

Supplemental Figure S1.

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GmJ8 : ATGGCTGCTCAACTGTGGAGTTGTTGGTGGAAATGG----- : 38
MtJ8 : ATGGCTGCTACTACTGTGTGGTATTATTGGTGGTAA----- : 38
RcJ8 : ATGGCAACTGCATCTGTGGTATGATTGGAGGAAACGGCTGTGC : 44

GmJ8 : TTCGTCCTG---CTTCTTGGATGCGATTCAA---AGGCAAGGAAA : 76
MtJ8 : TGGAT-----CTTCAATGGATGCAATTTGG---TAGAAAGGAAA : 73
RcJ8 : TGGGTCTTCATCTTCTTGGTTCCAGATCAAGAATAAGGAAGAGA : 88

GmJ8 : GGAAGCAAACCAAGATGAACAAGTCAAGGATTAAGTTGCTCTTAT : 120
MtJ8 : AGAAGCAAATAAGATGAATAAAGTTAGGGTTTGTGCTCTAAA : 117
RcJ8 : AGAATCAAATGGCGAGAGATAGAGTCAAGTTTCTTGCCTTCT : 132

GmJ8 : TCT-----TCTTCTGTGATGGATCCTTACAAGACCTT : 152
MtJ8 : TCTAAATCTAAA TCTTCTTCTGTGATGGATCCTTAAGACTTT : 161
RcJ8 : TCT-----TCTTCTGCTGTATGGATCCTTACAAGACTTCT : 167

GmJ8 : AAGAATACAACCGGGTGCCTCTGAATCTGAGGTCAGGAAGGCTT : 196
MtJ8 : AAGAATTCACACTGGTGCCTCTGAATCTGATGTTAGAAAGGCIT : 205
RcJ8 : CAGGATTCACACTGGTGCCTCTGAATCCGAAGTCAAGAAAGCCT : 211

GmJ8 : TCAGGCAGCTTGCTCTGCAGTACCATCCAGACGTGTGCAGAGGG : 240
MtJ8 : TTAGACAACCTTGCTTTGCAGTACCATCCAGATGATGTTGCAAGGA : 249
RcJ8 : TTAGGCAGCTTGCTTTGCAGTATCATCCAGATGATGCAGAGGA : 255

GmJ8 : AGCAATTGTGGGGTGCAGTTTCAAGAAATCAACGAGGCTTATGA : 284
MtJ8 : AGAGATTGTGGGGTGCAGTTTCACTTATGATCAATGAGGCTTATGA : 293
RcJ8 : AGCAATTGCGGAGTGCAGTTTAGTCCGATTAAAGAGCCTATGA : 299

GmJ8 : TACTGTGATGCCCAACTTAAGAGGAGAAATCAAATGCGACAGAAAT : 328
MtJ8 : TATTGTGATGCTCCAATTTGAGAGAA-----AATGTGATAGAAA : 331
RcJ8 : TATTGTGATGAGCAGTTTGGAGAGGAGAAAGCAGATGAATCGCAGC : 343

GmJ8 : CGTATGAGGCTTA---T-----TATGATGCTGGAATTGATGAG : 363
MtJ8 : CTTATGAGACCAC---TACCACATATAATGAGAACAATGATGAG : 372
RcJ8 : TCTTTGAGTCATCATA TGAACCCATATGATCAAGGAGTCGATGAG : 387

GmJ8 : CCACCTGAGGGGAATGAACGATCCAGATTGGGACATGTGGGAGGA : 407
MtJ8 : TCATTTAGAGGAATGAATGATCCAGATTGGGCTATTGGGAAGA : 416
RcJ8 : CCAATGAGGGGAATGSAAGACCCTGATTGGGACATGTGGGAAGA : 431

GmJ8 : GTGGATGGGCTGGGAAGGAGCAGGAATCTGTGACTACTCTCTTC : 451
MtJ8 : ATGGATGGCTTGGGAAGGAGCAGGAATCCGTGACTACTCTCTTC : 460
RcJ8 : GTGGATGGCATGGGAAGGAGCTGGAATTAGAGACTACACATCC : 475

GmJ8 : ATATTAATCCTTACATTTGA : 471
MtJ8 : ATATTAATCCTTACATTTAA : 480
RcJ8 : ATATTAATCCTTACATTTGA : 495

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Supplemental Figure S1.
Alignment of *J8* cDNA
sequences from *Medicago*
(*MtJ8*), soybean (*GmJ8*) and
castor bean (*RcJ8*). Position of
the PsJ8-R2 primer used for
initial amplifications of *PsJ8*
from pea is shown as a blue line
with an arrowhead indicating the
direction of the primer. Stop
codons of *J8* cDNAs are
marked.

stop



Supplemental Figure S2

start
PsJ8a : ATGGCGGCTACTACTAATT-----GTTGTTGGTGGTAACGGAGCTGGTGTTCATGGATGCAATTTGGAAGAAAGGA : 71
PsJ8b : ATGGCGGCTACTACTACTGCTGGTGTATTGGTGGTATAGGATCTGGTGTTCATGGATGCAATTTGGAAGAAAGGA : 77
TOC12 : ATGGCGGCTACTACTACTGCTGGTGTATTGGTGGTATAGGATCTGGTGTTCATGGATGCAATTTGGAAGAAAGGA : 77

PsJ8a : AACGAAACAAAATAAGATGAATAGAGTTAGAGTTGTTGCTCATCTTATCTTCTTCTGTGACAGATCCTTATAAGA : 148
PsJ8b : AACGAAACAAAATAAGATGAACAGAGTTAGAGTTGTTGCTCATCTTATCTTCTTCTGTGACAGATCCTTATAAGA : 154
TOC12 : AACGAAACAAAATAAGATGAACAGAGTTAGAGTTGTTGCTCATCTTATCTTCTTCTGTGACAGATCCTTATAAGA : 154

PsJ8a : TATTAAAGATTCAACCAGATGCTTCTGAATCTGATGTTAGAAAGGCTTTAGACAGCTTGCTTTCAGTATCATCCA : 225
PsJ8b : TATTAAAGATTCAACCAGATGCTTCTGAATCTGATGTTAGAAAGGCTTTAGACAGCTTGCTTTCAGTATCATCCA : 231
TOC12 : TATTAAAGATTCAACCAGATGCTTCTGAATCTGATGTTAGAAAGGCTTTAGACAGCTTGCTTTCAGTATCATCCA : 231

2nd Exon ← → 3rd Exon

PsJ8a : GATGTTTGCAGAGGAAAAGATTGTGATGTGCCGTTTCACGTAATCAATGAGGCTTATGTTGTTGCGATTACAAATTT : 302
PsJ8b : GATGTTTGCAGAGGAAAAGATTGTGATGTGCCGTTTCACGTAATCAATGAGGCTTATGTTGTTGCGATTACAAATTT : 308
TOC12 : GATGTTTGCAGAGGAAAAGATTGTGATGTGCCGTTTCACGTAATCAATGAGGCTTATGTTGTTGAGTTATTATTATCT : 308

PsJ8a : GAGAGAAGAAGCAAAGAAGAGAGA--AACATATGAGAAGGAGAAGAAGAAGAAGATGTGTTATGATGATGAACCATTT : 378
PsJ8b : GAGAGAAGAAGCAAAGAAGAGAGA--AACATATGAGAAGGAGAAGAAGAAGAAGAGCTGTTATGATGATGAACCATTT : 384
TOC12 : ATAA----- : 312

stop codon of Toc12
PsJ8a : AGAGGAAAAGAAATGATCCAGATTGGGGATATTGGGAAGAAATGGATGGGTTATGAAGGAGCAGGAATTAGTAATAGTGA : 455
PsJ8b : AGAGGAAAAGAAATGATCCAGATTGGGGATATTGGGAAGAAATGGATGGGTTATGAAGGAGCAGGAATTAGTAATAGTGA : 461
TOC12 : ----- : -

stop

PsJ8a : CTTCTCTAATCATATTAATCCTTTTCATTTGATAAAGTTTATGTTAGG-----TAGTGATAGT--AATCTTTAAGT-G : 524
PsJ8b : CTTCTCTAATCATATTAATCCTTTTCATTTGATCAACTTTTATGTTAATGTAATTAGTATTAATTAACCCCTTTATTA : 538
TOC12 : ----- : -

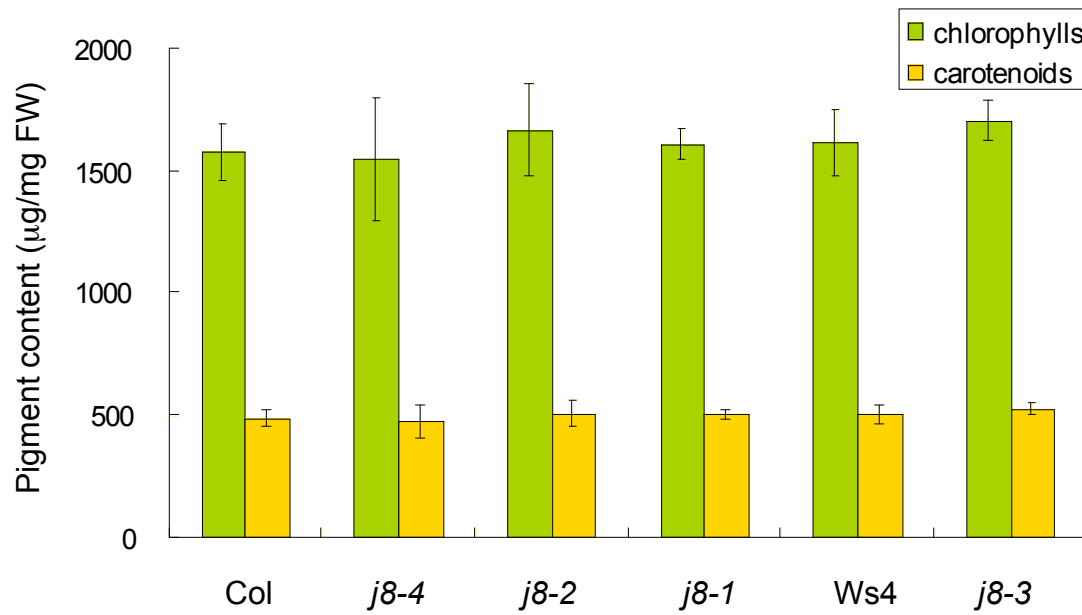
PsJ8a : CATATTATTACTATTAT--AGTTAAAATGGGTCTACTCTAGACCTACTACTGTGCCCATCCATCCAAAAGTGGTTG : 600
PsJ8b : TATGTTTATGTCATTAT--AGTTAAAATGGGT----TCTAGACCTACTACTGTGCCCATCCATAAAGTGGTTGGTTG : 611
TOC12 : ----- : -

PsJ8a : GTGCTTTGTATATA--TTCGTCCATGTTGATGTCAATTTTCTCTGCAT--ATTGAAAGTTCGCTTGTATTGTTATGCGT : 677
PsJ8b : GTGCTTTGTATATA--GTCCAAGTTGATGTCAATTTTCTCTGCAT--ATTGAAAGTTCAG--AATATT----TGGC : 679
TOC12 : ----- : -

PsJ8a : TAAATACAAATAAAGTATTGTTACATAAAAAAAAAAAAAAAAA : 720
PsJ8b : TTGTTATGATTTCTATTGTTATCTTTAAAAAAAAAAAAAAAA- : 721
TOC12 : ----- : -

Supplemental Figure S2. Sequence alignment of *PsJ8a* and *PsJ8b* cDNA and *TOC12*. Sequences of *PsJ8a* and *PsJ8b* from the start codon to the poly-A tail are shown. Stop codons of *PsJ8s* and *TOC12* are indicated in red and blue, respectively. The junction between the second and the third exons is also indicated.

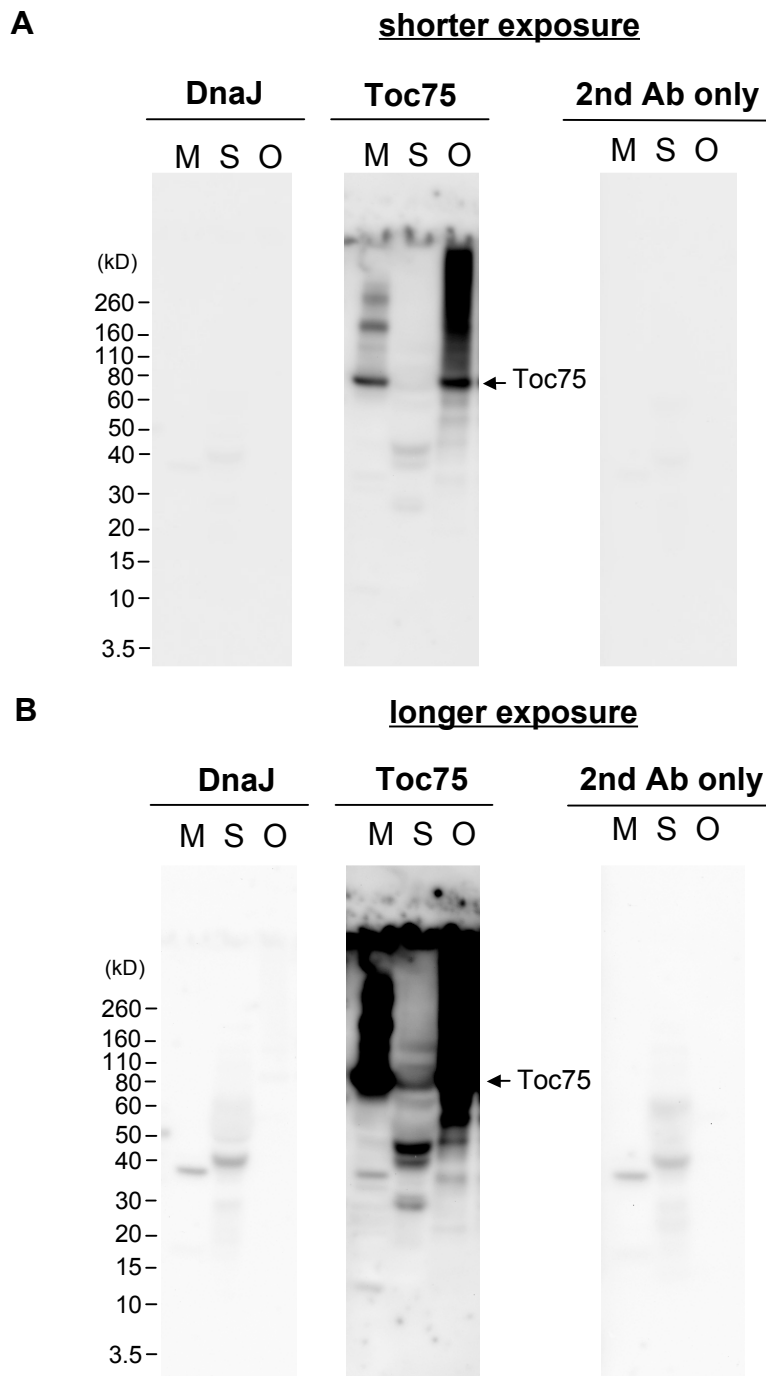
Supplemental Figure S3



Supplemental Figure S3. Pigment contents of *Arabidopsis j8* mutants.

Chlorophyll and carotenoid contents of 14-day-old wild type and *j8* mutants grown on MS medium were measured. Data are means \pm S. D., $n=8$.

Supplemental Figure S4



Supplemental Figure S4. The antibody against *E. coli* DnaJ failed to detect specific proteins in various fractions from pea chloroplasts.

Total membrane (M) and soluble (S) proteins (35 μ g of proteins in each lane), and the purified outer envelope membranes (O, 12.5 μ g of proteins) from pea chloroplasts were analyzed by SDS-PAGE followed by immunoblotting with rabbit antibodies against *E. coli* DnaJ (Stressgen, SPA-410) or pea Toc75, or with the HRP-conjugated donkey-anti-rabbit secondary antibody alone. Proteins were visualized using the HRP-conjugated secondary antibody and the ImmobilonWestern Chemiluminescent HRP system (Millipore), with the UVP BioSpectrum 600 Image System (Ultra Violet Products).

(A) Shorter exposure time of the blots.

(B) Longer exposure time of the blots.

Supplemental Table S1. Sequences of primers used in this study.

Primer Name	Primer Sequence (from 5' to 3')
PsJ8-F1	ggaaagcttttctgggttggtgaggttttggattttaaggatggcggctac
PsJ8-F6	tatgtgtgagttattattatcta
PsJ8-F8	tgctggctgtggcattcgcct
PsJ8-R2	tcaaatgtaaggattaatg
PsJ8-R7	tggttcacatcataacacc
PsJ8b-M1x-F	gccgcatggccgctggattattcggctactactactgctggtg
PsJ8b-M1x-R	caccagcagtagtagtagccgcaataatcccgcggccatggcggc
Toc12-3'UTR-R	ctaaattcaattcactatttgc
Toc12-R/T-F	caaaataagatgaacacagttacagtttggctcatct
Toc12-R/T-R	agatgagcaacaaactgtaactgtgttcatttatttg
Toc12MM-F	ttgtgagttattattatctaataatgtaaaatttcgttgcaata
Toc12MM-R	tatttgccaacgaaaatttaccatcattagataataataactcaca
AtJ8-KpnI-F1	cccggctaccatgacaattgctttaacgatc
AtJ8-EcoRI-R1	cccgaattctcaagcgtaggattaacgtg
AtJ8-F4	atgacaattgctttaacgatcggag
AtJ8-R5	atacacattggatgattctttcga
AtJ8-R6	gctcaacacaatatacgaagcttc
AtUBQ10-F	ggatctcactcgcgaccg
AtUBQ10-R	cttcttaagcataacagagacgag
AtJ8-Q-F1	tgttcttctcatctctgtaatgga
AtJ8-Q-R1	atfttaattgctcaacacaatcgtg
AtUBQ10-Q-F	cttcgcaagacttgaccg
AtUBQ10-Q-R	cttcttaagcataacagagacgag
AtJ8geno-F2	caaattgtctcaaagtattactgtgggtc
AtJ8geno-R2	acaagctaagggaagaagtgatacagaa
Salk-LBa1	tggttcacgtagtgggcatcg
Flag-LB4	cgtgtgccaggtgccacggaatagt
Wisc-L4	tgatccatgtagattcccggacatgaag
Gabi-LB	cccattggacgtgaatgtagacac

Supplemental Table S2. Primers used for amplification of PsJ8s and Toc12 cDNA and genomic DNA and site-directed mutagenesis.

Gene Amplified	Primers Used
PsJ8a/b cDNA ^a	PsJ8-F1 + PsJ8-R2
PsJ8a genomic	PsJ8-F1 + PsJ8-R2
PsJ8b genomic	PsJ8-F1 + Toc12-3'UTR-R; PsJ8-F6 + PsJ8-R7; PsJ8-F8 + PsJ8-R2
PsJ8b-M1x	PsJ8b-M1I-F + PsJ8b-M1I-R
Toc12RR ^b cDNA	PsJ8-F1 + Toc12-3'UTR-R
Toc12 (Toc12RR → TT ^c)	Toc12-R/T-F + Toc12-R/T-R
Toc12MM ^d	Toc12MM-F + Toc12MM-R
AtJ8 cDNA	AtJ8-KpnI-F1 + AtJ8-EcoRI-R1
UBQ10 (RT-PCR)	AtUBQ10-F + AtUBQ10-R
UBQ10 (Q-PCR)	AtUBQ10-Q-F + AtUBQ10-Q-R
AtJ8 (Q-PCR)	AtJ8-Q-F1 + AtJ8-Q-R1

^a After the initial amplification of PsJ8a/b cDNA using the primers listed, the authentic C-terminus sequence and 3' UTR was obtained by 3' RACE as described in the text. The sequences submitted to the GenBank were the corrected sequences after the 3' RACE.

^b Toc12RR is the Toc12 we amplified using primers indicated. Residues at position 34 and 36 in Toc12RR are both arginine, but in the published Toc12, these two residues are threonines.

^c This pair of primers are used for mutating the arginines at positions 34 and 36 in Toc12RR into threonine, which generated the Toc12 with sequence identical to the published Toc12 sequence.

^d This pair of primers are used for adding two methionine residues to the C terminus of Toc12.