# Supplementary Table S1

**A**

| | Arabidopsis | Rice | Tomato | Maize | Soybean | *N. benthamiana* |
|---|---|---|---|---|---|---|
| | *A. thaliana* | *O. sativa* | *S. lycopersicum* | *Z. mays* | *G. Max* | *N. benthamiana* |
| CCT1 | AT3G20050 | LOC_Os04g46620.1 | Solyc01g090750.2.1 | GRMZM2G039263_P01 | Glyma05g29870.1 | Niben101Scf00397g00001.1 |
| | | | | GRMZM2G110626_P01 | Glyma08g12970.1 | Niben101Scf08606g00014.1 |
| CCT2 | AT5G20890 | LOC_Os03g42220.1 | Solyc11g069000.1.1 | GRMZM2G058276_P01 | Glyma12g09250.1 | Niben101Scf05890g02025.1 |
| | | LOC_Os05g48290.3 | | | Glyma11g19220.1 | Niben101Scf04560g03018.1 |
| CCT3 | AT5G26360 | LOC_Os06g34690.1 | Solyc05g056310.2.1 | GRMZM2G175510_P01 | Glyma16g33380.1 | Niben101Scf01791g03001.1 |
| | | LOC_Os02g14929.1 | | GRMZM2G069765_P01 | Glyma09g28650.2 | Niben101Scf32212g00014.1 |
| | | | | | Glyma20g35760.1 | Niben101Scf04375g08004.1 |
| CCT4 | AT3G18190 | LOC_Os02g22780.1 | Solyc03g118910.1.1 | GRMZM2G122767_P01 | Glyma08g05470.1 | Niben101Scf08653g02001.1 |
| | | LOC_Os10g37060.1 | | GRMZM2G085909_P01 | Glyma05g34190.1 | Niben101Scf03550g00017.1 |
| | | | | | Glyma07g18110.1 | |
| CCT5 | AT1G24510 | LOC_Os06g36700.1 | Solyc05g013990.2.1 | GRMZM2G070542_P01 | Glyma11g37630.2 | Niben101Scf02949g03001.1 |
| | | | | GRMZM2G043383_P01 | Glyma18g01580.1 | Niben101Scf12680g01003.1 |
| CCT6 | AT5G16070 | LOC_Os05g05470.1 | Solyc02g085790.2.1 | GRMZM2G109425_P01 | Glyma18g53590.1 | Niben101Scf00894g01011.1 |
| | AT3G02530 | | Solyc02g063090.2.1 | | Glyma08g47920.1 | Niben101Scf02772g05001.1 |
| | | | | | | Niben101Scf02156g02014.1 |
| CCT7 | AT3G11830 | LOC_Os06g47320.1 | Solyc06g065520.2.1 | GRMZM2G009871_P01 | Glyma14g04770.1 | Niben101Scf06180g00016.1 |
| | | | | | Glyma02g44080.1 | |
| CCT8 | AT3G03960 | LOC_Os03g59020.1 | Solyc01g088080.2.1 | GRMZM2G083095_P01 | Glyma16g26920.1 | Niben101Scf12801g00001.1 |
| | | | | | Glyma02g07910.1 | Niben101Scf03572g02005.1 |

**B**

| Gene name | Loci number |
|---|---|
| *Tap46* | AT5G53000 |
| *PPX1* | AT4G26720 |
| α-tubulin isoform 6 (*TUA6*) | AT4G14960 |
| β-tubulin isoform 5 (*TUB5*) | AT1G20010 |
| β-tubulin isoform 6 (*TUB6*) | AT5G12250 |

**Supplementary Table S1**. Accession and loci numbers of CCT subunit genes (A) and other genes (B) used in this study.

There are eight CCT subunit genes in yeast, while there are nine subunit genes in *Arabidopsis*, tomato, and humans, due to an additional copy of the *CCT6* gene. Other plant species analyzed have higher numbers of CCT subunit genes. There are 11 CCT subunit genes in rice, with two genes each for *CCT2*, *CCT3* and *CCT4*, while there are 12 genes in maize, with two genes each for *CCT1*, *CCT3*, *CCT4*, and *CCT5*. Soybean and *N. benthamiana* respectively have 18 and 17 CCT subunit genes in the genome, more than twice the number of CCT subunit genes in yeast. This may be due to allopolyploidy in soybean and allotetraploidy in *N. benthamiana*.

**Supplementary Table S2**. List of primers used in this study.

| Primer name | Nucleotide sequences (5' → 3') |
|---|---|
| **Primers for GFP-CCTx** | |
| GFP/Flag-CCT1 F | GGA TCC ATG TCG ATC TCC GCC CAA |
| GFP/Flag-CCT1 R | CCA TGG TTA TTC TTC GCC TTG GCT |
| GFP/Flag-CCT2 F | GGA TCC ATG CCG ATC GAT AAG ATC |
| GFP/Flag-CCT2 R | CCA TGG TCA CAT CCT GTC TTC TCT |
| GFP/Flag-CCT3 F | GGA TCC ATG CAC GCA CCG GTA CTC |
| GFP/Flag-CCT3 R | CCA TGG TTA GTC GGG AAG AAT TTG |
| GFP-CCT4 F | CCC GGG AAT GGC GGC GGT AGC GGC A |
| GFP-CCT4 R | CCC GGG CTA CCT CAC AGT TAC GAT |
| GFP-CCT5 F | CCC GGG AAT GGC GCT GGC GTT CGA T |
| GFP-CCT5 R | CCA TGG TCA GTA TTC AGA ATT GGA |
| GFP-CCT6-1 F | GGA TCC ATG TCA GTG CGA GTT CTG |
| GFP-CCT6-1 R | ACT AGT TCA AGC AGT AGG CTT CCT |
| GFP/Flag-CCT6-2 F | GGA TCC ATG TCT GTG CGT GTA CTG |
| GFP-CCT6-2 R | ACT AGT TTA AGT AGG CTT CCT CAT |
| GFP-CCT7 F | CCC GGG AAT GGC ATC GAT GAT GCA A |
| GFP-CCT7 R | CCA TGG TTA TCG CCT TCG CAT TCC |
| GFP-CCT8 F | GGA TCC ATG GTG GGT ATG TCG ATG |
| GFP-CCT8 R | ACT AGT TTA GTC TTC CTC CGC ACC |
| **Primers for Flag-CCTx** | |
| Flag-CCT4 F | GAA TTC TAA TGG CGG CGG TAG CGG CA |
| Flag-CCT4 R | GAA TTC CTA CCT CAC AGT TAC GAT |
| Flag-CCT5 F | CCC GGG AAT GGC GCT GGC GTT CGA T |
| Flag-CCT5 R | CCA TGG TAT CAG TAT TCA GAA TTG GA |
| Flag-CCT6-1 F | GGA TCC ATG TCA GTG CGA GTT CTG |
| Flag-CCT6-1 R | AGA TCT GTC AAG CAG TAG GCT TCC T |
| Flag-CCT6-2 R | AGA TCT GTT AAG TAG GCT TCC TCA T |
| Flag-CCT7 F | CCC GGG AAT GGC ATC GAT GAT GCA A |
| Flag-CCT7 R | CCA TGG TAT TAT CGC CTT CGC ATT CC |
| Flag-CCT8 F | GAA TTC TAA TGG TGG GTA TGT CGA TG |
| Flag-CCT8 R | GAA TTC TTA GTC TTC CTC CGC ACC |
| **Primers for VIGS constructs** | |
| TRV2:CCT2 F | GGA TCC GTT TAT TCT TGA CAA GAA |
| TRV2:CCT2 R | GGT ACC CTT CTA TAA GCT TGC AAT |
| TRV2:CCT3 F | GGA TCC GGT AGA CAT TAA AAA GTA |
| TRV2:CCT3 R | GGT ACC TGT TCA CAA TTA CTG CTC |
| TRV2:TUA6 F | TCT AGA GAT GTA CCG TGG TGA TGT AGT C |
| TRV2:TUA6 R/TUA6-Myc R | CCCGGGA GTATTCCTCTCCTTCATC |
| TRV2:Tap46 F | GGATTCATGGGTGGTTTGGCTATG |
| TRV2:Tap46 R | GGTACCTTCGATCAGCAGGTGCAC |
| TRV2:PPX1/2 F | GGA TCC TTC CTG CGG AGA GGA GCT |
| TRV2:PPX1/2 R | GGT ACC TCC TTC AAA GCC TCG CAG C |
| TRV2:PPX1/2 mid F | ATT GAG CGA ATC GGA ACA GAG AGT TTG GGG |
| TRV2:PPX1/2 mid R | CCC CAA ACT CTC TGT TCC GAT TCG CTC AAT |

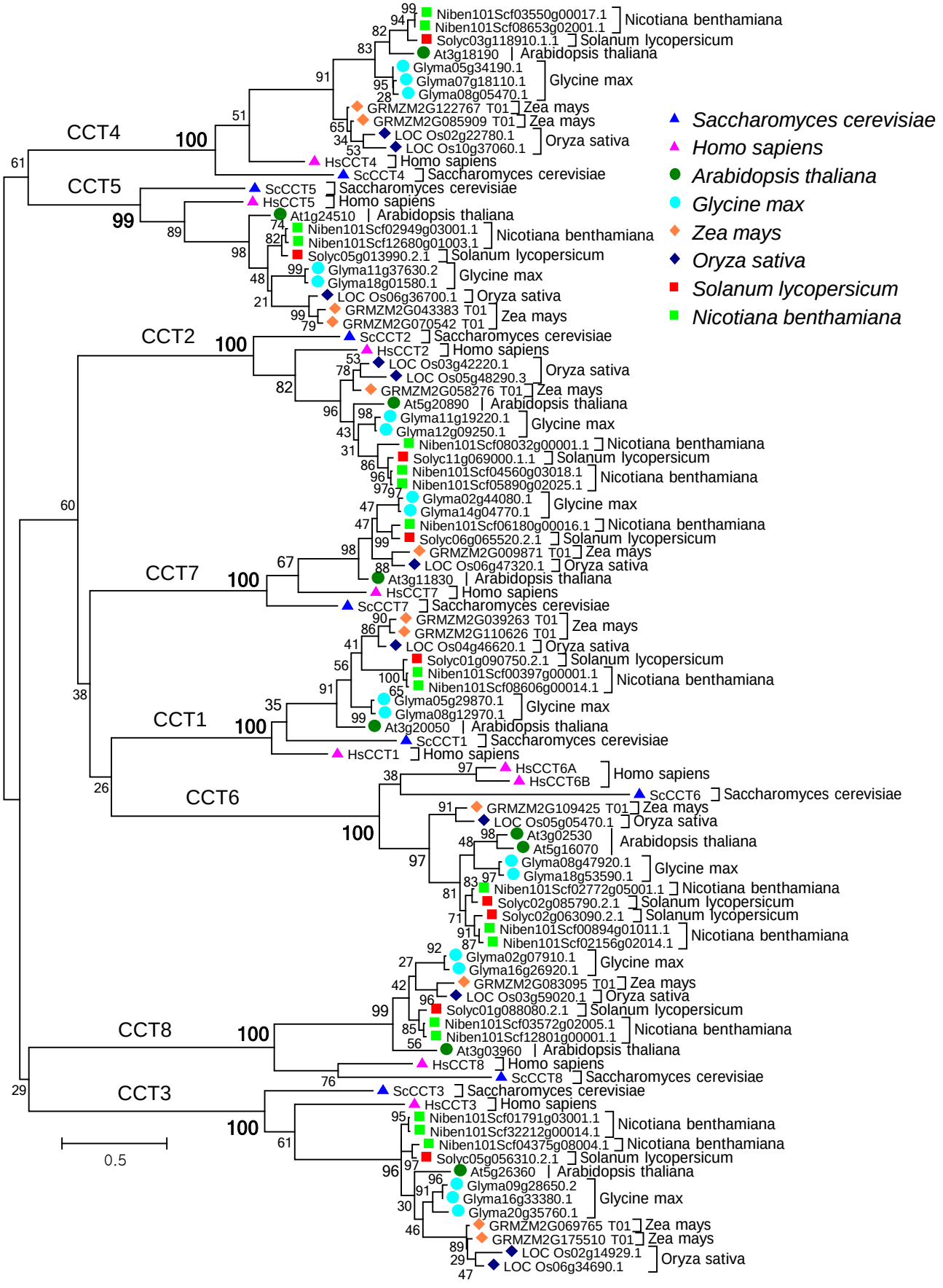
# Supplementary Table S2 (Cont.)

| Primer name | Nucleotide sequences (5' → 3') |
|---|---|
| **Primers for Myc constructs** | |
| TUA6-Myc F | GTC GAC ATG AGA GAG TGC ATT TCG ATC |
| TUB5-Myc F | CCC GGG ATG AGA GAG ATC CTT CAC |
| TUB5-Myc R | CCA TGG TGA GTC TCA TAA TCT CCC TCC |
| Tap46-Myc F | GAA TTC TAA TGG GTG GTT TGG CTA TG |
| Tap46-Myc R | CCA TGG TCA GCC ACA AGG TGT GAG |
| Tap46-N-Myc R | CCA TGG TTC CTT TAT TGC AGA GAG CAT TT |
| Tap46-C-Myc F | GAA TTC TAA GAC AGT TGA AGG ATG GA |
| PPX1-Myc F | GTC GAC ATG TCA GAC CTA GAT CGG |
| PPX1-Myc R | CCC GGG TTA TAG GAA GTA ATC AGG |
| **Primers for BiFC constructs** | |
| CCT2-SPYNE F | CTC GAG ATG CCG ATC GAT AAG ATC |
| CCT2-SPYNE R | CCC GGG CAT CCT GTC TTC TCT CCT |
| CCT1-SPYCE F | GTT AAC ATG TCG ATC TCC GCC CAA AAT CC |
| CCT1-SPYCE R | CCCGGGTTCTTCGCCTTGGCTCTC |
| CCT3-SPYCE F | CTC GAG ATG CAC GCA CCG GTA CTC |
| CCT3-SPYCE R | CCC GGG GTC GGG AAG AAT TTG TTC |
| CCT4-SPYCE F | CCC GGG ATG GCG GCG GTA GCG GCA |
| CCT4-SPYCE R | CCC GGG CCT CAC AGT TAC GAT GTC |
| CCT5-SPYCE F | CTC GAG ATG GCG CTG GCG TTC GAT |
| CCT5-SPYCE R | CCC GGG GTA TTC AGA ATT GGA GAT |
| CCT6-1-SPYCE F | TCT AGA ATG TCA GTG CGA GTT CTG |
| CCT6-1-SPYCE R | CCC GGG AGC AGT AGG CTT CCT CAT |
| CCT6-2 SPYCE F | TCT AGA ATG TCT GTG CGT GTA CTG AA |
| CCT6-2 SPYCE R | CCC GGG AGT AGG CTT CCT CAT ATT |
| CCT7 SPYCE F | CTC GAG ATG GCA TCG ATG ATG CAA CCG |
| CCT7 SPYCE R | CCC GGG TCG CCT TCG CAT TCC ACG |
| CCT8 SPYCE F | TCT AGA ATG GTG GGT ATG TCG ATG CAG C |
| CCT8 SPYCE R | ACT AGT GTC TTC CTC CGC ACC AGC AC |
| Tap46-SPYN/CE F | GGA TCC ATG GGT GGT TTG GCT AT |
| Tap46-SPYN/CE R | CTC GAG GCC ACA AGG TGT GAG TTT |
| PPX1-SPYN/CE F | ATC GAT ATG TCA GAC CTA GAT CGG |
| PPX1-SPYN/CE R | CCC GGG TAG GAA GTA ATC AGG GGC |
| **Primers for semi-quantitave RT-PCR** | |
| UBC21 RT-PCR F | CAAGAGCGCGACTGTTTAAA |
| UBC21 RT-PCR R | TCCTTTCTTAGGCATAGCGG |
| **Primers for Tap46-His recombinant protein** | |
| Tap46-His F | GGA TCC ATG GGT GGT TTG GCT ATG |
| Tap46-His R | CTC GAG GCC ACA AGG TGT GAG TTT |

# Supplementary Table S2 (Cont.)

| Primer name | Nucleotide sequences (5' → 3') |
|---|---|
| **Primers for RT-qPCR** | |
| CCT1 qPCR F | GTC GGC CTC GAC AAG ATG |
| CCT1 qPCR R | CGA CCT CCA ACA TCC TAA GAA |
| CCT2 qPCR F | CTA AGA TTC ACC CTA TGA CCA TCA |
| CCT2 qPCR R | TTC AGT AAA GCA TTA CGA GCA CA |
| CCT3 qPCR F | CGC TCA TCC TGC AGC TAA GT |
| CCT3 qPCR R | ACC AGC TAG AAC AAT AAC AGA CGT T |
| CCT4 qPCR F | AGT GAG CAA AAG GTT TTT GAT TG |
| CCT4 qPCR R | ACC GAG CTG CCT TGA GAG |
| CCT5 qPCR F | TTG CTC ACT TGC GGT TGA T |
| CCT5 qPCR R | GCA AAC GCC CTA ATT GCA TA |
| CCT6-1 qPCR F | GAA AGC CTG AGG AAG CTA TTG A |
| CCT6-1 qPCR R | GAA CAA GCC CCT CAA CCA |
| CCT6-2 qPCR F | AGT GCC TAA GAC GCT TGC TG |
| CCT6-2 qPCR R | TCC TTT GTC ATG CTC ACT CG |
| CCT7 qPCR F | TCC CAA GAC TCC GAG GTG |
| CCT7 qPCR R | GGC TTC GCT TC TTC AAA |
| CCT8 qPCR F | GTC GCC CAT TTG AAG CTT AG |
| CCT8 qPCR R | CTC GTT TCT TGC AAT CGT GA |
| TUA6 qPCR F | GAA GAA TGT TTC TTA AAA ATT GGA TTT T |
| TUA6 qPCR R | ACC AGC AAG AGA TAG AAA TAT AGG TTT AG |
| TUB6 qPCR F | GTG AAA AGA GCT GAT ATT ACC GA |
| TUB6 qPCR R | TAC ACA CAG ACC ATC ACA AAC AA |
| PPX1/2 qPCR F | ATT CCG TGT GTT TGA TGC AG |
| PPX1/2 qPCR R | CGA AGT TTT GCC CAT TAT AGG A |
| UBC10 qPCR F | ATG GGT CCT TCA GAG AGT CCT |
| UBC10 qPCR R | CTT GGT CCT AAA GGC CAC CT |

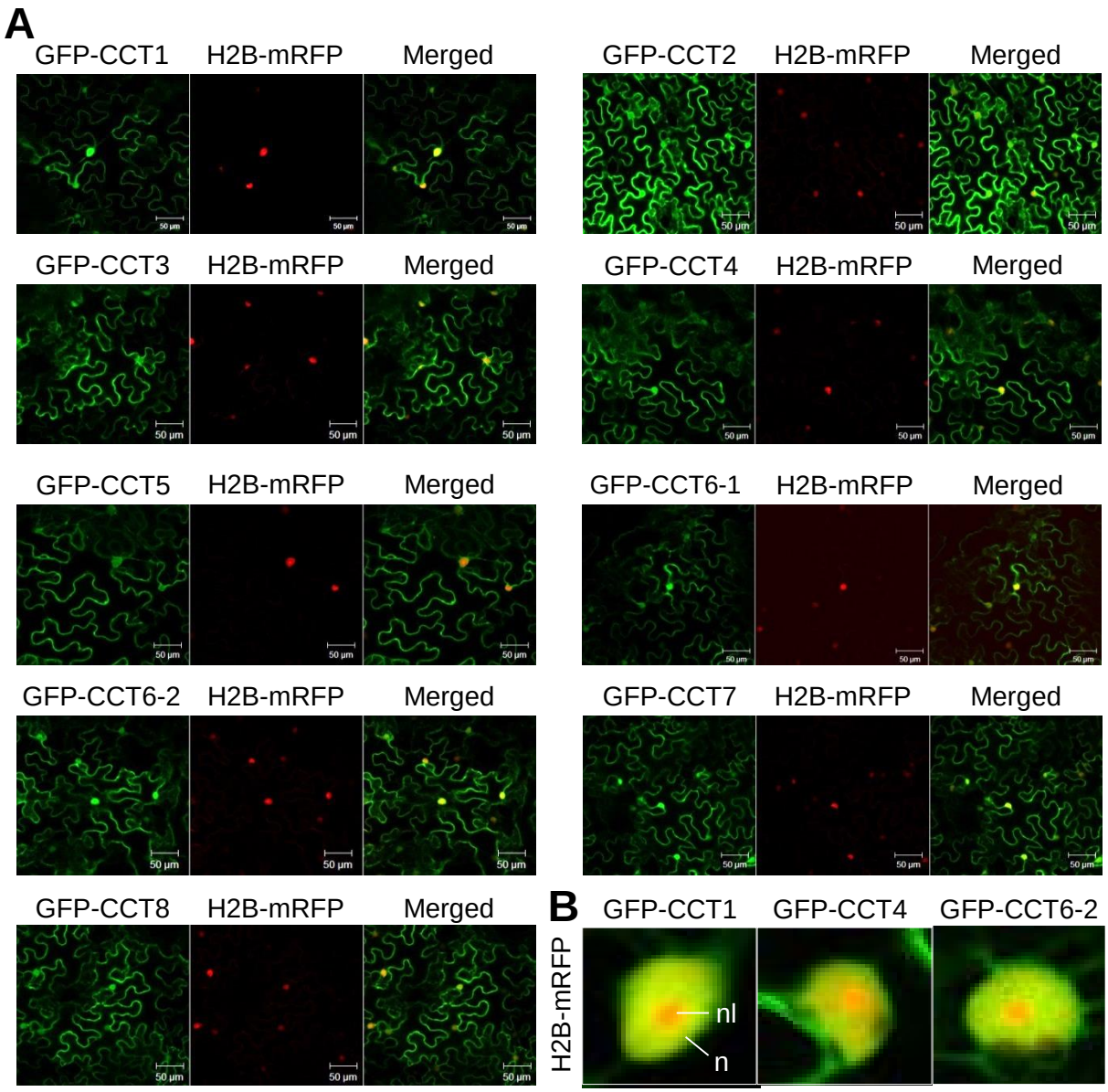
# Supplementary Figure S1



**Supplementary Fig. S1.** Phylogenetic tree analysis of CCT subunits.

Phylogenetic tree of CCT subunits were generated using the software MEGA7 (Kumar et al., 2016) with nucleotide sequences from *Saccharomyces cerevisiae*, *Homo sapiens, A. thaliana*, *G. max*, *Z. mays*, *O. sativa*, *S. lycopersicum*, and *N. benthamiana*. The tree branches are labeled with a bootstrap score based on 1,000 bootstrap replicates. The branch lengths were measured as the number of substitutions per site. Bootstrap values for each cluster of CCT subunits are shown in *bold*. The scale bar represents 0.5 amino acid substitutions per site in the primary structure.

# Supplementary Figure S2



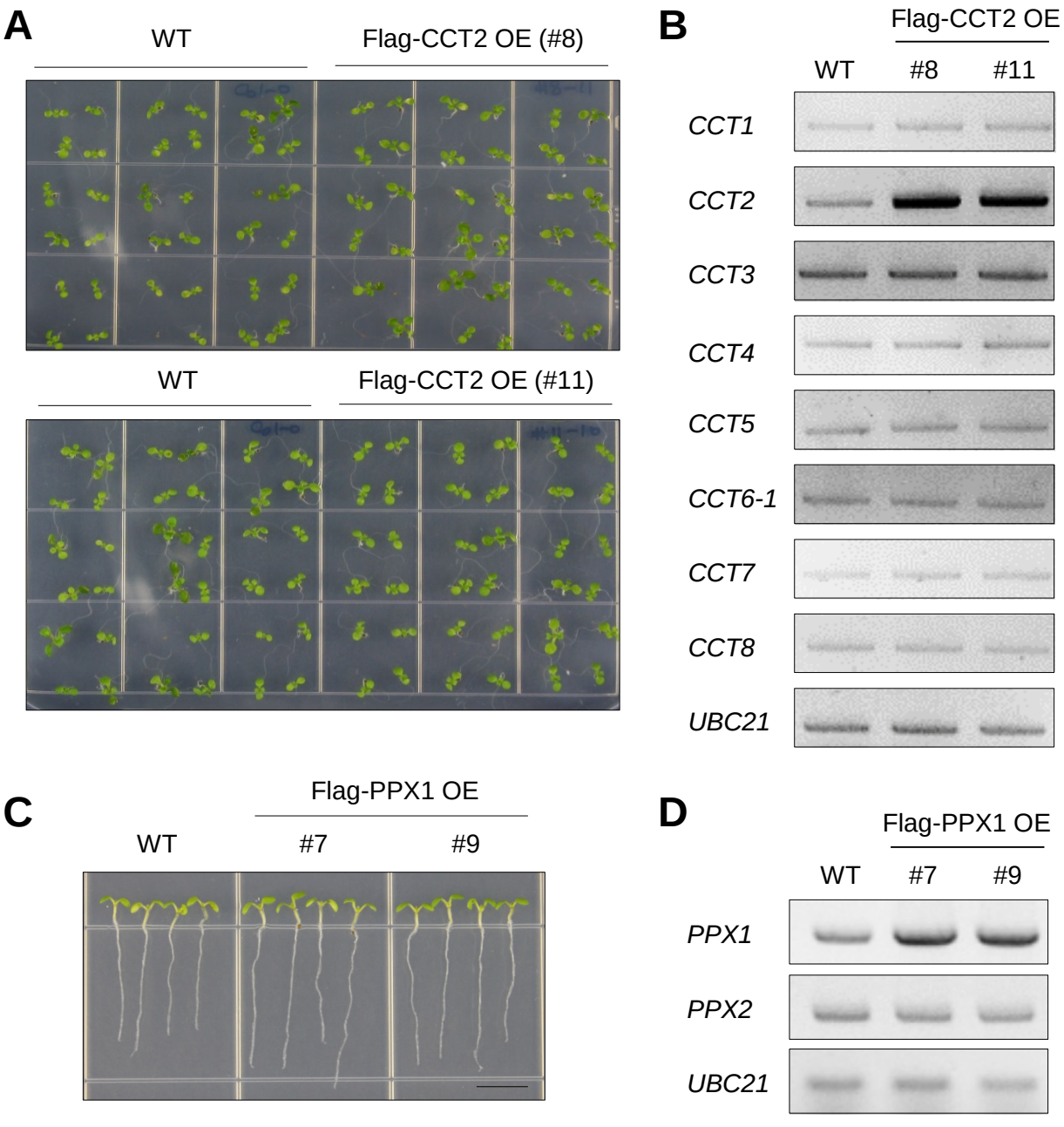
**Supplementary Fig. S2.** Subcellular localization of *Arabidopsis* CCT subunits.

To investigate the cellular functions of the CCT complex in plants, we first determined the subcellular localization of *Arabidopsis* CCT subunits. GFP was fused to the N-terminus of each CCT subunit and transiently expressed in *N. benthamiana* leaves via agroinfiltration, along with mRFP-tagged histone 2B (H2B-mRFP) as a nuclear marker. Confocal laser scanning microscopy of leaf epidermal cells showed that all CCT subunits were mainly localized to the cytosol, but were also detected in the nucleoplasm, but not in the nucleolus. Many of these subunits were predicted to possess nuclear localization signals within their sequences, supporting this observation.

(A) *Arabidopsis* CCT subunits as GFP-fusion proteins were expressed in *N. benthamiana* leaves together with the nuclear marker H2B-mRFP. Fluorescence signals were observed 2 days post-infiltration (DAI). Merged images are shown on the right.

(B) Enlargement of selected images of nuclei from (A). Merged images of GFP-CCT and H2B-mRFP are shown. *n*, nucleus; *nl*, nucleolus.

# Supplementary Figure S3



**Supplementary Fig. S3.** Characterization of *Arabidopsis* Flag-CCT2 overexpression (OE) and Flag-PPX1 OE plants under the control of CaMV35S promoter.

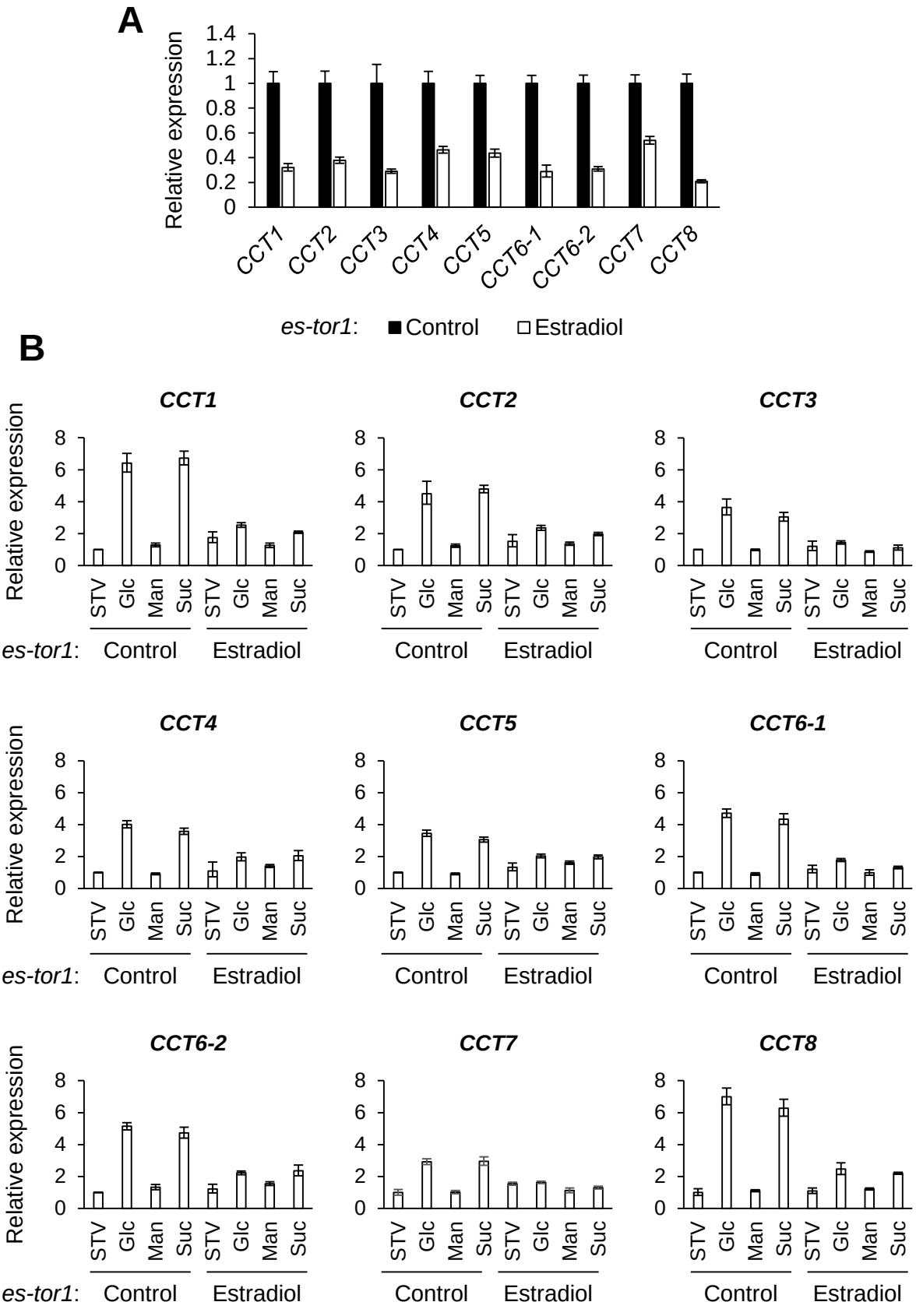
(A) Seedling phenotypes of Flag-CCT2 OE plants (T4 progeny; lines #8 and #11) at 7 days after germination (DAG) in comparison with those of WT (Col-0). The line #8 was used for further analyses.

(B) Semi-quantitative RT-PCR of CCT subunit genes in WT and Flag-CCT2 OE (lines #8 and #11) seedlings. *UBC21* mRNA served as the loading control.

(C) Seedling phenotypes of Flag-PPX1 OE plants (T4 progeny; lines #7 and #9) at 7 DAG in comparison with those of WT (Col-0). The line #7 was used for further analyses. Scale bar = 5 mm.

(D) Semi-quantitative RT-PCR of *PPX1* and *PPX2* in WT and Flag-PPX1 OE (lines #7 and #9) seedlings. *UBC21* mRNA served as the loading control.

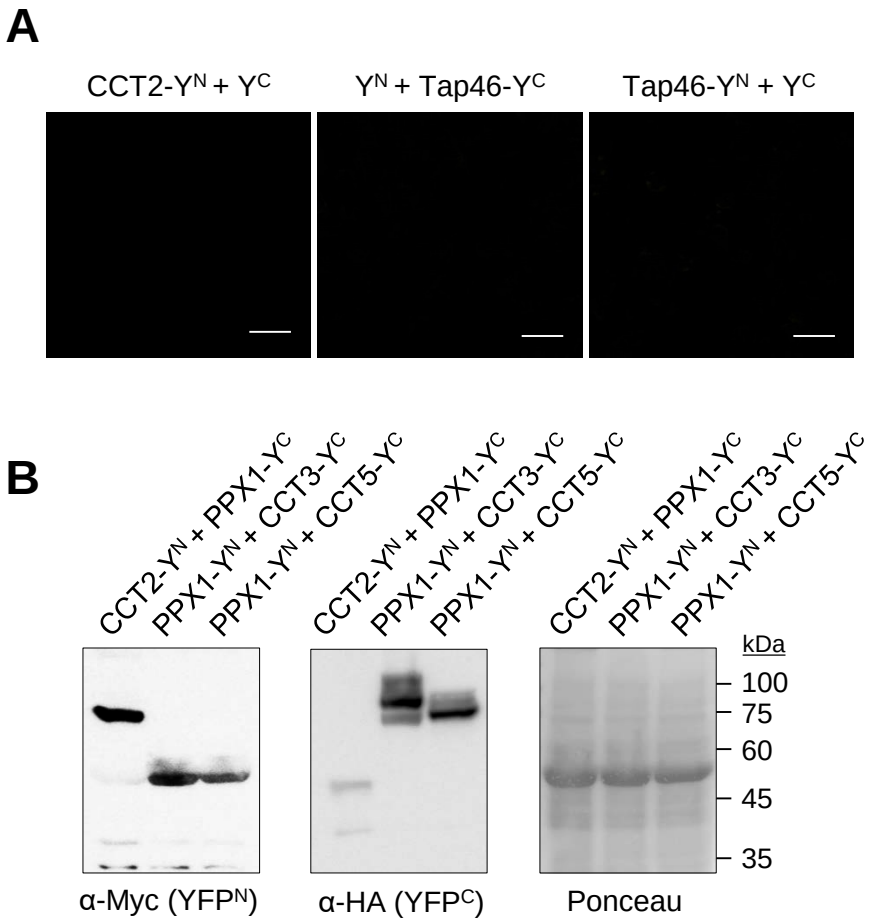
Supplementary Figure S4



**Supplementary Fig. S4.** Expression of *Arabidopsis* CCT subunit genes in estradiol-inducible TOR RNAi (*es-tor1*) plants.

(A) RT-qPCR of CCT subunit genes in *TOR*-silenced seedlings. The *es-tor1* seedlings were grown for 7 days and treated with ethanol (control) or 10 μM estradiol for 3 days. Transcript levels are normalized to those of *UBC10* mRNA, and expressed relative to those of control samples. Error bars represent SE from two biological replications. To explore a possible relationship between the CCT complex and the TOR signaling pathway, we tested whether *CCT* subunit genes are transcriptionally regulated by TOR activity, using *Arabidopsis* estradiol-inducible *TOR* RNAi (*es-tor1*) lines. Estradiol treatment and subsequent *TOR* silencing decreased mRNA levels of all *CCT* subunit genes.

(B) RT-qPCR of CCT subunit genes in *TOR*-silenced seedlings in response to sugar feeding. The *es-tor1* seedlings grown for 5 days were treated with ethanol (control) or 10 μM estradiol for 2 days. Then they were transferred to glucose-free medium for 24-h incubation (Stv), and then fed with 30 mM glucose (Glc), mannitol (Man), and sucrose (Suc) for 4 h. Transcript levels are normalized to those of *UBC10* mRNA, and expressed relative to those of Stv samples. Error bars represent SE from two biological replications. Since TOR plays a central role in glucose and energy signaling through rapid transcriptome reprogramming, we tested TOR- and sugar-dependent expression of CCT subunit genes. RT-qPCR analysis revealed that feeding with glucose or sucrose, but not with mannitol, boosted transcription of all *CCT* subunit genes in control seedlings, suggesting that *CCT* gene expression is modulated by cellular energy availability. However, *TOR* silencing by estradiol treatment attenuated the transcriptional changes of the *CCT* genes. These results suggest that *CCT* gene expression under normal conditions and in response to sugar feeding is modulated by TOR.

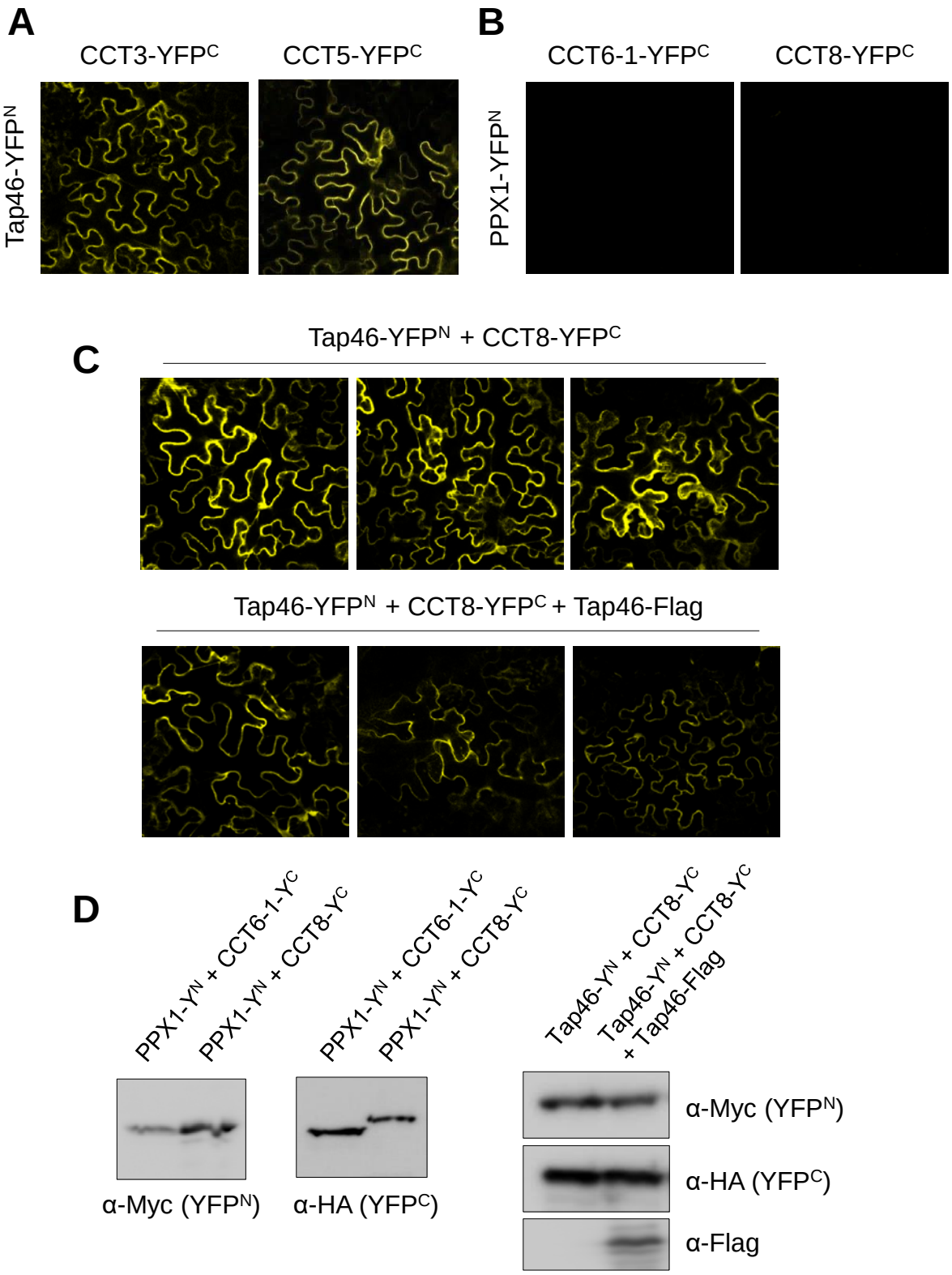



**Supplementary Fig. S5.** Negative controls and immunoblotting analyses for BiFC experiments shown in Fig. 4B.

(A) Negative control for BiFC. Combinations of CCT2-YFP$^N$ (Y$^N$) and YFP$^C$ (Y$^C$), YFP$^N$ and Tap46-YFP$^C$, and Tap46-YFP$^N$ and YFP$^C$ were expressed in *N. benthamiana* leaves via agroinfiltration. Confocal images were photographed at 2 DAI.

(B) Immunoblotting to detect protein expression in BiFC experiments. YFP$^N$-and YFP$^C$-fusion proteins in *N. benthamiana* leaves were detected using anti-Myc and anti-HA antibodies, respectively. Ponceau-stained rbcL served as the loading control.

Supplementary Figure S6



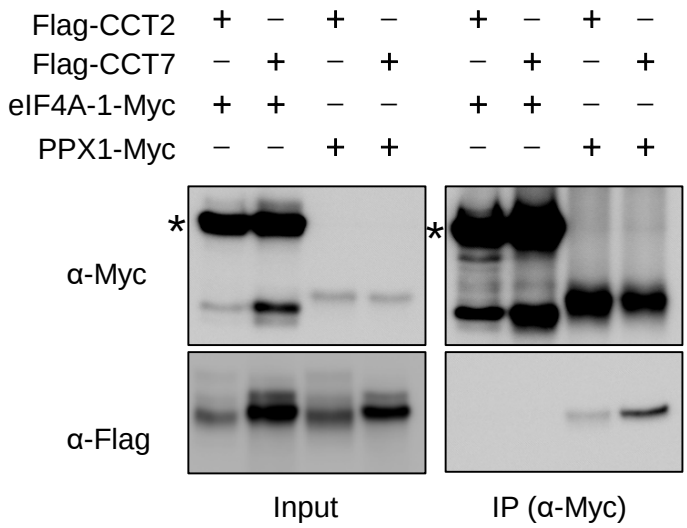
**Supplementary Fig. S6.** BiFC analyses of CCT interactions with Tap46 and PPX1.

(A) BiFC between Tap46 and CCT subunits. $YFP^N$ and $YFP^C$ constructs were expressed in *N. benthamiana* leaves via agroinfiltration. YFP signals were observed from *N. benthamiana* leaf epidermal cells at 2 DAI by confocal microscopy.

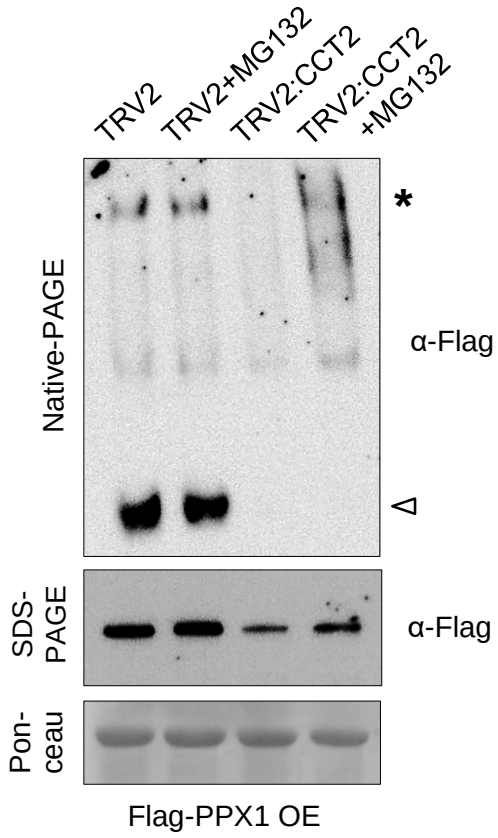
(B) BiFC between PPX1 and CCT subunits. No yellow fluorescence was detected in any combinations.

(C) BiFC between Tap46 and CCT8 with overexpressed Tap46-Flag proteins. Tap46-Flag protein was co-expressed with Tap46-$YFP^N$ and CCT8-$YFP^C$, which reduced the BiFC signals.

(D) Immunoblotting to detect protein expression in BiFC experiments. $YFP^N$- and $YFP^C$-fusion proteins in *N. benthamiana* leaves were detected using anti-Myc and anti-HA antibodies, respectively.

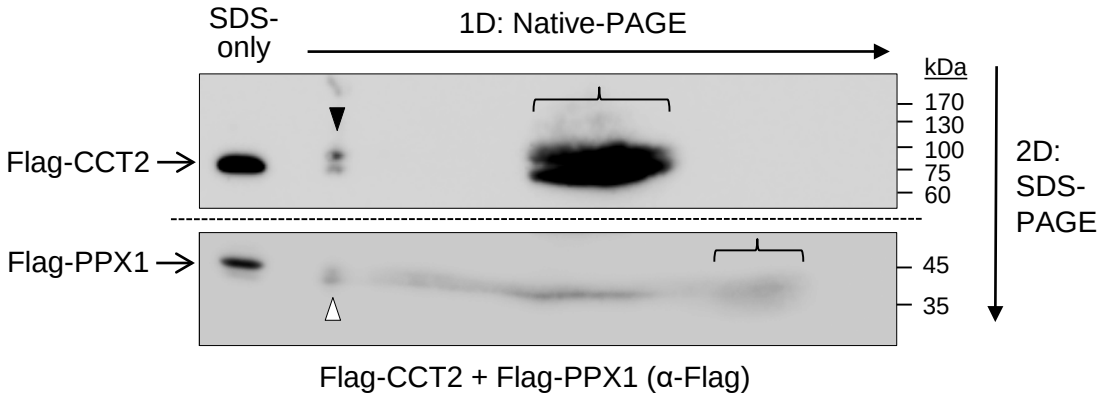



**Supplementary Fig. S7.** Control experiments for co-IP between PPX1 and CCT subunits. PPX1-Myc and eIF4A-1-Myc were co-expressed with Flag-CCT2 and Flag-CCT7 in *N. benthamiana* leaves. Immunoprecipitation was performed with anti-Myc antibody-conjugated resin, followed by immunoblotting with anti-Myc and anti-Flag antibodies to detect immunoprecipitated and co-immunoprecipitated proteins, respectively. CCT2 and CCT7 were immunoprecipitated with PPX1-Myc, but not with eIF4A-1-Myc (*asterisks*), despite abundant expression and successful immunoprecipipitation of eIF4A-1-Myc. These results suggest specific interactions between PPX1 and CCT subunits.




**Supplementary Fig. S8.** Native-PAGE and SDS-PAGE of Flag-PPX1 OE plants after *CCT2* VIGS with or without MG132 treatment.

Immunoblotting was performed with anti-Flag antibody with or without MG132 treatment (20 μM, 4-h). The *asterisk* and *white arrowhead* indicate the CCT complex-associated form and the mature form of Flag-PPX1, respectively. Ponceau-stained rbcL served as the loading control.




**Supplementary Fig. S9.** 2-dimensional PAGE using *N. benthamiana* extracts that co-expressed Flag-CCT2 and Flag-PPX1.

After native-PAGE (1D) and SDS-PAGE (2D), immunoblotting was performed with anti-Flag antibody. Flag-CCT2 and Flag-PPX1 detected at the position of the CCT complex are marked with *black* and *white arrowheads*, respectively. Monomeric Flag-CCT2 and Flag-PPX1 are marked with *brackets*. The band intensity of Flag-PPX1 was substantially lower than that of Flag-CCT2, and to match the band intensity between the two proteins, the membrane was cut (*dashed line*) and exposed for different time periods. In the "SDS-only" lane, cell extracts co-expressing Flag-CCT2 and Flag-PPX1 were loaded onto the 2D gel (SDS-PAGE) for immunoblotting to mark the position of Flag-CCT2 and Flag-PPX1.