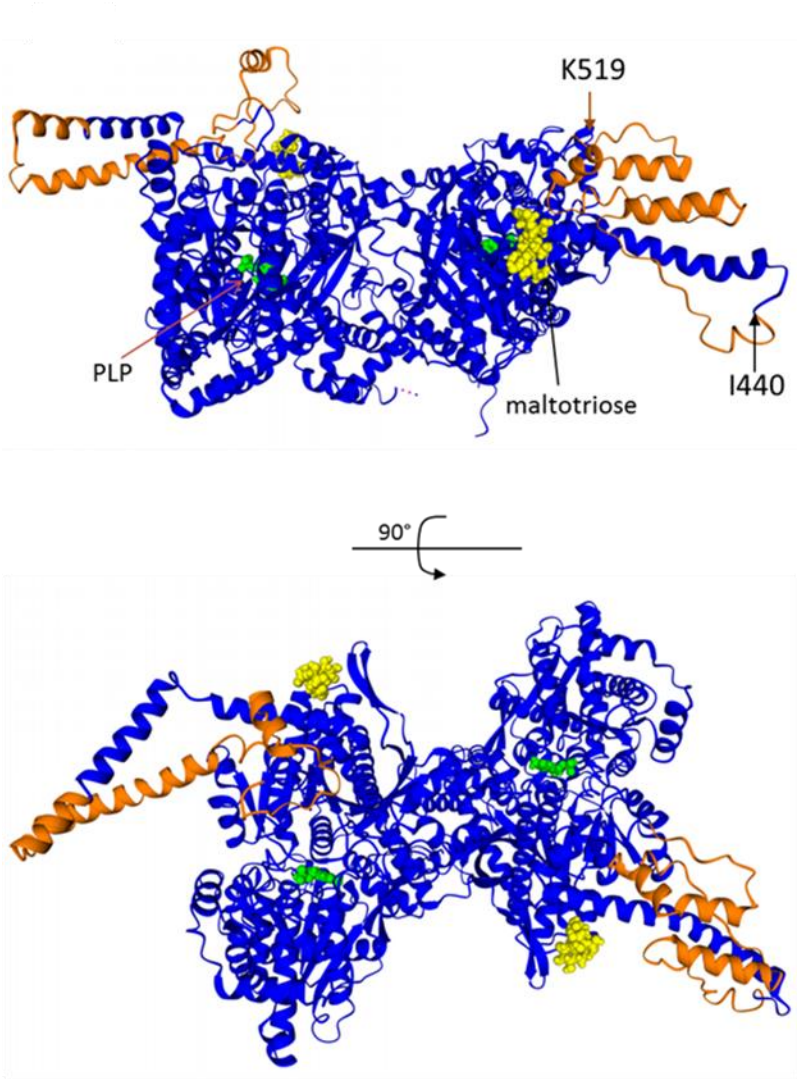
Supplemental Table and Figures

Table S1. Oligonucleotide primers used in this study.

Primer name	Sequence	Restriction sites
Halo-Bm	5'ATCGGATCCGAAATCGGTACTGGCTTTCCATTCGAC3'	*Bam*HI
Halo-NcSc	5'TTAGAGCTCGGCCATGGCGTTATCGCTCTGAAAGTACAGATC3'	*Sac*I, *Nco*I
Pho1-ATG	5'TTCCATGGCTAGCGTGGCGAGCGATCGGGGCGTGCA3'	*Nco*I
274seq-r	5'ATCCAGATGGAGTTCTGAGGTCAT3'	
DPE1-F	5'ATCCCACGGCACCGTCCACGTGTAGCCGTGCAA3'	
DPE1-Sal-R	5'GAAGTCGACTTAGAGCCGGTTGTACAGTCCAAGCAATTC3'	*Sal*I
DPE1-Bam-F	5'CGCGGATCCATGGCGGTGGCGCCCGACAAGGAGGAG3'	*Bam*HI
DPE1_650-F	5'GAGAGCTCAGAACGCAATATGACTGCTT3'	
DPE1_1210-F	5'TCTGAGTCAAAAGTAGCGCTGGTTGGAAGCT3'	
DPE1-Sal	5'GGAGTCGACGCGGTGGCGCCCGACAAGGAGGAG3'	*Sal*I
DPE1-Kpn	5'CAGGGTACCTTAGAGCCGGTTGTACAGTCCAAG3'	*Kpn*I

Figure S1. Modeled structure of rice Pho1. The structure was modeled on the X-ray crystal structure of *Arabidopsis thaliana* Pho2 using YASARA Structure (version 14.12.2)(Krieger et al. 2002). L80 region is denoted by orange color and itsr starting and ending amino acid residues are indicated. Maltotriose is shown in yellow and pyridoxal 5-phosphate (PLP) in green.



Reference

Krieger, E., Koraimann, G., and Vriend, G. (2002) Increasing the precision of comparative models with YASARA NOVA--a self-parameterizing force field. Proteins **47**, 393-402

Figure S2. DNA and protein sequences of HaloTag fusion proteins. Sequences in green, red, blue, and black denote polyhistidine (His_6) tag, HaloTag, fused proteins (GFP, OsPho1, OsPho1ΔL80, and OsDpe1), and part of the expression plasmid pSH582, respectively. Some important restriction enzyme sites are also shown.

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pSH583: DNA sequence of His_6-HaloTag-GFP

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                                                                    BamHI
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CGCGATGGCACCCCTGTGCTGTTCCTGCACGGTAACCCGACCTCCTCCTACGTGTGGCGCAACATCATCCCGCATGT
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NcoI
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                                                    KpnI  SacI  KpnI SmaI
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SalI PstI   HindIII
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pSH583: protein sequence of His_6-HaloTag-GFP (565 aa; 63.8 kDa)

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pSH585: DNA sequence of His_6-HaloTag-OsPho1

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KpnI SacI SalI
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TCGTCTTCAC

pSH585: Protein sequence of His_6-HaloTag-OsPho1 (1250 aa; 141.1 kDa)

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pSH642: DNA sequence of His_6-HaloTag-OsPho1ΔL80

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pSH642: protein sequence of His_6-HaloTag-OsPho1ΔL80 (1170 aa; 132.1 kDa)

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pSH757: DNA sequence of His_6-HaloTag-OsDpe1

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pSH757: protein sequence of His_6-HaloTag-OsDpe1 (876 aa; 98.5 kDa)

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Figure S3. Specificities of anti-OsPho1 and anti-OsDpe1 antibodies. Soluble protein extracts were prepared from immature seeds of rice TC65 (wildtype) and BMF136 (pho1 null mutant) as described in Experimental Procedures. Seed extracts (20 μg each) and antigen proteins (2 and 0.5 μg for CBB staining; 0.4 and 0.1 μg for immunoblot analysis) were resolved on a 12% SDS-polyacrylamide gel. Antibody titers used are as indicated below the immunoblot results.

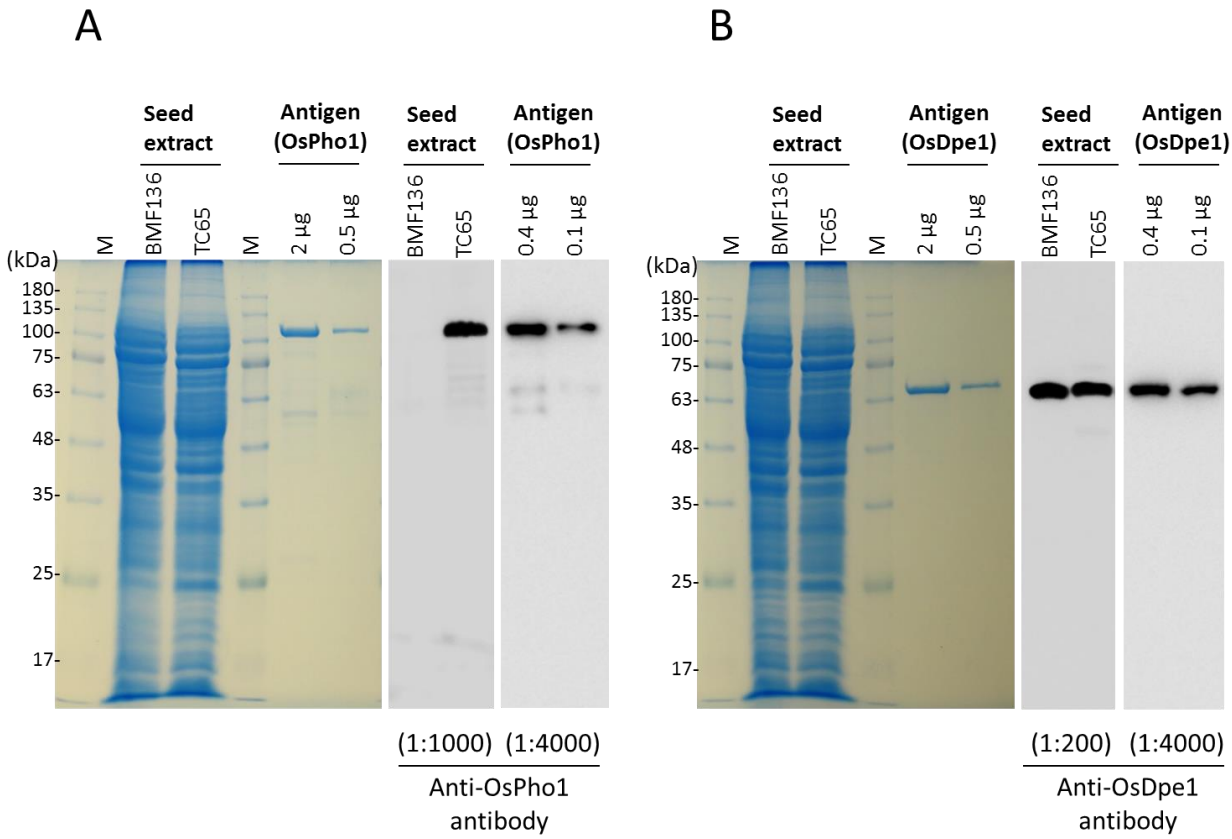

Figure S4. Molecular weight determination of OsDpe1 (*A*) and OsPho1:OsDpe1 complex (*B*) by Superdex-200 column chromatography. Size markers used were: a, bovine albumin (66 kDa); b, alcohol dehydrogenase (150 kDa); c, β-amylase (200 kDa); d, OsPho1 (210 kDa); e, apoferritin (443 kDa); f, thyroglobulin (669 kDa); g, OsDpe1. v, elution volume; v_0, void volume of the column.

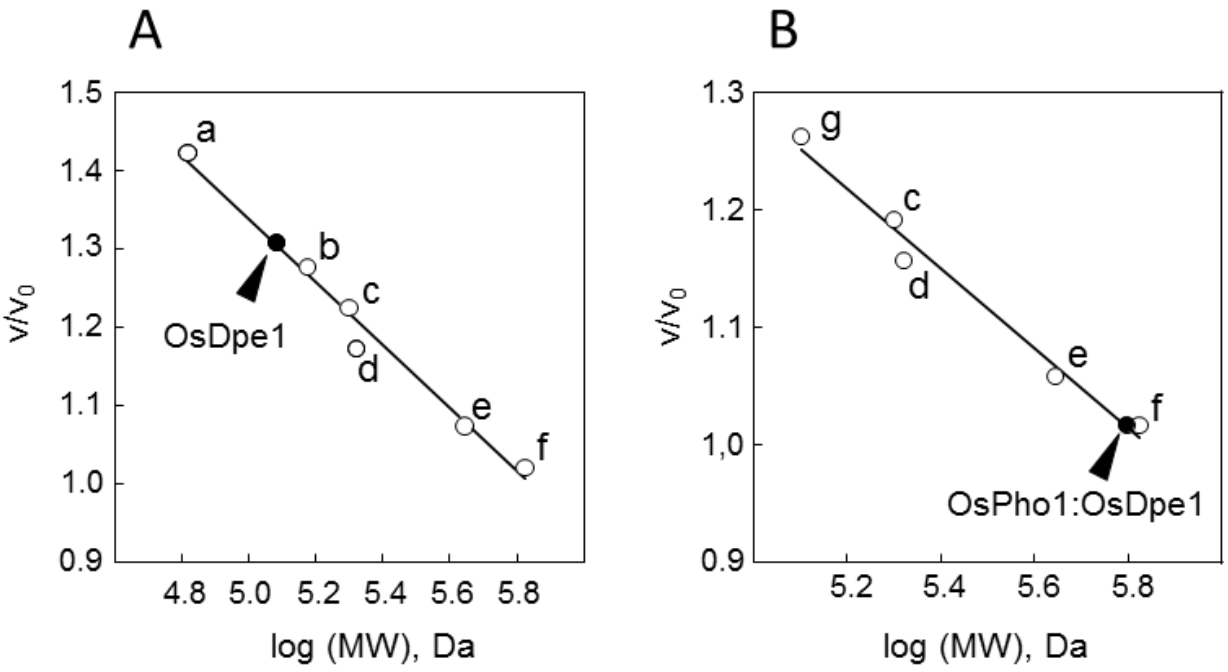

Figure S5. Elution profiles of mixtures of OsPho1 and OsDpe1 recombinant proteins at different molar ratios on Superdex-200 column chromatography. (From top to bottom) 1:2 ratio, a mixture of 340 μg OsPho1 and 400 μg of OsDpe1; 1:1 ratio, a mixture of 340 μg OsPho1 and 200 μg of OsDpe1; 2:1 ratio, a mixture of 680 μg OsPho1 and 200 μg of OsDpe1. The fractions were analyzed by SDS-PAGE. Shown are CBB-stained SDS-polyacrylamide gels.

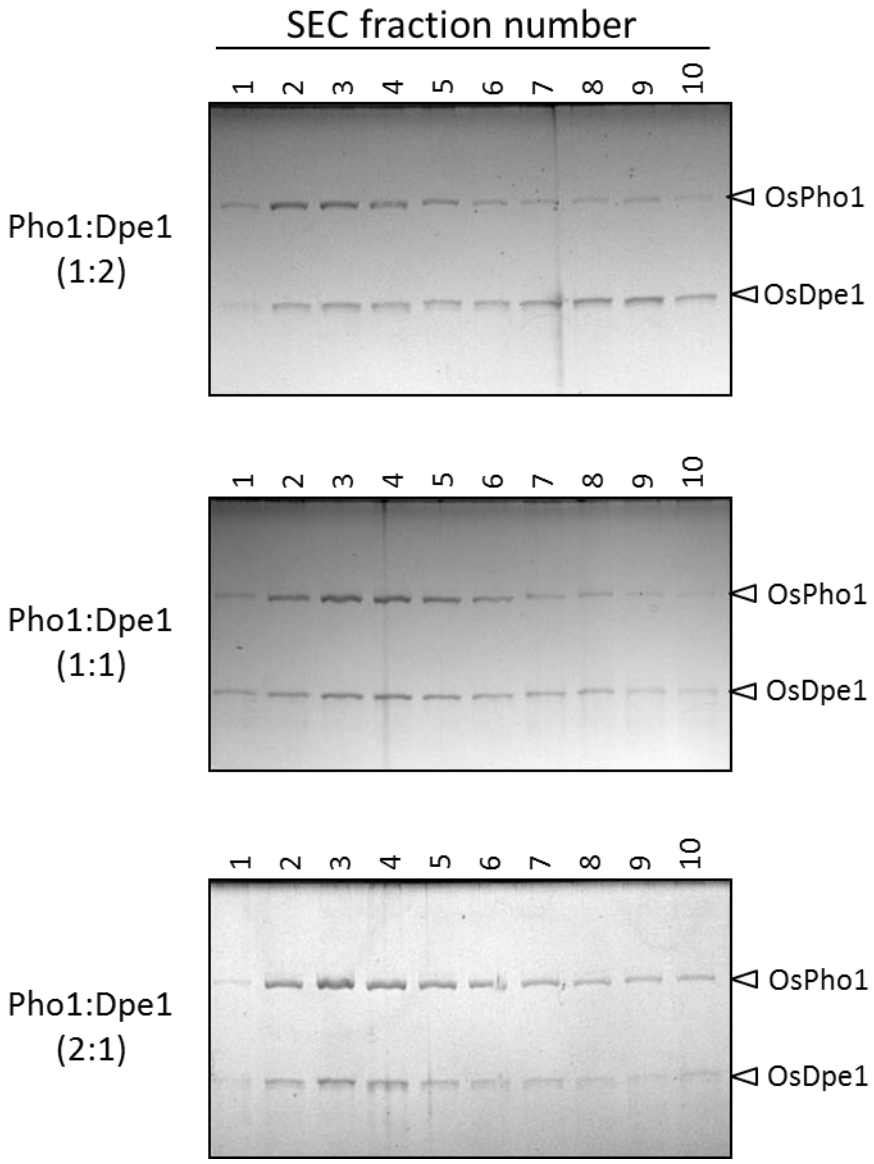

Figure S6. Reaction profiles of OsDpe1 (0.5 µg) in the presence of equal molar amounts of G5 alone (upper panel) and G5 and G1 (lower panel). FACE was performed with aliquots of the reaction products. The first and last lanes contain size markers.

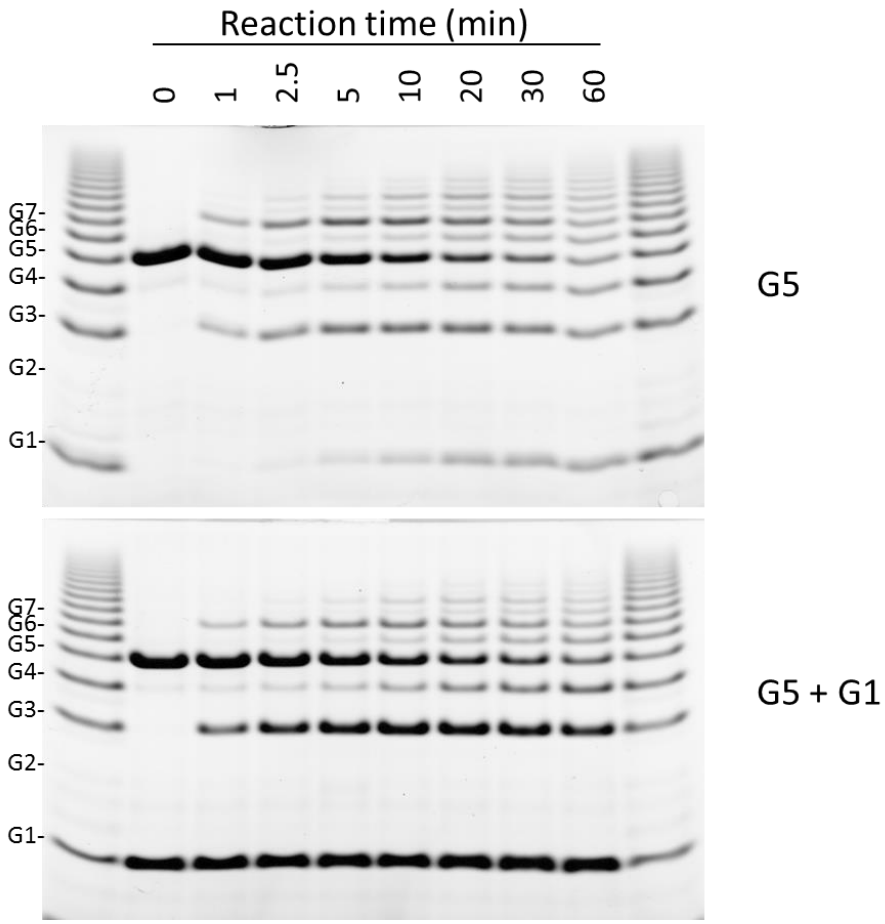

Figure S7. Phosphoprotein assay of the recombinant proteins. 2 μg each of the purified proteins (OsPho1, OsPho1ΔL80, OsDpe1, a mixture of OsPho1 and OsDpe1, HT-GFP, HT-OsPho1, and HT-OsDpe1) used in this study were resolved on 12% SDS-polyacrylamide gel and phosphoprotein was detected using Pro-Q Diamond phosphoprotein stain (Invitrogen). Bovine serum albumin (BSA, fraction V) and ovalbumin (two serine residues/molecule for phosphorylation; see below for reference) from Sigma-Aldrich were used as negative and positive controls, respectively. (*A*) CBB-stained SDS-polyacrylamide gel; (*B*) Pro-Q Diamond stained gel under ultraviolet light; (*C*) Normalized fluorescence of the signals (arbitrary unit) = fluorescence strength of band/(CBB staining intensity of band x Molecular weight of monomeric protein).

Reference

Kinoshita-Kikuta E1, Kinoshita E, Koike T (2012) Separation and identification of four distinct serine-phosphorylation states of ovalbumin by Phos-tag affinity electrophoresis. Electrophoresis 33:849-855. doi: 10.1002/elps.201100518