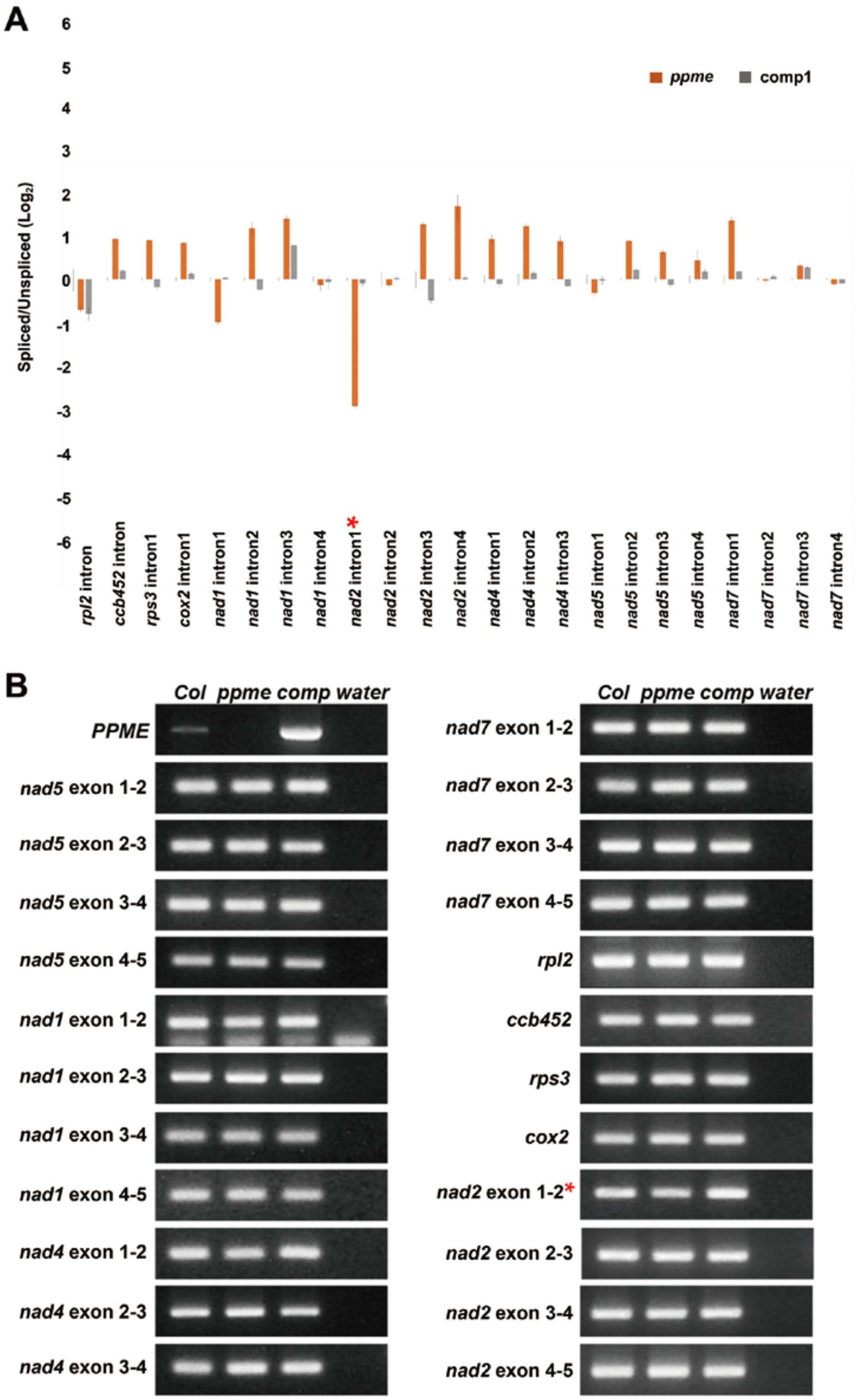
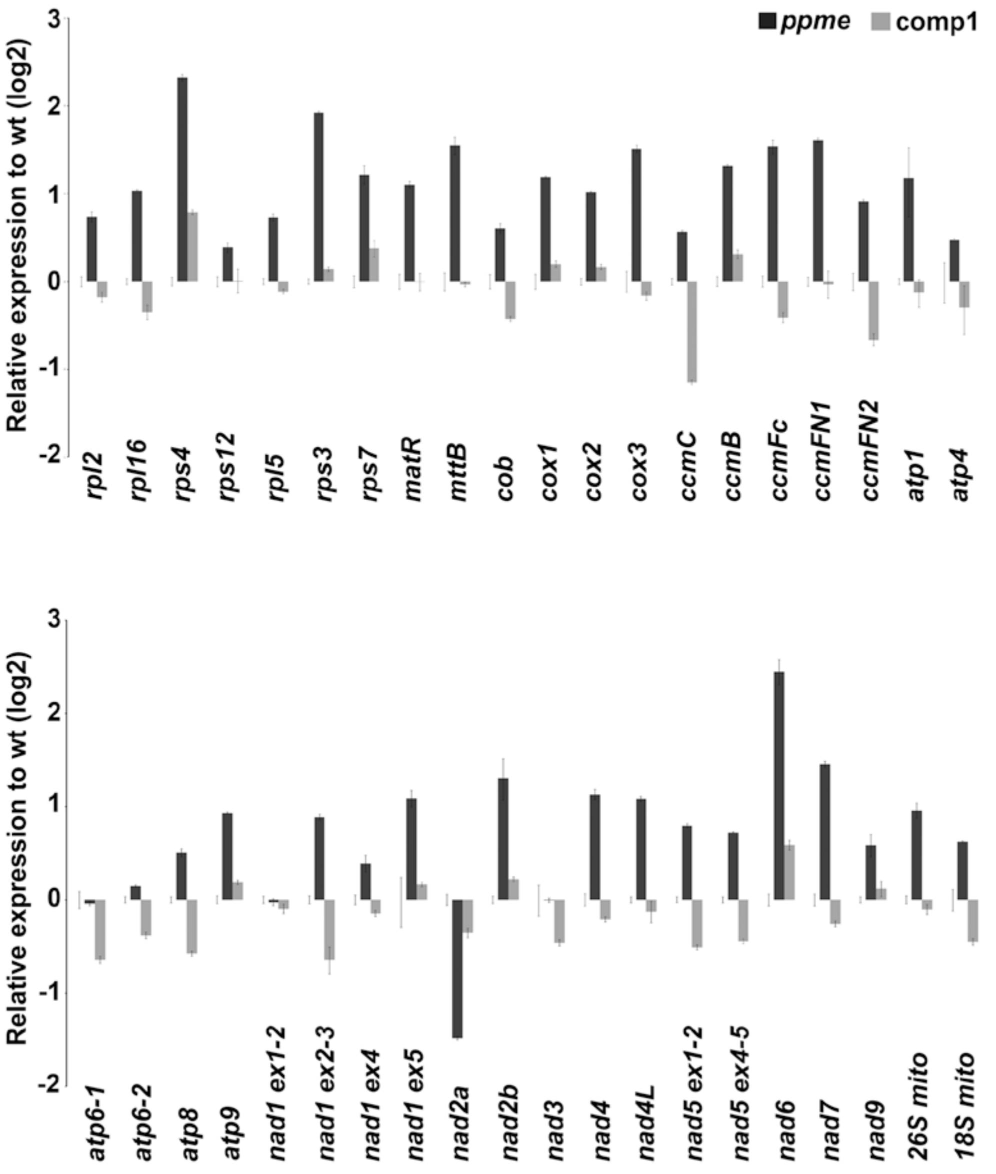
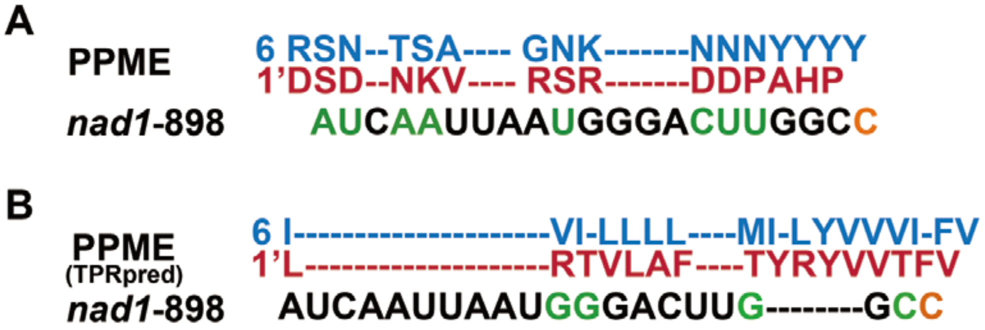


Supplemental Figure 1. Comparison of mitochondrial splicing efficiency.
(A) The splicing efficiency of each intron in whole mitochondrial intron containing genes. The transcript levels of exon-exon (Spliced) and intron-exon (Unspliced) of each intron from 14-day-old wild-type, homozygous *ppme*-/-, and complementation line seedlings were obtained by qRT-PCR. The ratio of the spliced to unspliced transcripts indicates the splicing efficiency of each intron. The red asterisk indicates that only *nad2* intron 1 displayed obviously lower splicing efficiency.
(B) Detection of individual mitochondrial joined exons after splicing. The primer pairs were designed in the exon near each exon-intron junction, and spliced products were detected by RT-PCR. The orange asterisk indicates the accumulation of spliced *nad2 exon 1-2* after removing *nad2 intron1*, even though the splicing efficiency was lower.



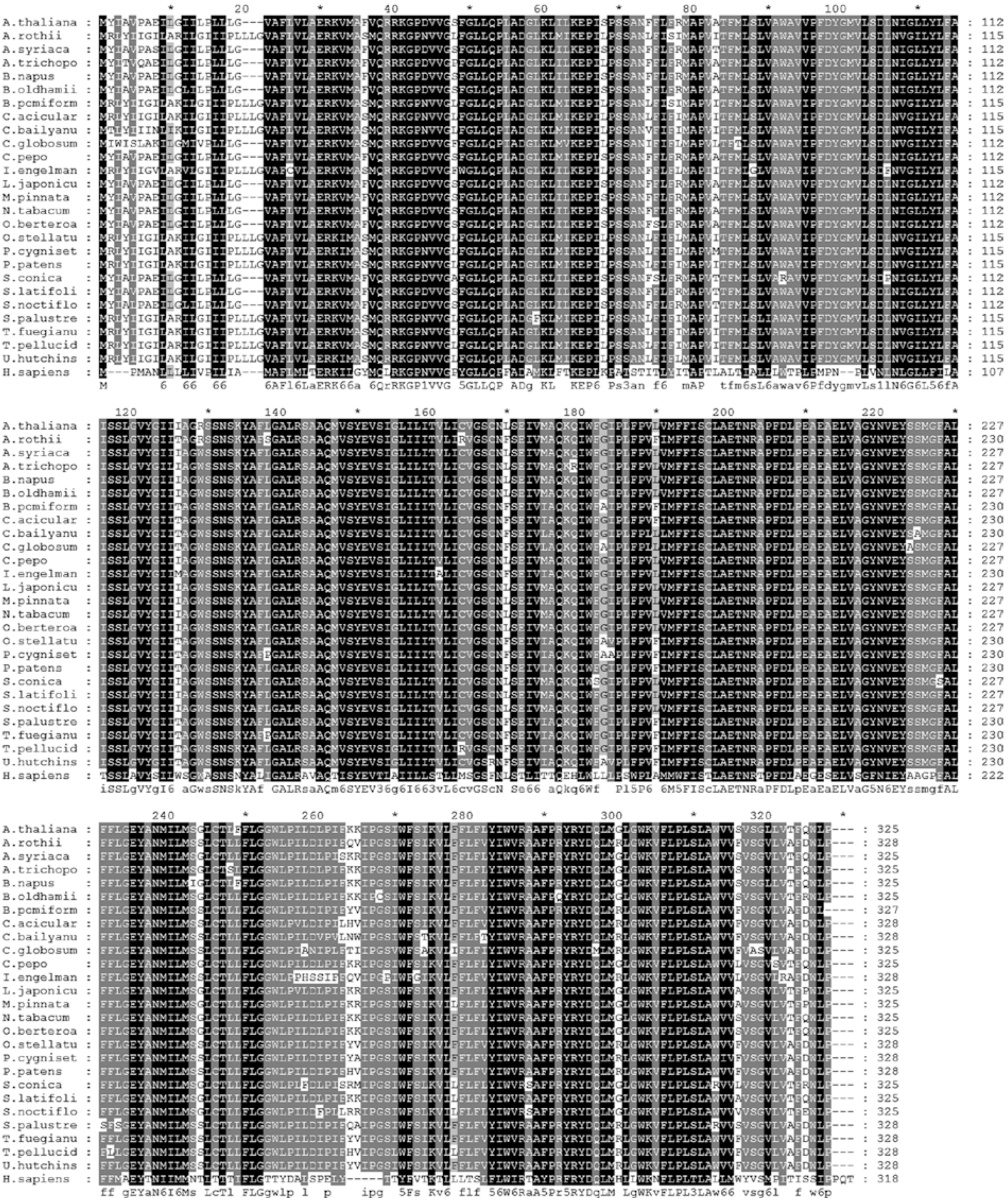
Supplemental Figure 2. The transcript abundance of whole mitochondrial genes.

The total RNAs extracted from 14-day-old wild-type, homozygous *ppme*-/- and 35S::*PPME-GFP*/*ppme* complementation line seedlings were used for qRT-PCR to monitor the expression levels of individual mitochondrial genes.



Supplemental Figure 3: Predicated PPR combination code between PPME and *nad1*-898.

(A) Analysis of the putative PPR combination code between PPME and *nad1-898*. The combination of the sixth position (6) of each PPR motif and the first position (1') of the following PPR motifs was extracted from 16 PPR motifs of PPME, which was then matched to the -20 upstream region in *nad1*-898. The black labeled nucleotide reveals a mismatched conserved code, and the green labeled nucleotide shows a matched code of the PPR combination between the 6-1' pair for this nucleotide located upstream of *nad1*-898. (B) The 17 PPR motifs predicted by the TPRpred tool showed much lower conservation between PPME and *nad1*-898 compared to (A).



Supplemental Figure 4. Protein alignment mitochondrial NAD1 among different species.

NAD1 is highly conserved among all tested species. Protein sequence alignment of Arabidopsis mitochondrial NAD1 and its homologs in different species was generated using CLUSTALW and GeneDoc software. The black, gray, and white regions indicate identical, highly conserved, and lack of conserved amino acids, respectively.

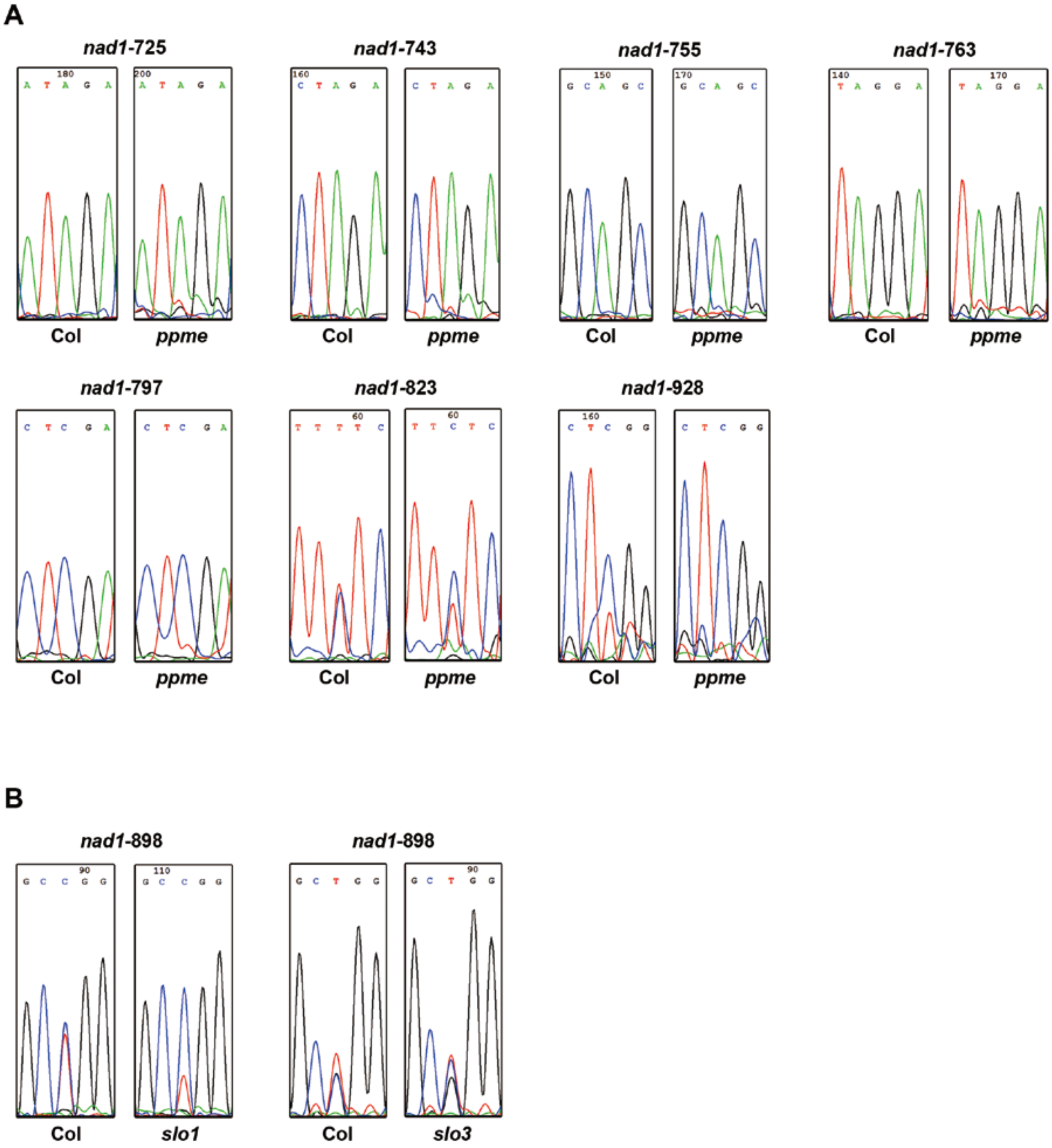


Figure S5. RNA editing efficiency of the nad1 editing sites in *ppme* and *slo* mutants.

(A) The raw sequence data for each editing site near the *nad1*-898 editing site. The middle C/T and G/A nucleotides of each panel indicate the editing efficiency of each editing site.
(B) RNA editing efficiency of *nad1*-898 in the *slo1* and *slo3* mutants.