Supplementary Information

Chloroplast SRP43 autonomously protects chlorophyll biosynthesis proteins against heat shock

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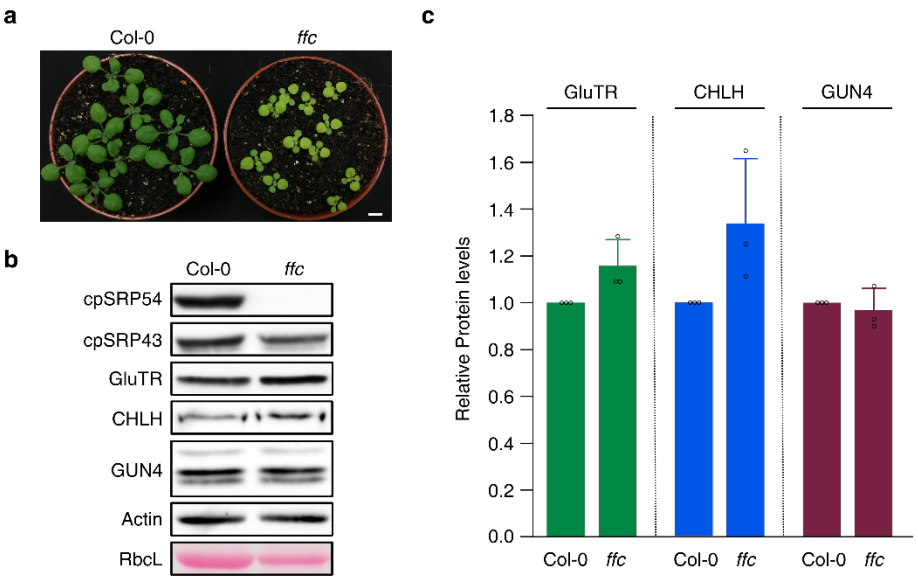
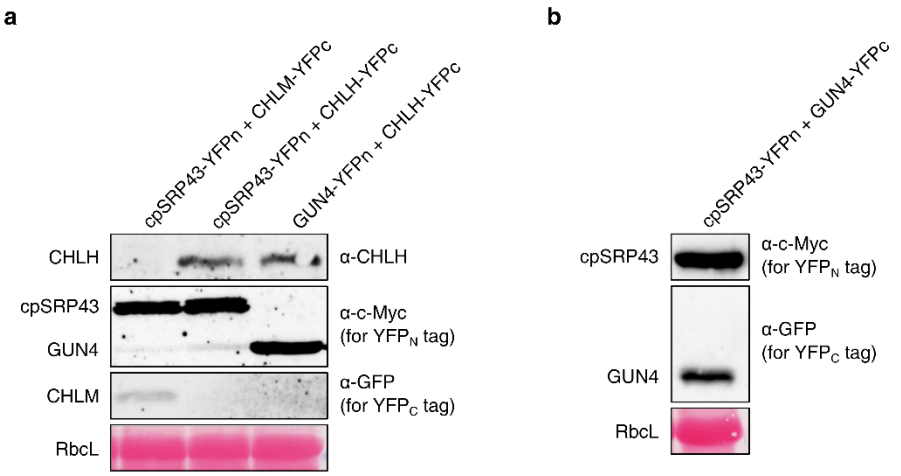


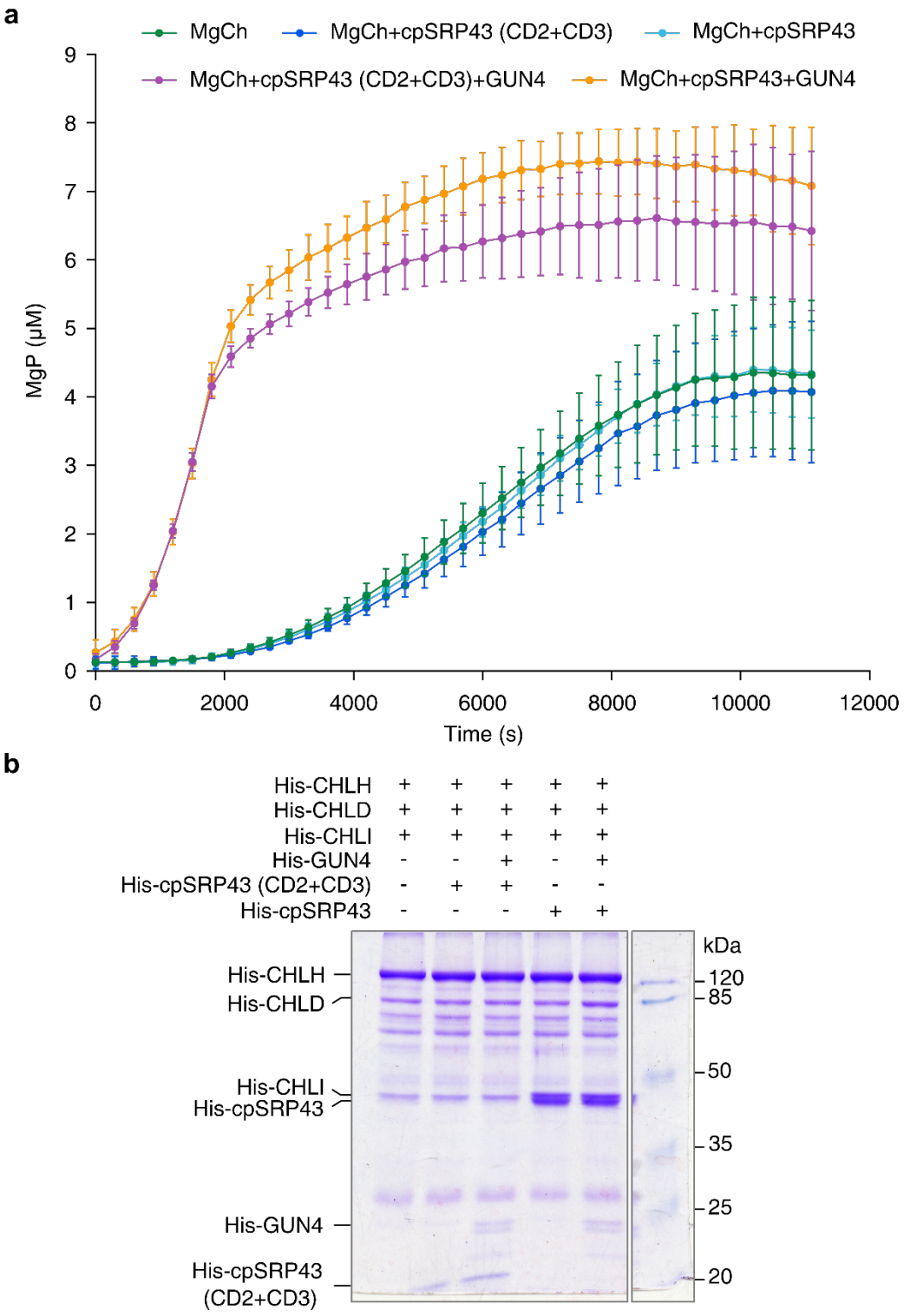
Supplementary Fig. 1. cpSRP54 deficiency does not compromise the abundance of GluTR, CHLH, and GUN4.

a, Phenotype of 18-day-old *cpsrp54* knock-out mutant (*ffc*) and wild-type (Col-0) in long-day (18 h light/ 6 h dark). Scale bar, 1 cm. **b**, Steady-state levels of TBS proteins in the wild-type and *ffc* were detected by immunoblotting using the indicated antibodies. Ponceau S stained large subunit of RubisCo (RbcL) and Actin are shown as loading controls. **c,** Semiquantitative analysis with Image J software (NIH) of the immunoblots in (**b**) from three biological replicates. The relative amounts of GluTR, CHLH and GUN4 in *ffc* were normalized to the levels in Col-0. The data are plotted as means ± s.d. (n = 3). The statistical analyses were performed using two-tailed Student's t-tests. No asterisk indicates no significant differences compared to protein levels in Ler-0: * $P < 0.05$, ** $P < 0.01$



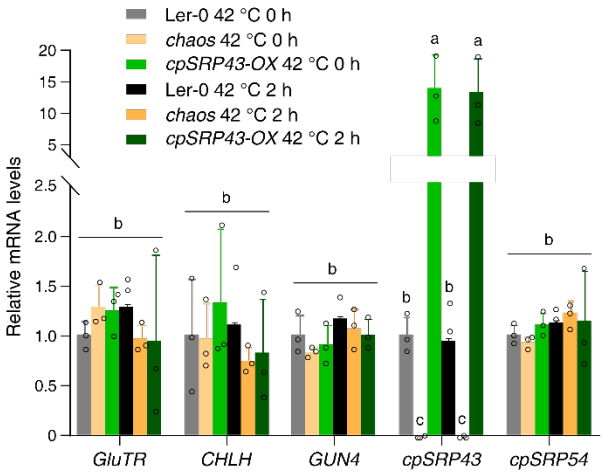
Supplementary Fig. 2. Physical interaction of cpSRP43 with CHLH and GUN4.

a, Immunoblotting analyses of protein extracts after the BiFC assay (shown in Fig. 2a) confirmed expression of cpSRP43-YFPn, GUN4-YFPn (c-Myc antibody) and YFPc tagged CHLM (GFP antibody) and CHLH (CHLH antibody). **b**, Immunoblotting analyses confirmed expression of cpSRP43-YFPn and GUN4-YFPc shown in Fig. 2a.



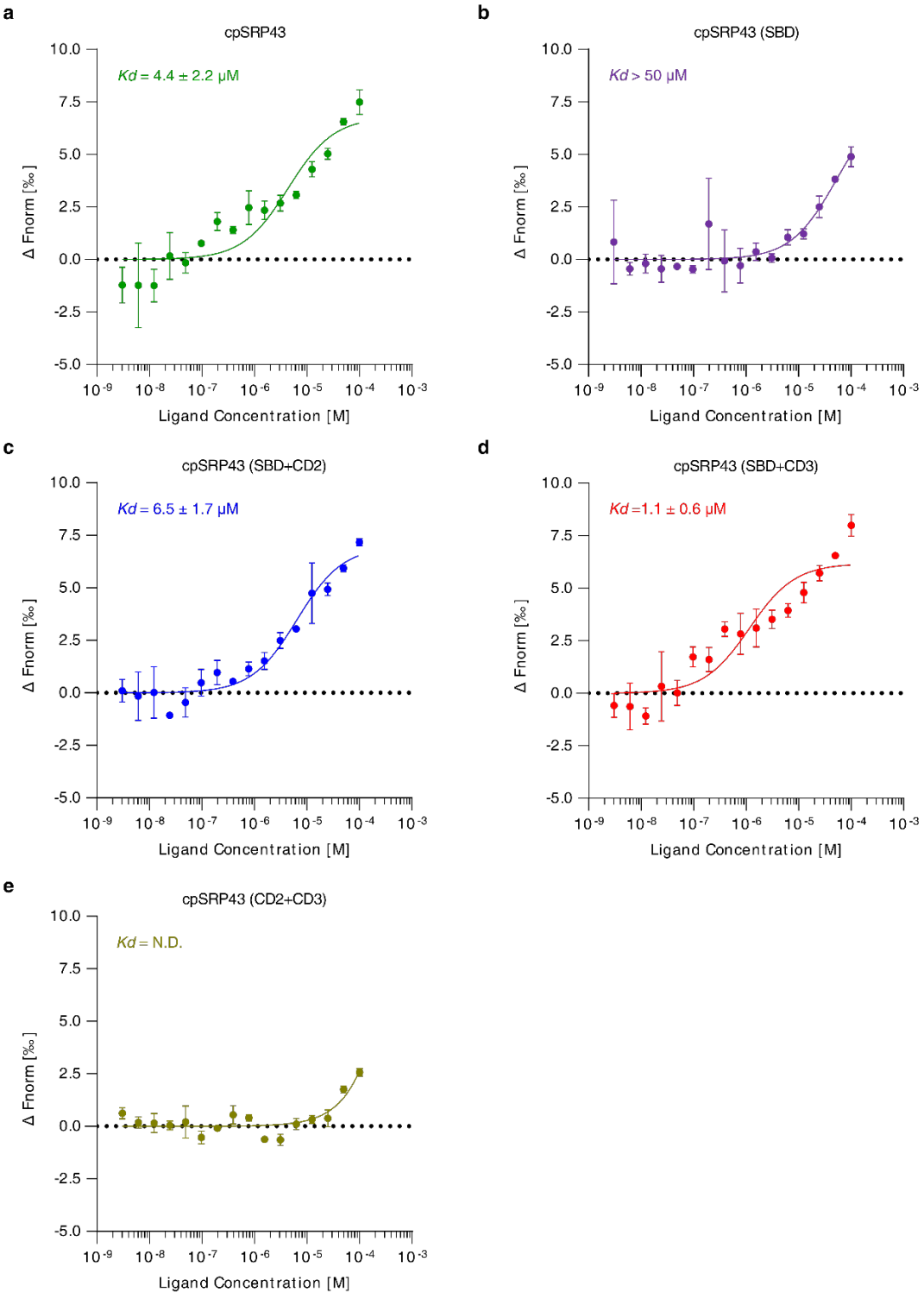
Supplementary Fig. 3. *In vitro* enzymatic assays of MgCh.

a, Time course curves of the MgCh enzymatic assay with fluorescence detection of MgP (Ex 416 nm/Em 595 nm). The data are plotted as mean ± s.d. (n = 3). **b**, Recombinant proteins used in the assay were stained by Coomassie Brilliant Blue.



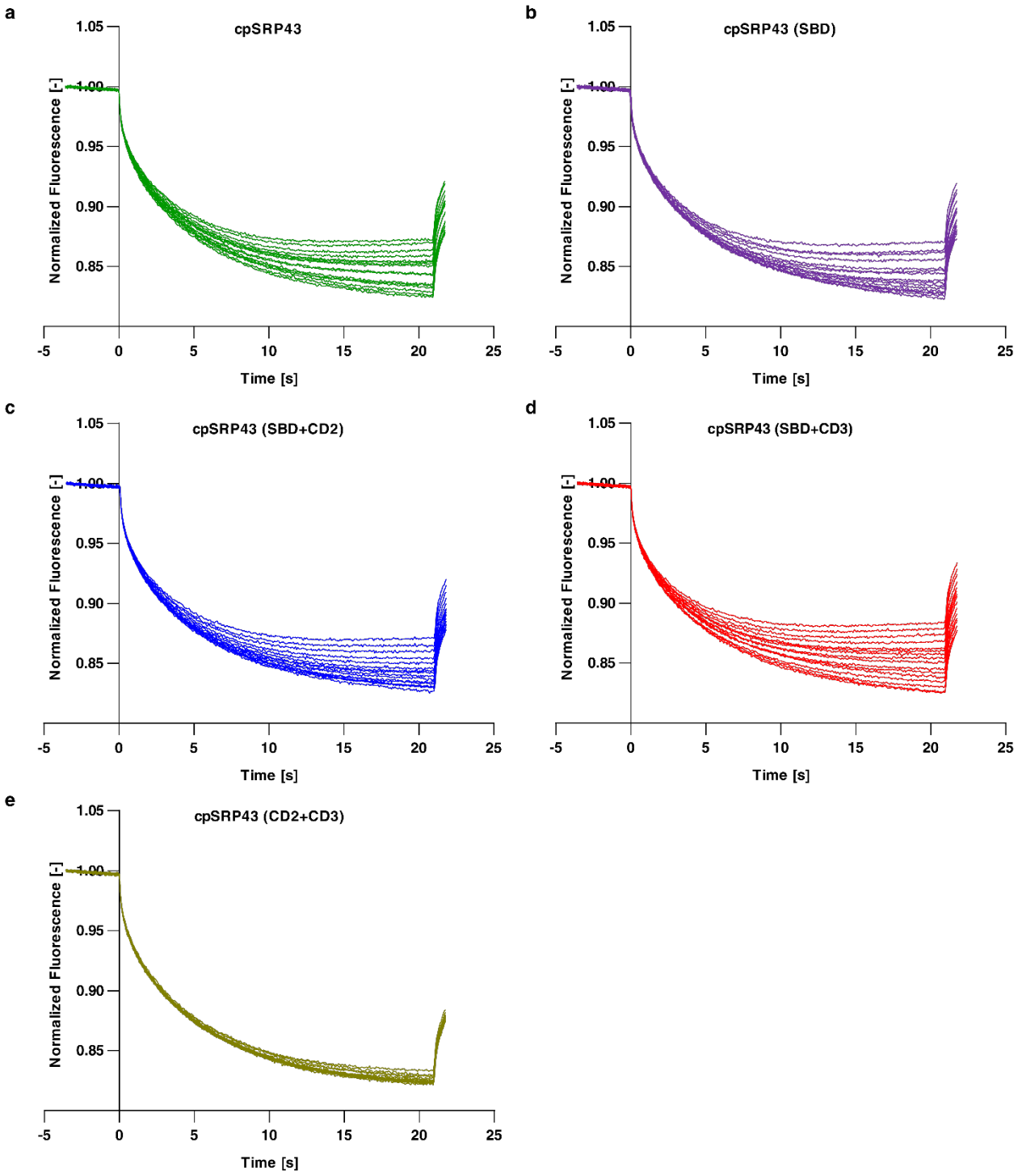
Supplementary Fig. 4. Short-term heat stress does not alter the transcriptional levels of *GluTR*, *CHLH*, *GUN4*, *cpSRP43* and c*pSRP54*.

Relative mRNA levels of indicated genes in 18-day-old Ler-0, *chaos* and *cpSRP43-OX* were measured prior to and after 2 h heat treatment at 42 °C. Gene expression was calculated relative to the Ler-0 and *SAND* was used as the reference gene. The data are plotted as mean ± s.d. (n = 3). The small open circles represent the individual values. Letters above histograms indicate significant differences as determined by two-way ANOVA with Tukey test ($P < 0.05$).



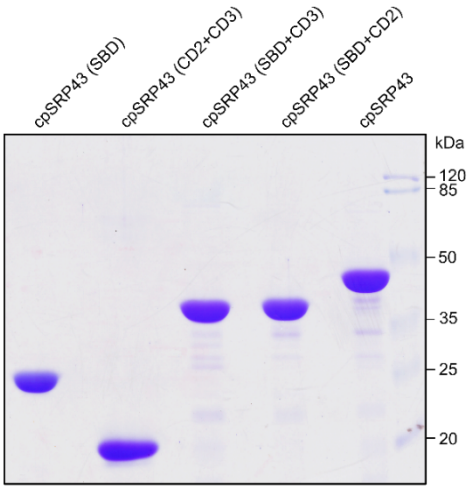
Supplementary Fig. 5. Measurement of the binding affinity of cpSRP43 with GUN4 using microscale thermophoresis (MST).

The fluorophore-labelled GUN4 were used at constant concentration (20 nM), while the concentration of the non-labelled wild-type (**a**) or truncated cpSRP43 (**b**-**e**) were varied between 3.05 nM and 100 µM. N.D., not detectable. The data are plotted as mean ± s.d. (n = 3)



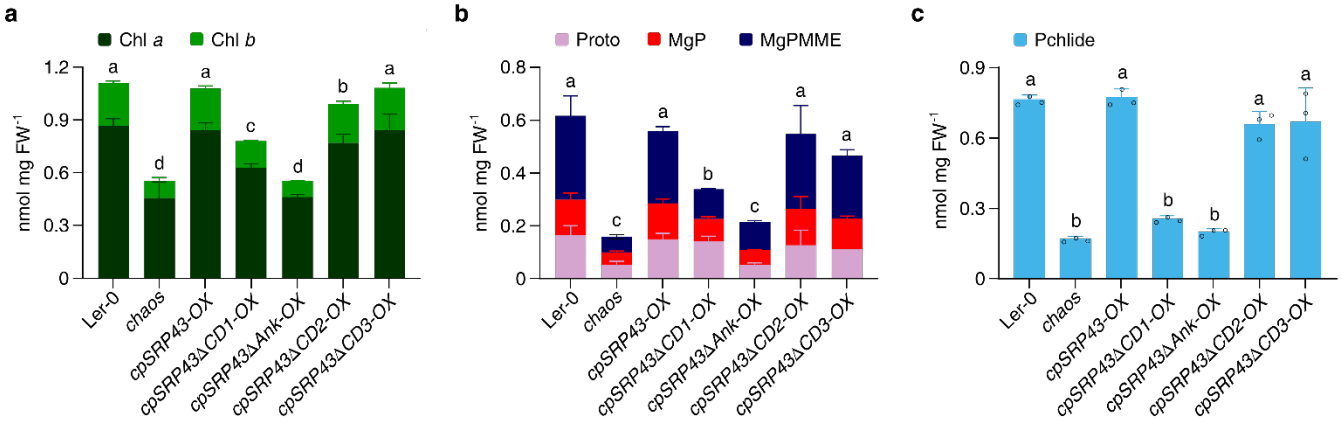
Supplementary Fig. 6. Raw MST data.

MST traces for the determination of the binding affinity of cpSRP43 with GUN4 refer to the binding curves shown in Supplementary Fig. 5. The recombinant proteins used for microscale thermophoresis are shown in Supplementary Fig. 7.



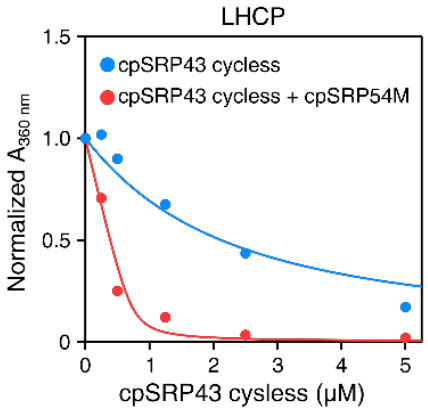
Supplementary Fig. 7. Commaassie stained SDS-PAGE gel of purified recombinant wild-type cpSRP43 and cpSRP43 truncation mutants used for the MST analyses.

The indicated recombinant His-tagged proteins were purified as described in the Methods. Three micrograms of each protein was analyzed by SDS-PAGE and stained with Coomassie Brilliant Blue.



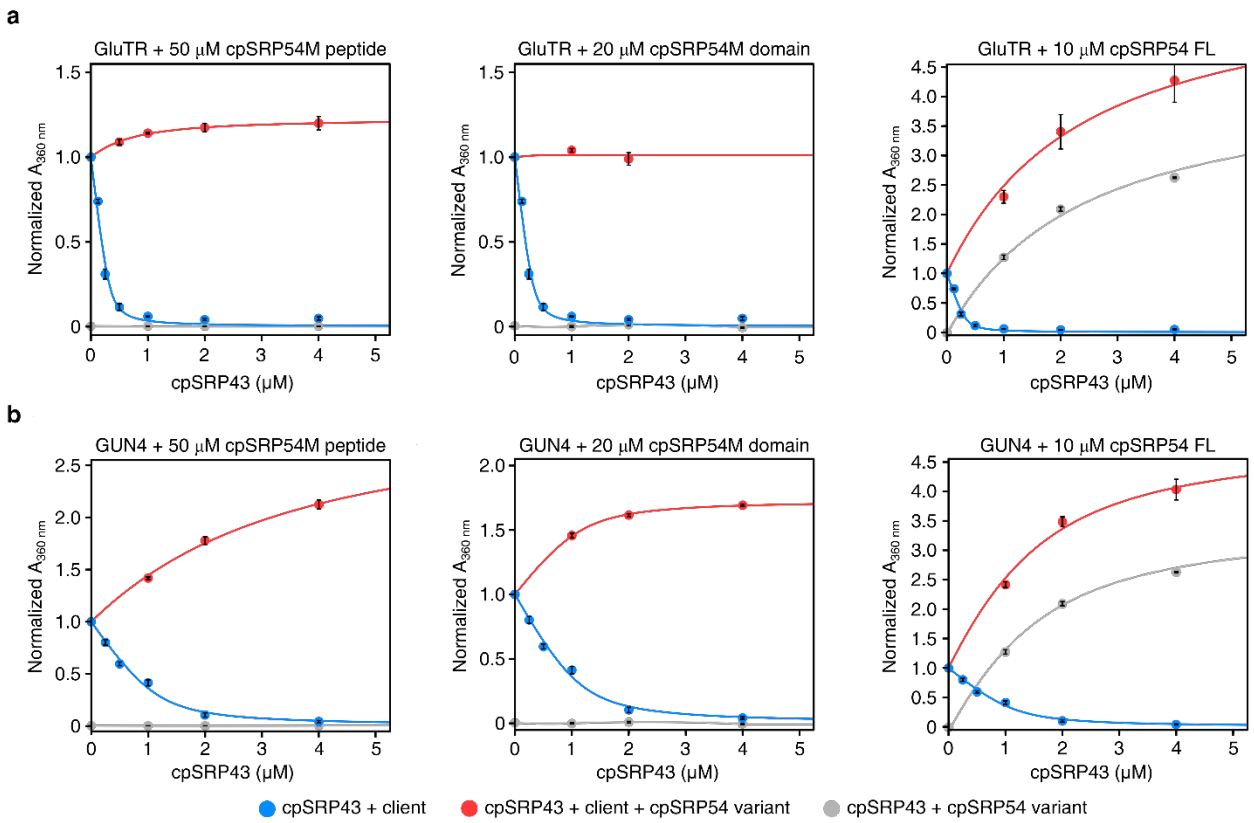
Supplementary Fig. 8. Accumulation of Chl and Chl precursors in various *chaos* complementation lines.

HPLC analyses of Chl (**a**), Proto, MgP and MgPMME (**b**) and Pchlide (**c**) in the seedlings of 18-day-old Ler, *chaos* and a set of *chaos* complementation lines. FW, fresh weight. The data are plotted as mean ± s.d. (n = 3), the small open circles represent the individual values. Letters above histograms indicate significant differences as determined by one-way ANOVA with Tukey test ($P < 0.05$)



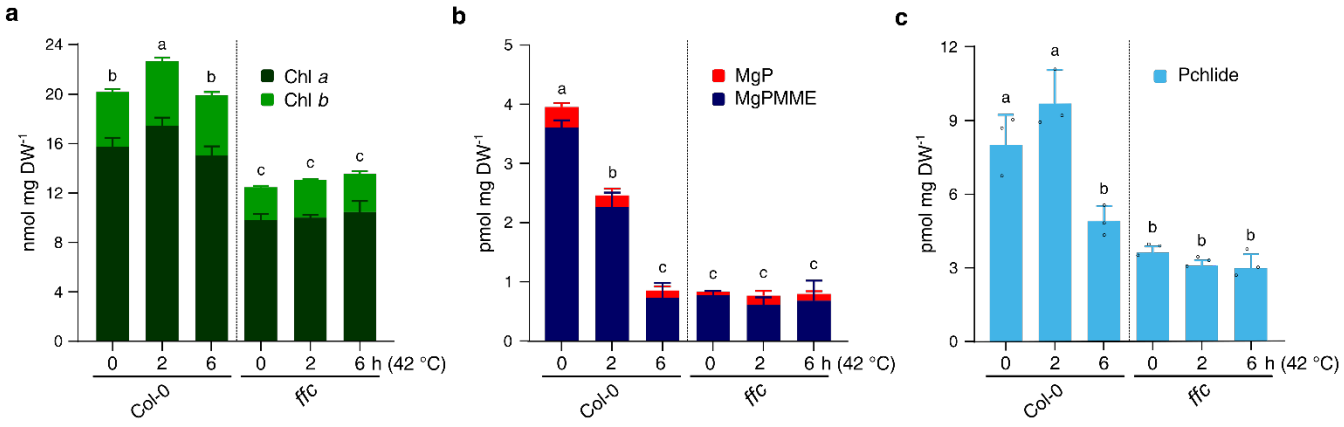
Supplementary Fig. 9. The cpSRP54M peptide stimulates the chaperone activity of cpSRP43 cysless towards LHCP.

The turbidity of 1 μM LHCP diluted out of 8 M urea was measured in the presence of cysteine-mutated cpSRP43 (cpSRP43 C175A/C297S) with (red line) or without (blue line) 50 μM cpSRP54M peptide present. The lines are fits of the data to Eq. 1 in the Methods, and the obtained K_{sol} values were 2 ± 0.4 μM and 0.03 ± 0.09 μM for apo cpSRP43 and cpSRP43 plus cpSRP54M peptide, respectively.



Supplementary Fig. 10. Comparison of cpSRP43 chaperone activity towards GUN4 and GluTR in the presence of cpSRP54M peptide, cpSRP54M domain, and full-length mature cpSRP54.

Heat-induced aggregation of GluTR (**a**) and GUN4 (**b**) was monitored in the presence of apo cpSRP43 (blue lines in each panel) and compared to cpSRP43 with 50 μM cpSRP54M peptide (red lines in the left panels), 20 μM cpSRP54M domain (red lines in the middle panels), or 10 μM full length (FL) mature cpSRP54 (red lines in the right panels). Additional experiments were carried out with cpSRP43 and each cpSRP54 variant in the absence of either client (grey lines in each panel). The turbidity at 360 nm was normalized to the value at 0 μM cpSRP43 under each condition, plotted as mean ± s.e. (n = 3), and fit to Eq. 1 in the Methods. Data and K_{sol} values for apo cpSRP43 are the same as in Fig. 3a and 3c. K_{sol} values for cpSRP43 in the presence of cpSRP54M variants were not obtained, as no protection was observed.



Supplementary Fig. 11. cpSRP54 deficiency does not compromise Chl biosynthesis during heat treatment.

HPLC analyses of Chl (**a**), MgP and MgPMME (**b**) and Pchlide (**c**) in 18-day-old Col-0 and *ffc* prior to and after 2 or 6 h heat treatment at 42 °C. DW, dry weight. The data are plotted as mean ± s.d. (n = 3). The small open circles represent the individual values. Letters above histograms indicate significant differences as determined by two-way ANOVA with Tukey test (P< 0.05).

Supplementary Table 1. List of primers used in this study.

qRT-PCR	Forward (5'-3')	Reverse(5'-3')
SAND	AACTCTATGCAGCATTTGATCCACT	TGATTGCATATCTTTATCGCCATC
GluTR	TTGCTGCCAACAAAGAAGAC	CCGTCTCCAATGAATCCCTC
CHLH	CTGGTCGTGACCCTAGAACAG	GATTGCCAGCTTCTTCTCTG
GUN4	TGATGGTAGATTCGGATACAGC	CAAGAAGCTTCATCCACTCAAC
cpSRP43	CTGCACATGGCGGCTGGTT	CGTCTTTGCCTTTCCCTCGTT
cpSRP54	GCTTCAGATAGATAAAGGCATG	GCACCACCTCTTGAATCACC
Plant transformation		
pGL1-cpSRP43	TCTAGAATGCAAAAGGTCTTCTTGG	CCCGGGTCACTTGTCATCATCGTCCTTGTAGTCTTCATTCATTGG
pGL1-cpSRP43ΔCD3	TCTAGAATGCAAAAGGTCTTCTTGG	CCCGGGTCACTTGTCATCATCGTCCTTGTAGTCAGCGTACTCCAG
Intermediate primers for cloning of truncated cpSRP43		
cpSRP43ΔCD1	CATCATCATCGTACGCTAGAAAAGCCG	TCGGCTTTTCTAGCGTACGATGATGATG
cpSRP43ΔAnk	CCCTGGTGGACGGCACAAGTGTTCGAGTAC	GTACTCGAACACTTGTGCCGTCCACCAGG
cpSRP43ΔCD2	CAAGTGTTCGAGTACGTAGCGGAGAGTGT	GTACTCGAACACTTGTCCTTCCAGG
BiFC constructs		
pDonor-cpSRP43	CAAAAAAGCAGGCTGAATGCAAAAGGTCTTCTT	CAAGAAAGCTGGGTGTTCATTCATTGGTTGTTGT
pDonor-GUN4	CAAAAAAGCAGGCTGAATGATGGCGACCA CAAAC	CAAGAAAGCTGGGTG GAAGCTGTAATTTGTTTT
Heterologous protein expression constructs		
pET28a-cpSRP43	GACATATGGCCGCCGTACAAAG	GCCTCGAGAGCGTACTCCAGCCCAT
pET28a-GluTR	ATCATATGGCTTCTTCTGATTCTGC	CTGAATTCTTACTTCTGTTGTTGTT