*SI Appendix*, Figures S1 to S15, Tables S1, and Materials and Methods.

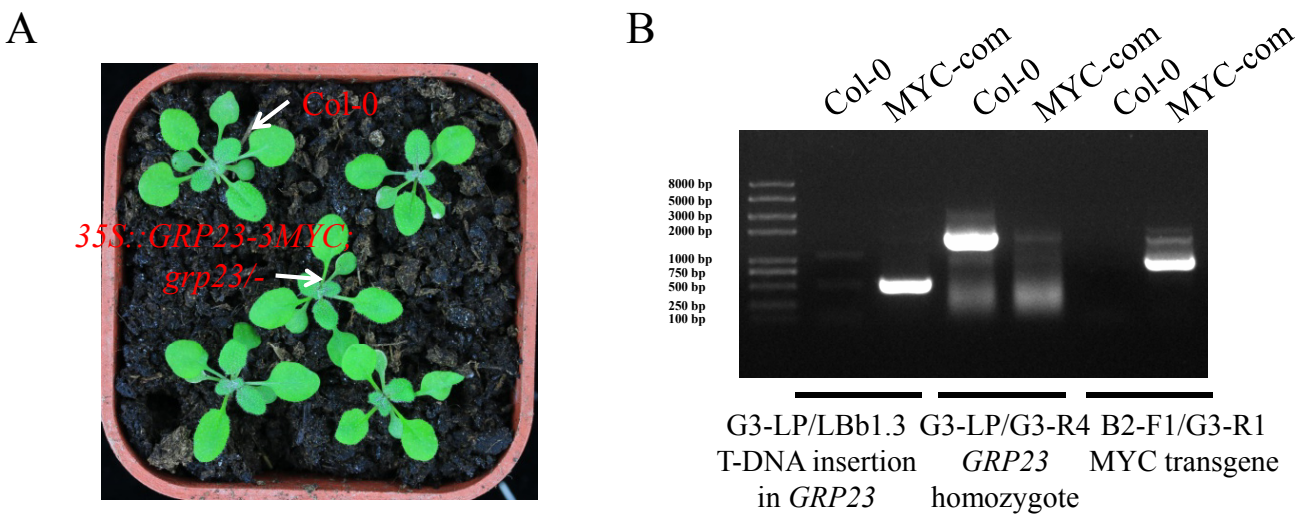



**Fig. S1. Complementation of *grp23* mutants with *35S::GRP23-3MYC*.**
(A) Phenotypes of the wild type and the *grp23* mutant complemented by *35S::GRP23-3MYC*. Images of 3-week-old seedlings were taken. (B) Genotype of the complemented *grp23* mutant. Primer pair G3-LP/LBb1.3 is used to identify the T-DNA insertion in *grp23*; G3-LP/G3-R4 is used to identify the *grp23* homozygote; B2-F1/G3-R1 is used to identify the *GRP23-3MYC* transgene.

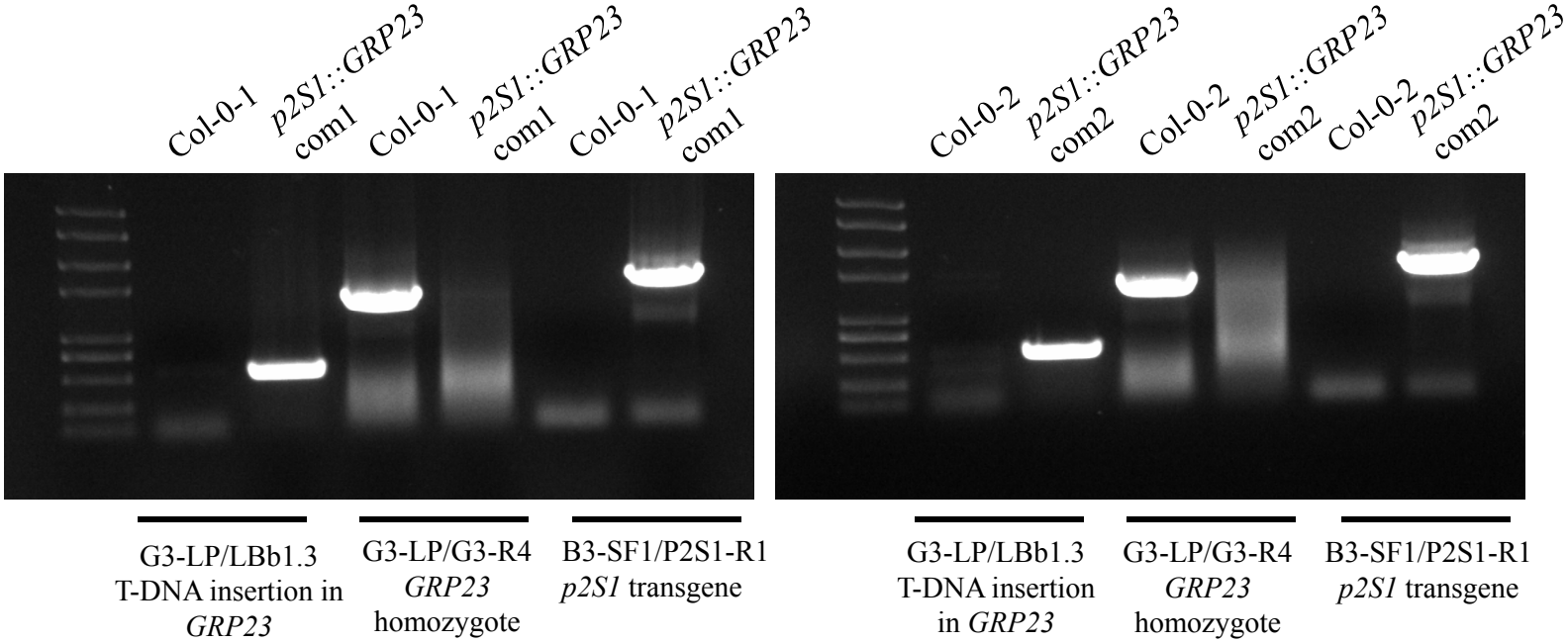



**Fig. S2. Genotype of the *grp23* mutants complemented by *p2S1::GRP23*.**
Primer pair G3-LP/LBb1.3 is used to identify the T-DNA insertion in *grp23*; G3-LP/G3-R4 is used to identify the *grp23* homozygote; B3-SF1/P2S1-R1 is used to identify the *p2S1::GRP23 transgene*.

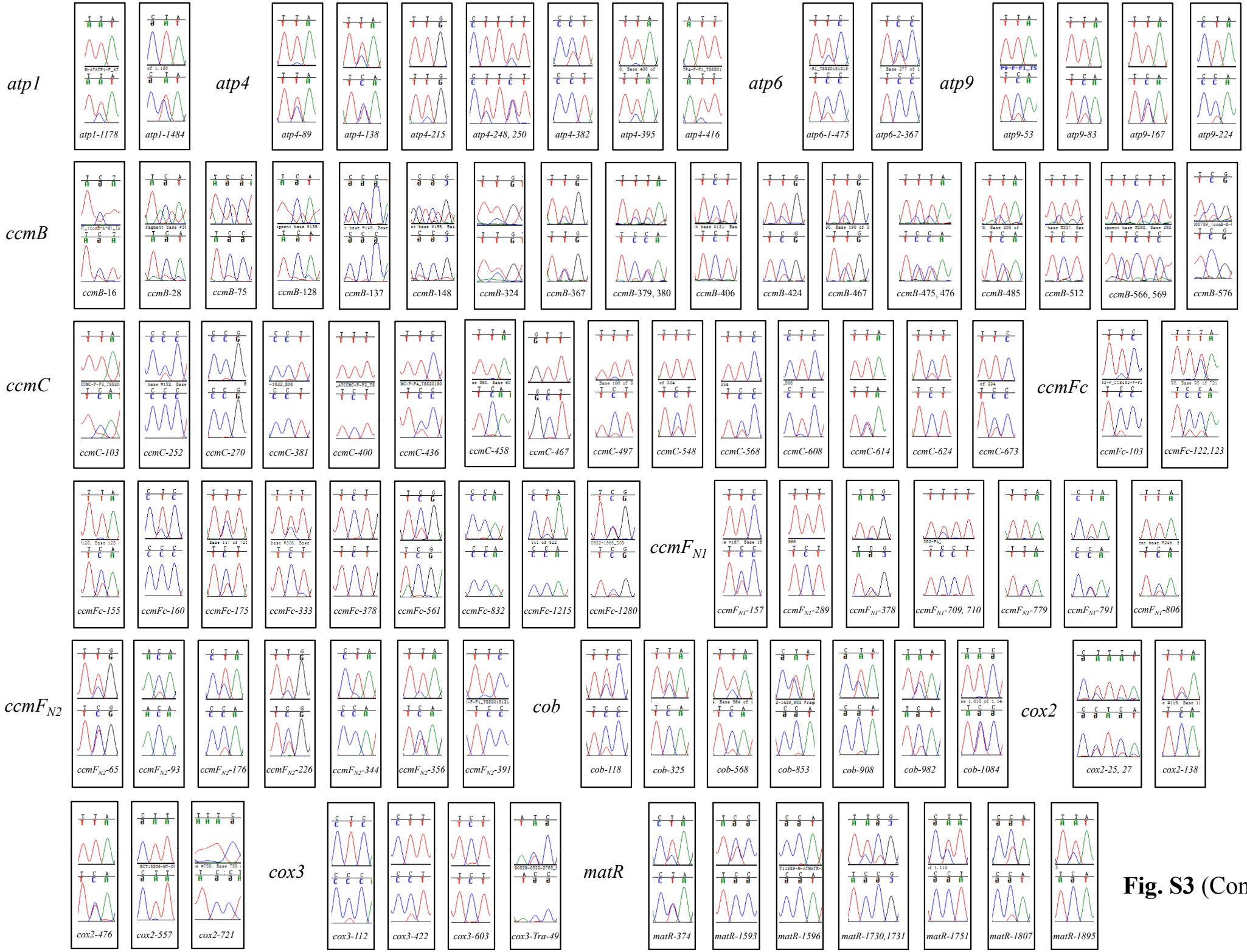



**Fig. S3** (Continued)


**Fig. S3** (Continued)

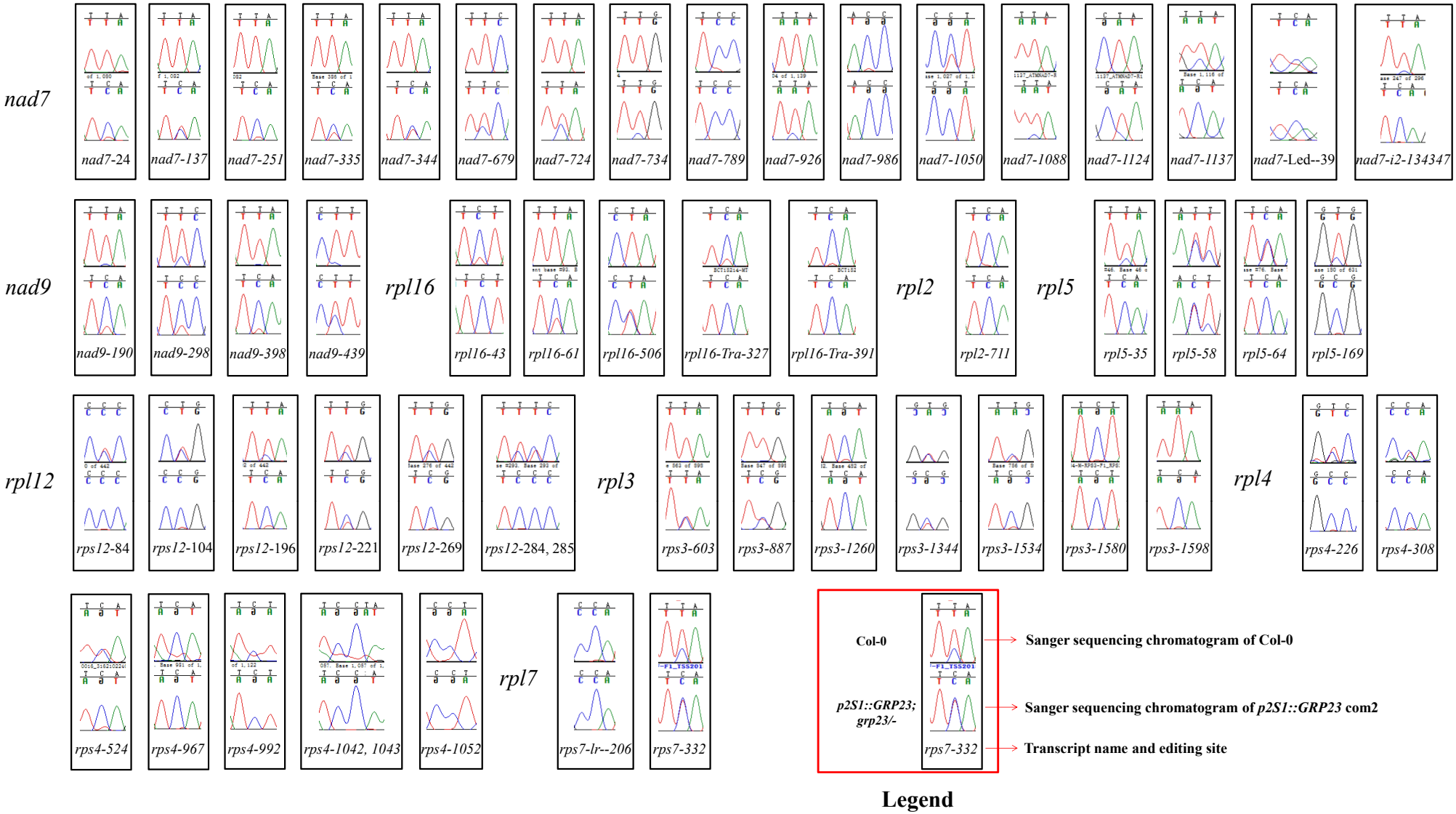



**Fig. S3. Editing analysis of *p2S1::GRP23* com2 by Sanger sequencing of targeted RT-PCR products.**

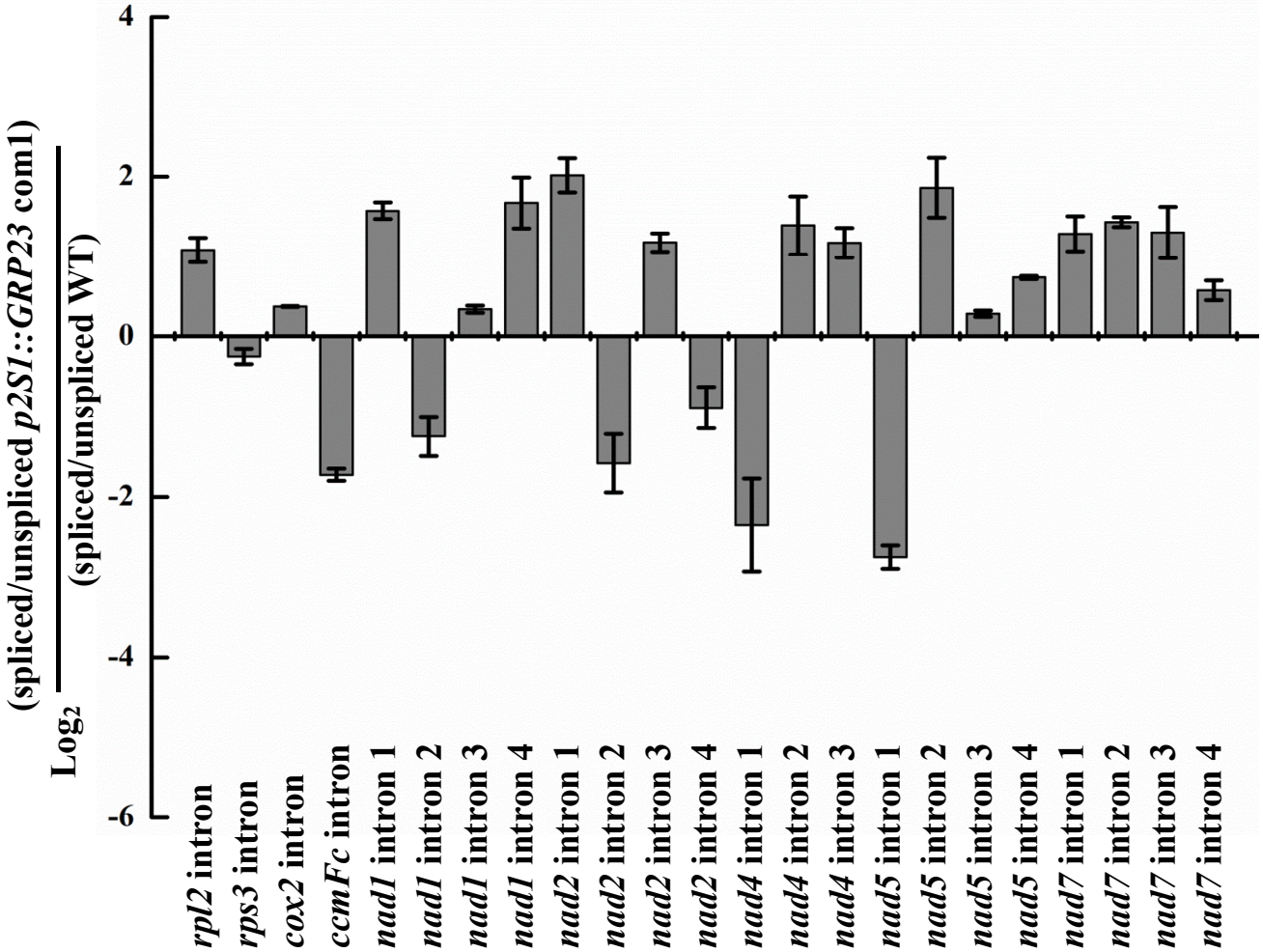



**Fig. S4. Splicing efficiency of mitochondrial introns in the *p2S1*::*GRP23* com1 mutants.**
The splicing efficiency of 23 introns of mitochondrial genes in the *p2S1*::*GRP23* com1 was detected by quantitative RT-PCR. Values are the mean ± SE of three biological repeats.

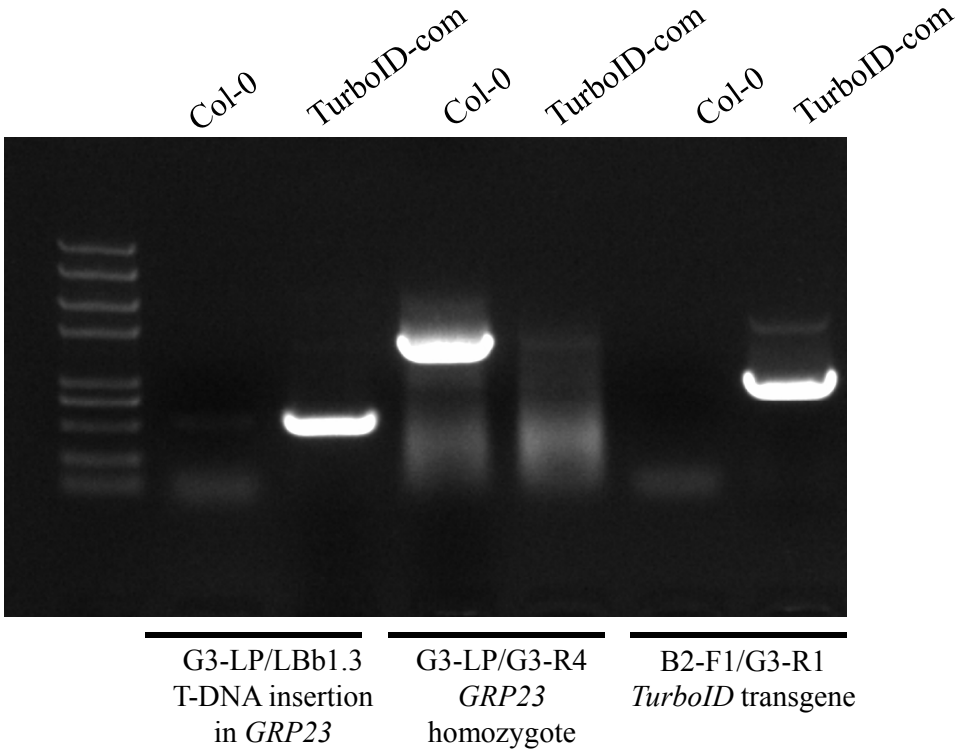
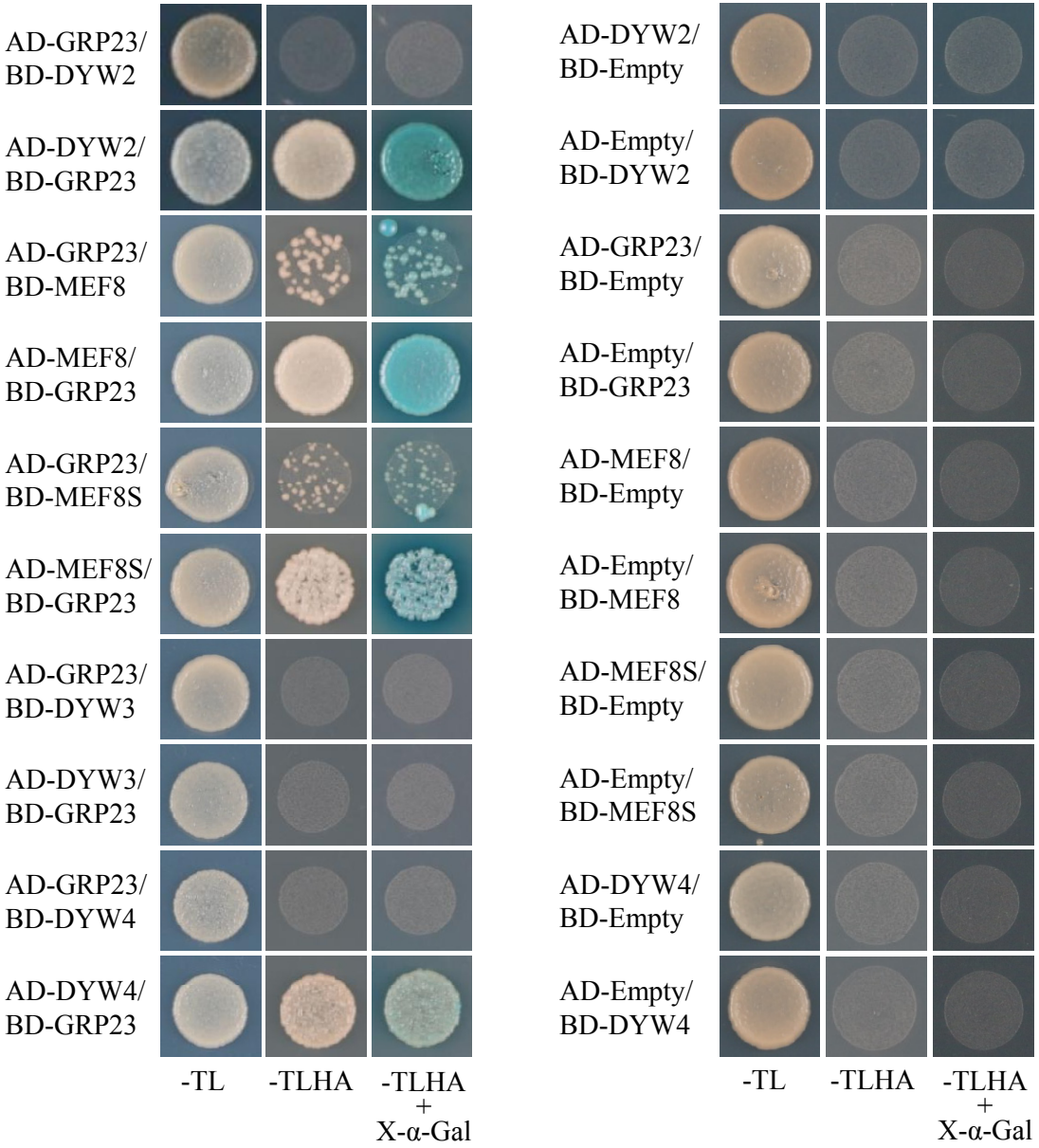



**Fig. S5. Genotype of the *grp23* mutant complemented by *p35S::GRP23-TurboID*.**
Primer pair G3-LP/LBb1.3 is used to identify the T-DNA insertion in *grp23*; G3-LP/G3-R4 is used to identify the *grp23* homozygote; B2-F1/G3-R1 is used to identify the *GRP23-TurboID* transgene.

A




B

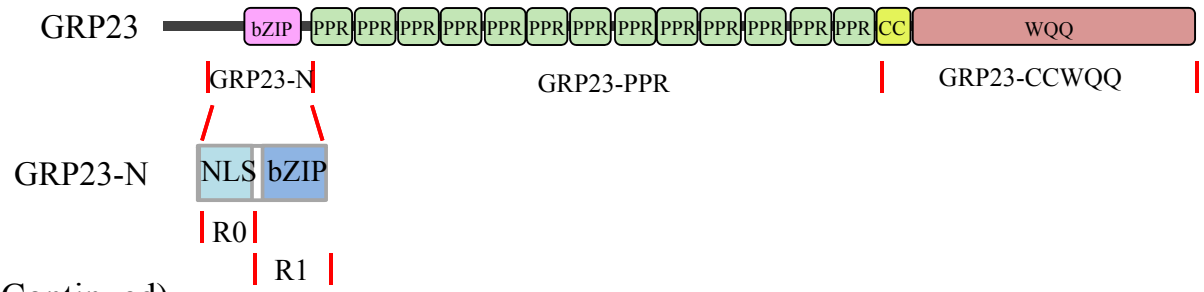



**Fig. S6.** (Continued)

C


**Fig. S6.** (Continued)

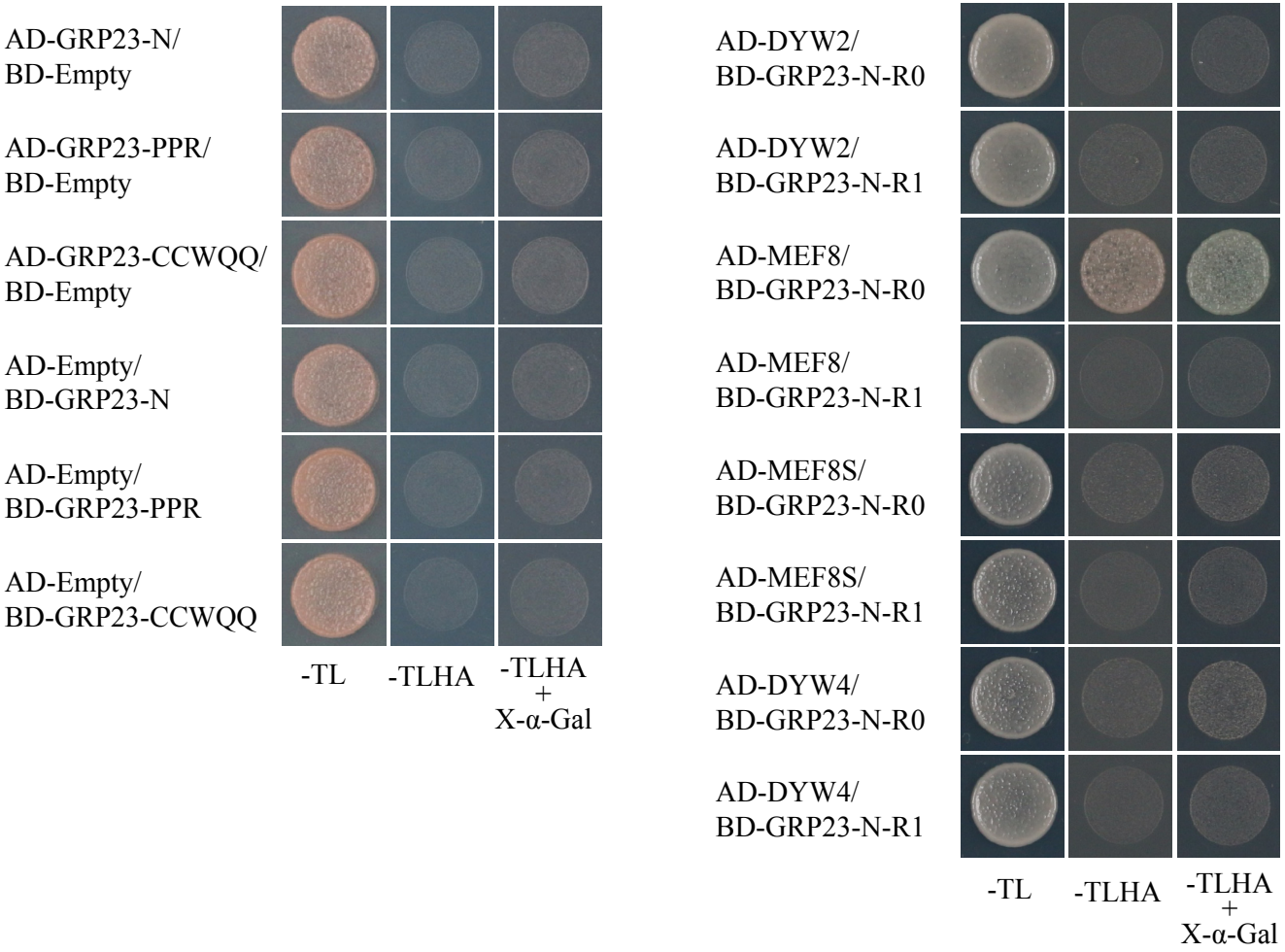



**Fig. S6.** (Continued)

D

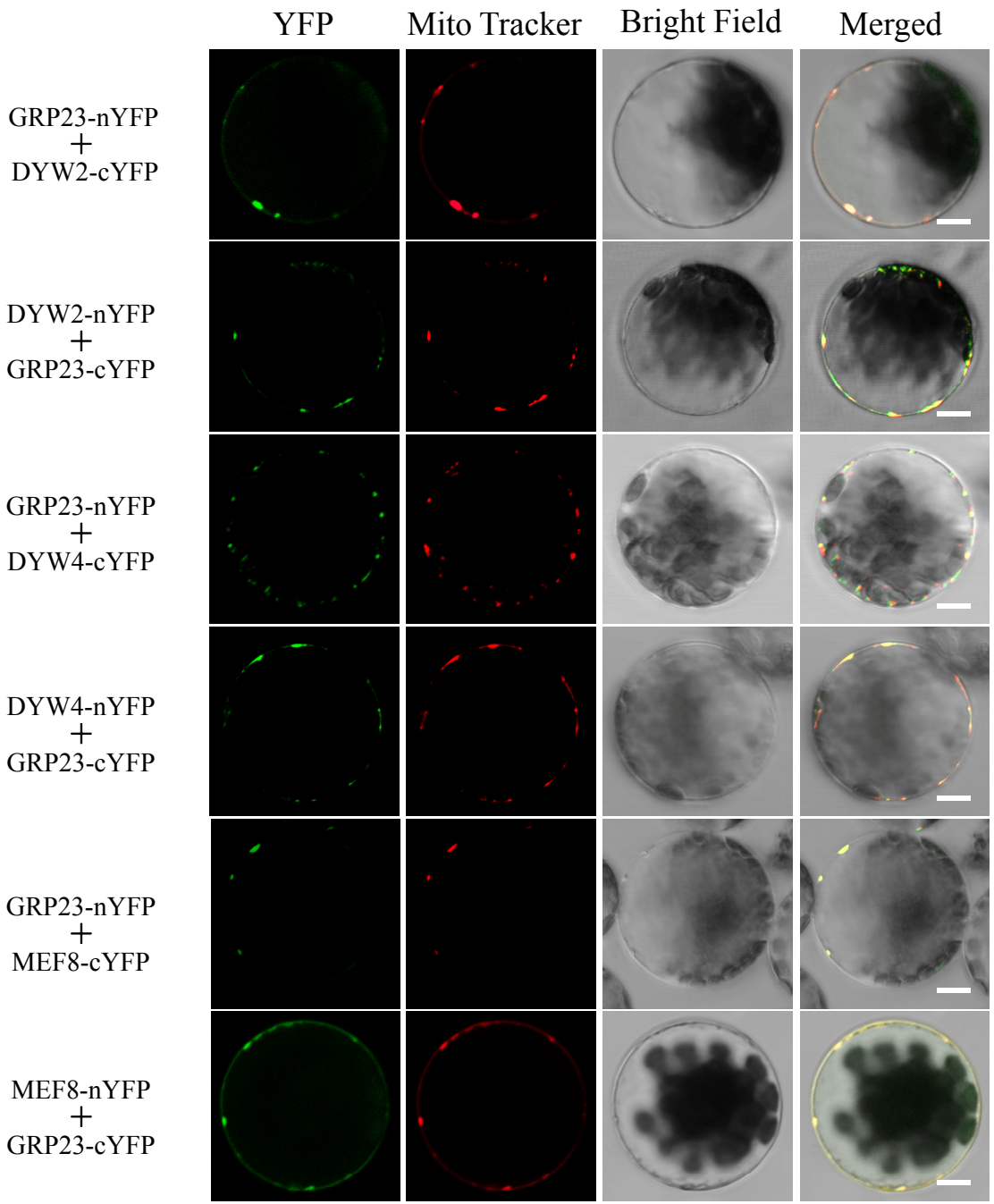



**Fig. S6.** (Continued)

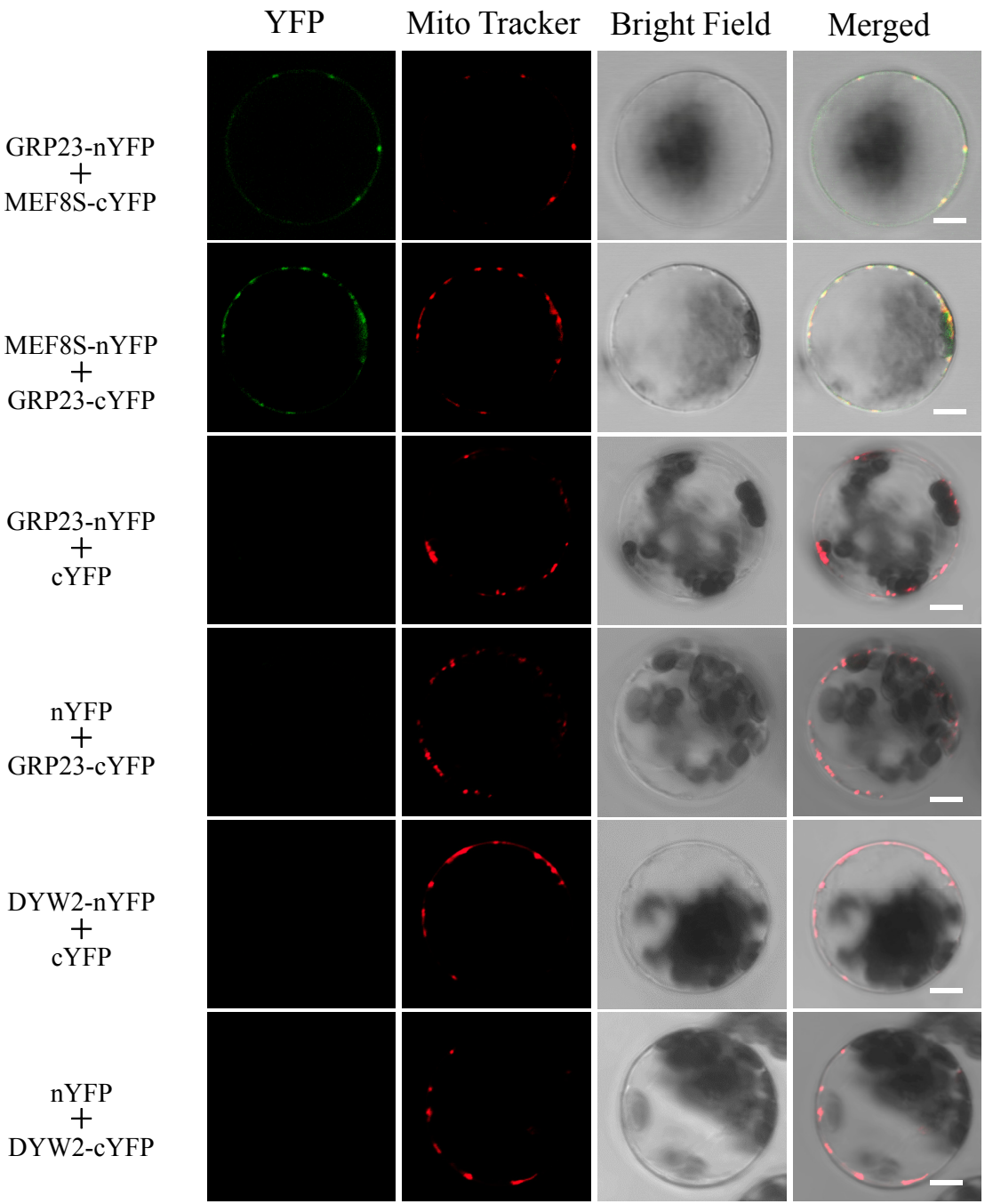



**Fig. S6.** (Continued)

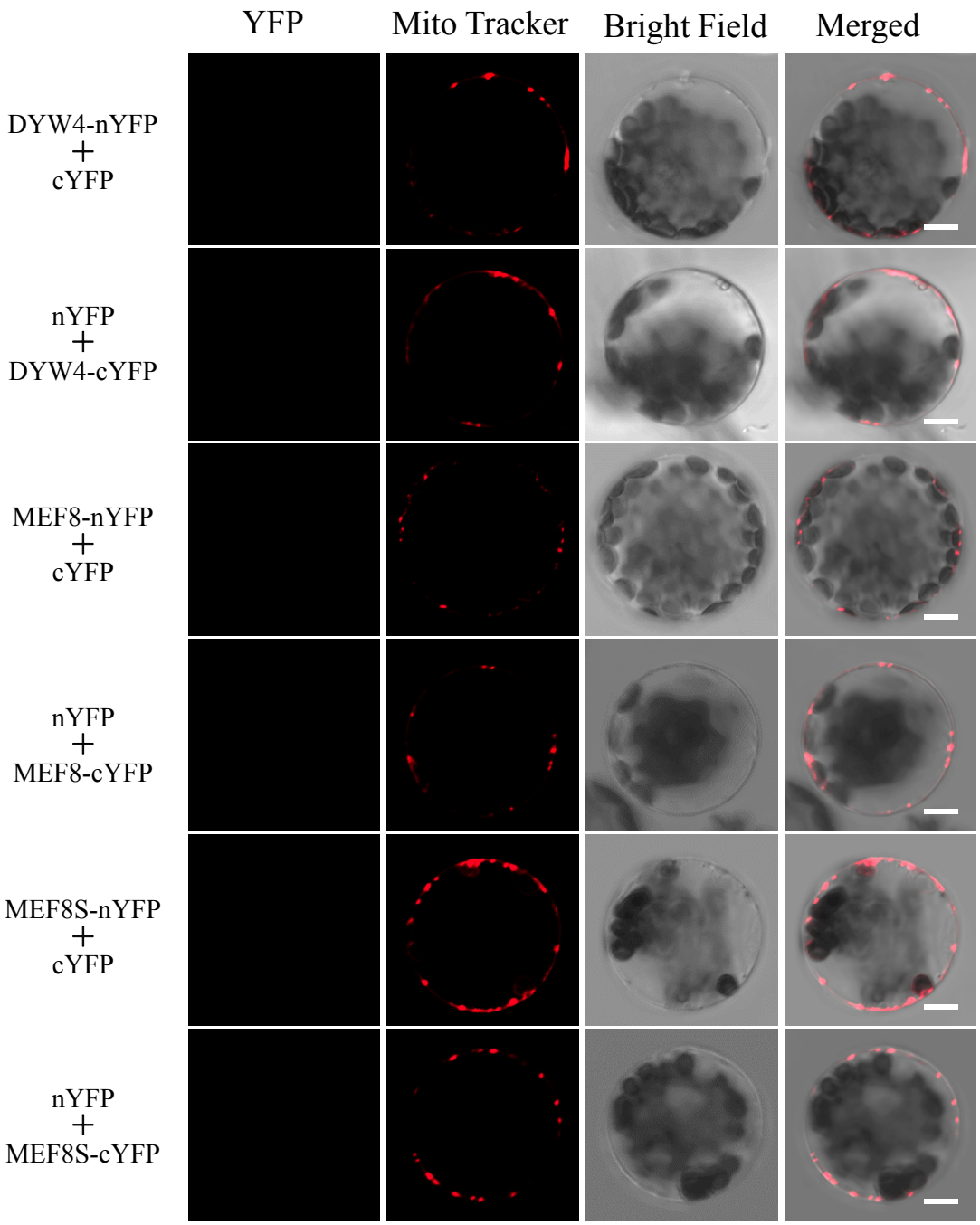



**Fig. S6.** (Continued)

**Fig. S6. GRP23 interacts with atypical PPR-DYWs in yeast and Arabidopsis protoplasts.**
(A) GRP23 interacts with DYW2, MEF8, MEF8S, and DYW4 in yeast. -TL, -TLHA and -TLHA+X-α-Gal indicate SD/-Trp-Leu, SD/-Trp-Leu-His-Ade, and SD/-Trp-Leu-His-Ade containing X-α-Gal dropout plates, respectively. AD-Empty, pGADT7 empty vector. BD-Empty, pGBKT7 empty vector. Images were taken after 3 days of incubation at 30 ℃. (B) Diagram of the truncations of GRP23 used in Y2H. (C) The N-terminal region and PPR motif of GRP23 interact with DYW2, MEF8, MEF8S, and DYW4. (D) BiFC analysis shows the interactions between GRP23 and DYW2, MEF8, MEF8S, and DYW4 in Arabidopsis mitochondria. Scale bars = 5 μm.

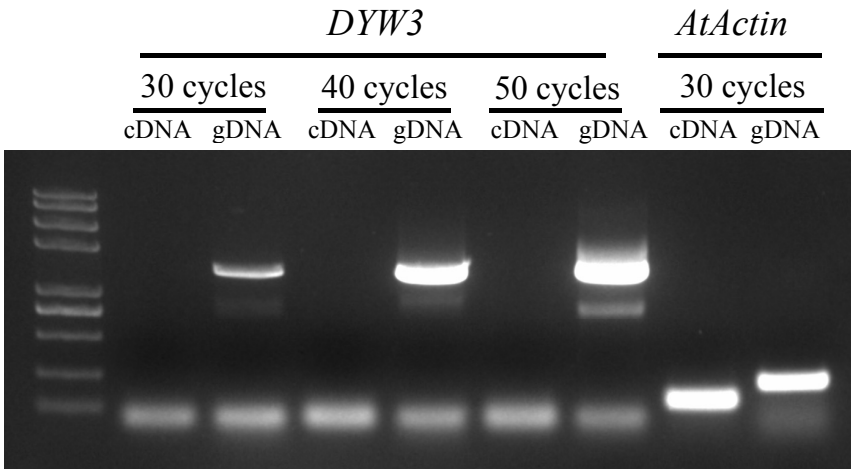



**Fig. S7. *DYW3* is not expressed in Arabidopsis.**
The expression of *DYW3* was detected by RT-PCR. Reverse transcription reactions were performed using 1 μg of total RNA from total seedling with random hexamers by SuperScript II Reverse Transcriptase (Invitrogen). PCR was performed with 0.1 μg Arabidopsis genomic DNA as a positive control.

A

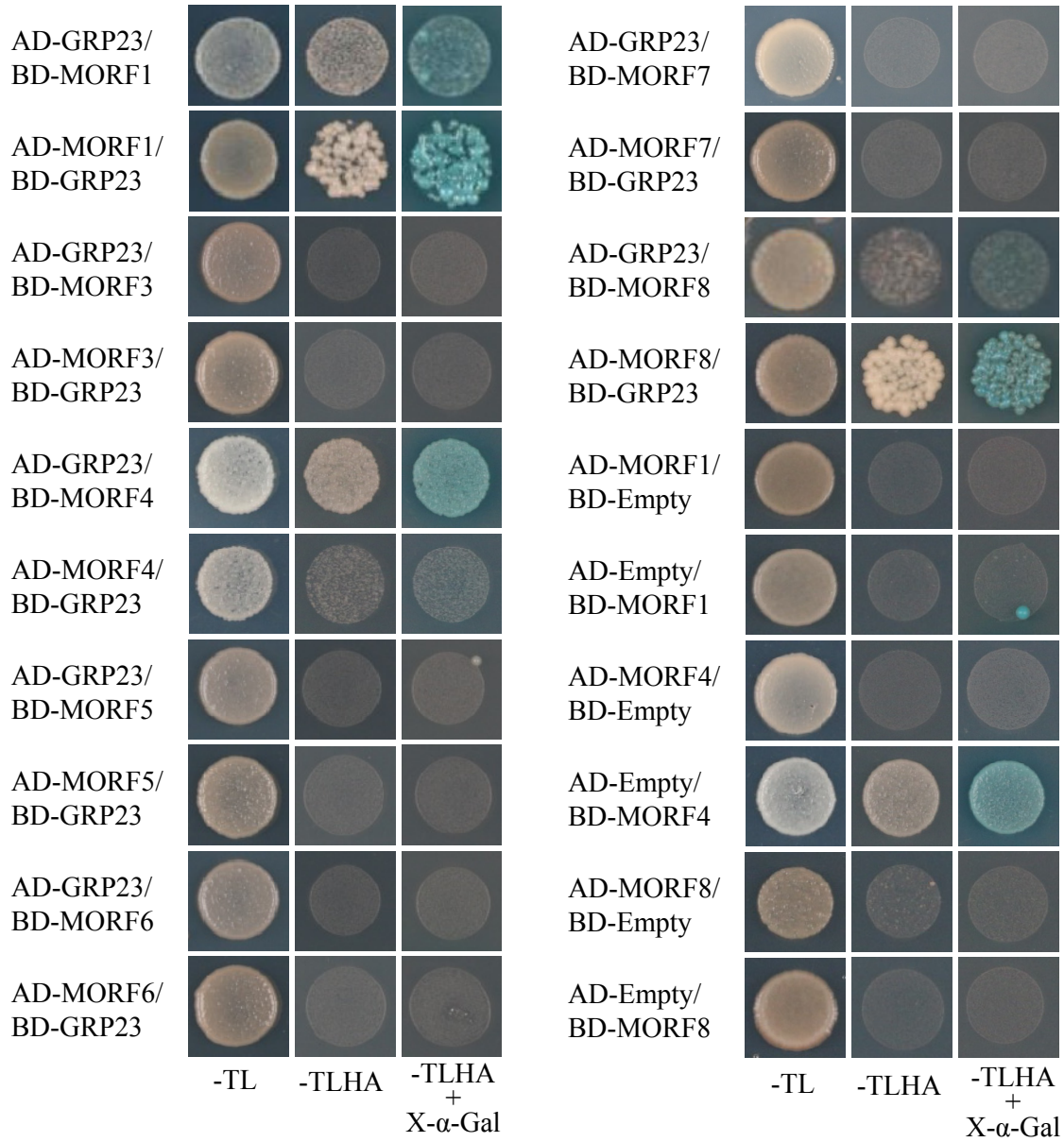



**Fig. S8** (Continued)

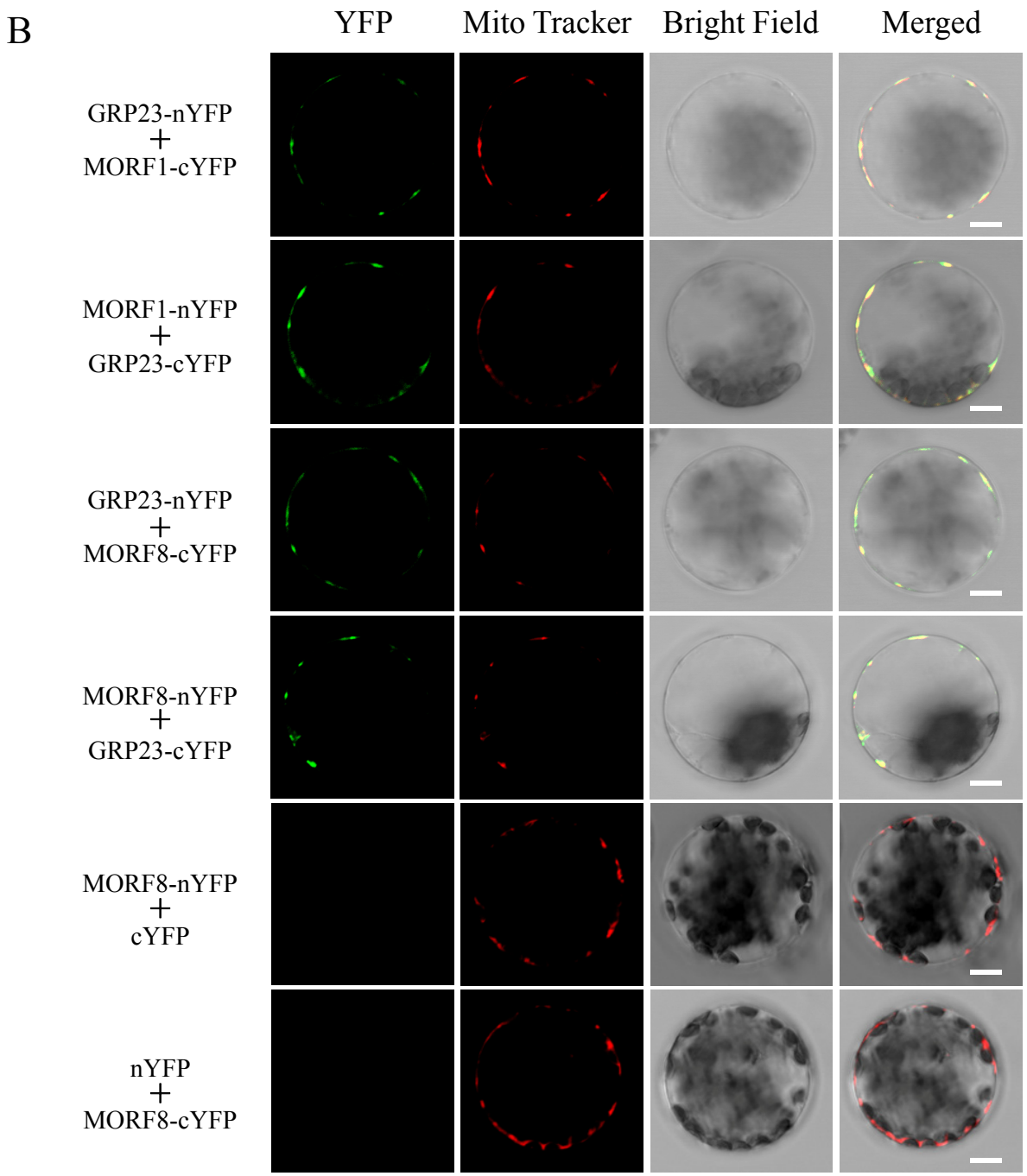



**Fig. S8** (Continued)

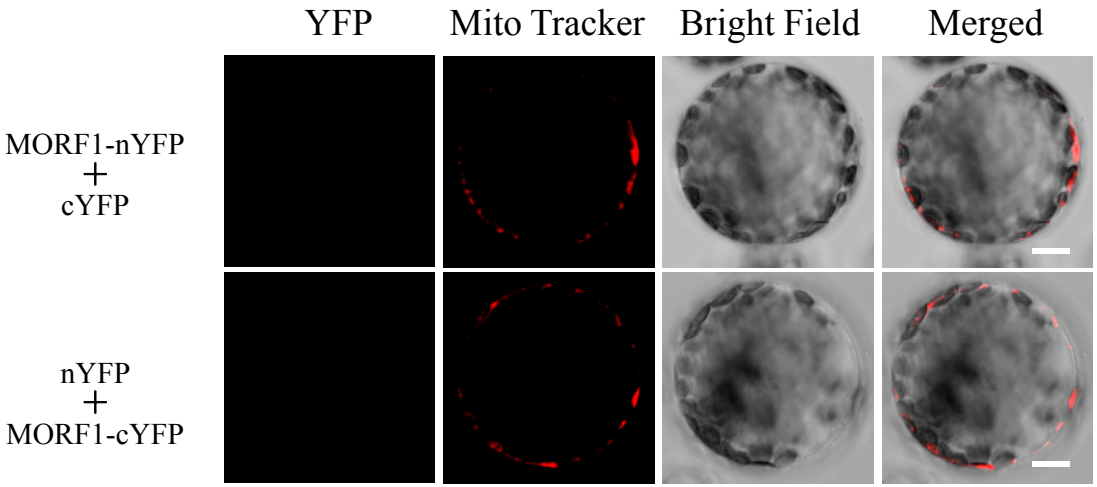



**Fig. S8. GRP23 interacts with MORF1 and MORF8 in yeast and Arabidopsis protoplasts.** (A) Detection of the interactions between GRP23 and MORFs in yeast. MORF4 fused to BD shows strong auto-activation for HIS and ADE reporters. Images were taken after 3 days of incubation at 30 ℃. -TL, -TLHA, and -TLHA+X-α-Gal indicate SD/-Trp-Leu, SD/-Trp-Leu-His-Ade, and SD/-Trp-Leu-His-Ade containing X-α-Gal dropout plates, respectively. B. BiFC analysis shows the interactions between GRP23 and MORF1 and MORF8 in Arabidopsis mitochondria. Scale bars = 5 μm.

A

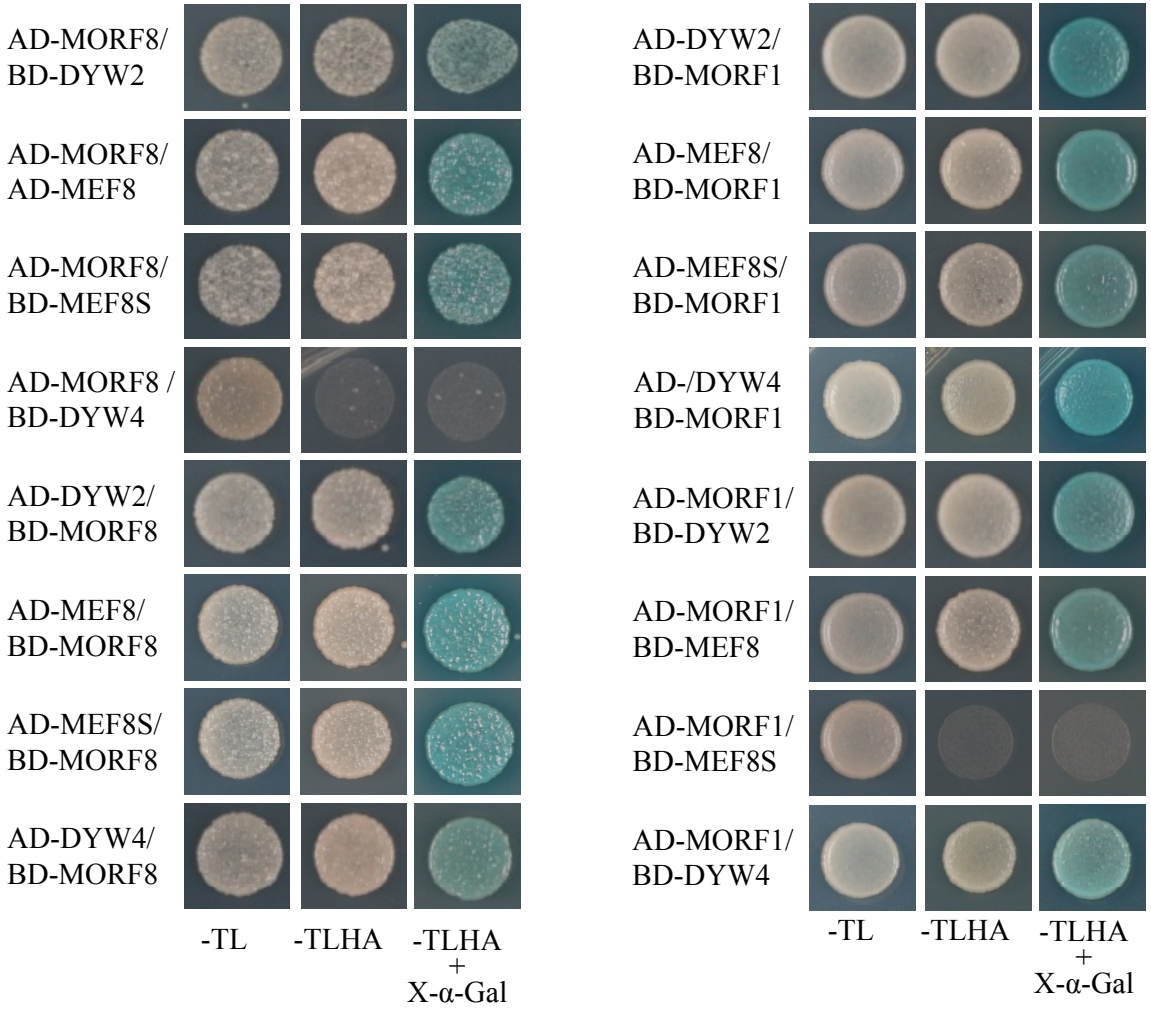



**Fig. S9** (Continued)

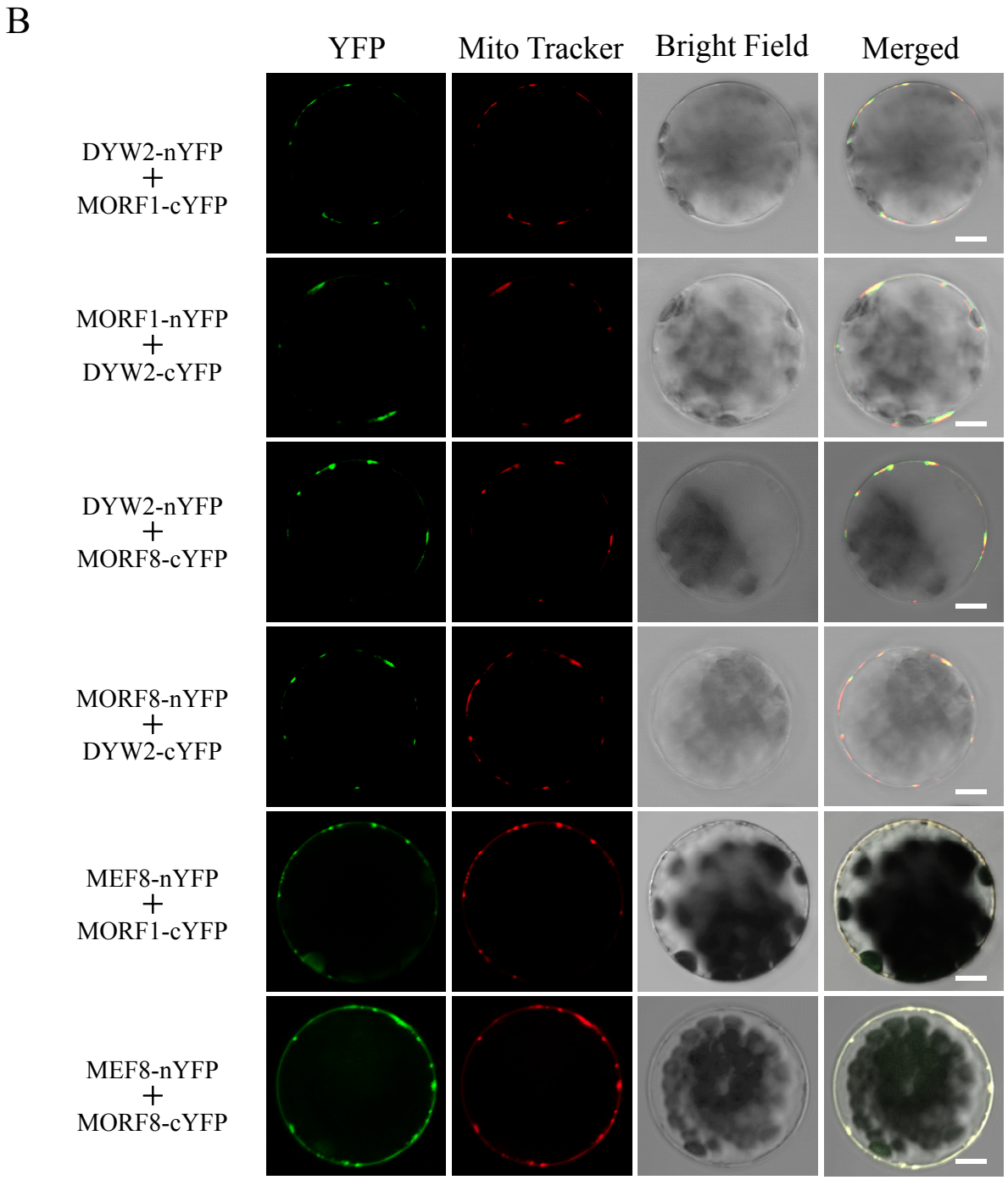



**Fig. S9** (Continued)

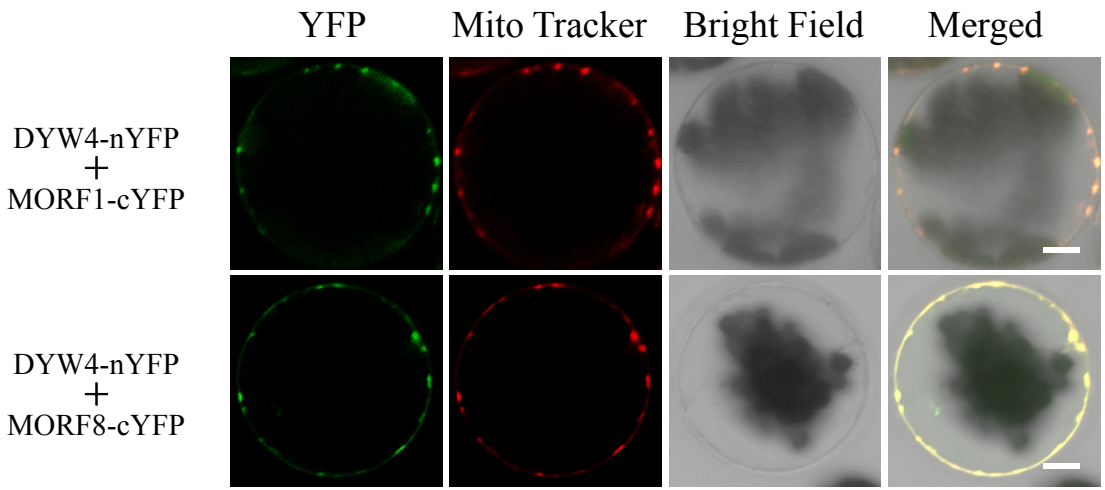



**Fig. S9. MORF1 and MORF8 interact with atypical PPR-DYW proteins DYW2, MEF8, MEF8S, and DYW4.**

(A) MORF1 and MORF8 interact with PPR-DYW proteins DYW2, MEF8, MEF8S, and DYW4 in yeast. -TL, -TLHA, and -TLHA+X-α-Gal indicate SD/-Trp-Leu, SD/-Trp-Leu-His-Ade, and SD/-Trp-Leu-His-Ade containing X-α-Gal dropout plates, respectively. (B) BiFC analysis shows the interactions between MORF1 and MORF8 with atypical PPR-DYWs DYW2, MEF8, and DYW4 in Arabidopsis mitochondria. Scale bars = 5 μm.

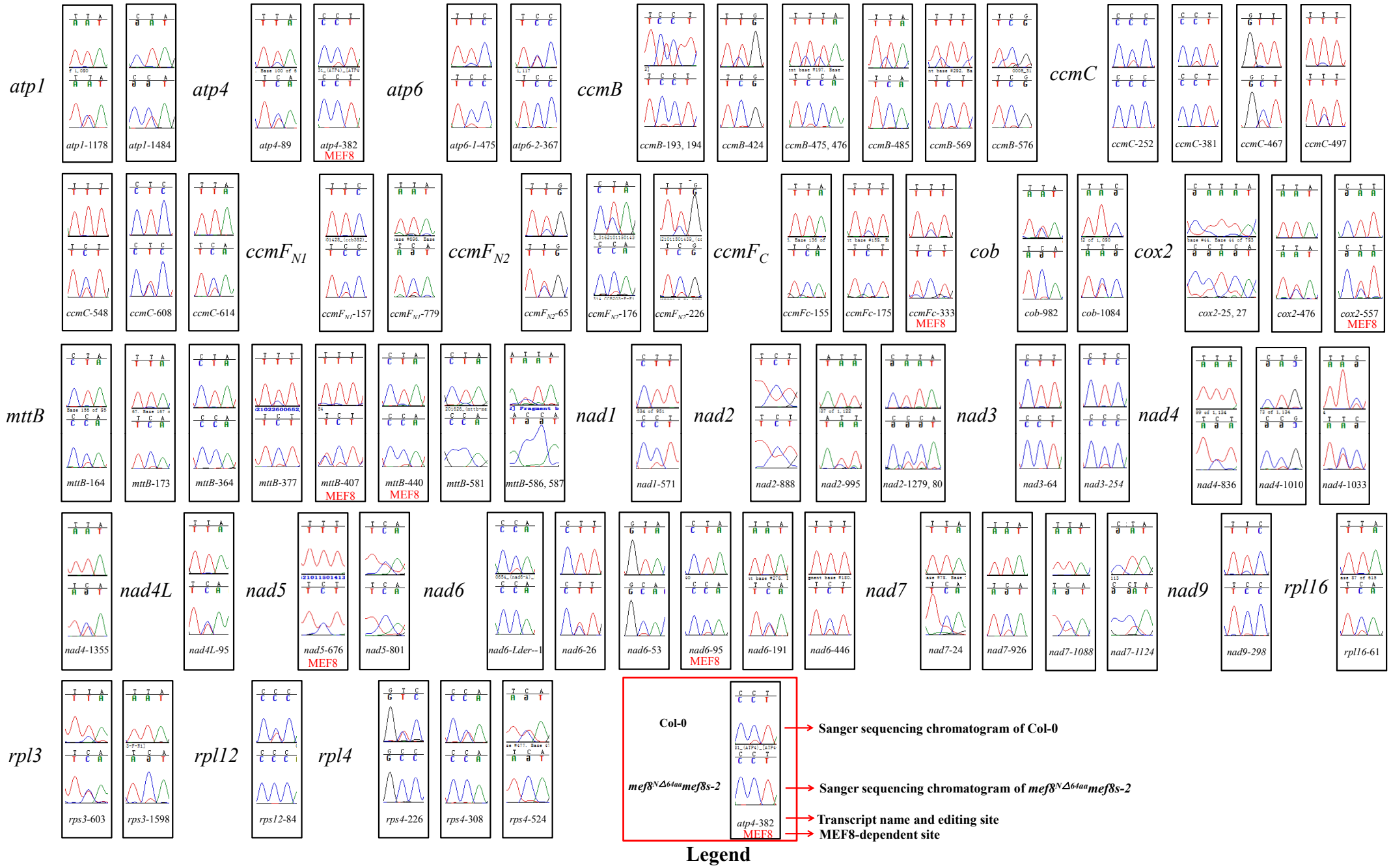



**Fig. S10. Editing analysis of *mef8*$^{N\Delta 64aa}$*mef8s-2* by Sanger sequencing of targeted RT-PCR products.**

A

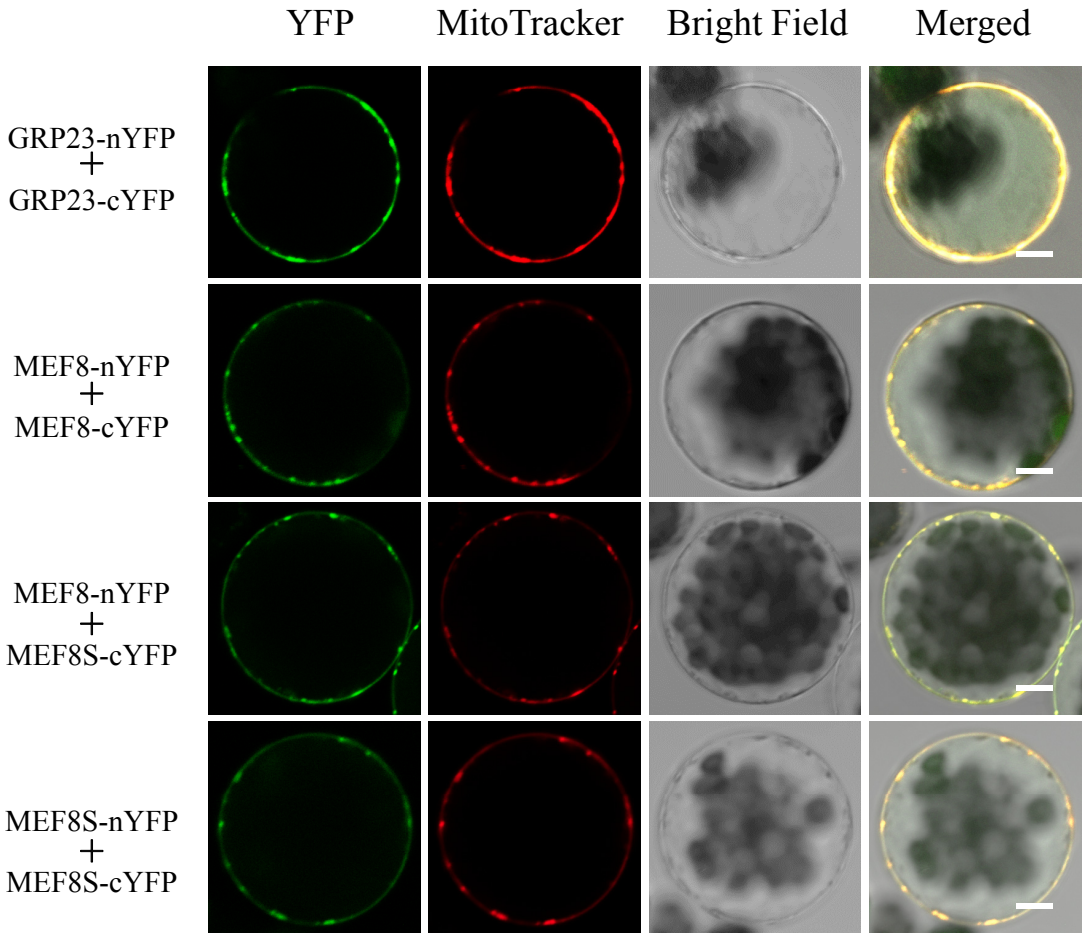



**Fig. S11** (Continued)

B

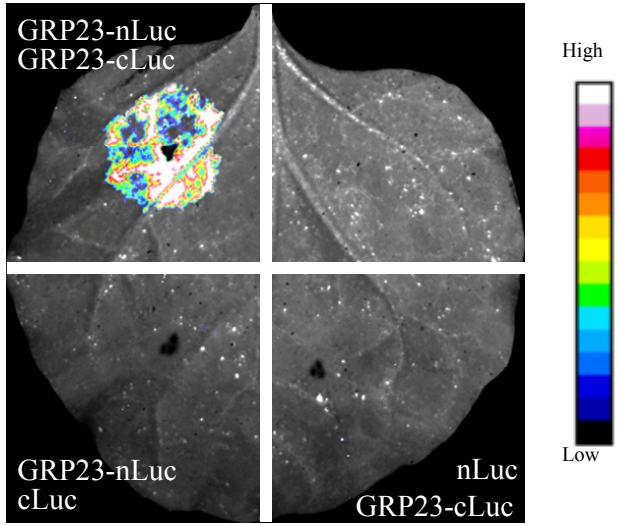



**Fig. S11. GRP23 and MEF8/MEF8S form dimers.**
(A) Detection of the dimerization of GRP23 and MEF8/MEF8S by BiFC assay. Arabidopsis protoplasts were transfected with the combination of GRP23-nYFP/GRP23-cYFP, MEF8-nYFP/MEF8-cYFP, MEF8-nYFP/MEF8S-cYFP, and MEF8S-nYFP/MEF8S-cYFP constructs and observed by confocal microscopy 18-22 hours after transfection. Scale bars = 5 μm. (B) Detection of GRP23 homodimer by LUC assay. Tobacco leaves were infiltrated with Agrobacterium containing GRP23-NLuc and GRP23-CLuc constructs. The signals were captured by a Lumazone Pylon 2048B system 2 days after infiltration.

```
                                   NLS
GRP23  78   IPYPPIPHRTMAFSSAEEAAAERRRRKRRLRIEPPLHALRRD----PSAPPPKRDPNAPR  133
            +P P +  R M+F++ EEAAAERRRRKRRLR+EPP+++  R        P P ++PN P+
NUWA   24   LPQPFLAVRYMSFATQEEAAAERRRRKRRLRMEPPVNSFNRSQQQQSQIPRPIQNPNIPK  83
                        bZIP                                  PPR
GRP23  134  LPDSTSALVGQRLNLHNRVQSLIRASDLDAASKLARQSVFSNTRPTVFTCNAIIAAMYRA  193
            LP+S SALVG+RL+LHN +  LIR +DL+ A+   R SV+SN RPT+FT N ++AA  R
NUWA   84   LPESVSALVGKRLDLHNHILKLIRENDLEEAALYTRHSVYSNCRPTIFTVNTVLAAQLRQ  143
                                      PPR
GRP23  194  KRYSESISLFQYFFKQSNIVPNVVSYNQIINAHCDEGNVDEALEVYRHILANAPFAPSSV  253
             +Y   + L   F  Q+ I PN+++YN I  A+ D    + ALE Y+  + NAP  PS
NUWA   144  AKYGALLQL-HGFINQAGIAPNIITYNLIFQAYLDVRKPEIALEHYKLFIDNAPLNPSIA  202
                                      PPR
GRP23  254  TYRHLTKGLVQAGRIGDAASLLREMLSKGQAADSTVYNNLIRGYLDLGDFDKAVEFFDEL  313
            T+R L KGLV    +  A  +  +M  KG   D  VY+ L+ G +   D D  ++ + EL
NUWA   203  TFRILVKGLVSNDNLEKAMEIKEDMAVKGFVVDPVVYSYLMMGCVKNSDADGVLKLYQEL  262
                                      PPR
GRP23  314  KSKC--TVYDGIVNATFMEYWFEKGNDKEAMESYRSLL--DKKFRMHPPTGNVLLEVFLK  369
            K K    V DG+V    M+ +F K  +KEAME Y   +  + K RM     N +LE   +
NUWA   263  KEKLGGFVDDGVVYGQLMKGYFMKEMEKEAMECYEEAVGENSKVRMSAMAYNYVLEALSE  322
                                      PPR
GRP23  370  FGKKDEAWALFNEMLDNHAPPNILSVNSDTVGIMVNECFKMGEFSEAINTFKKVGSKVTS  429
             GK DEA  LF+ +   H PP  L+VN  T  +MVN     G+F EA+  F+++G    S
NUWA   323  NGKFDEALKLFDAVKKEHNPPRHLAVNLGTFNVMVNGYCAGGKFEEAMEVFRQMGDFKCS  382
                                      PPR
GRP23  430  KPFVMDYLGYCNIVTRFCEQGMLTEAERFFAEGVSRSLPADAPSHRAMIDAYLKAERIDD  489
                 D L + N++ + C+  +L EAE+ + E   +++  D  ++  ++D   K  +ID+
NUWA   383  P----DTLSFNNLMNQLCDNELLAEAEKLYGEMEEKNVKPDEYTYGLLMDTCFKEGKIDE  438
                                      PPR
GRP23  490  AVKMLDRMVDVNLRVVADFGARVFGELIKNGKLTESAEVLTKMGEREPKPDPSIYDVVVR  549
                   MV+ NLR       R+  +LIK GKL ++      M  +  K D   Y  ++R
NUWA   439  GAAYYKTMVESNLRPNLAVYNRLQDQLIKAGKLDDAKSFFDMMVSK-LKMDDEAYKFIMR  497
                        PPR                   coiled-coil
GRP23  550  GLCDGDALDQAKDIVGEMIRHN-VGVTTVLREFIIEVFEKAGRREEIEKILNSVARPVRN  608
             L +   LD+   IV EM+  + V V+  L+EF+ E   K GR  ++EK++    R
NUWA   498  ALSEAGRLDEMLKIVDEMLDDDTVRVSEELQEFVKEELRKGGREGDLEKLMEEKERLKAE  557

GRP23  609  A-------------GQSGNTPPRVPAVFGTTPAAPQQPRDRAP--WTSQGVVHSNSGWAN  653
            A               QS N    +P      P A ++ ++ A   W ++      +
NUWA   558  AKAKELADAEEKKKAQSINIAALIP------PKAVEEKKETAKLLWENEAGGVEEADVVE  611

GRP23  654  GTAGQTAGGAYKANNGQNP  672
               G  AGG+    NGQ+P
NUWA   612  MAKGVEAGGS----NGQDP  626
```

**Fig. S12. Alignment of partial GRP23 and NUWA protein sequences.**

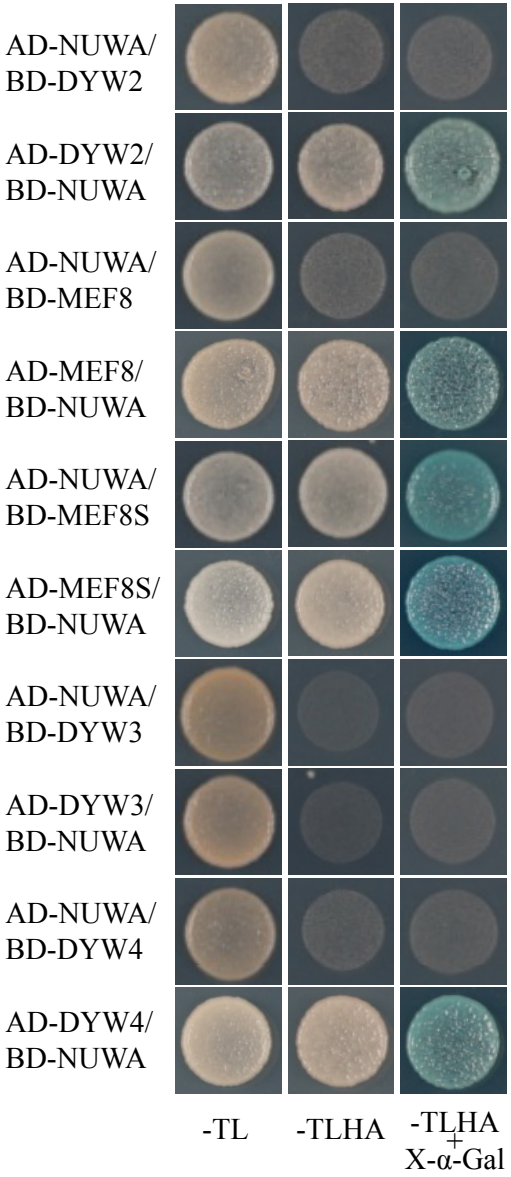



**Fig. S13. NUWA interacts with atypical PPR-DYWs in yeast.**
-TL, -TLHA and -TLHA+X-α-Gal indicate SD/-Trp-Leu, SD/-Trp-Leu-His-Ade, and SD/-Trp-Leu-His-Ade containing X-α-Gal dropout plates, respectively.

```
E+ domain      VHEFVAGDKKESHPESEEIYEKLEELIEEMK
MEF8     492   MQEFRAGDTNL..PENDELFQLLRNLKMHMV  520
MEF8S    414   IRYMAAGDISR..PENRELYMALKSLKEHMI  442
```

**Fig. S14. Comparison of the E+ domains of MEF8 and MEF8S with a consensus sequence of E+ domain.**

Red shaded boxes indicate identical residues. Red letters indicate similar residues. The consensus sequence of E+ domain was obtained using the HMMER package based on alignments of 148 E+ domain from Arabidopsis (13).

```
        62                                                                  125
MEF8     RTGSSGEVSESIHTQSQSLGSNQGRNEQSWKQSPSLSNSQVQSQYQGNWYGTNSDYQNGVGSSW

        62                                                                  125
MEF8S    QSLGGFQQNSYEQSLNPVSGQNPTNRFYQNGYNRNQSYGEHSEIINQRNQNWQSSDGCSSYGTT

        62                                                                  125
DYW2     PQSGSPSQHQRPYPPQSFDSQNQTNTNQRVPQSPNQWSTQHGGQIPQYGGQNPQHGGQRPPYGG
```

**Fig. S15. The N-terminal regions of MEF8, MEF8S, and DYW2 are rich in glutamine residue.** The N-terminal regions from 62aa to 125aa of MEF8, MEF8S, and DYW2 are shown. Glutamine residues are labeled in red.

**Table S1. Primers used in this study**

| Primer Name | sequence | Purpose |
|---|---|---|
| AtNad9-F-F1 | TGAAGAGCAAGAAGCGGAAC | STS-Seq |
| AtNad9-F-R1 | TTGATTTGTCCCCTGGACTG | STS-Seq |
| AtRpl16-F-F1 | GGTCGATTAAAAGACGCAGC | STS-Seq |
| AtRpl16-F-R1 | AGATTCTCGGCCCGCTTTAT | STS-Seq |
| Atccb452-F-F1 | GCGCCTTCTCTTCTTTAG | STS-Seq |
| Atccb452-F-R1 | GTCGAAGAAGTGCCTTGA | STS-Seq |
| Atrpl5-F-F1 | GAGTAGGAGGAGTCAGCTTA | STS-Seq |
| Atrpl5-F-R1 | CGCGAGCAATCTACGTTTGT | STS-Seq |
| AtCob-F-F1 | GGTGGGTGAACAAGAGTTGT | STS-Seq |
| AtCob-F-R1 | CTGCTTGTCAATTCTTGGTG | STS-Seq |
| AtNad6-F-F1 | GCTGAAGCAAGAACTAGCATG | STS-Seq |
| AtNad6-F-R1 | CTCATCTGCTCACGAATTGG | STS-Seq |
| AtAtp6-F-F1 | TTGACGGAGTGAAGCTGTCT | STS-Seq |
| AtAtp6-F-R1 | TCCATCCCTCGCTTTTGTTC | STS-Seq |
| AtAtp8-F-F1 | AAGCTGTCTGGAGGGAATCA | STS-Seq |
| AtAtp8-F-R1 | TCGAGTGCTTTACCTACCAG | STS-Seq |
| AtMatR-F-F1 | AAGGGGAGCAAAAAACGAGC | STS-Seq |
| AtMatR-F-R1 | AGTAAACGCCTGTTCGCATC | STS-Seq |
| AtCox3-F-F1 | AGGCCCCAAAGATAAAGAGC | STS-Seq |
| AtCox3-F-R1 | TCTTTGGAAAAGCCCGGTTC | STS-Seq |
| AtCcb382-F-F1 | ACCGGATGATGGGAATAACG | STS-Seq |
| AtCcb382-F-R1 | AGTCGAGTCATTAGGGTTCC | STS-Seq |
| AtCcb203-F-F1 | TGCTCCCTACGACGGTAAAT | STS-Seq |
| AtCcb203-F-R1 | AGCCAATTGCTGGCTCTGAA | STS-Seq |
| AtRps12-F-F1 | GTGATAGGGCACAAAATGGG | STS-Seq |
| AtRps12-F-R1 | TGATTGTTCCACCGACTGAC | STS-Seq |
| AtNad3-F-F1 | TTCGATATGCCGCTTCTTCG | STS-Seq |
| AtNad3-F-R1 | CCCATTTTGTGCCCTATCAC | STS-Seq |
| AtAtp9-F-F1 | GTAAGAATCGACGAGGAATC | STS-Seq |
| AtAtp9-F-R1 | GAGGTGCTTGCTTTATGAGA | STS-Seq |
| AtAtp6-2-F-F1 | AGTGTTCGGGTACAGTAGCT | STS-Seq |
| AtAtp6-2-F-R1 | AGACATCCATCCCTCGCTTT | STS-Seq |
| AtAtp1-F-F1 | AAAGCGGTATTCCTCCTTGC | STS-Seq |
| AtAtp1-F-R1 | GCCTCTCCAGTCTTTGCTTA | STS-Seq |
| AtRps7-F-F1 | TCGTCATCGAAAGCGGCTT | STS-Seq |
| AtRps7-F-R1 | CTGACTGAATGACGAAGAGC | STS-Seq |
| AtCox1-F-F1 | CTAACCCCTCTCTGATAAGG | STS-Seq |
| AtCox1-F-R1 | AGGTTCTTAGTAGCAGTCGG | STS-Seq |
| Atnad2-F-F1 | CGCTGAAGACCGTAACGTAA | STS-Seq |
| STS-d5-i2-F | CGTACACTTTAGTGGCAATCG | STS-Seq |
| STS-d5-i2-R | TTAACATCACTACGGTCGGG | STS-Seq |
| STS-d7-i2-F | GGGCTCCTATTGAAAGGCTT | STS-Seq |

| | | |
|---|---|---|
| STS-d7-i2-R | CTCGTAATGGTACCTCGCAA | STS-Seq |
| AtNad6-Lder-F1 | GGGCTTGGAAGAAGAAAATG | STS-Seq |
| AtRpl16-Tler-F | GGTTAATGGGGATAAAGCGG | STS-Seq |
| AtRpl16-Tler-R | GAGAGTTCCTTCTCCATAC | STS-Seq |
| AtRps14-F-F1 | GAGACTTTACCACTGTGGAG | STS-Seq |
| AtRps14-F-R1 | TTACCTTGCTTGTGGACCAG | STS-Seq |
| AtRps7-Lder-F1 | CTTGTTGGGAGAGGTTGTGA | STS-Seq |
| AtRps7-Lder-R1 | CGCAATCGTTACTGTCCATC | STS-Seq |
| orf114-F-F1 | CATCCAACGGATGGCTCTAT | STS-Seq |
| orf114-F-R1 | GGAACACCGAGTAGGATCAA | STS-Seq |
| AtCox2-F-F1 | GAAGGAACCTTTGCTTTG | STS-Seq |
| AtCox3-Tler-F1 | GGGAGGTATATGAAGGAACG | STS-Seq |
| AtCox3-Tler-R1 | GACCAAGATCTAATTTCGGG | STS-Seq |
| AtccmB-EF2 | GTAAGGAAATGAGACGAC | STS-Seq |
| AtccmB-ER2 | GTAACATGGGAAAACCAC | STS-Seq |
| ATMNAD5-F1 | ATGTATCTACTTATCGTATTTTTGCCC | STS-Seq |
| ATMNAD5-R1 | TTATTCTTGACTTGACTTTGTATAAAAAC | STS-Seq |
| ATMNAD1-F1 | ATGTACATAGCTGTTCCAGCTGAAAT | STS-Seq |
| ATMNAD4-F1 | ATGTTAGAACATTTCTGTGAATGC | STS-Seq |
| ATMNAD4-R1 | TCAATGAAATTTGCCATGTTGCAC | STS-Seq |
| Atnad7-F2 | TTGGTACTGTCACTGCACAG | STS-Seq |
| ATMNAD7-R1 | CTATCTATCCACCTCTCCAAACAC | STS-Seq |
| qAtrpl2-e1-2-F | CCGAAGACGGATCAAGGTAA | qRT-PCR |
| qAtrpl2-i1-F | TGCTTCTCTAATAGCCCCGT | qRT-PCR |
| qAtrpl2-e1-2-R | CGCAATTCATCACCATTTTG | qRT-PCR |
| qAtrps3-e1-2-F | CCGATTTCGGTAAGACTTGG | qRT-PCR |
| qAtrps3-i1-F | TCTACGGCGGGGTCACTAT | qRT-PCR |
| qAtrps3-e1-2-R | AGCCGAAGGTGAGTCTCGTA | qRT-PCR |
| qAtcox2-e1-2-F | TGATGCTGTACCTGGTCGTT | qRT-PCR |
| qAtcox2-i1-F | AGCAGTACGAGCTGAAAGGC | qRT-PCR |
| qAtcox2-e1-2-R | TGGGGGATTAATTGATTGGA | qRT-PCR |
| qAtccb452-e1-2-F | CACATGGAGGAGTGTGCATC | qRT-PCR |
| qAtccb452-i1-R | CCCGGATCGAATCAGAGTT | qRT-PCR |
| qAtccb452-e1-2-R | GTGGGTCCATGTAAATGATCG | qRT-PCR |
| qAtnad1-e1-2-F | TTGCCATATCTTCGCTAGGTG | qRT-PCR |
| qAtnad1-i1-F | CGTGCTCGTACGGTTCATAG | qRT-PCR |
| qAtnad1-e1-2-R | GACCAATAGATACTTCATAAGAGACCA | qRT-PCR |
| qAtnad1-e2-3-F | TCTGCAGCTCAAATGGTCTC | qRT-PCR |
| qAtnad1-i2-R | GGTTGGGTTAGGGGAACATC | qRT-PCR |
| qAtnad1-e2-3-R | ATTCAGCTTCCGCTTCTGG | qRT-PCR |
| qAtnad1-e3-4-F | TCCGTTTGATCTCCCAGAAG | qRT-PCR |
| qAtnad1-i3-F | GGGAGCTGTATGAGCGGTAA | qRT-PCR |
| qAtnad1-e3-4-R | AAAAGAGCAGACCCCATTGA | qRT-PCR |
| qAtnad1-e4-5-F | TCTTCAATGGGGTCTGCTC | qRT-PCR |
| qAtnad1-i4-F | ACGGAGCTGCATCCCTACT | qRT-PCR |
| qAtnad1-e4-5-R | AGCCCGGGATCTTCTTGA | qRT-PCR |

| | | |
|---|---|---|
| qAtnad2-e1-2-F | GGATCCTCCCACACATGTTC | qRT-PCR |
| qAtnad2-i1-F | CCCATTCCTAACCAGTGGAG | qRT-PCR |
| qAtnad2-e1-2-R | GCGAGCAGAAGCAAGGTTAT | qRT-PCR |
| qAtnad2-e2-3-F | AATATTTGATCTTAGGTGCATTTTC | qRT-PCR |
| qAtnad2-i2-R | CCCGATCCGATAGTTTACAA | qRT-PCR |
| qAtnad2-e2-3-R | AAAGGAACTGCAGTGATCTTGA | qRT-PCR |
| qAtnad2-e3-4-F | CTATGGGTCTACTGGAGCTACCC | qRT-PCR |
| qAtnad2-i3-F | GGCGAATTTCAAACTTGTGG | qRT-PCR |
| qAtnad2-e3-4-R | GCGCAATAGAAAGGAATGCT | qRT-PCR |
| qAtnad2-e4-5-F | TATTTGTTCTTCGCCGCTTT | qRT-PCR |
| qAtnad2-i4-R | CTTATTCGTGGCAACCTTCC | qRT-PCR |
| qAtnad2-e4-5-R | CAAAGGAGAGGGGTATAGCAA | qRT-PCR |
| qAtnad4-e1-2-F | ATTCTATGTTTTTCCCGAAAGC | qRT-PCR |
| qAtnad4-i1-F | CCGTATGATGCGGAAGTCTC | qRT-PCR |
| qAtnad4-e1-2-R | GAAAAACTGATATGCTGCCTTG | qRT-PCR |
| qAtnad4-e2-3-F | AATACCCATGTTTCCCGAAG | qRT-PCR |
| qAtnad4-i2-F | GCGGAACGACCAGAAAAATA | qRT-PCR |
| qAtnad4-e2-3-R | TGCTACCTCCAATTCCCTGT | qRT-PCR |
| qAtnad4-e3-4-F | TTCCTCCATAAATTCTCCGATT | qRT-PCR |
| qAtnad4-i3-F | TCTAGCTTGGTTCGGAGAGC | qRT-PCR |
| qAtnad4-e3-4-R | TGAAATTTGCCATGTTGCAC | qRT-PCR |
| qAtnad5-e1-2-F | CCATGGATCTCATCGGAAAT | qRT-PCR |
| qAtnad5-i1-F | TTCGCAAATAGGTCCGACT | qRT-PCR |
| qAtnad5-e1-2-R | TGGACCAAGCTACTTATGGATG | qRT-PCR |
| qAtnad5-e2-3-F | CTGGCTCTCGGGAGTCTCTT | qRT-PCR |
| qAtnad5-i2-F | GTACGATCGTGTCGGGTGA | qRT-PCR |
| qAtnad5-e2-3-R | TACCTAAACCAATCATCATATC | qRT-PCR |
| qAtnad5-e3-4-F | GATATGATGATTGGTTTAGGTA | qRT-PCR |
| qAtnad5-i3-F | GCCGTGTAATAGGCGACCA | qRT-PCR |
| qAtnad5-e3-4-R | AACTCGGATTCGGCAAGAA | qRT-PCR |
| qAtnad5-e4-5-F | GTTCCTGCGTTTCGGATAT | qRT-PCR |
| qAtnad5-i4-F | CCTGTAAACCCCCATGATGT | qRT-PCR |
| qAtnad5-e4-5-R | AACATTGCAAAGGCATAATGA | qRT-PCR |
| qAtnad7-e1-2-F | ACCTCAACATCCTGCTGCTC | qRT-PCR |
| qAtnad7-i1-F | ACGGTTTTTAGGGGGATCTG | qRT-PCR |
| qAtnad7-e1-2-R | AAGGTAAAGCTTGAAGATAAGTTTTGT | qRT-PCR |
| qAtnad7-e2-3-F | GAGGGACTGAGAAATTAATAGAGTACA | qRT-PCR |
| qAtnad7-i2-F | AGTGGGAGAGCCGTGTTATG | qRT-PCR |
| qAtnad7-e2-3-R | TGGTACCTCGCAATTCAAAA | qRT-PCR |
| qAtnad7-e3-4-F | ACTGTCACTGCACAGCAAGC | qRT-PCR |
| qAtnad7-i3-F | TAAAGTGAAGTGGTGGGCCT | qRT-PCR |
| qAtnad7-e3-4-R | CATTGCACAATGATCCGAAG | qRT-PCR |
| qAtnad7-e4-5-F | GATCAAAGCCGATGATCGTAA | qRT-PCR |
| qAtnad7-i4-F | CGGCCAAATGACTACAGGAT | qRT-PCR |
| qAtnad7-e4-5-R | AGGTGCTTCAACTGCGGTAT | qRT-PCR |
| G3-LP | GTCGGAAGTAAGGTCACCTCC | Genotyping |

| | | |
|---|---|---|
| LBb1.3 | ATTTTGCCGATTTCGGAAC | Genotyping |
| G3-R4 | AAGGGCAACGAACGTAGTAG | Genotyping |
| B2-F1 | ATTTGGAGAGAACACGGGG | Genotyping |
| G3-R1 | ATCTCCGATCCTTCCAGCTT | Genotyping |
| B3-SF1 | TGTGGAATTGTGAGCGGATA | Genotyping |
| P2S1-R1 | GCAGACGAGGAACAACTTGT | Genotyping |

## Materials and Methods

### Complementation of Mutants

To complement the mutants under the control of seed specific promoter, the 2000 bp promoter region of *At2S1* was fused with the coding regions of *GRP23* and *MEF8S* at their 5' termini by PCR and the resulting fragments were cloned into pGWB1 vectors. To complement *grp23* mutant with GRP23-3MYC fusion, the coding region of *GRP23* was cloned into pGWB17 vector. To complement *grp23* with GRP23-TurboID fusion, the coding region of *GRP23* was fused with ORF of TurboID at 3' terminus by PCR and the resulting fragments were cloned into pGWB1 vector. The constructs were transferred into *Agrobacterium tumefaciens* EHA105 and transformed into the corresponding heterozygous T-DNA insertion plants by floral dip (1). Transformed plants were selected on MS plats containing hygromycin B resistance. T2 or T3 homozygous plants were used for experiments.

### Y2H

The sequences of interest genes encoding the mature proteins were cloned into the pGADT7 (AD) and pGBKT7 (BD) vectors. Y2HGold yeast strain was co-transformed with AD-interest and BD-interest constructs. Empty vectors were used as negative controls. Transformed yeast cells were selected on agar medium plates lacking leucine and tryptophan (SD-TL). Single transformants were grown in liquid SD-TL media overnight before they were diluted with liquid SD-TL media to $OD_{600}$= 0.5. 7 µl of dilutions were dropped onto the agar medium plates lacking leucine, tryptophan, histidine, and adenine (SD-TLHA) and SD-TLHA supplemented with X-α-Gal. The results were recorded after 3 days of incubation at 30 °C.

### BiFC and Subcellular Localization

The complete coding sequences of interest were cloned into BiFC vectors pSPYNE and pSPYCE (2), respectively, to enable the expression of proteins of interest fused either to the N-terminal 155 amino acids of YFP (nYFP) or to the C-terminal 86 amino acids of YFP (cYFP). Constructs were co-transfected into Arabidopsis protoplasts as previously described (3). The protoplasts were incubated under weak light for 18-22 hours, and the YFP fluorescence was determined using a Zeiss LSM 880 confocal microscope under the same setting. Mitochondria were stained with MitoTracker Red. Each combination was repeated three independent times with similar results.

The full coding sequence of GRP23 was cloned into pGWB5 vector to express GRP23-GFP fusion under the control of CaMV 35S promoter. The resulting construct was transferred into EHA105. Agrobacterium cells containing pGWB5-*GRP23* was adjusted with infiltration buffer (10 mmol/L MgCl2, 10 mmol/L MES (pH 5.6), 200 mmol/L Acetosyringone) to $OD_{600}$= 0.5 before infiltration. Young leaves of 4-week-old *N. benthamiana* were infiltrated with the cultures. At 22-24 hours after infiltration, small pieces of infiltrated leaves were excised, soaked with PBS containing 100 nM Mito Tracker Red and 10µg/ml DAPI for 1 hour, and observed under a Zeiss LSM 880 confocal microscope.

### Organelle purification and Western Blot

To obtain mitochondrial proteins, 10g roots of 35S::GRP23-3MYC complemented *grp23* plants or

Col-0 were ground at 4°C in extraction buffer (0.3 M Suc, 5mM tetrasodium pyrophosphate, 10mM $KH2PO_4$, pH 7.5, 2 mM EDTA, 1% [w/v] polyvinylpyrrolidone 40, 1% [w/v] BSA, 5 mM Cys, and 20 mM ascorbic acid). The homogenate was centrifuged for 5 min at 3,000 g to remove cell debris, and the supernatant was centrifuged for 10 min at 20,000 g. The pellet was resuspended in ice-cold wash buffer (0.3 M Suc, 1 mM EGTA, and 10 mM MOPS/KOH, pH 7.2) twice and crude mitochondria were solubilized in 2×SDS sample buffer.

To obtain chloroplast proteins, 10g leaves of 35S::GRP23-3MYC complemented *grp23* plants or Col-0 were ground at 4°C in extraction buffer (50 mM HEPES-NaOH, pH 8, 2 mM EDTA, 0.3 M mannitol, 1% (w/v) bovine serum albumin, and 2 mM DTT). Chloroplasts were isolated using a 50% Percoll gradient at 6,500 g for 15 min. Intact chloroplasts were collected, washed twice in wash buffer (50 mM HEPES-NaOH, pH 8, 2 mM EDTA, and 0.3 M mannitol), and solubilized in 2×SDS sample buffer.

To obtain nuclear proteins, 10g leaves of 35S::GRP23-3MYC complemented *grp23* plants or Col-0 were ground at 4°C in ice-cold homogenization buffer (0.01M trizma base, 0.08M KCl, 0.01M EDTA, 1Mm spermidine, 1 mM spermine, final pH 9.5). The homogenate was centrifuged for 5 min at 500 g, and Triton X-100 at a final concentration of 0.5% was added to the supernatant. The homogenate was pelleted by centrifugation at 1800 g for 20 min, and then the pellet was washed by ice-cold wash buffer twice. Nuclei were solubilized in 2×SDS sample buffer.

Proteins were separated by SDS-PAGE, transferred to nitrocellulose membranes, and subjected to immunoblotting using antibodies against the MYC tag (Sigma), the nuclear histone H3, the chloroplast D1 (4), and the mitochondrial Nad9 (5).

**TurboID-mediated Proximity Labeling**

The *grp23* mutants complemented by 35S::GRP23-TurboID were treated by 50 μM biotin for 4 hour. 2 g roots of treated plants were ground to fine powder with liquid nitrogen, and then the powder was suspended with 2 ml lysis buffer (50 mM Tris, pH 7.5, 300 mM sucrose, 1% Triton X-100, cOmplete EDTA-free protease inhibitors (Roche), and centrifuged at 12,000 g for 15 min. The supernatant was subjected to Zeba Spin Desalting Columns to remove free biotin. The resulting proteins were incubated with 100 μL Dynabead M-280 Streptavidin beads (Thermo Fisher Scientific, Waltham, MA) at 4 °C for 2 hours, and then the beads were washed 3 times with wash buffer (50 mM Tris, pH 7.5, 0.1% Triton X-100, cOmplete EDTA-free protease inhibitors (Roche)). The biotinylated proteins were eluted by 2×SDS sample buffer and separated by SDS-PAGE. The entire gel lane was cut and digested with trypsin, and the extracted peptides were analyzed by a Q-Exactive mass spectrometry (Thermo Fisher Scientific) coupled to an EASY-nLC 1000 (Thermo Fisher Scientific).

**RNA editing analysis**

Total RNA was extracted from leaves or entire seedlings using the Qiagen Plant RNeasy kit (Qiagen) according to the manufacturer's instructions and followed by a treatment with DNase I (New England BioLabs) to remove genomic DNA contamination. Reverse transcription reactions were performed with random hexamers by SuperScript II Reverse Transcriptase (Invitrogen). Primers to amplify the mitochondrial and plastid transcripts are described in Table S2. The RT-PCR amplicons were mixed in equimolar proportions and then sheared by sonication to generate 350 bp fragments. Sequencing libraries were generated using Truseq Nano DNA HT Sample preparation

Kit (Illumina USA) following manufacturer's recommendations. Sequencing was performed by Illumina NovaSeq platform and 150 bp paired-end reads were generated with insert size around 350 bp. Sequencing data was analyzed by VarScan2 program. The difference threshold for editing extent is defined as: (T/(C+T)% in mutant-T/(C+T)% in WT) ≥20%.

The amplicons were directly sequenced to verify their editing extents as well. The editing efficiency between the mutant and Col-0 was compared by SEQUENCHER 4.14 software (SoftGenetics, Cambridge, MA, USA).

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