	OsPHO2		_	
HvPHO2	79.06%/85.35%	HvPHO2		_
ZmPHO2	77.00%/85.81%	77.80%/83.75%	ZmPHO2	
AtPHO2	45.10%/62.16%	44.15%/61.53%	44.72%/62.57%	AtPHO2
NbPHO2	45.42%/62.20%	44.77%/61.66%	45.68%/62.08%	49.22%/66.08%

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813,814       822       845       874         LVKDBHTRAQHVLAACAAYMEGVPVGSSANLQGNSTN-STGFKIMLSKLYPKLEAFSEIGPDCVQEIGPES 907       AtPHO2         CNTRAHTCCAPHILDACAAYLGGDLVGHARDSAYISDDCCNNSSTGFKIMLAKLLPKLVTTFSEAGTPCSP       876       OsPHO2         :**::******:****:*****:*****:*********	*::**:*****	*****:***	****:*****	*:*:*:***:*::*****:	**************	:*:*:****::***	***::**** :*:*****	**
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OsTryh4 LEPKUNIMETSSSSS 134	OsTryh4	LERKUNMETS	SSSS <b>134</b>	L				

Supplemental Figure S1. Alignments of amino acid sequences.

**A**, The identity/similarity matrix for plant PHO2 proteins from *Oryza sativa* (Os), *Hordeum vulgare* (Hv), *Zea mays* (Zm), *Arabidopsis thaliana* (At), and *Nicotiana benthamiana* (Nb).

**B**, Amino acids sequences alignment of PHO2 proteins in Arabidopsis and rice. UBC domains and cysteine residues are highlighted.

**C**, Amino acid sequences alignment between OsTrxh1 and OsTrxh4. Cysteine residues in the conserved active domain "WCGPC" are indicated.

Α



**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 =  $10 \mu m$ .



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;2mCherry fusion proteins in rice protoplasts. The ER/Golgi markers were AtWAK2/GmMan1-mCherry, respectively. Bars =  $10 \mu m$ .

APP	pBT3-STE	pBT3-STE	pBT3-N	pBT3-N	pBT3-STE	pBT3-N
	OsPHO1;2	OsPHF1-	Cub-	Cub-	OsPHO2-	Cub-
	-Cub	Cub	OsPHO1;2	OsPHF1	Cub	OsPHO2
pBT3-STE	pBT3-STE	pBT3-STE	pBT3-N	pBT3-N	pBT3-N	
OsPT2-Cub	OsPT6-Cub	OsPT8-Cub	Cub-OsPT2	Cub-OsPT6	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://genevestigator.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.

OsPHO2 HvPHO2 ZmPHO2 AtPHO2 NbPHO2	416 NIENS 393 NIDKS 415 NIDKS 413 EGKFDPNADT 417 DQVFSLDGKSMYSEMI : :	EANLTNGTVAVS EADLTNGTMTVS EADPTNGSLAS- VATEAKHLLT INSQLKNIDKRR :	SRESLDTSSAFLS SRKSLDTSSGFLS KESLVTSSGFLS ESDYSGAYFLS NSDFTGYDHLP :* :*.	IGNVLGYNDEGLEV IGNVLGYKDDGIEV TGNVLGYKDNGIEV IGVVTGFKNGSVKV IGIIVGFEDGNIEV * : *::: .::*	QWASG 464 QWASG 441 QWANG 462 KWANG 464 KWATG 476 :**.*	
OsPHO2 Osativa LOC Zmays GRW Zmays GRW Athaliana A Crubella Ca Mtruncatul: Gmax Glym Gmax Glym Gmax Glym Csativus Cu Stuberosun Egrandis Eu Vvinifera GS Rcommunis Ptrichocarp	C_Os05g48390 LOC_Os05g48390.1 IZM2G381709 GRMZM2G381709_T01 IZM2G464572 GRMZM2G36464572_T01 IZG33770 AT2G33770.1 rubv10022607m.g Carubv10022607n a Medtr4g020620 Medtr4g020620.1 a.13G239100 Glyma.13G239100.1 a Medtr4g088835 Medtr4g088835.1 a.07G196500 Glyma.07G196500.1 a.13G1796000 Glyma.13G179600.1 csa.046210 Cucsa.046210.1 n PGSC0003DMG400029724 PGSC000 cgr.D00620 Eucgr.D00620.1 SVIVG01005206001 GSVIVT010052060  29851.t000060 29851.m002418 a Potri.011G052600 Potri.011G05260	426          425          423          423          405       NVE-         401       LNVE-         433       FNAE-         433       FNAE-         433       FNAE-         434       MNAE-         435       FNAE-         436       FNAE-         437       MNES-         3       FNAE-         419       KAEE-         427       MGET-         421       MNAC-		-NGTVAVSRESLD -NGSLA-SKESLV ATEAKHLLTESDY TTEAKHLLTESDY KNLYQISYPNNSV LINWDQISYPNNSV LKNGNQMNYQDDF TKNGNQMSYQDEF PKNGNQMSYQDEF NEGNRIINNNM LKNVDTGKUNLG LKNVDTGKUNLG LKVCTCSKDQNEY IEGKECGKDQSDY LEGSDHGKDQVDY	TSSAFLSCIGN TSSGFLSCTGN TSSGFLSCTGN SGAYFLSSIGV TGAYCLSSIGV GNCRLSCIGN VDNSYLSCIGN PDDCYLRCWGT PDNHFMSCIGS PDNYFMSCIGS PDNYFMSCIGS PDNYFMSCIGN SDKYYSSHIGN PCGYLSCIGY	VLGYNDEGLEVQ VLGYKDNGIEVQ VLGYKDNGIEVQ VTGFKNGSVKVK VTGFEDGDVEVK VTGFEDGDVEVK VTGFEDGDVEVK VTGFEDGDVEV VTGFEDGDVEVT VTGFKDGDVEVT VGFKDGDVEVT VGFKDGDVEVK VGFKDGAVEVK VSGFKDGAVEVT

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