Supplemental data

The following supplemental data are available

Supplemental Figure S1. Identification of *cr-osatp4* and *rps8* editing defects in *cr-osatp4*

Supplemental Figure S2. Subcellular localization of OsATP4-YFP when transiently expressed in rice protoplasts

Supplemental Figure S3. RNA editing of *rps8-182* in *ppr676-1*

Supplemental Figure S4. *rps8* editing defects in the W104 and C015 rice accessions

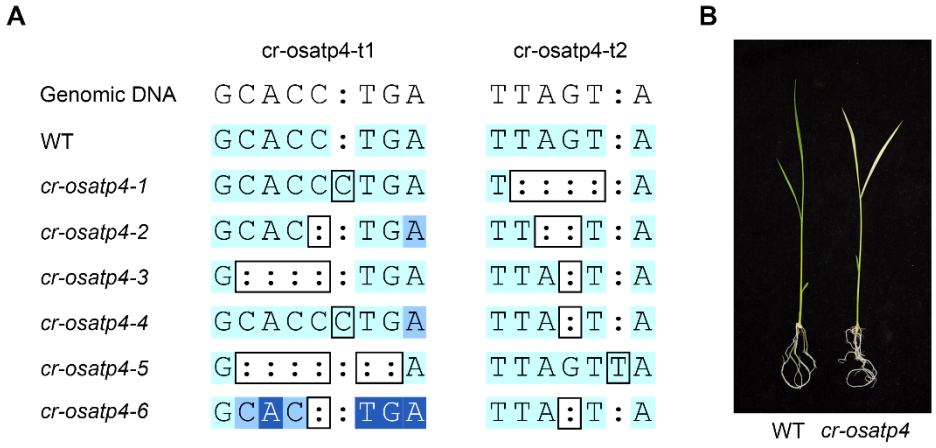
Supplemental Figure S5. RNA gel blot hybridization of *rpl16* transcripts in C015

Supplemental Figure S6. Accumulation of rRNAs in WT and *osatp4*

Supplemental Table S1. The editing efficiency of WT and *osatp4* under both 30℃ and 20℃ conditions

Supplemental Table S2. List of oligonucleotides used in this study

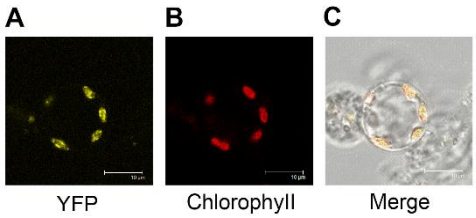
Supplemental Figure S1.



Supplemental Figure S1. Identification of *cr-osatp4* mutants

(A) CRISPR/CAS9 mutant lines of *OsATP4* (abbreviated as *cr-osatp4-1*, *cr-osatp4-2*, *cr-osatp4-3*, *cr-osatp4-4*, *cr-osatp4-5*, and *cr-osatp4-6*, respectively). The mutant lines were verified by PCR-based sequencing. Sequenced PCR product of the sgRNA target regions (cr-osatp4-t1 and cr-osatp4-t2) of WT and the CRISPR/CAS9 mutants were aligned with the genomic DNA sequence by Sequencher software. The missing/inserted base(s) are marked with black box. The background blue shading of bases indicates confidence levels of the nucleotide identity at that position: the darkest blue shading indicates low confidence and the lightest blue indicates high confidence. **(B)** The phenotype of *cr-osatp4* mutant at 20 °C.

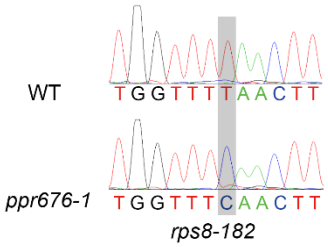
Supplemental Figure S2.



Supplemental Figure S2. Subcellular localization of OsATP4-YFP when transiently expressed in rice protoplasts

(A) Yellow fluorescence signals of P35S:OsATP4:YFP. **(B)** Chlorophyll autofluorescence signals. **(C)** Merged images of (A) and (B).

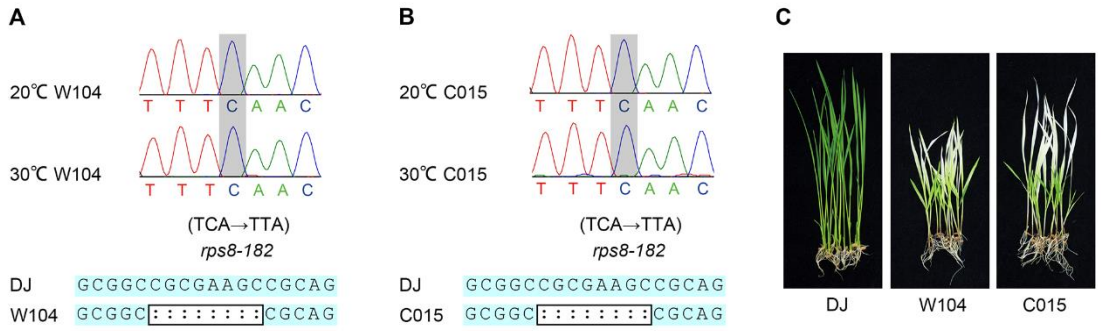
Supplemental Figure S3.



Supplemental Figure S3. RNA editing of *rps8-182* in *ppr676-1*

Sequence chromatograms of nucleotide sequences including the RNA editing site of *rps8-182* in *ppr676-1*, which was generated by CRISPR/Cas9 system in a Nipponbare background.

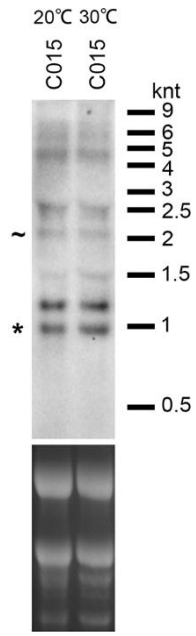
Supplemental Figure S4.



Supplemental Figure S4. *rps8* editing defects in the W104 and C015 rice accessions

The sequence chromatograms of nucleotide sequences including the RNA editing sites of *rps8-182* in W104 **(A)** and C015 **(B)** at 30 °C and 20 °C. The editing site of *rps8-182* is shaded in gray. *rps8-182* was unedited in W104 and C015 under both conditions. The *DUA1* gene in W104 and C015 were aligned with genomic DNA sequence of DJ (Dongjin) by Sequencher software. The 8 bp deletion base(s) in W104 and C015 are marked with black box. **(C)** Phenotypes of Dongjin (DJ), W104 and C015 at 20 °C.

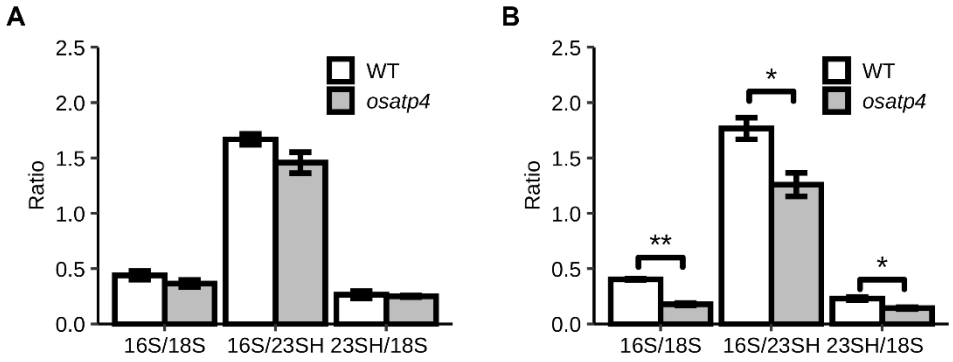
Supplemental Figure S5.



Supplemental Figure S5. RNA gel blot hybridization of *rpl16* transcripts in C015

Total leaf RNA (20 µg/lane) of C015 was analyzed by RNA gel blot using a *rpl16* exon 2 probe shown in Fig 6. The positions of RNA size markers are shown on the right side. ~ and * indicate the unspliced and spliced forms of processed *rpl16-rpl14* transcripts, respectively, according to previous results of maize *atp4* (Zoschke et al., 2013b). The ethidium bromide stained gels are shown below to indicate equal sample loading.

Supplemental Figure S6.



Supplemental Figure S6. Accumulation of rRNAs in WT and *osatp4*

The ratios of plastid 16S rRNA (component of the 30S subunit) to cytosolic 18S, plastid 16S rRNA to plastid 23SH rRNA (component of the 50S subunit), and plastid 23SH rRNAs to cytosolic 18S rRNA are shown for the WT and *osatp4*. "23SH" is a 1.1 kb fragment of the 23S rRNA (post-transcriptionally cleaved) in the 50S subunit of the chloroplast ribosome ("hidden break" product of the 23S rRNA) (Tiller et al. 2012). Data were obtained from three biological replicates for each plant line, and error bars indicate SE. Significant differences are marked with asterisks according to t-test (**: P<0.01; *: represent P<0.05).

(A) rRNA ratio in the WT and *osatp4* grown at 30 °C.

(B) rRNA ratio in the WT and *osatp4* grown at 20 °C.

Supplemental Table S1.

Supplemental Table S1. The editing efficiency of WT and *osatp4* under both 30℃ and 20℃ conditions

editing site	30℃ condition		20ºC treatment	
	WT	*osatp4*	WT	*osatp4*
atpA-1148	0.92	0.93	0.95	0.94
ndhA-473	0.94	0.96	0.99	0.95
ndhA-563	0.99	0.98	0.99	0.99
ndhA-1070	0.55	0.54	0.88	0.85
ndhB-467	0.99	0.99	0.99	0.99
ndhB-586	0.80	0.80	0.85	0.85
ndhB-611	0.80	0.80	0.90	0.90
ndhB-704	0.93	0.91	0.90	0.93
ndhB-737	0.50	0.50	0.70	0.70
ndhB-830	0.10	0.10	0.10	0.10
ndhB-836	0.89	0.90	0.90	0.90
ndhB-1481	0.71	0.78	0.77	0.83
ndhD-878	0.95	0.96	0.98	0.99
ndhF-62	0.63	0.71	0.80	0.86
ndhG-5-UTR-10	0.54	0.61	0.88	0.90
rpl2-2	0.59	0.61	0.66	0.66
Rps14-80	0.70	0.70	0.90	0.90
rpoB-467	0.16	0.23	0.47	0.43
rpoB-545	0.40	0.40	0.48	0.55
rpoB-560	0.60	0.60	0.80	0.80
rpoC2-4106	0.77	0.77	0.79	0.81
rps8-182	0.87	0.08	0.98	0.06
ycf3-185	0.84	0.84	0.86	0.82

Table S2 List of oligonucleotides used in this study

Primer name	**primers**	**Application**
P1	AGCCAAACCTGGTCGTGTAC	Genotyping
P2	TTGAGATGCGACTCGAACAC	
P3	CCACAGTTTTCGCGATCCAGACTG	
q-F	GGGCATCCTGAACGAGAT	qRT-PCR of OsATP4
q-R	TAGCAACGGATGAGCGAG	
Pcom-F	GGGGTACCCACTGGCCAAGTTACCAATTT	Complementation of *osatp4*
Pcom-R	GGGGTACCGGGGTATGCTTGGACTGAAA	
PsmrT1-U3-F	GGCAGCAGTGGTCGCTGCACCTGA	CRIPSR-Cas9 constructs
PsmrT1-U3-R	AAACTCAGGTGCAGCGACCACTGC	
PsmrT2-U6a-F	GCCGAAATCGGCAGAGTTAGTAG	
PsmrT2-U6a-R	AAACCTACTAACTCTGCCGATTT	
Hpt-F	AGAATCTCGTGCTTTCAGCTTCGA	Southern blot analysis
Hpt-R	TCAAGACCAATGCGGAGCATATAC	
Adapter attB1	GGGGACAAGTTTGTACAAAAAAGCAGGC T	Subcellular localization analysis
Adapter attB2	GGGGACCACTTTGTACAAGAAAGCTGGGT	
PtargetATP4-F	AAAAAGCAGGCTTCACCATGGCTTCCCCTT CCTCCCT	
PtargetATP4-R	AGAAAGCTGGGTGCACCGCTACTAACTCTG CCGA	
editing-ndhB-1F	GGAAATAGCATGGAATAAGGTTTG	RNA editing analysis
editing-ndhB-1R	TTCCTGGGGAGTTATACATTTGTG	
editing-ndhB-2F	ATTTACTCATGGGTGGGGCA	
editing-ndhB-a	TCCTTCGTAGACGTCAGGAG	

editing-ndhB-b	CCCACTCCAGTCGTTGCTTT	
editing-ndhB-2R	ATTACCCTAGCGCCCATTCC	
editing-rpoB-F	GGATGCTGTGTATGAATCAC	
editing-rpoB-R	CTTGGCTTGTTGTATCCGTC	
editing-rps8-F	ACCTCTATAAGAAACGCGGACA	
editing-rps8-R	AGACTTCTCCCCCAATTCTGTT	
editing-rps14-F	CCATCTAGGATTAGAACCGT	
editing-rps14-R	ATCTTGTTGCACCCGGTAAC	
AtpA-1148-F	ACGTTGGATGGGGTATTTCCGTTTCCAGAG	
AtpA-1148-R	ACGTTGGATGCCGCGAATTGAGCTAATTCC	
AtpA-1148-E	AAACAAGTAGCTGGCAAAT	
ndhA-473-F	ACGTTGGATGATAGATTGAGCGGCAGCTCG	
ndhA-473-R	ACGTTGGATGATTGGTCTTCTTATGGCAGG	
ndhA-473-E	GCTCGTAGACCACCT	
ndhA-563-F	ACGTTGGATGGCTGCCGCTCAATCTATTAG	
ndhA-563-R	ACGTTGGATGGACTGTGCTTCAACTATATC	
ndhA-563-E	CTATCTCTACTATCCAACAGTT	
ndhA-1070-F	ACGTTGGATGCTATTTCCCTGGGCAATCTC	
ndhA-1070-R	ACGTTGGATGTTGAGAGGACTTTTGTGTTG	
ndhA-1070-E	GCAATCTCTTATTAACAACTTCTT	
ndhB-704-F	ACGTTGGATGCCCCAGGAATTTCAATTGCG	
ndhB-704-R	ACGTTGGATGTAGACGTCAGGAGTCCATTG	
ndhB-704-E	GAATTTCAATTGCGCTTATAT	

ndhB-836-F	ACGTTGGATGGATGCCATTCGTTTGATGAG	
ndhB-836-R	ACGTTGGATGCACTCCAGTCGTTGCTTTTC	
ndhB-836-E	AGAATTCGCGTGGCT	
ndhB-1481-F	ACGTTGGATGGACTGTATGTGTGATAGCATC	
ndhB-1481-R	ACGTTGGATGTAAAAGAGGGTATCCTGAGC	
ndhB-1481-E	CGTATGTGTGATAGCATCTACTATAC	
ndhD-878-F	ACGTTGGATGGGAATTGTTACCTCATGCTC	
ndhD-878-R	ACGTTGGATGTTGAAATTGCGTTGGCCAAG	
ndhD-878-E	GATGCAAATAATCTATGCAGCTT	
ndhF-62-F	ACGTTGGATGAATCCCTCTTCTCCCACTTC	
ndhF-62-R	ACGTTGGATGAAAGCCCATATGCGACGAAG	
ndhF-62-E	CCCACTTCCAGTTATTATGT	
ndhG-5-UTR-10-F	ACGTTGGATGGTATTGGCCCAGGTAAATCC	
ndhG-5-UTR-10-R	ACGTTGGATGCCTAATCCCTTTTTTCTTCC	
ndhG-5-UTR-10-E	CCAGGTAAATCCATTATGGATAA	
rpl2-2-F	ACGTTGGATGTGCTCGGGATAGGTGTTTTG	
rpl2-2-R	ACGTTGGATGACCCTTCAACCGGGTTATTC	
rpl2-2-E	CTTCGTTTTGTATAAATGTTTCGCC	
rpoB-467-F	ACGTTGGATGCACCGGGACTATAATATCAG	
rpoB-467-R	ACGTTGGATGACGCGAGCCCATATCCTTTC	
rpoB-467-E	TAAGATTGGGGAGGAAGAT	
rpoB-545-F	ACGTTGGATGGGCTCGCGTGAGTAGAAAAC	
rpoB-545-R	ACGTTGGATGTCAGGGTAGGAAACATTATC	

rpoB-545-E	ACAAAAGATATCTATTCTAGTTCTAT	
rpoC2-4106-F	ACGTTGGATGGGGTTTCCGAAGATGGAATG	
rpoC2-4106-R	ACGTTGGATGATCGATTCATCCAAAGCCCG	
rpoC2-4106-E	AAGATGGAATGTCTAATGTTTTTT	
rps8-182-F	ACGTTGGATGGTTCGGAAACATCAGGAAAG	
rps8-182-R	ACGTTGGATGTTCCCTTTCTAGTCTTTCTC	
rps8-182-E	ATGAAAGTAACAGATATTTCTTGGTTT	
ycf3-185-F	ACGTTGGATGCGATCATAGGGATCACTTTC	
ycf3-185-R	ACGTTGGATGATGTTGGCTCAATCCGAAGG	
ycf3-185-E	ATATGGATCACTTTCTAGTCGC	
Nor-rps8-F	ATGGGCAAGGACACTATTGC	Northern blot analysis
Nor-rps8-R	TTACCATATATAACATAAGACTTCTCCCC	
Nor-rpl16-F	CCAAAAGAACCAGATTTCGTAAACA	
Nor-rpl16-R	ACCGAAGAAATTGACTTCGTATGG	

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