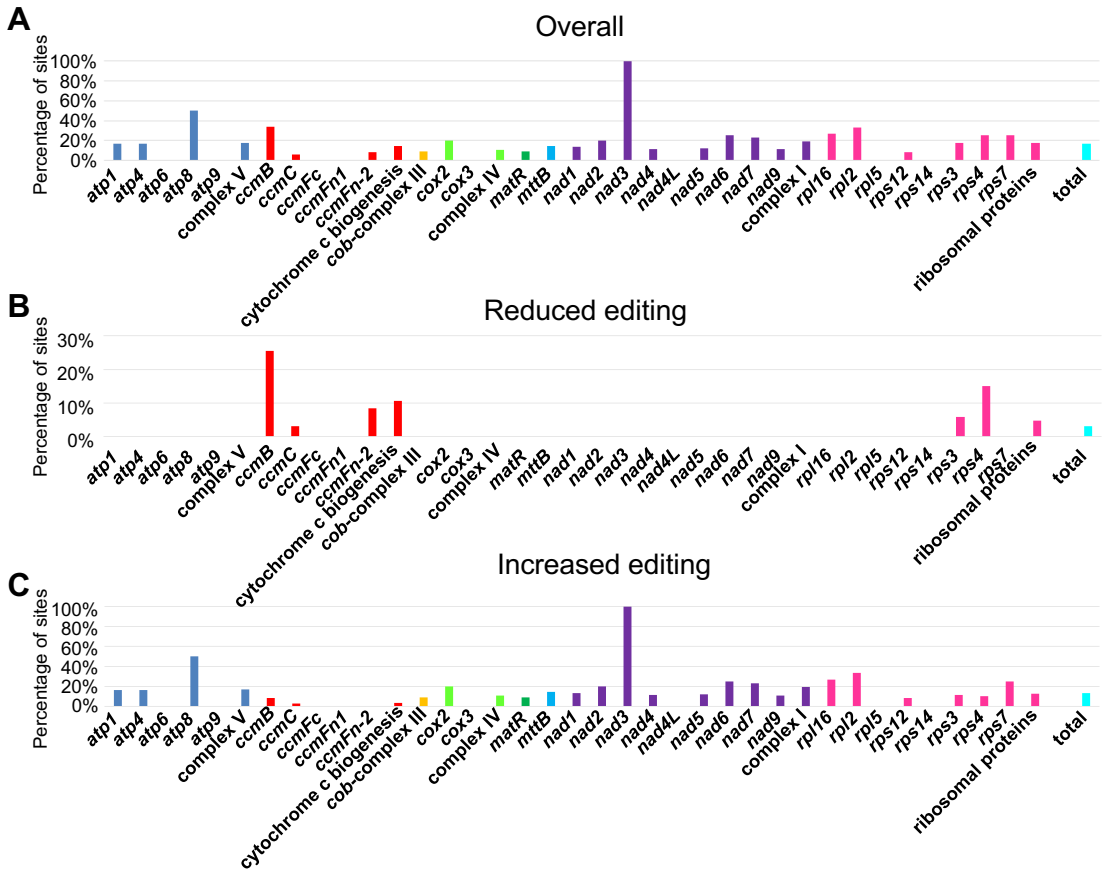
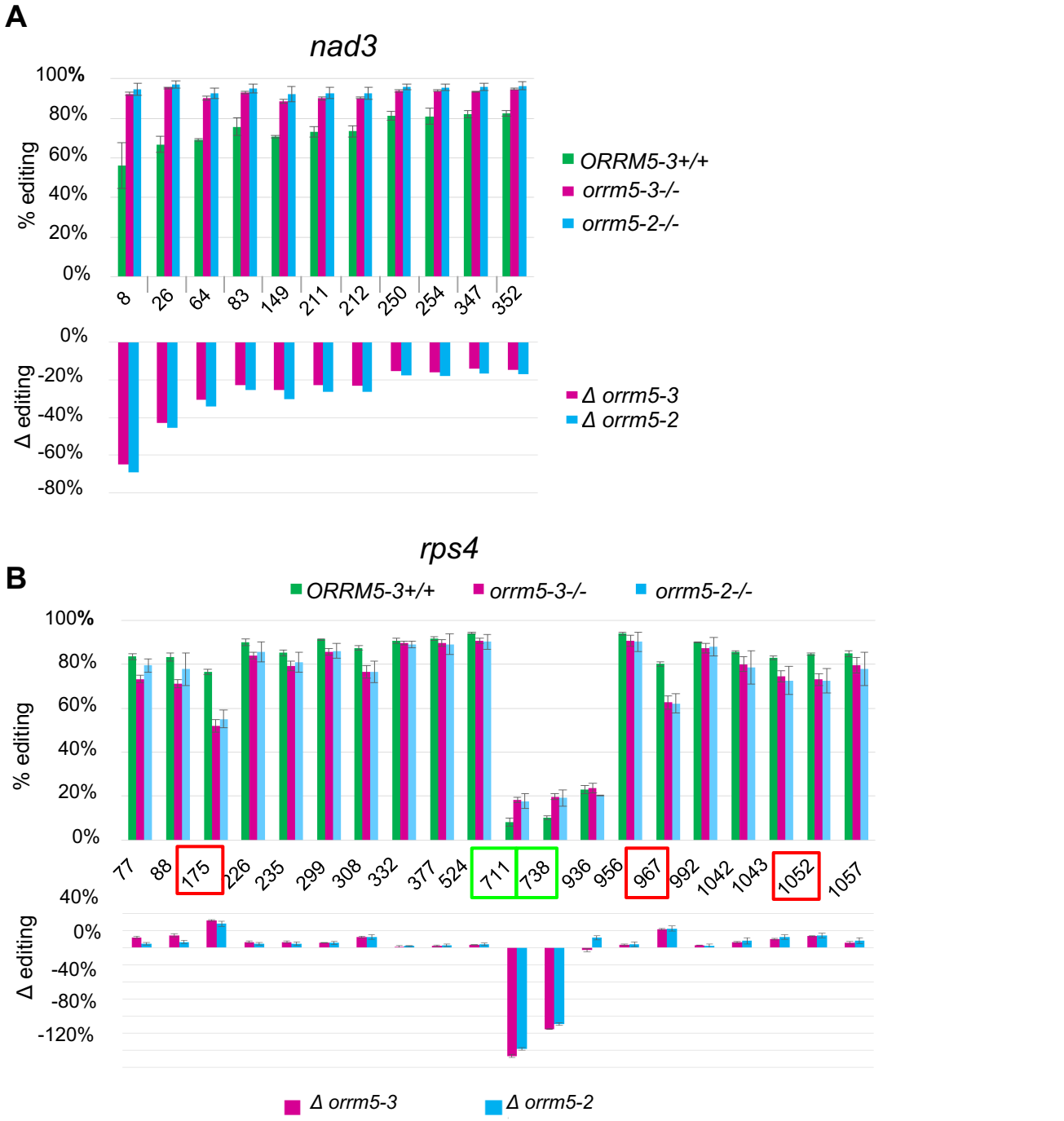
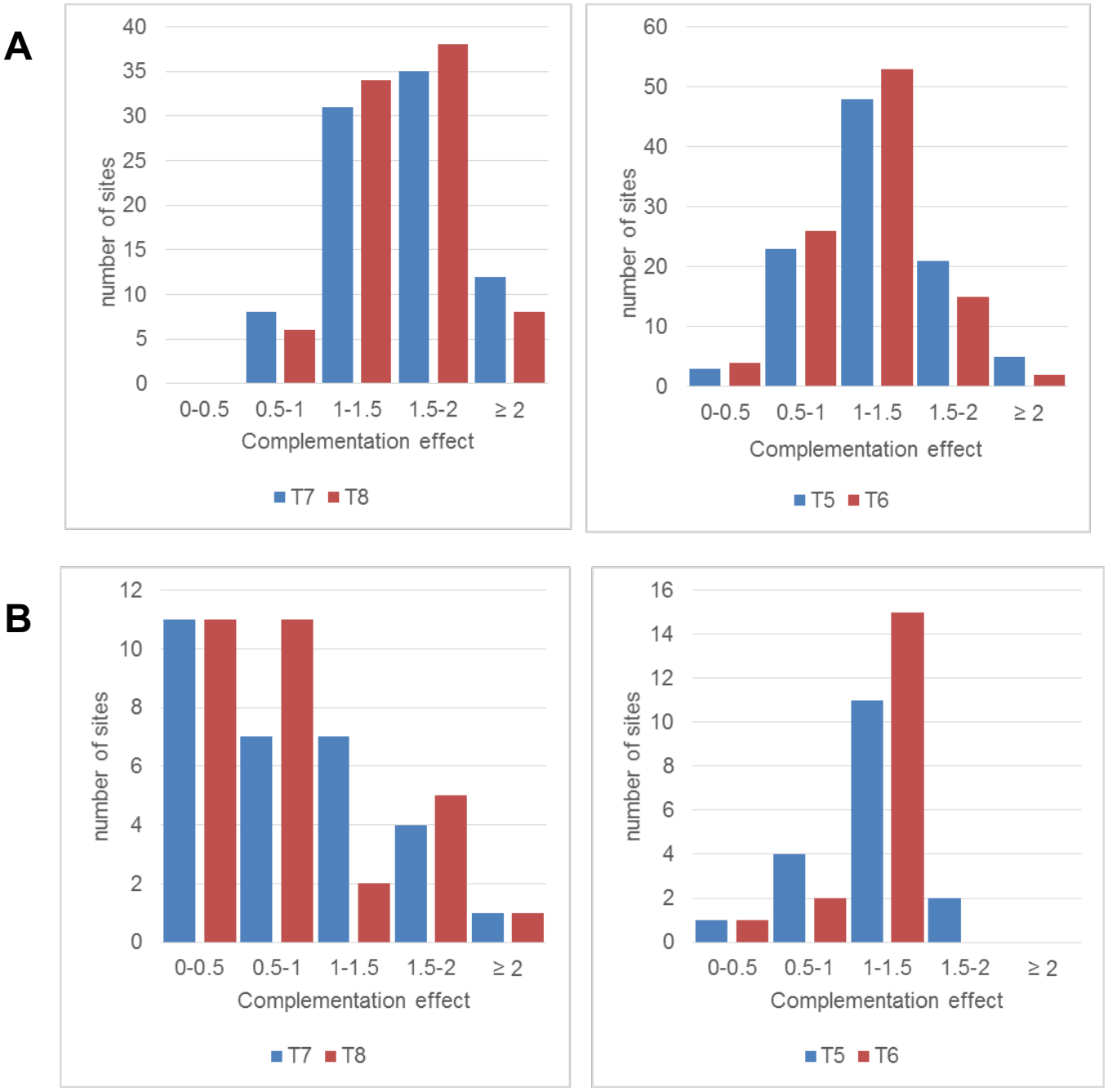


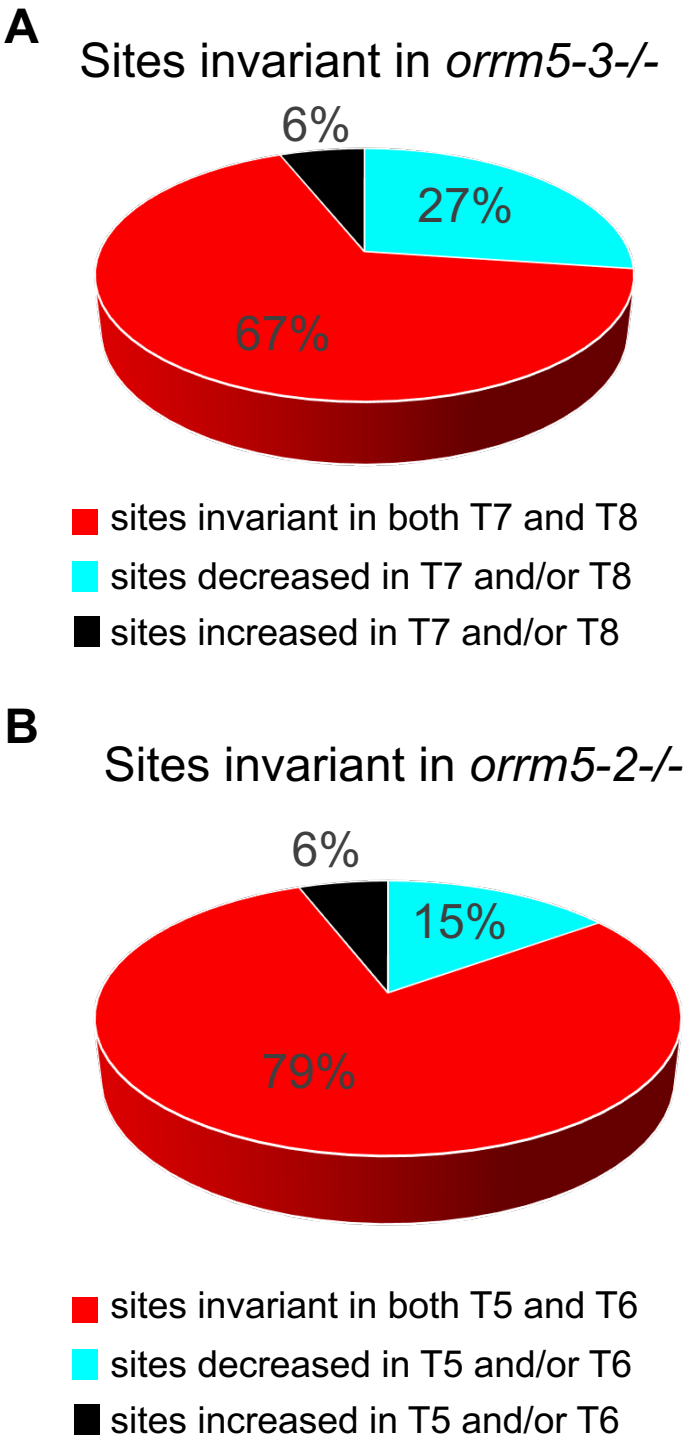

**Supplementary Figure S1.** Percentage of affected edited sites/transcript in the *orrm5* mutants. **(A)** Percentage of sites affected by *ORRM5* mutations on each transcript. Each bar represents a transcript color-coded according to the complex to which it belongs. **(B)** Percentage of sites experiencing reduced editing extents by *ORRM5* mutations on each transcript. **(C)** Percentage of sites showing increased editing efficiency on each transcript as a result of *orrm5* mutations.



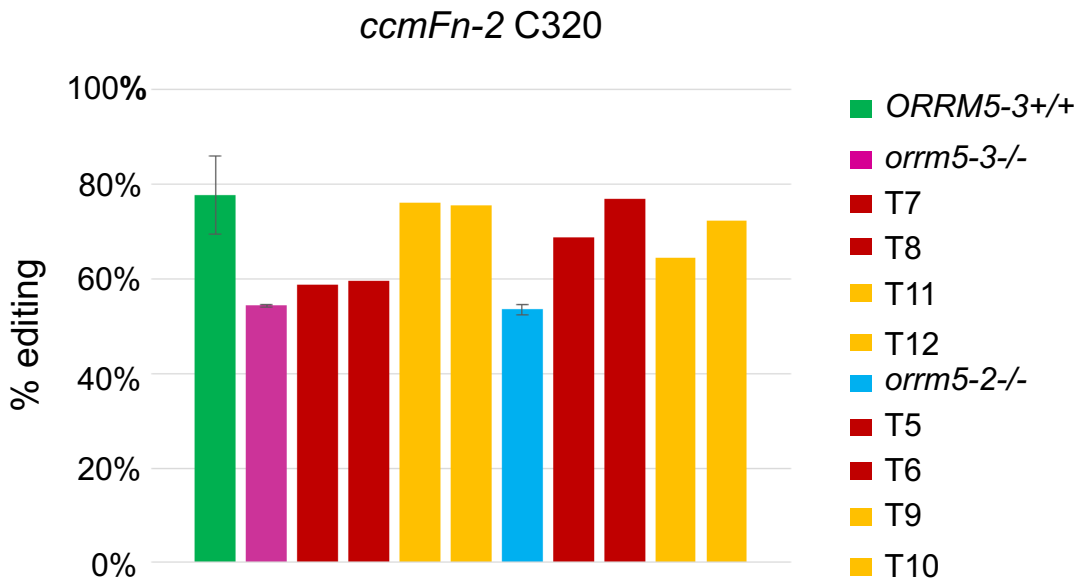

**Supplementary Figure S2.** Editing on the *nad3* and *rps4* transcripts is affected by *ORRM5* mutation. *ORRM5-3*+/+, wild-type siblings of *orrm5-3* mutants; *orrm5-3* -/-, *orrm5-3* homozygous mutants; *orrm5-2*-/-, *orrm5-2* homozygous mutants (n=2). **(A)** *ORRM5* mutations result in increased editing extent at all the mitochondrial C targets on the *nad3* transcript. X-axis represents position of different editing sites on the *nad3* transcript. The lower panel represents the variation in editing extent (Δ editing); Δ*orrm5-2*, (% editing of *ORRM5-3*+/+ minus % editing of *orrm5-2*-/-)/ % editing of *ORRM5-3*+/+; Δ*orrm5-3*, (% editing of *ORRM5-3*+/+ minus % editing of *orrm5-3*-/-)/ % editing of *ORRM5-3*+/+. **(B)** Effects of *ORRM5* mutations on mitochondrial RNA editing on the *rps4* transcript. Like in Figure 4A, the upper panel represents editing extent at different sites on the transcript with the location on the X axis. Sites experiencing a significant reduction of editing extents (Δ ≥ 10%, P < 1.6e-6) are in red squares, whereas sites showing an increase of editing extents are in green squares (Δ ≥ 10%, P < 1.6e-6). The lower panel represents the variation in editing extent (Δ editing) with Δ*orrm5-2* and Δ*orrm5-3* calculated as in Figure 4A.



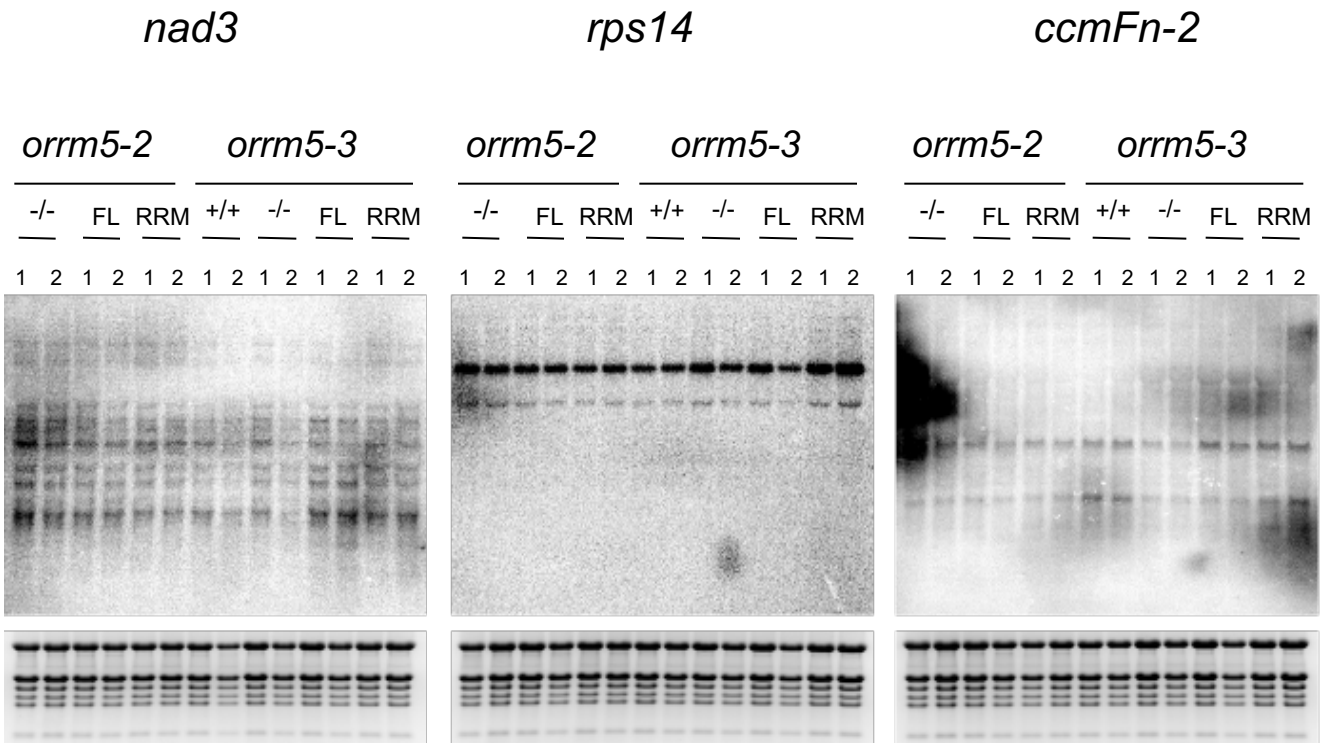

**Supplementary Figure S3.** Distribution of the editing sites that showed a significant change of editing extent in the mutant lines (vs. the wild-type) in the transgenic lines transformed with the full length *ORRM5* according to the complementation effect. The complementation effect is calculated as (% editing in transgenic-% editing in mutant)/(% editing in wild-type-% editing in mutant). The complementation effect is a measure of the response observed in the transgenic lines in relation to the one observed in the wild-type; when this metric equals 1 the response in the transgenic is similar to the one observed in the wild-type. **(A)** Distribution for the sites that were significantly increased in the mutant plants**. (B)** Distribution for the sites that were significantly decreased in the mutant plants. T7 and T8, *orrm5-3* transgenic lines, T5 and T6, *orrm5-2* transgenic lines.



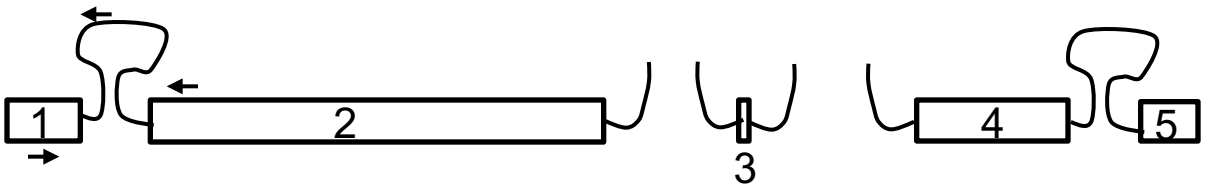

**Supplementary Figure S4.** Distribution and behavior of the editing sites that did not show any significant change in the mutant lines (vs. the wild-type) in the transgenic lines transformed with the full length *ORRM5*. **(A)** Distribution in T7 and T8, *orrm5-3* transgenic lines**. (B)** Distribution in T5 and T6, *orrm5-2* transgenic lines.




**Supplementary Figure S5**. Editing defect at the *ccmFn-2* C320 site in the *orrm5* mutants and its complementation by expressing *ORRM5* or the N-terminal RRM domain of *ORRM5* (*nORRM5*). Editing extents were measured by STS-PCRseq. T7 and T8: *orrm5-3*-/- w/35S:: *ORRM5*; T11 and T12: *orrm5-3*-/- w/35S:: *nORRM5*; T5 and T6: *orrm5-2*-/- w/35S:: *ORRM5*; T9 and T10: *orrm5-2*-/- w/35S:: *nORRM5*. Both *orrm5-3* and *orrm5-2* mutants show a decrease of editing extent compared to the *ORRM5-3* wild-type. In *orrm5-3* transgenic plants, only the RRM (T11, T12) is able to complement the editing defect to wild-type level. In *orrm5-2* transgenic plants, both the full length *ORRM5* (T5, T6) and the RRM (T9, T10) are able to complement the editing defect to wild-type level or lower. Values represent mean ± SD for *ORRM5-3*+/+, *orrm5-3*-/-, and *orrm5-2*-/- (n=2).




**Supplementary Figure S6.** Transcript abundance of *nad3*, *rps14* and *ccmFn-2* in the *orrm5* mutants versus the controls examined by RNA blots. *orrm5-2*, plants in an *orrm5-2* homozygous mutant background; *orrm5-3*, plants in an *orrm5-3* homozygous mutant background; +/+, wild-type siblings of *orrm5-3* mutants. -/-, mutant plants that do not contain the *ORRM5* or *nORRM5* transgene; FL, mutant plants transformed with the coding sequence of *ORRM5* under a 35S promoter; RRM, mutant plants transformed with the N-terminal RRM motif of *ORRM5* under a 35S promoter. The negative of the ethidium bromide staining gel in the bottom panel serves as a loading control.




**Supplementary Figure S7.** Representation of the complex structure of the *nad5* transcript. The five exons are symbolized by squares drawn to scale and numbered 1 to 5. The introns are sketched as solid lines. The maturation of *nad5* transcript necessitates two *cis*-splicing events to join exon1 and exon2 and exon4 and exon5. In addition, two *trans*-splicing events are needed to join exon3 to exon2 and exon4. The arrows represent the primers used in the qRT-PCR assay to determine the splicing efficiency of intron1.

| | *orrm5* up | *orrm5* other | |
|---|---|---|---|
| *orrm2* down | 16<br>*5.13*<br>(23.03) | 23<br>*33.87*<br>(3.49) | 39 |
| *orrm2* other | 57<br>*67.87*<br>(1.74) | 459<br>*448.13*<br>(0.26) | 516 |
| | 73 | 482 | 555 |

$\chi^2 = 28.528$ $P = 9$ e-8

| | *orrm5* up | *orrm5* other | |
|---|---|---|---|
| *orrm3* down | 21<br>*7.63*<br>(23.44) | 37<br>*50.37*<br>(3.55) | 58 |
| *orrm3* other | 52<br>*65.37*<br>(2.73) | 445<br>*431.63*<br>(0.41) | 497 |
| | 73 | 482 | 555 |

$\chi^2 = 30.134$ $P = 4$ e-8

| | *orrm5* up | *orrm5* other | |
|---|---|---|---|
| *orrm4* down | 19<br>*29.73*<br>(3.87) | 207<br>*196.27*<br>(0.59) | 226 |
| *orrm4* other | 54<br>*43.27*<br>(2.66) | 275<br>*285.73*<br>(0.40) | 329 |
| | 73 | 482 | 555 |

$\chi^2 = 7.518$ $P = 6$ e-3

**Supplementary Figure S8.** Contingency tables of the number of mitochondrial sites experiencing an increase in *orrm5* mutant (*orrm5* up) or no increase in *orrm5* mutant (*orrm5* other) and a decrease in other mitochondrial editing factors (*orrm2*, *orrm3*, or *orrm4* down) or no decrease in these mutants or silenced tissues (*orrm2*, *orrm3*, or *orrm4* other). The results with *orrm2* were obtained on silenced tissues. The observed number of sites are in plain character while the expected values are in italics, and the individual $\chi^2$ are displayed in parentheses. Below each contingency table is given the $\chi^2$ value for a test of independence and the p value. The total number of mitochondrial sites commonly surveyed between this study and the previous studies on ORRM2. ORRM3, and ORRM4 is 555.

**A**

| | T7,T8 down | T7,T8 other | |
|---|---|---|---|
| *orrm2* down | 15<br>*5.54*<br>(16.13) | 6<br>*15.46*<br>(5.79) | 21 |
| *orrm2* other | 103<br>*112.46*<br>(0.80) | 323<br>*313.54*<br>(0.29) | 426 |
| | 118 | 329 | 447 |

$\chi^2 = 23$ $P = 2\text{ e-}6$

| | T7,T8 down | T7,T8 other | |
|---|---|---|---|
| *orrm3* down | 28<br>*9.5*<br>(36) | 8<br>*26.5*<br>(12.9) | 36 |
| *orrm3* other | 90<br>*108.5*<br>(3.15) | 321<br>*302.5*<br>(1.13) | 411 |
| | 118 | 329 | 447 |

$\chi^2 = 53.2$ $P = 0$

**B**

| | T5,T6 down | T5,T6 other | |
|---|---|---|---|
| *orrm2* down | 11<br>*2.74*<br>(24.88) | 9<br>*17.26*<br>(3.95) | 20 |
| *orrm2* other | 50<br>*58.26*<br>(1.17) | 375<br>*366.74*<br>(0.19) | 425 |
| | 61 | 384 | 445 |

$\chi^2 = 30.19$ $P = 4\text{ e-}8$

| | T5,T6 down | T5,T6 other | |
|---|---|---|---|
| *orrm3* down | 22<br>*4.25*<br>(74.15) | 9<br>26.75<br>(11.78) | 31 |
| *orrm3* other | 39<br>*56.75*<br>(5.55) | 375<br>*357.25*<br>(0.88) | 414 |
| | 61 | 384 | 445 |

$\chi^2 = 92.36$ $P = 0$

**Supplementary Figure S9.** Contingency tables of the number of mitochondrial sites experiencing either a decrease in transgenic lines, (T7, T8 down) or (T5, T6 down), or no decrease in transgenic lines, (T7, T8 other) or (T5, T6 other), and a decrease in other mitochondrial editing factors (*orrm2* or *orrm3* down) or no decrease in these mutants or silenced tissues (*orrm2*, *orrm3* other). The results with *orrm2* were obtained on silenced tissues. This analysis is restricted to the invariant sites that did not show significant change in the mutant plants either *orrm5-3*, or *orrm5-2* when compared to the wild-type. The observed number of sites are in plain character while the expected values are in italics, and the individual $\chi^2$ are displayed in parentheses. Below each contingency table is given the $\chi^2$ value for a test of independence and the p value. **(A)** Contingency tables obtained with *orrm5-3* transgenic lines. **(B)** Contingency tables obtained with *orrm5-2* transgenic lines