Supplementary data

A lipid transfer protein variant with a mutant eight-cysteine motif causes photoperiod- and thermo-sensitive dwarfism in rice

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Figure S1 The CDS sequence of *PTD1* (*Os01g0822900*)

The CDS is highlighted in yellow, and 5'UTR and 3'UTR in grey. Sequence between the two exons is the 86-bp intron. The box marks the mutation site, where the sequence of *PTD1* is "GTG", of *Ptd1* is "TT". The bold triplets are start codon (ATG) and stop codons (TAA for *Ptd1*; TAG for *PTD1*). The sequence is based on Os01t0822900-03 from：https://rapdb.dna.affrc.go.jp/viewer/gene_detail/irgsp1?name=Os01t0822900-03

Lipid-binding assay of the recombinant PTD1 and Ptd1 proteins

(1) Prokaryotic expression of recombinant PTD1 and Ptd1 proteins

The coding sequences of PTD1 and Ptd1 (excluding the 27-aa signal peptide) were amplified from stem cDNA of wild-type (*PTD1*/*PTD1*) and mutant homozygote (*Ptd1*/*Ptd1*), respectively, using primer pair PTD1/Ptd1-pET32a-F/-R. The nsLTP positive control *OsC6*-specific fragment was amplified from anther cDNA of a rice cultivar Taichung65 (T65) using primer pair OsC6-pET32a-F/-R. The fragments were subsequently cloned into pET-32a (QIAGEN, Germany) prokaryotic expression vector via *Nco*I and *Eco*RI cloning sites. The resultant constructs were transformed into *Escherichia coli* strain transB (DE3) (Transgen Biotech, China). Prokaryotic expression and protein purification were performed as the manufacturer's instruction.

(2) Lipid-binding assay of the recombinant PTD1 and Ptd1 proteins

Lipid binding assay was performed following the published protocols (Zachowski *et al*., 1998; Zhang *et al*., 2010). The purified proteins (1 μmol/L in 10 mmol/L MOPS) were mixed with different concentrations of *p*-96 (Invitrogen, USA) and maintained at 25°C. The emission spectrum was measured at 378 nm (excitation at 343 nm) using a Synergy Mx multimode microplate reader (BioTek, USA). The recombinant OsC6 protein (Zhang *et al*., 2010) was used as a positive control. The pET32a protein (1 μmol/L in 10 mmol/L MOPS) expressed from the empty vector pET-32a and the MOPS buffer (10 mmol/L) were used as negative controls.

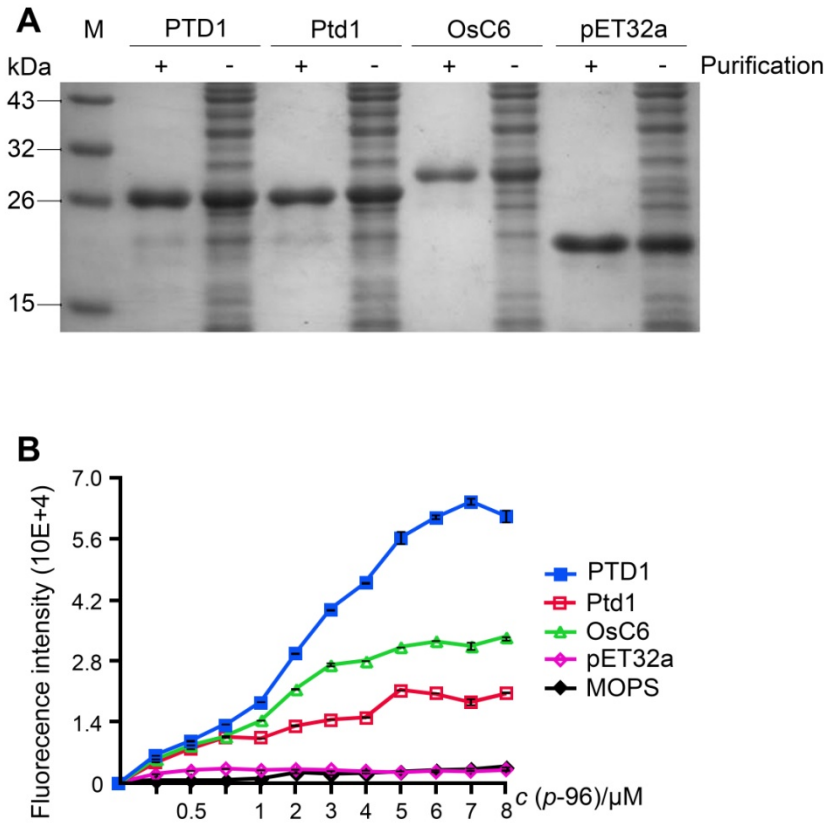


Figure S2 Recombinant PTD1 protein displays lipid-binding activity

(A) The expression and purification of recombinant protein PTD1 (MW 26 kDa), Ptd1 (MW 26 kDa) and OsC6 (MW 30 kDa). pET32a (MW 17 kDa) is a control protein expressed from the empty vector pET-32a. Lane M, Standard protein marker; lane -, bacterial lysate; lane +, purified protein. **(B)** Lipid-binding activity of the recombinant PTD1 and Ptd1 proteins. The purified proteins were mixed with varied concentrations of *p*-96, and relative fluorescence intensities were measured (n = 3). Blue and red squares stand for the recombinant PTD1 and Ptd1 proteins, respectively. The pET32a protein (purple rhombus) and MOPS buffer (black rhombus) were used as negative controls. Recombinant rice OsC6 protein (Zhang *et al*., 2010) was used as the positive control (green triangle).

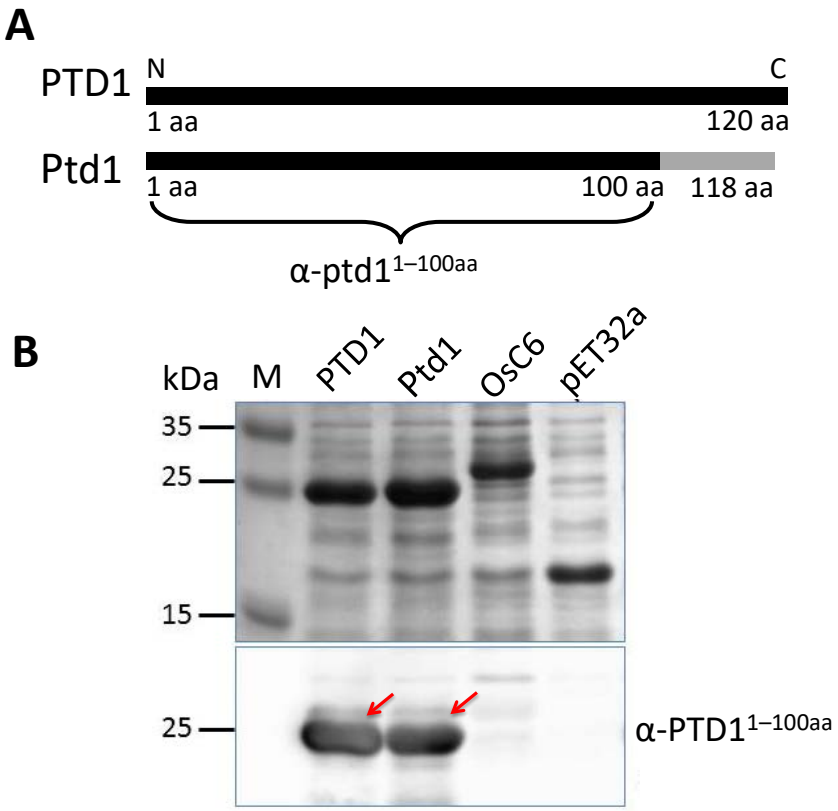


Figure S3 Verification of the specificity of antibody α-PTD1$^{1\text{-}100aa}$ by western blotting

(A) The 1–100th aa sequence shared by Ptd1 and PTD1 was used as the antigen to produce an anti-PTD1/Ptd1 polyclonal antibody (named α-PTD1$^{1–100aa}$) in rabbits (Beijing Genomics Institute, Shenzhen, China). The wild-type PTD1 protein contains 120 aa, while the Ptd1 mutant protein contains 118 aa, of which the grey bar represents the frame-shifted C terminus (101-118th aa) due to a point mutation at the 101st codon in *PTD1*. N, N terminus; C, C terminus.

(B) Verification of the antibody specificity by western blotting. The expressed proteins in the pET-32a prokaryotic expression system were used for western blot assay with the antibody α-PTD1$^{1–100aa}$. Specific bands (arrowed) were detected in the samples containing PTD1 and Ptd1 protein, but not in with the control nsLTP protein OsC6 (Zhang et al, 2010), or in the negative control sample expressed from the empty vector pET-32a. M, marker.

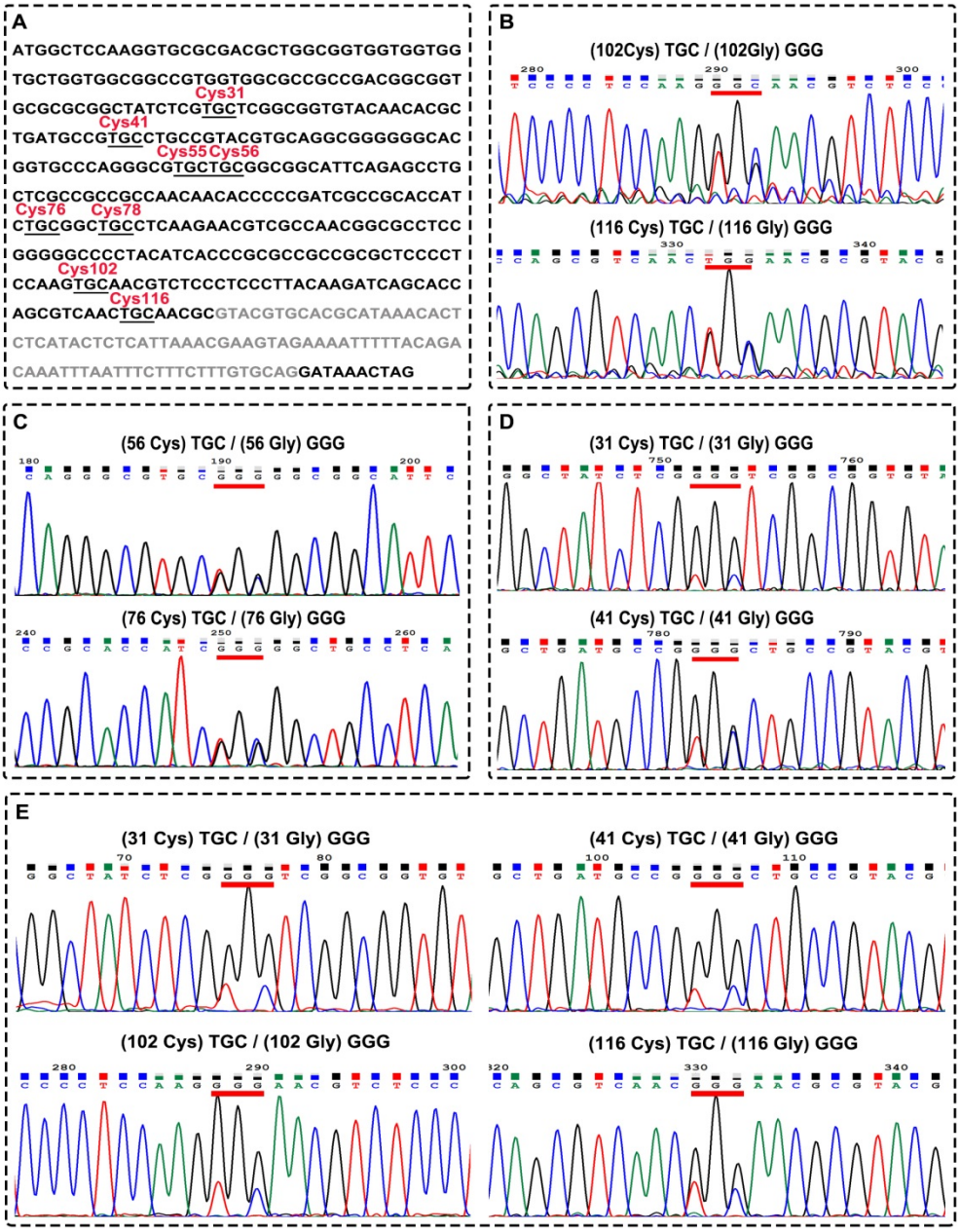


Figure S4 Sequencing verification of the transgenic plants carrying modified *PTD1* constructs with codon substitutions for the conserved cysteine sites

(A) Genomic DNA sequence of *PTD1*. The underlined bases are codons for the eight conserved Cys. Bases in grey are intron sequence. (**B, C)** Sequencing chromatograms of the transgenic plants carrying modified PTD1 without both 3rd and 4th disulfide bonds due to the Cys-to-Gly substitutions of $PTD1^{C102G/C116G}$ (B) and $PTD1^{C56G/C76G}$ (C). (**D)** Sequencing chromatograms of the transgenic plant carrying modified PTD1 without both the 1st and 2nd disulfide bonds due to the Cys-to-Gly substitutions of $PTD1^{C31G/C41G}$. (**E)** Sequencing

chromatograms of the transgenic plant carrying modified PTD1 without all four disulfide bonds due to the Cys-to-Gly substitutions of PTD1$^{C31G/C41G/C102G/C116G}$. Red lines mark the positions of the substituted codons. Due to mixture PCR products of the endogenous TGC (Cys) and the transgenic GGG (Gly), these positions display overlapped chromatogram patterns. The phenotypes of these transgenic plants are shown in Figure 6.

Table S1 List of primers used in this study

Primer name	Primer sequences (5'-3')
Omega PCR	
omega-C102G&C116G-F	GGGAACGTCTCCCTCCCTTACAAGATCAGCACCAGCGTCAACGGGAACGCGTACGT
omega-C102G&C116G-R	CCCGTTGACGCTGGTGCTGATCTTGTAAGGGAGGGAGACGTTCCCCTTGGAGGGGAG
omega-C31G&C41G-F	TCGGGGTCGGCGGTGTACAACACGCTGATGCCGGGGCTGCCGTACGTGCAGGCG
omega-C31G&C41G-R	CAGCCCCGGCATCAGCGTGTTGTACACCGCCGACCCCGAGATAGCCGCGCGCAC
omega-C56G-F	CCCAGGGCGTGCGGGGGCGGCATTCAGAGCCTGCTCGCCGCC
omega-C56G-R	CTGAATGCCGCCCCCGCACGCCCTGGGCACCGTGCCCCCCGC
omega-C76G-F	CGCCGCACCATCGGGGGCTGCCTCAAGAACGTCGCCAACGGC
omega-C76G-R	CTTGAGGCAGCCCCCGATGGTGCGGCGATCGGGGGTGTTGTT
omega-Ptd1:FLAG-F	GCAGGAGACTACAAAGACGATGACGACAAGTAAACTGAGGCGGCCGGCCTGATGC
omega-Ptd1:FLAG-R	GTTTACTTGTCGTCATCGTCTTTGTAGTCTCCTGCACAAAGAAAGAAATTAAATTTG
Truncated constructs	
PTD1Δ102-120-F	CCGGGGGCCCCTACATCACCCGCGCCGCCGCGCTCCCCTCCAATTAGCAACG
PTD1Δ109-120-F	GCGCTCCCCTCCAAGTGCAACGTCTCCCTCCCTTACTAGATCAG
adapter-F	CCGGGGGCCCCTACATCACCCGCGCCGCCGCGCTCCCCTCCAAGTGCAAC
Bam HI R	TTATGGATCCGCGGCATTGTTGCTGTCGGGTTC
Overlaping PCR	
PTD1-20aa-F	CCTCCGGGGGCCCCTACATCAC
PTD1-20aa-R	GGCCGGCCGCCCTAGTTTATCC
18aa-F	GATAAACTAGGGCGGCCGGCCAACAGCACCAGTCCGAGCCTAACTAGGAGTGCTCCTG
18aa-R	CGGGCACACACGTACGCATCAGCGAGTAGCTGTACTGGCAGGAGCACTCCTAGTTAGGC

PTD1-Terminator-F	CGC TGATGCGTACGTGTGTGCCCG
Bam HI R	TTATGGATCCGCGGCATTGTTGCTGTCGGGTTC
CRISPR/Cas9 vector construction	
Cas9-PTD1-U3-F	GGCAAGGTGCGCGACGCTGGCGG
Cas9-PTD1-U3-R	AAACCCGCCAGCGTCGCGCACCT
qRT-PCR	
PTD1-qR-F	ACAAGATCAGCACCAGCGT
PTD1-qR-R	TGCACAATATAGCTCCACCATC
Actin-F	CACATTCCAGCAGATGTGGA
Actin-R	ACCACAGGTAGCAATAGGTA
Cloning	
pro-F	TTATGGATCC GCGGCATTGTTGCTGTCGGGTTC
pro-R	ATTAGAATTC CTCCTGCTGCTGGCCTCACAGAC
Genotyping	
HPT-F	ATTTGTGTACGCCCGACAGT
HPT-R	GTGCTTGACATTGGGGAGTT
Cas9-F	CTGACGCTAACCTCGACAAG
Cas9-R	CCGATCTAGTAACATAGATGACACC
endo-PTD1-F	GTTTATGCGTGCACGTACGCGTTG
endo-PTD1-R	GCATGCATACGATCGAGACGTAC
Ptd1-FLAG-F	CCTCAAGAACGTCGCCAAC
Ptd1-FLAG-R1	GTCAACCGTCCATTGTTCTGC
Ptd1-FLAG-R2	GGATCGTATGTCTCCACAG

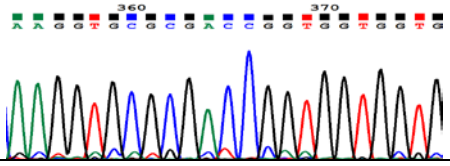
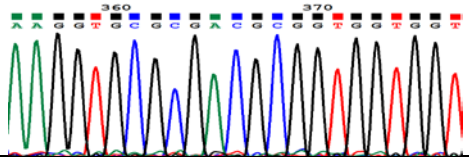
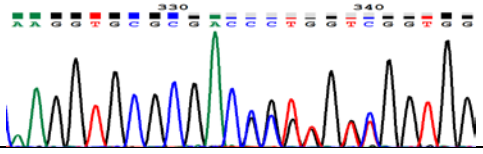
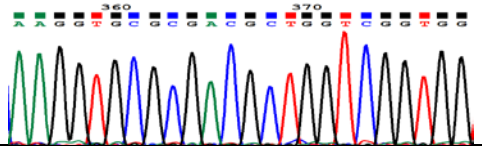
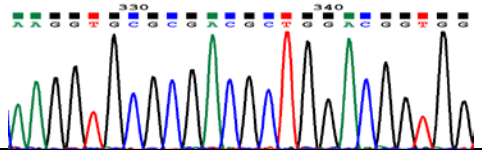
HRM

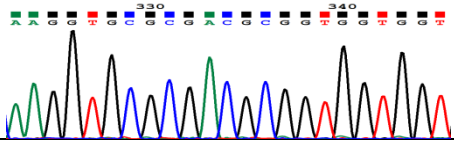
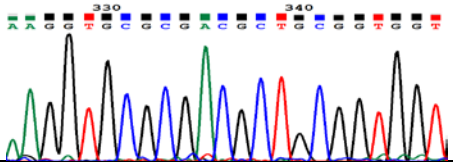
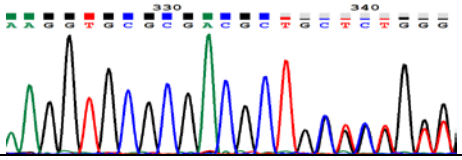
HRM-P1	CGTACGCGTTGCAGTTGAC
HRM-P2	CCTCAAGAACGTCGCCAAC
HRM-P3	TGGTGCTGATCTTGTAAG
HRM-PTD1-P4	GCGCTATTCTCCAAGTG
HRM-Ptd1-P4	GCGCTATTCTCCAATTCA

Prokaryotic expression vectors

PTD1/Ptd1-pET32a-F	CATGCCATGGCTATCTCGTGCTCGG
PTD1/Ptd1-pET32a-R	AGGCGAATTCCCCTAGTTTATCGCGTTGCAG
OsC6-pET32a-F	CATGCCATGGCGCCGTCCAAGTCCACAGCCG
OsC6-pET32a-R	AGGCGAATTCCTCATATATACTCAGGCAGAT

Table S2 Genotypes and sequencing chromatograms of the CRISPR/Cas9 knockout mutants of *PTD1* and *Ptd1*

Gene	Line	Target mutation		Mutation type
PTD1	1	Ref	AAGGTGCGCGACGCTGGCGGTGG	Homozygous
		Allele1	AAGGTGCGCGAC-----CGGTGG	
		Allele2	AAGGTGCGCGAC-----CGGTGG	
				
	2	Ref	AAGGTGCGCGACGCTGGCGGTGG	Homozygous
		Allele1	AAGGTGCGCGACG----CGGTGG	
		Allele2	AAGGTGCGCGACG----CGGTGG	
				
	3	Ref	AAGGTGCGCGACGCTGG-CGGTGG	Biallelic
		Allele1	AAGGTGCGCGAC------CGGTGG	
		Allele2	AAGGTGCGCGACGCTGGTCGGTGG	
				
Ptd1	1	Ref	AAGGTGCGCGACGCTGG-CGGTGG	Homozygous
		Allele1	AAGGTGCGCGACGCTGGTCGGTGG	
		Allele2	AAGGTGCGCGACGCTGGTCGGTGG	
				
	2	Ref	AAGGTGCGCGACGCTGG-CGGTGG	Homozygous
		Allele1	AAGGTGCGCGACGCTGGACGGTGG	
		Allele2	AAGGTGCGCGACGCTGGACGGTGG	
				

3	Ref	AAGGTGCGCGACGCTGGCGGTGG	Homozygous
	Allele1	AAGGTGCGCGACGC----GGTGG	
	Allele2	AAGGTGCGCGACGC----GGTGG	
			
4	Ref	AAGGTGCGCGACGCTGGCGGTGG	Homozygous
	Allele1	AAGGTGCGCGACGCTG-CGGTGG	
	Allele2	AAGGTGCGCGACGCTG-CGGTGG	
			
5	Ref	AAGGTGCGCGACGCTGG-CGGTGG	Biallelic
	Allele1	AAGGTGCGCGACGCTGGTCGGTGG	
	Allele2	AAGGTGCGCGACGCTG--CGGTGG	
			

Note: Ref, target reference sequences; underlined bases indicate protospacer adjacent motif (PAM); red letter indicates base insertion; red dash indicates base deletion; black dash is for alignment. The phenotypes of the knockout plants are shown in Figure 4. The mutant allelic sequences of the sequencing chromatograms were analyzed using the DSDecode program (Liu et al., 2015; Xie et al., 2017).

References

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