Supporting Information

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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 L*er* with wild-type Col-0. Other lines used in this study were: *flu AAA:LUC*$^{+}$ (2); *flu AAA:LUC*$^{+}$ *caa39*, *flu caa39*, obtained by a backcross of *flu AAA:LUC*$^{+}$ *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 $\mu mol \cdot m^{-2} \cdot s^{-1}$) 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*$^{+}$ *caa39* in Col-0 with *flu AAA:LUC*+ in L*er*. Of 1,700 F2 plants, 500 homozygous *flu AAA:LUC*+ *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 SSLP (simple sequence length polymorphism) markers listed in The *Arabidopsis* Information Resource database (TAIR, 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.
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3. Clough SJ, Bent AF (1998) Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J* 16:735–743.
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.
5. Hellemans J, Mortier G, De Paepe A, Speleman F, Vandesompele J (2007) qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. *Genome Biol* 8:R19.
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.

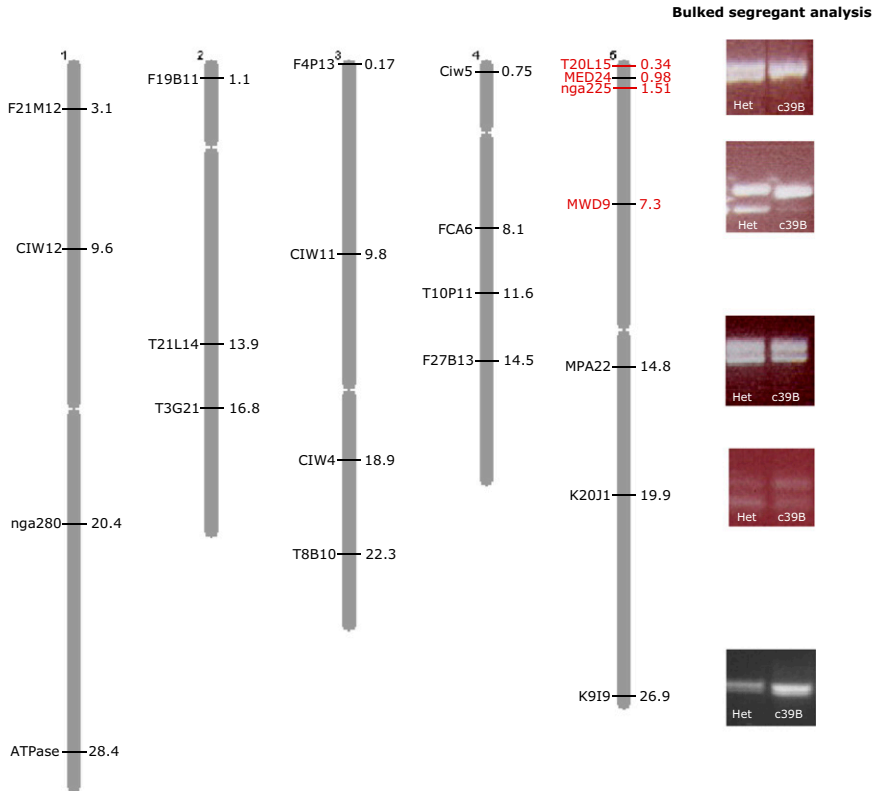

Fig. S1. *Arabidopsis* chromosome map showing the Arabidopsis Genome Initiative (AGI) map positions (Mbp) of the polymorphic markers used for the rough mapping of the *caa39* mutation by bulk segregant analysis (1). Names of the polymorphic markers or the corresponding BAC clones are indicated. On the right side, results of bulked segregant analysis for markers localized on chromosome 5 are presented. Images of PCR-based analysis for *flu AAA:LUC*$^{+}$ *caa39* DNA bulk sample (c39B) and heterozygous Col/Ler (Het) DNA sample; markers MED24 and MWD9 showed genetic linkage to the *caa39* locus. Position of the *caa39* locus in this region was confirmed by low recombination rate for two flanking markers, T20L15 and nga225, located upstream and downstream of MED24, respectively.

1. Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. *Proc Natl Acad Sci USA* 88:9828–9832.

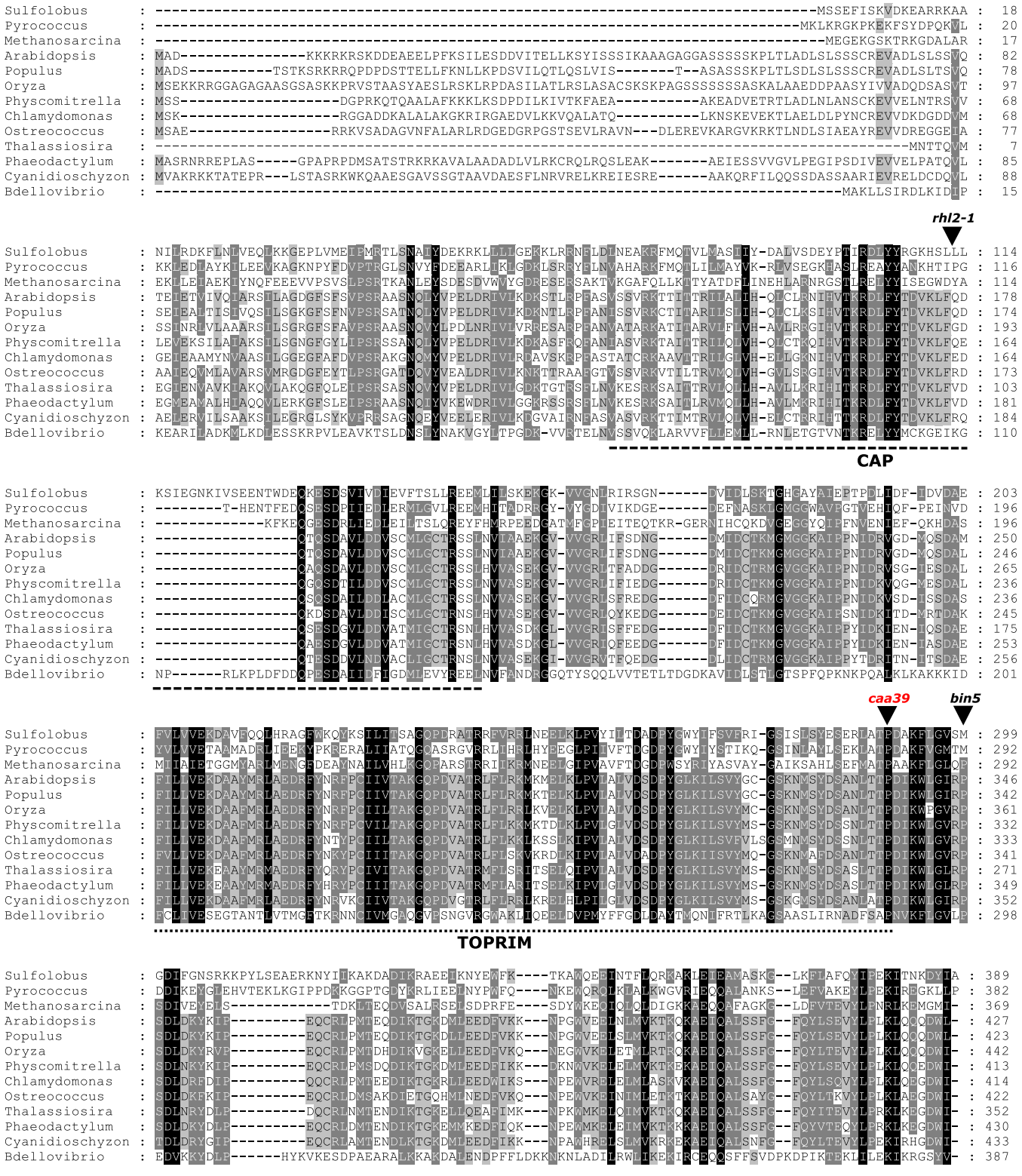

Fig. S2. Multiple alignment of TOP6A/SPO11-3 proteins from archea, plants, green algae, diatoms, red algae, and proteobacteria. The proteins were aligned using CLUSTALW. Conserved CAP and topoisomerase-primase (TOPRIM) domains are indicated. Arrows show position of the amino acid exchange caused by *caa39* mutation and positions of *rhl2-1* 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 tricornutum* XP_002177352.1; *Cyanidioschyzon merolae* CMQ111C; *Bdellovibrio bacteriovorus* CAE80644.1.

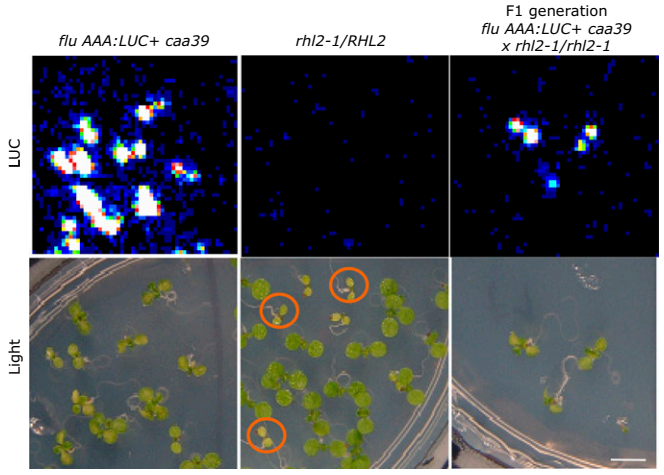

Fig. S3. Allelism test of *flu AAA:LUC*$^{+}$ *caa39* with *rhl2-1* mutant. *flu AAA:LUC*$^{+}$ *caa39* mutant was crossed with the homozygous *rhl2-1* mutant selected based on the mutant phenotype from the segregating *rhl2-1/RHL2* F2 generation that was grown under long-day conditions. F1 generation 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*$^{+}$ *caa39* seedlings, F2 segregating *rhl2-1/RHL2* population, and F1 seedlings from the *flu AAA:LUC*$^{+}$ *caa39* × *rhl2-1/rhl2-1* cross-over are presented. Orange circles mark homozygous *rhl2-1* plants. (Scale bar, 0.5 cm.)

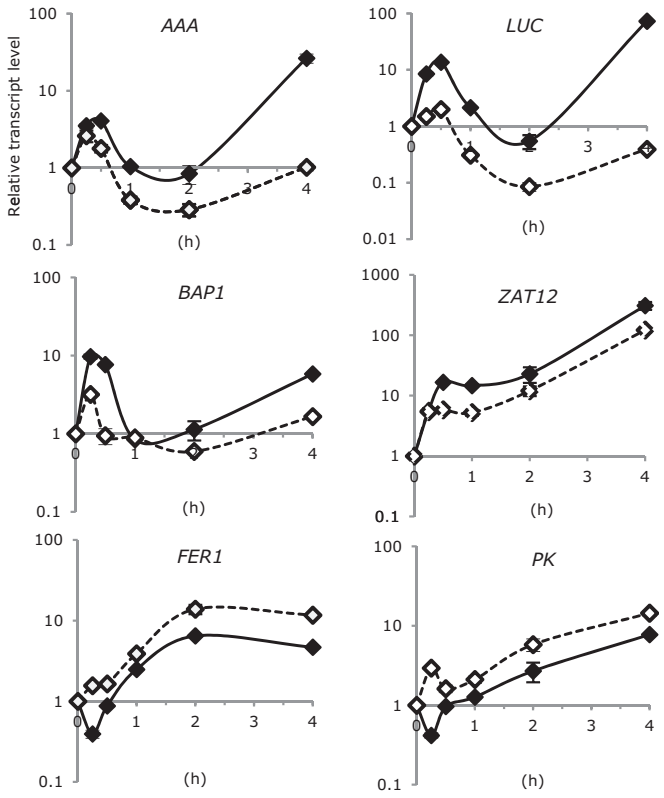

Fig. S4. Response of the *flu AAA:LUC*$^{+}$ *caa39* mutant to high light stress conditions. Relative transcript levels of the $^{1}O_2$-responsive *AAA-ATPase, LUC* and *BAP1* genes, the H_2O_2-responsive *FER1* and *PK* genes and the general reactive oxygen species (ROS)-responsive marker gene *ZAT12* were analyzed in *flu AAA:LUC*$^{+}$ (closed symbols) and *flu AAA:LUC*$^{+}$ *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 μmol·m^{-2}·s^{-1}) treatment. Before HL stress, seedlings were grown for 6 d in low light (LL, 12 μmol·m^{-2}·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 ± SE.

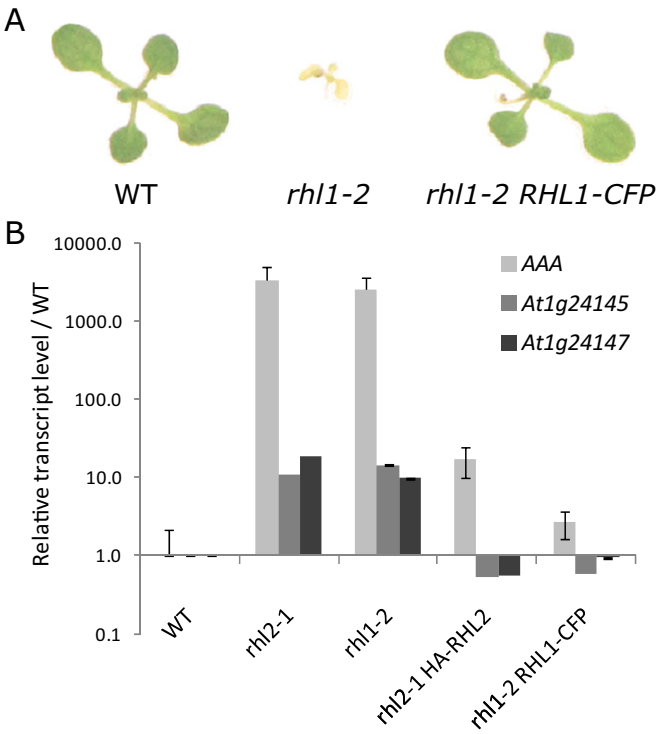

Fig. S5. Functional complementation of *rhl2-1* and *rhl1-2* by HA-RHL2 and RHL1-CFP respectively. (*A*) Morphological phenotype of wild-type, homozygous *rhl1-2*, and double homozygous *rhl1-2/rhl1-2 RHL1-CFP/RHL1-CFP* 10-d-old plants. Similar complementation of the morphological phenotype of the *rhl2-1* homozygous mutant was obtained with HA-RHL2. (*B*) HA-RHL2 and RHL1-CFP restored a near wild-type level of transcripts in *rhl2-1* and *rhl1-2,* respectively. Results represent mean values of two biological replicates ± SE.

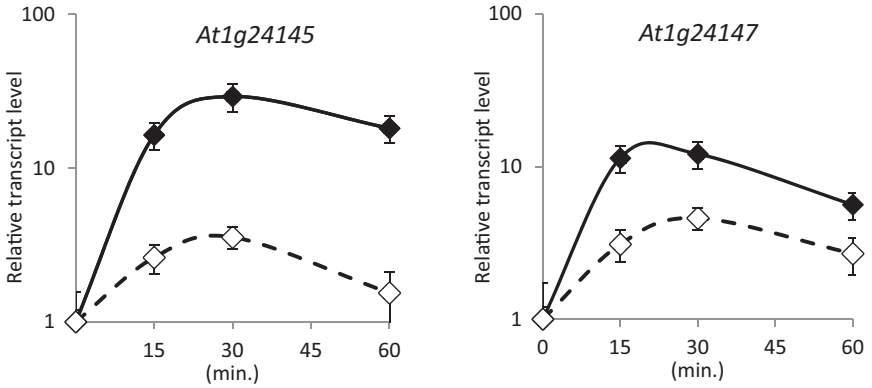

Fig. S6. Activation of the *At1g24145* and *At1g24147* genes in response to high light stress in wild-type (closed symbols) and *caa39* (open symbols). Relative transcript levels were analyzed in seedlings that were grown for 6 d in low light (LL, 12 μmol·m^{-2}·s^{-1}) and then transferred to moderate high light (HL, 1,050 μmol·m^{-2}·s^{-1}) for 15 min, 30 min, and 1 h. Relative transcript levels were determined by quantitative RT-PCR and expressed relative to the levels in LL. Results represent mean values of two biological replicates ± SE.

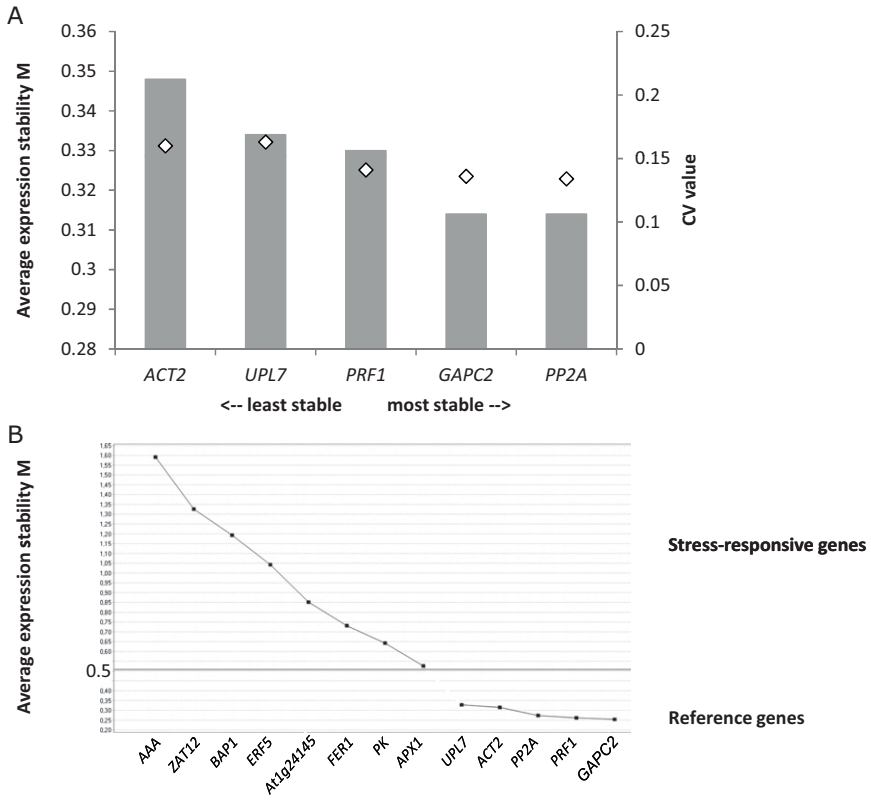

Fig. S7. Evaluation of reference genes in *Arabidopsis* WT and *caa39* seedlings before and after exposure to high light stress. (*A*) Average expression stability (M values) of the five candidate reference genes was analyzed by geNorm across 12 cDNA samples from *Arabidopsis* wild-type and *caa39* seedlings before and after exposure to high light stress. Reference genes are ranked from the least to the most stable (left to right) according to their M values (histograms) and coefficient variance (CV) values (open symbols). A lower M value (<0.5) and a lower CV value (<0.25) indicate a more stable expression. (*B*) Comparison of the expression stability (M < 0.5) of the reference genes with the unstability (M > 0.5) of the stress-responsive genes.

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 *caa39* mutation				
I	3164003	F21M12	GGCTTTCTCGAAATCTGTCC	TTACTTTTTGCCTCTTGTCATTG
	9621344	CIW12	AGGTTTTATTGCTTTTCACA	CTTTCAAAAGCACATCACA
	20877364	NGA280	GGCTCCATAAAAAGTGCACC	CTGATCTCACGGACAATAGTGC
	28185746	ATPase	GTTCACAGAGAGACTCATAAACCA	CTGGGAACGGTTCGATTCGAGC
II	1078851	F19B11	CCAAAGCTTTTGCTCTTGC	TGGGTTTTGATGTTTTTCCTTT
	13875000	T21L14	TTCAAAGTTTCCACCTCATGC	TATGTGTGAGGCCAAGAACC
	16885036	T3G21	CTCGTGTTCGTGCGTAGC	AAGCCAAGCAAACGCATC
III	178000	F4P13	CAAGCCAAAAGGTCTTCACC	TAATCCAGGCCTCGCAAC
	9775545	CIW11	CCCCGAGTTGAGGTATT	GAAGAAATTCCTAAAGCATTC
	18901818	CIW4	GTTCATTAAACTTGCGTGTGT	TACGGTCAGATTGAGTGATTC
	22309429	T8B10	AGTGCTATTTTGTAATCGCT	TCAACATTTCATTTTTTCCA
IV	747854	CIW5	GGTTAAAAATTAGGGTTACGA	AGATTTACGTGGAAGCAAT
	8110273	FCA6	CATGTTATCAGGACAATCTAACG	GCTTCGGCACCAGACTTGTA
	1164575	T10P11	TCATGATAACACAGGGCGTAA	TTCGCATATCTCATGCCATC
	1617020	ATA22	CTTTAGATGCTATACTAGGTT	CGATTTTGCATTGTTTTTTATG
V	980000	MED24	TGCCTCCTTGGGAAAGTG	GGCCCAAGCACACCTACA
	7428725	MWD9	CTCTTACTAAATTTTCAGCT	TACGAATGTATTCACATGCA
	14952821	MPA22	GGTCTTGAATCGCAGCATA	GATGATAGTGACACCTTCTCATGT
	19493000	K20J1	TGGGAAGACGATGATGGA	CCGCATGATGCATAGCAA
Polymorphic markers used for fine mapping of the *caa39* mutation				
T20L15	340947	SSLP	AGATTGTTTCTTCTCAGTGCTC	AGATTGTTTCTTCTCAGTGCTC
T7H20	436559	SSLP	GAGGAGTTGTGCAAGTTGAG	TTAGTACCTAGCTAACGGACCT
T22P11	586645	CAPS	AGGCGTAACCATTGCACAC	ACGACGATCAGAGAAACTAAGC
F9G14-1	620980	CAPS	TAAGAGGAACTAAAGAAGCCTG	CCCATATTCTATAGTCAATCGG
F9G14	660584	SSLP	ACAAATTCAT AAGTTTCGTC ACT	TATGCCGTTTACTGATAATCC
F9G14-2	661303	CAPS	CTCTTGAACAACTTCCGAAC	AATGTTTATCATCTGCGGGATG
F15A17	698509	SSLP	GAGAGGTTTATGACCCAACGAC	CTACAAAGCAAATATTCACGCC
F15A17-1	709015	CAPS	GAACACGAGGTCTTGCCTTG	TCAGATGAATTCAGATCAGGATC
F17C5	967107	SSLP	GTTTACCCTTAAGAACCCGA	TGCCGGTACTAGAATATGTT
MED24	1034836	SSLP	GCTTCTATTGGATGATGAGAC	CTACAAGTCCGATTGATTCCA
NGA225	1507038	SSLP	CAGAGGAGAATCGAATTGGAG	CTCATGATTTGGTGTGGTCG

Table S2. Oligonucleotides used for cloning

Gene name	Forward primer 5′ to 3′	Reverse primer 5′ to 3′
AT5G02820	GATGGGCCCTTGCACATTCGTCTTAC	CTACACTAGTCTGCCTCCGACTG

The underlined bases indicate the ApaI 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 (%)
PP2AA3	*At1g13320*	CAGTATCGCTTCTCGCTCCAG	GTTCTCCACAACCGCTTGGTC	95.2
UPL7	*At3g53090*	CTTCTGGGAGGTCATGAAAGG	CTCCAATAGCAGCCCAAAGAG	96.3
Actin2 (*ACT2*)	*At3g18780*	CATTCTTGCTTCCCTCAGCAC	CCCCAGCTTTTTAAGCCTTTG	97.0
Profilin 1 (*PRF1*)	*At2g19760*	AGAGCGCCAAATTTCCTCAG	CCTCCAGGTCCCTTCTTCC	98.1
GAPC2	*At1g13440*	GGCCATCAAGGAGGAATCTG	CTTGGCATCGAAAATGCTTG	100.0
AAA-ATPase (*AAA*)	*At3g28580*	GGCAATCTTTCTCGTTTTACCC	GCTCTATCGTCTTCCCTTCTCTC	108.9
At1g24145	*At1g24145*	AATGGAACATCGCAAACTCG	GTTGCAATTTTGGAGCCTTG	107.2
AT1G24147	*At1g24147*	CGCAAACTCAAAGAGATGATCAC	TCAGGAAGATAAGTGGTGACGA	112.3
BAP1	*At3g61190*	GGTGATAAGTGTGGGATCGTC	GTCTCTAATCTCGGCCTCCA	96.5
ERF5	*At5g47230*	TTATGTGACTGGGATTTAACGGG	TCAAACAACGGTCAACTGGG	99.0
FER1	*At5g01600*	CGTTCACAAAGTGGCCTCAG	CAAACTCCGTGGCCTTTGC	91.8
PK	*At3g49160*	CGGAGTTCCAACTAGAGCTG	AGCTTCAACGATATTCTTGCCT	99.5
APX1	*At1g07890*	TGCCTTTTTCGCTGATTACG	CAAAAACAGCCATGACTCTCG	110.2
ZAT12	*At5g59820*	TGCGAGTCACAAGAAGCCTA	GTGTCCTCCCAAAGCTTGTC	91.4
Luciferase		TTACACGAAATTGCTTCTGGTG	CCTCGGGTGTAATCAGAATAGC	

Table S4. Oligonucleotides used for ChIP-PCR

Locus name	AGI	Forward primer 5′ to 3′	Reverse primer 5′ to 3′
Pseudogene	*At4g03760*	GAAGCGAGACTTTCTGCTCGG	CCGAGGCGGTTGTTGTGCTAC
At1g24145	*At1g24145*	GATCCCATTTGACCGAGTAAAC	TACCGCCATCTTAGAAGTGAATG
At1g24147	*At1g24147*	TAAATGCGTAAACGTGAGTCGG	CATGAAGTGAAAACACGAGGAC
AAA-ATPase	*At3g28580*	TTGTGTACCAGAACCACCATC	GGCTTAGGGCTTTGGAAGAG

Table S5. Oligonucleotides used for genotyping

Description	Forward primer 5′ to 3′	Reverse primer 5′ to 3′
AtSPO11-1-3 WT	TTTCAGTGTAGTCGGTACAACTTGAATGTG	CCACAACCAGTATGTACTCAGCTAAGCTAAC
AtSPO11-1-3 T-DNA	TTTCAGTGTAGTCGGTACAACTTGAATGTG	GCGTGGACCGCTTGCTGCAACT
AtSPO11-2 WT	GGGACTTGAAGCATACAGATACGGTAAAG	CTCGAGTTATATGTATTTGCCTTGCACGATCTTGG
AtSPO11-2 T-DNA	GGGACTTGAAGCATACAGATACGGTAAAG	TAGCATCTGAATTTCATAACCAATCTCGATACA

Other Supporting Information Files

Dataset S1 (XLSX)