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Supplementary Information for

GUN1 interacts with MORF2 to regulate plastid RNA editing during retrograde signaling

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Datasets S1 to S2

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Supporting information

SI Materials and Methods

Plant Materials and Growth Condition. *Col6-3* wild type, *gun1-8* and *gun1-9* mutants (1) were from our lab seed stock. *otp81* (*SALK_092402*), *otp84* (*SALK_120902*) and *ys1* (*SALK_123515*) mutants were ordered from ABRC (2). *otp81otp84ys1* triple mutant was obtained by crossing the corresponding single mutants. Seeds were surface sterilized using chlorine gas for 4 hours and placed on 1/2 Linsmaier and Skoog (LS) medium (Caisson Laboratories) plate with 0.8% micropropagation type-1 agar (Caisson Laboratories). After a 4-day stratification in the dark at 4 °C, plates were moved to 24 h constant light condition under 100 μmol.m^{-2}.s^{-1} light intensity at 22 °C for 5 days. For norflurazon treatment, norflurazon (NF) (Sigma Aldrich-SUPELCO) was added to the 1/2 LS medium at 5 μM final concentration. For lincomycin treatment, lincomycin (Linc) (Sigma Aldrich) was added to the 1/2 LS medium at 220 μg/mL final concentration. To eliminate the influence of interplay between sucrose and retrograde signaling pathway (3), no additional sucrose was supplied in the NF, Linc treatment or normal growth conditions. The AGI locus number of each gene is: *GUN1* (AT2G31400), *MORF2* (AT2G33430), *OTP81* (AT2G29760), *OTP84* (AT3G57430), *YS1* (AT3G22690), *LHCB1.2* (AT1G29910), *PLASTOCYANIN* (AT1G76100), *CA1* (AT3G01500), *LHCB2.2* (AT2G05070), *CP12* (AT3G62410), *PPI2* (AT4G02510) and *PP2AA3* (AT1G13320). All the primers used for cloning and vectors construction are listed in Table S2.

RNA Isolation and Analysis of Plastid RNA Editing. Total RNA was isolated from pooled whole seedlings (Grown under 24 h light condition with 100 μmol.m^{-2}.s^{-1} light intensity at 22 °C with 5 μM NF or 220 μg/mL Linc treatment for 5 days as indicated) using the RNeasy Plant Mini Kit (Qiagen) with dsDNase I treated. The first-strand cDNA was synthesized using Maxima First Strand cDNA Synthesis Kit for RT-qPCR (Thermo). PCR fragments containing chloroplast RNA editing sites were obtained with specific primers (Table S3) surrounding editing sites by RT-PCR using One*Taq*® 2× Master Mix with Standard Buffer (NEB). The following thermal condition was used for RT-PCR: 94 °C for 3 min, 33 cycles of 94 °C for 30 s, 52 °C for 45 s, 72 °C for 1 min. PCR products were then purified and used as templates for Sanger DNA sequencing (Sequencing was carried out by Eton Bioscience, San Diego, USA). The sequencing primer for each site is listed in Table S3. The "C" to "T" (equal C to U in RNA) editing level of each site was measured by the relative height of the peak of the nucleotide in sequence chromatograms and calculated by the height of "T" divided by the sum of the height of "T" and "C". Statistical significances are calculated using two-tailed *Student's t*-Test in Excel.

Yeast Two-Hybrid Assay. The yeast two-hybrid assay was performed following the manual of Matchmaker™ Gold Yeast Two-Hybrid System (Clontech) with modifications. Generally, the coding sequence (CDS) of each gene was cloned by RT-PCR using Phusion® High-Fidelity DNA Polymerase (NEB) and vectors were linearized by restriction enzymes EcoRI (for GUN1) or NdeI

and BamHI. Then insert genes were transferred to target plasmids using In-Fusion® HD Cloning Plus kit (Clontech). Combinations of GAL4 DNA binding domain (pGBKT7) and GAL4 activation domain (pGADT7) fusions of corresponding genes were co-transformed into the yeast strain AH109 (Clontech). Co-transformants were placed on SD/–Leu/–Trp dropout plates under 30 °C in dark for 5 days to verify successful co-transformation and then on SD/–Ade/–His/–Leu/–Trp/X-α-Gal dropout plates under 30 °C in dark for 5 days to verify the interaction.

Bimolecular Fluorescence Complementation (BiFC) and Firefly Luciferase Complementation Imaging (LCI) Assay. For BiFC assay, the CDS of each gene without stop codon was cloned by RT-PCR using Phusion® High-Fidelity DNA Polymerase (NEB) and transfer to target plasmids: pUC-pSPYNE173 for N- terminal YFP fusion or pUC-pSPYCE(M) for C-terminal YFP fusion (4). Vectors were digested by XbaI and SalI. Transfection-grade plasmid DNA was prepared using QIAGEN Plasmid Maxi Kit (Qiagen). For control plasmids, the coding sequence of transit peptide of OTP81 was cloned by PCR and ligated to the N terminal of YFP to test chloroplast localization. Then, the transit peptide of OTP81 was transferred to pUC-pSPYNE173 or pUC-pSPYCE(M) for N or C-terminal YFP fragment fusions targeting to chloroplasts, respectively. 20 μg of each transfection-grade plasmid was cotransformed into *Arabidopsis* protoplasts isolated from *Col-0* as previously described (5). Protoplasts were then incubated under constant 100 $\mu mol.m^{-2}.s^{-1}$ light at 22 °C for 16-20 h and the fluorescence signal was determined using a Zeiss LSM 710 confocal laser scanning microscope.

For LCI assay, the CDS of each gene without stop codon was cloned and transfer to target plasmids: pUC19-NLuc for N-terminal luciferase fusion or modified pUC19-CLuc (cLuc is fused to the C-terminal of each gene) for C-terminal luciferase fusion (6). Vectors were digested by SacI or KpnI and SalI. 20 μg of each transfection-grade plasmid were cotransformed into *Arabidopsis* protoplasts and incubated under constant 100 $\mu mol.m^{-2}.s^{-1}$ light condition at 22 °C for 16-20 h. Then, the D-luciferin (BIOSYNTH) was added to protoplasts at a final concentration of 1 mM and protoplasts were kept in dark for 10 min to quench the fluorescence. The relative luciferase activity was recorded using the Tecan Safire2™ plate reader.

Plant Transformation and Expression Level Detection. For overexpression experiment, the whole fragment containing the cauliflower mosaic virus (CaMV) 35S promoter, the CDS of MORF2 (without stop codon) and the 3×HA-3×FLAG tag was cloned by PCR using Phusion® High-Fidelity DNA Polymerase (NEB) from MORF2 BiFC assay vector and then transferred to the modified binary vector pEarleyGate 101 (7) digested by restriction enzymes MluI and SpeI. In the final vector, the CDS of MORF2 (without stop codon) was driven by CaMV 35S promoter and fused with 3×HA-3×FLAG tags in the C-terminal. Plasmids were then transformed into *Agrobacterium tumefaciens* strain GV3101 to transform *Arabidopsis Col6-3* wild type plants using the floral-dip method (8). Transgenetic plants with Basta resistance selected on Glufosinate-ammonium (Sigma) plates were further propagated and T3 homozygous seeds were chosen for further study. At least two independent lines were examined with similar results, and one

representative line was shown. The expression level of MORF2 in *MORF2OX* lines were examined by qPCR and western blot. Total RNA or protein was extracted from whole seedlings grown on 1/2 LS medium under 24 h constant light (100 μmol.m^{-2}.s^{-1}) at 22 °C for 5 days. The qPCR analysis was as below and primers for *MORF2* were: MORF2-qF: 5'-ATCTCGGTTAGGTTGTTC-3' and MORF2-qR: 5'-ATCATCTGCTGCTTAGTC-3'. The antibodies used for western blot were Anti-FLAG® M2-Peroxidase (HRP) antibody (A8592, Sigma) and Anti-Actin (plant) antibody (A0480, Sigma).

Coimmunoprecipitation and Mass Spectrometric Analysis. GUN1 overexpression line fused with GFP tag in the background of *gun1-9* (1) was crossed with *MORF2OX(w)* (fused with 3×HA and 3×FLAG tags) line to construct coexpression lines. F3 homozygous plants were used for the co-immunoprecipitation assay. Generally, 5 g total seedlings grown on 5 μM NF plate under 24 h light (100 μmol.m^{-2}.s^{-1}) at 22 °C for 5 days were collected. Then 25 mL immunoprecipitation (IP) buffer (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 5 mM $MgCl_2$, 10% glycerol, 0.1% NP-40, 0.5 mM DTT, 1 mM PMSF, 1×complete protease inhibitor mixture (Roche), 10 μM MG132) was added to the grinded samples and rotated at 4 °C for 10 min. The supernatants were separated by centrifugation for 30 min at 4℃, 13,500 rpm and incubated with 100 μL Anti-FLAG® M2 Magnetic Beads (Sigma) for 2.5 h at 4 ℃ with rotation. Washed beads with 25 mL IP buffer once followed by five times wash with 1 ml IP buffer for 5 min each. Then binding proteins were eluted twice using 100 μL of 3×FLAG peptide (Sigma) (150 ng/μL) at 25 ℃ with rotation for 15 min each.

The Mass spectrometric analysis was performed at the Mass Spectrometry Core of the Salk Institute for Biological Studies. Generally, eluted protein complexes from co-immunoprecipitation were precipitated by methanol/chloroform. Dried pellets were dissolved in 8 M urea/100 mM TEAB, pH 8.5. Proteins were reduced with 5 mM tris (2-carboxyethyl) phosphine hydrochloride (TCEP, Sigma-Aldrich) and alkylated with 10 mM chloroacetamide (Sigma-Aldrich). Proteins were digested overnight at 37 ° C in 2 M urea/100 mM TEAB, pH 8.5, with trypsin (Promega). Digestion was quenched with formic acid, 5% final concentration. Digested samples were analyzed on a Fusion Orbitrap tribrid mass spectrometer (Thermo). Protein and peptide identification were done with Integrated Proteomics Pipeline - IP2 (Integrated Proteomics Applications). Tandem mass spectra were extracted from raw files using RawConverter (9) and searched with ProLuCID (10) against UniProt database with reversed sequences. The search space included all fully-tryptic and half-tryptic peptide candidates. Data were searched with 50 ppm precursor ion tolerance and 600 ppm fragment ion tolerance. Identified proteins were filtered to using DTASelect (11) and utilizing a target-decoy database search strategy to control the false discovery rate to 1% at the protein level (12).

***gun* Phenotype Assay with qPCR Analysis.** For *gun* phenotype assay, retrograde signaling marker genes expression were checked by qPCR. RNA samples were isolated from pooled whole seedlings grown under 24 h light condition with 100 μmol.m^{-2}.s^{-1} light intensity at 22 °C with 5

μM NF or 220 μg/mL Linc treatment for 5 days. The first-strand cDNA was synthesized using Maxima First Strand cDNA Synthesis Kit for RT-qPCR (Thermo). qPCR was performed on a CFX384™ Real-Time PCR Detection System (Bio-rad) using iTaq™ Universal SYBR® Green Supermix (Bio-rad). The following thermal condition was used: 95 °C for 3 min, 45 cycles of 95 °C for 10 s and 60 °C for 30 s. Expression levels for all assayed genes were normalized using *PP2AA3* (AT1G13320) (13) as the internal control. Primers used for qPCR are listed in Table S4. Statistical significances are calculated using two-tailed *Student's t* test in Excel.

RNA-sequencing and Data Analysis. Total RNA samples for RNA-sequencing (RNA-seq) were prepared as same as used in the RNA editing analysis. Two biological replicates of each genotype were sequenced. 500 ng total RNA of each sample was used to prepare RNA-seq libraries using the TruSeq Stranded mRNA Library Prep Kit (Illumina). Single-end sequencing was run on Illumina HiSeq 2500 sequencing machine (Illumina). BRB-SeqTools (https://brb.nci.nih.gov/seqtools/) was used to calculate the raw count of each gene. In detail, raw reads were aligned to *Arabidopsis* reference genome (TAIR10) using TopHat version 2.1.1 (14) with bowtie2-2.3.1 (15) and gene-level raw counts data files were generated using HTSeq version 0.6.1 (16). Chloroplast and mitochondria genes were excluded from further analysis. Raw counts were applied to Bioconductor package edgeR (17) in R language to get the Reads Per Kilobase of transcript per Million mapped reads (RPKM) of each gene and to identify differentially expressed genes (DEGs). When identifying DEGs, a gene was retained only if it was expressed at a count-per-million (CPM) above 0.5 in at least two samples. Genes had a log2-converted fold change ≥ 1 or ≤ -1 with a False Discovery Rate (FDR) ≤ 0.05 were considered as DEGs. Venn diagrams were generated using interactivenn (http://www.interactivenn.net/) (18) and modified manually. The significance of overlaps in Venn diagrams was calculated using the hypergeometric distribution method in R language. The gene ontology (GO) term enrichment was analyzed using agriGO v2.0 (http://systemsbiology.cau.edu.cn/agriGOv2/) (19) and terms with an FDR < 0.01 were retained for further analysis. Heatmaps were generated using the pheatmap program (package version 1.0.8) in R language. The list of photosynthesis genes was according to Sun et al, 2013 (20). The RNA-seq raw data have been deposited into the NCBI Gene Expression Omnibus database under the accession number GSE110125.

Mutant Genotyping. Homozygous T-DNA insertion mutant lines were identified using mutant gene-specific primers and T-DNA left-border primer (LBb1.3, 5'-ATTTTGCCGATTTCGGAAC-3'). Mutant gene-specific primers used are: *otp81*, 5'-GTCGTTCCATGTAATTGGGTG-3' and 5'-GCTAAGAACAGGGTCTGGTCC-3'; *otp84*, 5'-GTCATGTCCTCTTGCCTTCAC -3' and 5'- GGAATTCTCATCCAAAGAGCC -3'; *ys1*, 5'-TCAAACAAACTTCCTTGCACC-3' and 5'- TCGAGGATATGCATCTTCTGG-3'. PCR was carried out using the OneTaq® 2× Master Mix with Standard Buffer (NEB). The following thermal condition was used: 94 °C for 5 min, 35 cycles of 94 °C for 30 s, 52 °C for 45 s, 72 °C for 1 min.

Supplemental Figures

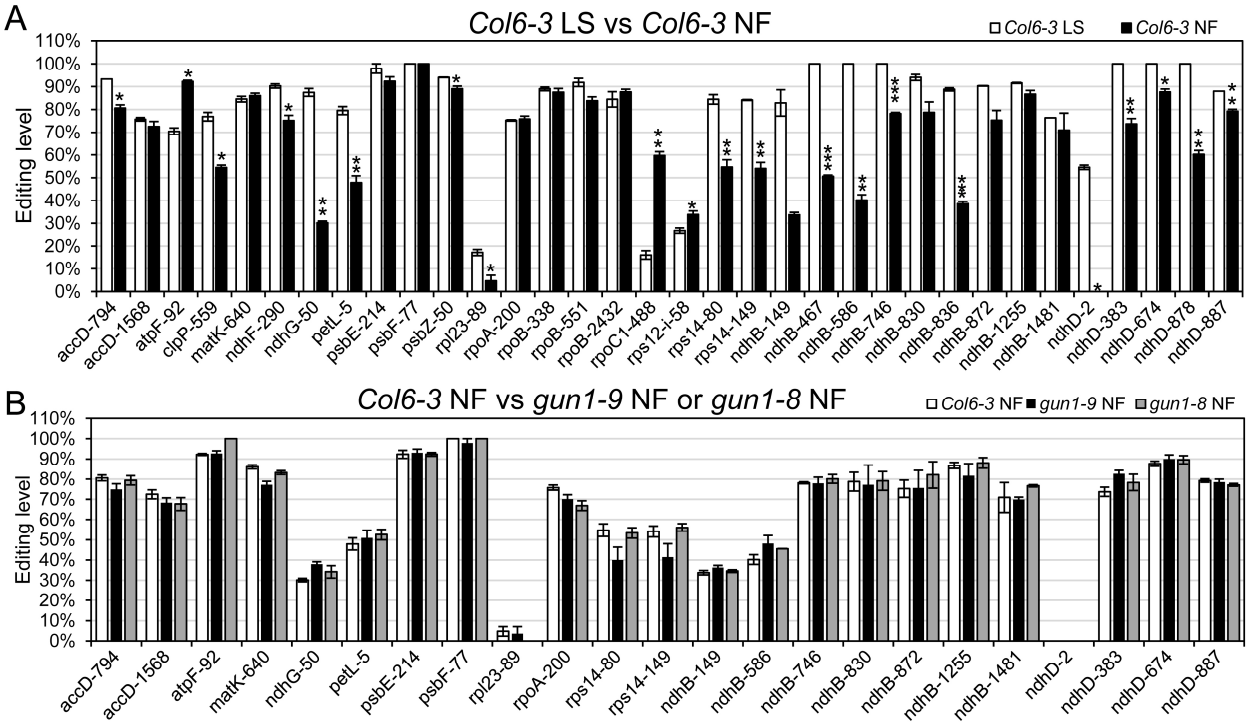


Fig. S1. Plastid RNA-editing profiles in wild type and *gun1* mutants under NF treatment. The *x* axis indicates different RNA-editing sites. The *y* axis represents the editing level of each site. Data are mean ± SEM from two (LS) or three (NF) biological replicates. *Col6-3* is the wild type. (*A*) The comparison of RNA-editing level changes in wild type *Col6-3* between with (NF) and without NF (LS) treatments. Asterisks represent significance level $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ (two-tailed Student's *t* test) compared with in *Col6-3* LS. (*B*) The RNA-editing profile of sites that were not significantly affected in *gun1* mutants compared with wild type under NF treatment.

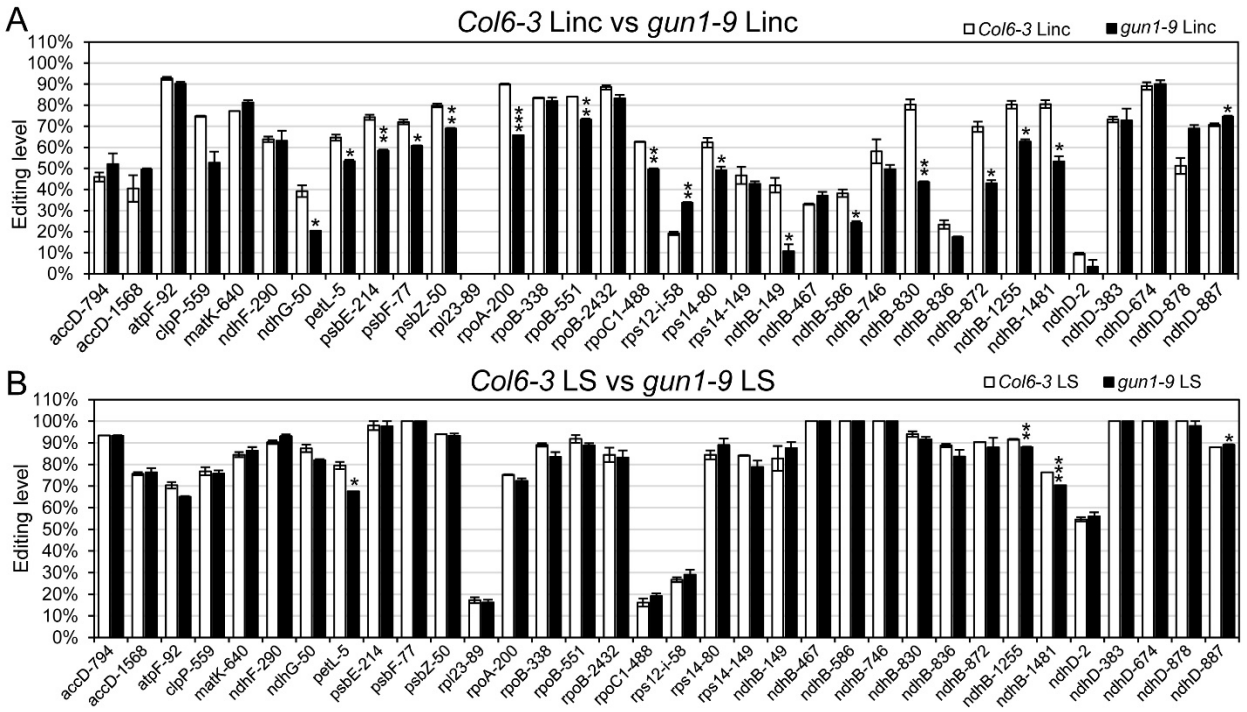
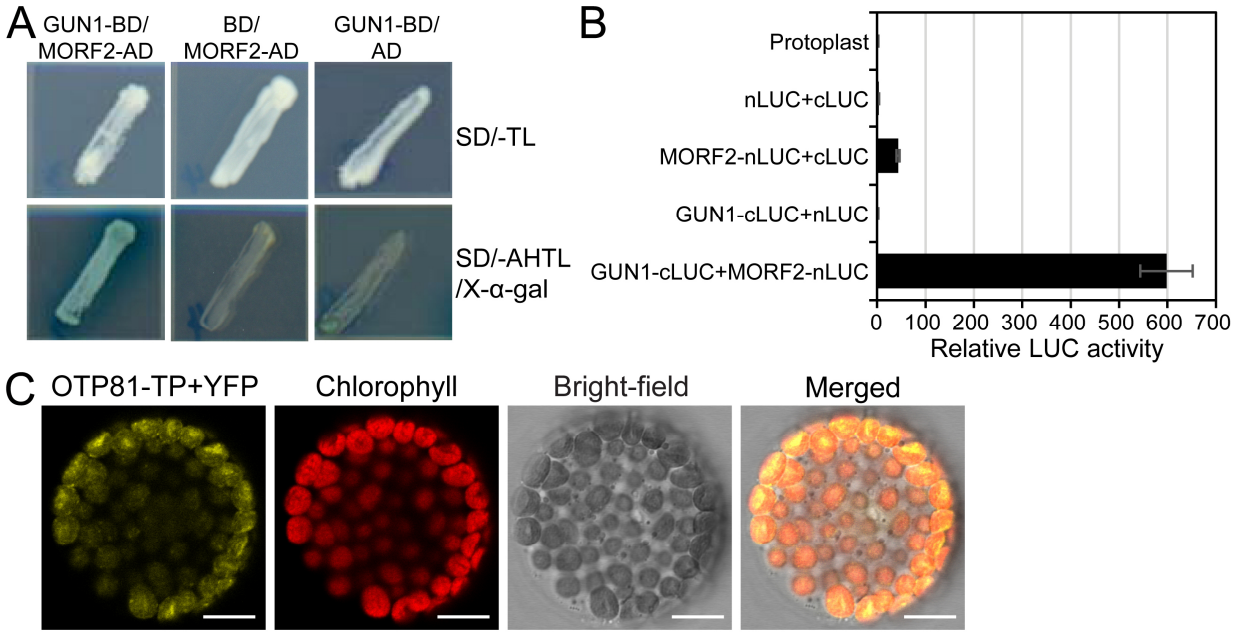


Fig. S2. Plastid RNA-editing profiles in Linc-treated or untreated wild-type and *gun1-9* seedlings. The *x* axis indicates different RNA-editing sites. The *y* axis represents the editing level of each site. Data are mean ± SEM from two biological replicates. *Col6-3* is the wild type. (*A*) The comparison of RNA-editing profile between wild type and *gun1-9* under Linc treatment. Asterisks represent significance level *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (two-tailed Student's *t* test) compared with in wild type. (*B*) The comparison of RNA-editing profile between wild type and *gun1-9* under normal growth condition (LS). Asterisks represent significance level *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (two-tailed Student's *t* test) compared with in wild type.



D

Uniprot accession	Protein	Peptide counts				Sequence coverage (%)				Description
		Col6-3	*35S:GUN1-GFP*	*MORF2 OX(s)*	*35S:GUN1-GFP/ MORF2OX(w)*	*Col6-3*	*35S:GUN1-GFP*	*MORF2 OX(s)*	*35S:GUN1-GFP/ MORF2OX(w)*	
A0A1P8B1 Q9	GUN1	0	0	0	22	0	0	0	16	Genomes uncoupled 1
O82380	OTP81	0	0	28	27	0	0	17.2	19.9	Pentatricopeptide repeat-containing protein At2g29760
Q7Y211	OTP84	0	0	26	27	0	0	21.3	29	Pentatricopeptide repeat-containing protein At3g57430
Q9LUJ2	YS1	0	0	6	17	0	0	6.5	12.9	Pentatricopeptide repeat-containing protein At3g22690

Fig. S3. Protein interaction assays show that GUN1 interact with MORF2. (*A*) Y2H assays show the interaction between GUN1 and MORF2 in yeast. AD and BD represent the GAL4 activation and DNA binding domain, respectively. SD/-TL and SD/-AHTL/X-α-gal indicate the SD/-Trp/-Leu and SD/-Ade/-His/-Trp/-Leu/X-α-gal dropout plates, respectively. The growth of colonies on SD/-TL indicates the successful cotransformation. The growth as well as the blue color of the colonies on SD/-AHTL/X-α-gal plate showing the reporter genes activity indicate the interaction. (*B*) The LCI assay confirms the interaction between GUN1 and MORF2 in plant. The *x* axis represents the relative luciferase activity. The *y* axis represents different co-transformations. Data are mean ± SEM from three biological replicates. nLUC: N-terminal luciferase, cLUC: C-terminal luciferase. Co-transformation leading to LUC complementation showing relative high luciferase activities indicate interactions. Target proteins coexpressed with corresponding empty vectors as well as protoplast are negative controls. (*C*) The YFP protein can be targeted to chloroplasts by the transit peptide of OTP81. OTP81-TP+YFP: YFP fused with the OTP81 transit peptide. Red auto-fluorescence from chlorophyll indicates the localization of chloroplasts. Bright-field images correspond to protoplast cells. Merged images show the colocalization of YFP and chloroplasts. (Scale bar, 10 μm). (*D*) The Mass Spectrometric analysis of partial proteins identified by coimmunoprecipitation of MORF2.

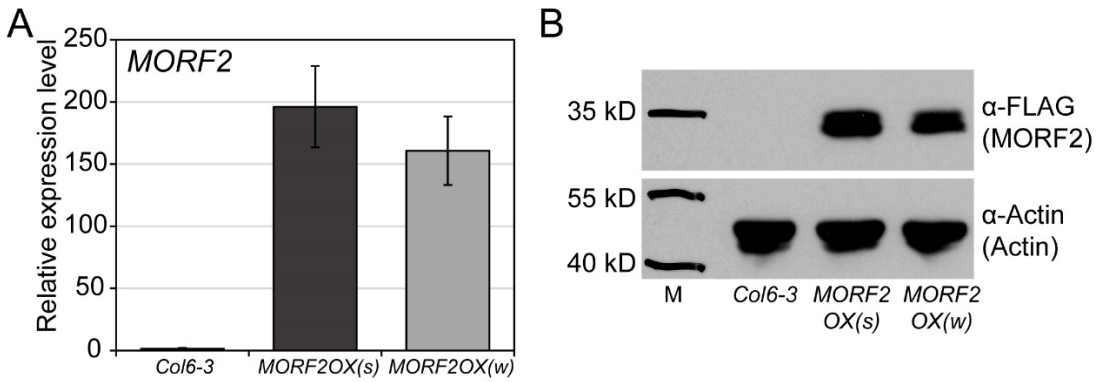


Fig. S4. Expression levels of *MORF2* in *MORF2* overexpression lines. (*A*) Elevated expressions of *MORF2* in *MORF2OX(s)* and *MORF2OX(w)* lines. Total RNAs were isolated from whole seedlings grown on 1/2 LS medium under 24 h light (100 μmol.m^{-2}.s^{-1}) at 22 °C for 5 days. The *x* axis indicates samples. The *y* axis represents the relative expression level and the expression level in *Col6-3* is set to one. Data are mean ± SEM (three biological replicates). (*B*) MORF2 protein expression levels in *MORF2OX(s)* and *MORF2OX(w)* lines. MORF2 protein levels were detected by FLAG antibodies (top panel). The bottom panel showing expression levels of Actin works as the loading control. M: protein marker. kD, kilo Dalton.

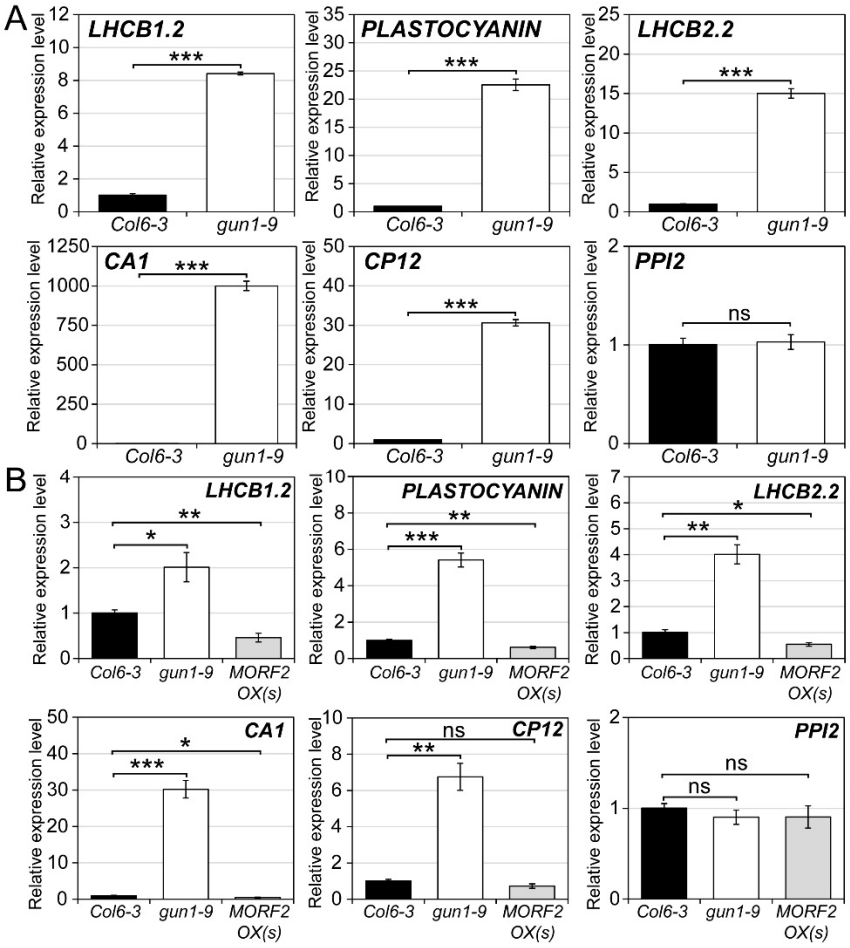


Fig. S5. Expression levels of retrograde signaling marker genes in NF-treated *gun1-9* mutant seedlings as well as in Linc-treated *gun1-9* and *MORF2OX(s)* seedlings. *Col6-3* is the wild type. Total RNAs were isolated from whole seedlings grown under 5 µM NF or 220 µg/mL Linc treatment under 24 h light (100 µmol.m^{-2}.s^{-1}) at 22 °C for 5 days. The *x* axis indicates samples. The *y* axis represents the relative expression level and the expressions of each gene in *Col6-3* are set to one. The *PPI2* gene is the negative control. Data are mean ± SEM (three biological replicates) (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, (ns) not significant, two-tailed Student's *t* test). (*A*) The qPCR analysis of retrograde signaling marker genes expressions in NF-treated *gun1-9* mutant seedlings. (*B*) The qPCR analysis of retrograde signaling marker genes expression in Linc-treated *gun1-9* and *MORF2OX(s)* seedlings.

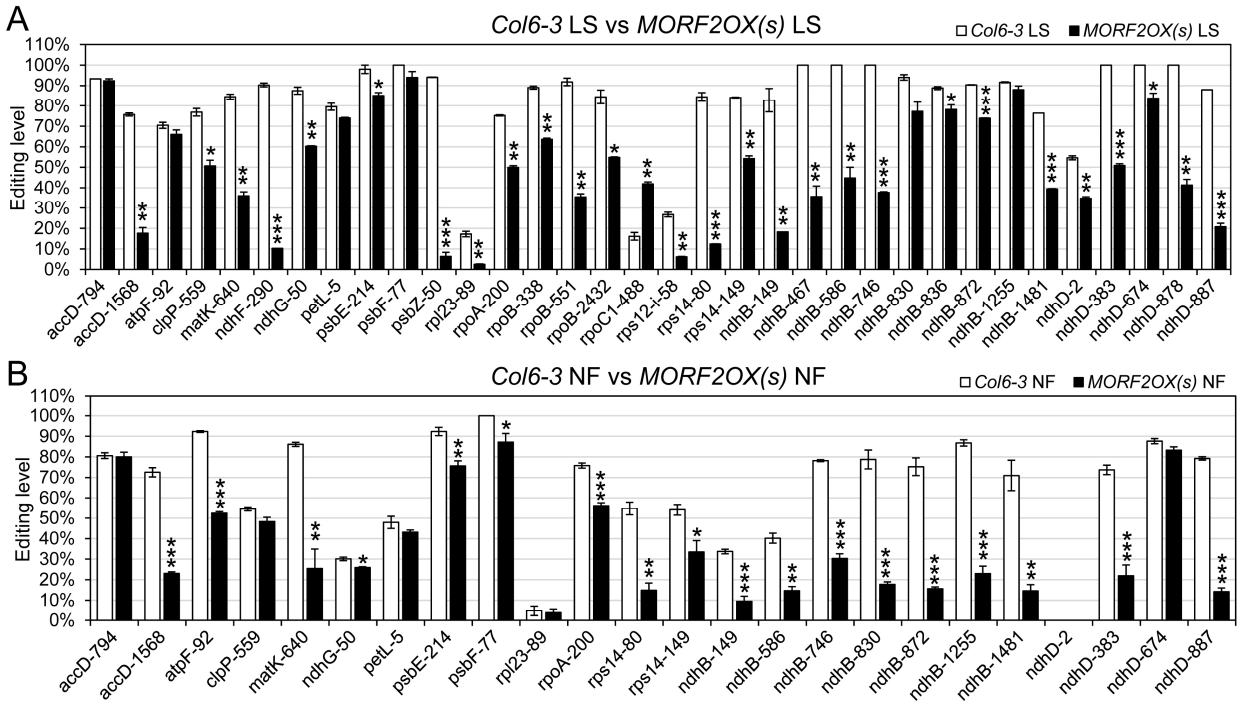


Fig. S6. Plastid RNA-editing profiles in untreated (LS) and NF-treated (NF) *MORF2OX(s)* seedlings. The *x* axis indicates the different RNA-editing sites. The *y* axis represents the editing level of each site. Data are mean ± SEM from two (LS) or three (NF) biological replicates. *Col6-3* is the wild type. (*A*) The plastid RNA-editing profile in untreated *MORF2OX(s)* seedlings. Asterisks represent significance level *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (two-tailed Student's *t* test) compared with in *Col6-3* LS. (*B*) The plastid RNA-editing profile in NF-treated *MORF2OX(s)* seedlings. The sites that are not overlapped with *gun1* mutation affected sites are listed. Asterisks represent significance level *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (two-tailed Student's *t* test) compared with in *Col6-3* NF.

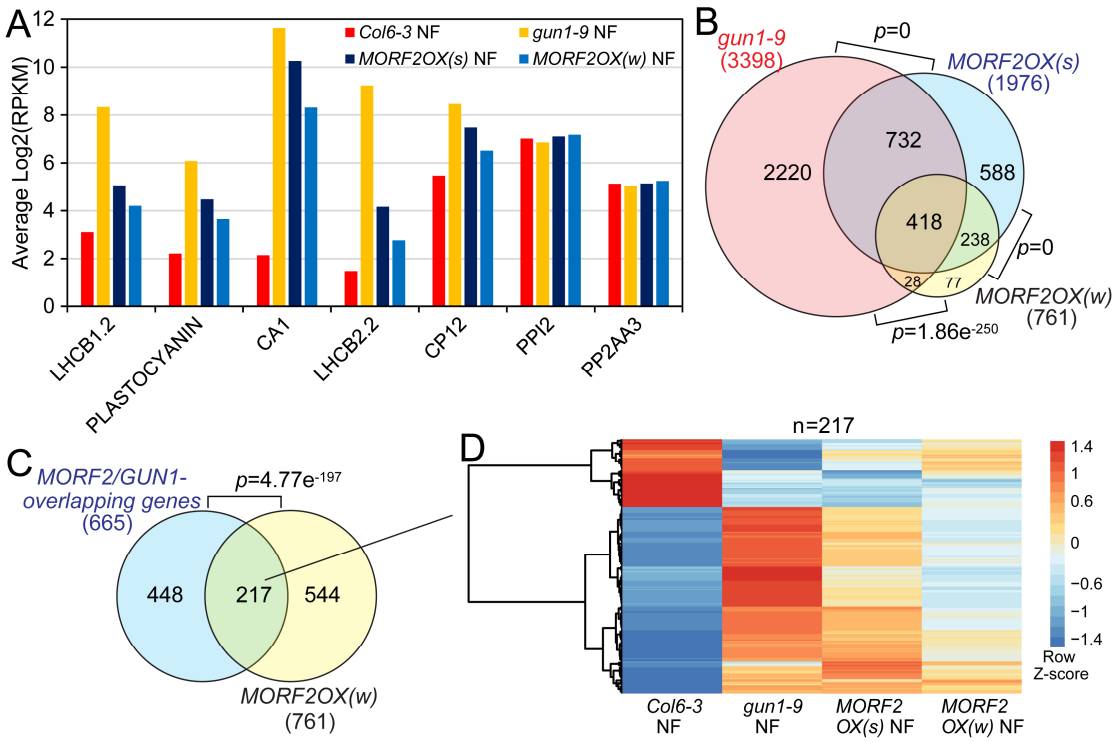


Fig. S7. RNA-sequencing data show *gun* phenotypes of *MORF2OX(s)* and *MORF2OX(w)* lines. (*A*) Expression levels of retrograde signaling marker genes from RNA-sequencing data. *PPI2* is the negative control gene and *PP2AA3* is the internal control gene used in the qPCR analysis. The *x* axis indicates different genes. The *y* axis represents the log2-transformed average RPKM (Reads Per Kilobase of transcript per Million mapped reads) of each gene. (*B*) The Venn diagram shows DEGs overlap among *gun1-9*, *MORF2OX(s)* and *MORF2OX(w)* (*MORF2OX(w)* NF vs *Col6-3* NF) under NF treatment. (*C*) The Venn diagram shows the overlap of MORF2/GUN1-overlapping retrograde signaling genes (MORF2/GUN1-overlapping genes) and *MORF2OX(w)* DEGs. *P* values in *B*-*C* show the statistical significance of the overlap between two groups of genes in Venn diagrams. (*D*) The heatmap displays the expression profile of 217 overlapping genes (from Fig. S7*C*, including all retrograde signaling marker genes) in different samples under NF treatment showing *MORF2OX(w)*'s relatively weaker *gun* phenotype. The heatmap shows the Z-score value of log2-transformed average (RPKM+0.001) of each gene. A background 0.001 is added to the RPKM of each gene to avoid minus infinity.

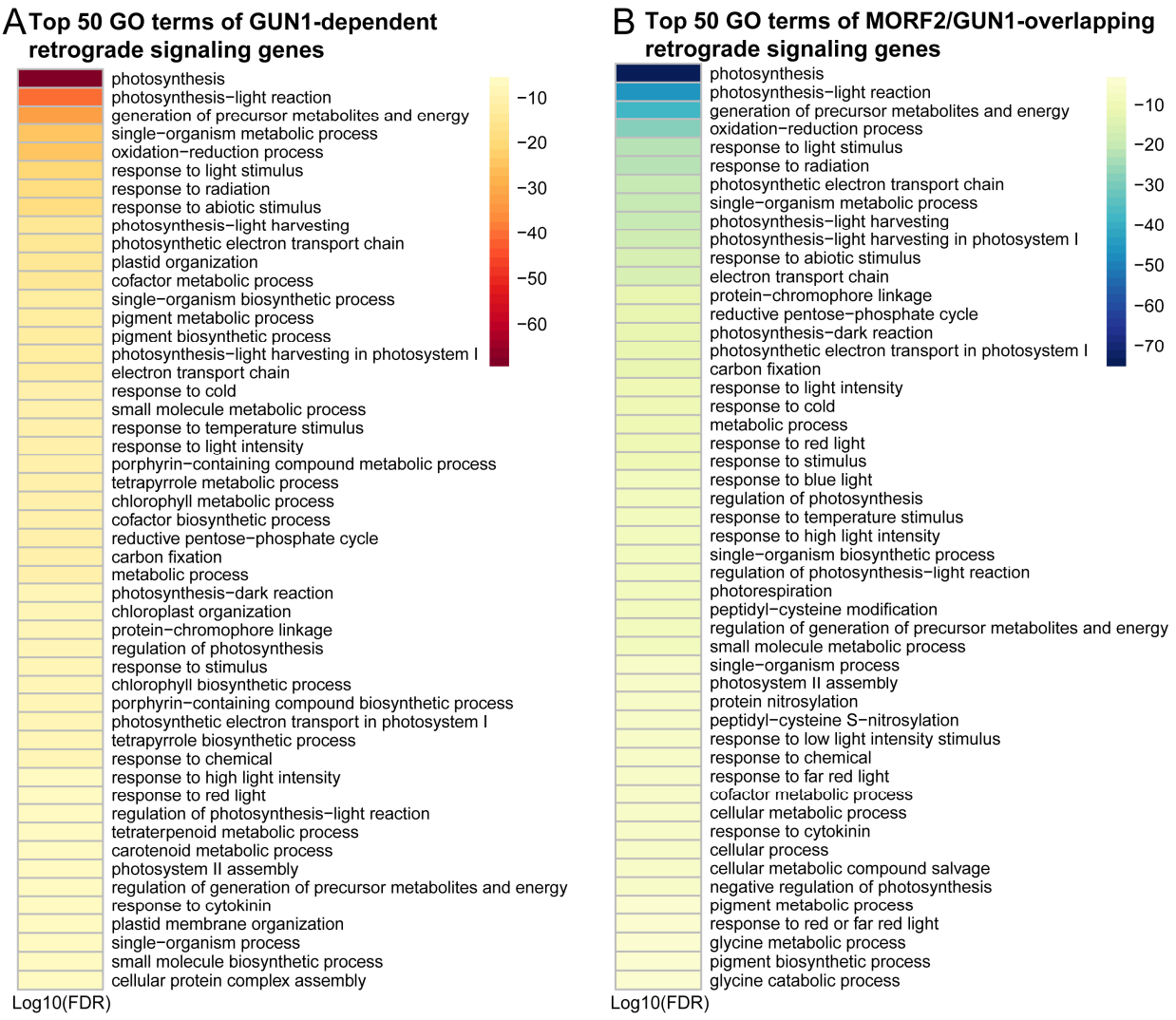


Fig. S8. Top 50 Gene Ontology (GO) terms show the similar enrichment between GUN1-dependent retrograde signaling genes and MORF2/GUN1-overlapping retrograde signaling genes. GO terms are ordered based on the log10-transformed FDR (false discovery rate) of each GO term. (*A*) Top 50 GO terms of GUN1-dependent retrograde signaling genes. (*B*) Top 50 GO terms of MORF2/GUN1-overlapping retrograde signaling genes.

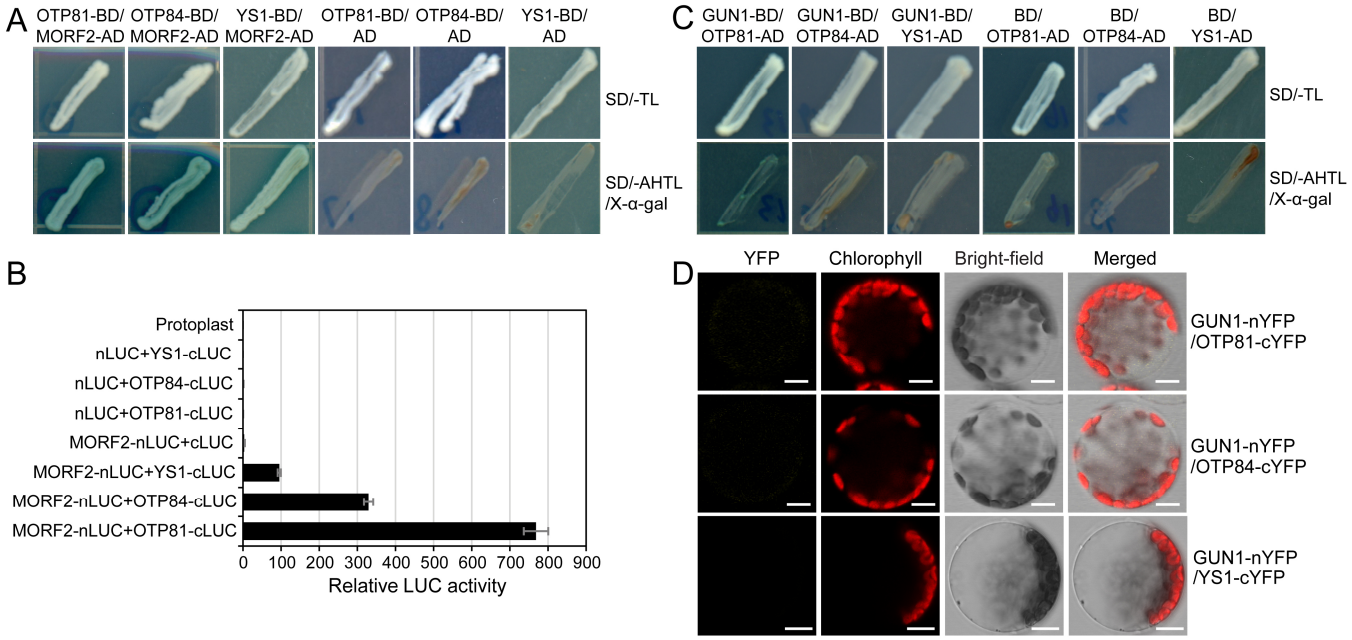


Fig. S9. Protein interaction assays show that MORF2 can interact with OTP81, OTP84, and YS1 but GUN1 cannot interact with OTP81, OTP84, or YS1. (*A*) Y2H assays show that MORF2 interact with OTP81, OTP84, and YS1 in yeast. AD and BD represent the GAL4 activation and the DNA binding domain, respectively. SD/-TL and SD/-AHTL/X-α-gal indicate SD/-Trp/-Leu and SD/-Ade/-His/-Trp/-Leu/X-α-gal dropout plates, respectively. The growth of colonies on SD/-TL indicates the successful cotransformation. The growth, as well as the blue color of colonies on SD/-AHTL/X-α-gal plates showing reporter genes activity, indicate interactions. (*B*) LCI assays confirm interactions of MORF2 with OTP81, OTP84, and YS1 in plant. The *x* axis represents the relative luciferase activity. The *y* axis indicates different cotransformations. Data are mean ± SEM from three biological replicates. nLUC: N-terminal luciferase, cLUC: C-terminal luciferase. Co-transformation leading to LUC complementation showing relative high luciferase activities indicate the interactions. Target proteins coexpressed with corresponding empty vectors as well as protoplast are negative controls. (*C*) Y2H assays show that GUN1 cannot interact with OTP81, OTP84, or YS1 in yeast. (*D*) BiFC assays show that GUN1 cannot interact with OTP81, OTP84, or YS1 in plants. nYFP: N-terminal YFP, cYFP: C-terminal YFP. Chlorophyll red auto-fluorescence indicates the localization of chloroplasts. Bright-field images correspond to protoplast cells. Merged images show colocalizations of YFP and chloroplasts. (Scale bar, 10 μm).

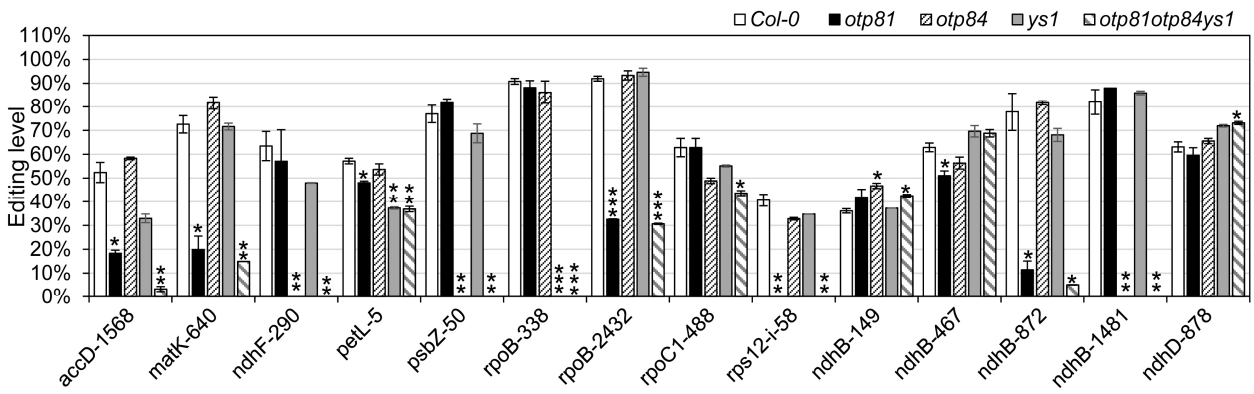


Fig. S10. Plastid RNA-editing profiles in NF-treated *otp81*, *otp84*, and *ys1* single and *otp81otp84ys1* triple mutant seedlings. The *x* axis indicates different RNA-editing sites. The *y* axis represents the editing level of each site. Data shown are the mean ± SEM from two biological replicates. *Col-0* is the wild type. Asterisks represent significance level *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (two-tailed Student's *t* test) compared with in *Col-0*.

Table S1 The expression profile of genes for *gun1* mutation-affected site associated PLS-PPR proteins and broad-effect plastid RNA-editing factors in NF-treated *gun1-9* seedlings.

Type	Name	LOC	Log2(FC) *gun1-9* vs *Col6-3*	Differentially expressed?*	FDR (false discovery rate)
PLS-PPR	*CLB19*	AT1G05750	0.23	No	0.377174007
	CRR22	AT1G11290	1.63	Yes	2.527E-16
	CRR28	AT1G59720	1.74	Yes	1.29259E-10
	DOT4	AT4G18750	0.02	No	0.936900458
	OTP81/QED1	AT2G29760	0.20	No	0.350009251
	OTP82	AT1G08070	-0.01	No	0.972989224
	OTP84	AT3G57430	0.80	No	0.000190692
	YS1	AT3G22690	-0.69	No	0.009531494
MORF/RIP	*MORF2/RIP2*	AT2G33430	0.19	No	0.281081422
	MORF8/RIP1	AT3G15000	-0.38	No	0.010482583
	MORF9/RIP9	AT1G11430	0.66	No	0.000608298
ORRM	*ORRM1*	AT3G20930	0.61	No	1.59749E-05
OZ	*OZ1*	AT5G17790	0.71	No	1.35848E-06
	PPO1	AT4G01690	0.97	No	2.33762E-08

*Genes had a log2-converted fold change ≥ 1 or ≤ -1 with a False Discovery Rate (FDR) ≤ 0.05 were considered as differentially expressed genes.

Table S2 Primers used in the cloning and vector construction.

Primer name	Vectors	Primer sequence: 5' - 3'	Assay	Restriction Enzymes
GUN1-BKIF-F	pGBKT7	CATGGAGGCCGAATTCATGGCGTCAACGCCGC	Y2H	EcoRI
GUN1-BKIF-R		GGATCCCCGGGAATTCCTACAAAAGAAGAGGCTGTAAAGCA	Y2H	EcoRI
GUN1-ADIF-F	pGADT7	GGAGGCCAGTGAATTCATGGCGTCAACGCCGC	Y2H	EcoRI
GUN1-ADIF-R		CACCCGGGTGGAATTCCTACAAAAGAAGAGGCTGTAAAGCA	Y2H	EcoRI
MORF2-BKF	pGBKT7	AGGAGGACCTGCATATGATGGCTTTGCCTTTGTCTG	Y2H	NdeI
MORF2-BKR		GCAGGTCGACGGATCCTCATCTTGTGTTTTCTCTGC	Y2H	BamHI
MORF2-ADF	pGADT7	CAGATTACGCTCATATGATGGCTTTGCCTTTGTCTG	Y2H	NdeI
MORF2-ADR		CGAGCTCGATGGATCCTCATCTTGTGTTTTCTCTGC	Y2H	BamHI
OTP81-BKF	pGBKT7	AGGAGGACCTGCATATGATGGCTATCTTCTCCACAG	Y2H	NdeI
OTP81-BKR		GCAGGTCGACGGATCCTCACCAGAAATCGTTACAGG	Y2H	BamHI
OTP81-ADF	pGADT7	CAGATTACGCTCATATGATGGCTATCTTCTCCACAG	Y2H	NdeI
OTP81-ADR		CGAGCTCGATGGATCCTCACCAGAAATCGTTACAGG	Y2H	BamHI
OTP84-BKF	pGBKT7	AGGAGGACCTGCATATGATGTCATGTCCTCTTGCCT	Y2H	NdeI
OTP84-BKR		GCAGGTCGACGGATCCTCACCAATAGTCTCCACAG	Y2H	BamHI
OTP84-ADF	pGADT7	CAGATTACGCTCATATGATGTCATGTCCTCTTGCCT	Y2H	NdeI
OTP84-ADR		CGAGCTCGATGGATCCTCACCAATAGTCTCCACAG	Y2H	BamHI
YS1-BKF	pGBKT7	AGGAGGACCTGCATATGATGGCGATGTTGGGTAATGTTC	Y2H	NdeI
YS1-BKR		GCAGGTCGACGGATCCTCACCAGAAGTCACCGCAAG	Y2H	BamHI
YS1-ADF	pGADT7	CAGATTACGCTCATATGATGGCGATGTTGGGTAATGTTC	Y2H	NdeI
YS1-ADR		CGAGCTCGATGGATCCTCACCAGAAGTCACCGCAAG	Y2H	BamHI
GUN1-BCF	pSPYNE173 & pSPYCE(M)	GGACTAGTATGGCGTCAACGCCGC	BiFC	SpeI
GUN1-BCR		ACGCGTCGACCAAAAGAAGAGGCTGTAAAGCAAAC	BiFC & LCI	SalI
MORF2-BCF	pSPYNE173 & pSPYCE(M)	GGACTAGTATGGCTTTGCCTTTGTCTGG	BiFC	SpeI
MORF2-BCR		ACGCGTCGACTCTTGTGTTTTCTCTGCGGC	BiFC & LCI	SalI
OTP81-BCF	pSPYNE173 & pSPYCE(M)	GGACTAGTATGGCTATCTTCTCCACAGCAC	BiFC	SpeI
OTP81-BCR		ACGCGTCGACCCAGAAATCGTTACAGGAACACT	BiFC & LCI	SalI
OTP84-BCF	pSPYNE173 & pSPYCE(M)	GGACTAGTATGTCATGTCCTCTTGCCTTCA	BiFC	SpeI
OTP84-BCR		ACGCGTCGACCCAATAGTCTCCACAGGAGCAA	BiFC & LCI	SalI
GUN1-BCF	pSPYNE173 & pSPYCE(M)	GGACTAGTATGGCGTCAACGCCGC	BiFC	SpeI
GUN1-BCR		ACGCGTCGACCAAAAGAAGAGGCTGTAAAGCAAAC	BiFC & LCI	SalI
YS1-BCF	pSPYNE173 & pSPYCE(M)	GCTCTAGAATGGCGATGTTGGGTAATG	BiFC	XbaI
YS1-BCR		CCGCTCGAGCCAGAAGTCACCGCAAGA	BiFC & LCI	XhoI
OTP81-TPF	pSPYNE173 & pSPYCE(M)	GCTCTAGAATGGCTATCTTCTCCACAGCACAACCTCTCTCTCT GCCACGTCATCCAAACTTCTCCAATCCGAATCAACCAACTAC CAATAACGAACGCTCCCGCCACATTTCTCTCATCGAACGATG TGTGTCTACTAGTCC	BiFC	XbaI SpeI
OTP81-TPR		GGACTAGTAGACACACATCGTTCGATGAGAGAAATGTGGCG GGAGCGTTCGTTATTGGTAGTTGGTTGATTCGGATTGGAGAA GTTTGGATGACGTGGCAGAGAGAGAGGTTGTGCTGTGGAGA AGATAGCCATTCTAGAGC	BiFC	SpeI XbaI
GUN1-LCIF	pUC19-NLuc & pUC19-CLuc	GGGGTACCATGGCGTCAACGCCGC	LCI	KpnI
MORF2-LCIF	pUC19-NLuc & pUC19-CLuc	CGGAGCTCATGGCTTTGCCTTTGTCTGG	LCI	SacI
OTP81-LCIF	pUC19-NLuc & pUC19-CLuc	CGGAGCTCATGGCTATCTTCTCCACAGCAC	LCI	SacI
OTP84-LCIF	pUC19-NLuc & pUC19-CLuc	GGGGTACCATGTCATGTCCTCTTGCCTTCA	LCI	KpnI
YS1-LCIF	pUC19-NLuc & pUC19-CLuc	CGGGGTACCATGGCGATGTTGGGTAATG	LCI	KpnI
35S-OXF	pEarleyGate 101	CCGACGCGTAGCTTGCATGCCTGCAG	Plant transformation	MluI
MORF2-OXR	pEarleyGate 101	GGACTAGTTTACTTATCGTCGTCATCCTTGTAATCCTTATCGT CGTCATCCTTGTAATCCTTATCGTCGTCATCCTTGTAATCAAA TTCGAGCTCAGCGTAATCTGGAACATCGTATGGGTAAGCGTA ATCTGGAACATCGTATGGGTAAGCGTAATCTGGAACATCGTA TGGGTAGGTACCCTCGAGGTCGACTCTTGTGTTTTCTCT	Plant transformation	SpeI

Table S3 PCR and sequencing primers used in the RNA editing analysis.

No.	Editing site	Forward primer	Primer sequence (F) 5' - 3'	Reverse primer	Primer sequence (R) 5' - 3'	Sequencing primer name	Sequencing primer sequence 5' - 3'
1	accD-794	accD-F	GTGGATTCAATGCGACAAT	accD-R	ATATGCAAGCAAGGGAGG	accD-956SR	CCGTTTAGTTGACCTGTACCTGTTT
2	accD-1568					accD-1285SL	GCATTTGCGGGTAAAAGAGT
3	atpF-92	atpF-92F	CCGATTCTTTCGTTTACTTG	atpF-92R	AGGGTTCCTATAGCTCCTTG	atpF-92F	CCGATTCTTTCGTTTACTTG
4	clpP-559	clpP-559F	ATGATCCATCAACCCGCTAG	clpP-559R	TATTGAACCGCTACAAGATC	clpP-397SL	TATGAGGCACAAACGGGAGA
5	matK-640	matK-640F	CGTTACCGGGTAAAAGATGC	matK-640R	AGCGGCGTATCCTTTGTTGC	matK-813SL	TTTTCCATAGAATACAATTCGCTCA
6	ndhF-290	ndhF-290F	AAAACCTTCGCCGCATGTGG	ndhF-290R	GCATTCGCTGCAATAGGTCG	ndhF-290R	GCATTCGCTGCAATAGGTCG
7	ndhG-50	ndhG-50F	ATAATGGATTTGCCTGGACC	ndhG-50R	CTTATTAAATCTTGCTCTAGA ATCTGGTT	ndhG-312SR	ACAAACCAACGAAGTAATCCCA
8	petL-5	petL-5F	AGGGAAGTACTTTAAGAATC	petL-5R	ATTAGACCTAAGACGATTCC	petL-175SR	ACACGGTAAGGAACTATCGAACA
9	psbE-214	psbE-214F	ACAGGAGAACGTTCTTTTGC	psbE-214R	CGTTGGATGAACTGCATTGC	psbE-214F	ACAGGAGAACGTTCTTTTGC
10	psbF-77						
11	psbZ-50	psbZ-50F	AGAACATAGCCCTATGAGTTAATACGA	psbZ-50R	GATAAGAGAATTAAGGATACC CACCA	psbZ-SL	GCTATGAGTTAATACGATCCCTA
12	rpl23-89	rpl23-89F	AATTCCTACTGGATGCACGC	rpl23-89R	AAGAGGTGGAATAGAATAACCCG	rpl23-89R	AAGAGGTGGAATAGAATAACCCG
13	rpoA-200	rpoA-200F	CGGACACTACAGTGGAAGTG	rpoA-200R	ATGAATACAGCATCGATAGG	rpoA-384SR	TTCCACAGCGGGCGGTAA
14	rpoB-338	rpoB-338F	TATCGGTTTATTGATCAGGG	rpoB-338R	GCAGCTGCTAACACATCTCG	rpoB-193SL	AAAGAACGAGATGCTGTCTATGAA
15	rpoB-551						
16	rpoB-2432	rpoB-2432F	AACACCTCAAGTGGCGAAAG	rpoB-2432R	GTCCTACATTCATGCGTGAG	rpoB-2244SL	GGCGAAAGAATCCTCCTATGC
17	rpoC1-488	rpoC1-488F	TTTTCTTTTGCTAGGCCCATAA	rpoC1-488R	TTCGCAAATCTAAATCGGCT	rpoC1-593SR	GCACCCGCCCCAGTAGAA
18	rps12-i-58	rps12-intF	CAAGACAGCCAATCCGAAAC	rps12-intR	CTTGTACAATTCACATTCTTTGGC	rps12-int-SR	TTTACCCTGTTAGTCCGTTCTTTTC
19	rps14-80	rps14-80F	TTGATTTATAGGGAGAAGAAGAG	rps14-149R	TACCAGCTTGATCTTGTTGC	rps14-266SR	GCCTGAACCATTTCCCGAAG
20	rps14-149						
21	ndhB-149	ndhB-1F	GCCTTTCATTTGCTTCTCTT	ndhB-1R	TCCTTCGTATACGTCAGGA	ndhB-346SR	CGGATAGAGGAATACAGAGAGTTGA
22	ndhB-467					ndhB-271SL	TTCCAAACGAACAATTTCAACG
23	ndhB-586						
24	ndhB-746						
25	ndhB-830	ndhB-2F	CGTATACGAAGGATCTCCCAC	ndhB-2R	CTAGAAGCTAAAAAGGGTATCCT	ndhB-1041SR	CCACCATTTGAGTCTCCAACA
26	ndhB-836						
27	ndhB-872						
28	ndhB-1255					ndhB-1018SL	ATTGTTGGAGACTCAAATGGTGG
29	ndhB-1481						
30	ndhD-2	ndhD-F	TTGAGTACGCGTTCTTTGGAC	ndhD-R	AATAGCTCCATTAAGTCCAGG	ndhD-252SR	TCCATCTATTCCCATTCTCCAGTA
31	ndhD-383					ndhD-303SL	TTTAGCGGCTTTTCCAGTTAC
32	ndhD-674						
33	ndhD-878						
34	ndhD-887						

Table S4 Primers used in the qPCR.

Locus	Gene name	Forward primer 5' - 3'	Reverse primer 5' - 3'
AT1G29910	LHCB1.2	AGGCTACAGAGTCGCAGGAAAT	TCTCTATCGGTCCCTTACCAGTG
AT1G76100	PLASTOCYANIN	CAACGCAGGGTTCCCACAT	CGCACAATAGAAACCGTAAGAGC
AT3G01500	CA1	GACTTTCAGCCAGGAGATGCC	TAAGGTGTAAGACCGCGTATTCA
AT2G05070	LHCB2.2	TTGACCCGCTTTATCCCG	AGGCGTTGTTAGCCACAGG
AT3G62410	CP12	AGCCGATTAAAGCAGCACCG	GCTAAGTTCTTCAACCTCGTCCC
AT4G02510	PPI2	TGAGGCTGAGGGCAACGA	CATCAATGGACGCAATCTGGT
AT1G13320	PP2AA3	CATGCAATGGTTACAAGACAAGGTT	CGAGAAGCGATACTGCACGAA

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Dataset S1. Differentially expressed genes in *Col6-3* after NF treatment (*A*), in *gun1-9* compared with *Col6-3* under NF treatment (*B*), in *MORF2OX(s)* compared with *Col6-3* under NF treatment (*C*) and in *MORF2OX(w)* compared with *Col6-3* under NF treatment (*D*).

Dataset S2. The expression pattern of GUN1-dependent retrograde signaling genes under NF treatment (*A*) and the expression profile of nuclear encoded photosynthesis genes under NF treatment (*B*).