Supplementary Figure legends

Figure S1. The RNA helicase RH33 affects the processing of various protein-encoding transcripts in Arabidopsis mitochondria.
(A) Scheme of the RNA Helicase 33 (RH33), encoded by the At2g07750 gene locus. Black boxes represent exons, lines indicate to intron regions, while grey boxes show the 5′ and 3′ UTRs. The different T-DNA insertions are indicated above the gene structure as triangles.
(B) Growth and developmental phenotypes associated with the *rh33* mutants. Seeds of wild type (Col-0) and the three T-DNA insertional lines, *rh33-1* (Sail_604_A01), *rh33-2* (Salk_119034), and *rh33-3* (Salk_119725), were germinated on MS-agar plates supplemented with 1% sucrose. The picture shows 3-week-old seedlings grown under optimal growth conditions (i.e., short-day conditions, 10:14 light to dark, with a light intensity of ~150 $\mu E \cdot m^{-2} \cdot s^{-1}$, with 50% relative humidity (RH), under either 22°C (optimal condition) or 28°C (restrictive condition).
(C) Transcript abundance in *rh33* mutants. Real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR) analyses of mitochondrial genes. Total RNA was extracted from 3-week-old seedlings of Col-0 and mutant plants were reverse-transcribed, and the relative steady-state levels of cDNAs corresponding to the different organellar transcripts were evaluated by qPCR with primers that specifically amplified mtRNAs. Specific oligonucleotides used in the RT-qPCRs are indicated in Ref's [1, 2].

Figure S2. Analysis of the splicing efficiencies of mitochondrial group II-type introns in *rh33-1* mutant.
The splicing efficiencies of the 23 group-II introns in Arabidopsis wild type (Col-0) and *rh33-1* mutant plants were evaluated by RT-qPCR, with RNA extracted from *3*-week-old wild-type (Col-0) and *rh33-1* mutant plants. The splicing efficiencies were estimated from the relative accumulation (pre-RNA/mRNA ratio's) of organellar transcripts in the mutant line versus the wild type plants by RT-qPCR with specific oligonucleotides designed to intron-exon regions (pre-RNAs) and exon-exon (mRNAs). Specific oligonucleotides used in the RT-qPCRs are indicated in Ref's [1, 2].

References

1. Best, C.; Mizrahi, R.; Edris, R.; Tang, H.; Zer, H.; Colas des Francs-Small, C.; Finkel, O. M.; Zhu, H.; Small, I. D.; Ostersetzer-Biran, O., MSP1 encodes an essential RNA-binding pentatricopeptide repeat factor required for nad1 maturation and complex I biogenesis in Arabidopsis mitochondria. *New Phytol* **2023,** 238, (6), 2375-2392.
2. Shevtsov-Tal, S.; Best, C.; Matan, R.; Chandran, S. A.; Brown, G. G.; Ostersetzer-Biran, O., nMAT3 is an essential maturase splicing factor required for holo-complex I biogenesis and embryo development in *Arabidopsis thaliana* plants. *Plant J* **2021,** 106, (4), 1128-1147.