Supplemental Data. Hammani et al., (2009). A study of new *Arabidopsis* chloroplast RNA editing mutants reveals general features of editing factors and their target sites.



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otp81















otp84





otp84



otp85, otp86





otp85, otp86

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otp85, otp86





**Supplemental Figure 1.** High resolution melting screen of *otp80*, *otp81*, *otp84*, *otp85* and *otp86* mutants.

Real-time PCR was done using primers surrounding the 34 known editing sites of Arabidopsis in the presence of a fluorescent double-strand-specific dye. At the end of the amplification, amplicons were denatured, renatured and then melted using precise incremental increases in temperature. Melting of DNA duplexes releases the dye, causing a decrease in fluorescence. The presence of less thermostable heteroduplexes in a sample alters the shape of the melting curves. For each editing site, the melting curve of a control consisting of genomic DNA (g) from the wt sample was compared to the melting curves of a mix of genomic DNA and cDNA (gc) from the mutant lines. Editing produces cDNA with a nucleotide change and thus the mix of genomic DNA and cDNA exhibits a different melting curve from that of genomic DNA alone. If an editing site is unedited in the mutant, then the melting curves from the genomic DNA sample and the mixed genomic/cDNA sample are identical.



**Supplemental Figure 2**. Analysis of subcellular localization of the OTP80 (A), OTP81 (B), OTP84 (C), OTP85 (D) and OTP86 (E) proteins.

Chimeric proteins consisting of the first 100 amino acids of OTP80, OTP81, OTP84, OTP86 or the first 72 amino acids of OTP85 fused to GFP were targeted to chloroplasts in transformed Arabidopsis cells. Fluorescence of the green fluorescent protein (GFP construct, shown in green), fluorescence of the red fluorescent protein in fusion with the small subunit of the ribulose biphosphate carboxylase (SSU-RFP, shown in magenta), and the overlay of the two fluorescence images are shown (merged).



**Supplemental Figure 3.** Phenotype of *otp80*, *otp81*, *otp84*, *otp85*, *otp86* and wild type (WT) *Arabidopsis* plants.

Three- (A) and seven- (B) week-old plants were grown under medium day conditions.



**Supplemental Figure 4.** *In vivo* analysis of electron transport activity using light intensity dependence of ETR (electron transport rate). ETR is plotted relative to  $\phi_{PSII} \times$  light intensity (µmol photons m<sup>-2</sup> s<sup>-1</sup>), where  $\phi_{PSII}$  is quantum yield of photosystem II. Light intensity dependence of NPQ (non-photochemical quenching) of chlorophyll fluorescence. All values represent the mean ± SD (n=3).

Chlorophyll fluorescence was measured with a MINI-PAM portable chlorophyll fluorometer (Walz, Effeltrich, Germany) in ambient air at room temperature (25°C). Minimum fluorescence at open PSII centers in the dark-adapted state (Fo) was excited by a weak measuring light (wavelength 650 nm) at a PFD of 0.05-0.1 µmol m<sup>-2</sup> s<sup>-1</sup>. A saturating pulse of white light (800 ms, 3,000 µmol photons m<sup>-2</sup> s<sup>-1</sup>) was applied to determine the maximum fluorescence at closed PSII centers in the dark-adapted state (Fm) and during AL illumination (Fm'). The steady state fluorescence level (Fs) was recorded during AL illumination (15-1,000 µmol photons m<sup>-2</sup> s<sup>-1</sup>). These photosynthetic parameters were determined 2 min after the change of AL intensity. NPQ was calculated as (Fm-Fm')/Fm'. The quantum yield of PSII (PSII) was calculated as (Fm'-Fs)/Fm' (Genty et al., 1989). ETR was calculated as PSIIxPFD. The transient increase in chlorophyll fluorescence after AL had been turned off was monitored as described (Shikanai et al., 1998).