Supporting Information

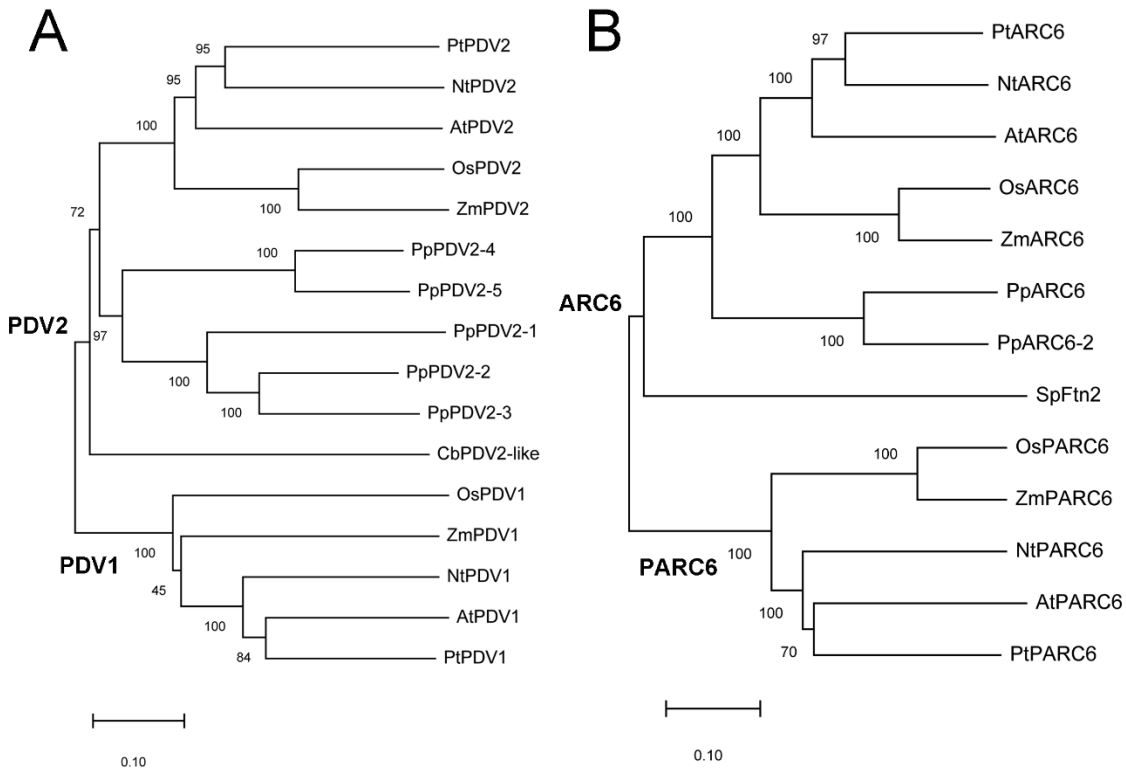

Fig. S1. Phylogenetic analysis of PDV1, PDV2, ARC6 and PARC6. (*A*) A phylogenetic tree of PDV1 and PDV2 in various plants. *At*, *Arabidopsis thaliana*; *Nt*, *Nicotiana tabacum*; *Pt*, *Populus trichocarpa*; *Os*, *Oryza sativa*; *Zm*, *Zea mays*; *Pp*, *Physcomitrium* (*Physcomitrella*) *patens*. *Cb*, *Chara braunii*. (*B*) A phylogenetic tree of PARC6 and ARC6 in various species. Plant species used for the analysis are mostly the same as those in (*A*). *Sp*, *Synechocystis sp. PCC 6714*. Accession numbers of the sequences used for the analysis are listed in Table S1.

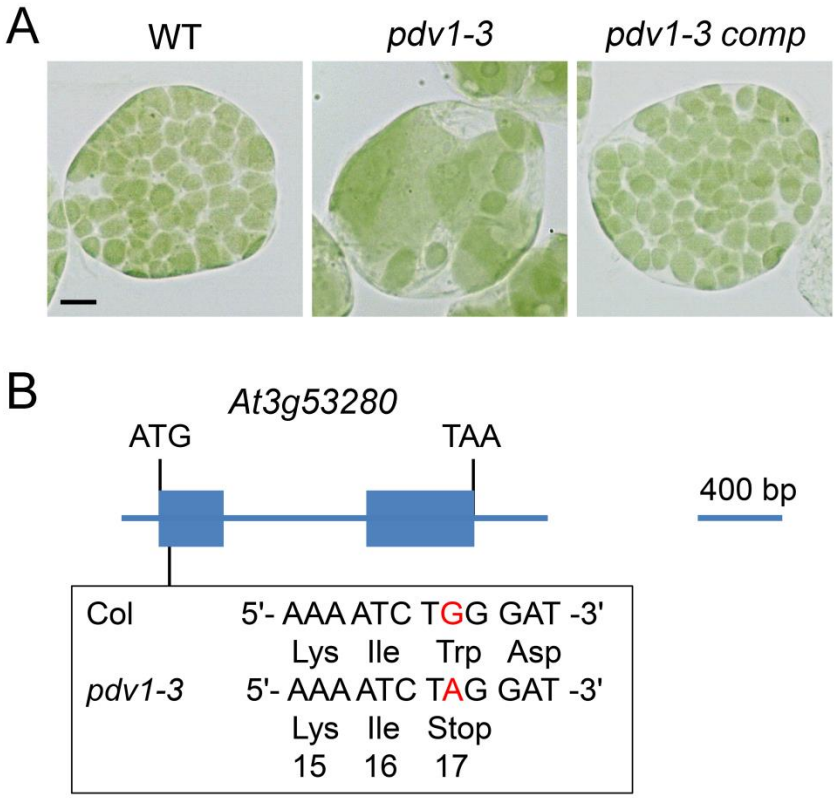

Fig. S2. Identification of a new mutant allele of *PDV1*, *pdv1-3*. (*A*) Chloroplast phenotypes of mesophyll cells in 4-week-old plants. WT, wild-type; *pdv1-3*, a new mutant allele of *PDV1*; *pdv1-3 comp*, *pdv1-3* complemented with a wild-type *PDV1* transgene. Scale bar (10 μm) applies to all the images. (*B*) Gene structure and the mutation site of *pdv1-3* in *PDV1* gene. Blue boxes indicate the coding sequences and the straight line between blue boxes represents intron. Mutation site and the corresponding site in the wild type (Col) are indicated with red font color. ATG, start codon; TAA, stop codon.

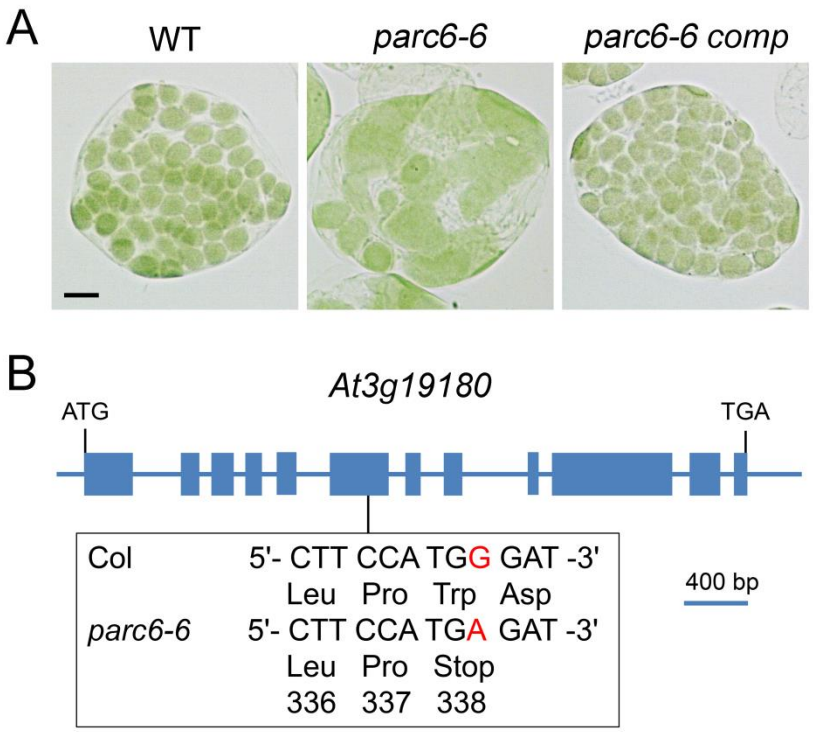

Fig. S3. Identification of a new mutant allele of *PARC6*, *parc6-6*. (*A*) Chloroplast phenotypes of mesophyll cells in 4-week-old plants. WT, wild-type; *parc6-6*, a new mutant allele of *PARC6*; *parc6-6 comp*, *parc6-6* complemented with a wild-type *PARC6* transgene. Scale bar (10 μm) applies to all the images. (*B*) Gene structure and the mutation site of *parc6-6* in *PARC6* gene. Blue boxes indicate exons and the straight lines between blue boxes represent introns. Mutation site and the corresponding site in the wild type (Col) are indicated with red font color. ATG, start codon; TGA, stop codon.

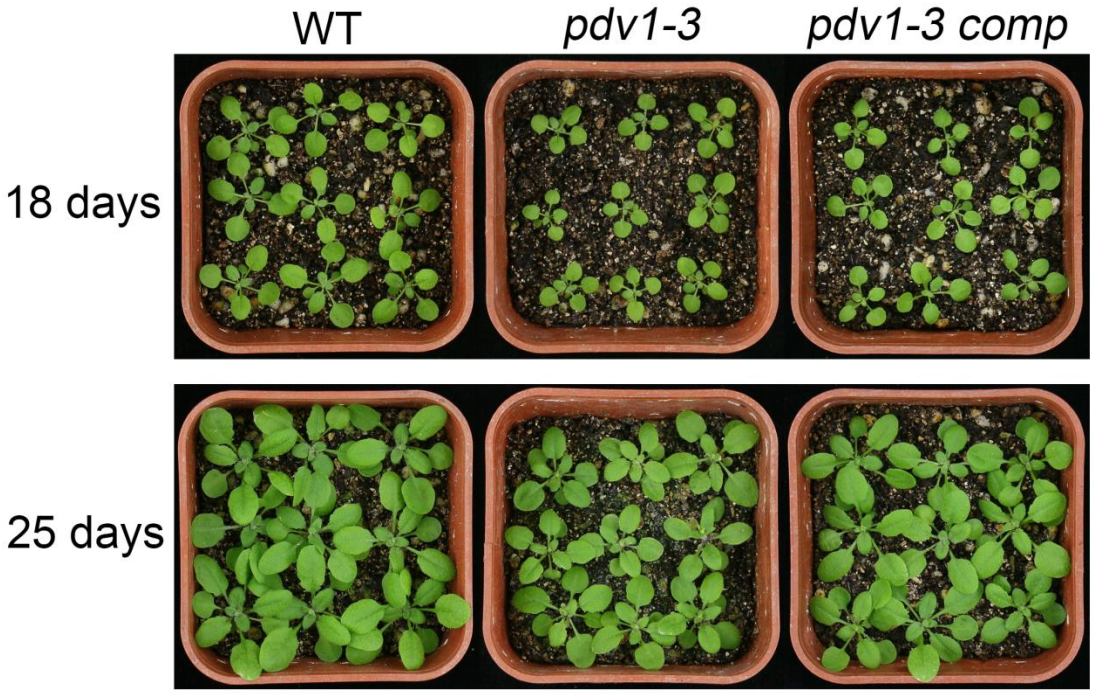

Fig. S4. Arabidopsis plants of 18- and 25-day old. The plants of wild-type, *pdv1-3* and *pdv1-3 comp* (a complementation line) were grown in soil for 18 days (upper panel, fast-growing) and 25 days (lower panel, close to mature).

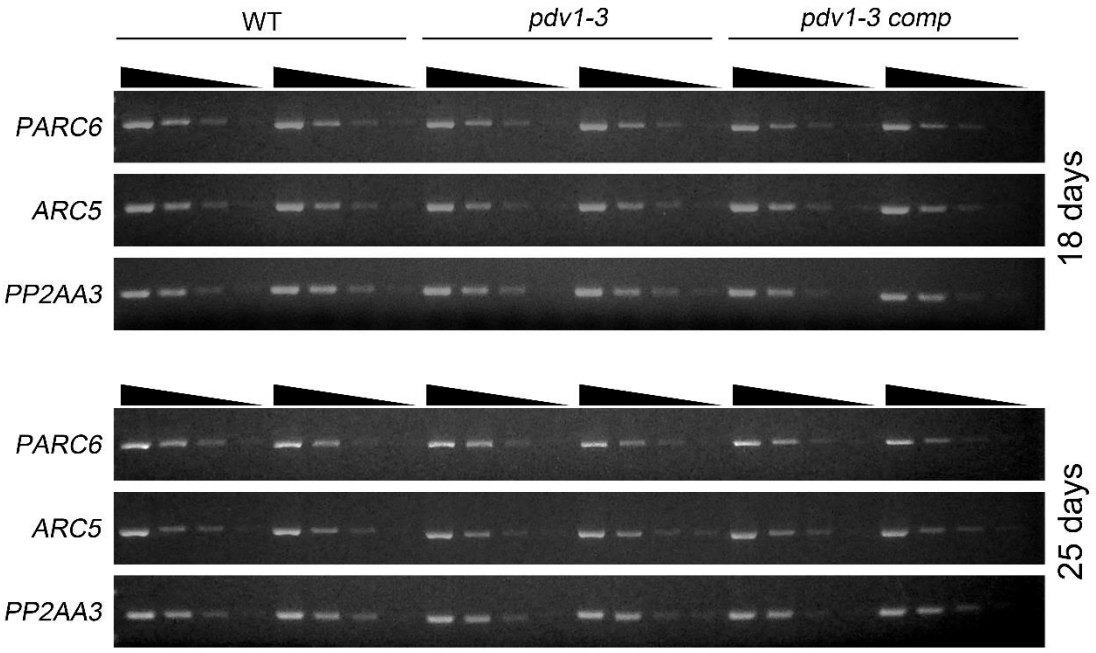

Fig. S5. Semi-quantitative RT-PCR analysis of the transcription level of *PARC6* and *ARC5* gene in WT, *pdv1-3* and *pdv1-3 comp* plants. Total RNA was isolated from the leaves of 18-day-old (upper panel) or 25-day-old (lower panel) plants. The black triangle indicates that the quantity of cDNA was diluted three times continuously, and the dilution factor was four (from left to right). *PP2AA3* was used as a control.

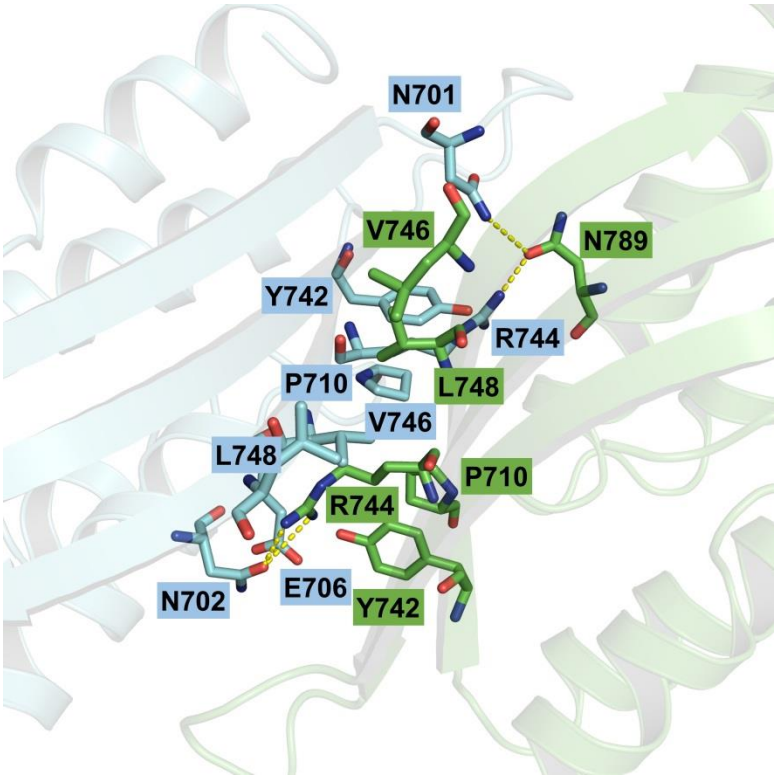

Fig. S6. Interaction at the dimerization interface of two PARC6C protomers. The two PARC6C protomers are shown on the left and right, and colored gray blue and gray green, respectively. Residues involved in interactions are shown as stick models. The hydrogen bonds are shown as yellow dashed lines.

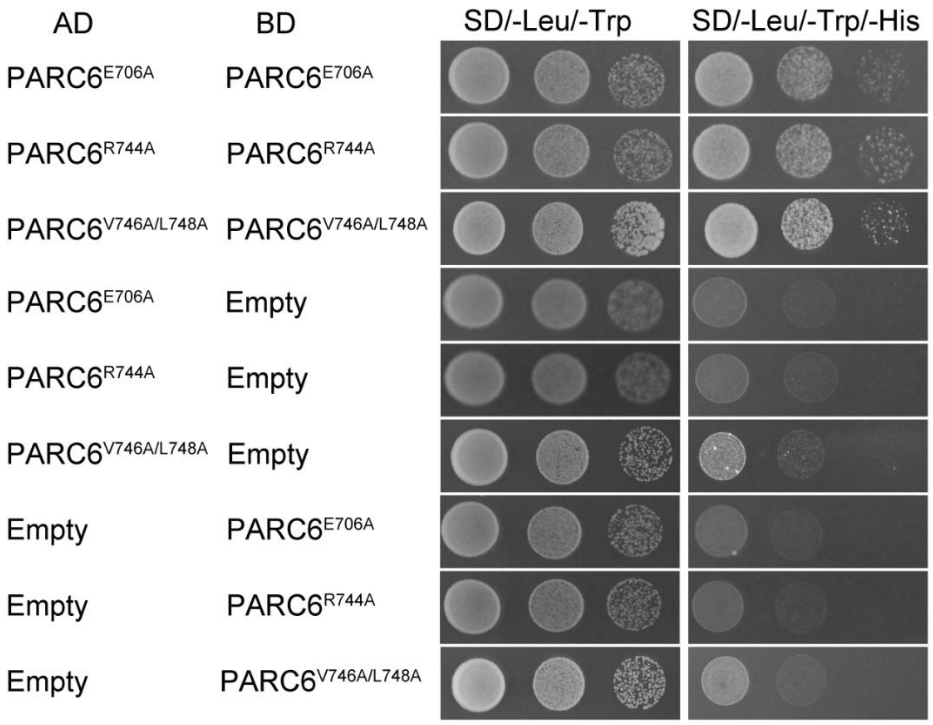

Fig. S7. Yeast two-hybrid assays of the effect of mutations on the self-interaction of PARC6. The two PARC6 molecules could still interact with each other with the mutations PARC6^{E706A}, PARC6^{R744A} and PARC6$^{V746A/L748A}$, but with slightly lower capability. Cotransformed yeast grown in the presence (left panel) or absence (right panel) of histidine are shown. Tenfold serial dilutions were performed from the same starting culture. AD, activation domain; BD, binding domain; SD, synthetic dropout medium; Empty, empty pGADT7 or pGBKT7 vector.

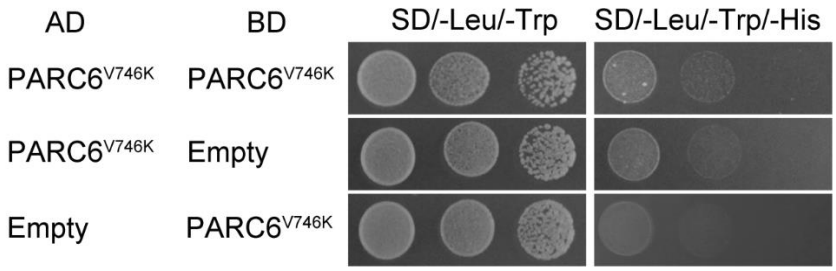

Fig. S8. Yeast two-hybrid assays of the effect of PARC6^{V746K} on PARC6 self-interaction. The PARC6^{V746K} mutation abolished the self-interaction PARC6. Cotransformed yeast grown in the presence (left panel) or absence (right panel) of histidine are shown. Tenfold serial dilutions are performed from the same starting culture. AD, activation domain; BD, binding domain; SD, synthetic dropout medium; Empty, empty pGADT7 or pGBKT7 vector.

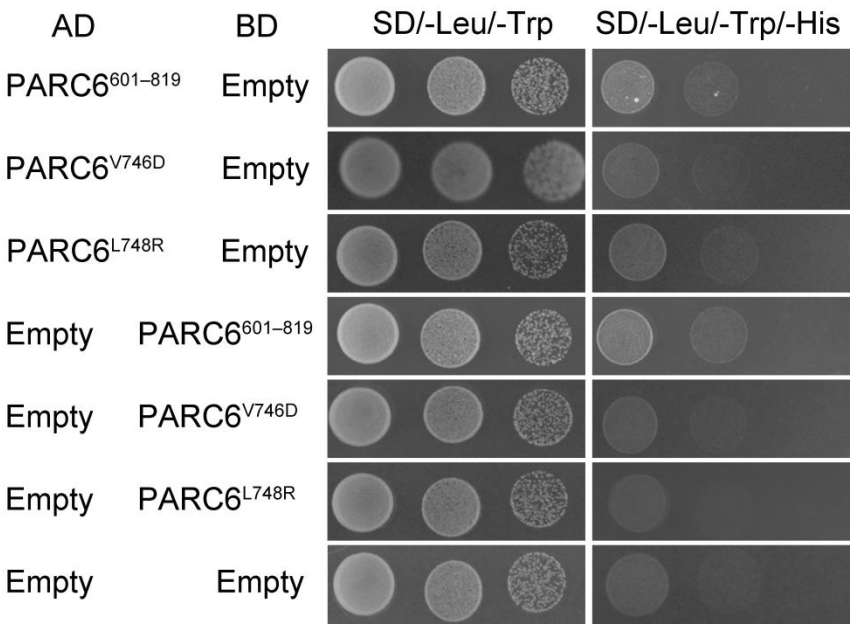

Fig. S9. Negative controls of the yeast two-hybrid assays in Fig. 4*D*. Cotransformed yeast grown in the presence (left panel) or absence (right panel) of histidine are shown. Tenfold serial dilutions were performed from the same starting culture. AD, activation domain; BD, binding domain; SD, synthetic dropout medium; Empty, empty pGADT7 or pGBKT7 vector.

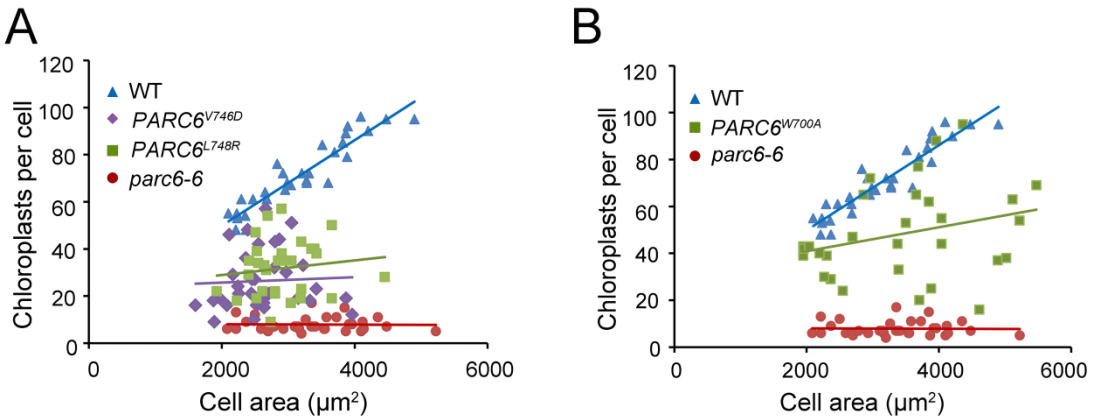

Fig. S10. Quantitative analysis of chloroplast number versus mesophyll cell plan area of four-week-old plants. (*A*) Relationship between chloroplast number and mesophyll cell plan area in WT, *parc6-6*, *PARC6*^{V746D} and *PARC6*^{L748R} shown in Fig. 4*E*. Slopes and R^2 values of the best-fit lines are as follows: WT, 0.0181 (R^2=0.8715); *PARC6*^{V746D}, 0.0011 (R^2=0.0023); *PARC6*^{L748R}, 0.0031 (R^2=0.0172); *parc6-6*, 0.0003 (R^2=0.0049). (*B*) Relationship between chloroplast number and mesophyll cell plan area in WT, *parc6-6*, *PARC6*^{W700A} shown in Fig. 5*E*. Slopes and R^2 values of the best-fit lines are as follows: WT, 0.0181 (R^2=0.8715); *PARC6*^{W700A}, 0.0051 (R^2=0.0793); *parc6-6*, 0.0003 (R^2=0.0049). n ≥ 30 cells for each sample.

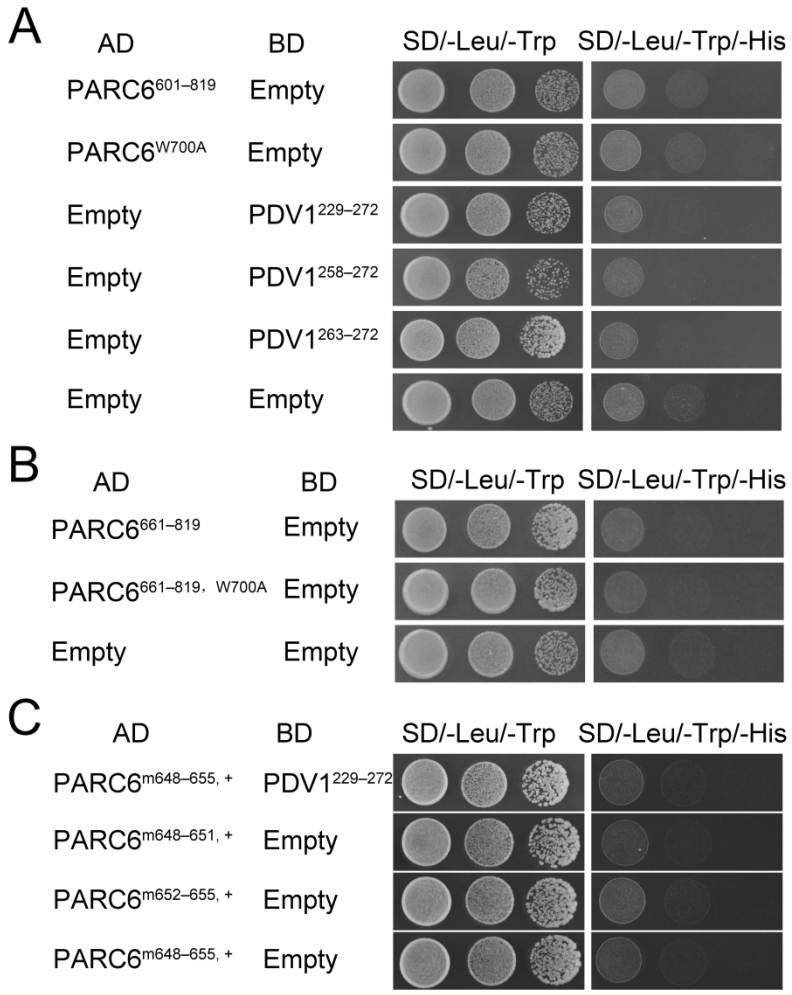

Fig. S11. Negative controls of the yeast two-hybrid assays in Fig. 5*D*, *G* and *H*, respectively. Cotransformed yeast grown in the presence (left panel) or absence (right panel) of histidine are shown. Tenfold serial dilutions were performed from the same starting culture. PARC6$^{m648–655, +}$ represents the mutant with mutations V648S, L649D, I650S, D651A, M652S, L653D, K654A, M655S, and W700A in PARC6$^{601–819}$. AD, activation domain; BD, binding domain; SD, synthetic dropout medium; Empty, empty pGADT7 or pGBKT7 vector.

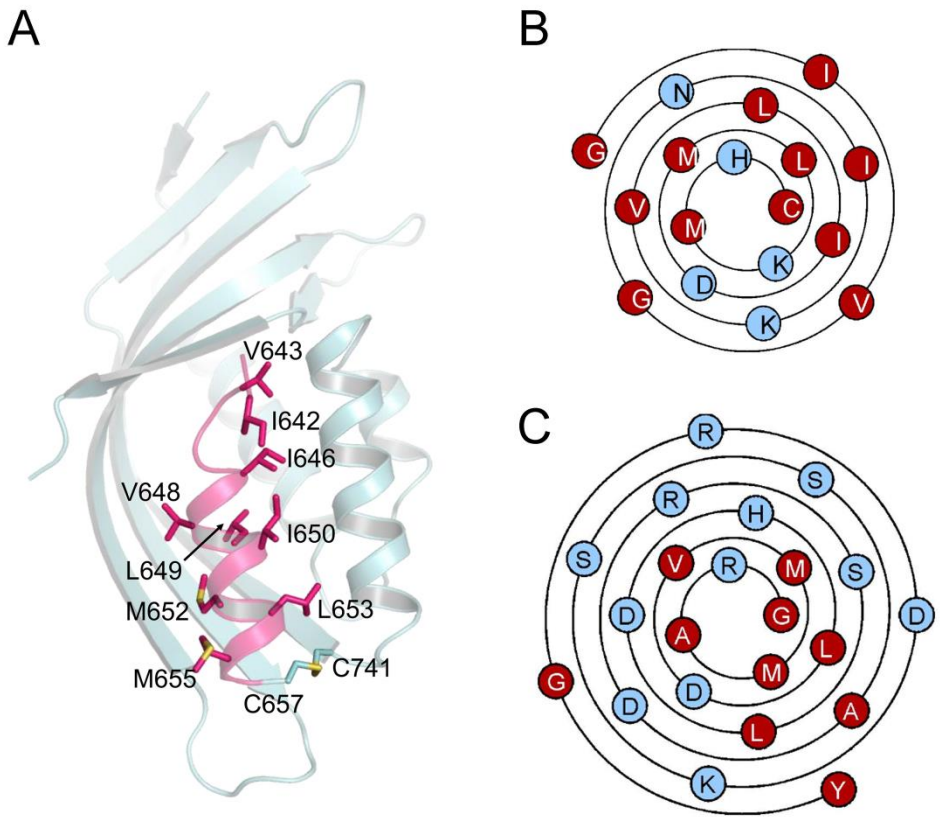

Fig. S12. The lid of PARC6C pocket and the C-terminus of PDV1 both have a hydrophobic side. (*A*) A structural view of the lid of PARC6C pocket. The hydrophobic side chains are shown as red sticks. (*B*) A wenxiang diagram of the lid of PARC6C pocket (residues 641–657). Hydrophobic residues are shown with a red background. (*C*) A wenxiang diagram of the C-terminus of PDV1 (residues 251–272). Hydrophobic residues are shown with a red background.

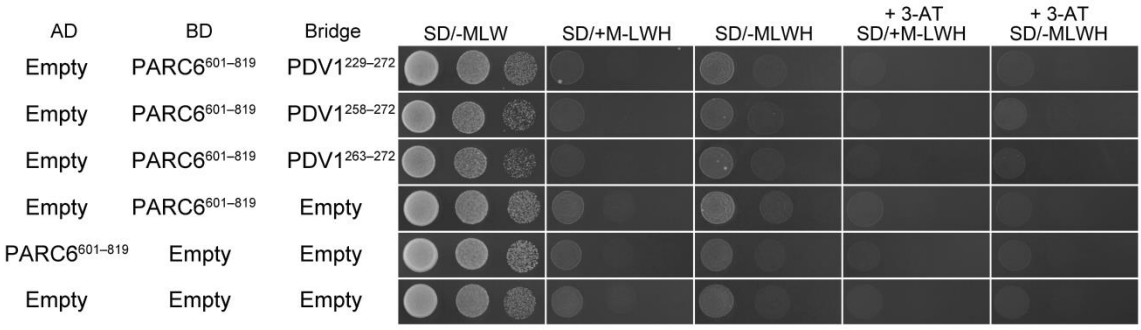

Fig. S13. Negative controls of yeast three-hybrid assays in Fig. 6*A*. As "bridge" proteins, the coding sequences of the C terminal regions of PDV1 (PDV1$^{229–272}$, PDV1$^{258–272}$ or PDV1$^{263–272}$) were cloned into the second multiple clone site of pBridge under the control of the *Met25* promoter. The expression of PDV1 was suppressed by methionine in the growth medium. 3 mM 3-AT was used for a higher stringency.

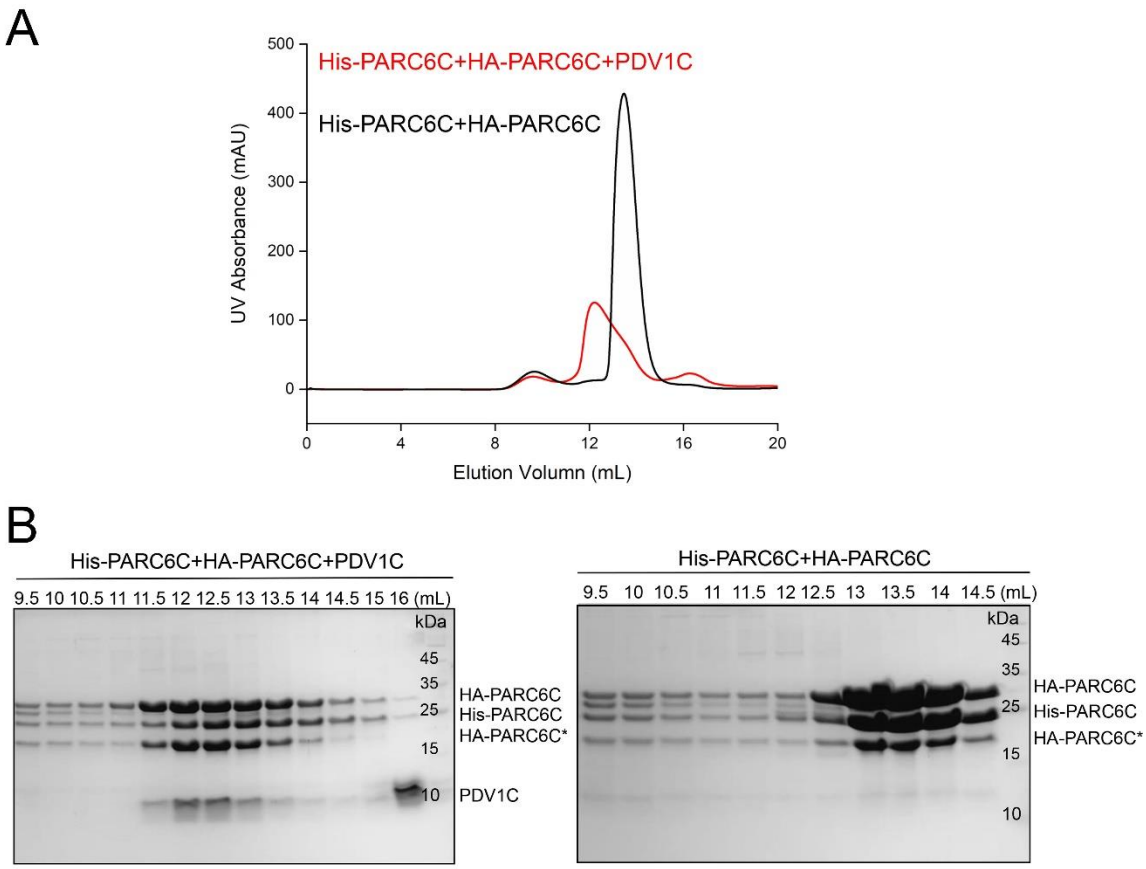

Fig. S14. PDV1C induced the dimerization of PARC6C. (*A*) Gel filtration analysis of the mix of His-PARC6C/HA-PARC6C, and the mix of His-PARC6C/HA-PARC6C/PDV1C (residues 226–272). (*B*) Coomassie Brilliant Blue staining of the peak fractions shown on an SDS–PAGE. HA-PARC6C* indicates the degraded form of HA-PARC6C protein.

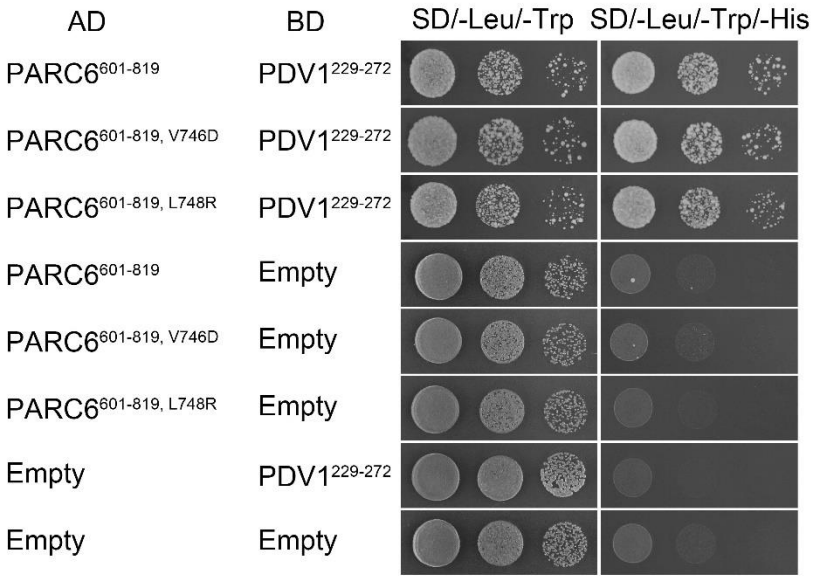

Fig. S15. Yeast-two-hybrid analysis of the effect of PARC6^{V746D} and PARC6^{L748R} mutations on the interaction between PARC6 and PDV1. In Fig. 4D, the mutations of V746D and L748R were shown to abolish the self-interaction of PARC6.

Table S1. The corresponding species and NCBI accession numbers of the sequences used in the phylogenetic analyses in Fig. S1.

Names	Species	Accession numbers
PtPDV2	Populus trichocarpa	XP_002306076.1
NtPDV2	*Nicotiana tabacum*	XP_016455399.1
AtPDV2	*Arabidopsis thaliana*	NP_028242.1
OsPDV2	*Oryza sativa Indica Group*	EAZ02618.1
ZmPDV2	*Zea mays*	NP_001143832.1
PpPDV2-1	*Physcomitrium patens*	PNR34934.1
PpPDV2-2	*Physcomitrium patens*	XP_024360080.1
PpPDV2-3	*Physcomitrium patens*	XP_024404227.1
PpPDV2-4	*Physcomitrium patens*	XP_024402881.1
PpPDV2-5	*Physcomitrium patens*	XP_024393078.1
CbPDV2-like	*Chara braunii*	GBG86295.1
OsPDV1	*Oryza sativa Indica Group*	EAY73086.1
ZmPDV1	*Zea mays*	NP_001182834.1
AtPDV1	*Arabidopsis thaliana*	NP_200140.1
PtPDV1	*Populus trichocarpa*	XP_002318370.2
NtPDV1	*Nicotiana tabacum*	XP_016475564.1
PtARC6	*Populus trichocarpa*	XP_006383731.2
NtARC6	*Nicotiana tabacum*	XP_016465359.1
AtARC6	*Arabidopsis thaliana*	NP_199063.1
OsARC6	*Oryza sativa (indica cultivar-group)*	DAA01472.1
ZmARC6	*Zea mays*	NP_001148117.1
SpFtn2	*Synechocystis sp. PCC 6714*	AIE75001.1
PpARC6-1	*Physcomitrium patens*	XP_024361348.1
PpARC6-2	*Physcomitrium patens*	XP_024359405.1
OsPARC6	*Oryza sativa Japonica Group*	XP_015636653.1
ZmPARC6	*Zea mays*	NP_001346328.1
AtPARC6	*Arabidopsis thaliana*	NP_188549.2
PtPARC6	*Populus trichocarpa*	XP_024438100.1
NtPARC6	*Nicotiana tabacum*	XP_016514193.1

Table S2. Data collection and refinement statistics.

	PARC6$^{640–819}$	**PARC6$^{685–819}$ – PDV1$^{263–272}$**
Data collection		
Space group	$C222_1$	$C222_1$
Cell dimensions		
a, *b*, *c* (Å)	44.128, 121.669, 130.206	91.454, 95.827, 196.566
α, β, γ (°)	90.00, 90.00, 90.00	90.00, 90.00, 90.00
Resolution (Å)	50-2.534	50-2.894 (2.997-2.894)*
R_{sym} or R_{merge} (%)	18.1 (90.1)	19.4 (89.0)
$I/\sigma(I)$	13.2 (2.5)	11.3 (2.6)
Completeness (%)	99.9 (100.0)	99.7 (99.9)
Redundancy	10.7 (9.7)	7.1 (6.1)
Refinement		
Resolution (Å)	44.45-2.534	47.91-2.894
No. reflections	11333	18678
R_{work} / R_{free}	0.219/0.271	0.223/0.271
No. atoms	2460	4583
Protein	2447	4583
Ligand/ion	0	0
Water	13	0
B factors	40.54	46.05
Protein	40.59	46.05
Ligand/ion		
Water	31.45	
R.m.s. deviations		
Bond lengths (Å)	0.005	0.011
Bond angles (°)	1.18	1.55
Ramachandran plot (%)		
Favored	96.91	97.79
Allowed	2.41	2.03
Outliers	0.69	0.18

*For each structure one crystal was used. *Values in parentheses are for highest-resolution shell.

Table S3. Sequences of primers used in this study.

Primers	Sequences (5'-3')
Primers for constructing plasmids used in Y2H assays	
PDV1Y2H-1	CCTCATATGGCTACTAGTGAACATCATCTGCAG
PDV1Y2H-2	CCTCATATGGCTGACAGAAGTTTGGATCACTTG
PDV1Y2H-3	CCTCATATGGATCACTTGGACGTAATGATGGCTC
PDV1Y2H-4	CCTGGATCCGCCGAGAGATCTTGTGATTTACAAG
PARC6Y2H-1	CCTCATATGGGTAGACTACAGAGTATGCCTATATC
PARC6Y2H-2	CCTCATATGCCGGATGCCCTGTATCTGAAAAG
PARC6Y2H-3	CCTGGATCCGACAAATCCTGTGGGTTACTAAGG
PARC6-745Up	GAACCTCCAATAACAGGATTTC
PARC6-V746D	GATCTGCTTCATCTTGAGGTTTTG
PARC6-747Up	CAGAACGAACCTCCAATAACAG
PARC6-L748R	AGGCATCTTGAGGTTTTGCAAGC
PARC6-699Up	CTGTCTCACAAGCTCTTCC
PARC6-W700A	GCGGAAAATGTTAAGGCTGAAGC
PARC6-648-51Mu	GGCAGAGTCCGATTTGATGTTTCCCACGATACC
PARC6-652down	ATGTTAAAGATGCATTGTGGCG
PARC6-651up	GTCAATGAGCACTTTGATGTTTCC
PARC6-652-55 Mu	TCTGATGCGTCCCATTGTGGCGAACATCCG
PARC6-705Up	AGCCTTAACATTTTCCCACTG
PARC6-E706A	GCAGCTCTTGGACCAACACATC
PARC6-743Up	CCAATAACAGGATTTCGCC
PARC6-R744A	GCTTTCGTTCTGCTTCATCTTGAG
PARC6-V746A/L748A	GCTCTGGCACATCTTGAGGTTTTGCAAGC
PARC6-V746K	AAACTGCTTCATCTTGAGGTTTTG
Primers for constructing plasmids used in Y3H assays	
ARC6H-8	GTTGGATCCACCTGAGACTTTCCTACACCG
ARC6H-20	CCTGAATTCGGTAGACTACAGAGTATGCC
PDV1-34	CCTGCGGCCGCTGCTACTAGTGAACATCATCTGC
PDV1-35	CCTAGATCTGATTTACAAGAAAGCCTAACCAC
PDV1-36	CCTGCGGCCGCTGACAGAAGTTTGGATCACTTG
PDV1-37	CCTGCGGCCGCTCACTTGGACGTAATGATGG
Primers for constructing plasmids used in plant transformation	
ARC6H-10	CATGGATCCTCAGCCTCATCTTGACCTA
ARC6H-11	CATACGCGTCAAACCTGAGACTTTCCTACAC
PARC6-745Up	GAACCTCCAATAACAGGATTTC
PARC6-V746D	GATCTGCTTCATCTTGAGGTTTTG

PARC6-747Up	CAGAACGAACCTCCAATAACAG
PARC6-L748R	AGGCATCTTGAGGTTTTGCAAGC
PARC6-699Up	CTGTCTCACAAGCTCTTCC
PARC6-W700A	GCGGAAAATGTTAAGGCTGAAGC
PDV1-15	CTTACGCGTGTGTAGACAAGTTCGATCTCTGG
PDV1-28	CCTGGATCCCTGTTTCAAAAGGCCGAAC
Primers for semi-quantitative RT-PCR analysis	
PARC6-652down	ATGTTAAAGATGCATTGTGGCG
PARC6-789Up	TTTTGCGTTTTTGGGCTGAG
ARC5-4	CCTTGCTCACGGTATCCAGC
ARC5-5	CCTTGCTCACGGTATCCAGC
PP2AA3-1	CCAAGCGGTTGTGGAGAAC
PP2AA3-2	GAACCAAACACAATTCGTTGCTG
Primers for constructing plasmids used in protein expression	
PARC6-Nde640f	GGGAAACATATGAATGGTATCGTGGGAAA
PARC6-Xho819rstop	CCGCTCGAGTCACTTCTGTATTTGAAT
PARC6-Nde685f	GGGAAACATATGAAGAGACCAATGGATAC
PARC6-819-linker-f	GATATTCAAATACAGAAGTCTACCGGAAACGCCT
linker-addPDV1EcoRr	CGGAATTCTTAACCACGAGCCATCATTACGTCCAAGTGATCAGAT CCGACGGTTCCGTCTCCT
PARC6-Nde642f	ATACATATGATCGTGGGAAACATCAAAGTGCTCATTGACATGTTA AAGATGCATTCTGGCGA
PARC6-W700A-f	AAGAGCTTGTGAGACAGGCGGAAAATGTTAAGGCTGA
PARC6-W700A-r	TCAGCCTTAACATTTTCCGCCTGTCTCACAAGCTCTT
PDV1-Nde226f	GGAATTCCATATGCACCAAGTGGCTACTAGTGAA
PDV1-Xho272rstop	CCGCTCGAGTTAACCACGAGCCATCAT
PARC6-640f1	GATGTTCCAGATTATGCATATCCCTATGACGTACCCGACTATGCA AATGGTATCGTGGG
PARC6-Ndef2	ATACATATGTACCCATACGATGTTCCAGATTACGCTTACCCATACG ATGTTCCAGATTA
PARC6-685f1	GATGTTCCAGATTATGCATATCCCTATGACGTACCCGACTATGCA AAGAGACCAATGGA