

## Supporting Information Appendix

### **RIP1, a member of an Arabidopsis protein family, interacts with the protein RARE1 and broadly affects RNA editing**

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#### **Supplementary Materials and Methods**

**Generation of  $\alpha$ -RARE1 Antibody.** A 159 aa polypeptide spanning the last short PPR motif, the E domain and the beginning of the DYW domain of RARE1 (1), was expressed in *E. coli* strain Rosetta (DE3) (EMD Novagen, Madison WI) by cloning into vector pGEX-6p3. Primers Rare1-159F and Rare1-159/194R (SI appendix, Table 3) were used to amplify the fragment by PCR, which was cloned into vector pCR2.1/TOPO (Invitrogen, Carlsbad, CA), before subcloning the EcoRI-Sall fragment into pGEX6p3. Following sonic disruption of the cells, the GST-RARE1 fusion protein was purified on Glutathione-Agarose (Sigma-Aldrich, St. Louis, MO) according to the manufacturer's recommended protocol, except after binding, RARE1 was proteolytically cleaved from GST using PreScission Protease (GE Healthcare, Piscataway, NJ). The eluted protein was used as an antigen for production of rabbit polyclonal antisera (PRFAL, Canadensis, PA). A 194 aa recombinant polypeptide, including the 159 aa region above, but with an additional PPR repeat on the N-terminus, was produced in a similar fashion, using instead as a forward primer Rare1-194F (SI appendix, Table 3). Immunoaffinity chromatography using the SulfoLink kit (Thermo Fisher Pierce, Rockford, IL) was used to purify  $\alpha$ -RARE1 according to the manufacturer's recommended protocol.

#### **Generation of Transgenic Plants Expressing Affinity-tagged RARE1.**

Transformation vector pBI121 (2) was modified to contain an affinity tag C-terminally fused to a Gateway cassette in place of the GusA gene. The affinity tag we used contains a sequence encoding the the 3xFLAG epitope (Sigma) 5' to a sequence encoding the StrepII epitope (IBA, St. Louis, MO) with a 4 aa V-G-A-G linker (3). Two rounds of PCR with overlapping primers were used to generate the fusion tag: first 3xFLAG-StrepIIF1 and 3xFLAG-StrepIIR and secondly with 3xFLAG-StrepIIF2 and 3xFLAG-StrepII R (SI appendix, Table 3). The resulting 117 nt fragment was cloned into pCR2.1/TOPO, and a SmaI-SacI fragment was used to replace the GusA of pBI121 cut with the same two enzymes. For overexpression (35S promoter) constructs, the GWb cassette (Invitrogen) was inserted at the SmaI site. For native promoter constructs, the CaMV 35S promoter was first removed using HindIII and XbaI before inserting the GWb cassette.

Full-length *RARE1* for overexpression was cloned by PCR using primers Rare1F and Rare1\_+2259R for untagged constructs or L5- Rare1\_+2256R for making fusion proteins with a 5 aa linker (L5) encoding G-S-G-G-G, which had been successfully used in (4). For native promoter constructs, 311 bp 5' of the start codon was amplified using Rare1\_-311F in combination with the primers above (SI appendix, Table 3). All *RARE1* PCR products were cloned to pCR8/GW/TOPO (Invitrogen) and fragments were

recombined into the modified pBI121 vectors above using LR Clonase II (Invitrogen). After sequence verification, the plasmids were transformed into *Agrobacterium tumefaciens* GV3101 and floral dip transformation of *rare1* homozygous mutants (WiscDsLox330H10) or (GABI\_167A04) was performed as in (5). Transgenic plants were selected on MS agar plus 50 µg/ml kanamycin and 100 µg/ml carbenicillin. Sequencing of the transgene in the plants containing RARE1-3xFS under the control of the native promoter revealed that after the 3xFLAG sequences, a frameshift had occurred that affected the StrepII sequence. Thus only the FLAG sequences were used as an epitope tag.

**Immunoblotting.** 10 or 12% Tris-Glycine (Protogel, National Diagnostics, Atlanta, GA), or 4-12% Bis-Tris NuPAGE (Invitrogen) polyacrylamide gels were used for SDS-PAGE (6). Proteins were electroblotted to nitrocellulose using a Mini-Protean II cell (BioRad, Hercules, CA), blocked with 5% powdered milk. When probed with α-RARE1 or α-Rubisco LSU (7) goat α-Rabbit IgG-HRP (GE Healthcare) secondary antibody was used for detection; otherwise, α-FLAG M2-HRP (Sigma-Aldrich) was used according to manufacturer's protocol.

**Size Exclusion Chromatography.** Stromal protein (0.5mg) was prepared as in (8), dialyzed against KEX buffer (30 mM HEPES-KOH, pH 8.0, 200 mM KOAc, 10 mM Mg(OAc)<sub>2</sub>, and 5 mM DTT) (9), clarified by micro-centrifugation and 0.4 µm filtered before fractionation over Superdex-200 resin (GE Healthcare) with KEX buffer. Flow was maintained by use of a peristaltic pump and fractions of approximately 0.3 ml were collected. As KEX buffer was found to precipitate in 2X Laemmli sample buffer (6), protein from individual fractions was purified using the SDS-Page Sample Prep Kit (Thermo Fisher Pierce), and 50% of the indicated fractions were subjected to SDS-Page. Calibration of the Superdex column was performed with standards from Sigma MWGF1000 Kit, including carbonic anhydrase, bovine serum albumin, alcohol dehydrogenase, β-Amylase, apoferritin, thyroglobulin and Blue Dextran corresponding to 29, 66, 150, 200, 443, 669 and 2,000 kDa, respectively. Standards were run one at a time over the column, and protein concentration was measured by measuring absorbance at 260 nm. For size exclusion chromatography of 3xFLAG-tagged RARE1, the buffer used was RIPA (formulation in immunoprecipitation section), and 1 mg total leaf protein prepared in this buffer was fractionated.

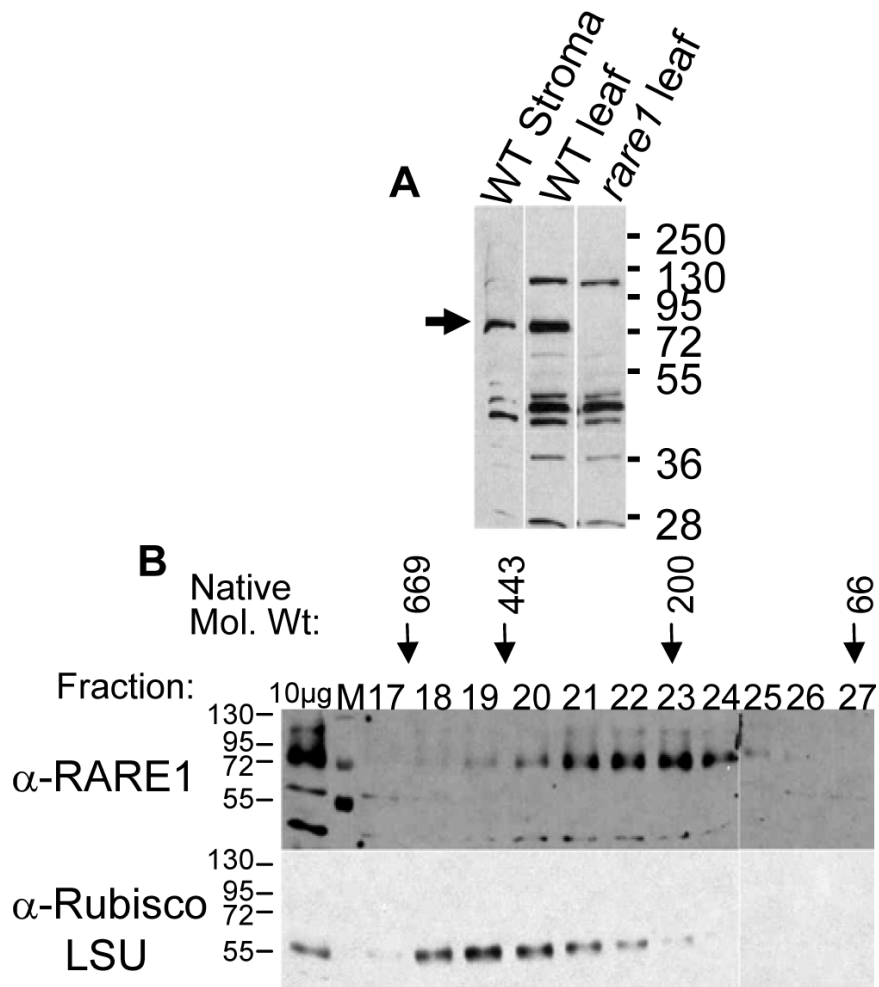
**Immunoprecipitation.** For immunoprecipitation with the α-RARE1 antibody, the Dynabeads Protein-A Kit (Invitrogen) was used according to manufacturer's protocol. Antibody was crosslinked to the beads using 5 mM Bis(sulfosuccinimidyl)suberate (Thermo Fisher, Waltham, MA) prior to addition of 2 mg leaf extract per immunoprecipitation. Total leaf protein extracts were prepared by powdering with a mortar and pestle in liquid nitrogen prior to extraction in RIPA lysis/binding buffer (50mM Tris-HCl, pH 7.4, 150mM NaCl, 1mM EDTA, 1% TritonX-100, 25 mM 2-mercaptoethanol and 1X Complete Protease Inhibitor Cocktail [Roche, Indianapolis, IN]) and subsequent pelleting of insoluble material by centrifugation. After washing with supplied Wash Buffer, the immunoprecipitate was eluted in NuPAGE LDS Sample Buffer plus Reducing Reagent (Invitrogen).

3xFLAG immunoprecipitation was performed as in (10), except  $\alpha$ -FLAG M2 Magnetic Agarose (Sigma-Aldrich) was mixed with 10 mg total leaf extract prepared as above (without 2-mercaptoethanol) and elution was done with 2 M  $MgCl_2$ , 50 mM Tris pH 8, 150 mM NaCl, and 0.5 % CHAPS (addition of CHAPS as in (11)).  $MgCl_2$  concentration was reduced 3-fold by the addition of TBS, and proteins were precipitated by adding 3 volumes of acetone. Proteins were resuspended in 2X Laemmli sample buffer and were resolved by SDS-PAGE as above. Staining was performed with SilverSNAP (Thermo Fisher) or SyproRuby protein gel stain (Invitrogen).

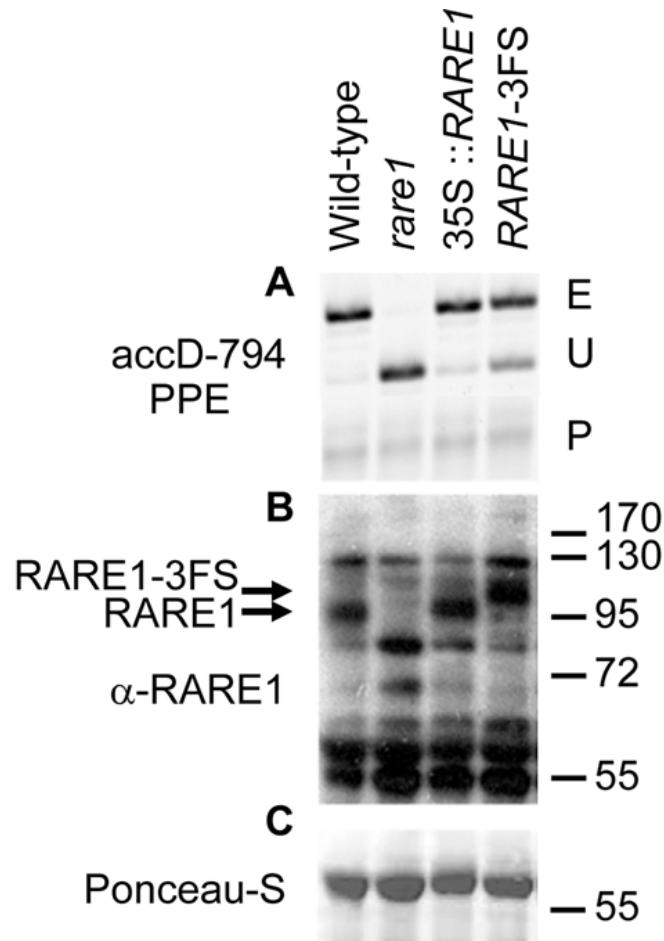
**Proteome Analysis by NanoLC-LTQ-Orbitrap Mass Spectrometry.** Each gel lane was cut in seven slices. Proteins were digested with trypsin and the extracted peptides were analyzed by nanoLC-LTQ-Orbitrap mass spectrometry using data dependent acquisition and dynamic exclusion, as described in (12). Peak lists (mgf format) were generated using DTA supercharge (v1.19) software (<http://msquant.sourceforge.net/>) and searched with Mascot v2.2 (Matrix Science) against the Arabidopsis genome (ATH v8) supplemented with the plastid-encoded proteins and mitochondrial-encoded proteins. Details for calibration and control of false positive rate can be found in (12). Mass spectrometry-based information of all identified proteins was extracted from the Mascot search pages and filtered for significance (e.g. minimum ion scores, etc), ambiguities and shared spectra as described in (12).

**Protein-protein Interaction Verification *in vivo*.** Yeast two-hybrid analysis was performed with the ProQuest Two-Hybrid System (Invitrogen), using Gateway-ready cDNA clone G67651 for At3g15000 obtained from the Arabidopsis Biological Resource Center (ABRC, The Ohio State University). Additionally At3g15000 was cloned without a putative transit peptide of 56 aa, using primers At3g15000\_+169F and At3g15000\_+1188R (SI appendix, Table 3). These clones were used for LR Clonase II recombination reactions with pDEST22, generating GAL4 transcriptional activation domain fusions with each. RARE1 without a putative transit peptide of 33 aa was cloned using RARE1\_+100F and RARE1\_+2259R primers (SI appendix Table 3) and TOPO cloned in pCR8/GW/TOPO before recombination into pDEST32, thereby fusing it to the GAL4 DNA-binding domain. *Saccharomyces cerevisiae* strain Mav203 was transformed using the recommended protocol and transformants were selected on SD dropout media lacking leucine and tryptophan (Sunrise Science Products, San Diego, CA). The X-Gal reporter assay was done according to the suggested protocol.

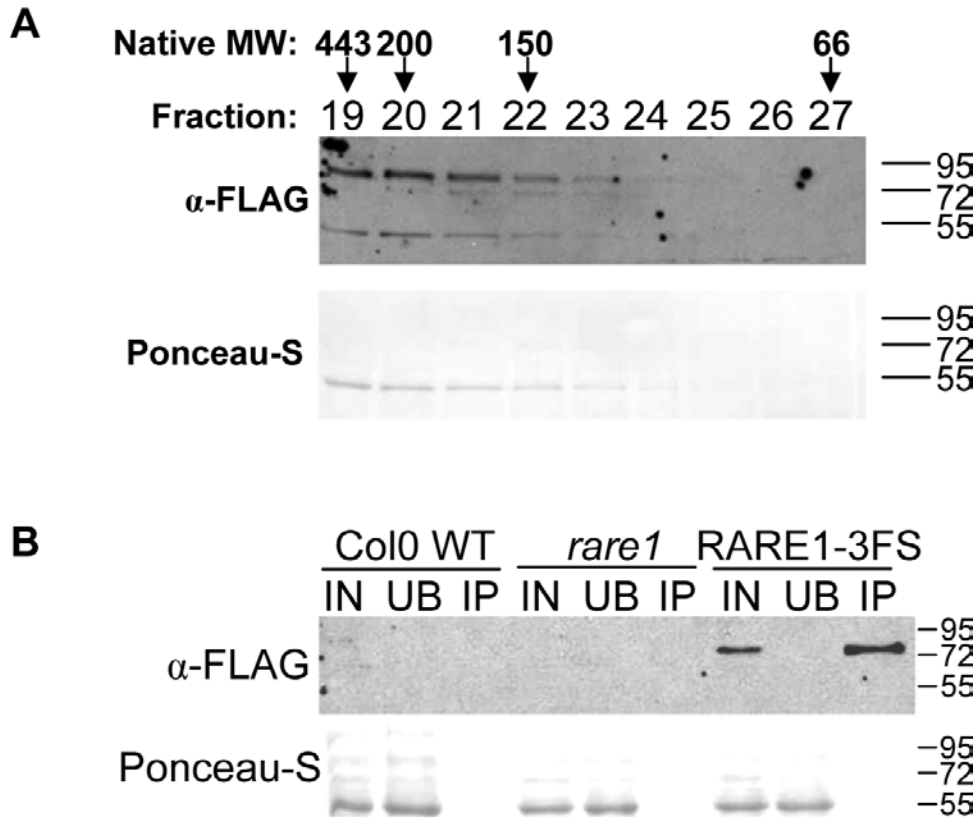
Supplementary Figures



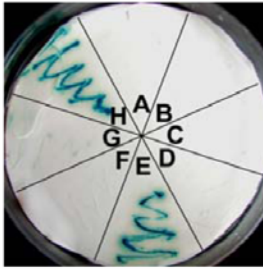
**Fig. S1.** RARE1 is part of a protein complex. (A) Immunoblot of wild-type and *rare1* protein extracts using  $\alpha$ -RARE1 antibody.  $\alpha$ -RARE1 antibody reacts with a 75 kDa protein in wild-type stroma and leaf, which is absent in *rare1* leaf. Arrow indicates RARE1 protein. Loading for all plant protein samples is 20  $\mu$ g/lane. (B) Size exclusion chromatography fractions of wild-type stroma probed with  $\alpha$ -RARE1 antibody or  $\alpha$ -Rubisco LSU antibody. An equal volume of each fraction was loaded. An arrow indicates the fraction containing the greatest amount of each size standard.



**Fig. S2.** A tagged version of RARE1 partially restores the *accD-794* editing defect in the *rare1* mutant. (A) Acrylamide gel separating the poisoned primer extension (PPE) products obtained from the wild-type, the *rare1* mutant, and two transgenic *rare1* lines transformed with different versions of *RARE1*, 35S :: *RARE1*: wild-type allele under the control of the 35S promoter, *RARE1-3xF*: tagged *RARE1* with 3xFLAG under the control of the native promoter. The PPE products E (edited), U (unedited), and P (Primer) are 34, 30 and 22 nt, respectively. The two constructs, 35S :: *RARE1* and *RARE1-3xF*, restore *accD-794* editing extent with a decreasing efficiency, as shown by the increasing intensity of the unedited band in the respective lanes. (B) Immunoblot in which 20  $\mu$ g total leaf protein from each sample was probed with  $\alpha$ -RARE1 antibody to determine the relative abundance of RARE1 protein in the individual lines. (C) Ponceau-S stain of Rubisco large subunit demonstrates approximately equal loading.



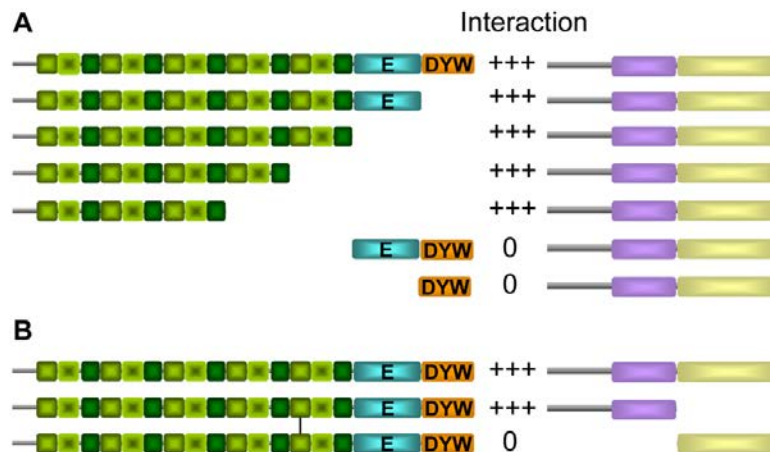
**Fig. S3.** Separation and Immunoprecipitation of the RARE1-3x $\text{F}$  complex. (A) Extracts of chloroplast stroma in RIPA buffer contain a RARE1-3x $\text{F}$  complex of similar size as the previously observed RARE1 complex extracted in KEX Buffer (Fig. 1). Size exclusion chromatography fractions of wild-type stroma were probed with  $\alpha$ -FLAG antibody, with the peak fraction indicated where the size standards eluted. Due to the different buffer used for the RARE1-3x $\text{F}$  extracts, the particular fraction(s) in which size standards and RARE1 complexes eluted are not identical to the chromatography with the native complex. (B) Leaf extracts were treated with  $\alpha$ -FLAG antibody, immunoprecipitates were separated by SDS-PAGE, and the immunoblot was probed with  $\alpha$ -FLAG antibody. As expected, neither the wild-type nor the mutant react with the  $\alpha$ -FLAG antibody. The RARE1-3x $\text{F}$  protein is present in the input (IN) and immunoprecipitate (IP) fractions from the transgenic line and depleted in the unbound (UB) fraction. Ponceau-S stain of Rubisco shows equal loading of control and transgenic samples.



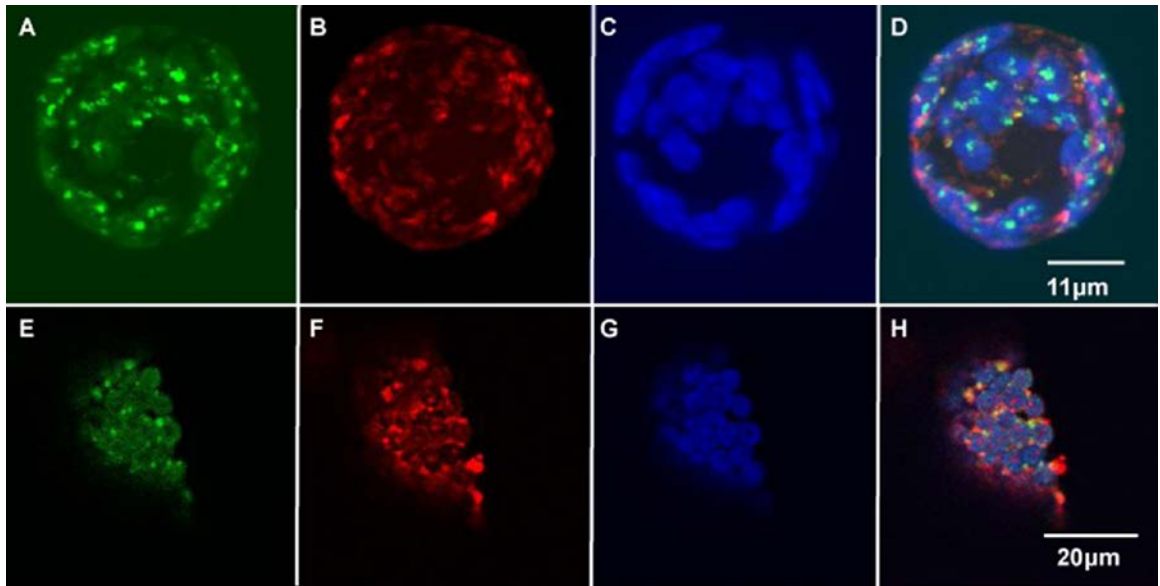
**B**

	pDEST32	pDEST22	Interaction
A	empty	RIP1FL	0
B	empty	RIP1ΔcTP	0
C	RARE1ΔcTP	empty	0
D	RARE1ΔcTP	RIP1FL	0
E	RARE1ΔcTP	RIP1ΔcTP	+++
F	Krev1	RalGDS-m2	0
G	Krev1	RalGDS-m1	+
H	Krev1	RalGDS-wt	+++

**Fig. S4.** RIP1 interacts with RARE1 *in vivo*. (A) X-gal reporter assay of lacZ transcriptional activation as proof of interaction in a yeast two-hybrid experiment. (B) Table describing the constructs tested for interaction in the yeast two-hybrid analysis and relative degree of lacZ expression. F-H contain control plasmids included with ProQuest kit for a negative, weak and strong protein-protein interaction in F, G and H, respectively. Unless otherwise indicated, pDEST22 and pDEST32 are empty vectors used to demonstrate that there is no autoactivation of lacZ expression when only RARE1- or RIP1-fusion proteins are expressed. RIP1FL denotes full-length RIP1 without cTP removal and RIP1ΔcTP indicates removal of a TargetP-predicted 56 aa cTP.

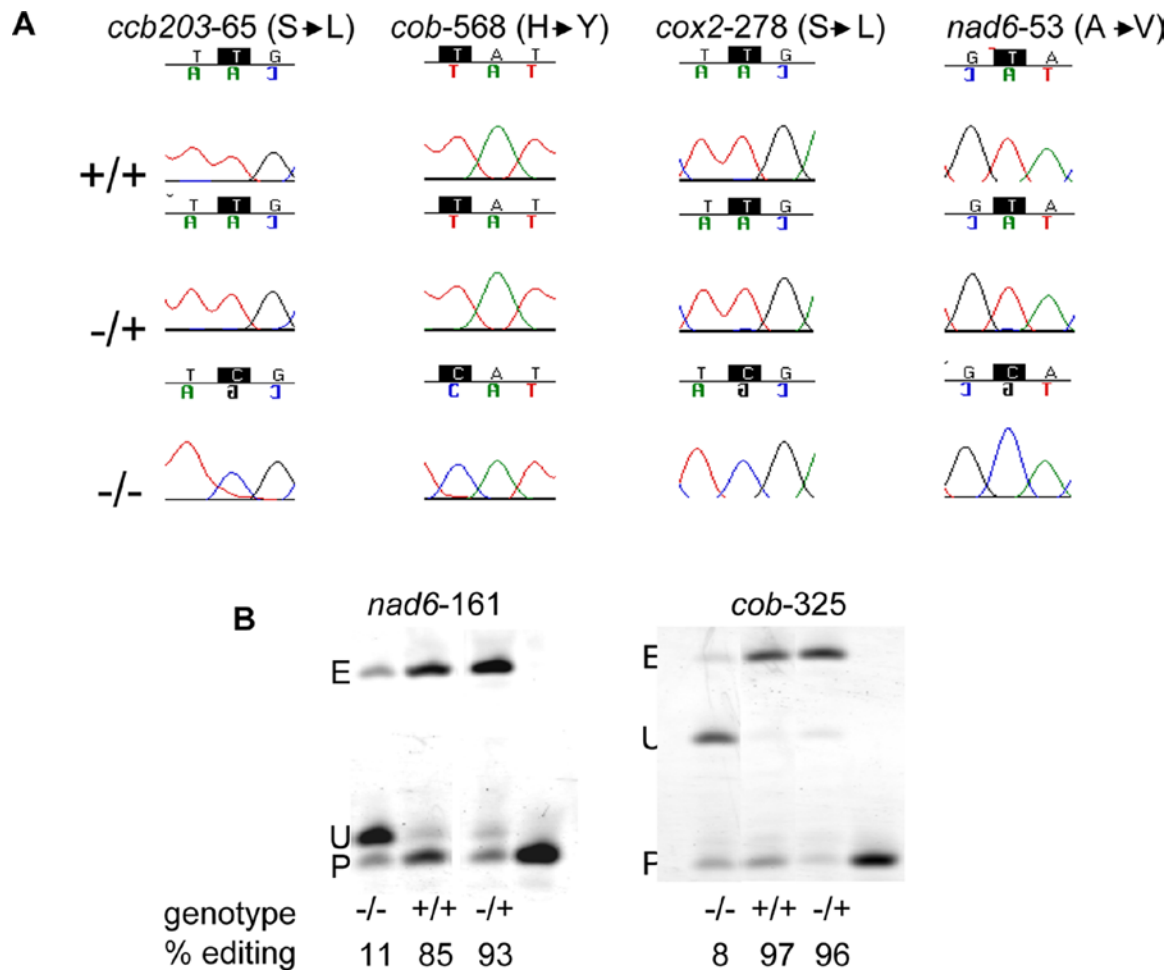


**Fig. S5.** Specific regions of RARE1 and RIP1 are responsible for their interaction *in vivo*. (A) Diagram of the serial deletions of RARE1 (left) that were tested in by yeast two-hybrid analysis with RIP1 (right), which is divided into N-terminal (purple) and C-terminal (yellow) regions. Pentatricopeptide motifs: P ■ L ■ S ■. All the proteins were expressed without predicted transit peptides. Relative degree of lacZ expression indicated by + signs.

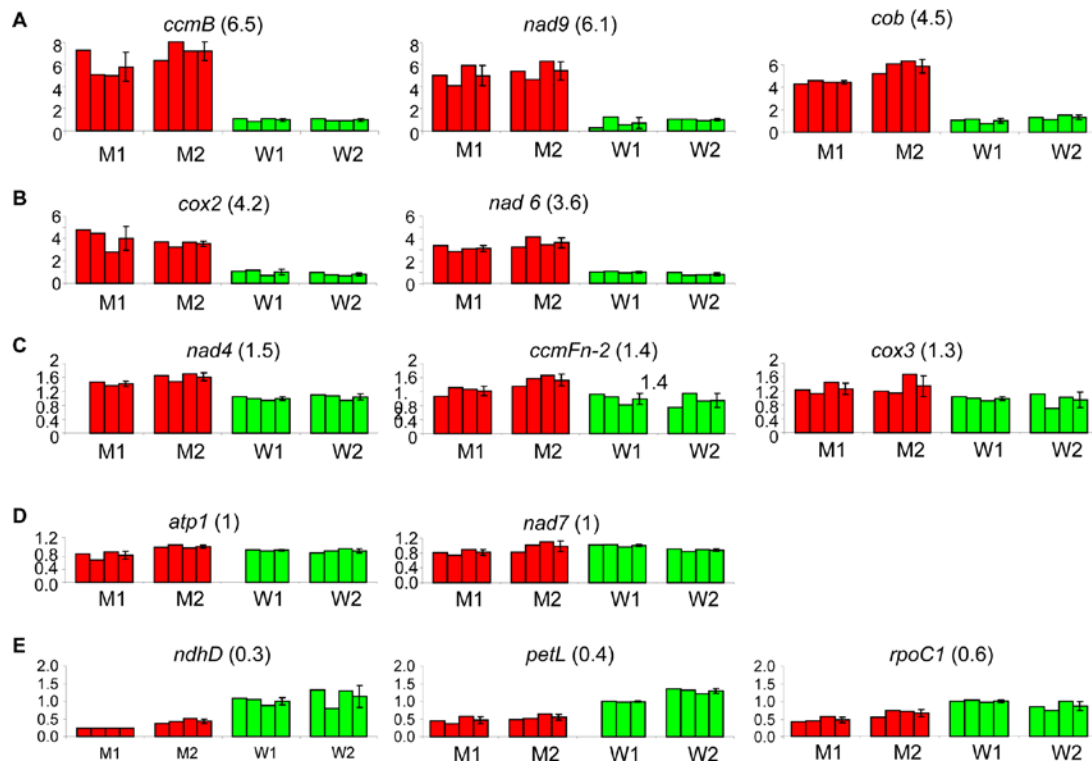


**Fig. S6** RIP1 is dual-targeted to mitochondria and chloroplasts (A-D) and co-localizes with plastid nucleoids (E-H). Protoplasts prepared from leaves of *N. benthamiana* were transfected with a construct encoding a RIP1-GFP fusion protein under the control of a 35S promoter. (A, E) Protoplasts were examined for GFP fluorescence 16 h after incubation with the construct. (B) Mitochondria were detected with Mitotracker Orange. (C, G) Chlorophyll autofluorescence is shown as blue. (D) Merged image shows GFP co-localization within mitochondria (yellow) spots or in chloroplasts (turquoise). (F) DAPI staining of DNA in chloroplast nucleoids (red). (H) Merged images of DAPI and GFP signals (yellow) shows RIP1 to co-localize with nucleoids

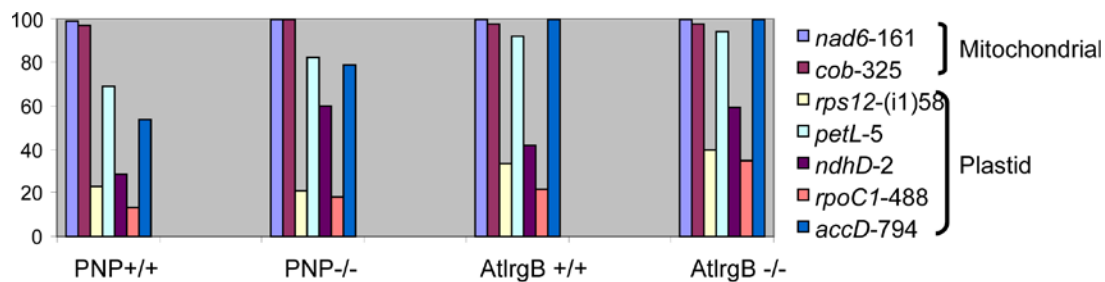




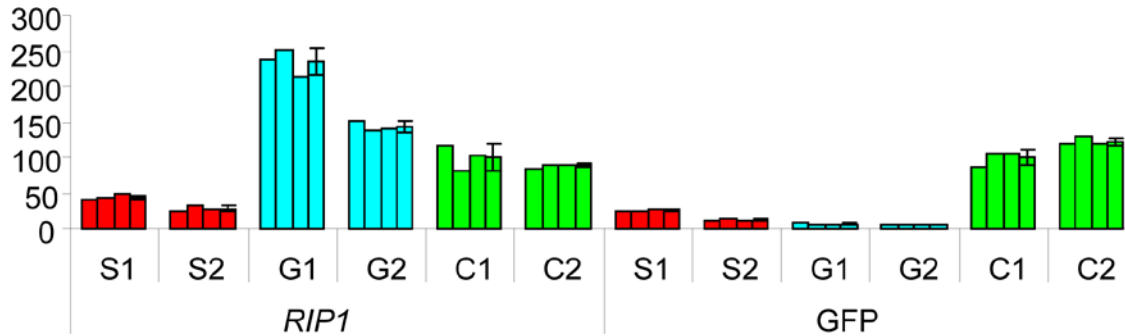
**Fig. S7.** *rip1* mutation is recessive in its effect on mitochondrial editing extent. (A) Bulk sequencing electrophoretograms following RT-PCR are shown for 4 sites in different mitochondrial transcripts and for the 3 genotypic classes, top row: homozygous wild-type (+/+), middle row: heterozygous (-/+), bottom row: homozygous mutant (-/-). Above the electrophoretograms is given the name of the editing site (the position of the site is given after the name of the transcript to which it belongs) followed by the aa change upon editing. The edited position is highlighted by a black shade. No difference can be detected between the electrophoretograms of the RT-PCR products derived from wild-type homozygous (+/+) and the heterozygous (-/+) plants. (B) PPE assay confirms *rip1* mutation to be recessive in its effect on mitochondrial site editing extent. No significant difference is found between the editing extent of heterozygous and homozygous wild siblings for sites *nad6-161* and *cob-325*, two sites that show a very strong reduction of editing extent in the homozygous mutant. P: primer, U: unedited, E: edited



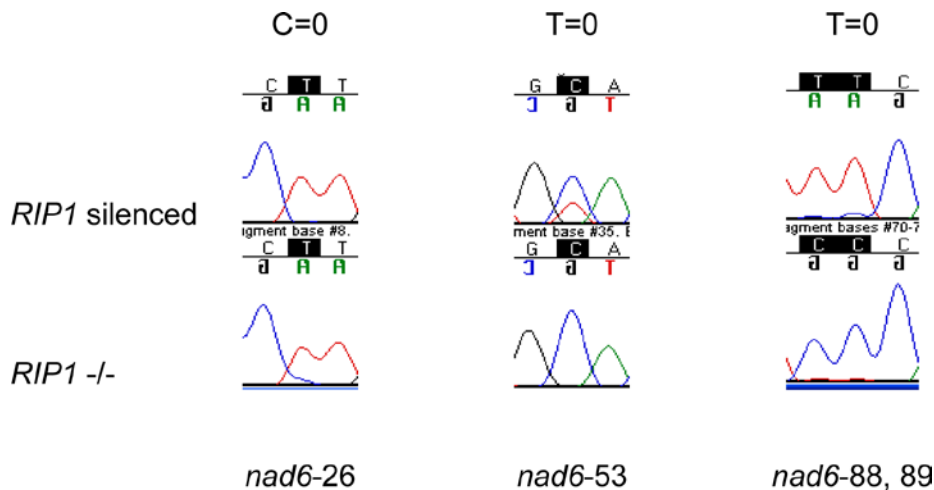
**Fig. S8.** Transcript abundance is differentially affected in *rip1* organelles. (A-D) qRT-PCR shows a variable steady state level of mitochondrial transcripts in *rip1* (M1, M2) relative to wild-type (W1, W2). The level of *rip1* mitochondrial transcripts is markedly increased (A-B), slightly increased (C) or unchanged (D) when compared to wild-type. (E) By contrast to mitochondrial transcripts, qRT-PCR indicates a significantly decreased amount of transcript in *rip1* for plastid genes *ndhD*, *petL* and *rpoC1*. The values are means of three replicates normalized to W1, with error bars representing S.D. The number in parenthesis close to the gene refers to the ratio of transcript expression (M/W).



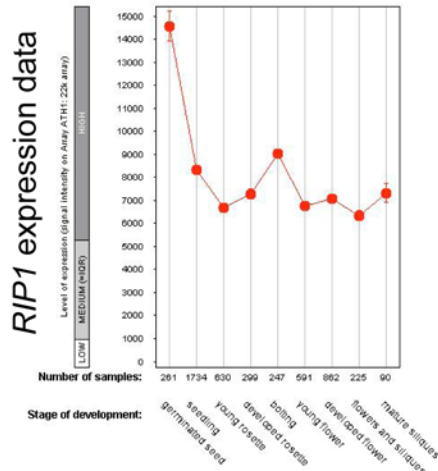
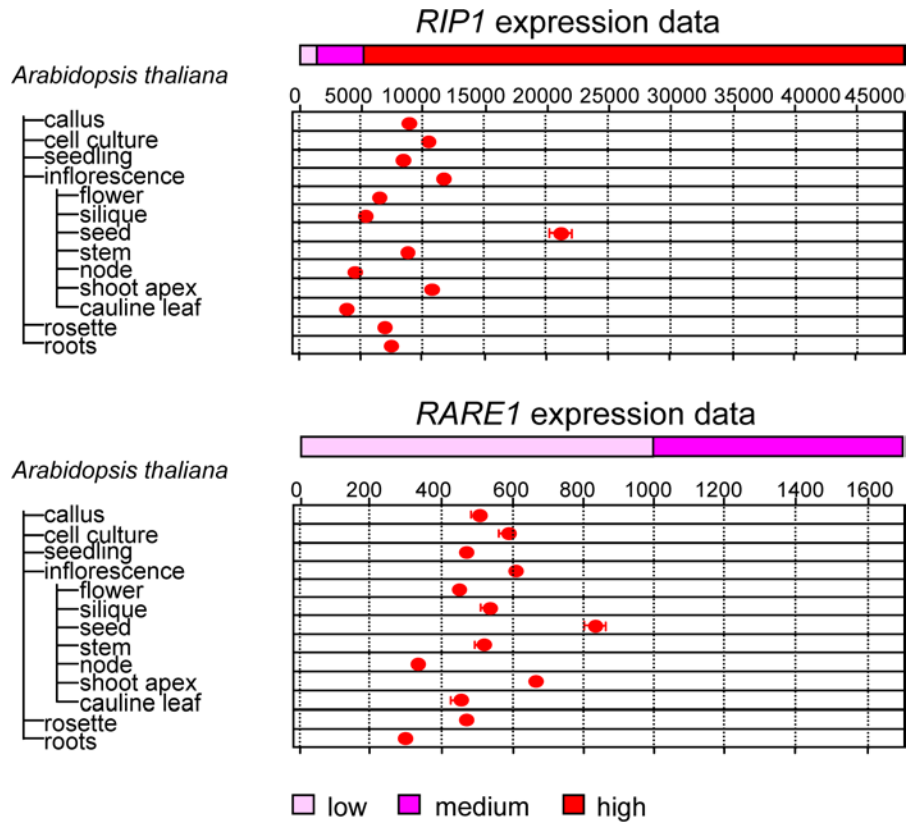
**Fig. S9.** Editing extent of some plastid sites show an increase in *pnp* and *IrgB* null mutants that are impaired in plastid RNA metabolism and plastid biogenesis, respectively. PPE assay reveals an invariant level of editing extent at two mitochondrial sites in *pnp* and *IrgB* mutants, while plastid *ndhD-2* shows a marked increase of editing extent in both mutants. Editing extents of *petL-5* and *accD-794* are increased only in the *pnp* mutant, whereas *rpoC1-488* editing extent increases only in the *IrgB* mutant.



**Fig. S10.** *RIP1* silencing results in a significant decrease of *RIP1* transcript level. Virus induced gene silencing (VIGS) of *RIP1* results in a significant decrease of *RIP1* transcript level in silenced plants (S) relative to control plants (G, C). Quantitative RT-PCR measured the level of *RIP1* and GFP expression in two *RIP1*-silenced plants (S1, S2), two GFP-silenced plants (G1, G2) and two uninoculated plants (C1, C2). The level of *RIP1* and GFP cDNAs was arbitrarily fixed at 100 for C1. GFP is used as a marker for silencing in VIGS experiment and as such is significantly decreased in both *RIP1* silenced and GFP silenced plants. *RIP1* transcript level is significantly decreased only in *RIP1* silenced plants.



**Fig. S11.** *RIP1* silencing affects only sites exhibiting a strong *RIP1* dependence in the mutant. *RIP1* silencing affects only the editing extent of sites showing a strong *RIP1* dependence in the mutant (T=0), but the reverse is not true; some sites showing strong *RIP1* dependence are not affected by *RIP1* silencing. On the left is shown an electrophoretogram of a *RIP1*-independent site whose editing extent is not affected by *RIP1* mutation nor by *RIP1* silencing. In the middle is an electrophoretogram of a *RIP1* dependent site whose editing extent is both affected by *RIP1* mutation and silencing. On the right the electrophoretogram of two *RIP1* dependent sites, *nad6-88* and *89*, show no detectable reduction of editing extent in a *RIP1* silenced plant but still exhibit a total loss of editing in the mutant.



**Fig. S12.** *RIP1* is highly expressed throughout plant development. Expression data were obtained from Genevestigator, a database and web-browser data mining interface for Affymetrix GeneChip data (<https://www.genevestigator.ethz.ch>). Although their gene products interact, *RIP1* is expressed at much higher level than *RARE1*.

## Supplementary tables

**Table S1. MS/MS based identification of RIP1 (At3g15000.1) in the co-immunoprecipitate from FLAG-tagged RARE1 (At5g13270.1)**

Peptide (a)	Modification (b)	SearchType (c)	# matched MS/MS spectra
<b>RARE1 - At5g13270.1</b>			
ACASLEELNLGK		Full_Tryptic	7
AGLCSNTSIETGIVNMYVK	Oxidation (M)	Full_Tryptic	5
AGVSVSSYSYQCLFEACR		Full_Tryptic	4
AVGLFSGMLASGDKPPSSMYTTLLK	2 Oxidation (M)	Full_Tryptic	2
ELSCSWIQEK		Full_Tryptic	4
FIVGDKHHPQTQEIYEK		Full_Tryptic	1
HVSLVTGHEIVIR		Full_Tryptic	6
KLNEAFEFLLQEMDK	Oxidation (M)	Full_Tryptic	1
KPVACTGLMVGYTQAGR	Oxidation (M)	Full_Tryptic	7
LAIAFGLISVHGNAPAPIK		Full_Tryptic	3
LFDEMSELNAVSR	Oxidation (M)	Full_Tryptic	4
LKEFDGFMEGDMFQCNMTER	3 Oxidation (M)	Full_Tryptic	2
LNEAFEFLLQEMDK	Oxidation (M)	Full_Tryptic	4
NLELGEIAGEELR		Full_Tryptic	7
SGLLDEALK		Full_Tryptic	3
SLIGSQYGESALITMYSK	Oxidation (M)	Full_Tryptic	4
TTMISAYAEQGILDK	Oxidation (M)	Full_Tryptic	5
<b>RIP1 - At3g15000.1</b>			
TLAQIVGSEDEAR	none	Full_Tryptic	10

The mass spectral data were searched using MASCOT ( $p < 0.01$ ; error  $< 6$  ppm for precursor ions) against the Arabidopsis database (v.8) downloaded from TAIR. Neither proteins were identified in control samples

(a) Matched peptide sequence from MS/MS spectra, within 6 ppm mass accuracy

(b) Variable peptide methionine oxidation

(c) Only full tryptic peptide are allowed

**Table S2. Effect of FLAG\_150D11 insertion on RNA editing of chloroplast C-targets, ranked by degree of change in editing and grouped by known trans-factors**

Trans-factor (if known)	Chloroplast C-target	Genotype			P value	$\Delta$ Editing -/-::+/+
		+/+	-/+	-/-		
	<i>rps12-(i1)58</i>	16 ± 1	18	42	0.001 **	<b>162.5</b>
	<i>petL-5</i>	87 ± 1	87 ± 1	35	0.0008 ***	<b>-60.0</b>
CRR4	<i>ndhD-2</i>	57 ± 2	58	26 ± 2	0.004**	<b>-55.4</b>
CRR28	<i>ndhB-467</i>	85 ± 1	85 ± 3	68 ± 2	0.006 **	<b>-20.6</b>
CRR28	<i>ndhD-878</i>	91 ± 1	91 ± 1	70 ± 9	0.08 ns	-23.0
	<i>rpoC1-488</i>	62 ± 2	57 ± 1	74 ± 4	0.002 **	<b>18.4</b>
RARE1	<i>accD-794</i>	98 ± 1	98 ± 1	83 ± 1	0.015 *	<b>-14.9</b>
	<i>ndhB-586</i>	94	93	84	0.0004***	<b>-10.8</b>
	<i>rpoB-2432</i>	83 ± 2	85 ± 3	91 ± 1	0.03 *	<b>9.6</b>
OTP84	<i>ndhB-1481</i>	94	96	89 ± 1	0.04*	<b>-5.4</b>
OTP84	<i>ndhF-290</i>	98	98 ± 1	95 ± 1	0.029*	<b>-3.6</b>
OTP84	<i>psbZ-50</i>	94 ± 1	94 ± 2	90 ± 3	0.27 ns	-3.5
CRR21	<i>ndhD-383</i>	98	98 ± 1	94 ± 1	0.049*	<b>-3.6</b>
OTP82	<i>ndhB-836</i>	95	95 ± 1	92	0.03*	<b>-2.8</b>
OTP82	<i>ndhG-50</i>	77 ± 3	82 ± 1	72 ± 1	0.18 ns	-5.9
	<i>ndhB-830</i>	98 ± 1	98	95	0.03*	<b>-2.7</b>
CRR22	<i>ndhB-746</i>	98	97 ± 1	96	0.02*	<b>-1.2</b>
CRR22	<i>ndhD-887</i>	88 ± 2	88 ± 2	73 ± 9	0.16 ns	-16.7
CRR22	<i>rpoB-551</i>	50 ± 9	50 ± 3	50 ± 13	0.99 ns	1.0
OTP80	<i>rpl23-89</i>	69 ± 2	70 ± 8	60 ± 3	0.07 ns	-13.3
OTP85	<i>ndhD-674</i>	92 ± 1	92 ± 1	82 ± 6	0.15 ns	-10.8
CLB19	<i>rpoA-200</i>	72 ± 4	67 ± 8	80 ± 3	0.16 ns	10.2
CLB19	<i>clpP-559</i>	92 ± 1	92 ± 2	93	0.33 ns	1.2
	<i>ndhB-149</i>	98 ± 4	97 ± 1	90 ± 4	0.1 ns	-8.0
	<i>rps14-80</i>	79 ± 1	77 ± 1	73 ± 1	0.05 ns	-7.6
	<i>matK-640</i>	69 ± 6	64 ± 6	73 ± 7	0.58 ns	5.9
LPA66	<i>psbF-77</i>	83	83 ± 3	86 ± 1	0.078 ns	3.6
	<i>ndhB-1255</i>	91 ± 5	93 ± 1	88 ± 2	0.5 ns	-3.2
	<i>ndhB-872</i>	87 ± 3	86 ± 1	85	0.39 ns	-2.7
	<i>psbE-214</i>	98	98	96 ± 2	0.21 ns	-2.3
	<i>atpF-92</i>	93 ± 3	97	95 ± 1	0.44 ns	2.3
	<i>accD-1568</i>	60 ± 2	58 ± 7	61 ± 1	0.57 ns	2.2
YS1	<i>rpoB-338</i>	97 ± 1	97	95 ± 1	0.22 ns	-1.5
OTP86	<i>rps14-149</i>	80	80 ± 1	80	0.25 ns	-0.7

The variation in editing is = 100\* (editing extent in -/- - editing extent in +/+)/editing extent in +/+. Minus sign indicates that the editing extent is decreased in the mutant. Significant editing extent variation is given in bold.

<b>Table S3.</b>	<b>Oligonucleotides used in this study</b>	
Name	Sequence (5' to 3')	Purpose
Rare1-159F	CCCGAATTCCCACTATTGATCATTATGATTGT	159 aa F
Rare1-159/194R	CGAGTCGAGGTCAATCAAGAAGCTGTTCTCTTCT	159/194 aa R
Rare1-194F	5'CCCGAATTCCCACTATTGATCATTATGATTGT	194 aa F
Rare1_+100F	GGATCCATGTCGAGCACTTCTTCTCCGTCT	Δ 33 aa cTP
3XFLAG-StrepII F1	TAAAGATCATGACATCGATTACAAGGATGACGATGACAAGGTCGGCGCCGGTT	PCR1 F
3XFLAG-StrepII F2	CCCGGGGACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGATT	PCR2 F
3XFLAG-StrepII R	TGACAAGGTCGGCGCCGGTTGGTCTCATCCTCAATTTGAAAAATAAGAGCTC	PCR1/2 R
Rare1F	TCCATCAACTATGACGATTCTCACTGT	Full-length F
Rare1_+2259R	TCACCAGTAATCGTTGCAAGAACA	Full-length R
L5-Rare1_+2256R	ACCTCCACCAGATCCCCAGTAATCGTTGCAAGAAC	L5-3FS fusion
Rare1_-311F	GCCGCCATTTGAGAGGAGG	Native promoter
Rare1_+1933F	CACCATCCACAAACTCAGGAG	genotyping
At3g15000_-442F	GTCACACATTTTCACCAAATTGACC	genotyping
At3g15000_+99R	GGCGAGAGGAGCAGATGAAG	genotyping
FLAG_LB4	CGTGTGCCAGGTGCCACGGAATAGT	genotyping
At3g15000_+856F	GGTAGTTGCTTTGCTCGTCC	genotyping
At3g15000_+1334R	GGCCTCCTGCCATGTTCT	genotyping
FLAG_Tag3	CTGATACCAGACGTTGCCCGCATAA	genotyping
At3g15000_VF	ACCCCCACAGAACAACAA	Rip1-VIGS F
At3g15000_VR	AATCCCGTTTAATGCAGAA	Rip1-VIGS R
At3g15000_+169F	ATGGGCGGCCTTGTGTCTGTC	Δ 56 aa cTP
At3g15000_+1F	ATGGCGACGCATACCATTCTCG	Full-length F
At3g15000_+1188R	TTAACCTGGTAGGGGTTGCC	Full-length R
At3g15000_+1185R	ACCACCACCAGAACCCTGGTAGGGGTTGCCACT	RIP1-stop
cob-118	GCAATCTTAGTTATTGGTGGGGGTTCCGG	PPE
cob-325	TTGTGGTTTACCTTCATATTTTTTCGTGGTC	PPE
nad6-26	CAACCATCAAACCAGAGACCAAAGC	PPE
nad6-161	GGTCTCGACTTCTTCGCTATGATCTTCC	PPE
RARE1_+1956R	CTAGATCTCCTGAGTTTGTGGATGGTG	Y2H
RARE1_+1933F	CACCATCCACAAACTCAGGAGATC	Y2H
RARE1_+1674R	CTAGCTCATTGCATCAGGTTAAAAGG	Y2H
RARE1_+1651F	CCTTTTGAACCTGATGCAATGAGC	Y2H
RARE1_+1050R	CTACCATGAAAATATCGGGGTTGTC	Y2H
RARE1_+1368R	CTAGTTTGGCTCACGGATTTCTTGAAATGC	Y2H
At3g15000_+702R	CTATCTTCTCCTCTCAAAGTTTCTGCT	Y2H
At3g15000_+703F	TCTTCTCCTCTCAAAGTTTCTGCT	Y2H
RIP1-F1	ATGGCGACGCATACCATTCTCG	qRT-PCR
RIP1-R1	ACGCCGAGATTTGGCGAGAG	qRT-PCR
RIP1-F2	ACCGGCGAAATCTCTTTCGTTTCT	qRT-PCR
RIP1-R2	ACAAGGCCGCCACGGAAAAC	qRT-PCR
RIP1-F3	GCTTTTGGGGCACTTGTGTCAGAA	qRT-PCR
RIP1-R3	CAGCCTTCCCATCGATGAAAGGTT	qRT-PCR
RIP1-F4	GGAGCACCCCCACAGAACAACAA	qRT-PCR
RIP1-R4	GTAGGGGTTGCCACTGCCATCC	qRT-PCR
At2g28390-F	AACTCTATGCAGCATTGATCCACT	qRT-PCR
At2g28390-R	TGATTGCATATCTTTATCGCCATC	qRT-PCR
GFP-F	ATGGCCCTGTCCTTTTACCAGACA	qRT-PCR

GFP-R	TAATCCCAGCAGCTGTTACAAACTCAAG	qRT-PCR
atp1-F1	TCCCGCGGGAAAGGCTATGCT	qRT-PCR
atp1-R1	TCCCAGGGGCTTTCACTTCGACA	qRT-PCR
ccb203-F1	TCCGGATTGCTAGCTCCCGTTCAT	qRT-PCR
ccb203-R1	CTTCGCGCCACAACCATCTCTTTT	qRT-PCR
ccb206-F1	GATTCGGATCCCTCCGTTGTTTC	qRT-PCR
ccb206-R1	GAATAACCCGGTGACCCACCAA	qRT-PCR
cob-F1	TGGGGGTTTCGGTCCGTTAGCT	qRT-PCR
cob-R1	GCAACCAGCCCCCTTCAACATC	qRT-PCR
cox2-F1	TACCCCGTCCCCTGCGCAATAGT	qRT-PCR
cox2-R1	AGTGGCGCCTAGCCGTTGAGAGC	qRT-PCR
cox3-F1	GTGGCGCGATGTTCTACGTGAAT	qRT-PCR
cox3-R1	TCTACCGCAGGTGCCAAAGAAGA	qRT-PCR
nad4-F1	TTTCGCCGTCAAAGTGCCTATG	qRT-PCR
nad4-R1	CGCTTCGGGAAACATGGGTATT	qRT-PCR
nad6-F1	TCGCGACACTTCAGGTTTACTTC	qRT-PCR
nad6-R1	TCTTCGTGAATCTCCGCTATTTG	qRT-PCR
nad7-F1	CCGGCAACCGTATCTGGAAACA	qRT-PCR
nad7-R1	TTCGCGAATCCCAGCATAACC	qRT-PCR
nad9-F1	TGCGGAGTTGATCATCCCTCTCGA	qRT-PCR
nad9-R1	CCGGCCGGCTGATGGAAATAGA	qRT-PCR
ndhD-F1	CAACATCTCCCGGTCAACGTAATT	qRT-PCR
ndhD-R1	CAGCGCCAATAAATCCATGAGAA	qRT-PCR
petL-F1	AAAAAAACATATTTTATTGAGTCCCTTCATG	qRT-PCR
petL-R1	GACCAATAAACAGAACTGAGGTTATAG	qRT-PCR
rpoC1-F1	GGGCGGGTGCTATCCGAGAAC	qRT-PCR
rpoC1-R1	TCCCGTAGGCCCTTCTTCTCC	qRT-PCR

## References to Supplementary Data

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