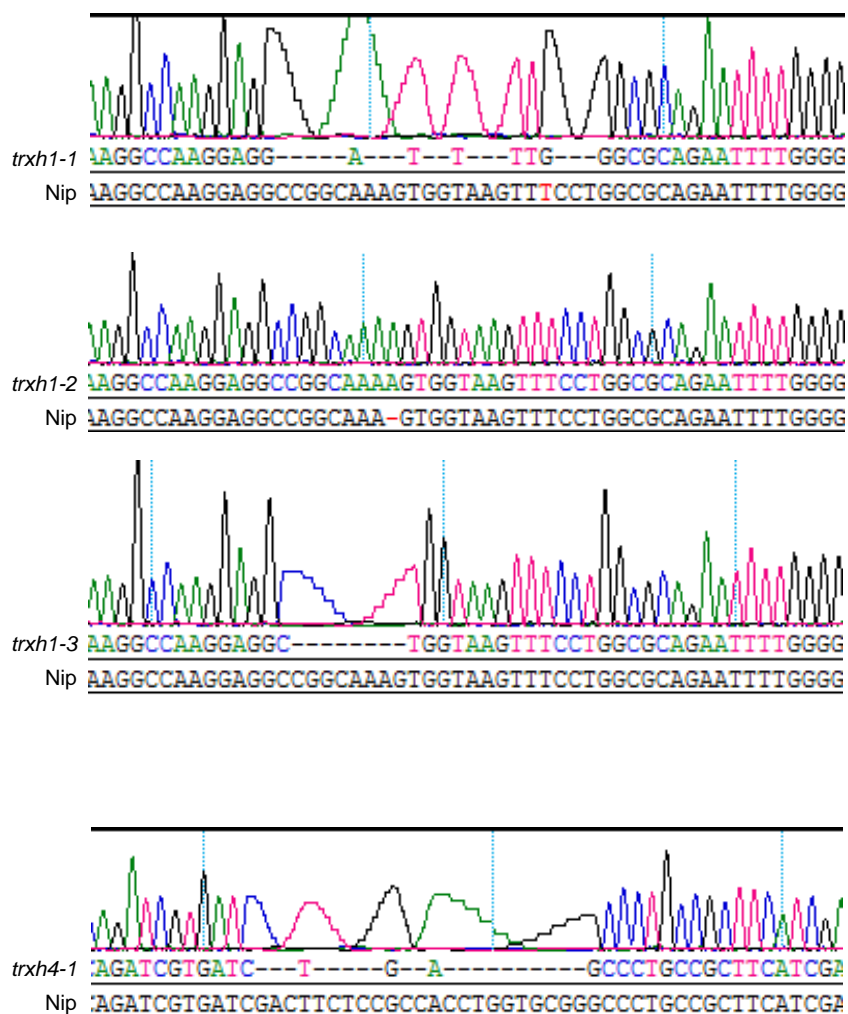
APP	pBT3-STE OsPHO1;2 -Cub	pBT3-STE OsPHF1- Cub	pBT3-N Cub- OsPHO1;2	pBT3-N Cub- OsPHF1	pBT3-STE OsPHO2- Cub	pBT3-N Cub- OsPHO2
pBT3-STE OsPT2-Cub	pBT3-STE OsPT6-Cub	pBT3-STE OsPT8-Cub	pBT3-N Cub-OsPT2	pBT3-N Cub-OsPT6	pBT3-N Cub-OsPT8	



**Supplemental Figure S6.** Validation of auto activation activities of bait vectors in split-ubiquitin yeast two-hybrid assays. The coding sequences of OsPHO2/OsPHF1/OsPHO1;2 and OsPT2/6/8 were cloned in frame into both pBT3-STE and pBT3-N bait vectors with Cub. The yeast strain NMY51 cells were transformed with these bait constructs and grown on SD/-L (lacking Leu) plates. The auto activation activities of reporter genes were assessed by the growth of yeast colonies on SD/-LHA (lacking Leu, His, and Ade) plates for 3 days.

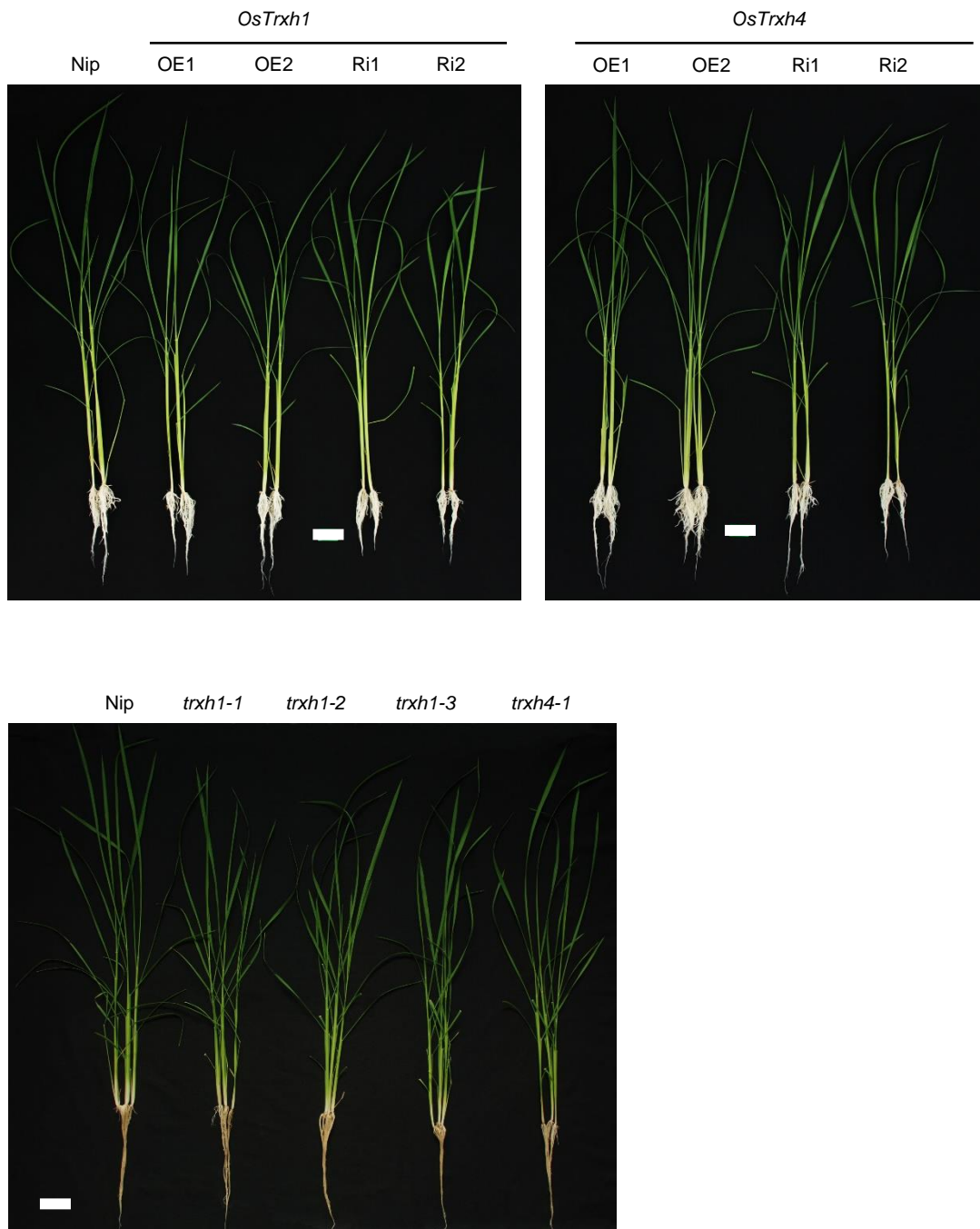


**Supplemental Figure S7.** Expression levels of *OsTrxh1*, *OsTrxh4* and *OsPHO2* in **(A)** different tissues and **(B)** different development stages based on microarray data retrieved from GENEVESTIGATOR (<https://geneinvestigator.com/gv/>).

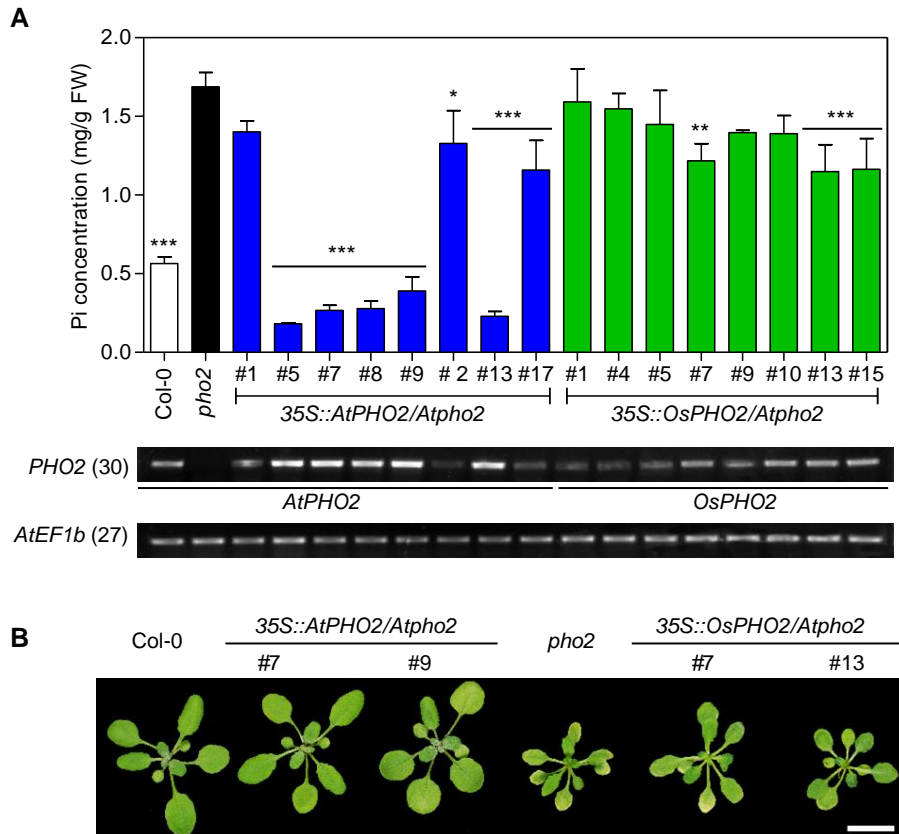


**Supplemental Figure S8.** Sequencing results of the CRISPR/Cas9 mutants of *OsTrxh1* and *OsTrxh4*. PCR products were amplified using genomic DNA as template. The primers used to amplify the fragments for sequencing are listed in Table S2.





**Supplemental Figure S9.** Morphological appearance of Nipponbare (Nip), overexpression (OE), RNA interference (Ri) and CRISPR/Cas9 mutants of *OsTrxh1* and *OsTrxh4*. Rice seedlings were grown in a Pi-sufficient solution (0.32 mM Pi) for 28 days. Bars = 4 cm.

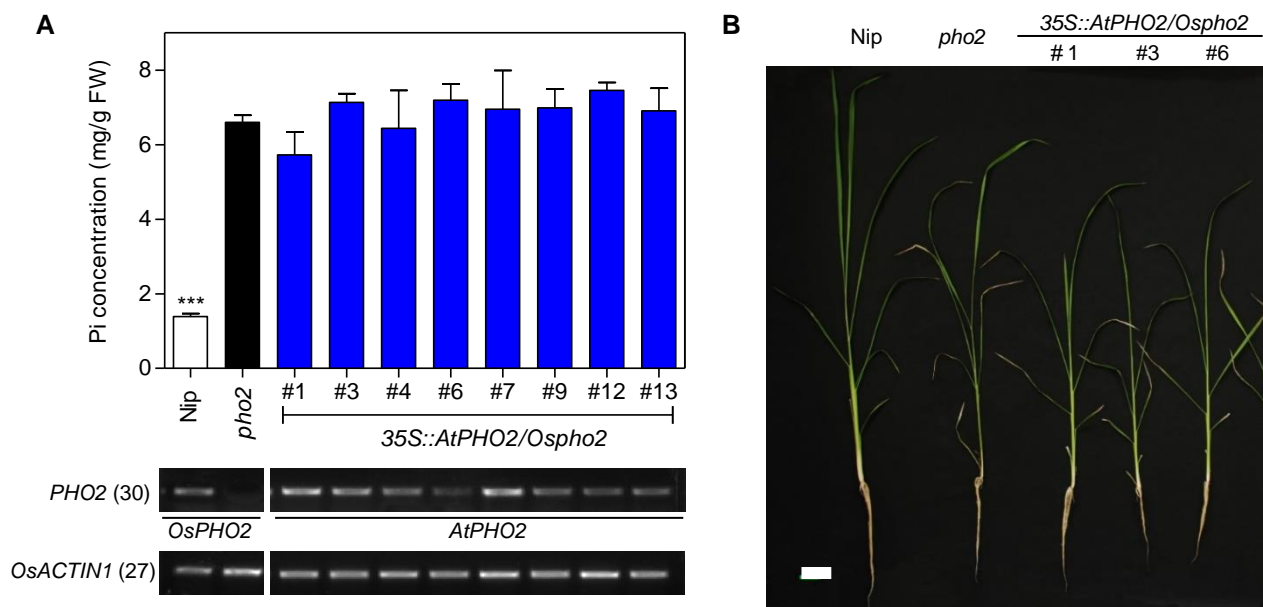


**Supplemental Figure S10.** Over-expression of *AtPHO2* could restore Arabidopsis *pho2* mutant, whereas over-expression of *OsPHO2* could not.

Transgenic Arabidopsis seedlings were germinated on MS medium with 18 mg/L Hygromycin B and grown for 9 days, then transferred into MS medium for further 9-day-growth before sampled.

**A,** The Pi concentration in shoots of *AtPHO2* or *OsPHO2* over-expressing transgenic lines in Arabidopsis *pho2* background. Errors bars indicate SD and n = 3. The transcript level of *PHO2* in the wild type (Col-0), *pho2* mutant and transgenic lines over-expressing *AtPHO2* or *OsPHO2* were analyzed by RT-PCR. Expression of *AtEF1b* served as the internal control. Data significantly different from the *pho2* were indicated by asterisks (Dunnett's ANOVA test; \*P<0.05, \*\*P<0.01, \*\*\*P<0.001).

**B,** Growth performance of 18-day old seedlings. Bar = 4 cm.



**Supplemental Figure S11. Rice *pho2* mutants could not be recovered by expressing 35S::AtPHO2.**

Rice seedlings were germinated and grown in a Pi-sufficient hydroponic solution (0.32 mM Pi) for 24 days before sampled.

**A**, The Pi concentration in shoots of *AtPHO2* over-expressing transgenic lines in rice *pho2* background. Errors bars indicate SD and n = 3. The transcript level of *PHO2* in the wild type Nip (*Nipponbare*), *pho2* and *AtPHO2*-over-expressing transgenic lines were analyzed by RT-PCR. Expression of *OsACTIN1* served as the internal control. Data significantly different from the *pho2* were indicated by asterisks (Dunnett's ANOVA test; \*\*\*P<0.001).

**B**, Growth performance of 24-day-old seedlings. Bar = 4 cm.

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OsPHO2 416 N-----IENSEANLTNGTVAVSRESLDTSSAFLSCIGNVLGYNDEGLEVQWASG 464
HvPHO2 393 N-----IDKSEADLTNGTMTVSRKSLDTSSGFLSCIGNVLGYKDDGIEVQWASG 441
ZmPHO2 415 N-----IDKSEADPTNGSLAS-KESLVTSSGFLSCTGNVLGYKDNGIEVQWANG 462
AtPHO2 413 EGKFDPN-----ADTIVATEAKHLLT---ESDYSGAYFLSSIGVVTGFKNGSVKVKWANG 464
NbPHO2 417 DQVFSLDGKSMYEMDINSQLKNIDKRRDNSDFTGYDHLPCIGIIVGFEDGNIEVKWATG 476
      :           :           :           : * : . * . . * : * : : . : * : * : *

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OsPHO2 Osativa|LOC_Os05g48390|LOC_Os05g48390.1 426 -----NGTVAVSRESLDTSSAFLSCTIGNVLGYNDEGLEVQ
Zmays|GRMZM2G381709|GRMZM2G381709_T01 425 -----NGSLA-SKESLVTSSGFLSCTIGNVLGYKDNGIEVQ
Zmays|GRMZM2G464572|GRMZM2G464572_T01 425 -----NGSLA-SKESLVTSSGFLSCTIGNVLGYKDNGIEVQ
AtPHO2 Athaliana|AT2G33770|AT2G33770.1 423 -----IVATEAKHLLTESDYSGAYFLSSIGVVTGFKNGSVKVK
Crubella|Carubv10022607m.g|Carubv10022607m 423 -----IVTTEAKHLLTESDYTGAYCLSSIGVVTGFKNGAVEVK
Mtruncatula|Medtr4g020620|Medtr4g020620.1 405 NVE-----APLKNLYQISYPNNSVGNCRLSCIGNVTGFKDGHVEVK
Gmax|Glyma.15G074200|Glyma.15G074200.1 430 LNVE-----VPLINWDQISYPNKSVNSYLSCIGNVTGFEDGDVEVK
Gmax|Glyma.13G239100|Glyma.13G239100.1 431 LNVE-----VPLINWDQISHPNKYADNSYLSCIGNVTGFEDGDVEVK
Mtruncatula|Medtr4g088835|Medtr4g088835.1 434 MNAE-----AALKNGNQMNYQDDFPDDCYLRCVGTVIGFKDGDVEVK
Gmax|Glyma.07G196500|Glyma.07G196500.1 433 FNAE-----AVTKNGNQMSYQDEFPDNHFMSCIGSVTGFODGDVEVT
Gmax|Glyma.13G179600|Glyma.13G179600.1 433 FNAE-----AVPKNGNQMSYQDEFPDNYFMSCIGSVTGFKDGDVEVT
Csativus|Cucsa.046210|Cucsa.046210.1 421 MNES-----AQNECNRIIN--NMMIMDNFLSFIGNVTGFKDGAVEVK
Stuberosum|PGSC0003DMG400029724|PGSC0003 437 MDSN-----TDLKNVDTGKDNLDFPKYDHLSCIGIIVGFKDGDLEVK
Egrandis|Eucgr.D00620|Eucgr.D00620.1 419 KAEE-----ADLEIEKFRDHRGDPNNFYLSCIGNVEGFKDGAVEVK
Vvinifera|GSVIVG01005206001|GSVIVT010052060 441 MGKE-----IPLKETCSKDQNEYSDKYYSSHIGNVVGFKDGGVKVK
Rcommunis|29851.t000060|29851.m002418 427 MGET-----VAIEGKECGKDQSDYPCDGYLSCIGYVSGFKDGAVEVT
Ptrichocarpa|Potri.011G052600|Potri.011G052600. 431 MNAD-----APLEGSDHGKDQVDY-----LCCIGVTGFEDGSVEVT

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Supplemental Figure S12. Amino acids alignment of PHO2 and PHO2-like proteins in multiple plant species.