Supplemental Data Flores-Pérez et al. (2008) A mutant impaired in the production of plastome-encoded proteins uncovers a mechanism for the homeostasis of isoprenoid biosynthetic enzymes in Arabidopsis plastids.

SUPPLEMENTAL FIGURES

Figure S1. Quantification of FSM resistance of *rif1* seedlings.

Wild-type (Col) and homozygous *rif1* plants were germinated on MS plates supplemented with the indicated concentrations of fosmidomycin (FSM) and grown under LD conditions. **(A)** Quantification of pigment levels. Chlorophylls (CHL) and carotenoids (CRT) were extracted from 5-day-old seedlings and quantified by HPLC as described (Rodríguez-Concepción *et al*. 2004). The mean and standard deviation values from at least two independent experiments with two replicas each are represented. **(B)** Quantification of seedling establishment rates. The percentage of 15-day-old seedlings that developed green true leaves (seedling establishment) in the presence of FSM was calculated relative to the value observed on plates without the inhibitor (which was considered as 100%). Values represent the mean and standard deviation from populations of more than 50 individuals in at least two independent experiments.

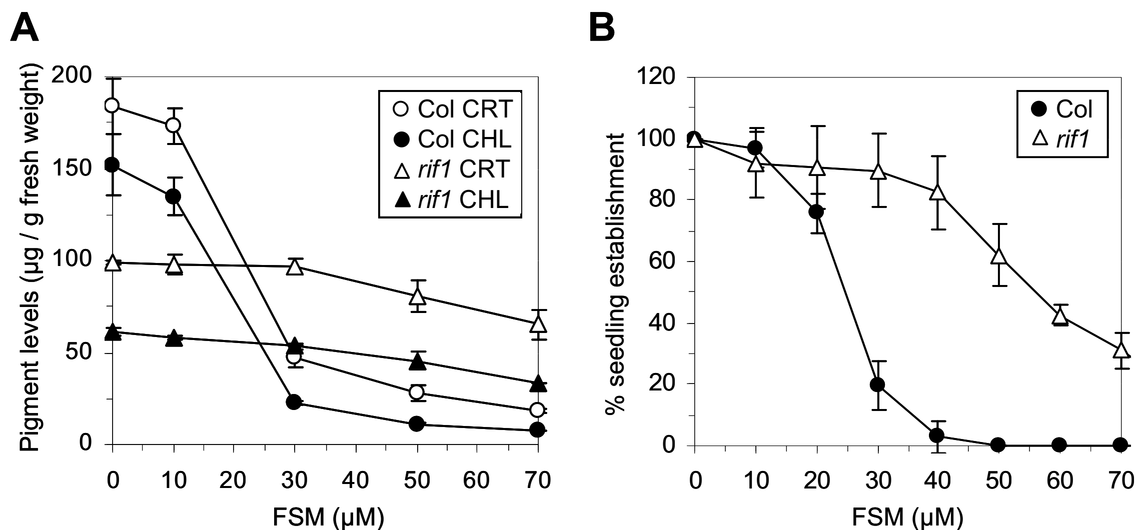


Figure S2. Multiple alignment of the *Bacillus subtilis* YqeH protein with the Arabidopsis closest homologues.

The N-terminal sequence of RIF1/NOS1/NOA1 is predicted to target the protein to mitochondria (TargetP, Predotar) or plastids (Psort, ChloroP) according to the indicated algorithms available at www.expasy.org. The plastid targeting peptide predicted with ChloroP is boxed. The GTP-binding domain is underlined in black and a putative Zn-binding domain (CxxCx[26-34]CxxC) is underlined in gray.

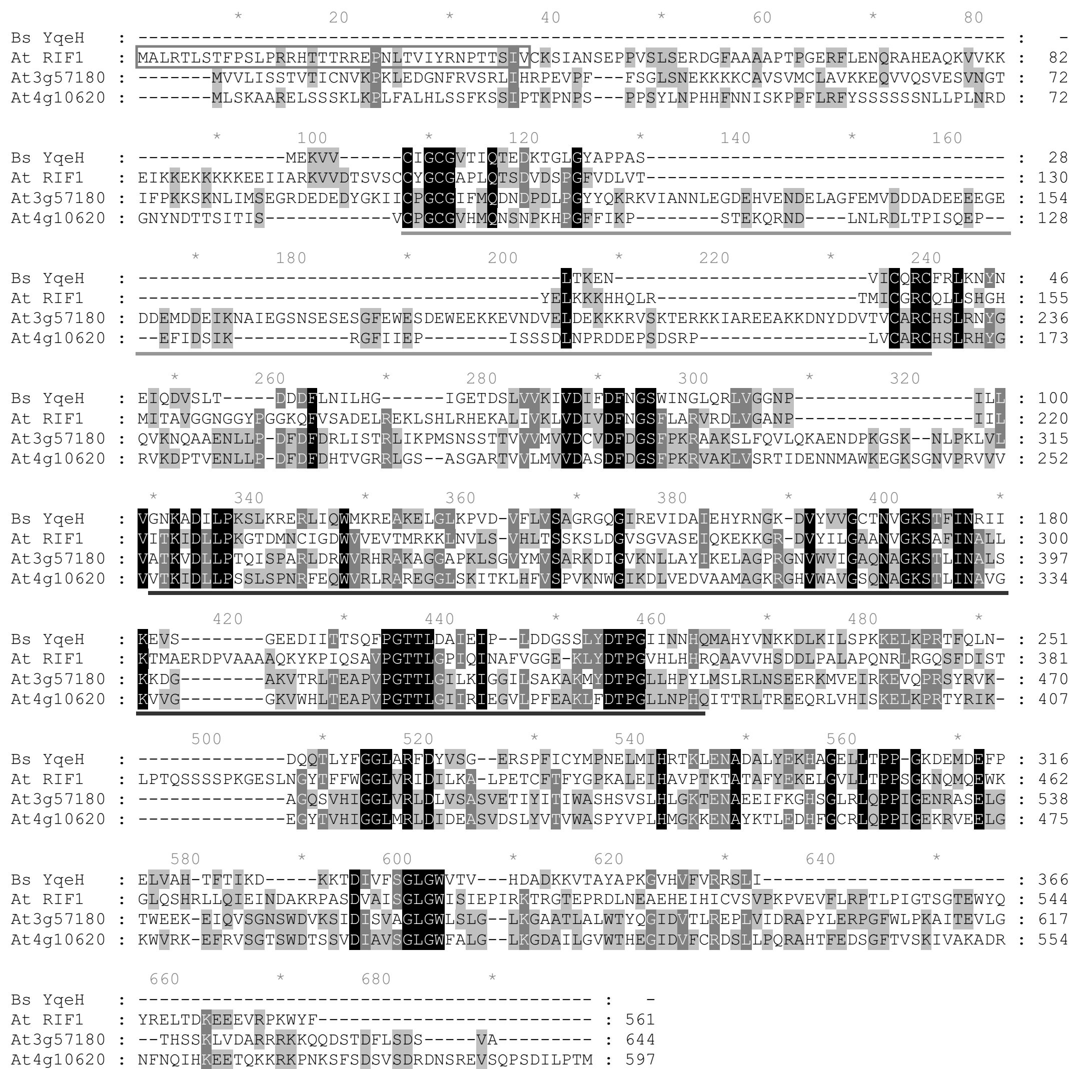


Figure S3. Phenotype of *rif1* plants constitutively expressing a plastid-targeted bacterial YqeH protein fused to GFP (PYG).

(A) Phenotype of representative seedlings of the indicated genotypes grown under LD for 5 days on MS plates supplemented (+) or not (-) with 50 µM FSM. Chlorophyll autofluorescence (in red) of is also shown (right panels). All panels are to the same scale. **(B)** Young leaves from transgenic *rif1-1 P35S:PYG* plants were used for confocal microscopy to detect green fluorescence of the recombinant PYG protein (upper panel). Red autofluorescence of chlorophyll in the same area of the leaf is shown (central panel). Overlapping green and red fluorescence is also shown in yellow (lower panel). All panels in each section are to the same scale.

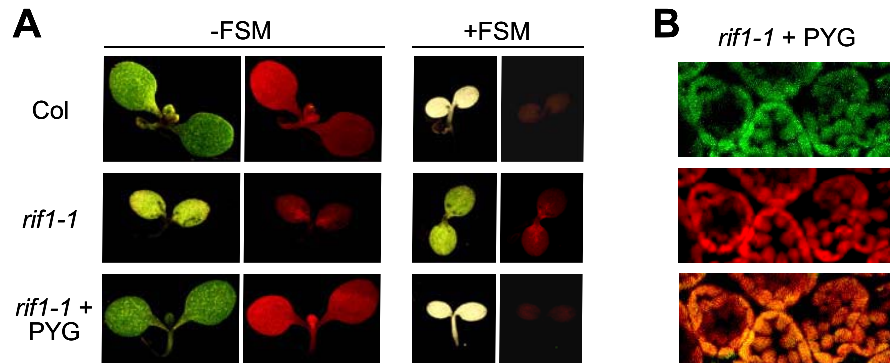
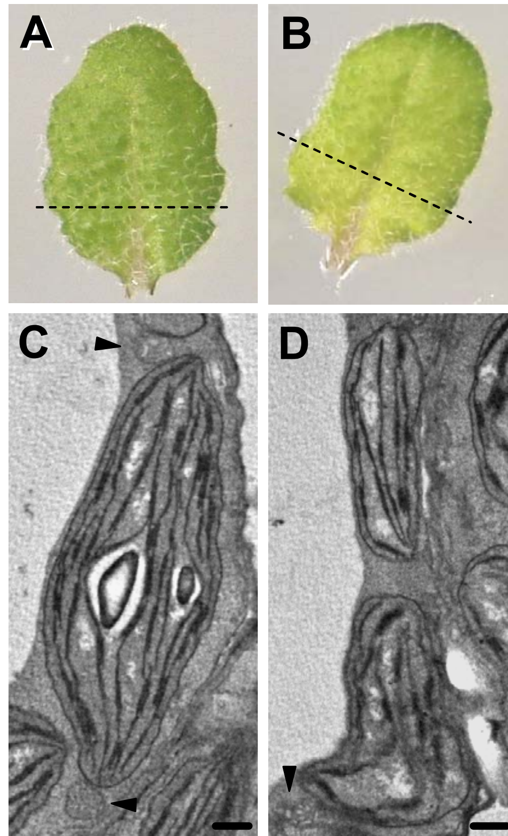


Figure S4. Ultrastructure of chloroplasts in wild-type and *rif1* leaves.

Young leaves were collected from the inner whorls of the rosette of soil-grown Col (**A**) or *rif1-1* (**B**) plants like those shown in Figure 2. Dashed lines mark the area in the basal part of the leaves used for transmission electron microscopy. Representative mesophyll chloroplasts from Col (**C**) and *rif1-1* (**D**) leaves are shown. Mitochondria are indicated with arrowheads. Bars correspond to 1 µm.



SUPPLEMENTAL METHODS

Constructs for the production of transgenic lines

A cDNA population was obtained from wild-type 10-day-old seedling RNA with Superscript reverse transcriptase (GibcoBRL) and used to amplify the sequence encoding the full-length Arabidopsis RIF1 protein by PCR with Pfu DNA polymerase (Promega) and primers RIF1-2F-NcoI (5'- T C T C A G C *C* A T G G C G C T A C G A A C -3'; the original A nucleotide in the position indicated in italics was mutated to C to introduce a *Nco*I site containing the translation start codon) and RIF1-1R-SalI (5'- A A G A A G G T C *G A C* A A A G T A C C A T T T G G –3'; the positions of the original stop codon, indicated in italics, were substituted to create a *Sal*I site). The amplified 1.7 kb product was digested with *Nco*I and *Sal*I and cloned into a modified version of the pGFP-MRC plasmid (Rodríguez-Concepción et al., 1999) in which a *Sal*I site had been added next to the the *Nco*I site. The generated construct, which was sequenced to confirm its integrity, encoded a chimeric protein (RIF1-GFP) with the full-length RIF1 polypeptide fused in frame to the N-terminus of a synthetic green fluorescent protein (GFP). The cDNA encoding RIF1-GFP was then recovered after *Nco*I and *Sma*I digestion and cloned into the *Nco*I and *Pml*I sites of the pCAMBIA-1303 vector under the transcriptional control of the cauliflower mosaic virus *35S* promoter. The resulting plasmid was named pP35S:RIF1-GFP.

The gene encoding YqeH was PCR-amplified from *Bacillus subtilis* colonies using Pfu DNA polymerase and primers YqeH-5F-SpeI (5`- T A G T A T G G A A A A G G T T G T T T G T A T C G -3'; the translation start codon is underlined) and YqeH-3R-XbaI (5'- C C C C T T T C T *A G* A A A T T A A T G A A C G C -3'; the positions of the original stop codon, indicated in italics, were substituted to create a *Xba*I site). The *ca.* 1.2 kb product was used as a template for nested PCR with Pfu DNA polymerase and primers YqeH-5F-NcoI (5'- G T C C A T G G A G A C T A G T A T G G A A A A G G -3'; introduced *Nco*I and *Spe*I sites are underlined) and YqeH-3R-XbaI. After digestion with *Nco*I and *Xba*I, the resulting sequence was cloned into the *Nco*I and *Spe*I sites of pCAMBIA-1302 under the transcriptional control of *35S* promoter to generate plasmid pP35S:YG, in which the full-length YqeH protein was fused in frame to the N-terminus of GFP. A cDNA encoding the plastid-targeting sequence of the Arabidopsis HDS/GCPE protein (Querol et al., 2002) was then amplified with primers HDS-5F-NcoI (5'- A G A A *C C* A T G G C G A C T G G A G T A T T G C -3'; the nucleotides in italics were mutated to introduce a *Nco*I site containing the translation start codon) and HDS-5R-XbaI (5'- G C C T T G *T C* T *A* G A A T T C C G G A T A A C C –3'; the positions in italics were substituted to create a *Xba*I site), digested with *Nco*I and *Xba*I,

and cloned into pCAMB-YG previously digested with *Nco*I and *Spe*I. The resulting plasmid was named pP35S:PYG.

Chloroplast isolation.

Chloroplast isolation was performed as described (Kubis et al., 2007). A linear Percoll gradient was used to recover intact chloroplasts (lower band in the gradient), which were washed and resuspended in HMS buffer (50 mM HEPES-NaOH pH 8.0, 3 mM $MgSO_4$, 0.3 M sorbitol). Chloroplast yield was then calculated using a haemocytometer. About 10^7 chloroplasts were used for import assays in a 150 µL mix with HMS buffer containing 20 mM gluconic acid, 10 mM $NaHCO_3$, 0.2% (w/v) bovine serum albumin, 5 mM Mg-ATP, 10 mM methionine, and 15 µL of the transcription/translation mixture (10% of reaction volume). Import was performed in white light at 25°C for 10 minutes. Thermolysin treatment and detection of imported polypeptides was carried out as described (Aronsson and Jarvis, 2002).

Microscopy.

Chlorophyll autofluorescence in whole seedlings was observed with a Leica MZ FLIII fluorescence stereomicroscope equipped with a 546/10 nm excitation filter and a 590 nm barrier filter and recorded using a Leica DC250 digital camera with the IM1000 1.10 software. Subcellular localization of the GFP fusion proteins in cells of transgenic plants was observed by direct examination of the tissue samples with a Leica TCS 4D Confocal Laser Scanning Microscope (CSLM). Green fluorescence corresponding to the fusion proteins was detected using a BP515-525 filter after excitation with blue light at 488 nm. Red autofluorescence from chlorophyll was detected using a LP590 filter after excitation with green light at 568 nm.

For transmission electron microscopy (TEM), samples from cotyledons of light-grown seedlings were collected after germination of seeds on MS plates and incubation for three days under LD conditions. In the case of etiolated seedlings, plates were kept for three days in the dark following stratification and a one-hour pulse of white light to stimulate germination. Cotyledon samples were fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in 100 mM sodium cacodylate buffer (pH 7.4) for 2 hours at room temperature and kept at 4ºC overnight. Following four washes for 10 min at 4ºC in cacodylate buffer, samples were post-fixated in 1 % buffered OsO_4 for 1 h,

rinsed in water, dehydrated in a gradient of acetone, and embedded in Spurr's resin. After microtomy, ultrathin sections were stained with 2% uranyl acetate for 30 min followed by 2.6% lead citrate for 10 min. Specimens were observed with a Hitachi H800 MT transmission electron microscope and images were recorded using the Digital Micrograph 3.3.0 (Gatan Ltd.) software.