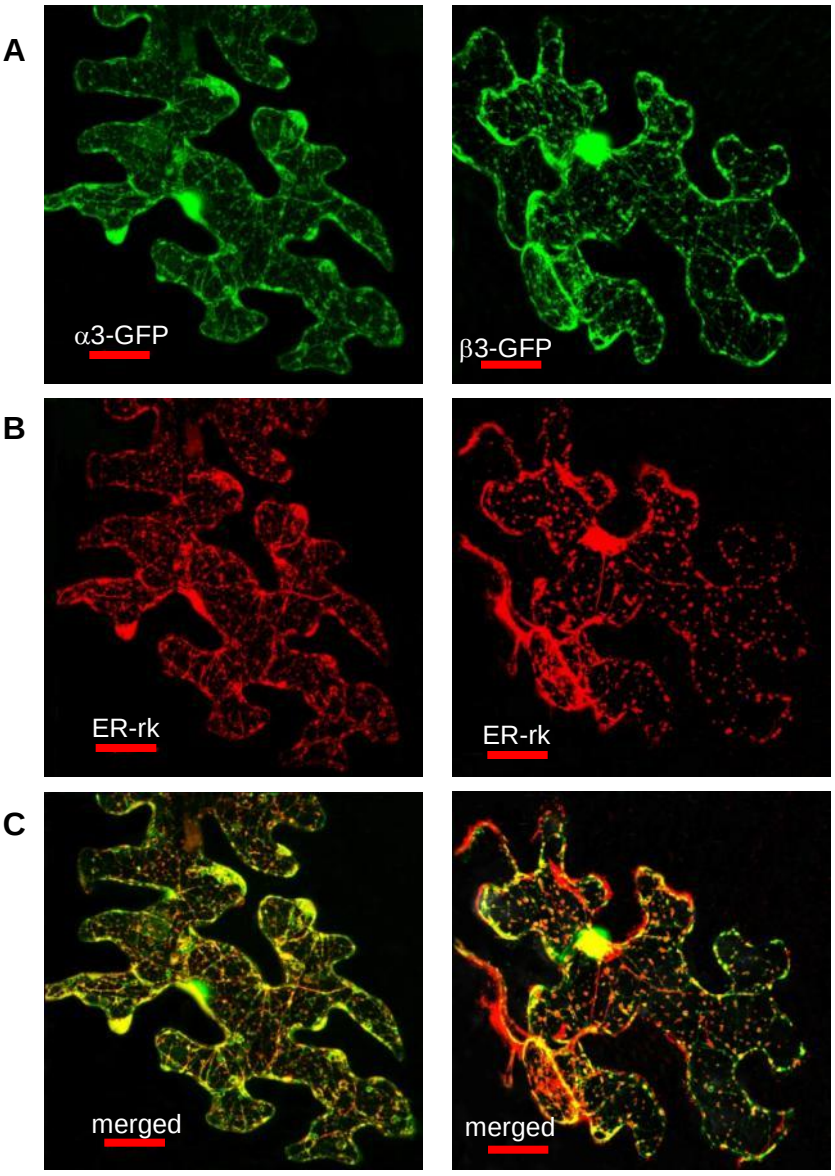
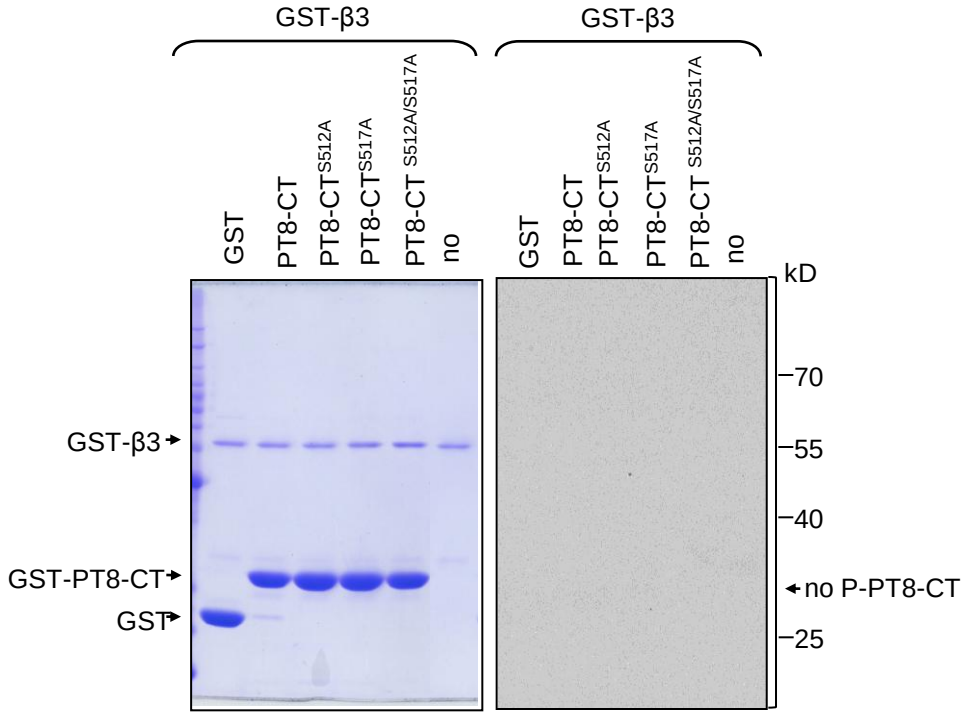
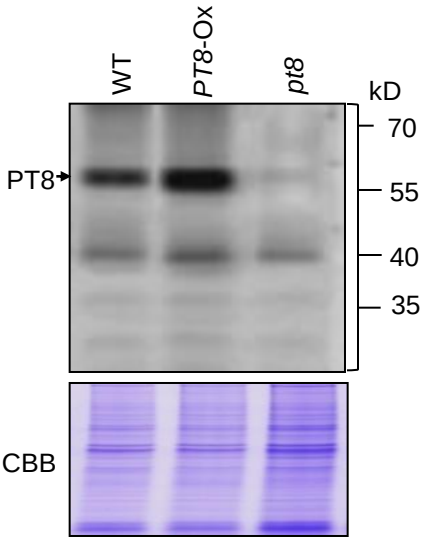


Supplemental Figure 1. Subcellular localization of CK2α3/β3.

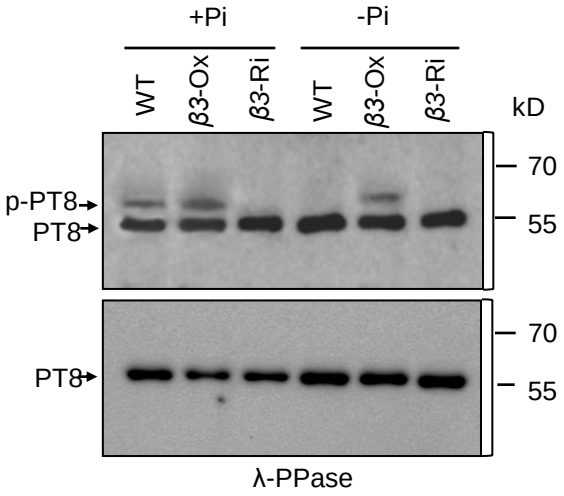
Colocalization of CK2α3/β3-GFP (*35S-α3/β3-GFP*) and ER-rk in tobacco leaves. The displayed image is a maximum projected z-stack of 24 confocal sections (total z depth = 28 µm). Bars = 20 µm.



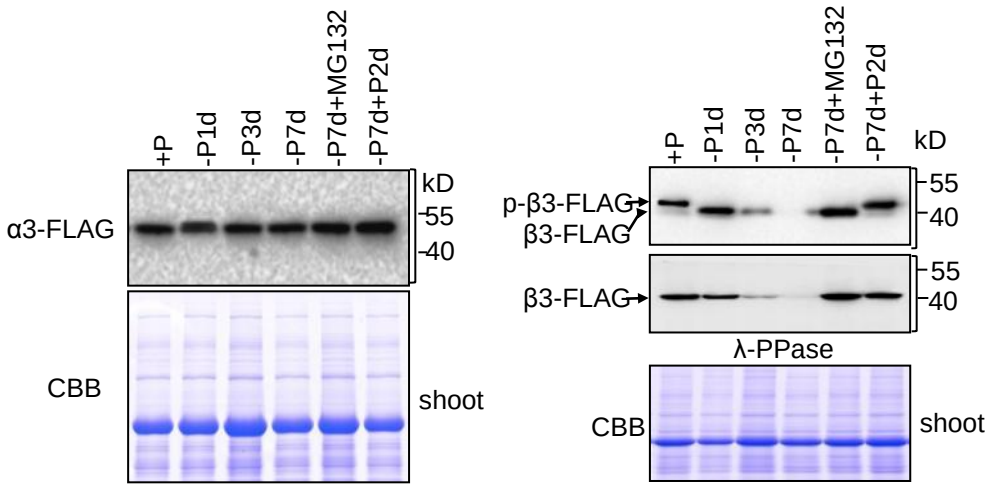
Supplemental Figure 2. CK2β3 is unable to phosphorylate the C-terminal Ser-517 residue of PT8. Equal amounts of GST-tagged PT8-CT and its Ser-to-Ala mutant proteins were incubated with CK2β3 in the presence of γ-^{32}P-ATP. The reactions were stopped by the addition of SDS-loading buffer. After electrophoresis, proteins were visualized by Coomassie blue staining (left panel) and the phosphorylated proteins were visualized by autoradiography (right panel). Control reactions with GST as a substrate (first lane) and no substrate (last lane) were carried out side-by-side.



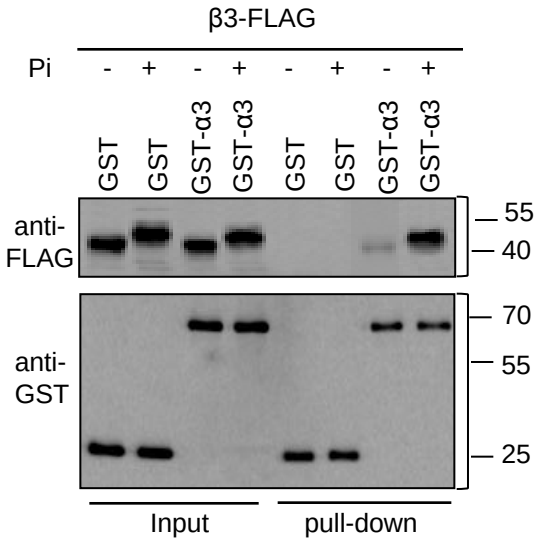
Supplemental Figure 3. Specificity of the rice PT8 polyclonal antibody. Immunoblotting of plasma membrane protein from roots of the wild type (WT; NIP), plants displaying *PT8*-overexpression (*PT8*-Ox) and *pt8* mutant showed a specific band of expected size of PT8 (59.1 kDa). The immunoblots were developed with anti-PT8 in SDS-PAGE. Coomassie brilliant blue (CBB) staining was used as loading control.



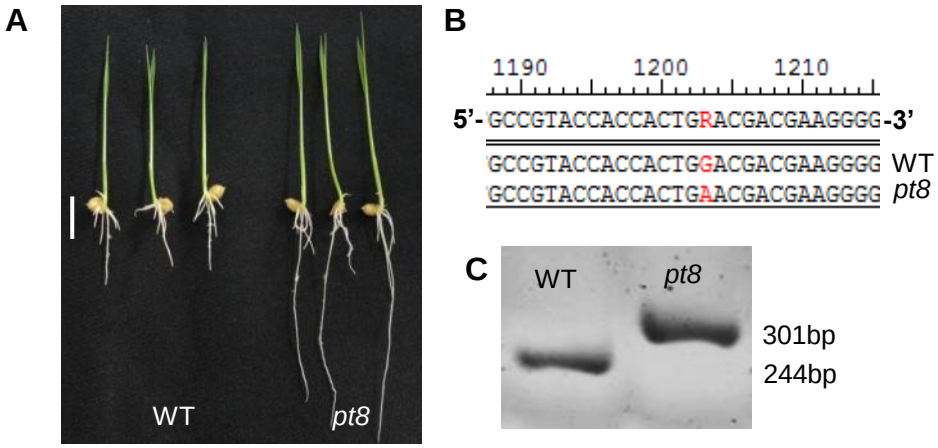
Supplemental Figure 4. Effect of CK2β3 overexpression on PT8 phosphorylation *in vivo*. Mobility of bands was observed in the wild type (WT), *CK2β3*-overexpression (*β3*-Ox) and *CK2β3*-knockdown (*β3*-Ri) plants (upper). Sensitivity of these bands to λ-phosphatase treatment (λ-PPase; lower) was also tested. The immunoblots were developed with anti-PT8 in PhosTag SDS-PAGE, with 10 μg protein loaded in each lane.



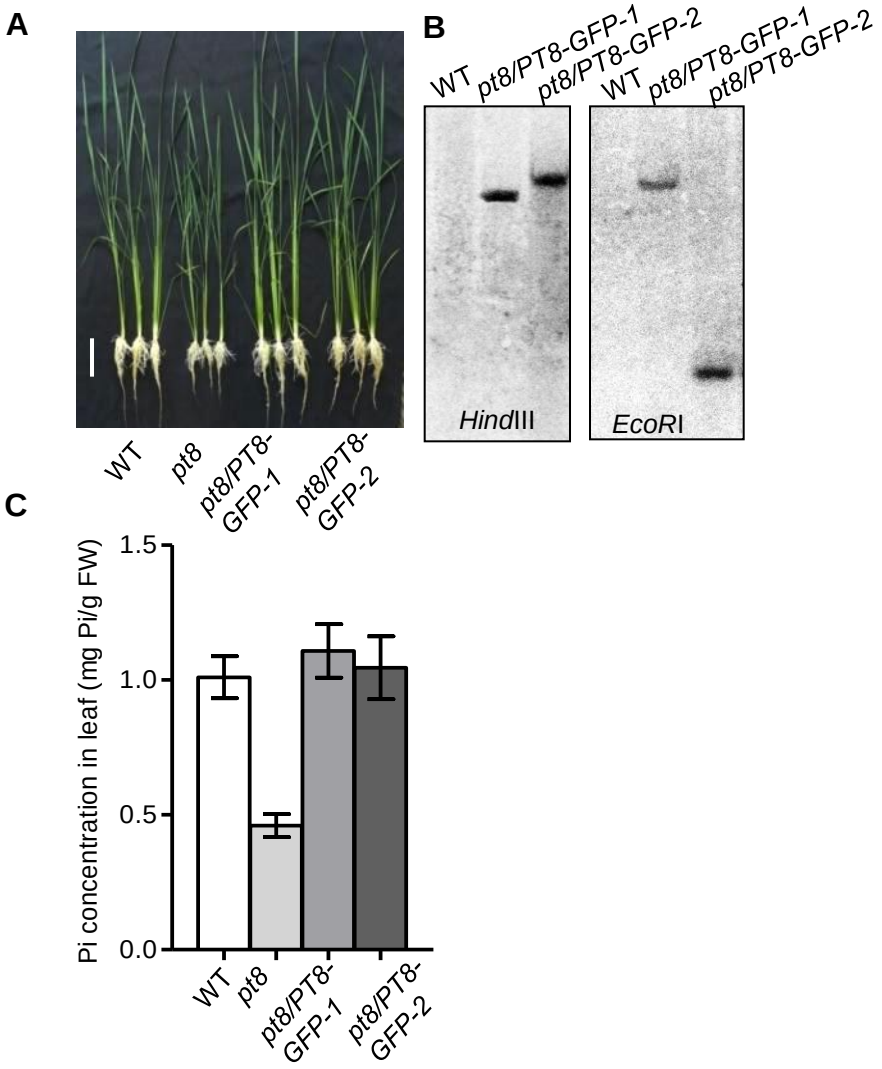
Supplemental Figure 5. Immunoblotting analysis for CK2α3/β3 protein level in plants grown under Pi-sufficient and Pi-starvation conditions. CK2α3/β3 levels in shoots. Proteins from shoots of the transgenic plants grown for 10 d and then subjected to Pi starvation for different periods as indicated, to Pi re-supply after Pi starvation, or to Pi starvation with MG132 (10 μM) were detected by immunoblotting in Phostag SDS-PAGE using an anti-FLAG antibody. Equal amounts of protein (15 μg) were used for immunoblotting. Coomassie brilliant blue (CBB) staining was used as loading control.



Supplemental Figure 6. Cellular Pi sensitivity of the interaction between CK2β3 and CK2α3. Equal amounts of β3-FLAG (50 ng) purified from transgenic plants grown under +P or –P1d, and GST-α3 purified in *E. coli* were subjected to GST pull-down assays. Immunoblots were developed using tag-specific antibodies. Purified GST-α3 and β3-FLAG proteins were loaded as the input lane.

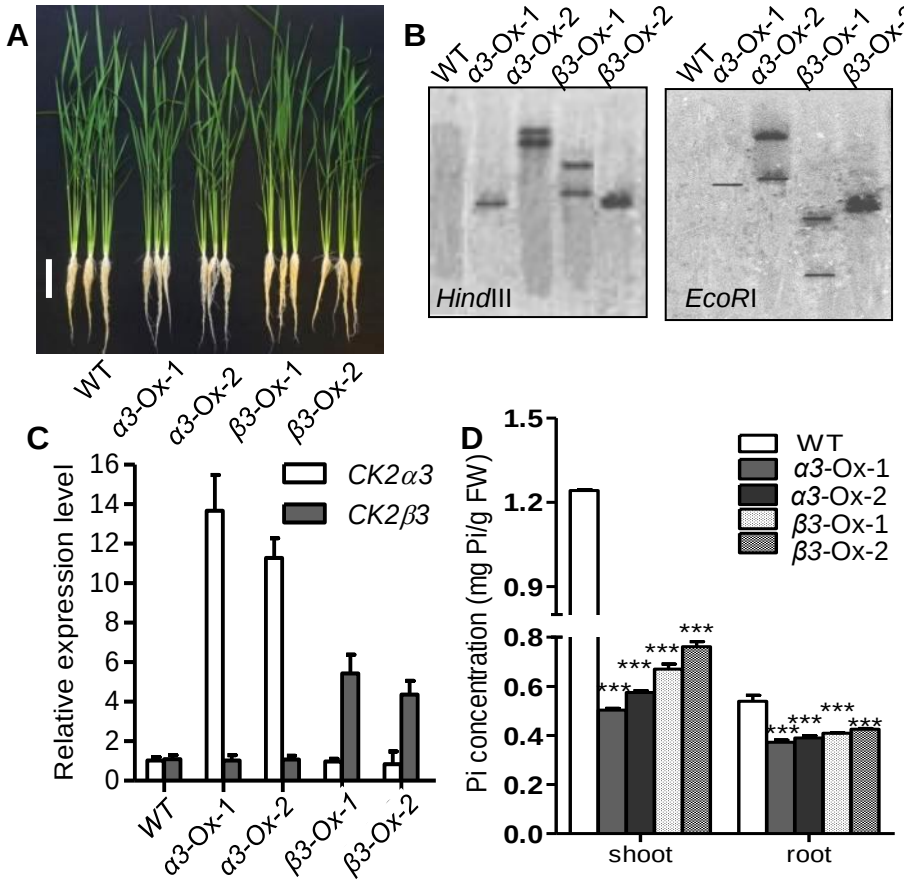


Supplemental Figure 7. Isolation of *pt8* mutants in Nipponbare background.
(A) Tolerance of *pt8* mutant to arsenate (As; 30 µM). Seedlings of the wild-type (cv. Nipponbare) and *pt8* mutant after growth in solution containing 30µM As for 5 d. Bar = 1 cm. (B) Representative sequences of mutant allele identified from a single plant of the *pt8* mutant. (C) PCR analysis using a pair of dCAPS primers to confirm the point mutation in *pt8*. The PCR products of *PT8* from the wild type and *pt8* from the mutant were digested with *SalI*. dCAPS primers are listed in Supplemental Table 1.

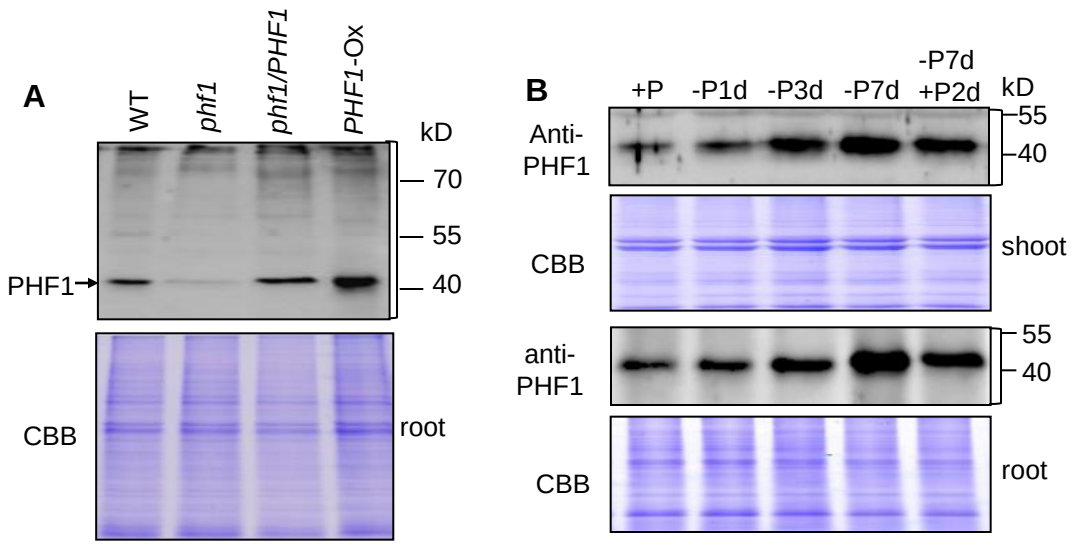


Supplemental Figure 8. Functional PT8-GFP rescued *pt8* mutant.

(A) Phenotype of 30-d-old plants of wild type, *pt8* mutant, and two independent transgenic lines of the *pt8* mutant complemented with *PT8-GFP* construct (*PT8p-PT8-GFP*) grown in solution culture with 200 µM Pi. Bar = 5 cm. (B) DNA gel blot analysis of the two independent transgenic lines. (C) Cellular Pi concentration in leaves of 21-d-old wild type, *pt8* mutant, and the transgenic lines of the *pt8* mutant complemented with *PT8-GFP* grown in solution culture supplied with 200 µM Pi. Error bars represent SD (n = 3).

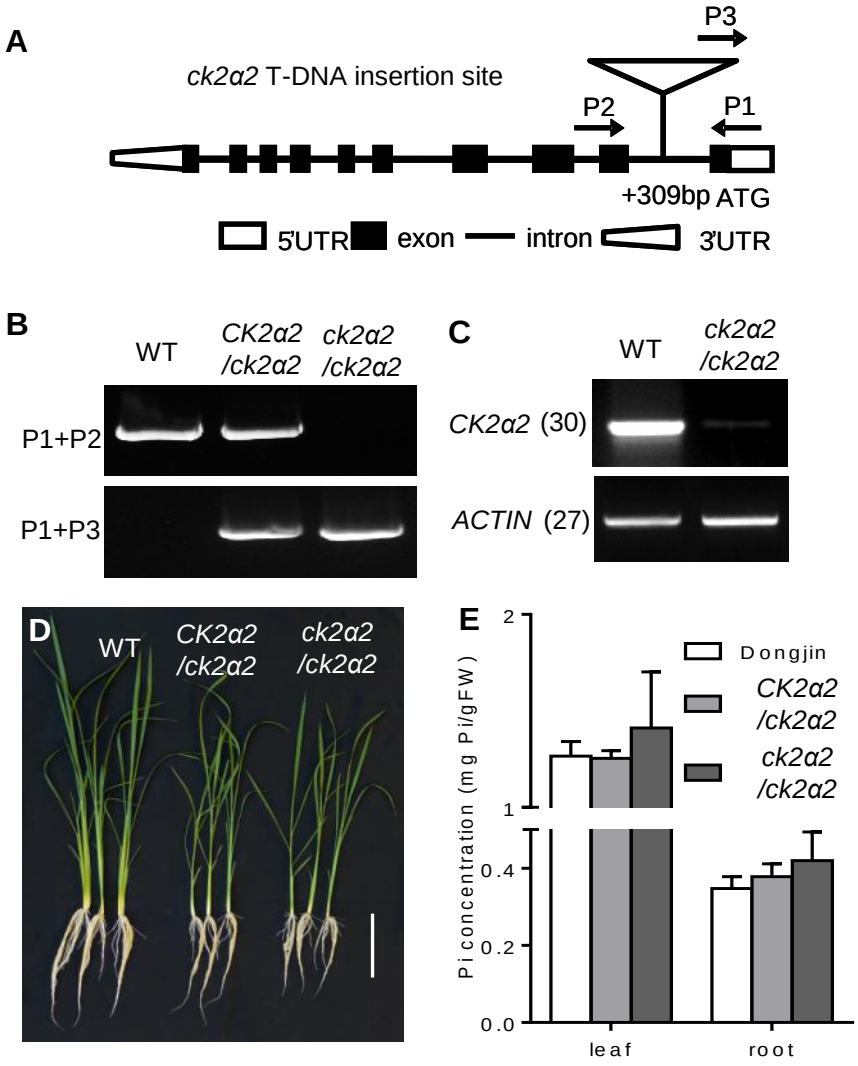


Supplemental Figure 9. Phenotype and Pi concentration of transgenic plants overexpressing *CK2α3/β3.* (A) Phenotypes of the wild type (WT; cv. Nipponbare) and two independent lines displaying 35S promoter driven overexpression of *CK2α3* (*α3*-Ox-1/2) or *CK2β3* (*β3-Ox-1*/2). The plants were grown for 30 d in a high Pi regime (200 μM Pi). Bars = 10 cm. (B) DNA gel blot analysis of the two independent transgenic lines. (C) Relative expression of *CK2α3/β3* in the transgenic plants. (D) Cellular Pi concentration of shoots and roots of the plants shown in (A). Error bars represent SD (*n* = 3 for C and D). Data significantly different between WT and the corresponding plants are indicated (** $P < 0.05$; *** $P < 0.001$; Student's *t* test). FW, Fresh weight.

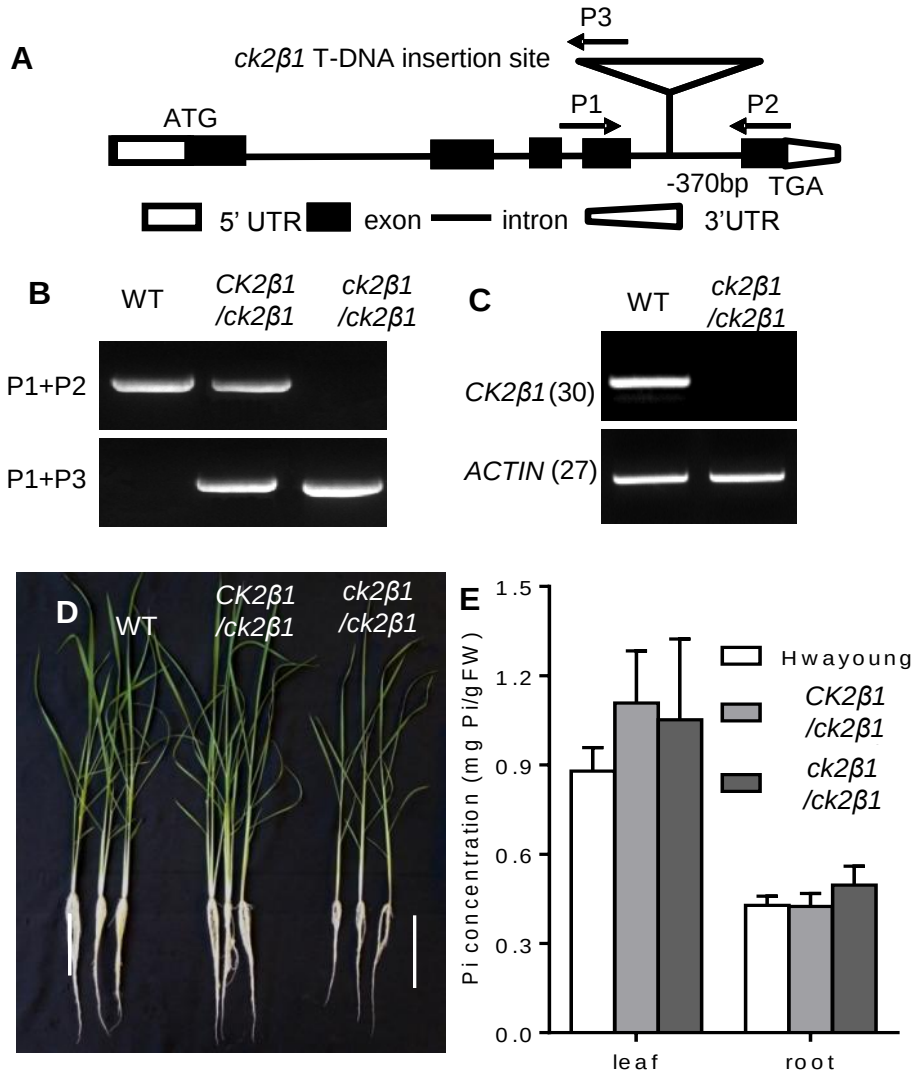


Supplemental Figure 10. Specificity of the PHF1 polyclonal antibody.

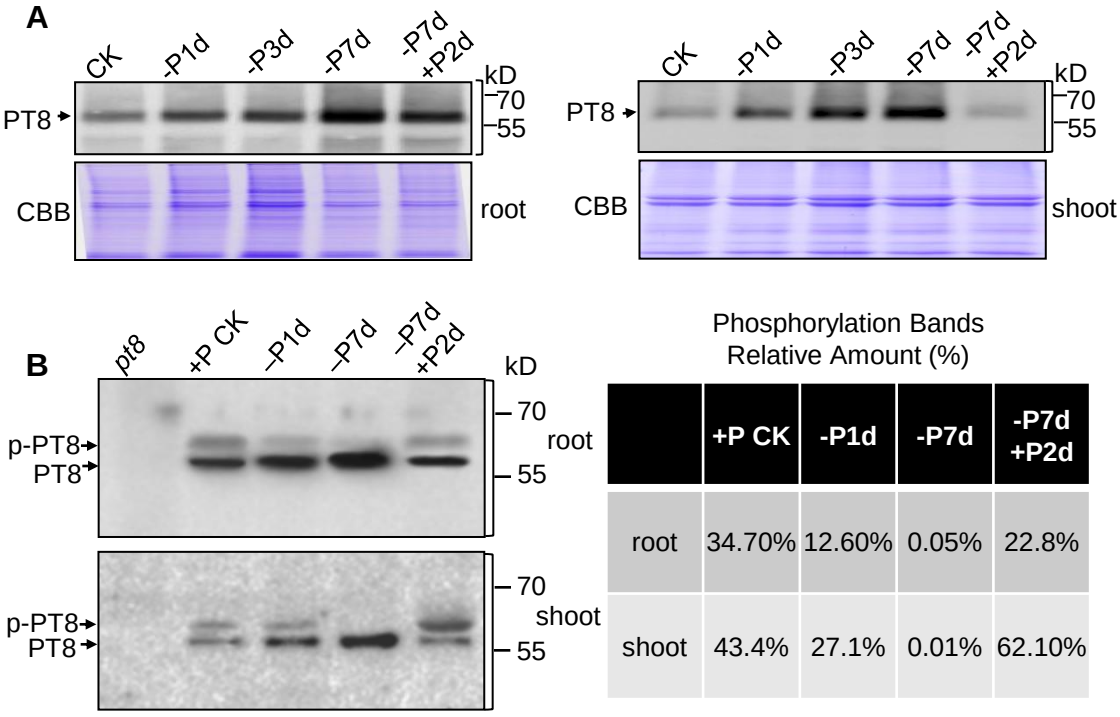
(A) Immunoblotting of membrane protein from roots of the wild type (WT; cv. Nipponbare), *phf1* mutant, one transgenic line of *phf1* complemented with a fragment encompassing 3702 bp before the ATG of *PHF1* and the *PHF1* ORF (*phf1/PHF1*), as well as transgenic plants overexpressing *PHF1* (*PHF1*-Ox). A specific band of the expected size of PHF1 (43 kDa) was detected with the expected intensity according to the genetic constitution of the plants corresponding to each lane. (B) The PHF1 level in shoots (top panel) and roots (bottom panel) of 10-d-old seedlings grown under –P conditions for an additional 7 d. The immunoblots were probed with anti-PHF1. Equal amounts of microsomal protein (10 μg) were used for immunoblotting. Staining by Coomassie blue indicates the similar amounts of proteins loaded.



Supplemental Figure 11. Phenotype and Pi content analysis of *α2* T-DNA insertional mutant. (A) Diagrammatic representation of the T-DNA insertion site at the first intron of *α2* at 309 bp after the ATG determined by sequencing and PCR analysis (B). (C) RT-PCR analysis of *α2* transcript in WT and homozygous (*ck2α2*/*ck2α2*) mutant plants. (D) Phenotype of the wild type (WT, Dongjin), heterozygous (*CK2α2*/*ck2α2*) and homozygous (*ck2α2*/*ck2α2*) mutant plants. The plants were grown in solution culture (200 μM Pi) for 30 d. Bar = 10 cm. (E) Pi concentration of plants shown in (D). Error bars represent SD (*n* = 3). Primers are listed in the Supplemental Table 1.



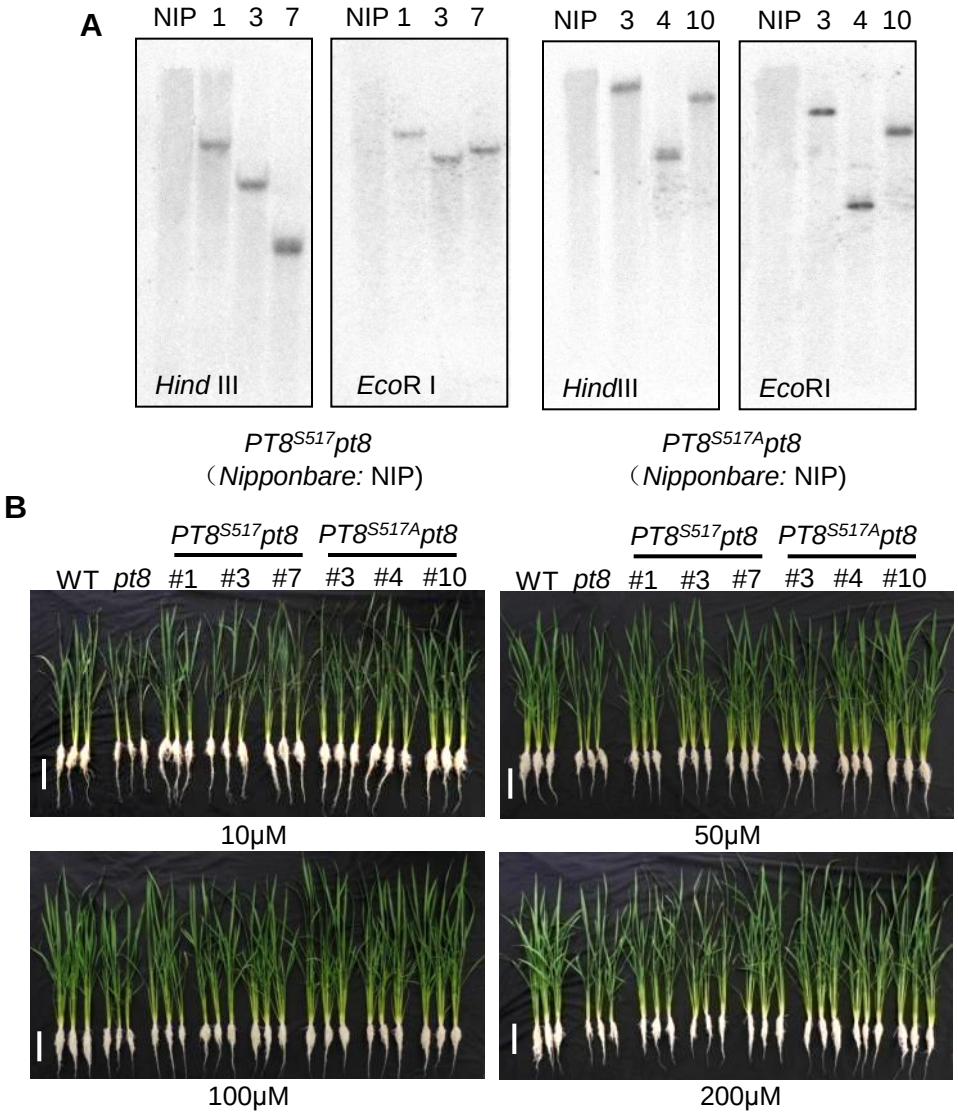
Supplemental Figure 12. Phenotype and Pi analysis of *β1* T-DNA insertional mutant. (A) Diagrammatic representation of the T-DNA insertion site at 370 bp upstream of the last exon of *β1* determined by sequencing and PCR analysis (B). (C) RT-PCR analysis of *β1* transcript in WT and homozygous (*ck2β1*/*ck2β1*) mutant plants. (D) Phenotype of the wild-type (WT, Hwayoung), heterozygous (*CK2β1*/*ck2β1*) and homozygous (*ck2β1*/*ck2β1*) mutant plants. The plants were grown in solution culture (200 μM Pi) for 30 d. Bar = 10 cm. (E) Pi concentration of plants shown in (D). Error bars represent SD (*n* = 3). Primers are listed in the Supplemental Table 1.



Phosphorylation Bands Relative Amount (%)

	+P CK	-P1d	-P7d	-P7d +P2d
root	34.70%	12.60%	0.05%	22.8%
shoot	43.4%	27.1%	0.01%	62.10%

Supplemental Figure 13. PT8 protein accumulation and phosphorylation levels differ in roots and shoots. (A) PT8 protein level in plasma membrane-enriched fractions of shoot and root. Proteins were detected by immunoblotting using an anti-PT8 antibody in roots (left panel) and shoots (right panel) of transgenic plants grown for 10 d and then subjected to Pi starvation for different periods as indicated, or to Pi re-supply after Pi starvation. Equal amounts of plasma membrane protein (10 μg) were used for immunoblotting. Staining by Coomassie blue indicates the similar amounts of proteins loaded. (B) Total membrane PT8 protein level and phosphorylation in roots and shoots. The total membrane protein was extracted from plant samples as in (A) and equal amounts of total membrane protein (10 μg) were used for immunoblotting. The immunoblots were developed with anti-PT8 in PhosTag SDS-PAGE. The percentage of phosphorylated PT8 versus the total quantity of PT8 protein was quantified (right panel).



Supplemental Figure 14. DNA gel blot analysis and plant phenotype of independent transgenic lines of the *pt8* mutant transformed with *PT8^{S517}* and *PT8^{S517A}* expressing constructs. (A) DNA gel blot analysis of three independent transgenic lines for each construct. (B) Phenotype of the wild type (WT), *pt8* mutants and transgenic *pt8* mutant plants expressing *PT8 (PT8^{S517})* or the non-phosphorylatable *PT8^{S517A}* mutant from the PT8 promoter. Dry weight and Pi content of shoots and roots of NIP, *pt8* and transgenic plants grown under different Pi regimens are shown in Figure 7. Bar = 10 cm.

Supplementary Table 1 Primers used in this study.

Primer	Sequence(5'-3')
PT2-pBT3-STE-U	ATTAACAAGGCCATTACGGCCATGGCGGGATCGCAGCTCAA
PT2-pBT3-STE-L	AACTGATTGGCCGAGGCGGCCCCCGCTTGGGCGATCGCTTCTT
PT8-pBT3-STE-U	ATTAACAAGGCCATTACGGCCATGGCGCGGCAGGAGCAGCAGCAG
PT8-pBT3-STE-L	AACTGATTGGCCGAGGCGGCCCCCGCCGTCTGCGGCCGCACG
CK2α2-pPR3-N-U	ATTAACAAGGCCATTACGGCCATGTCCAAGGCCAGGGTCTACAC
CK2α2-pPR3-N-L	AACTGATTGGCCGAGGCGGCCCTATTGTGCACGAGGCCTACTGTTC
CK2α3-pPR3-N-U	ATTAACAAGGCCATTACGGCCATGTCGACGGCCCGCGTCTA
CK2α3-pPR3-N-L	AACTGATTGGCCGAGGCGGCCTTACGTTCTCATTCTGCAGTCTTCT
CK2β1-pPR3-N-U	ATTAACAAGGCCATTACGGCCATGAGCGGCGCGTACAGG
CK2β1-pPR3-N-L	AACTGATTGGCCGAGGCGGCCTCATGACTGCTTGTGAAGTTTGAAG
CK2β3-pPR3-N-U	ATTAACAAGGCCATTACGGCCATGTACAAGCAGGGGGGAGGA
CK2β3-pPR3-N-L	AACTGATTGGCCGAGGCGGCCTCATGGCTTGTGGAGCTTGAAT
PT2-GFP-U	GCTCTAGAATGGCGGGATCGCAGCTCAACGTTT
PT2-GFP -L	GCTCTAGACGCTTGGGCGATCGCTTCTTGG
PT8-GFP-U	CCCTCTAGAATGGCGCGGCAGGAGCAGCAG
PT8-GFP -L	CCCCTCGAGCGCCGTCTGCGGCCGCACG
CK2α2-HA-U	CCCTTAATTAACATGTCCAAGGCCAGGGT
CK2α2-HA-L	TTGGCGCGCCCTTGTGCACGAGGCCTACT
CK2α3-Flag-U	GCTCTAGAATGTCGACGGCCCGCGT
CK2α3-Flag-L	GCTCTAGACGTTCTCATTCTGCAGTCTTCTGCT
CK2β1-HA-U	CCCTTAATTAACATGAGCGGCGCGTACA
CK2β1-HA-L	TTGGCGCGCCCTGACTGCTTGTGAAGTTTGAA
CK2β3-pC-TAPa-U	GAAGATCTATGTACAAGCAGGGGGGAGG
CK2β3-pC-TAPa-L	GAAGATCTTCATGGCTTGTGGAGCTTGAA
PT2-CT-Y3H-U	AGGAATTCCCGGAGTCCAAGGGCTTGTCGC
PT2-CT-Y3H-L	CGAGGATCCTCACGCTTGGGCGATCGCTTCTTG
PT8-CT-Y3H-U	GGAATTCCCGGAGTCGAAGGGGAAGT
PT8-CT-Y3H-L	GGAATTCCTACGCCGTCTGCGGCC
CK2β3-Y3H-U	GAAGATCTATGTACAAGCAGGGGGGAGG
CK2β3-Y3H-L	GAAGATCTTCATGGCTTGTGGAGCTTGAA
PT8-CT-GFP-U	GGAATTCATGCCGGAGTCGAAGGGGAAGT
PT8-CT-GFP-L	GGAATTCCTACGCCGTCTGCGGCC
CK2α3-GFP-U	GCTCTAGAATGTCGACGGCCCGCGT
CK2α3-GFP-L	GCTCTAGACGTTCTCATTCTGCAGTCTTCTGCT
CK2β3-GFP-U	GCTCTAGAATGTACAAGCAGGGGGGA
CK2β3-GFP -L	GCTCTAGATGGCTTGTGGAGCTTGAATCCAAAT
GST-CK2α3-U	CGGGATCCATGTCGACGGCCCGCGT
GST-CK2α3-L	CGGGATCCTTACGTTCTCATTCTGCAGTCTTCT
GST-CK2β3-U	CGGGATCCATGTACAAGCAGGGGGGAGGAGGGG

GST-CK2β3-L	ACGCGTCGACTCATGGCTTGTGGAGCTTGAATCCA
GST-PT8-CT-U	AAAGAATTCCCGGAGTCGAAGGGGAAGTCGCT
GST-PT8-CT-L	AAAGTCGACCTACGCCGTCTGCGGCCGC
GST-PT8-CT^{S512A}-U	GGAATTCCCGGAGTCGAAGGGGAAGGCCCTGGAGGAG
GST-PT8-CT^{S512A}-L	GCGTCGACCTACGCCGTCTGCGGCCGCACGGC
GST-PT8-CT^{S517A}-U	GGAATTCCCGGAGTCGAAGGGGAAGTCGCTGGAGGAGATGGCCGGCGAG GCG
GST-PT8-CT^{S517A}-L	GCGTCGACCTACGCCGTCTGCGGCCGCACGGCGGCGCCACCG
GST-PT8-CT$^{S512A/517A}$-U	GGAATTCCCGGAGTCGAAGGGGAAGGCCCTGGAGGAGATGGCCGGCGAG GCG
GST-PT8-CT$^{S512A/517A}$-L	GCGTCGACCTACGCCGTCTGCGGCCGCACGGCGGCGCCACCGC
35S-CK2β3-FLAG-U	GCTCTAGAATGTACAAGCAGGGGGGA
35S-CK2β3-FLAG-L	GCTCTAGATGGCTTGTGGAGCTTGAATCCAAAT
35S-CK2α2-U	GCTCTAGAATGTCCAAGGCCAGGGTCTACACCG
35S-CK2α2- L	GCTCTAGATTGTGCACGAGGCCTACTGTTCTCC
35S-CK2α3-U	GCTCTAGAATGTCGACGGCCCGCGT
35S-CK2α3-L	GCTCTAGACGTTCTCATTCTGCAGTCTTCTGCT
35S-CK2β1-U	GCTCTAGAATGAGCGGCGCGTACA
35S-CK2β1-L	GCTCTAGATGACTGCTTGTGAAGTTTGAAGCCA
35S-CK2β3-U	GCTCTAGAATGTACAAGCAGGGGGGA
35S-CK2β3-L	GCTCTAGATGGCTTGTGGAGCTTGAATCCAAAT
PT8 promoter-U	ACGCGTCGACTGTCGTGCTCTCCCGTGTCTTCGTT
PT8 promoter-L	GCTCTAGACGCCGTCTGCGGCCGCAC
PHF1-pBT3-N-U	ATTAACAAGGCCATTACGGCCATGGCAGGCGGCGGAGGT
PHF1-pBT3-N-L	AACTGATTGGCCGAGGCGGCCTCACCAGGGGTTCTGGTCCTCAGG
PT8-pPR3-STE-U	ATTAACAAGGCCATTACGGCCTTGCGCGGCAGGAGCAGCAGCAG
PT8-pPR3-STE-L	AACTGATTGGCCGAGGCGGCCCCCGCCGTCTGCGGCCGCACG
PT8^{S517A}-pPR3-STE-P1	ATTAACAAGGCCATTACGGCCTTGCGCGGCAGGAGCAGCAGCAGCA
PT8^{S517A}-pPR3-STE-P2	CTCCGCCTCGCCCGCCATCTCCTCC
PT8^{S517A}-pPR3-STE-P3	GGAGGAGATGGCGGGCGAGGCGGAG
PT8^{S517A}-pPR3-STE-P4	AACTGATTGGCCGAGGCGGCCCCCGCCGTCTGCGGCCGCACG
PT8^{S517D}-pPR3-STE-P1	ATTAACAAGGCCATTACGGCCTTGCGCGGCAGGAGCAGCAGCAGCA
PT8^{S517D}-pPR3-STE-P2	CTCCGCCTCGCCGTCCATCTCCTCC
PT8^{S517D}-pPR3-STE-P3	GGAGGAGATGGACGGCGAGGCGGAG
PT8^{S517D}-pPR3-STE-P4	AACTGATTGGCCGAGGCGGCCCCCGCCGTCTGCGGCCGCACG
PT8-GFP-U	CCCTCTAGAAT GGCGCGGCAGGAGCAGCAG
PT8-GFP -L	CCCCTCGAGCGCCGTCTGCGGCCGCACG
pt8 dCAPS-U	GGCCTCGCCGTGCCGTACCACCAGTC
pt8 dCAPS-L	CGGCGAGCACGAACAGCGAGTT
ck2α2 T-DNA-P1	TTGCATCACAATCAATCAGGA
ck2α2 T-DNA-P2	AATTGATGTTTTGGACAATGG
ck2α2 T-DNA-P3	ACGTCCGCAATGTGTTATTAA
ck2β1 T-DNA-P1	AAGGAGCCATTTCCAATAACC
ck2β1 T-DNA-P2	CCAAAGTACGCTCCGTCAAT

ck2β1 T-DNA-P3	AACGCTGATCAATTCCACAG
CK2α2-RT-U	GGGCTGCGGAGAACAGTAGG
CK2α2-RT-L	AATCCACGTTTGCGGTTAAG
CK2β1-RT-U	AGCGTACTTTGGGACAACAT
CK2β1-RT-L	GCAATTACAGCGAGATTCA
ACTIN-RT-U	CAACACCCCTGCTATGTACG
ACTIN-RT-L	CATCACCAGAGTCCAACACAA
CK2α3-RNAi-U	GCATCATTAAAATTCTCAAGCCT
CK2α3-RNAi-L	AATGACAGTAATCCAATGCCTT
CK2β3-RNAi-U	TATGGGCTGATTCATGCACGAT
CK2β3-RNAi-L	AACAGGTGCGGGAATGT
CK2α3-QRT-U	AAAATAGGCATCTTGTCTCTCCA
CK2α3-QRT-L	AAAGTAGGGATGCGCCATAG
CK2β3-QRT-U	GGGAGTCTGAATCTGATAGTGAA
CK2β3-QRT-L	CTGCTAAGCCCACAGAGGT
ACTIN-QRT-U	CAACACCCCTGCTATGTACG
ACTIN-QRT-L	CATCACCAGAGTCCAACACAA