

# Supporting Information

Šimková et al. 10.1073/pnas.1202041109

## SI Materials and Methods

**Plant Material and Growth Conditions.** All experiments were performed with *Arabidopsis thaliana* ecotype *Columbia* (Col-0). The *flu* Col-0 line used in this work had been obtained by five backcrosses of *flu1-1* (1) in *Ler* with wild-type Col-0. Other lines used in this study were: *flu* AAA:*LUC*<sup>+</sup> (2); *flu* AAA:*LUC*<sup>+</sup> *caa39*, *flu* *caa39*, obtained by a backcross of *flu* AAA:*LUC*<sup>+</sup> *caa39* to *flu* Col-0 and *caa39* obtained by backcrosses to wild-type Col-0; *spo11-1-3* (SALK\_146172); *spo11-2* (SAIL\_551\_F05); *bin5*, kindly provided by J. Chory (The Salk Institute, San Diego); *rhl2-1*, *hyp6*, *rhl1-2*, and *bin4-1*, kindly provided by K. Sugimoto-Shirasu (Riken Institute, Yokohama, Japan); and *RHL1-CFP* and *HA-RHL2* transgenic lines, kindly provided by Viktor Kirik (Carnegie Institution of Washington, Pasadena, CA). Sequences of primers used for genotyping the mutant lines are listed in Table S5. Seeds were surface-sterilized and grown on Murashige and Skoog (MS) medium (without sucrose) including vitamins and 2-(N-morpholino)ethanesulfonic acid (MES) buffer (M0255; Duchefa) and 0.8% (wt/vol) agar (Sigma-Aldrich) at 20 °C in continuous light (80–100 μmol·m<sup>-2</sup>·s<sup>-1</sup>) unless otherwise indicated. High-light stress experiments were performed using FYTO-LED light panels (SL3500; Photon Systems Instruments).

**Identification and Complementation of the *caa39* Mutation.** A segregating F2 mapping population was generated from a cross of *flu* AAA:*LUC*<sup>+</sup> *caa39* in Col-0 with *flu* AAA:*LUC*<sup>+</sup> in *Ler*. Of 1,700 F2 plants, 500 homozygous *flu* AAA:*LUC*<sup>+</sup> *caa39* mutants were selected based on high constitutive luciferase expression in continuous light. The *CAA39* locus was mapped using CAPS (cleaved amplified polymorphic sequence) or SLP (simple sequence length

polymorphism) markers listed in The *Arabidopsis* Information Resource database (TAIR, [www.arabidopsis.org](http://www.arabidopsis.org)). Additional markers used for mapping were designed based on the collection of predicted *Arabidopsis* SNP and small insertions/deletions in the publicly available *Columbia* and *Landsberg erecta* sequences generated by Monsanto (<http://www.arabidopsis.org/Cereon>) and are provided in Table S1. For complementation, the full-length coding sequence of *AtTOP6A* amplified by PCR (Table S2) was cloned in pCAMBIA1302 binary vector under the control of the CaMV 35S promoter and introduced into *flu* *caa39* via *Agrobacterium*-mediated transformation as described (3). Positive transformants were selected on hygromycin-containing media.

**RNA Isolation, Quantitative RT-PCR, and Microarray Analysis.** Quantitative RT-PCR was performed as described previously (2). Sequences and efficiencies of primers used for qRT-PCR are listed in Table S3. Validation of the reference genes *PRF1*, *ACT2*, *PP2AA3*, *GAPC2*, and *UPL7* (4) was performed with geNorm (5) and is presented in Fig. S7. Microarray experiments were performed with three biological replicates, and full-genome Affymetrix *Arabidopsis* AGRONOMICS1 microarrays (Affymetrix) were used. Labeling of samples, hybridizations, and measurements were performed as previously described (6, 7). Signal values were derived using the RMA algorithm implemented in the statistical language R (8). Differentially expressed genes were selected using LIMMA (9) followed by multiple testing correction according to ref. 10. Genes were considered as differentially expressed if *P* < 0.05 and fold-change at least 1.5. The microarray data have been submitted to ArrayExpress with the experiment number E-TABM-1076.

1. Meskauskiene R, et al. (2001) FLU: A negative regulator of chlorophyll biosynthesis in *Arabidopsis thaliana*. *Proc Natl Acad Sci USA* 98:12826–12831.
2. Baruah A, Šimková K, Apel K, Laloi C (2009) *Arabidopsis* mutants reveal multiple singlet oxygen signaling pathways involved in stress response and development. *Plant Mol Biol* 70:547–563.
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4. Czechowski T, Stitt M, Altmann T, Udvardi MK, Scheible WR (2005) Genome-wide identification and testing of superior reference genes for transcript normalization in *Arabidopsis*. *Plant Physiol* 139:5–17.
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6. Hennig L, Menges M, Murray JA, Gruissem W (2003) *Arabidopsis* transcript profiling on Affymetrix GeneChip arrays. *Plant Mol Biol* 53:457–465.
7. Rehrauer H, et al. (2010) AGRONOMICS1: A new resource for *Arabidopsis* transcriptome profiling. *Plant Physiol* 152:487–499.
8. R Development Core Team (2009) *R: A Language and Environment for Statistical Computing* (R Foundation for Statistical Computing, Vienna, Austria).
9. Smyth GK (2004) Linear models and empirical bayes methods for assessing differential expression in microarray experiments. *Stat Appl Genet Mol Biol*, 3:Article3.
10. Storey JD, Tibshirani R (2003) Statistical significance for genomewide studies. *Proc Natl Acad Sci USA* 100:9440–9445.



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Sulfolobus      : -----MSSEFISKVDKEARRKAA : 18
Pyrococcus     : -----MKLKRKPKKFKFSYDPQKWL : 20
Methanosarcina : -----MEGEKGSKTRKGDALAR : 17
Arabidopsis    : MAD-----KKKRRKSKDDEAEELPFKSILESDDVITELLKSYISSSIKAAAGAGASSSSSKPLTLADLSLSSSSCREVADLSLSSV : 82
Populus        : MADS-----TSTKSRKRQPPDPSTTELLFKNLLKPSVILQTLQSLVIS-----T--ASASSSKPLTSLDLSLSSSSCREVADLSLSSV : 78
Oryza          : MSEKRRRGAGAGAASGSASKPRVTAASYAESLRSLKRPDASILATLRLSASCSKSPAGSSSSSSSASKALAAEDDPAASYIVVADQDSASVT : 97
Physcomitrella : MSS-----DGPRKQTQAALAFKKKLSKDPDILKIVTKFAEA-----AKEADVETRTLADLNLANSCKEVEVNLNTRSVV : 68
Chlamydomonas : MSK-----RGGADDKALALAKGKRIRGAEDVLKKVQALATQ-----LKNKSEVKTLLAEDLDPYNCREVVDDKGGDDMV : 68
Ostreococcus  : MSAB-----RRKVSADAGVNFALARLRDGEDGRPGSTSEVLRVAVN-----DLEREVKARGVKRKTLDLSIAEAYREVDVGGEEIA : 77
Thalassiosira : -----MNTTQVM : 7
Phaeodactylum : MASRNRREPLAS----GPAPRPDMSATSTRKRKAVALAADLVLKRCRQLRQSLSEAK-----AEIESSVVGLPEGIPSDIVEVVELPATQWL : 85
Cyanidioschyzon : MVAKRKKTATEPR---LSTASRKKWQAESGAVSSGTAADVAESFLNRVRELKREIESRE-----AAKQRFILQSSDASSAARIEVELDCDQVL : 88
Bdellovibrio  : -----MAKLLSIRDLKIDIP : 15
    
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**rhl-2**

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Sulfolobus      : NIIRDKFLINVEQIKKGEPLVMEIEMTLNANLYDEKRLKLLIGEKLRLENLDINEAKRFMQIVLMASTIY-DALVSDPEYFPIRDIYRGRKSHLL : 114
Pyrococcus     : KKLEDAAYKILEEVKAGKNPYFDVPTRGLSNVYFDEEARLKLKGDRLSRRYELNVAHAKRFMQLIMAYVK-RIVSEGKIASLREAYANKHTIPG : 116
Methanosarcina : EKLELEAKIYNQFEEVVPVSVLPSPTKANLAYSDESVDVWYGDRESESAKTVKGAQQLLKYATYDFLINEHLARNGRSTLRELYYISEGWDYA : 114
Arabidopsis    : TELETIVIVQIARSLLAGDGFSEFVPSRAASNOLYVPELDRIVKDRSTLPEASVSSVRKTTITRILLALIH-QLCLRNIHYTKRDLFYDVKLFQD : 178
Populus        : SELEALITISIVQSLILSGKGFSEFVPSRAASNOLYVPELDRIVKDRNTLPEANLSSVRKCTITARILSLIH-QLCLRNIHYTKRDLFYDVKLFQD : 174
Oryza          : SSINRVLAAARSLISGRGFSEFVPSRAASNOLYVPELDRIVVRESANPQANVATARKATTIARVLFVHV-AVLRRIIGHVTKRDLFYDVKLFQD : 193
Physcomitrella : LEVKSILAKSILSGNGFGYLLPSRSSANOLYVPELDRIVKDRASFPQANASVRRKTAITRRLQVHV-QLCTKQIHYTKRDLFYDVKLFQD : 164
Chlamydomonas : GELAAAMYNVAASILGGEGFAFVPSRAAGNOLYVPELDRIVRDAVSKPEASTATCRKAAVTTRILGHVH-ELLGKNIHYTKRDLFYDVKLFQD : 164
Ostreococcus  : AALEQVMIAVARSMRGGDFEYTPSRGATDQVYVEALDRIVKNTTRAAEAGTVSSVRKVTILTRVMQVHV-GVLSGEGHYTKRDLFYDVKLFQD : 173
Thalassiosira : EGENAVKIAKQVLAQKGFQLEIPSRASNOYVPELDRIVLGDKTGTSEFLNPKESRKSATITRVLQVHL-AVLLKRIHYTKRDLFYDVKLFQD : 103
Phaeodactylum : EGVEAALHIAQVLERKGFSLIPSRASNOLYVPELDRIVLGGKRSSEFLNPKESRKSATITRVMQVHL-AVLMKRIHYTKRDLFYDVKLFQD : 181
Cyanidioschyzon : AELERVILSAKSLLEGRGLSYKVPRESAGNOYVPELELERVVKDVAISN-ASVASVRKTTIMTRVLQVHV-ELCTRIHYTKRDLFYDVKLFQD : 184
Bdellovibrio  : KEARIADKVLKDESSKRPVLEAVKTSLDMSLYNAKVGYLTPGDIVVTELVNVSVOELARVVFLEMLL-RNLETGTVMNPKRELYYMCKGEIKG : 110
    
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**CAP**

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Sulfolobus      : KSIEGNKIVSEENTWDECKFSDSVIVLIEVFTSLRREMLILSKEKGGK-VGENTRIRSGN-----DVIDLSKTHGAYATEPTPLIDF-DVDAE : 203
Pyrococcus     : -----T-HENTFEDCSFSDPIIEELERMLQVLRREEMHITDRRGY-VYGDIVIKDGE-----DEFNASKLGGGAVVGTVEHIOF-PEINVD : 196
Methanosarcina : -----KFKCCGSDRLIELEILTLTSLQREYFHMPEEDGATVFGPIETIEQTKR-GERNIHCQKVDGEGGYQIFNVENIEF-QKHDS : 196
Arabidopsis    : -----QTQSDAVLDDVSCMLGCTRSSNLVIAAEKGV-VVGRLIIFSDNG-----DMIDCTKMGVGGKAI-PNIDRVGD-VQSDAM : 250
Populus        : -----QTQSDAVLDDVSCMLGCTRSSNLVIAAEKGV-VVGRLIIFSDNG-----DMIDCTKMGVGGKAI-PNIDRVGD-VQSDAL : 246
Oryza          : -----CAQSDAVLDDVSCMLGCTRSSNLVIAAEKGV-VVGRLIIFSDNG-----DRIDCTKMGVGGKAI-PNIDRVSG-VESDAL : 265
Physcomitrella : -----CGQSDIILDDVSCMLGCTRSSNLVIAAEKGV-VVGRLIIFEDG-----DRIDCTKMGVGGKAI-PNIDRVGD-VESDAL : 236
Chlamydomonas : -----CSQSDAILDDIACMLGCTRSSNLVIAAEKGV-VVGRLIIFEDG-----DFIDCTKMGVGGKAI-PNIDRVGD-VESDAS : 236
Ostreococcus  : -----QKQSDAVLDDVSCMLGCTRSSNLVIAAEKGV-VVGRLIQKEDG-----DEIDCTKMGVGGKAI-PNIDKIDT-VRTDAK : 245
Thalassiosira : -----CSQSDVLDVDTMIGCTRSSNLVIAAEKGL-VVGRLIIFEDG-----DFIDCTKMGVGGKAI-PYIDKIEH-VQSDAE : 175
Phaeodactylum : -----CAQSDVLDVDTMIGCTRSSNLVIAAEKGL-VVGRLIQEEEDG-----DFIDCTKMGVGGKAI-PYIDKIEH-VASDAE : 253
Cyanidioschyzon : -----QTQSDVLDVDTMIGCTRSSNLVIAAEKGI-VVGRVITQEDG-----DFIDCTKMGVGGKAI-PYIDKIEH-VTSDAE : 256
Bdellovibrio  : NP-----RLKPLDFDDCPESDAILFIDGMLIEVYREELNVFANDRGGQYSQQLVVTETLTDGDKAVILDLSTLGTSPFPQKPKQALKAKKID : 201
    
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**caa39**

**bin5**

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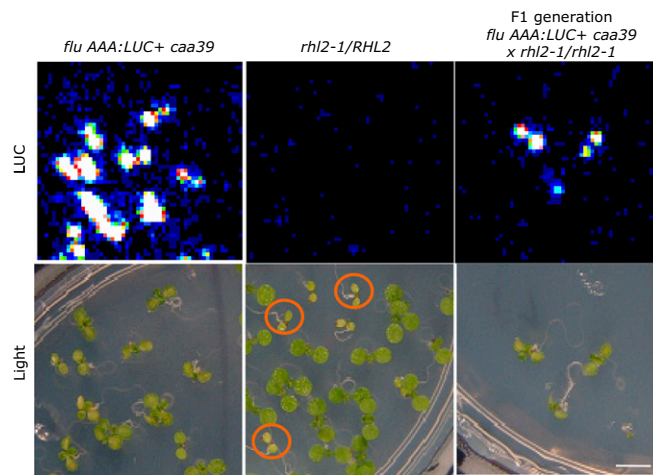
Sulfolobus      : FVLLVEKDAEYVQQLHRAGFVKQIKSLLISAGOPDRAIRREVRRLNEELKLPVYIITDADYEWYIFSVHRI-GSISLSVESERTATPDKWGLV : 299
Pyrococcus     : YVLLVETAMADRLIEEKYPKRERALLIATQCGASRGVRRILRHLHYEGLPLIIVTDDGDFYQWYLYSTIKQ-GSINILAMLEKATPAKFGMTM : 292
Methanosarcina : MIILAIETGGMYARLIMNGEADANALLVHLKQGPARTRIIKRMNEEIGPVAVETDGDPEWSYR-YASVAV-GALKSAHLSEFVAIPAAKFGLQ : 292
Arabidopsis    : FILLVEKDAAYMRLAEDRFVNRFPCCIIVTAKGQPDVATRFLLRKKMTELKLPVLAIVDSDDFYGLKILSVYGC-GSKNMSYDSANLTPDIKWGLV : 346
Populus        : FILLVEKDAAYMRLAEDRFVNRFPCCIIVTAKGQPDVATRFLLRKKMTELKLPVLAIVDSDDFYGLKILSVYGC-GSKNMSYDSANLTPDIKWGLV : 342
Oryza          : FILLVEKDAAYMRLAEDRFVNRFPCCIIVTAKGQPDVATRFLLRKLVEKLPVLAIVDSDDFYGLKILSVYMC-GSKNMSYDSANLTPDIKWGLV : 361
Physcomitrella : FILLVEKDAAYMRLAEDRFVNRFPCCIIVTAKGQPDVATRFLLRKKMTELKLPVLAIVDSDDFYGLKILSVYMS-GSKNMSYDSANLTPDIKWGLV : 332
Chlamydomonas : FILLVEKDAAYMRLAEDRFVNRFPCCIIVTAKGQPDVATRFLLRKLKSSLKIPVLAIVDSDDFYGLKILSVFVLSGSMNMSYDSANLTPDIKWGLV : 333
Ostreococcus  : FVLLVEKDAAYMRLAEDRFVNRFPCCIIVTAKGQPDVATRFLLSKVRDLKIPVLAIVDSDDFYGLKILSVYMC-GSKNMAFDSANLTPDIKWGLV : 341
Thalassiosira : FILLVEKDAAYMRLAEDRFVNRFPCCIIVTAKGQPDVATRFLLRKLVEKLPVLAIVDSDDFYGLKILSVYMS-GSKNMSYDSANLTPDIKWGLV : 271
Phaeodactylum : FILLVEKDAAYMRLAEDRFVNRFPCCIIVTAKGQPDVATRFLLRKLVEKLPVLAIVDSDDFYGLKILSVYMS-GSKNMSYDSANLTPDIKWGLV : 349
Cyanidioschyzon : FILLVEKDAAYMRLAEDRFVNRFPCCIIVTAKGQPDVATRFLLRKLVEKLPVLAIVDSDDFYGLKILSVYMS-GSKNMSYDSANLTPDIKWGLV : 352
Bdellovibrio  : FCLLVESEGTANTLVTMGFTKRNNQIVMGAQGVESVNGVGRKRLIQEEDVPMYFFGDADAYTMQNI-FRTLKAGSAAASLIRNADFSPANNKFLVGLV : 298
    
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**TOPRIM**

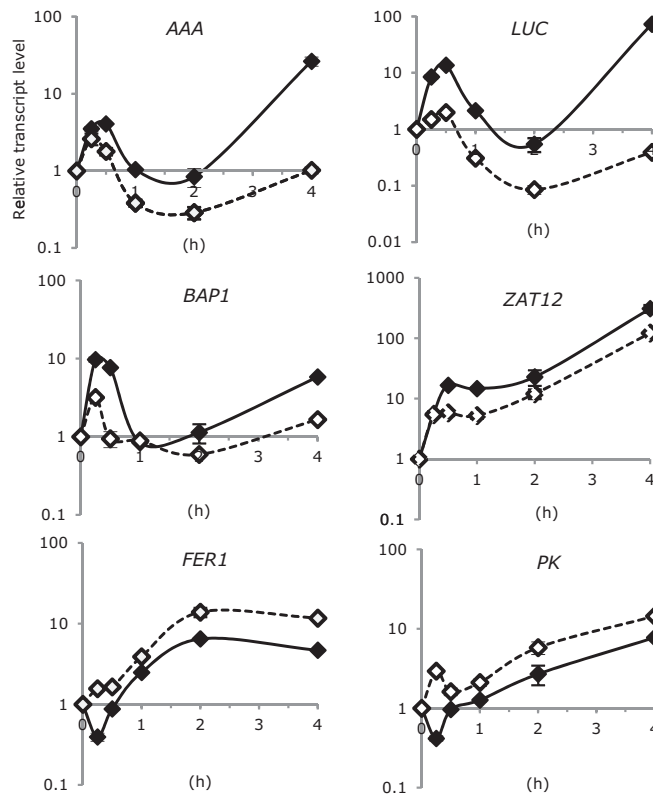
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Sulfolobus      : GDIFGSRKPKYLSEAERKNYIKAKDADIKRAEIKNYEWFK---TKAQBEINTFQORAKLETEAMASKG---LKFIAFOYIPEKITNKDYIA : 389
Pyrococcus     : DDIKENYGLHEVTEKLRGIPDPKGGPTGYKRLIEENYFPWFQ---NKEWROLKIALKGVVRIEQQALANKS---LEFVAKELYLPEKTRFGKLLP : 382
Methanosarcina : SDLIVEYELIS-----TDKLEQDYSALRSEISDRPFE---SDYKKBQIQHQLDIGKKAQQQFAGK---LDFVTEVYLLRNREKFMGMT : 369
Arabidopsis    : SLDLKYKIF-----EQCRIPMTEEQDIKTGKMLLEEDFVKK---NPGWVEELSLMVKTRKQKAEIQALSSFG---FOYLSVYLLKLLKQQEDWI : 427
Populus        : SLDLKYKIF-----EQCRIPMTEEQDIKTGKMLLEEDFVKK---NPGWVEELSLMVKTRKQKAEIQALSSFG---FOYLSVYLLKLLKQQEDWI : 423
Oryza          : SLDLKYKIF-----EQCRIPMTEEQDIKTGKMLLEEDFVKK---NEGWVKELETLVTRKQKAEIQALSSFG---FOYLTVEYLLKLLKQQEDWI : 442
Physcomitrella : SLDLKYKIF-----EQCRIPMTEEQDIKTGKMLLEEDFVKK---NKNWVKELETLVTRKQKAEIQALSSFG---FOYLSVYLLKLLKQQEDWI : 413
Chlamydomonas : SLDLDRDIF-----EQCRIPMTEEQDIKTGKMLLEEDFVKK---NPEWVRELELMLASVKAETIQALSSFG---FOYLSVYLLKLLKQQEDWI : 414
Ostreococcus  : SLDLDRDIF-----EQCRIPMTEEQDIKTGKMLLEEDFVKK---NPEWVRELELMLASVKAETIQALSSFG---FOYLTVEYLLKLLKQQEDWI : 422
Thalassiosira : SLDLNRVDF-----DQCRINMTENDIKTGKMLLEEDFVKK---NPKMKKELQMVKTRKQKAEIQALSSFG---FOYLTVEYLLKLLKQQEDWI : 352
Phaeodactylum : SLDLKYKIF-----EQCRIPMTEEQDIKTGKMLLEEDFVKK---NPEWVKELETLVTRKQKAEIQALSSFG---FOYLTVEYLLKLLKQQEDWI : 430
Cyanidioschyzon : TDLDRYGF-----EQCRIPMTEEQDIKTGKMLLEEDFVKK---NPAWVRELELMLASVKAETIQALSSFG---FOYLTVEYLLKLLKQQEDWI : 433
Bdellovibrio  : EDVKKYDLE-----HYKVESDPAEARALKKAKDALENDPFLDKKNKNLADILRLWIKERTRCBQCSFFSVDPKDPKIKLLEKIKRGSYV : 387
    
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**Fig. S2.** Multiple alignment of TOP6A/SPO11-3 proteins from archae, plants, green algae, diatoms, red algae, and proteobacteria. The proteins were aligned using CLUSTALW. Conserved CAP and topoisomerase-primase (TOPRIM) domains are indicated. Arrows show position of the amino acid exchange caused by *caa39* mutation and positions of *rhl-2* and *bin5* mutations. Accession numbers are as follows: *Sulfolobus shibatae* CAA71605.1; *Pyrococcus furiosus* AAL81702.1; *Methanosarcina mazei* NP\_634442.1; *Arabidopsis thaliana* NP\_195902.1; *Populus trichocarpa* XP\_002326329.1; *Oryza sativa* CAD79468.1; *Physcomitrella patens* XP\_001763679.1; *Chlamydomonas reinhardtii* XP\_001701722.1; *Ostreococcus tauri* CAL53806.1; *Thalassiosira pseudonana* XP\_002294481.1; *Phaeodactylum tricoratum* XP\_002177352.1; *Cyanidioschyzon merolae* CMQ111C; *Bdellovibrio bacteriovorus* CAE80644.1.



**Fig. S3.** Allelism test of *flu AAA:LUC<sup>+</sup> caa39* with *rh12-1* mutant. *flu AAA:LUC<sup>+</sup> caa39* mutant was crossed with the homozygous *rh12-1* mutant selected based on the mutant phenotype from the segregating *rh12-1/RHL2* F2 generation that was grown under long-day conditions. F1 generation seeds were plated on MS agar plates and grown under constant light (CL) conditions. Luciferase image (LUC) and visible picture (Light) of *flu AAA:LUC<sup>+</sup> caa39* seedlings, F2 segregating *rh12-1/RHL2* population, and F1 seedlings from the *flu AAA:LUC<sup>+</sup> caa39* × *rh12-1/rh12-1* cross-over are presented. Orange circles mark homozygous *rh12-1* plants. (Scale bar, 0.5 cm.)



**Fig. S4.** Response of the *flu AAA:LUC<sup>+</sup> caa39* mutant to high light stress conditions. Relative transcript levels of the  $^{1}O_2$ -responsive *AAA-ATPase*, *LUC* and *BAP1* genes, the  $H_2O_2$ -responsive *FER1* and *PK* genes and the general reactive oxygen species (ROS)-responsive marker gene *ZAT12* were analyzed in *flu AAA:LUC<sup>+</sup> caa39* (closed symbols) and *flu AAA:LUC<sup>+</sup> caa39* (open symbols) seedlings at the onset and 15 min, 30 min, 1 h, 2 h, and 4 h after the initiation of the high light (HL,  $1,050 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) treatment. Before HL stress, seedlings were grown for 6 d in low light (LL,  $12 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ). Relative transcript levels were determined by quantitative RT-PCR and expressed relative to the levels in LL. Results represent mean values of two technical replicates  $\pm$  SE.





**Table S1. Polymorphic markers used for rough and fine mapping of the *caa39* mutation**

chromosome or name	Position (bp)	Name	Forward primer 5' to 3'	Reverse primer 5' to 3'
Polymorphic markers used for rough mapping of the <i>caa39</i> mutation				
I	3164003	F21M12	GGCTTTCTCGAAATCTGTCC	TTACTTTTTGCCTCTTGTCATTG
	9621344	CIW12	AGGTTTTATTGCTTTTCACA	CTTTCAAAGCACATCACA
	20877364	NGA280	GGCTCCATAAAAAGTGACC	CTGATCTCACGGACAATAGTGC
	28185746	ATPase	GTTACAGAGAGACTCATAAACCA	CTGGGAACGGTTCGATTCGAGC
II	1078851	F19B11	CAAAGCTTTTGCTCTTGC	TGGGTTTTGATGTTTTCTTT
	13875000	T21L14	TTCAAAGTTTCCACCTCATGC	TATGTGTGAGGCCAAGAACC
	16885036	T3G21	CTCGTTCGTGCGTAGC	AAGCCAAGCAAACGCATC
III	178000	F4P13	CAAGCCAAAAGGTCTTCACC	TAATCCAGCCTCGCAAC
	9775545	CIW11	CCCCGAGTTGAGGTATT	GAAGAAATTCCTAAAGCATT
	18901818	CIW4	GTTCAATAAACTGCGTGTGT	TACGGTCAGATTGAGTGATTC
	22309429	T8B10	AGTGCTATTTGTAATCGCT	TCAACATTTCATTTTTCCA
IV	747854	CIW5	GGTAAAAATTAGGGTTACGA	AGATTTACGTGGAAGCAAT
	8110273	FCA6	CATGTTATCAGGACAATCTAACG	GCTTCGGCACCAGACTTGTA
	1164575	T10P11	TCATGATAACACAGGGCGTAA	TTCGCATATCTCATGCCATC
	1617020	ATA22	CTTTAGATGCTATACTAGGTT	CGATTTTGCATTGTTTTTATG
V	980000	MED24	TGCCTCCTGGGAAAGTG	GGCCCAAGCACACCTACA
	7428725	MWD9	CTCTACTAAATTTTCAGCT	TACGAATGTATTCACATGCA
	14952821	MPA22	GGTCTTGAATCGCAGCATA	GATGATAGTGACACCTTCTCATGT
	19493000	K20J1	TGGGAAGACGATGATGGA	CCGCATGATGCATAGCAA
Polymorphic markers used for fine mapping of the <i>caa39</i> mutation				
T20L15	340947	SSLP	AGATTGTTTCTCTCAGTGCTC	AGATTGTTTCTCTCAGTGCTC
T7H20	436559	SSLP	GAGGAGTTGTGCAAGTTGAG	TTAGTACCTAGCTAACGGACCT
T22P11	586645	CAPS	AGGCGTAACCATTGCACAC	ACGACGATCAGAGAACTAAGC
F9G14-1	620980	CAPS	TAAGAGGAACTAAAGAAGCCTG	CCCATATTCTATAGTCAATCGG
F9G14	660584	SSLP	ACAAATTCAT AAGTTTCGTC ACT	TATGCCGTTTACTGATAATCC
F9G14-2	661303	CAPS	CTCTTGAACAACCTCCGAAC	AATGTTTATCATCTGCGGGATG
F15A17	698509	SSLP	GAGAGGTTTATGACCCAACGAC	CTACAAAGCAAATATTCACGCC
F15A17-1	709015	CAPS	GAACACGAGGTCTTGCCCTTG	TCAGATGAATTAGATCAGGATC
F17C5	967107	SSLP	GTTTACCCTTAAGAACCCGA	TGCCGGTACTAGAATATGTT
MED24	1034836	SSLP	GCTTCTATTGGATGATGAGAC	CTACAAGTCCGATTGATTCCA
NGA225	1507038	SSLP	CAGAGGAGAATCGAATTGGAG	CTCATGATTTGGTGTGGTGC

**Table S2. Oligonucleotides used for cloning**

Gene name	Forward primer 5' to 3'	Reverse primer 5' to 3'
<i>AT5G02820</i>	GAT <u>GGGCC</u> CTTGACATTCGTCTTAC	CTA <u>CTAGT</u> CTGCCTCCGACTG

The underlined bases indicate the Apal and SpeI restriction enzyme sites added for cloning purposes.

**Table S3. Oligonucleotides used for quantitative RT-PCR**

Gene name	AGI	Forward primer 5' to 3'	Reverse primer 5' to 3'	Efficiency (%)
<i>PP2AA3</i>	<i>At1g13320</i>	CAGTATCGTCTCTCGCTCCAG	GTTCTCCACAACCGTTGGTC	95.2
<i>UPL7</i>	<i>At3g53090</i>	CTTCTGGGAGGTGATGAAAGG	CTCCAATAGCAGCCCAAAGAG	96.3
<i>Actin2 (ACT2)</i>	<i>At3g18780</i>	CATTCTTGCTTCCCTCAGCAC	CCCAGCTTTTTAAGCCTTTG	97.0
<i>Profilin 1 (PRF1)</i>	<i>At2g19760</i>	AGAGCGCCAAATTTCTCAG	CCTCCAGGTCCCTTCTTCC	98.1
<i>GAPC2</i>	<i>At1g13440</i>	GGCCATCAAGGAGGAATCTG	CITGGCATCGAAAATGCTTG	100.0
<i>AAA-ATPase (AAA)</i>	<i>At3g28580</i>	GGCAATCTTTCTCGTTTTACCC	GCTCATCGTCTTCCCTCTCTC	108.9
<i>At1g24145</i>	<i>At1g24145</i>	AATGGAACATCGCAAACCTCG	GTTGCAATTTTGGAGCCTTG	107.2
<i>AT1G24147</i>	<i>At1g24147</i>	CGCAAACCTCAAAGAGATGATCAC	TCAGGAAGATAAGTGGTGACGA	112.3
<i>BAP1</i>	<i>At3g61190</i>	GGTGATAAGTGTGGGATCGTC	GTCTCTAATCTCGGCCTCCA	96.5
<i>ERF5</i>	<i>At5g47230</i>	TTATGTGACTGGGATTTAACGGG	TCAAACAACGGTCAACTGGG	99.0
<i>FER1</i>	<i>At5g01600</i>	CGTTCACAAAGTGGCCTCAG	CAAATCCGTGGCCTTTGC	91.8
<i>PK</i>	<i>At3g49160</i>	CGGAGTTCCAACACTAGAGCTG	AGCTTCAACGATATTTCTTGCT	99.5
<i>APX1</i>	<i>At1g07890</i>	TGCCTTTTCTCGTGATTACG	CAAAAACAGCCATGACTCTCG	110.2
<i>ZAT12</i>	<i>At5g59820</i>	TGCGAGTCACAAGAAGCCTA	GTGTCCTCCAAAGCTTGTG	91.4
<i>Luciferase</i>		TTACACGAAATTGCTTCTGGTG	CCTCGGGTGAATCAGAATAGC	

**Table S4. Oligonucleotides used for CHIP-PCR**

Locus name	AGI	Forward primer 5' to 3'	Reverse primer 5' to 3'
Pseudogene	<i>At4g03760</i>	GAAGCGAGACTTTCTGCTCGG	CCGAGGCGGTTGTTGTGCTAC
<i>At1g24145</i>	<i>At1g24145</i>	GATCCCATTTGACCGAGTAAAC	TACCGCCATCTTAGAAGTGAATG
<i>At1g24147</i>	<i>At1g24147</i>	TAAATGCGTAAACGTGAGTCGG	CATGAAGTGAAAACACGAGGAC
<i>AAA-ATPase</i>	<i>At3g28580</i>	TTGTGTACCAGAACCACCATC	GGCTTAGGGCTTTGGAAGAG

**Table S5. Oligonucleotides used for genotyping**

Description	Forward primer 5' to 3'	Reverse primer 5' to 3'
AtSPO11-1-3 WT	TTTCAGTGTAGTCGGTACAACCTGAATGTG	CCACAACCAGTATGTACTCAGCTAAGCTAAC
AtSPO11-1-3 T-DNA	TTTCAGTGTAGTCGGTACAACCTGAATGTG	GCGTGGACCGCTTGCTGCAACT
AtSPO11-2 WT	GGGACTTGAAGCATACAGATACGGTAAAG	CTCGAGTTATATGTATTTGCCTTGACGATCTTGG
AtSPO11-2 T-DNA	GGGACTTGAAGCATACAGATACGGTAAAG	TAGCATCTGAATTCATAACCAATCTCGATACA

## Other Supporting Information Files

[Dataset S1 \(XLSX\)](#)