PNAS www.pnas.org

Supplementary Information for

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

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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 μ mol.m⁻².s⁻¹ light intensity at 22 °C with 5 μ M NF or 220 μ g/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 Cterminal 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 pUCpSPYNE173 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 µmol.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 μmol.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 Safire2TM 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 μ M NF plate under 24 h light (100 μ mol.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 MgCl₂, 10% glycerol, 0.1% NP-40, 0.5 mM DTT, 1 mM PMSF, 1×complete protease inhibitor mixture (Roche), 10 μ M 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°C, 13,500 rpm and incubated with 100 μ L Anti-FLAG[®] M2 Magnetic Beads (Sigma) for 2.5 h at 4 °C 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 μ L of 3×FLAG peptide (Sigma) (150 ng/ μ L) at 25 °C 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 μ mol.m⁻².s⁻¹ light intensity at 22 °C with 5

 μ M NF or 220 μ g/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 CFX384TM Real-Time PCR Detection System (Bio-rad) using iTaqTM 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 2500 sequencing machine (Illumina). HiSeq **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 countper-million (CPM) above 0.5 in at least two samples. Genes had a log2-converted fold change ≥ 1 or \leq -1 with a False Discovery Rate (FDR) \leq 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 RNAseq 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 primers and **T-DNA** left-border primer (LBb1.3, 5'gene-specific 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.



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 \pm 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 \pm 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 significance level **p* < 0.05, ***p* < 0.01, ****p* < 0.001 (two-tailed Student's *t* test)



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-a-gal indicate the SD/-Trp/-Leu and SD/-Ade/-His/-Trp/-Leu/X-a-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 μ mol.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 μ M NF or 220 μ g/mL Linc treatment under 24 h light (100 μ mol.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* 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. S7C, 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.

A Top 50 GO terms of GUN1-dependent retrograde signaling genes

retrograde signaling genes			grade signaling genes			
nhotosynthesis			photosynthesis			
photosynthesis-light reaction	10		photosynthesis-light reaction			
apportion of procursor motobolitos and operation	-10		generation of precursor metabolites and energy	-10		
singlo-organism motabolic process			oxidation-reduction process			
single-organism metabolic process	-20		response to light stimulus	-20		
oxidation-reduction process			response to radiation			
	20		nhotosynthetic electron transport chain	-30		
response to radiation	-30		single-organism metabolic process	00		
response to abiotic stimulus			photosynthesis-light harvesting	40		
photosynthesis-light narvesting	-40		photosynthesis-light harvesting in photosystem I	-40		
photosynthetic electron transport chain			rosponso to abiotic stimulus			
plastid organization	50		electron transport chain	-50		
cofactor metabolic process	-50		pretein_shremenhere linkage			
single-organism biosynthetic process			protein-chromophore inikage	-60		
pigment metabolic process	-60		reductive pentose-phosphate cycle			
pigment biosynthetic process			photosynthesis-dark reaction	-70		
photosynthesis-light harvesting in photosystem I			photosynthetic electron transport in photosystem i	10		
electron transport chain			carbon fixation			
response to cold			response to light intensity			
small molecule metabolic process			response to cold			
response to temperature stimulus			metabolic process			
response to light intensity			response to red light			
porphyrin-containing compound metabolic process	6		response to stimulus			
tetrapyrrole metabolic process			response to blue light			
chlorophyll metabolic process		regulation of photosynthesis				
cofactor biosynthetic process			response to temperature stimulus			
reductive pentose-phosphate cycle		response to high light intensity				
carbon fixation		single-organism biosynthetic process				
metabolic process		regulation of photosynthesis-light reaction				
photosynthesis-dark reaction		photorespiration				
chloroplast organization		peptidyl-cysteine modification				
protein-chromophore linkage		regulation of generation of precursor metabolites and energy				
regulation of photosynthesis			small molecule metabolic process			
response to stimulus			single-organism process			
chlorophyll biosynthetic process			photosystem II assembly			
porphyrin-containing compound biosynthetic proce	200		protein nitrosylation			
photosynthetic electron transport in photosystem I			pentidyl-cysteine S-nitrosylation			
tetranyrrole biosynthetic process			response to low light intensity stimulus			
response to chemical			response to chemical			
response to high light intensity			response to far red light			
response to red light	response to high light intensity					
regulation of photosynthesis-light reaction			collular motabolic process			
tetraternenoid metabolic process			response to cytokinin			
cal deliber la scombly			cellular metabolic compound salvage			
procession of apparation of procursor metaboliton and apparation						
regulation of generation of precursor metabolites and energy			negative regulation of photosynthesis			
response to cytokinin			response to red or for red light			
plastid membrane organization			response to red or lar red light			
single-organism process			giveine metabolic process			
small molecule biosynthetic process			pigment biosynthetic process			
cellular protein complex assembly		glycine catabolic process				
Log10(FDR)		Log10(FDF	र)			

B Top 50 GO terms of MORF2/GUN1-overlapping

Fig. S8. Top 50 Gene Ontology (GO) terms show the similar enrichment between GUN1dependent 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.

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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 \pm SEM from three biological replicates. nLUC: N-terminal luciferase, cLUC: C-terminal luciferase. Cotransformation 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 autofluorescence 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 \pm 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*.

Type	Name	LOC	Log2(FC)	Differentially	FDR
1,100	1 (unit	Ecc	gun1-9 vs Col6-3	expressed?*	(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

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

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

GUN-BRIFF GUN-BRIFF GUN-ADIF-	Primer name	Vectors	Primer sequence: 5' - 3'	Assay	Restriction Enzymes
	GUN1-BKIF-F	nGBKT7	CATGGAGGCC <u>GAATTC</u> ATGGCGTCAACGCCGC	Y2H	EcoRI
CLUILADIE- GADIT7 CGADGCCADIGAATICATCGCCCCA CECK Construction MORE3-BKF PGADT7 CACCCGCGCGATICACTOCCTITICCTITIC Y2H EcoRI MORE3-BKF MORE3-BKF PGADT7 CACCGCGCGATICACTOCACTITICCTITI	GUN1-BKIF-R	pobler/	GGATCCCCGG <u>GAATTC</u> CTACAAAAGAAGAGGCTGTAAAGCA	Y2H	EcoRI
CONTAUNT-R FORKT CACCCOUNTINGENTIAL TRANSPORT Content of the second	GUN1-ADIF-F	pGADT7	GGAGGCCAGT <u>GAATTC</u> ATGGCGTCAACGCCGC	Y2H	EcoRI
NODE: POINT // ADDATAGE (CLANDER INDECTION CLIPTIC) 11 Note: NODE:: PGADT CCAOTECCEMATEGATECTION CLIPTIC) YII Bamili NOR:: ADGACTACE (CLANDER INDECTION CLIPTIC) YII Bamili NOR:: ADGACTACE (CLANDER INDECTIC) YII Bamili NOR:: BANILI CCAGTTACACCACAAATAGETC: YIII Bamili YSI-: PGADT CCAGTTACACCAAAGAAGEGCCECTACE CLACCACAAGETC YIII Bamili	GUNI-ADIF-K	T CDVT7		Y2H V2U	ECORI
MORE2-ADF PGADT7 PGADT7 CAGATTACGCTEXATATGATGGCTTTCCTTCTC YEI Nodal and PGADT7 OTBSI-BKF PGBKT7 PGGAGGACCTGATGATGCTTTCCTCTCC YEI Nodal BamHI OTBSI-BKF PGBKT7 PGGAGGACCTGATGATGCTACCTGATGATGGTACGAG CAGATTCGATGAGGACCTGCACCAATATGGTACAGGA CAGATTACGCTCACTAATATGGTCACCAGA CGAGTCGATGATGGATGCATGCTTCCCCACAG YEI Nodal BamHI OTBSI-ADF PGADT7 CAGATTACGCTCACTGATGATGCTTCCCACAG CGAGTCGATGGATGCATGCTCCCCCACAGA YEI Nodal BamHI OTBSI-ADF PGADT7 CAGATTACGCTCACTGACAGATCGTCCCCCCACA YEI Nadal BamHI OTBSI-ADF PGADT7 CGAGTTGCCGACGATGCTCCCCACAGA YEI Nadal BamHI OTBSI-ADF PGADT7 CGAGTTGCCGATGATGCTCCCACCAGA YEI Nadal BamHI YSI-BKF YSI-BKF PGADT7 CGAGTTGCCGATGATGCTCCACCAGA YEI Nadal BamHI YSI-BKF YSI-BKF PGADT7 CGAGTTGCGATGATGCTCCACCAGA YEI Nadal BamHI YSI-BKF YSI-BKF YSI-BKF PGADT7 CGAGTTGCGATGATGCTCCACCAGAG YEI Nadal BamHI GUNI-BCF PSPVEI73 & GGACTAGTATGCGCTCACAGAGCGCGCAAGGCAACGCACAG YEI Nadal YEI GUNI-BCF PSPVEE(M) CGCGCTGGACCCAAAAGAAGAGGCTGTAAAGCAACC BiFC Sel MORF2-BCF PSPVEE(M) CGCGCTGGACCCAAAAGAAGAGGCTGTAAAGCAACC BiFC Sel	MORF2-BKF	pGBK1/		Y2H V2H	Ndel BamHI
MORE2-ADR PGADT7 PGADT7 CGAGCTCGATEGATECTATCTTGTGTTTTCTCCCAG Y2H BamHI Model OTR81-BKR PGBKT7 PGGAGTCGATEGATEGATECTATCTTGTGTTTCTCCCAG Y2H BamHI Model OTR81-ADR PGADT7 CAGGTCGATEGATEGATECTTCTCCCAGAGAY Y2H BamHI Model OTR81-ADR PGADT7 CAGGTCGATEGATECTCACCAGAAATCGTTACAGG Y2H BamHI Model OTR81-ADR PGADT7 CAGGTCGATEGATECTCACCAGAAATCGTTCTCCCCAGG Y2H BamHI Model OTR81-ADR PGADT7 CAGGTCGATEGATECTCACCAGAAATCGTTCTCTCCCCAG Y2H BamHI Model OTR81-ADR PGADT7 CAGGTCGATEGATEGATECTCACCACAGAG Y2H BamHI Model OTR81-ADR PGADT7 CAGGTCGATEGATEGATEGATECTCACCAGAAG Y2H BamHI Model YSI-ADF PGADT7 CAGGTCGATEGATEGATEGATECTCACCAGAAGC Y2H BamHI Model YSI-ADF PGADT7 CAGGTCGATEGATEGATEGATECTCACCAGAAGC Y2H BamHI Model YSI-ADF PGADT7 CAGGTCGATEGATEGATECTCACCAGAAGC W2H BamHI Model YSI-ADF PGADT7 CAGGTCGATEGATECTCACCAGAAGCCAAATGTTCCCCAGCAGAAGC BFC Sel GUNI-BCF pSPYCEI0A CAGGCTCGATEGATECTCACCAGAAGGCCCAAAAC EGCAGCTCGATEGATEGATECTCACCAG	MORE 2-DRR		CAGATTACGCTCATATGATGGCTTTGCCTTTGTCTG	Y2H	Ndel
$ \begin{array}{llllllllllllllllllllllllllllllllllll$	MORF2-ADR	pGADT7	CGAGCTCGATGGATCCTCATCTTGTGTTTTCTCTGC	Y2H	BamHI
$\begin{aligned} & \text{OTPSI-BARR} & \text{pGADT7} & \text{GCAGGTCGACQGATCCTCACCAGAAATCGTTACAGG Y2H BamH1} \\ & \text{OTPSI-ADR} & \text{pGADT7} & \text{CAGATTACGTCAATQGTGATCTTCTCCCACAG Y2H BamH1} \\ & \text{OTPSI-ADR} & \text{pGADT7} & \text{CAGATTACGTCATTATGTGATGCTCTTTCTCCCACAG Y2H BamH1} \\ & \text{OTPSI-ADR} & \text{pGBK77} & \text{CAGATTACGTCATCGTCTCTTCTCCCTTGCT Y2H BamH1} \\ & \text{OTPSI-BARR} & \text{pGBK77} & \text{CCAGATCGCATQGATCCTCACCAATAGTCTCCTCTCGCT Y2H BamH1} \\ & \text{OTPSI-ADR} & \text{pGADT7} & \text{CAGATTACGTCATGTATCTCTTGCCTCTTGGCT Y2H BamH1} \\ & \text{STB-BKR} & \text{pGBK77} & \text{CGAGTTCCACGGATCCTCACCAATAGTCTCCTCTGCCT Y2H BamH1} \\ & \text{YSI-BKR} & \text{pGBK77} & \text{CCAGTTCACCGATCGATCGGATCCTCACCAATAGTCCCCTCTGCCACAG Y2H BamH1} \\ & \text{YSI-BKR} & \text{pGBK77} & \text{CCAGTTCACCGATCGATCGGATCCTCACCAATAGTCC Y2H BamH1} \\ & \text{YSI-ADR} & \text{pGADT7} & \text{CCAGTTCCACTGCATGGATCCTCACCGAAGG Y2H BamH1} \\ & \text{YSI-ADR} & \text{pGADT7} & \text{CCAGTTCCACTGATGGCTATGTGGGTAATGTC Y2H BamH1} \\ & \text{YSI-ADR} & \text{pGADT7} & CCAGTTCCACTGCACGCACGC BFC Spel DFSPYCE(M) & ACGCGTCGACTGCATTCTCTCCACGAGAACACCT & BFC Spel DFSPYCE(M) & ACGCGTCGACCAATAGTTCCCACGCACG BFC Spel DFSPYCE(M) & ACGCGTCGACCCAATAGTTCCCACGAGAAACC BFC Spel DFSPYCE(M) & ACGCGTCGACCCAATAGTTCCCACGAGAA BFC Spel DFSPYCE(M) & ACGCGTCGACCCAATAGTTCCCACGAGAAACC BFC Spel DFSPYCE(M) & ACGCGTCGACCCAATAGTTCCCACGGAGA BFC Spel DFSPYCE(M) & ACGCGTCGACCACAATAGAGAGGGCTGTAAAGAAACC BFC Spel DFSPYCE(M) & ACGCGTCGACCACAATAGCTCCCCAGAAACC BFC Spel DFSPYCE(M) & ACGCGTCGACCACAATAGCTCCCCAGAAACCCCCC BFC Spel DFSPYCE(M) & ACGCGTCGACCACAATAGCTCCCCAGAAACCCCCCC BFC Spel DFSPYCE(M) & ACGCGTCGACCACAATAGCTCCCCCAATAGCCCCC BFC Spel DFSPYCE(M) & ACGCGTCGACCACACACCCCCC BFC Spel DFC Spel DFSPYCE(M) & ACGCGTCGACCCAATAGCTCCCCCAATAGCCCCCC BFC Spel DFSPYCE(M) & ACGCGTCGACCCACACACCCCCC BFC Spel DFC Spel DFSPYCE(M) & ACGCGTCGACCCAATAGCTCCCCCAATAGCCCCCC BFC Spel DFSPYCE(M) & ACGCGTCGACCCACACACCCCCC BFC Spel SFSPYCE(M) & ACGCGTCGACCCACACACCCCCCC BFC Spel SFSPYCE(M) & ACGCGTCGACCCCCCCCCCCCCCCCCCCCCCCCCCCCCC$	OTP81-BKF	CDVTT	AGGAGGACCTGCATATGATGGCTATCTTCTCCACAG	Y2H	NdeI
OTP81-ADF OTP81-ADFpGADT7CAGATTACGCTEATAGATGCTATCTTCTCCACG CAGATTACGCTCATGATGCATCTTCCACGGY11NdelOTP84-BKF OTP84-BKF PGBKT7pGBKT7AGAGGACCTCATGATGATGCATGCTCTCTCCC CAGATTACGCTCCACGATGATGCATGCTCCACGA CAGATTACGCTCCACGACGATCTTCGCTTGCCTY11NdelOTP84-ADF YS1-BKFpGADT7CGAGCTCCATGGATGCTCCACGACGATGCTCCCACG CAGATTACGCTCCCACGATGATGCCTCCCACGACG Y11NdelYS1-BKF YS1-ADF PGADT7CGAGCTCCCATGGATCCCTCCACAAGTCCCCCACG CCGATGCATCCCCTCCCATGGATGCTCCCACGACG CCCACGCACGCACGY11NdelYS1-ADF YS1-ADF PGADT7CGAGCTCACCGACGCCCCCCCCCCCCAG CCGATCGCATCGCATCGCCCCCCCCCCCCCCCCCCCCCAG CGACTACGCTCCCCCCACGCCCCCCCCCCCCCCCCCCCC	OTP81-BKR	pGBK1/	GCAGGTCGAC <u>GGATCC</u> TCACCAGAAATCGTTACAGG	Y2H	BamHI
OTP81-ADRCGAGCTCGATGGATCCTCACCAGAAATCGTTACAGGY2HBamHIOTP84-BKRpGBKT7AGGAGCACCTGGATGCCCATGCCTCTCCCTY2HNodelOTP84-ADRpGADT7CAGATTACGTCGATGGATCCTCTGCCTCTY2HNodelOTP84-ADRpGBKT7CGAGCTCGATGGATCCTCACAGAGY2HBamHIYSI-BKRpGBKT7CGAGCTCGATGGATCCTCACAGAGTCTCCCACAGY2HBamHIYSI-ADRpGADT7CCAGCTCGATGGATCCTCACAGAGTCTCCCACAGY2HBamHIYSI-ADRpGADT7CCAGCTCGATGGATCCTCACAGACCCCCACAGY2HBamHIGUNI-BCRpSPYNE173 &GGACTAGTATGATGGCATAAGCTCACAGCCGCBiFCSpeIGUNI-BCRpSPYNE173 &GGACTAGTATGGCTTGGCTTAGCTGAAAGCAACCLCISallOTP81-BCRpSPYNE173 &GGACTAGTATGTCCTCTTGCCACAGGACAACLCISallOTP81-BCRpSPYNE173 &GGACTAGTATGTCCACAGCCCCBiFC &SpeIOTP84-BCRpSPYCE(M)ACCCCGTCGACCCAAAAGAAGAGGCTGTAAAGCAAACLCISallOTP84-BCRpSPYCE(M)ACCCGTCGACCCAAAAGAAGAGGCTGTAAAGCAAACLCISallYSI-BCRpSPYCE(M)ACCCGTCGACCCAAAGAAGAGGCGTGTAAAGCAAACBiFC &SpeIYSI-BCRpSPYCE(M)ACCCGTCGACCCAAAGAGAGGCGTGTAAAGCAAACBiFC &SallYSI-BCRpSPYCE(M)ACCCGTCGACCCAAAGAGAGGCGTGTGAAGGAAACBiFC &SpeIYSI-BCRpSPYCE(M)ACCCGTCGACCCAAAGGCGCGCBiFC &SpeIYSI-BCRpSPYCE(M)ACCCGTCGACCAAAGGCGCCGCAAGGCGCGCAAGGCGCGGGGGGGG	OTP81-ADF	pGADT7	CAGATTACGCT <u>CATATG</u> ATGGCTATCTTCTCCACAG	Y2H	NdeI
OTP84-BKF OTP84-BKF OTP84-ADF PGADT7 pGGAGTGCTGCATGGATCTCTCTGCCTTGCCT YH Ned- Bornitic OTP84-ADF OTP84-ADF PGADT7 CGAGTGCGACGGATCGTCTCCACGACGACGCC CAGATTACGCTCCTCACGATGGTCGCCACG CAGATTACGCTCCCCATGGTCGTCCTCCCCCACG YH Ned- Bornitic YSI-BKF YSI-BKF PGBKT7 PGBKT7 CGAGCTCCCCATGGTCGCTCCCCCACGACG CCAGCTCCCCCCCCCC	OTP81-ADR		CGAGCTCGAT <u>GGATCC</u> TCACCAGAAATCGTTACAGG	Y2H	BamHI
$\begin{split} & \text{OTP84-ADR} & \text{pGADT} & \text{CAGGTCGACQGATCTCCACAG} & \text{YH} & \text{BamHI} \\ & \text{OTP84-ADR} & \text{pGADT} & \text{CAGGTCGATQGATCTCTTGGCT} & \text{YH} & \text{Nolel} \\ & \text{OTP84-ADR} & \text{pGADT} & \text{CAGGTCGATQGATCTCCACAGG} & \text{YH} & \text{BamHI} \\ & \text{SI-BKR} & \text{pGBKT7} & \text{CAGGTCGATQGATCCTCACACAGTCTCCCTT} & \text{YH} & \text{Nolel} \\ & \text{CAGGTCGACGGATCCTCATATGATGGCCATATTCT} & \text{YH} & \text{Nolel} \\ & \text{YSI-BKR} & \text{pGBKT7} & \text{CAGGTCGATCGGATCCTCACAGAGTCACCGCCAAG} & \text{YH} & \text{BamHI} \\ & \text{CAGGTCGACGGATCCTCATATGATGGCGCATGTTGGGTAAGTCTC} & \text{YH} & \text{Nolel} \\ & \text{GAGTAGTTACGTCGATLTGATGGATGGTTGGGTAAGTCTC} & \text{YH} & \text{Nolel} \\ & \text{GAGTAGTTACGTCGATLTGATGGCGTCACGCCGC} & \text{BirC} & \text{Spel} \\ & \text{GUNI-BCR} & \text{pSPYEEIM} & \text{CAGGTCGACCCAAAAGAAGGAGGCTGTAAAGCAAAC} & LCI & \text{Sall} \\ & \text{ODRP2-BCR} & \text{pSPYCEIM} & \text{ACGCGTCGACCTCTTGTCTTGCCTTGCGCC} & \text{BirC} & \text{Spel} \\ & \text{MORP2-BCR} & \text{pSPYVEIM} & \text{ACGCGTCGACCCAAAAGAAGGAGCTGTAAAGCAAAC} & LCI & \text{Sall} \\ & \text{OTP81-BCR} & \text{pSPYVEIM} & \text{ACGCGTCGACCAAAAGCAACCTCTCCCCAAGGAGCAA} & LCI & \text{Sall} \\ & \text{OTP81-BCR} & \text{pSPYVEIM} & \text{ACGCGTCGACCAAAAGAAGGAGGCTGTAAAGCAAACC} & LCI & \text{Sall} \\ & \text{OTP81-BCR} & \text{pSPYVEIM} & \text{ACGCGTCGACCAAAAGAAGAGGGCTGTAAAGCAAACC} & LCI & \text{Sall} \\ & \text{OTP81-BCR} & \text{pSPYVEIM} & \text{ACGCGTCGACCAAAAGAAGAGGCGCGC} & BirC & \text{Spel} \\ & \text{BirC} & \text{Sall} & LCI & \text{Sall} \\ & \text{OTP84-BCR} & \text{pSPYVEIM} & \text{ACGCGTCGACCAAAGGAAGGCGCGC} & BirC & \text{Sall} \\ & \text{CGCTCGGACCAAAAGAAGAGGCCGCCCCAAGAACCTCCTCTCC} \\ & \text{GCCCTCGGACCAAAAGGAAGGCCGCCCAAGAACCCCCCCAAGGA & LCI & \text{Sall} \\ & \text{S1-BC} & \text{pSPYVEIM} & \text{ACGCGTCGACAACTCTCTCCCAAGGACAACCTCTCTCTC} \\ & \text{GCCACGTCATGGCATCTTGCACAGCACACCTCCTCTCC} \\ & \text{GCCCCTGGAACCCAGCAGAGGCCGCC} & BirC & \text{Spel} \\ & \text{BirC} & \text{Sall} \\ & \text{CGCCTCGGACCCAGAAGTCACCCGCAAGGA} & LCI & \text{Sall} \\ & \text{GCCCCTGGAACCCAGCGCACCACTTCCTCCAAGGACAACCTCTCTCC} \\ & \text{GCCCCGTCGAACCTCTCTGGCGTCAACCCACCTCCTCTC} \\ & \text{GCCCCGTCGAACCTCTTGGCGTCACCCCCAAGGAGGAAGGCTGTGGGGAGA} \\ & GCCCCGTCGACCCAGGCAGCCCCCCCCCCCCCCCCCCCC$	OTP84-BKF	pGBKT7	AGGAGGACCTG <u>CATATG</u> ATGTCATGTCCTCTTGCCT	Y2H	NdeI
OTF84-ADF pGADT7 CAGATTACGCTCATEGATIGATAGTCTCACCAG Y2H BamHil YSI-BKF pGBKT7 CGAGCTCGATGGATGGATAGTTCCCAGG Y2H Nael YSI-BKF pGBKT7 CGAGCTCGATGGATGGATGGTCGAAGGTCCCAGG Y2H Nael YSI-BKF pGBADT7 CCAGATTACCCTCCATGGATGGATGGCCAAGTCCCCCAG Y2H Nael YSI-ADF pGADT7 CGAGCTCGATGGATGCGATGGTCGAAGTCCCCCAAG Y2H Nael GUNI-BCF pSPYNE173 & GGACTAGTATGCCTCAATGGACGCCAAG BiFC Sel MORP2-BCF pSPYNE173 & GGACTAGTATGGCCTATCTTCTCTCCACAGCAC BiFC & Sel OTP81-BCF pSPYNE173 & GGACTAGTATGGCCTAACGCCCC BiFC & Sel OTP84-BCF pSPYNE173 & GCACTAGTATGGCCTAACGCCCC BiFC & Sall US1-BCF pSPYNE173 & GCACTAGTATGGCCTAACGCCCC BiFC & Sall US1-BCF pSPYNE173 & GCACTAGTATGGCCAAAGGCGGCAACGCCC BiFC & Sall US1-BCF pSPYNE173 & GCACTAGTATGGCCAAAGGCGGCAACGCCC </td <td>OTP84-BKR</td> <td>1</td> <td>GCAGGTCGAC<u>GGATCC</u>TCACCAATAGTCTCCACAG</td> <td>Y2H W2U</td> <td>BamHI</td>	OTP84-BKR	1	GCAGGTCGAC <u>GGATCC</u> TCACCAATAGTCTCCACAG	Y2H W2U	BamHI
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	OTP84-ADF	pGADT7		Y2H V2U	Ndel
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$ YSI-ADP \\ YSI-ADP \\ YSI-ADP \\ YSI-ADP \\ YSI-ADP \\ YSI-ADP$	VS1-BKR	pGBKT7	GCAGGTCGACGGATCCTCACCAGAAGTCACCGCAAG	V2H	BamHI
YSI-ADR GUNI-BCF pSPYNEI73 & GGACTAGTATGCCGTCAACGAGGTCAACGCCCAAGGTCACCGCAAG GGACTAGTATGCGTCAACGACGCCYI BamHI BiFC & BiFC & SelGUNI-BCR pSPYCE(M)pSPYCE(M)ACGCGTCGACCAAAGAAGGGCTGTAAAGCAAAC LCIBiFC & BiFC & SelMORF2-BCR pSPYNEI73 & GGACTAGTATGCCTTGGTGTTTCTCTGCGGCBiFC & BiFC & BiFC & SelSall LCIOTP81-BCF pSPYNEI73 & pSPYNEI73 & GGACTAGTATGCCTAGTGCCACCAGAAATCGTTCACGGACCAAGAACCBiFC & BiFC & SelSallOTP84-BCF pSPYNEI73 & GGACTAGTATGCCAAGGCCCAAAGCAACCTBiFC & BiFC & SelSallOTP84-BCF pSPYNEI73 & GGACTAGTAGTCATGTCCCAAGAACCCGCGBiFC & BiFC & SallSallGUNI-BCR VSI-BCF pSPYNEI73 & GGACTAGTAGCACCAAAAGAAGAGGCGTGTAAAGCAAACBiFC & BiFC & SallSallGUNI-BCR VSI-BCF pSPYNEI73 & GGACTAGTAGCACCAAAAGAAGGGCGTGTAAAGCAAACBiFC & BiFC & CCCCTCGAGCCAAAAGAAGGGCGTGTAAAGCAAACBiFC & CACGCGTCGACCAAAAGAAGGCGTGTAAAGCAAACYSI-BCF pSPYNEI73 & pSPYNEI73 & GCCCTCGAGCAGAGCAAGCACCCCGCCAAGGAGAAACCTCCTCTCTCCCACGCACAACTACCCACACTCBiFC & XbalOTP81-TPF OCTP81-TPFpSPYNEI73 & pSPYNEI73 & GGACTAGTAGCCAGCACCCCCCCCCCCCCCCCCCCCCCC	YS1-ADF		CAGATTACGCTCATATGATGGCGATGTTGGGTAATGTTC	Y2H	NdeI
GUNI-BCF GUNI-BCR $pSPYNE173$ $PSPYCE/M$ $GGACTAGTATGGCGTCAACGCCGCBFCACCCGTCGACCAAAAGAAGAGCGCTGTAAAGCAAACBFCLC1SelMORF2-BCFPSPYCE/MpSPYCE/MGGACTAGTATGGCTTTGCTTGGCTGGGCBiFCLC1SelMORF2-BCRPSPYCE/MpSPYCE/MACGCGTCGACCAAAAGAAAGCGCTTACACGCACBiFCLC1SelOTP81-BCRPSPYCE/MpSPYLT13ACGCGTCGACCCAAAATCGTTACAGGAACACTBiFCLC1SelOTP84-BCFOTP84-BCRPSPYCE/MGGACTAGTATGCATGTCCTTGCCTTCACAGGACAAACACGCGTCGACCCAAAAGAAGAGGCGTGTAAAGCAAACBiFCLC1SelOTP84-BCROTP84-BCRPSPYCE/MGGACTAGTATGCCGTCACCCAGAAATCGTTACAGGACAAACLC1BiFCLC1SelOTP84-BCROTP84-BCRPSPYCE/MGGACTAGTATGGCGGTCAACGCCGCBiFCLC1SelGUNI-BCRPSPYNE173PSPYNE173SS1-BCRGCTCTAGAATGGCGATGTTGGGTAATGPSPYNE173RCCCCGTCGAGCCAAAAGTCACCGCAAAGABiFCLC1SelYSI-BCFPSPYNE173PSPYNE173SCACGCTCGCACCAAACTCTCTCCCCCACAACTCTCTCCACGCACACCTCTCTCCGCTCGAGCCCAACTCTCTCCACAGCACACCCTCTCTCCACGCTCGATAGACCACCACCTCTCCCACACCCCCCCCCC$	YS1-ADR	pGADT7	CGAGCTCGATGGATCCTCACCAGAAGTCACCGCAAG	Y2H	BamHI
GUN1-BCR pSPYCE(M)ACGCGTCGACCAAAAGAAGAGAGGCTGTAAAGCAAAC LCIBiFC & LCISallMORF2-BCF pSPYNE173 & OTP81-BCFpSPYCE(M)ACGCGTCGACTTGCTTTGCTTTGCTGGBiFC & SpelOTP81-BCF pSPYNE173 & OTP81-BCRpSPYCE(M)ACGCGTCGACCAGAATCGTTCTCTCACAGCAC CGACTAGTATGCATCTTCTCCACAGGACACTBiFC & SpelOTP81-BCR pSPYCE(M)GGACTAGTATGGCTATCTTCTCCACAGGACACC ACGCGTCGACCCAGAAATCGTTACAGGACACACT BiFC & SpelBiFC & SpelOTP84-BCR GUN1-BCR pSPYCE(M)GGACTAGTATGGCGTCAACGCCGCBiFC & SpelGUN1-BCF pSPYCE(M)pSPYCE(M)ACGCGTCGACCCAATAGTCTCCACAGGAGCAA CCGTCGACCCAATAGTCTCCACAGGAGCAA CCGCTCGACCCAATAGTCGCGACAGA CCGCTCGACCAAAAGAAGAGGCGCTGTAAAGCAAAC LCISallGUN1-BCR VS1-BCR pSPYNE173 & pSPYNE173 & GCTCTAGAATGGCGATGTGGGTAATG pSPYCE(M)GCTCTAGAATGCCGAAGAGGCGCGAAAA CCGCTCGAGCCAGAAGTCTCCCACAGCACACCTCTCTCTC	GUN1-BCF	"SDVNE172 %	GG <u>ACTAGT</u> ATGGCGTCAACGCCGC	BiFC	SpeI
MORE2-BCF MORE2-BCRpsPYNE173 & psPYCE(M)GGACTAGTATGGCTTTGCTTTGCTTGGCBiFC LCISpelMORF2-BCRpsPYCE(M)ACGCGTCGACTCTTGTGTTTTCTCTGCGGCLLCISallOTP81-BCF psPYCE(M)psPYCE(M)GGACTAGTATGCCTATCTTCTCCACAGGAACACTBiFC BiFC SpelSpelOTP84-BCF psPYCE(M)psPYCE(M)GGACTAGTATGCCTCTGCCTTCABiFC BiFC SpelSpelOTP84-BCF psPYCE(M)psPYCE(M)GGACTAGTATGCCCAGAAAGGAGGACAACBiFC BiFC SpelSpelGUN1-BCF psPYCE(M)psPYCE(M)GGACTAGTATGGCGACCAAAAGAAGAGGGCTGTAAAGCAAAAC CCGCTCGAGCCAAAAGAAGAGGCAGATGTGGGAAATG CCGCTCGAGCCAAAAGAAGAGGCAGAATCACCCAGAAACAACCCTCTCTCT	GUN1-BCR	pSPYCE(M)	ACGC <u>GTCGAC</u> CAAAAGAAGAGGGCTGTAAAGCAAAC	BiFC & LCI	SalI
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OTP81-BCF OTP81-BCRpSPYNE173 & pSPYNE173 & pSPYNE173 & pSPYNE173 & pSPYNE173 & pSPYNE173 & PSPYNE173 & PSPYNE173 & OTP84-BCRGGACTAGTATGGCCTATGTCTCTGCCTTGABiFC BiFC & SallSall LCI CIGUN1-BCF QUN1-BCRpSPYNE173 & pSPYNE173 & PSPYNE173 & PSPYNE173 & PSPYNE173 & CCGCGTCGACCAAAGAAGAAGAGGCGTGTAAAGCAAACBiFC & BiFC & SallSallGUN1-BCR QUN1-BCRpSPYNE173 & pSPYNE173 & PSPYNE173 & PSPYNE173 & PSPYNE173 & CCGCGTCGACCAAAGAAGAAGAAGAAGCAGCGTGTAAAGCAAACBiFC & LCI SallSallGUN1-BCR VS1-BCRpSPYNE173 & pSPYNE173 & PS	MORF2-BCR	pSPYCE(M)	ACGC <u>GTCGAC</u> TCTTGTGTTTTCTCTGCGGC	BiFC & LCI	SalI
OTP81-BCR PSPYCE(M)ACGCGTCGAC CCAGAAATCGTTACAGGAACACTBiFC & LCI LCISall LCIOTP84-BCF pSPYCE(M)pSPYCE(M)GGACTAGTATGTCATGTCCTTGCCTTCABiFC & SelSall LCIGUN1-BCF pSPYCE(M)pSPYCE(M)GGACTAGTATGTCACGCCGCBiFC & SelSall LCIGUN1-BCF yS1-BCRpSPYCE(M)GGACTAGTATGGCGTCAACGCCGCBiFC & SelSall LCIYS1-BCR OTP81-TPFpSPYNE173 & pSPYNE173 & CGCTCTAGAATGGCCAAAGAAGAGAGGCTGTAAAGCAAACBiFC & XbalSall LCIOTP81-TPFpSPYNE173 & pSPYNE173 & pSPYNE173 & CGCTCTAGAATGGCTATCTTCTCCCACAGCACACCACCTCCTCTCT GCCACGTCATGCAAACGCACCCCGCACATTCTTCTCCCACAGCAACACACAC	OTP81-BCF	nSPYNE173 &	GG <u>ACTAGT</u> ATGGCTATCTTCTCCACAGCAC	BiFC	SpeI
OTP84-BCF OTP84-BCRpSPYNE173 & pSPYCE(M)GGACTAGTATGTCATGTCCTCTGCCTTCABiFC BiFC & SallGUN1-BCF QUN1-BCRpSPYNE173 & pSPYCE(M)GGACTAGTATGCCTCAACGCCGCBiFC & BiFC & SallSallGUN1-BCRpSPYCE(M)GCCCTAGACGCGAAAGAGAGGCGTGTAAAGCAAACLCISallYS1-BCRpSPYNE173 & pSPYNE173 & pSPYCE(M)GCTCTAGAATGGCGATGTTGGGTAATGBiFC & LCISallYS1-BCRpSPYNE173 & pSPYNE173 & 	OTP81-BCR	pSPYCE(M)	ACGC <u>GTCGAC</u> CCAGAAATCGTTACAGGAACACT	BiFC & LCI	SalI
OTP84-BCRpSPYCE(M)ACGCGTCGACCCAATAGTCTCCACAGGAGCAABiFC & Sall LCIGUN1-BCFpSPYNE173 & PSPYCE(M)GGACTAGTATGGCGTCAACGCCGCBiFC & SpelGUN1-BCRpSPYCE(M)ACGCGTCGACCAAAAGAAGAGGCTGTAAAGCAAACLCISallYS1-BCFpSPYNE173 & PSPYCE(M)GCTCTAGAATGGCGATGTTGGGTAATGBiFC & XbalYS1-BCRpSPYCE(M)GCTCTAGAATGGCCAACGCCACCACCTCTCTCTCCCGOTP81-TPFpSPYNE173 & PSPYCE(M)GCTCTAGAATGGCTATCTTCTCCACAGCAACACCTCTCTCT	OTP84-BCF	pSPYNE173 &	GG <u>ACTAGT</u> ATGTCATGTCCTCTTGCCTTCA	BiFC	SpeI
GUN1-BCF GUN1-BCRpSPYNE173 & PSPYCE(M)GGACTAGTATGGCGTCAACGCCGCBiFC ACGCGTCGACCAAAGAAGAAGAGGGCTGTAAAGCAAACSall LCIYS1-BCRpSPYNE173 & PSPYCE(M)GCTCTAGAATGGCGATGTTGGGTAATGBiFC BiFC CCG CCGACGCAGAAGTCACCGCAAGAXbal SprC SprCOTP81-TPFpSPYNE173 & PSPYNE173 & <b< td=""><td>OTP84-BCR</td><td>pSPYCE(M)</td><td>ACGC<u>GTCGAC</u>CCAATAGTCTCCACAGGAGCAA</td><td>BiFC & LCI</td><td>SalI</td></b<>	OTP84-BCR	pSPYCE(M)	ACGC <u>GTCGAC</u> CCAATAGTCTCCACAGGAGCAA	BiFC & LCI	SalI
GUN1-BCR YS1-BCFpSPYCE(M)ACGCGTCGACCAAAAGAAGAGGGCTGTAAAGCAAACIJC I ICISallYS1-BCR YS1-BCRpSPYNE173 & PSPYCE(M)GCTCTAGAATGGCGATGTTGGGTAATGBiFCXbal 	GUN1-BCF	pSPYNE173 &	GG <u>ACTAGT</u> ATGGCGTCAACGCCGC	BiFC	SpeI
YS1-BCF YS1-BCRpSPYNE173 & pSPYCE(M)GCTCTAGAATGGCGATGTTGGGTAATGBiFC BIFC BIFC CG CCGCTCGAGCCAGAAGTCACCGCAAGAXbal BIFC BIFC CCGOTP81-TPFpSPYNE173 & pSPYCE(M)GCTCTAGAATGGCTATCTTCTCCAATCCGAATCCAACCAA	GUN1-BCR	pSPYCE(M)	ACGC <u>GTCGAC</u> CAAAAGAAGAGGGCTGTAAAGCAAAC	LCI	SalI
YS1-BCRpSPYCE(M)CCGCTCGAGCCAGAAGTCACCGCAAGABiFC & XholOTP81-TPFGCTCTAGAATGGCTATCTTCCAAAGCAACCTCTCTCTCT GCCACGTCATCCAACCAACTTCCCAATCCGAATCAACCAAC	YS1-BCF	nSPYNE173 &	GC <u>TCTAGA</u> ATGGCGATGTTGGGTAATG	BiFC	XbaI
OTP81-TPFCCTCCTCCTPSPYNE173 & pSPYNE173 & pSPYCE(M)GGCACGTCATCCAAACTTCTCCCAATCCGAATCAACCAAC	YS1-BCR	pSPYCE(M)	CCG <u>CTCGAG</u> CCAGAAGTCACCGCAAGA	BiFC &	Xhol
OTP81-TPFGCCACGTCATCCAAACTTCTCCCAATCCGAATCAACCAAC			GCTCTAGAATGGCTATCTTCTCCACAGCACAACCTCTCTCT	LUI	
OTP81-TFFCAATAACGAACGCTCCCGCCACATTTCTCTCATCGAACGATGBifCSpelpSPYNE173 & pSPYCE(M)pSPYCE(M)GGACTAGTAGACACACTCGTTCGATGAGAGAAATGTGGCG GGACCGTTCGTTATTGGTAGTTGGTGGTTGATTCGGATGGAGAA GTTTGGATGACGACGCGGCAGAGAGAGAGAGGTTGTGGTGGTGGAGAA AGATAGCCATTCTAGAGCBiFCSpelOTP81-TPRpUC19-NLue & pUC19-NLue & gGGGTACCATGGCTATCGTCATGGCTATCTTCTCCACAGCACLCIKpnlOTP81-LCIF pUC19-NLue & pUC19-NLue & pUC19-CLueGGGGTACCATGGTAGCTAGCTGCTCTTGCATGCTTCA CGGGGTACCATGGTAGCGTAATG CGGGGTACCATGGTAGCGTAATG CGGGGTACCATGGTAGCTTGCATGCTTGCATGCTTGTATCCTTATCGT cGTCATCCTTGTAATCCTTATCGTCGTCATCCTTGTAATCCTTATCGT cGTCATCCTTGTAATCCTTATCGTCGTCATCCTTGTGATAGCGTA ATCTGGGACATCGTAAGCGTAATCTGGAACATCGTAAGCGTAA TTCGAGCTAGCCTAGCGTAATCTGGAACATCGTAGCGTAAGCGTA TTCGAGCTAGCCTAGCGTAATCTGGAACATCGTATGGGAACATCGTAGCGTA TTGGGACATCGTAGCGTAATCTGGAACATCGTATGGGTAAGCGTA TTGGGACATCGTATCCTTGTGTGTTTTCTCT<	OTD81 TDE		GCCACGTCATCCAAACTTCTCCAATCCGAATCAACCAACTAC	DEC	XbaI
pSPYNEI73 & pSPYCE(M)TGTGTCT <u>ACTAGT</u> CC GGACTAGTAGACACACACATCGTTCGATGAGAGAAATGTGGCG GGAGCGTTCGTATTGGATGAGAGAGAGAGAGAGAGAGAGA	01181-111		CAATAACGAACGCTCCCGCCACATTTCTCTCATCGAACGATG	DIPC	SpeI
pSPYCE(M)GGACCTAGTAGACACACATCGTTCGTTGGATGAGAGAAATGTGGGG GGAGCGTTCGTTATTGGAGCAGTGGTGATTGGAGAAABiFCSpel XbalOTP81-TPR PUC19-NLue & pUC19-CLueGGGGTACCATGGCGTCAACGCCGCLCIKpnIMORF2-LCIF PUC19-NLue & pUC19-NLue & pUC19-CLueCGGGTACCATGTCATGTCTTGGCTTCALCIKpnI35S-OXFpEarleyGate 101CCGACGCGTAGCTTGCATGCTGCAAGCTGAATCCTTATCGT CGTCATCCTTGTAATCCTTATCGTCGTCATCCTTGTAATCCTTATCGT CGTCATCCTTGTAATCCTTATCGTCGTCATCCTTGTAATCCTTATCGT CGTCATCCTTGTAATCCTTATCGTCGTCATCCTTGTAATCCAAA TTCGGAACATCGTAAGGTAACCTGGAACATCGTATGGAA		pSPYNE173 &	TGTGTCT <u>ACTAGT</u> CC		
OTP81-TPRGGAGGTTGACTGGCAGAGAGAGAGAGAGAGAGAGAGTGATGGGAGAGA GTTTGGATAGCCAT <u>TCTAGAG</u> CBiFCSpel XbalGUN1-LCIFpUC19-NLuc & pUC19-CLucGG <u>GGTACCATGGCCATGGCGTCAACGCCGC</u> LCIKpnlMORF2-LCIFpUC19-NLuc & pUC19-NLuc & pUC19-CLucGGGGTACCATGTCATGTCTTTGCCTTGCATGCCTTCALCIKpnlYS1-LCIFpUC19-NLuc & pUC19-CLucCGGGGTACCATGGCGATGTTGGGTAATGLCIKpnl35S-OXFpEarleyGate 101CCGACGCGTAGCTTGCATGCTGCAGCTGCAGCPlant transformMlul ationMORF2-OXRpEarleyGate 101TCGAGCTCAGCGTAATCGTATCGTCATCGTTGGAACATCGTAATCGTAATCGTA TCGGAACATCGTATGGGTAAGCGTAA ATCTGGAACATCGTATGGGTAAGCGTAATCGTAGGGTAACTCGTAACCTTATGGAACATCGTATGGAACATCGTA ATCTGGAACATCGTATGGGTAAGCGTAATCGTAGGTAACTCGTAACATGGAACATCGTATGGAACATCGTATGGAACATCGTATGGAACATCGTATGGAACATCGTATGGAACATCGTATGGAACATCGTATGGAACATCGTA transform ationSpel		pSPYCE(M)	GGACTAGTAGACACACATCGTTCGATGAGAGAAATGTGGCG		C I
GUN1-LCIFpUC19-NLuc & pUC19-NLuc & pUC19-NLuc & pUC19-CLucGGGGTACCATGGCGTCAACGCCGCLCIKpn1MORF2-LCIFpUC19-NLuc & pUC19-NLuc & pUC19-CLucCGGAGCTCATGGCTTTGCCTTTGTCTGGLCISacIOTP81-LCIFpUC19-NLuc & pUC19-NLuc & pUC19-CLucCGGAGCTCATGGCTATCTTCTCCACAGCACLCISacIOTP84-LCIFpUC19-NLuc & pUC19-NLuc & pUC19-CLucGGGGTACCATGTCATGTCTTGCCTTGCATGCLCIKpn1YS1-LCIFpUC19-NLuc & pUC19-CLucCGGGGTACCATGGCGATGTTGGGTAATGLCIKpn135S-OXFpEarleyGate 101CCGACGCGTAGCTTGCATGCTGCAGPlant transformMlu1 ation35S-OXFpEarleyGate 101CCGACGCGTAGCTTGCATCGTCGTCATCCTTGTAATCCTTATCGT CGTCATCCTTGTAATCCTTATCGTCGTCATCCTTGTAATCCTTATCGT CGTCATCCTTGTAATCCTTATCGTCGTCATCCTTGTAATCCTTATCGT CGTCATCCTTGTAATCCTTATCGTCGTCATCCTTGTAATCCTTATCGT TTCGAGCTCAGCGTAATCTGGAACATCGTATGGGAACATCGTATGGGAACATCGTAATCTGGAACATCGTA TGGGTAGGTAGCCTCAGGGTGAACTCTTGTGTTTTCTCTSpe1	OTP81-TPR		GTTTGGATGACGTGGCAGAGAGAGAGAGGTGTGTGCTGGGAGA	BiFC	Spei Xbal
GUN1-LCIFpUC19-NLuc & pUC19-CLucGGGGTACCATGGCGTCAACGCCGCLCIKpnIMORF2-LCIFpUC19-NLuc & pUC19-CLucCGGAGCTCATGGCTTTGCCTTTGTCTGGLCISacIOTP81-LCIFpUC19-NLuc & pUC19-NLuc & pUC19-CLucCGGAGCTCATGGCTATCTTCTCCACAGCACLCISacIOTP84-LCIFpUC19-CLucGGGGTACCATGTCATGTCCTCTTGCCTTCALCIKpnIYS1-LCIFpUC19-NLuc & 			AGATAGCCATTCTAGAGC		Abdi
GUNI-LCIFpUC19-CLucpUC19-CLucICIKpniMORF2-LCIFpUC19-NLuc & pUC19-CLucCGGAGCTCATGGCTTTGCCTTTGTCTGGLCISacIOTP81-LCIFpUC19-NLuc & pUC19-NLuc & GGGGTACCATGTCATGTCATGTCCTCTTGCCTTCALCISacIYS1-LCIFpUC19-NLuc & pUC19-CLucGGGGTACCATGTCATGTCATGTCTTTGCCTTCALCIKpniYS1-LCIFpUC19-NLuc & pUC19-CLucCGGGGTACCATGGCGATGTTGGGTAATGLCIKpni35S-OXFpEarleyGate 101CCCGACGCGTAGCTTGCATGCTGCAGCPlant transformMlul ation35S-OXFpEarleyGate 101CCGACGCGTAGCTTGCATGCTGCATCCTTGTAATCCTTATCGT CGTCATCCTTGTAATCCTTATCGTCGTCATCCTTGTAATCCTTATCGT GGACTAGCTAACCTCGTAATCTGGAACATCGTAATCCTTATCGT GGGACAATCGTAATCTGGAACATCGTAAGCGTA ATCTGGAACATCGTAAGCGTAATCTGGAACATCGTAAGCGTA ATCTGGAACATCGTAGGGTAAGCGTAATCTGGAACATCGTATGGGAACATCGTATGGGTAAGCGTA ATCTGGAACATCGTAGGGTAAGCGTAATCTGGAACATCGTATGGGAACATCGTATGGGAACATCGTATGGGAACATCGTATGGGAACATCGTATGGGTAAGCGTA ATCTGGAACATCGTAGGGTAAGCGTAATCTGGAACATCGTATGGGAACATCGTATGGGAACATCGTATGGGAACATCGTAGGGTAAGCGTA ATCTGGAACATCGTAGGGTAAGCGTAATCTGGAACATCGTATGGGAACATCGTATGGGAACATCGTATGGGAACATCGTATGGGAACATCGTAGGGTAAGCGTA ATCTGGAACATCGTAGGGTAAGCGTAATCTGGAACATCGTATGGGAACATCGTATGGGAACATCGTAGGGTAAGCGTA ATCTGGAACATCGTAGGGTAAGCGTAATCTGGAACATCGTATGGGAACATCGTAGGGTAAGCGTA ATCTGGAACATCGTAGGGTAAGCGTAATCTGGGAACATCGTAGGGTAAGCGTA ATCTGGAACATCGTAGGGTAAGCGTAATCTGGGAACATCGTAGGGTAAGCGTA ATCTGGAACATCGTAGGGTAAGCGTAATCTGGGAACATCGTAGGGTAAGCGTAATCTGGAACATCGTAGGGTAAGCTTGGAACATCGTAGGGTAAGCTTGGAACATCGTAGGAACATCGTAGGGAACATCGTAGGGAACATCGTAGGGAACATCGTAGGAACATCGTAGGAACATCG	CUNU LOIF	pUC19-NLuc &		LCI	VI
MORF2-LCIFpUC19-NLuc & pUC19-CLucCGGAGCTCATGGCTTTGCCTTTGTCTGGLCISaclOTP81-LCIFpUC19-NLuc & pUC19-NLuc & pUC19-NLuc & pUC19-NLuc & pUC19-NLuc & pUC19-NLuc & pUC19-CLucCGGAGCTCATGTCATGTCCTCTGCCTTCALCISaclYS1-LCIFpUC19-NLuc & pUC19-CLucGGGGTACCATGTCATGTCCTCTGCGTTACGLCIKpnI35S-OXFpEarleyGate 101CCGACGCGTAGCTTGCATGCCTGCAGPlant transform ationMlul ationMORF2-OXRpEarleyGate 101GGACTAGTTACTTATCGTCGTCATCCTTGTAATCCTTATCGT GGGACTAGCTCAGCGTAATCGGAACATCGTATGGGTAAGCGTA ATCTGGAACATCGTAGGGTAAGCGACATCTGGAACATCGTATGGGAACATCGTAGGGTAAGCGTA ationSpeI	GUNI-LUIF	pUC19-CLuc	GGGIACCAIGGCGICAACGCCGC	LCI	Kpni
OTP81-LCIFpUC19-NLuc & pUC19-CLucCGGAGCTCATGGCTATCTTCTCCACAGCACLCISaclOTP84-LCIFpUC19-NLuc & pUC19-CLucGGGGTACCATGTCATGTCCTCTTGCCTTCALCIKpnIYS1-LCIFpUC19-NLuc & pUC19-CLucCGGGGTACCATGGCGATGTTGGGTAATGLCIKpnI35S-OXFpEarleyGate 101CCGACGCGTAGCTTGCATGCCTGCAGPlant transformMlul ationMORF2-OXRpEarleyGate 101GGACTAGTTTACTTATCGTCGTCATCCTTGTAATCCTTATCAAA TTCGAACATCGTAAGCGTAATCTGGAACATCGTAAGCGTA ATCTGGAACATCGTAGGGTAAGCGTAATCTGGAACATCGTATGGGTAAGCGTA ATCTGGAACATCGTAGGGTAAGCGTAATCTGGAACATCGTATGGGTAAGCGTAPlant transformSpeI	MORF2-LCIF	pUC19-NLuc & pUC19-CLuc	CG <u>GAGCTC</u> ATGGCTTTGCCTTTGTCTGG	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 transform ation Mlul ation MORF2-OXR pEarleyGate 101 GGACTAGTTACTTATCGTCGTCATCCTTGTAATCCTTATCGT GGTCATCCTTGTAATCGTAGGGTAAGCGTAATCTGGAACATCGT TTCGAGCTCAGCGTAATCTGGAACATCGTATGGGTAAGCGTA ATCTGGAACATCGTATGGGTAAGCGTAATCTGGAACATCGTATGGGAACATCGT TGGGTAGGTAGCTCAGCGTAAGCGTAATCTGGAACATCGTATGGGAACATCGTA TGGGTAGGTAGCTCCTCGAGGTCGACTCTTGTGTTTTCTCT Plant transform SpeI	OTP81-LCIF	pUC19-NLuc &	CG <u>GAGCTC</u> ATGGCTATCTTCTCCACAGCAC	LCI	SacI
OTP84-LCIF pUC19-CLuc GGGGTACCATGTCATGTCATGTCCTTGCCTTCA LCI Kpnl YS1-LCIF pUC19-NLuc & pUC19-CLuc CGGGGTACCATGGCGATGTTGGGTAATG LCI Kpnl 35S-OXF pEarleyGate 101 CCGACGCGTAGCTTGCATGCCTGCAG Plant transform Mlul ation MORF2-OXR pEarleyGate 101 CCGACGCTAGCTCAGCGTAATCGTCGTCATCCTTGTAATCAAA Plant transform Spel MORF2-OXR pEarleyGate 101 TCGGACCTCAGCGTAATCTGGAACATCGTATGGGTAAGCGTA TTCGAACATCGTATGGGTAAGCGTAATCTGGAACATCGTATGGGTAAGCGTA Plant		pUC19-NLuc &		1.01	
YS1-LCIF pUC19-NLuc & pUC19-CLuc CGG <u>GGTACCATGGCGATGTTGGGTAATG</u> LCI KpnI 35S-OXF pEarleyGate 101 CCG <u>ACGCGT</u> AGCTTGCATGCCTGCAG Plant transform Mlul 35S-OXF pEarleyGate 101 CCG <u>ACGCGT</u> AGCTTGCATGCCTGCAG mation Plant MORF2-OXR pEarleyGate 101 TCGAGCTCAGCGTAATCCTTATCGTCGTCATCCTTGTAATCAAA Plant ation MORF2-OXR pEarleyGate 101 TCGGAGCTCAGCGTAATCTGGAACATCGTATGGGTAAGCGTA TCGAGCTCAGCGTAGCGTAGCGTA transform SpeI ATCTGGAACATCGTATGGGTAAGCGTAATCTGGAACATCGGAACATCGTGAGCGTA TGGGTAGGTAGCGTCAGCGTCTTGTGTTTTCTCT ation SpeI	OTP84-LCIF	pUC19-CLuc	GG <u>GGTACC</u> ATGICATGICCICITGCCITCA	LCI	Kpnl
35S-OXF pEarleyGate 101 CCG <u>ACGCGT</u> AGCTTGCATGCCTGCAG Plant 35S-OXF pEarleyGate 101 CCG <u>ACGCGT</u> AGCTTGCATGCCTGCAG mainon GG <u>ACTAGT</u> TTACTTATCGTCGTCATCCTTGTAATCCTTATCGT CGTCATCCTTGTAATCCTTATCGTCGTCATCCTTGTAATCAAA Plant MORF2-OXR pEarleyGate 101 TTCGAGCTCAGCGTAATCTGGAACATCGTAATGGGTAAGCGTA transform SpeI ATCTGGAACATCGTATGGGTAAGCGTAATCTGGAACATCGGAACATCGTA TGGGTAGGTAGGGTAGGCGACTCTTGTGTTTTCTCT ation SpeI	YS1-LCIF	pUC19-NLuc &	CGG <u>GGTACC</u> ATGGCGATGTTGGGTAATG	LCI	KpnI
35S-OXF pEarleyGate 101 CCGACGCGTAGCTTGCATGCCTGCAG transform ation MluI ation 35S-OXF pEarleyGate 101 CCGACGCGTAGCTTGCATGCCTGCAG transform ation MluI ation MORF2-OXR pEarleyGate 101 CGGCACTAGCTCATCCTTGTAATCCTTGTGAATCAAA Plant Fransform SpeI MORF2-OXR pEarleyGate 101 TTCGAGCTCAGCGTAATCTGGAACATCGTAATGCGTAAGCGTA transform ATCTGGAACATCGTATGGGTAAGCGTAATCTGGAACATCGTA ation SpeI		POOL CLue		Plant	
ation GG <u>ACTAGT</u> TTACTTATCGTCGTCATCCTTGTAATCCTTATCGT CGTCATCCTTGTAATCCTTATCGTCGTCATCCTTGTAATCAAA Plant MORF2-OXR pEarleyGate 101 TTCGAGCTCAGCGTAATCTGGAACATCGTATGGGTAAGCGTA transform SpeI ATCTGGAACATCGTATGGGTAAGCGTAATCTGGAACATCGTA ation TGGGTAGGTACCCTCGAGGTCGACTCTTGTGTTTTCTCT	35S-OXF	pEarleyGate 101	CCG <u>ACGCGT</u> AGCTTGCATGCCTGCAG	transform	MluI
GG <u>ACTAGT</u> TTACTTATCGTCGTCATCCTTGTAATCCTTATCGT CGTCATCCTTGTAATCCTTATCGTCGTCATCCTTGTAATCAAA Plant MORF2-OXR pEarleyGate 101 TTCGAGCTCAGCGTAATCTGGAACATCGTATGGGTAAGCGTA transform SpeI ATCTGGAACATCGTATGGGTAAGCGTAATCTGGAACATCGTA ation TGGGTAGGTACCCTCGAGGTCGACTCTTGTGTTTTCTCT				ation	
CGTCATCCTTGTAATCCTTATCGTCGTCATCCTTGTAATCAAA Plant MORF2-OXR pEarleyGate 101 TTCGAGCTCAGCGTAATCTGGAACATCGTATGGGTAAGCGTA transform SpeI ATCTGGAACATCGTATGGGTAAGCGTAATCTGGAACATCGTA ation TGGGTAGGTACCCTCGAGGTCGACTCTTGTGTTTTCTCT			GG <u>ACTAGT</u> TTACTTATCGTCGTCATCCTTGTAATCCTTATCGT		
MORE2-OAK pEarleyGate 101 ITCGAGCTCAGCGTAATCTGGAACATCGTATGGGTAAGCGTA transform Spel ATCTGGAACATCGTATGGGTAAGCGTAATCTGGGAACATCGTA ation TGGGTAGGTACCCTCGAGGTCGACTCTTGTGTTTTCTCT	MORF2-OXR	pEarleyGate 101	CGTCATCCTTGTAATCCTTATCGTCGTCATCCTTGTAATCAAA	Plant	C I
TGGGTAGGTACCCTCGAGGTCGACTCTTGTGTTTTCTCT				uransform	Spei
			TGGGTAGGTACCCTCGAGGTCGACTCTTGTGTTTTCTCT	anon	

 Table S2 Primers used in the cloning and vector construction.

No.	Editing	Forward	Primer sequence (F)	Reverse	Primer sequence (R)	Sequencing	Sequencing primer
	site	primer	5' - 3'	primer	5' - 3'	primer name	sequence 5' - 3'
1	accD-794	aaaD E	CTCCATTCA ATCCCACA AT	aaaD B	ATATGCAAGCAAGGGAG	accD-956SR	CTGTTT
2	accD-1568	accD-F	GIGGATICAAIGCGACAAI	accD-K	G	accD-1285SL	GCATTTGCGGGTAAAAGAG
3	atpF-92	atpF-92F	CCGATTCTTTCGTTTACTTG	atpF-92R	AGGGTTCCTATAGCTCC	atpF-92F	I CCGATTCTTTCGTTTACTTG
4	clpP-559	clpP-559F	ATGATCCATCAACCCGCTAG	clpP-559R	TATTGAACCGCTACAAG ATC	clpP-397SL	TATGAGGCACAAACGGGA GA
5	matK-640	matK-640F	CGTTACCGGGTAAAAGATGC	matK-640R	AGCGGCGTATCCTTTGT TGC	matK-813SL	TTTTCCATAGAATACAATT CGCTCA
6	ndhF-290	ndhF-290F	AAAACCTTCGCCGCATGTGG	ndhF-290R	GCATTCGCTGCAATAGG TCG	ndhF-290R	GCATTCGCTGCAATAGGTC G
7	ndhG-50	ndhG-50F	ATAATGGATTTGCCTGGACC	ndhG-50R	CTTATTAAATCTTGCTCT AGA ATCTGGTT	ndhG-312SR	ACAAACCAACGAAGTAAT CCCA
8	petL-5	petL-5F	AGGGAAGTACTTTAAGAATC	petL-5R	ATTAGACCTAAGACGAT TCC	petL-175SR	ACACGGTAAGGAACTATCG AACA
9 10	psbE-214 psbF-77	psbE-214F	ACAGGAGAACGTTCTTTTGC	psbE-214R	CGTTGGATGAACTGCAT TGC	psbE-214F	ACAGGAGAACGTTCTTTTG C
11	psbZ-50	psbZ-50F	AGAACATAGCCCTATGAGTT AATACGA	psbZ-50R	GATAAGAGAATTAAGGA TACC CACCA	psbZ-SL	GCTATGAGTTAATACGATC CCTA
12	rpl23-89	rpl23-89F	AATTCCTACTGGATGCACGC	rpl23-89R	AAGAGGTGGAATAGAAT AACCCG	rpl23-89R	AAGAGGTGGAATAGAATA ACCCG
13	rpoA-200	rpoA-200F	CGGACACTACAGTGGAAGTG	rpoA-200R	ATGAATACAGCATCGAT AGG	rpoA-384SR	TTCCACAGCGGGCGGTAA
14 15	гроВ-338 гроВ-551	rpoB-338F	TATCGGTTTATTGATCAGGG	rpoB-338R	GCAGCTGCTAACACATC TCG	rpoB-193SL	AAAGAACGAGATGCTGTCT ATGAA
16	rpoB-2432	rpoB-2432F	AACACCTCAAGTGGCGAAAG	rpoB-2432R	GTCCTACATTCATGCGT GAG	rpoB-2244SL	GGCGAAAGAATCCTCCTAT GC
17	rpoC1-488	rpoC1-488F	TTTTCTTTTGCTAGGCCCATAA	rpoC1-488R	TTCGCAAATCTAAATCG GCT	rpoC1-593SR	GCACCCGCCCCAGTAGAA
18	rps12-i-58	rps12-intF	CAAGACAGCCAATCCGAAAC	rps12-intR	CTTGTACAATTCACATTC TTTGGC	rps12-int-SR	TTTACCCTGTTAGTCCGTTC TTTTC
19 20	rps14-80 rps14-149	rps14-80F	TTGATTTATAGGGAGAAGAAG AG	rps14-149R	TACCAGCTTGATCTTGTT GC	rps14-266SR	GCCTGAACCATTTCCCGAA G
21	ndhB-149					ndhB-346SR	CGGATAGAGGAATACAGA GAGTTGA
22 23 24	ndhB-467 ndhB-586 ndhB-746	ndhB-1F	GCCTTTCATTTGCTTCTCTT	ndhB-1R	TCCTTCGTATACGTCAG GA	ndhB-271SL	TTCCAAACGAACAATTTCA ACG
25 26 27	ndhB-830 ndhB-836 ndhB-872	ndhB-2F	CGTATACGAAGGATCTCCCAC	ndhB-2R	CTAGAAGCTAAAAAGGG TATCCT	ndhB-1041SR	CCACCATTTGAGTCTCCAA CA
28 29	8 ndhB-1255 9 ndhB-1481					ndhB-1018SL	ATTGTTGGAGACTCAAATG GTGG
30	ndhD-2					ndhD-252SR	TCCATCTATTCCCATTCTCC AGTA
31 32 33 34	ndhD-383 ndhD-674 ndhD-878 ndhD-887	ndhD-F	TTGAGTACGCGTTCTTTGGAC	ndhD-R	AATAGCTCCATTAAGTC CAGG	ndhD-303SL	TTTAGCGGCTTTTCCAGTT AC

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

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).