

**Supplemental Figure 1.** Segregation of the *shot1* mutation, map based cloning, and RT-PCR in *shot1* mutants. (A) Segregation analysis of the suppressor phenotype and short hypocotyl phenotype. Hypocotyl growth of F2 individuals of a cross between *hot1-4* and *hot1-4 shot1-1*, before (blue bar) and after (red bar) heat treatment (38°C/3h), is shown on the y-axis. (B) Map-based cloning locates the suppressor gene on chromosome 3 (At3g60400). (C) Location of the missense allele, *shot1-1*, and the T-DNA insertion allele, *shot1-2*, on the *SHOT1* gene structure and RT-PCR of *SHOT1* gene in wild-type and *shot1* mutants. Note that the *SHOT1* gene has no introns, so no reverse transcriptase (-RT) control is included. Amplification from genomic DNA is also included as a control. RT-PCR reactions were performed to test whether the mutations affect the expression of

*SHOT1* with the following primers: P1, ATTGAAGAATCTGCCTTATGTGCT; P2, CTCCACAAGCCACTTAT GGAATCTA; P3, CTATGAAGGCCTTTTCGCTTG. M: DNA ladder. WT: Wild-type, *h1-4: hot1-4, s1-1: shot1-1, s1-2: shot1-2.* 



**Supplemental Figure 2.** SHOT1-GFP localizes to mitochondria. (A) Hypocotyl elongation assay shows that two independent homozygous T3 plants (Comp1 and Comp2) carrying *SHOT1-GFP* under the 35S promoter complement the phenotype of the *hot1-4 shot1-1*. Hypocotyl growth before (blue bars) and after (red bars) heat treatment (AC>45°C/1h) is shown. Error bars indicate standard deviation. N≥8. (B) *35Spro:SHOT1-GFP* construct was made by inserting the coding sequence of the *SHOT1* gene in front of GFP in the pCGTNG vector (GenBank accession number: DQ370425, provided by Dr. David Galbraith at the University of Arizona). The construct was transformed into *hot1-4 shot1-1* plants. T3 homozygous plants were obtained and confirmed to complement the *shot1-1* mutation. Leaves from homozygous plants were processed to isolate protoplasts

according to Sheen (Sheen, 1995).

Protoplasts were visualized under a confocal microscope (LSM model 510 META microscope, Zeiss). Mitochondria were stained with 0.5  $\mu$ M MitoTracker Orange CMTMRos (Invitrogen) in 10 mM MES/KOH buffer, pH5.7 for 30 min and washed twice with the buffer to remove excess dye.

The Meta module was used to perform spectral imaging at excitation wavelengths of 488 nm (for GFP) and 543 nm (for MitoTracker Orange) simultaneously. The images were processed for linear unmixing to discriminate GFP fluorescence from fluorescence that originated from autofluorescence and the MitoTracker dye.



**Supplemental Figure 3.** Complementation analysis and HSP101 protein level in *shot1* mutants. (A) Complementation of the growth phenotype of the *shot1-2* mutation by wild-type genomic DNA. All plants are three weeks old. (B) Complementation of the heat stress phenotype of *shot1-2* by wild-type genomic DNA. Hypocotyl length after heat treatment is shown relative to the length without heat treatment. The error bars

indicate standard errors of 12 samples. Asterisk indicates no growth after the heat treatment. C1 and C2 are two independent T3 homozygous transgenic plants transformed with *SHOT1* genomic DNA in *shot1-2* background. WT: wild-type. (C) HSP101 protein in *shot1* mutants accumulates to a similar level as in wild-type. Total protein extracts were prepared from 2.5-day-old dark-grown seedlings with (H) or without (C) heat treatment (AC>45°C/1h). (D) HSP101 protein level during recovery after heat treatment (AC>45°C/1h) is not significantly different between *hot1-4* (*h*) and *hot1-4 shot1-2* (*hs*) plants. Total protein was extracted before (C) and after the heat treatment at different recovery time points, R0: right after the heat treatment, R2: 2h after the heat treatment, R7: 7h after the heat treatment, R14: 14h after the heat treatment, and R23: 23h after the heat treatment.



**Supplemental Figure 4.** Heat stress phenotype of transgenic plants with altered AOX1a protein level. (A) Hypocotyl length after heat treatment is shown relative to the length without heat treatment. The error bars indicate standard errors of 12 samples. Asterisk indicates no growth after the heat treatment. (B) Heat tolerance test on light-grown seedlings. (C) Immunoblot analysis of AOX protein level. Protein was extracted from light-grown seedlings after heat treatment (AC>45°C/2h). Asterisk indicates cross-reacting bands for loading control. WT: Wild-type, AS-12: AOX1a antisense line, XX-2: AOX1a over-expression line.



**Supplemental Figure 5.** Plants with the *shot1-2* mutation have slightly higher manganese superoxide dismutase activity but lower catalase activity. (**A**) 10-day-old seedlings grown on agar plates were treated with or without heat shock (AC>45 °C/1h). 30  $\mu$ g of total protein was separated and stained for superoxide dismutase (SOD) activity as described in Miura et al. (2010). 10  $\mu$ g of total protein was separated for Coomassie blue staining of Rubisco. (**B**) 10-day-old seedlings grown on agar plates were treated with or without heat shock (AC>45 °C/1h). 10  $\mu$ g of total protein was separated and stained for coomassie blue staining of Rubisco. (**B**) 10-day-old seedlings grown on agar plates were treated with or without heat shock (AC>45 °C/1h). 10  $\mu$ g of total protein was separated and stained for catalase activity as described in Frugoli et al. (1996). The gel was later stained with Coomassie blue to determine equal loading. The experiments were performed twice (two biological replicates) with similar results. Comp: complemented *shot1-2* line, *s1-2: shot1-2, h1-3: hot1-3*.



**Supplemental Figure 6.** The chloroplast mTERF mutant *soldat10* does not show increased heat tolerance. Hypocotyl length after heat treatment (AC>45°C/120min) is shown relative to the length without heat treatment. Col: Columbia-0 wild-type for *hot1-3* and *shot1-2*. Ler: Landsberg erecta wild-type for *soldat10*. *hot1-3* Ler: *hot1-3* mutation introgressed into Ler eight times. Asterisk indicates no growth after heat treatment. The error bars indicate standard errors of at least 10 seedlings.



**Supplemental Figure 7.** Mitochondria appear normal in *shot1-2*. (A) Mitochondria from wild-type leaves. (B) Mitochondria from *shot1-2* leaves. Scale bars represent 500 nm. 10-day-old plate-grown seedlings were fixed with 2.5% glutaraldehyde, 2% paraformaldehyde, 0.1 M cacodylate (pH 7.3) under vacuum for 3 h at room temperature. Fixation was continued overnight at 4  $^{\circ}$ C, followed by a second fixation in 1% osmium tetroxide and 0.1 M cacodylate (pH7.3) in a microwave (85 Watts for total 6 min) under vacuum and 1 hr incubation at room temperature. The specimens were dehydrated through a series of ethanol concentrations and embedded in Spurr's resin. 70nm sections were cut onto uncoated 150 mesh copper grids, stained with 2% lead citrate for 2 min and observed with an FEI CM12S electron microscope operated at 80ky.



**Supplemental Figure 8.** Immunoblot analysis of cytochrome c level in the *shot1* mutants. A mitochondriaenriched fraction was separated by SDS-PAGE. Stained membrane is shown to check for transfer efficiency and equal loading. *s1-2: shot1-2*, Comp: complemented *shot1-2* line.



**Supplemental Figure 9.** Changes in transcript levels of genes involved in glycolysis, mitochondrial ETC and chloroplastic ETC of 22 °C-grown plants. MAPMAN analysis of transcripts associated with (**A**) glycolysis, (**B**) mitochondrial ETC, and (**C**) chloroplastic ETC. Each rectangle represents a single transcript. Red and blue colors indicate increase and decrease in *shot1-2* compared to wild-type, respectively. Scale bars represent log2 fold ratio of *shot1-2* to wild-type.

# Supplemental Table 1. GO analysis of differentially regulated transcripts in 22 °C-grown wild-type and *shot1-2* plants

A total of 700 differentially regulated genes were chosen for GO enrichment analysis based on the cut-off values, p<0.05 and log2 fold ratio > 0.5. The percentage of expected representation in the whole genome is also shown along with False Discovery Rate (FDR). Number of genes up- or down-regulated in *shot1-2* is shown in parenthesis.

GO Terms	Observed (%)	Expected	FDR
	(Up/Down)	(%)	
Biological Process			
response to stimulus (GO:0050896)	27.14 (109/81)	12.08	4.00E-23
response to stress (GO:0006950)	18.71 (77/54)	6.89	1.70E-21
response to chemical stimulus (GO:0042221)	15.86 (58/53)	6.17	3.50E-16
response to oxidative stress (GO:0006979)	4.86 (14/20)	1.00	1.80E-10
response to abiotic stimulus (GO:0009628)	9.57 (36/31)	4.19	3.60E-07
secondary metabolic process (GO:0019748)	4.71 (23/10)	1.50	7.40E-06
response to biotic stimulus (GO:0009607)	5.29 (32/5)	1.92	2.30E-05
response to other organism (GO:0051707)	5.00 (30/5)	1.78	2.90E-05
lipid transport (GO:0006869)	2.43 (11/6)	0.50	5.00E-05
lipid localization (GO:0010876)	2.43 (11/6)	0.57	0.00024
toxin metabolic process (GO:0009404)	1.43 (9/1)	0.18	0.00024
Molecular Function			
antioxidant activity (GO:0016209)	4.00 (10/18)	0.51	1.30E-12
peroxidase activity (GO:0004601)	3.57 (8/17)	0.41	1.60E-12
oxidoreductase activity, acting on peroxide as acceptor (GO:0016684)	3.57 (8/17)	0.41	1.60E-12
oxidoreductase activity (GO:0016491)	10.14 (33/38)	4.42	5.20E-08
heme binding (GO:0020037)	2.29 (3/13)	0.34	1.50E-06
water transmembrane transporter activity (GO:0005372)	1.57 (0/11)	0.14	3.30E-06
water channel activity (GO:0015250)	1.57 (0/11)	0.14	3.30E-06
electron carrier activity (GO:0009055)	3.71 (4/22)	0.99	3.30E-06
tetrapyrrole binding (GO:0046906)	2.43 (3/14)	0.44	4.40E-06
disulfide oxidoreductase activity (GO:0015036)	1.71 (5/7)	0.20	6.80E-06
iron ion binding (GO:0005506)	2.57 (4/14)	0.55	1.50E-05

oxidoreductase activity, acting on sulfur group of donors (GO:0016667)	2.00 (6/8)	0.34	2.20E-05
glutathione transferase activity (GO:0004364)	1.43 (9/1)	0.19	0.00014
lipid binding (GO:0008289)	2.86 (12/8)	0.83	0.00022
oxidoreductase activity, acting on phosphorus or arsenic in donors (GO:0030613)	0.86 (1/5)	0.05	0.00037

# Supplemental Table 2. GO analysis of differentially regulated transcripts in heat-stressed wild-type and *shot1-2* plants

Total of 484 differentially regulated genes were chosen for GO enrichment analysis based on the cut-off values, p<0.05 and log2 fold ratio > 0.5. The percentage of expected representation in the whole genome is also shown along with False Discovery Rate (FDR). Number of genes up- or down-regulated in *shot1-2* is shown in parenthesis.

GO Terms	Observed (%)	Expected (%)	FDR
	(Up/Down)		
Biological Process			
response to stress (GO:0006950)	23.35 (47/66)	6.89	1.30E-26
response to stimulus (GO:0050896)	29.96 (55/90)	12.08	2.60E-22
response to chemical stimulus (GO:0042221)	18.39 (25/64)	6.17	4.60E-17
response to abiotic stimulus (GO:0009628)	13.22 (30/34)	4.19	7.30E-13
response to oxidative stress (GO:0006979)	6.40 (10/21)	1.00	8.10E-13
response to temperature stimulus (GO:0009266)	6.40 (21/10)	1.37	1.50E-09
response to heat (GO:0009408)	3.72 (17/1)	0.50	3.90E-08
response to water (GO:0009415)	4.13 (5/15)	0.65	5.60E-08
response to water deprivation (GO:0009414)	3.93 (5/14)	0.62	1.30E-07
response to external stimulus (GO:0009605)	4.34 (8/13)	1.16	7.10E-05
secondary metabolic process (GO:0019748)	4.75 (10/13)	1.50	0.00028
response to inorganic substance (GO:0010035)	3.93 (10/9)	1.08	0.00032
response to endogenous stimulus (GO:0009719)	7.44 (9/27)	3.15	0.00032
response to biotic stimulus (GO:0009607)	5.37 (21/5)	1.92	0.00043
lipid localization (GO:0010876)	2.69 (6/7)	0.57	0.00065
lipid transport (GO:0006869)	2.48 (6/6)	0.50	0.00083
response to light intensity (GO:0009642)	1.86 (4/5)	0.27	0.00097

Molecular Function			
oxidoreductase activity (GO:0016491)	12.60 (18/43)	4.42	3.90E-10
water transmembrane transporter activity (GO:0005372)	2.27 (0/11)	0.14	1.60E-07
water channel activity (GO:0015250)	2.27 (0/11)	0.14	1.60E-07
oxidoreductase activity, acting on peroxide as acceptor (GO:0016684)	3.10 (1/14)	0.41	6.50E-07
peroxidase activity (GO:0004601)	3.10 (1/14)	0.41	6.50E-07
antioxidant activity (GO:0016209)	3.31 (2/14)	0.51	1.30E-06
heme binding (GO:0020037)	2.69 (1/12)	0.34	2.60E-06
tetrapyrrole binding (GO:0046906)	2.89 (2/12)	0.44	5.90E-06
electron carrier activity (GO:0009055)	3.93 (2/17)	0.99	4.90E-05
iron ion binding (GO:0005506)	2.89 (2/12)	0.55	6.00E-05
catalytic activity (GO:0003824)	38.64 (58/129)	29.64	0.00075

Locus ID	Description	Fold change
22 °C control		
AT3G04120	glyceraldehyde-3-phosphate dehydrogenase, cytosolic (GAPC)	2.04
AT4G37910	mitochondrial heat shock protein 70-1 (MtHSC70-1)	2.04
AT5G51440	23.5 kDa mitochondrial small heat shock protein (HSP23.5-MI)	1.74
AT4G21870	15.4 kDa nucleo-cytoplasmic small heat shock protein (HSP15.4-CV)	1.59
AT5G02500	heat shock cognate 70 kDa protein 1 (HSC70-1)	1.43
AT3G51910	heat shock transcription factor family protein HSFA7A	-1.58
AT1G54400	heat shock family protein AtAcd16.6	-1.63
Heat		
AT1G71000	DNAJ heat shock N-terminal domain-containing protein	2.71
AT5G51440	23.5 kDa mitochondrial small heat shock protein (HSP23.5-MI)	1.96
AT3G09440	heat shock cognate 70 kDa protein 3 (HSC70-3)	1.94
AT3G25230	peptidyl-prolyl cis-trans isomerase / FK506-binding protein (ROF1)	1.73
AT1G16030	heat shock protein 70B (HSP70B)	1.69
AT5G52640	heat shock protein 81-1 (HSP81-1)	1.68
AT5G09590	mitochondrial heat shock protein 70-2 (MtHSC70-2)	1.66
AT4G21870	15.4 kDa nucleo-cytoplasmic small heat shock protein (HSP15.4-CV)	1.64
AT1G59860	17.6 kDa class I heat shock protein (HSP17.6A-CI)	1.60
AT4G23100	glutamate-cysteine ligase / gamma-glutamylcysteine synthetase (GSH1)	1.60
AT2G47180	galactinol synthase	1.59
AT4G25200	23.6 kDa mitochondrial small heat shock protein (HSP23.6-MI)	1.57
AT5G56010	heat shock protein 81-3 (HSP81-3)	1.57
AT5G56030	heat shock protein 81-2 (HSP81-2)	1.53
AT3G12580	heat shock protein 70 (HSP70)	1.50
AT1G74310	heat shock protein 101 (HSP101)	1.49
AT1G07400	17.8 kDa class I heat shock protein (HSP17.8-CI)	1.48
AT3G04120	glyceraldehyde-3-phosphate dehydrogenase, cytosolic (GAPC)	1.45
AT1G20440	dehydrin cold regulated 47 (COR47)	-1.90

### Supplemental Table 3. Heat-responsive genes that are differentially regulated in *shot1-2*

**Supplemental Table 4.** Genes up-regulated in both *shot1-2* and *soldat10*. 257 genes are up-regulated in *shot1-2* (log2 fold ratio > 0.5) and 201 genes in *soldat10* (log2 fold ratio > 1) under control conditions. Eight commonly up-regulated genes are shown below.

Locus ID	ID Gene Description		Fold
Locus ID			(soldat10/Ler)
AT4G12470	Protease inhibitor/seed storage/lipid transfer protein (LTP) family protein	3.01	3.73
AT5G51440	23.5 kDa mitochondrial small heat shock protein (HSP23.5-MI)	1.74	3.43
AT4G12480	Protease inhibitor/seed storage/lipid transfer protein (LTP) family protein, PEARLI 1	3.59	2.72
AT1G80840	WRKY family transcription factor, WRKY40	1.98	2.50
AT2G43620	Chitinase family protein	1.53	2.84
AT5G43450	Putative 2-oxoglutarate-dependent dioxygenase	1.64	2.84
AT4G21870	26.5 kDa class P-related heat shock protein (HSP26.5-P)	1.59	2.63
AT1G72900	Toll-Interleukin-Resistance (TIR) domain-containing protein	1.55	2.11

Supplemental Table 5. List of genes and primer sequences used in estimation of mitochondrial and plastid genome copy numbers. Primer sequences are given in  $5^{2} \rightarrow 3^{2}$  orientation.

Gene (Locus ID)	Forward	Reverse
Nuclear genes		
AT1G19290	TTGTTCAAGCTTGGCGATTT	TGGCTTCATTGACCTTCTCC
AT3G48810	GGTAGATGGTGCGAAGAAGC	CAAACCAACCTCACACATCG
AT4G18750	CTCAGATCGGCATGTTGCTA	CCCGCTTGTCTCATTTGATT
<i>RPL5B</i> (AT5G39740)	CAGAAGACCTTTCCGTGCTC	CAAACACACGGTTTCCAGTG
POR (AT1G10390)	CTTCGGTGCCTCAAACTCTC	TTGAAGCTCCAAAAGCACCT
Mitochondrial genes		
nad3	CGAATGTGGTTTCGATCCTT	GCACCCCTTTTCCATTCATA
nad4L	GGGGAATCCTCCTTAATAGACG	AACGAAAATGGCTAACCCAATA
nad6	TATGCCGGAAAGGTACGAAG	GTGAGTGGGTCAGTCGTCCT
nad9	GGATGACCCTCGAAACCATA	CACGCATTCGTGTACAAACC
matR	AATTTTTGCGAGAGCTGGAA	TTGAACCCCGTCCTGTAGAC
Plastid genes		
petN	CGCATGGGCTGCTTTAAT	GAGTCCACTTCTTCCCCACA
ndhC	TATAGAACCGATCGGGGATG	AACTCATTGCCCACGGATAC
atpE	TCCACAAGAAGCTCAGCAAA	GTGTCCGAGCTCGTCTGAG
atpB	CCGTTTCGTACAAGCAGGAT	CGGGGTCAGTCAAATCATCT
petG	TCTAATTCCTATTACTTTGGCTGGA	CCAACTGATCACCACGTCTG

Locus ID	Gene Description	Fold (shot1-2/Col)	p-value
AT4G28510	PROHIBITIN 1 (PHB1), constituent of mitochondrial respiratory chain complex I	1.32	0.0448
AT4G05020	NAD(P)H Dehydrogenase B2 (NDB2)	1.57	0.0030
AT2G43400	Electron-Transfer Flavoprotein:Ubiquinone Oxidoreductase (ETFQO)	0.73	0.0311
AT3G22370	Alternative Oxidase 1a (AOX1a)	1.61	0.0019
AT3G10860	ubiquinol-cytochrome C reductase complex ubiquinone-binding protein	1.34	0.0246
AT4G10040	Cytochrome C-2 (CYTC-2)	1.30	0.0405
AT1G22840	Cytochrome C-1 (CYTC-1)	1.39	0.0248
AT1G53030	Cytochrome C Oxidase Copper Chaperone (COX17)	1.32	0.0318
AT1G69750	Cytochrome C Oxidase 19-2 (COX19-2)	1.31	0.0358
AT5G58970	Uncoupling Protein 2 (UCP2)	1.53	0.0440
AT3G54110	Plant Uncoupling Mitochondrial Protein (PUMP), Uncoupling Protein 1 (UCP1)	1.43	0.0124
AT5G13450	ATP Synthase delta subunit (ATP5)	1.32	0.0401
AT1G51650	ATP Synthase epsilon subunit	1.33	0.0381

### Supplemental Table 6. Changes in transcript levels of mitochondrial ETC components.

**Supplemental Table 7**. List of primer sequences used in the qRT-PCR for the validation of microarray experiments and generation of biotinylated complementary RNA (cRNA) probes. Primer sequences are given in

 $5' \rightarrow 3'$  orientation.

Locus ID	Forward	Reverse
MtHSC70-1 (AT4G37910)	GGGTACAAGTGGGACTGAGC	CATCCGGGACTCTCAATAGG
MtHSC70-2 (AT5G09590)	CACCCGAAATACAACCATCC	CCTCTTGGAGATGGAATGCC
HSFA2 (AT2G26150)	CAGCGTTGGATGTGAAAGTG	CCATAACTTAGACCGCAACAA
<i>HSC70-3</i> (AT3G09440)	GATGTTCCTCCTTCTGCCGG	CAAGCGAGCCCTCAAAATAG
HSP70B (AT1G16030)	TTGCAACCCAATCATCTCAA	TGGTCCAAAGCCACTCTTTC
HSP23.5-MI (AT5G51440)	GCGGAAATGAAGAATGGTGT	ACACATAGGACCGTGGGAAA
<i>HSP81-1</i> (AT5G52640)	GGAAGATGGTGATATGCCTGA	TGACACAAACCCAACCCTAGA
DnaJ (AT1G71000)	GCTCGTACGACGTTGGACTC	TTGCATTGATTGGTTCTGGA
HSP17.6A-CI (AT1G59860)	TGATTTGAGTTCTCTCTGGGTCT	CAAATACACACATTTCTCCACCA
Reference gene (AT2G32170)	ATCGAGCTAAGTTTGGAGGATGTAA	TCTCGATCACAAACCCAAAATG
ccmF <sub>C</sub>	ACTATTGAAATGGTTCGTCAGTAGAGATG	taatacgactcactatagggCGCTTCGCTGACCTA
		TCGC
coxl	GTAGGTAGCGGCACTGGGTG	taatacgactcactatagggATACCGAATCCAGG
		CAGAATGAG
atp9	CATAAGAGAAGACGAAGACGG	taatacgactcactatagggCTCACGCTTTGTCAT
		TCACTTA
psbA	AGGTTACAGATTCGGGCAAGA	taatacgactcactatagggAGCCTCAACAGCAG
		CTAGGTC
atpB	GAACCGCCAGGAGCTCGTATG	taatacgactcactatagggTGGCAGGTGCGGGG
		TCAGTC

Supplemental Table 8. List of primer sequences used in the qRT-PCR of mitochondrial transcripts. Primer sequences are given in  $5' \rightarrow 3'$  orientation.

Transcript (Locus ID)	Forward	Reverse
rpl2	CCGAAGACGGATCAAGGTAA	CGCAATTCATCACCATTTTG
rpl5	AAGGGGTTCGACAGGAAAGT	CGTATTTCGACCGGAAAATC
rpl16	GAGCATTTGCCAAACTCACA	CGGACACTTTCATCGTGCTA
rps3	CCGATTTCGGTAAGACTTGG	AGCCGAAGGTGAGTCTCGTA
rps4	ACCCATCACAGAGATGCACA	TCACACAAACCCTTCGATGA
rps7	CTCGAACTGAACGCGATGTA	AAGCTGCTTCAAGGATCCAA
rps12	AGCCAAAGTACGGTTGAGCA	TTTGGGTTTTTCTGCACCAT
matR	AATTTTTGCGAGAGCTGGAA	TTGAACCCCGTCCTGTAGAC
mttB	GGGGTCTTTCTTTGGAAACC	TCTCCCTCATTCCACTCGTC
cob	TGCCGGAATGGTATTTCCTA	GCCAAAAGCAACCAAAACAT
cox1	GTAGCTGCGGTGAAGTAGGC	CTGCCTGGATTCGGTATCAT
cox2	TGATGCTGTACCTGGTCGTT	TGGGGGATTAATTGATTGGA
cox3	CCGTAACTTGGGCTCATCAT	AAACCATGAAAGCCTGTTGC
ccmB	TCTTGGAATCACATCCAGCA	CGAGACCGAAATTGGAAAAA
ccmC	CGTGTCGTTCGTAATGGAAA	GCCGTGGCGATATAAACAAT
ccmFc	CACATGGAGGAGTGTGCATC	GTGGGTCCATGTAAATGATCG
ccmFN1	AGCTCTTGGCATTGCTTTGT	AGTGCCACAATCCCATTCAT
ccmFN2	CGTGTCGTTCGTAATGGAAA	TGATAAGCCCACCAACTTCC
atp1	TCACTTCGACACGTCTTTGC	GGAATGGCCTTGAATCTTGA
atp4	GGATCAGCTTGCGAATTTGT	GCAAATTGCTTCCCCACTAA
atp6-1	TCTTTTGCGAGTCAATGCAC	TCTCGCGTATCTCACATTGC
atp6-2	GCTTGGCAATCCTTGGTAGA	GACCAAGATGCAAGGGAAAA
atp8	CCGTCGACTTATTGGGAAAA	TTCCTTGGCCATGTACAACA
atp9	GGAGCTGCTATCGGTATTGG	TAGAGCAAAGCCCAAAATGG
nad1 ex1-2	GACCAATAGATACTTCATAAGAGACCA	TTGCCATATCTTCGCTAGGTG
nad1 ex2-3	TCTGCAGCTCAAATGGTCTC	ATTCAGCTTCCGCTTCTGG
nad1ex4	AAAAGAGCAGACCCCATTGA	GGGAGCTGTATGAGCGGTAA
nad1 ex5	AGCCCGGGATCTTCTTGA	ACGGAGCTGCATCCCTACT
nad2a	GGATCCTCCCACACATGTTC	GCGAGCAGAAGCAAGGTTAT
nad2b	TATTTGTTCTTCGCCGCTTT	CAAAGGAGAGGGGGTATAGCAA
nad3	CGAATGTGGTTTCGATCCTT	GCACCCCTTTTCCATTCATA

nad4	AATACCCATGTTTCCCGAAG	TGCTACCTCCAATTCCCTGT
nad4L	GGGGAATCCTCCTTAATAGACG	AACGAAAATGGCTAACCCAATA
nad5 ex1-2	TGGACCAAGCTACTTATGGATG	CCATGGATCTCATCGGAAAT
nad5 ex4-5	AACATTGCAAAGGCATAATGA	GTTCCTGCGTTTCGGATAT
nad6	TATGCCGGAAAGGTACGAAG	GTGAGTGGGTCAGTCGTCCT
nad7	ACTGTCACTGCACAGCAAGC	CATTGCACAATGATCCGAAG
nad9	GGATGACCCTCGAAACCATA	CACGCATTCGTGTACAAACC
18S mito	CGTCACCTGGGTCAAAAACT	GCTTGAAAACCGAAGTGAGC
26S mito	GACGAGACTTTCGCCTTTTG	CTTGGAGCGAATTGGATGAT
nucl 18S rRNA (AT3G41768)	AAACGGCTACCACATCCAAG	ACTCGAAAGAGCCCGGTATT
YLS8 (AT5G08290)	GGGATGAGACCTGTATGCAGATGGA	GCTCGTACATGGTGTTGAAGTCTGG
RPL5B (AT5G39740)	CAGAAGACCTTTCCGTGCTC	CAAACACACGGTTTCCAGTG
TUB6 (AT5G12250)	GGTGAAGGAATGGACGAGAT	GTCATCTGCAGTTGCGTCTT
<i>UBC</i> (AT5G25760)	TTCGTTCTCTTTGGGAAATTAGA	CTCGCTGTACCTCTTTGTATTCTTT
ACTIN2-8 (AT3G18780, AT1G49240)	GGTAACATTGTGCTCAGTGGTGG	AACGACCTTAATCTTCATGCTGC

Supplemental Table 9. List of primer sequences used in the qRT-PCR of plastid transcripts. Primer sequences

are given in 5'  $\rightarrow$  3' orientation.

Transcript (Locus ID)	Forward	Reverse
accD	TGTGGATTCAATGCGACAAT	TTTTGCGCAGAGTCAATACG
atpA	CGGAAATCTTACCTCGACCA	ATGGGTGACGGTTTGATGAT
atpB	CCGTTTCGTACAAGCAGGAT	CGGGGTCAGTCAAATCATCT
atpE	TCCACAAGAAGCTCAGCAAA	GTGTCCGAGCTCGTCTGAG
atpF	GCTCCTTCACGCAGTTCTTC	TACTTGGGTCACTGGCCATC
atpH	ATCCACTGGTTTCTGCTGCT	TTCCTTCTGCCTCAGGTTGT
atpI	ATTGGCAAATAGGGGGTTTC	GCCGTCAGTTGGAATTGTTT
ccsA	CACAATAACTGCGCCAAGTG	AACAGAGCGCCATAGCCTAA
cemA	TTTGCCCTGGTTGATCTCTC	TTGGATCGTTTCTTTGTGGA
ClpP1	GTCGGAGGAGCAATTACCAA	GTGATGGTTTCGCGAAGTTT
matK	ATCCTTTGTTGCCAGAATGC	TTTTTCTACGCAAGCGGTCT
ndhA	TTGACGCCACAAATTCCAT	TTAGGTGGTCTGCGAGCTG
ndhB	CCAGAAGAAGATGCCATTCA	TCATCAATGGACTCCTGACG
ndhC	TATAGAACCGATCGGGGGATG	AACTCATTGCCCACGGATAC
ndhD	TGGAGAATGGGAATAGATGGAC	TCCCGAGAAGAAAATGATCCTA
ndhE	TGGATTGATCACAAGTCGAAA	AGCGGCTGCAATTGCTATAA
ndhF	CGGCGGGTATTTTTCTTGTA	GGCTAAACCCCGCTTAATGT
ndhG	TTGCCTGGACCAATACATGA	ACATTTATGGCCCCCACATA
ndhH	ATGGGAAATTCAATGGCAAA	TCAAAGCCCCTGCTTTCTAA
ndhI	TTTGCCTGTTGTTGATTGGA	ATTGGTAAACGACCCAAAGC
ndhJ	CGTTTTCTGGGTTTGGAAAA	AGGCCACCCTATCCAACTCT
ndhK	GCAGTCCGCATATTGGAAAT	CGTGGGACGATACTGGACTT
petA	CAGAGGGCGAATCCATTAAA	GCCAAAACAACCGATCCTAA
petB	ATTGGGCGGTCAAAATTGTA	AGACGGCCGTAAGAAGAGGT
petD	TCCTTTTGCAACTCCTTTGG	CCGCTGGTACTGAAACCATT
petG	TCTAATTCCTATTACTTTGGCTGGA	CCAACTGATCACCACGTCTG
petI	TTTCGGTTTTCTACTAGCAGCTTT	TGCTTAGACCAATAAACAGAACTGA
petN	CGCATGGGCTGCTTTAAT	GAGTCCACTTCTTCCCCACA
psaA	GCCAAGAAATCCTGAATGGA	CATCTTGGAACCAAGCCAAT
psaB	GGACCCCACTACTCGTCGTA	ATTGCTAATTGCCCGAAATG
psaC	GAGCATGCCCTACAGACGTA	CAGGCGGATTCACATCTCTT

psaI	ACTTACCCTCTATTTTTGTGCCTTT	TGAATATGAAGAAATAAAGAAGCCATT
psaJ	ATGGTTCGGTTCGTTAGCAG	GGGAAATGTTAATGCATCTGG
psbA	GAGCAGCAATGAATGCGATA	CCTATGGGGTCGCTTCTGTA
psbB	CGTGCGACTTTGAAATCTGA	TAGCACCATGCCAAATGTGT
psbC	ACTTCCCCACCTAGCCACTT	AGCCCAAAACTGCAGAAGAA
psbD	CACAAATCTTTGGGGGTTGCT	CCATCCAAGCACGAATACCT
psbE	TGTCTGGAAGCACAGGAGAA	AACCGGTGCTGACGAATAAC
psbF	GGACCTATCCAATTTTTACAGTGC	GTTGGATGAACTGCATTGCT
psbH	TCTAGATCTGGTCCAAGAAGCA	CATTGCAACACCCATCAAAG
psbI	TTTCTCTCTTCATATTTGGATTCCT	TTCTTCACGTCCCGGATTAC
psbJ	CTGGAAGGATTCCTCTTTGG	CAGGGATGAACCTAATCCTGA
psbK	AGGCCTACGCCTTTTTGAAT	CGAAAACTTACAGCGGCTTG
psbL	CAATCAAATCCGAACGAACA	GAAATAATTCGAAAATAAAACAGCAA
psbM	TGCACTCTTCATTCTCGTTCC	TCATTTTGACTAACGGTTTTTACG
psbN	GGAAACAGCAACCCTAGTCG	CGTGTTCCTCGAATGGATCT
psbT	GGAAGCATTGGTTTATACATTTCTCT	AAATTTTAGGTGGTTCCCGAAA
psbZ	TGCTTTCCAATTGGCAGTTT	GTTACTCGACCAACCATCAGG
rbcL	GTGTTGGGTTCAAAGCTGGT	CATCGGTCCACACAGTTGTC
rpL14	AGCGGGGCTAGAGAATTGAT	ACTGCGGCATTGTCATCATA
rpL16	TGTACGACGTGGTGGAAAAA	GCATTTTTGATGCCGCTATT
rpL2	CGGACCTCTCCAGAAGGTAAT	AAATGGGAAATGCCCTACCT
rpL20	TCGGAGGCGTAGAACAAAAC	CGATGAGCCGAAACTAAAGC
rpL22	AAAGCTGAGGTGAACCAAGG	TGTCCCATAGGCCTCCACTA
rpL23	CGGTTATTGGGGAAAAATCA	TTTTAACCTTTCCGGGGGAGT
rpL32	CTCGAAAAAGCGTATTCGTAAAA	TGAAAAAGCTTTCAACGATGTC
rpL33	GCCAAGGGTAAAGATGTTCG	TTGATTTCCCCGTGAATTGT
rpL36	AAATAAGGGCTTCCGTTCGT	CCTCGGGTTGGAACAAATTA
rpoA	GCGATGCGAAGAGCTTTACT	CCAGGACCTTGGACACAAAT
rpoB	AAAAAGCACGGATACGGATG	CTTCTTGAATGCCCCGATTA
rpoC1	TCGGATACGAAGATATCAAATGG	TTAGTTATGGGCCTAGCAAAAGA
rpoC2	ATGGAGCCCGTAAAGGAGTT	CGTCTGCTAAGACACGACCA
rpS11	TACTTGTGGATTCCGGGGTA	CAGCTCGTTGCATACCTTGA
rpS12A	TCTCACACCGGGTAAATCCT	ATCCGAAACGTCACGAAATC
rps14	AATCCCCACCGCGTAATAGT	AACATGCCTGAACCATTTCC
rpS15	CAGGGGATCCGTTGAATTT	CGTTGACGTTTTCCCAGAAT

rp\$18	CAAGCGATCTTTTCGTAGGC	AAAGTCACTCTATTCACCCGTCT
rpS19	CACAATGATTGGCCATACGA	TTTGGCATGTCCTCGAAAAT
rpS2	GGGCTCGGTGTCATTATGTT	TCTTCAACACAGCTGCATCC
rpS3	CAATCCGTATGGGGATCCTA	GATCCATTCAACACGTGCAA
rps4	CGATTGGGTATGGCTTTGAC	ATGGTTTGGCAATTCCTCAG
rpS7	AAACTGCAAAATCCGATCCA	ATGAGTTGACCCGCCTACAC
rpS8	CGACCGGGTCTACGAATCTA	ATTTCTCCGCCGATTCTTTT
ycf1	TTTCGGAAGAAGGGGAAGAT	TTCGAACGTGGAATTCATCA
ycf15	GCGAACAACCGGAGCTATTA	CCGACATGCGTATTTTTGATT
ycf2.1	TAGCCCTCGGTCTATTGGTG	GGATCCACTTTTTGGGGGAAT
ycf3	TCCAATACTCAGCGGCTTG	TTCGGGCATTAGAACGAAAC
ycf4	TTTCTATGGGATCGCAGGTC	GGAAATCCCCAACGAAAAAT
rRNA 23S	GGGCGACTGTTTACCAAAAA	TTACCCGACAAGGAATTTCG
rRNA 16S	CGGTATCTGGGGAATAAGCA	GATTTGACGGCGGACTTAAA
nucl 18S rRNA (AT3G41768)	AAACGGCTACCACATCCAAG	ACTCGAAAGAGCCCGGTATT
YLS8 (AT5G08290)	GGGATGAGACCTGTATGCAGATGGA	GCTCGTACATGGTGTTGAAGTCTGG
<i>RPL5B</i> (AT5G39740)	CAGAAGACCTTTCCGTGCTC	CAAACACACGGTTTCCAGTG
<i>UBC</i> (AT5G25760)	TTCGTTCTCTTTGGGAAATTAGA	CTCGCTGTACCTCTTTGTATTCTTT
ACTIN2-8 (AT3G18780, AT1G49240)	GGTAACATTGTGCTCAGTGGTGG	AACGACCTTAATCTTCATGCTGC
Nucleoporin autopeptidase (AT1G10390)	CTTCGGTGCCTCAAACTCTC	TTGAAGCTCCAAAAGCACCT

#### **Supplemental References**

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