

## SUPPLEMENTAL FIGURE LEGENDS

### Figure S1. Design of vectors for BiFC assays in *A. thaliana* protoplasts.

(A) Oligonucleotides designed for PCR amplification of cDNAs, adding the appropriate restriction sites for cloning into the vectors, pSPYCE and pSPYNE, containing the N-terminal or the C-terminal ends of the YFP protein, respectively.

(B) Scheme of cDNAs cloning into BiFC vectors. Whereas Cc cDNA was fused with the cYFP domain (pSPYCE vector), Cc protein targets were cloned into the vector containing the nYFP fragment (pSPYNE vector). These targets were classified into five categories: type I, including the targets cloned with the *Sall/XhoI* restriction sites; type II, comprising the targets cloned with the *BamHI/XhoI* restriction sites; type III, including targets cloned with *BamHI/Sall* restriction sites; type IV, comprising the targets cloned with *BamHI/SmaI* restriction sites; type V, including targets cloned with *XhoI/SmaI* restriction sites. MCS, Multicloning site; c-myc, c-myc peptide epitope; HA, HA epitope; 35s, 35s promoter.

### Figure S2. Design of vectors for BiFC assays in HEK293T cells.

(A) Oligonucleotides designed for PCR amplification of cDNAs, adding the appropriate restriction sites for cloning into the vectors, cYFP and nYFP, containing the C-terminal or the N-terminal ends of the YFP protein, respectively.

(B) Scheme of the cDNAs cloning into BiFC vectors. Whereas Cc cDNA was fused with the cYFP domain (cYFP vector), Cc protein targets were cloned into the vector containing the nYFP fragment (nYFP vector). These targets were classified into three categories: type I, which includes the targets cloned with the *BamHI/XhoI* restriction sites; type II, which comprises the targets cloned with the *EcoRI/XhoI* restriction sites; type III, which includes targets cloned with *BamHI/NotI* restriction sites. MCS, Multicloning site; CMV, Cytomegalovirus promoter.

### **Figure S3. Proteomic workflow.**

Scheme of the proteomic workflow used to identify Cc-interacting proteins. Potential Cc partners were purified from untreated and H<sub>2</sub>O<sub>2</sub>-treated cell extracts through an affinity chromatography approach, using a column where the A111C mutant is covalently bound to the thiol-sepharose matrix (Cc TS-4B). Samples were dialyzed and analyzed with NanoLC-MS/MS. As controls, untreated and H<sub>2</sub>O<sub>2</sub>-treated cell extracts were loaded into a blank column (Blank TS-4B), devoid of Cc.

### **Figure S4. BiFC assays in *Arabidopsis* protoplasts before and after PCD induction.**

*A. thaliana* protoplasts were transfected with pSPYCE/pSPYNE vectors, as described in Sheen [34], to specifically confirm the *in vivo* interaction of Cc with its potential target BIP2 under PCD conditions. Images were captured 24 h following transient transfection before (*left panel*) and after 6 h of treatment with 35 mM H<sub>2</sub>O<sub>2</sub> (*right panel*). Reconstruction of eYFP yields green fluorescence, indicative of the interaction between Cc and BIP2. The nucleus was stained in blue by DAPI. Scale bar is 10 μm.

### **Figure S5. BiFC assays in HEK293T cells.**

HEK293T cells were transfected with the Cc-cYFP vector, along with another vector containing the N-terminal YFP fragment (nYFP) bound to each Cc protein partner. Images were captured 24 h after transient transfection using Lipofectamine 2000 (Invitrogen) following manufacturer's instructions and after 6 h of treatment with 10 μM CPT. Reconstruction of eYFP leads to the obtainment of fluorescence signal emission, indicative of interaction between Cc and its partners (YFP filter). Nucleus was stained in blue using DAPI (DAPI filter). Positive and negative controls were used, as described by Hu *et al.* [33]. The expression of Cc partners fused to the nYFP fragment was determined by immunoblotting with a rabbit anti-EGFP polyclonal antibody (BioVision). The

bands with the expected molecular weight did not appear in non-transfected cells. Scale bar = 5  $\mu$ m.

**Figure S6. Localization of Cc-cYFP construct in HEK293T cells.**

**(A)** HEK293T cells were transfected with the Cc-cYFP and empty nYFP vectors. Under homeostatic conditions (Upper Panel), the reconstitution of eYFP leads to a punctuate fluorescence pattern indicative of the mitochondrial localization of Cc, as reported by Goldstein *et al.* [44]. Nevertheless, after treating cells with 10  $\mu$ M CPT for 6 h (Lower Panel), the fluorescence showed a diffuse pattern, indicating Cc-cYFP to be properly released from mitochondria. Scale bar is 12  $\mu$ m.

**(B)** Western blot showing the band corresponding to the Cc-cYFP construct expression (right), immunoblotted using an anti-EGFP antibody (BioVision). This band did not appear in non-transfected cells (left).

**Supplemental Data 1.** Peptides identified by nanoLC-MS/MS for the novel 10 Cc-targets.

**Supplemental Data 2.** Association and dissociation rate constants ( $k_{on}$  and  $k_{off}$ , respectively) and dissociation equilibrium constants ( $K_D$ ) for plant cytochrome c interaction with its protein partners measured by a BiaCore 3000 system.