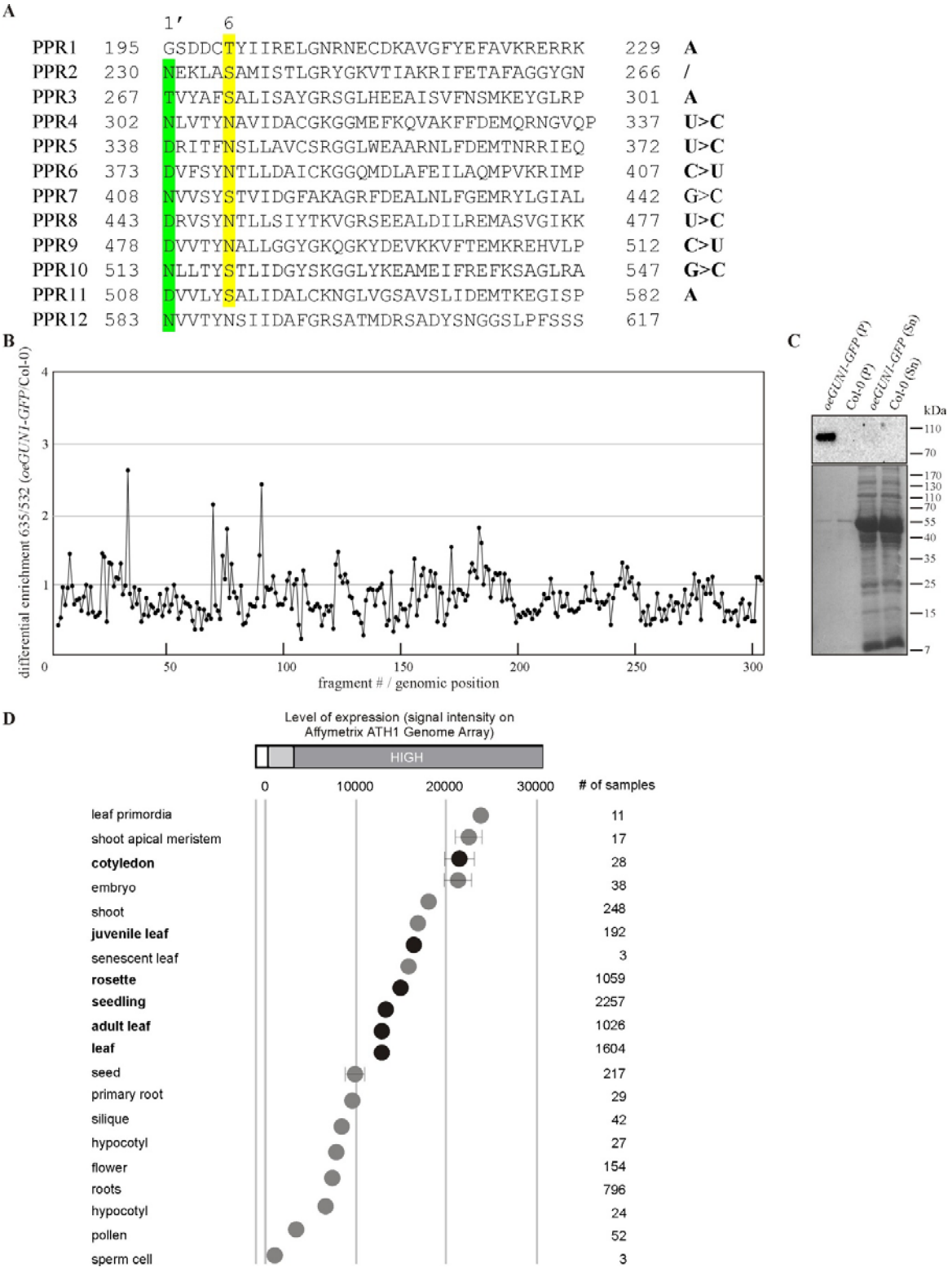
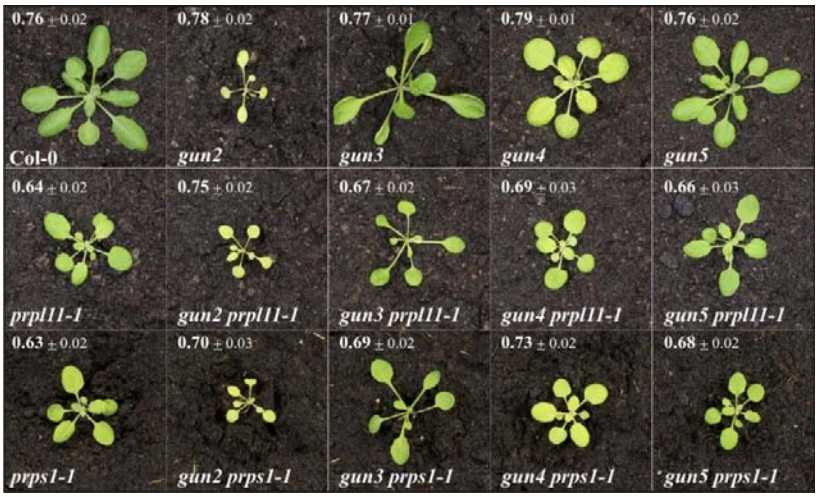
SUPPLEMENTAL DATA

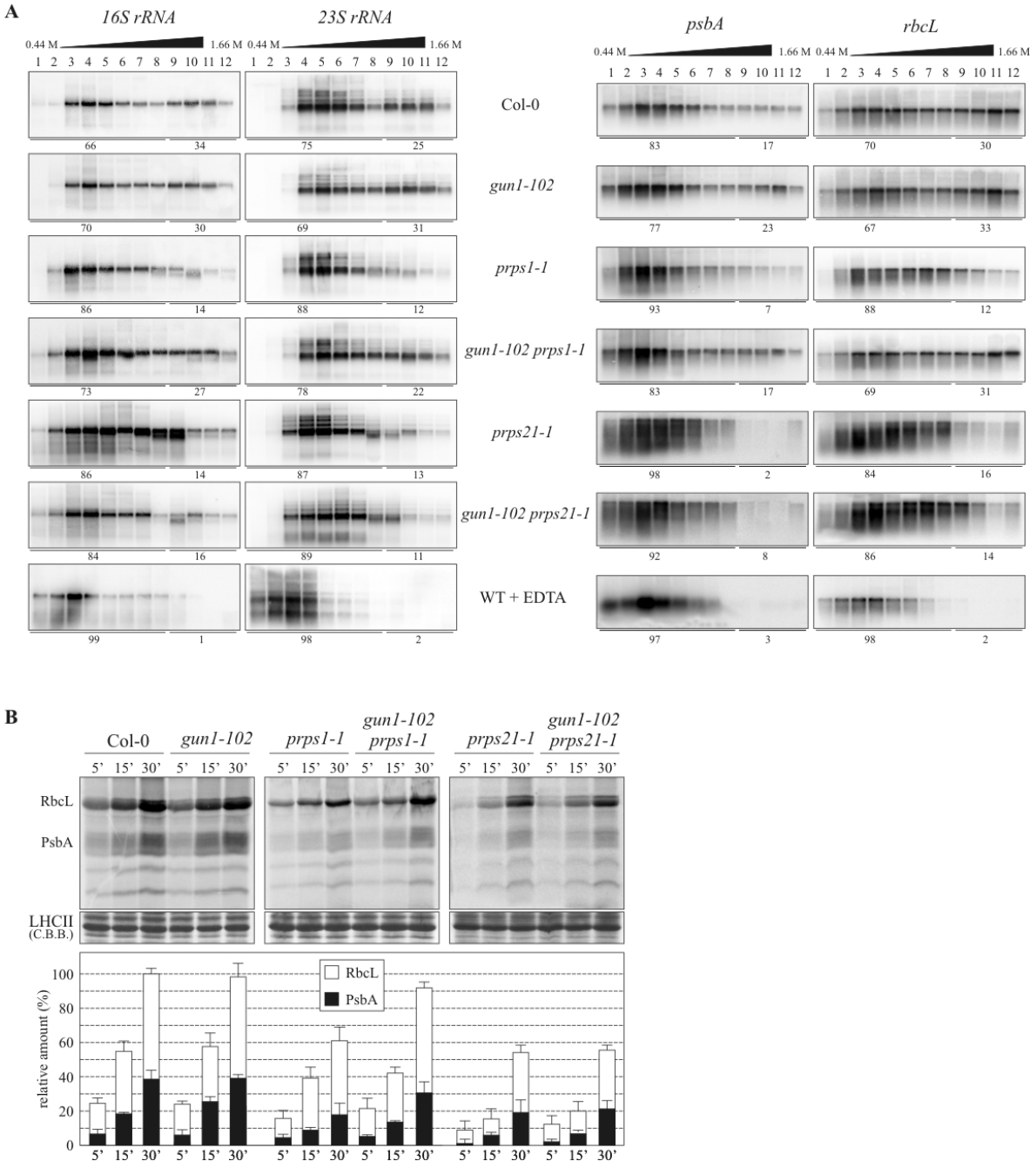


Supplemental Figure S1. The PPR protein GUN1 lacks obvious DNA- or RNA-binding activity and is expressed at several stages in development. A, PPR motifs in GUN1 were identified with the aid of

TPRpred (Karpenahalli et al., 2007). Note that the first two PPRs identified here have not been included in previous analyses and that the last PPR was considered to lack a canonical C-terminus (Koussevitzky et al., 2007). Additional C-terminal PPRs, separated by a non-PPR sequence tract, were also discovered by TPRpred, but were not taken into account here as they are unlikely to form a continuous RNA-binding surface with PPRs 1-11. The amino acid residues critical for sequence-specific RNA recognition (Barkan et al., 2012; Takenaka et al., 2013) are highlighted in green and yellow. The amino acid pairs found at position 6 of one and position 1 of the next PPR motif (named 1') allow one to infer the nature of the nucleotide base that is recognized. When the code developed for different 6-1' combinations is applied to the GUN1 repeats, the sequence ANAYYYSYYSAA emerges (for each repeat, the corresponding base is shown on the right). A more stringent version, based on the most likely nucleotide at each position (Barkan et al., 2012; Takenaka et al., 2013) reads ANAUUCGUCGAA. Note that for repeat 2 (with the amino acid combination S-T) no preferred ligand can be inferred on the basis of the current key. B, Differential enrichment ratios obtained by nucleotide immunoprecipitation (NIP)-chip analysis. The enrichment ratios (F_{635}/F_{532}) obtained from an assay of *oeGUN1-GFP* chloroplast stroma extract were normalized with respect to a control assay that used WT (Col-0) chloroplast stroma extract (both assays were performed in triplicate). The median-normalized values for replicate spots from the *oeGUN1-GFP* data were divided by the WT data, $\log_2$ transformed, and plotted according to fragment number. Fragments are numbered according to their chromosomal positions. Only fragments that (i) showed >2-fold enrichment relative to the WT control; (ii) hybridized with more than one genomic fragment on the array; and (iii) for which a t-test indicated significant enrichment ($P < 0.01$) can be considered to represent true DNA or RNA targets. Because none of the peaks fulfilled these criteria, direct interaction of GUN1 with chloroplast DNA or RNA is not supported by this assay. C, Immunoblot analysis of protein fractions obtained from immunoprecipitation experiments using GFP-trap and chloroplast stroma material from Col-0 and *oeGUN1-GFP* plants. Equal volumes of supernatant and pellet preparations were loaded onto the gel. Note that the supernatant from the GUN1-GFP immunoprecipitation gave no signal, implying quantitative precipitation of GUN1-GFP. The fact that no signal was obtained with Col-0 extracts demonstrates the specificity of the antibody. Ponceau S staining of the nylon membrane after transfer from SDS-PAGE was used to verify equal loading. P, pellet, Sn, supernatant. D, Expression profiling of *GUN1* in various organs of *A. thaliana* plants based on Genevestigator (https://genevestigator.com/gv/). *GUN1* mRNA expression data are displayed as signal intensities on Affymetrix *A. thaliana* ATH1 Genome arrays; # of samples indicates the number of microarrays covering the different categories. Stages of particular interest are highlighted in bold.

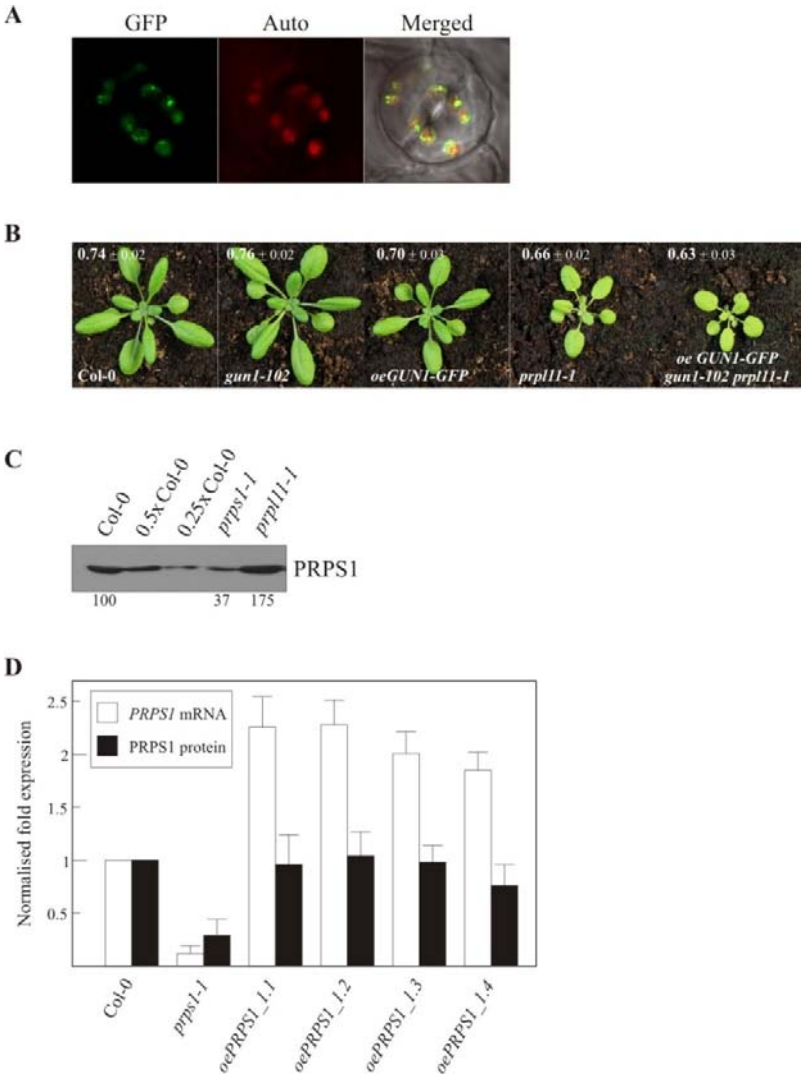


Supplemental Figure S2. Interactions between *gun2*-*gun5* mutations and two mutations affecting single ribosomal proteins (*prpl11-1* and *prps1-1*). The phenotypic characterization (including determination of Φ_{II}) of single (*prpl11-1*, *prps1-1*, *gun2*, *gun3*, *gun4* and *gun5*) and double (*gun2 prpl11-1*, *gun3 prpl11-1*, *gun4 prpl11-1*, *gun5 prpl11-1*, *gun2 prps1-1*, *gun3 prps1-1*, *gun4 prps1-1* and *gun5 prps1-1*) mutants was performed as in Fig. 2A. WT (Col-0) plants are shown as control.



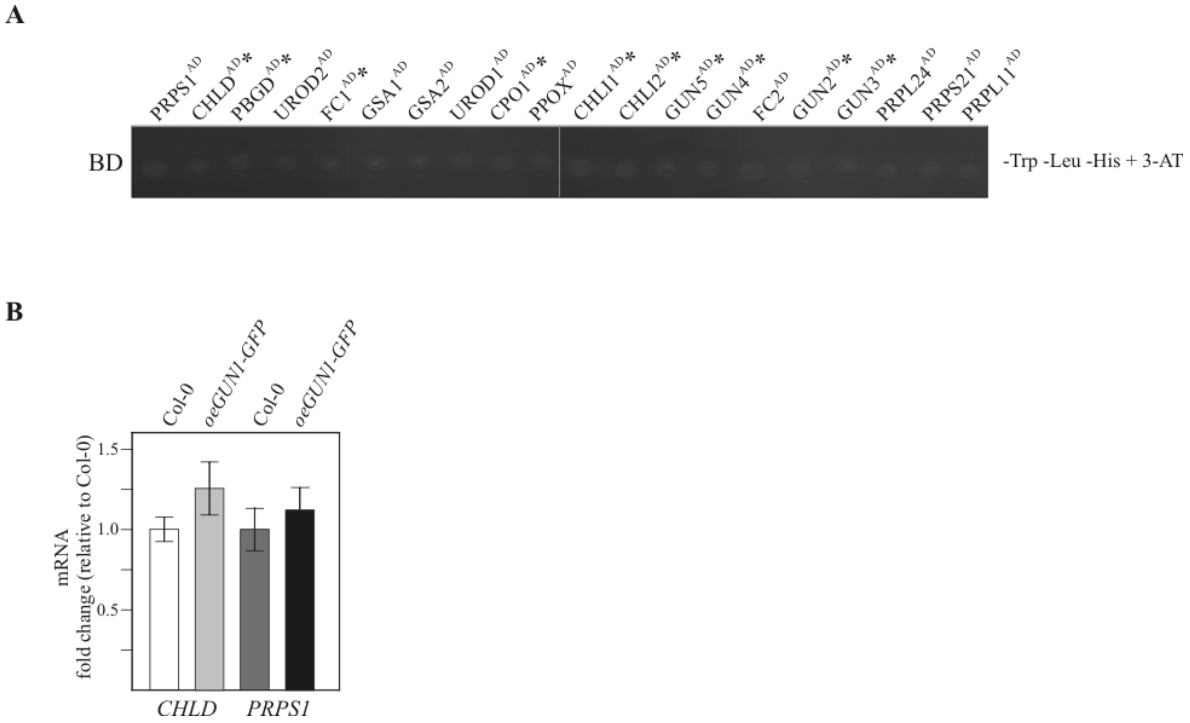
Supplemental Figure S3. Characterization of plastid translation efficiency in WT (Col-0) and mutant plants (*gun1-102*, *prps1-1*, *gun1-102 prps1-1*, *prps21-1* and *gun1-102 prps21-1*). A, Analysis of polysome loading. Polysomes isolated from leaves of four-week-old plants were centrifuged on sucrose gradients, and the 12 gradient fractions are numbered from top to bottom. Equal aliquots of extracted RNAs from all fractions were separated by denaturing agarose electrophoresis, transferred to nylon membranes and hybridized with specific probes to detect 23S and 16S rRNAs, *psbA* and *rbcL* mRNAs. To identify the

fractions that contain mainly polysomes (fractions 9-12), control gradients containing EDTA (causing polysome disassembly) were fractionated as above, and filters were hybridized with the same set of probes. Signal intensities were quantified (Image J) and compared. **B,** Translational efficiency of chloroplast-encoded mRNAs. Leaves isolated from four-week-old plants were pulse-labelled with [^{35}S]methionine under low-level illumination (20 μmol photons m^{-2} s^{-1}) for 5, 15, and 30 min in the presence of cycloheximide (to inhibit cytosolic protein synthesis). Total leaf proteins were then isolated, fractionated by SDS-PAGE, and detected by autoradiography. A portion of the Coomassie Brilliant Blue (C.B.B.)-stained gel, corresponding to the LHCII migration region, served as an internal standard for data normalization. Levels of [^{35}S]methionine incorporation into RbcL and D1 proteins were quantified (Image J) and are plotted in the histogram. Values are averages of five independent experiments and were normalized to the maximal signal intensities obtained in WT leaves after 30 min of labelling. A and B, Representative results from three independent experiments are shown.

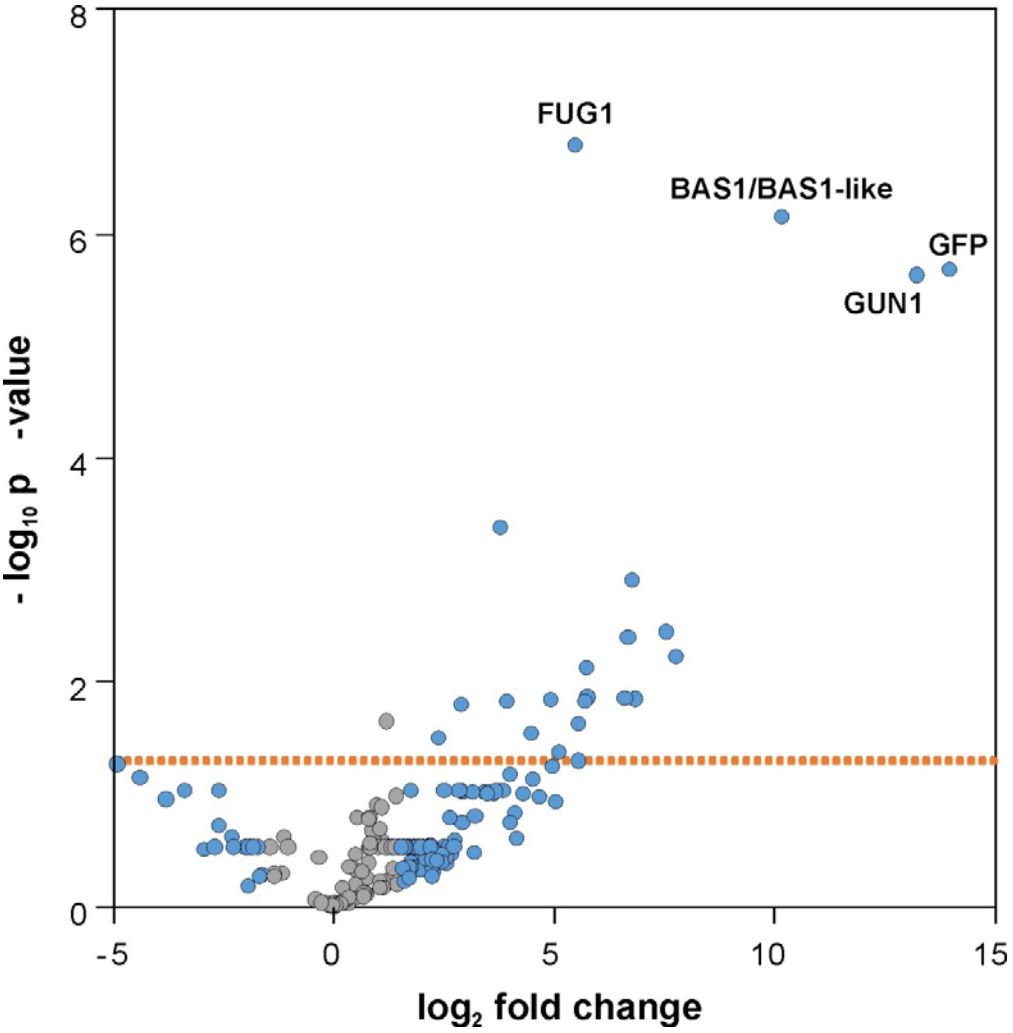


Supplemental Figure S4. *GUN1* and *PRPS1* overexpressor plants. A, Guard cells of stomata of *oeGUN1-GFP* lines analysed by confocal microscopy. The chimeric protein (GFP fluorescence, GFP) accumulates in distinct spots within the chloroplast (indicated by Chl autofluorescence, Auto), which were previously described as pTAC complexes (Koussevitzky et al., 2007). B, GUN1-GFP can functionally replace GUN1. While the combination of *gun1-102* and *prpl11-1* is lethal (see Fig. 2A), the *oeGUN1-GFP gun1-102 prpl11-1* mutant is viable and displays *prpl11-1*-like growth and photosynthesis. This confirms that the GUN1-GFP protein is functional and can replace GUN1. C, Immunoblot analyses of the PRPS1 protein in four-week-old leaves from WT (Col-0) and mutant (*prps1-1* and *prpl11-1*) plants. Proteins were extracted from equal amounts (fresh weight) of leaf tissue. D, Relative expression levels of *PRPS1* transcripts and protein in *prps1-1* and four *oePRPS1* (*35S:PRPS1 prps1-1*)(Romani et al., 2012) plants (Col-0 = 100%). *PRPS1* transcript accumulation (white bars) was measured by real-time PCR of leaf

cDNA. Immunoblot analyses were performed on the same material to quantify PRPS1 by Image J (black bars).



Supplemental Figure S5. Characterization of protein interactions of GUN1. A, The empty bait vector (BD) was used as control to verify the absence of prey autoactivation for the Y2H interactions tested in Fig. 5A. B, Quantitative real-time PCR analysis of *CHLD* and *PRPS1* mRNA expression in *oeGUN1-GFP* lines.



Supplemental Figure S6. Volcano plot of *P*-values against $\log_2$-transformed differences in abundances of co-immunoprecipitated proteins. Immunoprecitations were done in four independent experiments with antibodies against GFP on *oeGUN1-GFP* and WT control plants. Precipitated proteins were identified and quantified by nanoLC-MS/MS. Mean $\log_2$-ratios of abundances of proteins precipitated from GUN1-GFP expressing vs control lines are shown on the x-axis. The corresponding *P*-values of significance, derived by t-test statistics and subsequent adjustment to control the FDR are displayed on the y-axis. Proteins indicated by blue circles show a more than 1.5-fold difference in abundance between *oeGUN1-GFP* expressing and WT lines, abundances of proteins indicated by gray circles differ by less than the 1.5-fold cut-off value. Proteins plotted above the red dotted line fulfil the statistical significance criterion ($P \leq 0.05$).

SUPPLEMENTAL TABLES

Supplemental Table S1 (List of all proteins identified in co-immunoprecipitates of GUN1-GFP) is provided as separate Excel file.

Supplemental Table S2. Primers used in this study.

Locus	Gene	Sense primer (5´ to 3´)	Antisense primer (5´ to 3´)	Use	Nucleotides added at 5´ end
AT2G31400	*gun1-102*	GAGAGTAACAACCGAACGAC	AAAGTGCCAAAGCATGTCAG	Genotyping	/
AT3G27160	*prps21-1*	TCAATGATAGCTTGTGATGG	TTTCCAACTCACAATGTACC	Genotpying	/
ATCG00920	*16S rRNA*	AGTCATCATGCCCCTTATGC	CAGTCACTAGCCCTGCCTTC	NB	/
ATCG01180	*23S rRNA*	GTTCGAGTACCAGGCGCTAC	CGGAGACCTGTGTTTTTGGT	NB	/
ATCG00960	*4.5S rNA*	GAAGGTCACGGCGAGACGAGCC	GTTCAAGTCTACCGGTCTGTTAGG	NB	/
ATCG00970	*5S rRNA*	TATTCTGGTGTCCTAGGCGTAG	ATCCTGGCGTCGAGCTATTTTTCC	NB	/
ATCG00490	*rbcL*	CGTTGGAGAGACCGTTTCTT	CAAAGCCCAAAGTTGACTCC	NB	/
ATCG00020	*psbA*	CGGCCAAAATAACCGTGAGC	TATACAACGGCGGTCCTTATG	NB	/
AT5G30510	*PRPS1*	TTCTCGGGATTGAGATGTTC	CCAATGATGACAAACTCTTCC	NB	/
AT3G54890	*LHCA1*	GTCAAGCCACTTACTTGGGA	GGGATAACAATATCGCCAATG	NB	/
AT1G61520	*LHCA3*	AGGCTGGTCTGATTCCAGCA	ACTTGAGGCTGGTCAAGACG	NB	/
AT3G47470	*LHCA4*	TGAGTGGTACGATGCTGGGA	GTGTTGTGCCATGGGTCAGA	NB	/
AT1G29910	*LHCB1.2*	GACTTTCAGCTGATCCCGAG	CGGTCCCTTACCAGTGACAA	NB	/
AT2G05070	*LHCB2.2*	GAGACATTCGCTAAGAACCG	CCAGTAACAATGGCTTGGAC	NB	/
AT4G10340	*LHCB5*	CTGGTGCTTTGCTTCTTGATG	TCCAGCGATGACGGTAAGCA	NB	/
AT1G15820	*LHCB6*	GCATGGTTTGAAGCTGGAGC	ACAAACCAAGAGCACCGAGA	NB	/
AT4G28750	*PSAE1*	ATGGCGATGACGACAGCATC	TGTTGGTCGATATGTTGGCG	NB	/
AT1G30380	*PSAK*	ATGGTCTTCGAGCCACCAAA	CGTTCAGGTGCATGAGAATA	NB	/
AT1G08380	*PSAO*	ATGGCAGCAACATTTGCAAC	GTAATCTTCAGTCCTGCCCT	NB	/
AT4G21280	*PSBQ1*	ACAGATAACTCAGACCAAGC	GCTTGGCAAGAACATTGTTC	NB	/
AT1G67740	*PSBY*	ATGGCAGCAGCTATGGCAAC	CTCCGGAGGTGGAGTCAAAA	NB	/
AT5G38420	*RBCS*	ATGGCTTCCTCTATGTTCTC	CGGTGCATCCGAACAATGGA	NB	/
AT2G31400	*GUN1*	ATGAGGAAGCCATTAGTGTC	GCTCAATCCTTCTATTCGTC	Real-time	/
AT5G30510	*PRPS1*	TGGTATTGTACCTGGTATGG	AACGTTCCCAAGCAAGTTCG	Real-time	/
AT1G08520	*CHLD*	GTGCCTCCGCGAATGCTAC	GTCAGCATTGTACTCTATGC	Real-time	/
AT1G29910	*LHCB1.2*	CCGTGAGCTAGAAGTTATCC	GTTTCCCAAGTAATCGAGTCC	Real-time	/
AT4G36800	*RUB1*	CTGTTCACGGAACCCAATTC	GGAAAAAGGTCTGACCGACA	Real-time	/
AT2G31400	*GUN1*	**GAATTC**GCTCATCTTTCACAGACTACTC	**GGATCC**CACAGAGCCAAACATTGTTAGG	Yeast 2H	EcoRI/BamHI
AT2G31400	*GUN1-N*	**GAATTC**GCTCATCTTTCACAGACTACTC	**GGATCC**AATAGTTACTTTACCATATCTACCA	Yeast 2H	EcoRI/BamHI
AT2G31400	*GUN1-M*	**GAATTC**GCTAAGAGGATTTTCGAAACTG	**GGATCC**CCGACTGCAAGCATTCAG	Yeast 2H	EcoRI/BamHI

AT2G31400	*GUN1-C*	**GAATTC**TGTAACTCATTTGAAGATGCATCA	**GGATCC**CACAGAGCCAAACATTGTTAGG	Yeast 2H	EcoRI/BamHI
AT2G26670	*GUN2*	**CATATG**GTGGTTGCGGCTACTACTGC	**GAATTC**TCAGGACAATATGAGACGAAGT	Yeast 2H	NdeI/EcoRI
AT3G09150	*GUN3*	**CCCGGG**GTCTCTGCTGTGTCGTATAAGGAA	**ATCGAT**TTAGCCGATAAATTGTCCTGTT	Yeast 2H	XmaI/ClaI for Ad
AT3G09150	*GUN3*	**CCCGGG**GTCTCTGCTGTGTCGTATAAGGAA	**GTCGAC**TTAGCCGATAAATTGTCCTGTT	Yeast 2H	XmaI/SalI for Bd
AT3G59400	*GUN4*	**CATATG**AACGCCTCCGCCACAACT	**GGATCC**TCAGAAGCTGTAATTTGTTTTAAA	Yeast 2H	NdeI/BamHI
AT5G13630	*GUN5*	**GAATTC**GAGGCTCAGTACCAGTCTTCTC	**GAATTC**TTATCGATCGATCCCTTCG	Yeast 2H	EcoRI/EcoRI
AT5G30510	*PRPS1*	**GAATTC**GTTGCAATGTCTAGCGGTC	**GGATCC**CTAAATATCAACTGCAGAAGGAATG	Yeast 2H	EcoRI/BamHI
AT3G27160	*PRPS21*	**GAATTC**GAATCAATGGCGGTCGAAG	**GGATCC**TCAAGAAGGTACATCTCCACCAG	Yeast 2H / RT-PCR	EcoRI/BamHI
AT1G32990	*PRPL11*	**GAATTC**GCCATGGCTCCACCTAAACCC	**GGATCC**ATAGAAACTACCAACCAGGC	Yeast 2H	EcoRI/BamHI
AT5G54600	*PRPL24*	**GAATTC**CTTGCAAAGCTCAAGCGTTG	**GGATCC**CTAAGATGCGGAGGTAACTG	Yeast 2H	EcoRI/BamHI
AT5G26030	*FC1*	**GAATTC**TGCGATATAAAAGAGAGATCTTTCGG	**GAATTC**CTATAGGTTCCGGAACGCATGG	Yeast 2H	BamHI/BamHI
AT2G30390	*FC2*	**GAATTC**GCATTTGCTGCTACTTCATCAAAC	**GAATTC**TTATAATGAAGGCAAGATGCCCC	Yeast 2H	BamHI/BamHI
AT1G08520	*CHLD*	**GGATCC**GTGCCTCCGCGAATGCTAC	**GGATCC**GTATTGCAGACAAAATGAGGTCAAG	Yeast 2H	BamHI/BamHI
AT4G18480	*CHLI1*	**GAATTC**TCGGTTATGAATGTAGCCACTG	**GAATTC**TCAGCTGAAAATCTCGGCG	Yeast 2H	EcoRI/EcoRI
AT5G45930	*CHLI2*	**GGATCC**CTGTTATGAATGTCGCTACAGAG	**GGATCC**CTAAGTGAAAACCTCATAGAACTTC	Yeast 2H	BamHI/BamHI
AT5G08280	*PBGD*	**GAATTC**GCTCAAGCATACGAGACGC	**GGATCC**CTTCTTCGAATGGCTCAGTTG	Yeast 2H	EcoRI/BamHI
AT3G14930	*HEME1*	**GAATTC**GCTGCAAAAGGGCAAGCC	**GGATCC**TCAGACAACCAATTCAGGTTCAG	Yeast 2H	EcoRI/BamHI
AT2G40490	*UROD*	**GGATCC**GTTCCGTCGAGGGAACTAC	**GGATCC**TTAATATCTAATTTCTTGAGCAACCTC	Yeast 2H	BamHI/BamHI
AT5G63570	*GSA1*	**GGATCC**CCGTCGACGAGAAGAAGAAAAG	**GGATCC**CTAGATCCTACTCAGTACCCTC	Yeast 2H	BamHI/BamHI
AT3G48730	*GSA2*	**GAATTC**GCTTCTTCGTCGTCCAACC	**GGATCC**TCCAGAGACATTTTAGAGCCGAC	Yeast 2H	EcoRI/BamHI
AT1G03475	*HEMF1*	**GGATCC**TCTCAATTGAGAAAGAAGTTCCCG	**GGATCC**CAATGGGAAACACAGGCTAGATC	Yeast 2H	BamHI/BamHI
AT4G01690	*PPOX*	**GGATCC**CCACCATCACGACGGATTG	**GGATCC**ATTTACTTGTAAGCGTACCGTGACATG	Yeast 2H	BamHI/BamHI
AT2G31400	*GUN1*	*TCCTTTCAATGGCGTCAACG	**ACAAAAGAAGAGGCTGTAAAGCAAACG	BiFC	attB sites
AT5G30510	*PRPS1*	*ATGGCGTCTTTGGCTCAGC	**AAATATCAACTGCAGAAGGAATGTCG	BiFC	attB sites
AT1G08520	*CHLD*	*TTGAAAATGGCGATGACTCC	**AAGAATTCTTCAGATCAGATAGTGC	BiFC	attB sites
AT5G08280	*PBGD*	*TCGCTCCTCCACCTGAATCCATG	**CGTTGCCGAAGAAGCCAGGAC	BiFC	attB sites
AT2G40490	*UROD*	*ATGTCAATCCTTCAAGTCTCTAC	**AATATCTAATTTCTTGAGCAACC	BiFC	attB sites
AT5G26030	*FC1*	*ATGCAGGCAACGGCTTTATC	**ATAGGTTCCGGAACGCATGG	BiFC	attB sites
AT5G63570	*GSA1*	*ATGTCGGCGACGCTTACAG	**AGATCCTACTCAGTACCCTCTCAGC	BiFC	attB sites

NB, Northern Blot; real time, Real-time qPCR; *attB* sites:
GGGGACAAGTTTGTACAAAAAAGCAGGCT*; GGGGACCACTTTGTACAAGAAAGCTGGGT**

SUPPLEMENTARY MATERIAL AND METHODS

PPR domain predictions

PPR domains of GUN1 were predicted using the TPRpred tool (http://toolkit.tuebingen.mpg.de/tprpred) (Karpenahalli et al., 2007). Only consecutive PPR motifs were considered. The combination of amino acids at position 6 of one PPR and position 1 of the immediately following repeat (named 1') was used to determine the most likely nucleotide ligand for each PPR, based on a previously developed matrix (Barkan et al., 2012; Takenaka et al., 2013).

The BLASTN analysis (Altschul et al., 1990) was carried out on the NCBI server (http://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch&BLAST_SPEC=blast2seq&LINK_LOC=align2seq) using the stringent version of the target sequence and standard settings, except for a reduction in word size (word length 7), to identify similar sequences in the Arabidopsis chloroplast genome (NCBI Reference Sequence: NC_000932.1).

Genevestigator analysis

The Anatomy Tool in the Genevestigator database (https://genevestigator.com/gv/plant.jsp) (Hruz et al., 2008) was employed for *in silico* determination of *GUN1* mRNA expression patterns in different plant organs.

Bacterial one-hybrid (B1H) analysis

To test for a possible DNA-binding activity of GUN1, a B1H assay was performed according to a previously described protocol (Meng and Wolfe, 2006). *GUN1* was amplified from Col-0 cDNA with the primers 5'- GTGGTACCGCTCATCTTTCACAGACTACTC-3' and 5'-

GTTCTAGACACAGAGCCAAACATTGTTAGG-3'. The PCR product was cloned into the KpnI and XbaI sites of the pB1H2-pr2w2 vector using enzymes from New England Biolabs. The resulting plasmid was then transformed into the *E. coli* strain USOΔhisBΔpyrFΔrpoZ (Meng and Wolfe, 2006) . The 18-nt random library was generated by cloning the 5'-ACTGCGGCCGCTATCAGNNNNNNNNNNNNNNNNNGAATTCATACTACTA-3' sequence into the pH3U3-mcs vector. Self-activating sequences were eliminated by negative selection on 5-fluoro-orotic acid. The library vector was then introduced into the *E. coli* strain USOΔhisBΔpyrFΔrpoZ containing the pB1H-pr2w2-GUN1 vector, and the selection screen was performed on selective medium w/o histidine, containing appropriate antibiotics (100 µg/ml ampicillin, 50 µg/ml kanamycin and 10 µg/ml tetracycline), 10 µM IPTG, and increasing concentrations (0, 1, 2, and 4 mM) of 3-amino-triazole. Because no surviving colonies were obtained using this strategy, it can be concluded that GUN1 does not display DNA-binding activity in this assay. As a positive control for the B1H assay, we also carried out B1H experiments using the cDNA sequence coding for an Arabidopsis mTERF - a putative DNA-binding protein. Several clones were obtained in this control experiment, whose inserts were subsequently shown to reflect the target DNA sequences recognized by the full-length mTERF protein.

Nucleotide immunoprecipitation (NIP)-chip assay

Intact chloroplasts were isolated from WT and *oeGUN1-GFP* leaf tissue (10 g) and disrupted in 200 µL extraction buffer (2 mM DTT, 200 mM KOAC, 30 mM HEPES, pH 8.0, 10 mM MgOAc, and proteinase inhibitor cocktail) according to Kunst (1998). After centrifugation (16,000 g for 15 min), the supernatant (stroma extract) was mixed with 2 volumes of Co-IP buffer (150 mM NaCl, 20 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 0.5% (v/v) NP-40, 5 µg/ml aprotinin)

supplemented with 25 µl of magnetic GFP-trap beads (Chromotek, Planegg-Martinsried, Germany) and incubated (2 h, 4°C) on a rotator at 12 rpm. The beads were then washed several times with Co-IP buffer, and nucleic acids were isolated, both from the pellet and the first supernatant before washes, by extraction with phenol-chloroform.

Differential fluorescence labelling of nucleic acids that co-purified with GUN1-GFP or remained in the supernatant fraction was carried out as described (Schmitz-Linneweber et al., 2005) using the Kreatech ULS kit (Kreatech, Amsterdam, Netherlands). The labelled nucleic acids were hybridized to an array bearing DNA fragments representing the entire chloroplast genome of Arabidopsis patterned in a tiling fashion (Kupsch et al., 2012). Nucleic acid hybridization and data analysis were carried out with a Scanarray Gx microarray scanner (Perkin Elmer, Waltham, USA) and the Genepix Pro 7.0 analysis software (Molecular Devices, Sunnyvale, USA) as described before (Schmitz-Linneweber et al., 2005). Control experiments were performed using WT extracts.

Protein synthesis rate assay

The *in-vivo* translational assay was performed as described (Romani et al., 2012). Leaf discs of 4 mm diameter were vacuum-infiltrated with a 1 mM K_2HPO_4–KH_2PO_4 (pH 6.3) buffer containing 0.1 mCi ml^{-1} [^{35}S]methionine, 20 µg ml^{-1} cycloheximide, 0.1% (w/v) Tween-20. The leaf material was then exposed to light (20 µmol photons m^{-2} s^{-1}) and collected after 5, 15 and 30 min. Total proteins were extracted as described above and subjected to SDS-PAGE on a 12% PAA gel.

Polysomes were isolated from 200-mg (fresh weight) aliquots of frozen leaf material in the presence of 0.5 mg/ml heparin, 100 mM 2-mercaptoethanol, 100 µg/ml chloramphenicol and 25 µg/ml cycloheximide, as described previously (Barkan, 1998). The microsomal extract was solubilized with 1% (v/v) Triton X-100 and 0.5% (w/v) sodium deoxycholate. The solubilized

material was layered onto 0.44/1.6 M sucrose-step gradients and centrifuged at 250,000g for 65 min at 4°C. The gradient was fractionated, and the mRNA associated with polysomes was isolated by extraction with phenol/chloroform/isoamyl alcohol (25:24:1), followed by precipitation at room temperature with 95% ethanol. All samples were then subjected to RNA gel-blot analysis. The gene-specific radiolabelled probes were synthesized as described above; the corresponding primer sequences are listed in Supplemental Table S2.

SUPPLEMENTARY LITERATURE CITED

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol **215:** 403-410

Barkan A (1998) Approaches to investigating nuclear genes that function in chloroplast biogenesis in land plants. Photosynthesis: Molecular Biology of Energy Capture **297:** 38-57

Barkan A, Rojas M, Fujii S, Yap A, Chong YS, Bond CS, Small I (2012) A combinatorial amino acid code for RNA recognition by pentatricopeptide repeat proteins. PLoS Genet **8:** e1002910

Hruz T, Laule O, Szabo G, Wessendorp F, Bleuler S, Oertle L, Widmayer P, Gruissem W, Zimmermann P (2008) Genevestigator v3: a reference expression database for the meta-analysis of transcriptomes. Adv Bioinformatics **2008:** 420747

Karpenahalli MR, Lupas AN, Soding J (2007) TPRpred: a tool for prediction of TPR-, PPR- and SEL1-like repeats from protein sequences. BMC Bioinformatics **8:** 2

Koussevitzky S, Nott A, Mockler TC, Hong F, Sachetto-Martins G, Surpin M, Lim J, Mittler R, Chory J (2007) Signals from chloroplasts converge to regulate nuclear gene expression. Science **316:** 715-719

Kupsch C, Ruwe H, Gusewski S, Tillich M, Small I, Schmitz-Linneweber C (2012) Arabidopsis chloroplast RNA binding proteins CP31A and CP29A associate with large transcript pools and confer cold stress tolerance by influencing multiple chloroplast RNA processing steps. Plant Cell **24:** 4266-4280

Meng X, Wolfe SA (2006) Identifying DNA sequences recognized by a transcription factor using a bacterial one-hybrid system. Nat Protoc **1:** 30-45

Romani I, Tadini L, Rossi F, Masiero S, Pribil M, Jahns P, Kater M, Leister D, Pesaresi P (2012) Versatile roles of Arabidopsis plastid ribosomal proteins in plant growth and development. Plant J **72:** 922-934

Schmitz-Linneweber C, Williams-Carrier R, Barkan A (2005) RNA immunoprecipitation and microarray analysis show a chloroplast Pentatricopeptide repeat protein to be associated with the 5' region of mRNAs whose translation it activates. Plant Cell **17:** 2791-2804

Takenaka M, Zehrmann A, Brennicke A, Graichen K (2013) Improved computational target site prediction for pentatricopeptide repeat RNA editing factors. PLoS One **8:** e65343