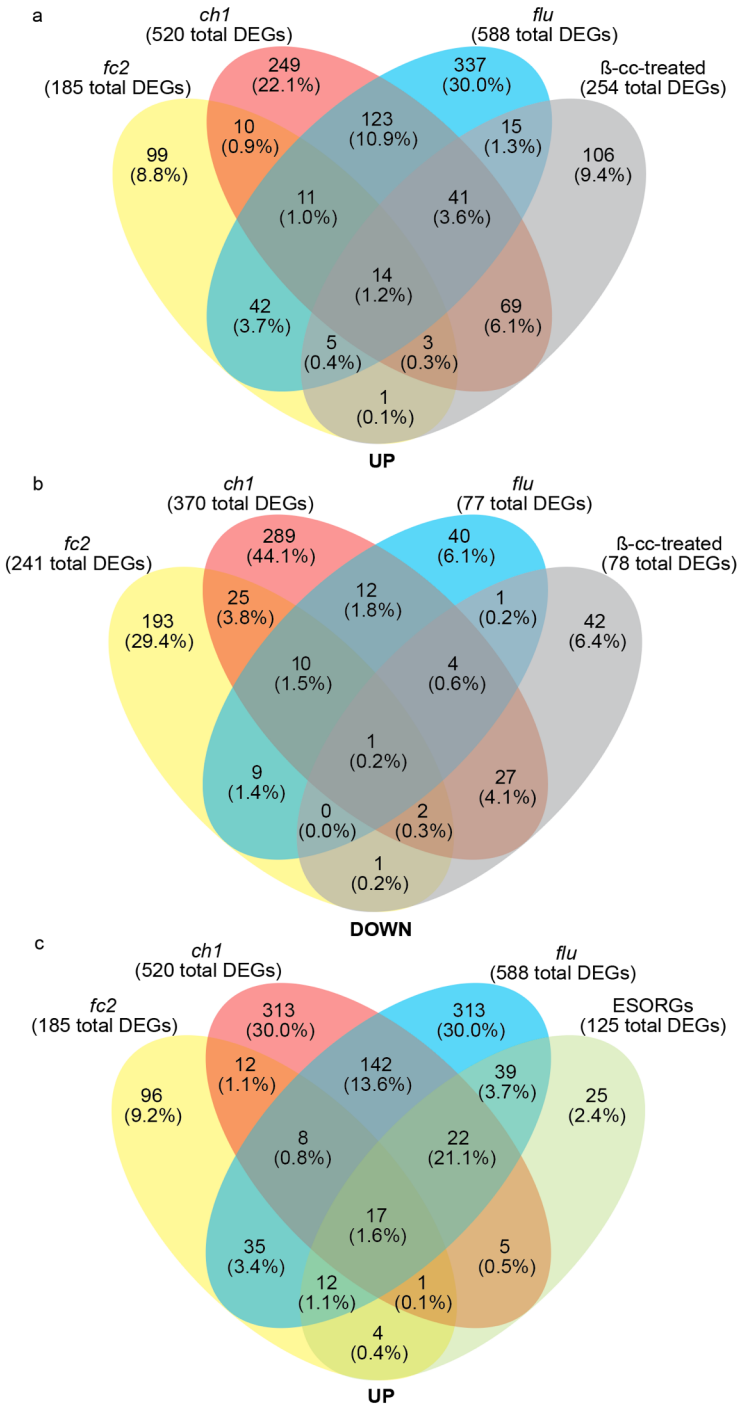
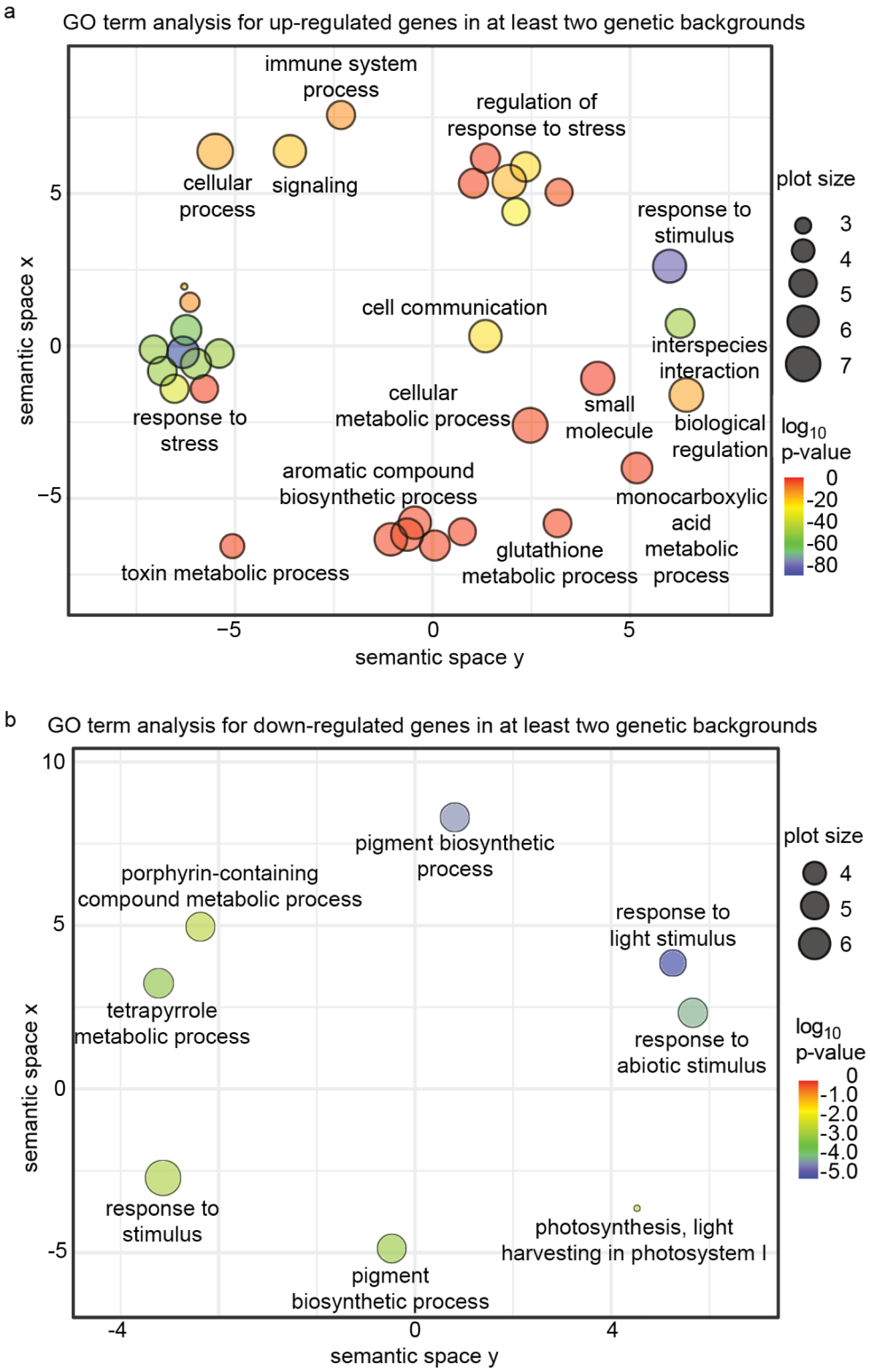
Figure S1. Meta-analysis of transcriptome expression data from three chloroplast $^1O_2$-producing mutant backgrounds.



Venn diagram depicting common differentially expressed genes (DEGs) **A)** up-regulated or **B)** down-regulated during chloroplast $^1O_2$ stress in the *fc2* (Woodson et al. 2015), *flu* (op den Camp

et al. 2003), and *ch1* (Ramel et al. 2013) genetic backgrounds compared to wt plants treated with β-cyclocitral (β-cc) (Ramel et al. 2012). **C)** Venn diagram showing common up-regulated genes during chloroplast $^1O_2$ stress in the *fc2* (Woodson et al. 2015), *flu* (op den Camp et al. 2003), and *ch1* (Ramel et al. 2013) genetic backgrounds compared to Early Singlet Oxygen Response Genes (ESORGs) in *flu* mutant seedlings (Dogra et al. 2017). The number of overlapping DEGs (and percent proportion to total DEGs in the analysis) is indicated within each colored area.

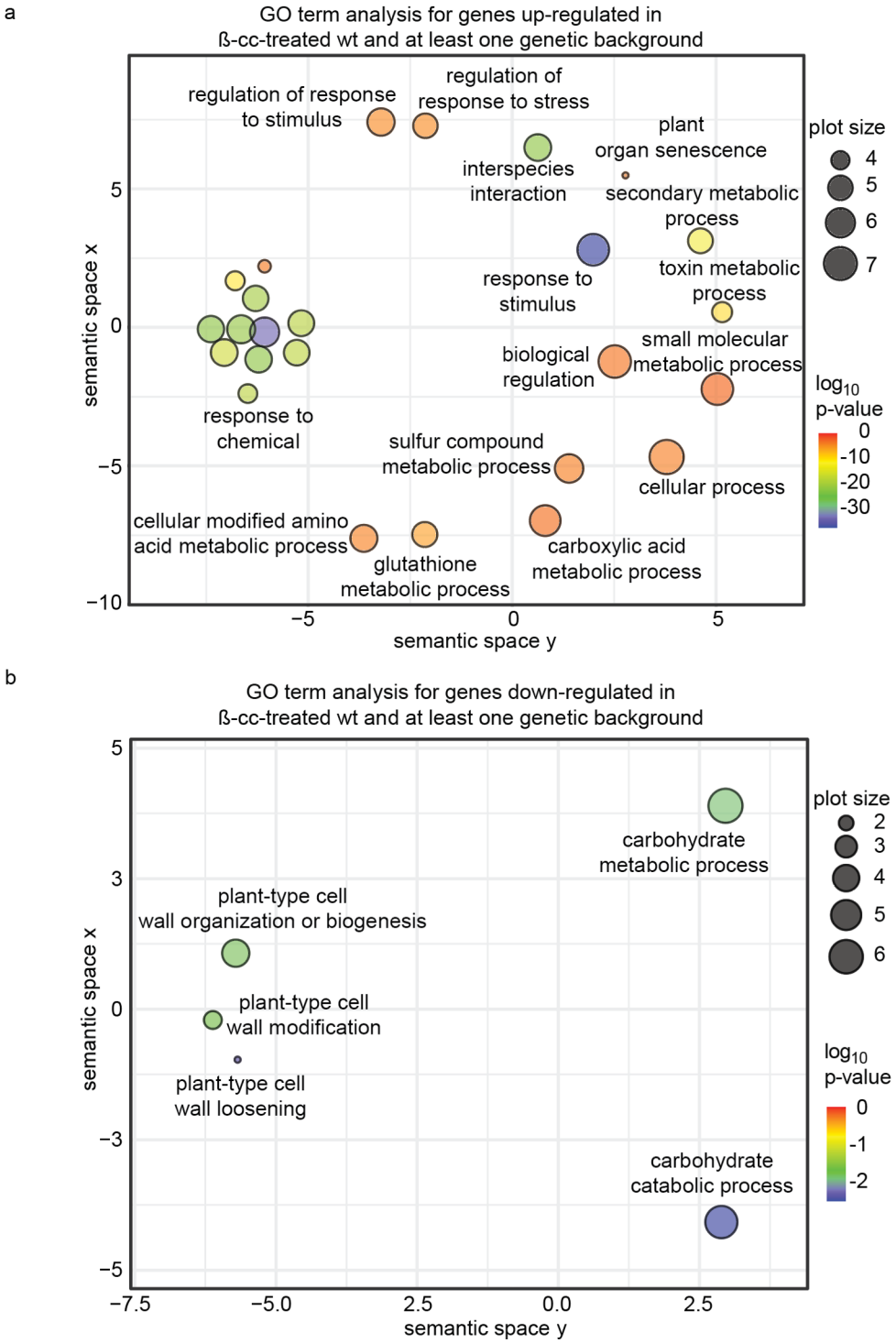
Figure S2. GO Term analysis for $^1O_2$-producing genetic backgrounds.



Scatterplot of gene ontology (GO) terms for differentially expressed genes shared among at least two chloroplast $^1O_2$ accumulating mutants; *fc2* (Woodson et al. 2015), *flu* (op den Camp et al.

2003), and *ch1* (Ramel et al. 2013). Shown are enriched GO terms from shared **A)** up-regulated or **B)** down-regulated genes. GO terms were identified using GO::TermFinder (https://go.princeton.edu/cgi-bin/GOTermFinder) (Boyle et al. 2004) and selected based on a p-value ≤ 0.01. Qualifying GO terms were exported to REVIGO ("small" option for filtering was applied) to create the scatterplot (http://revigo.irb.hr) (Supek et al. 2011), which shows the GO cluster representatives (i.e., remaining GO terms after removal of redundancies) as circles in a grid representing semantic similarities. The closer the circles are to each other, the more related the GO terms are. The size of the circle indicates the frequency of the GO term in the underlying GO annotation database. As such, larger circles represent more general terms. The color of the circles represents the $\log_{10}$ (p-value) of the GO term.

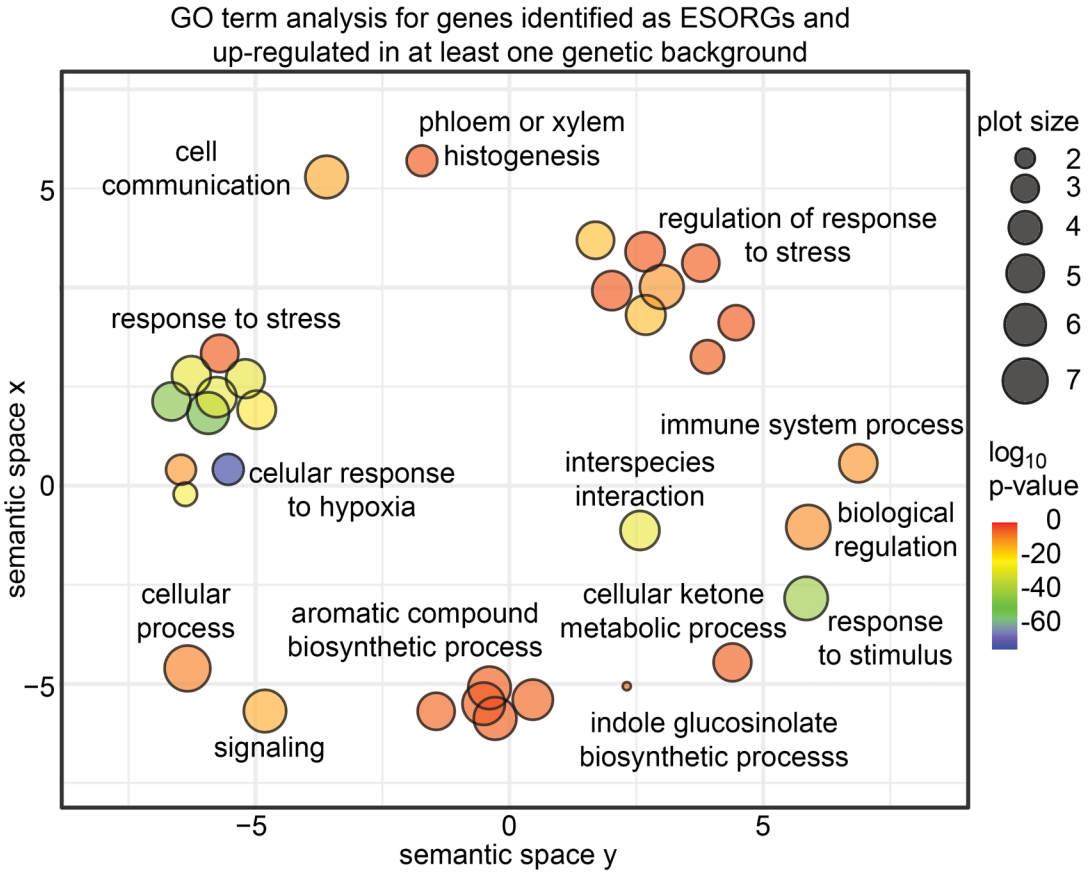
Figure S3. GO Term analysis for transcriptome overlap between $^1O_2$-producing genetic backgrounds and β-cyclocitral treatment.



Scatterplot of gene ontology (GO) terms for differentially expressed genes shared among β-cyclocitral (β-cc)-treated wt plants (Ramel et al. 2012) and at least one chloroplast $^1O_2$

accumulating mutant; *fc2* (Woodson et al. 2015), *flu* (op den Camp et al. 2003), and *ch1* (Ramel et al. 2013). Shown are enriched GO terms from shared **A)** up-regulated or **B)** down-regulated genes. GO terms were identified using GO::TermFinder (https://go.princeton.edu/cgi-bin/GOTermFinder) (Boyle et al. 2004) and selected based on a p-value ≤ 0.01. Qualifying GO terms were exported to REVIGO ("small" option for filtering was applied) to create the scatterplot (http://revigo.irb.hr) (Supek et al. 2011), which shows the GO cluster representatives (i.e., remaining GO terms after removal of redundancies) as circles in a grid representing semantic similarities. The closer the circles are to each other, the more related the GO terms are. The size of the circle indicates the frequency of the GO term in the underlying GO annotation database. Aa such, larger circles represent more general terms. The color of the circles represents the $\log_{10}$ (p-value) of the GO term.

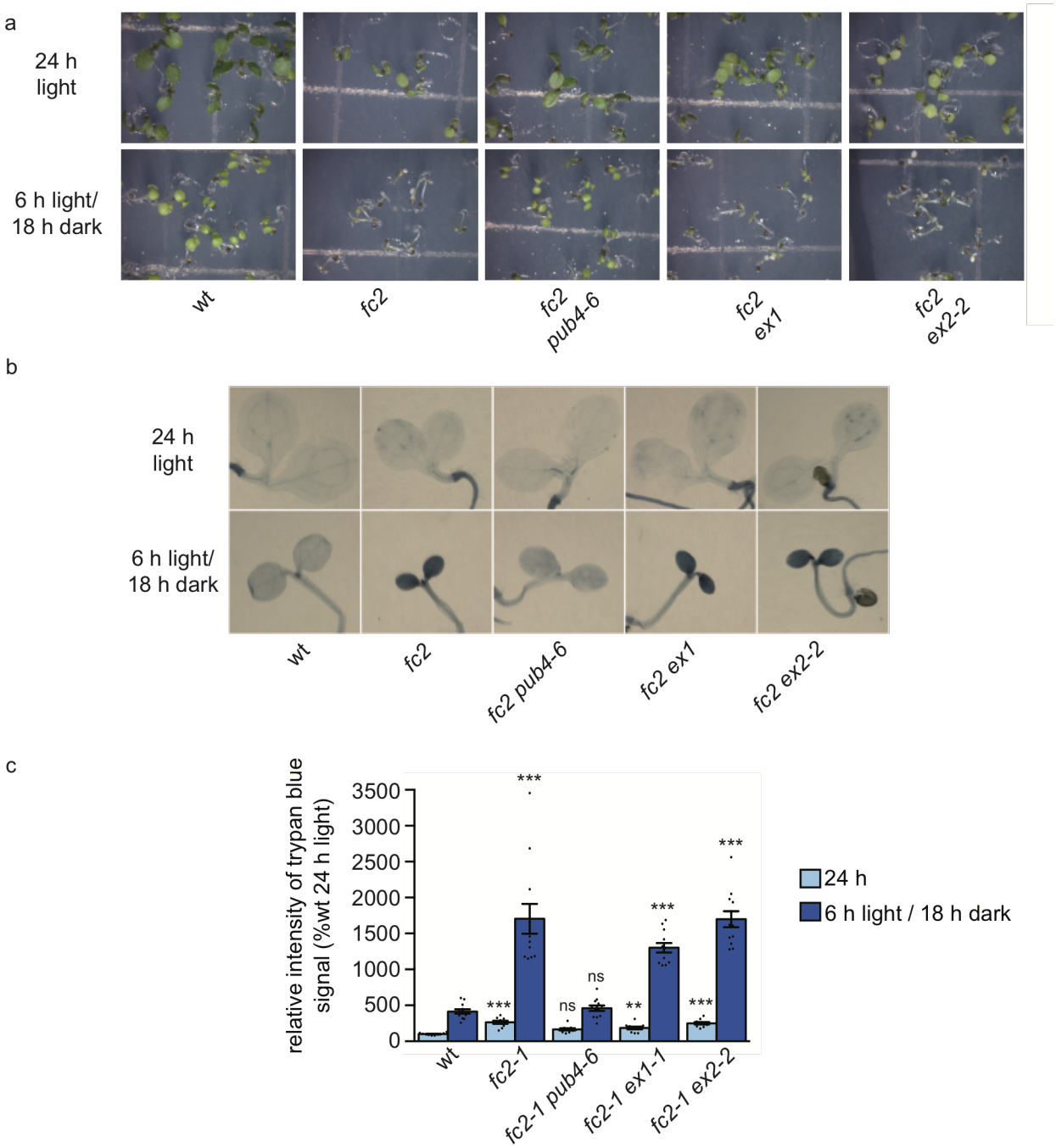
Figure S4. GO Term analysis for transcriptome overlap between $^1O_2$-producing genetic backgrounds and ESORGS.



Scatterplot of gene ontology (GO) terms for Early Singlet Oxygen Response Genes (ESORGs) in *flu* seedlings (Dogra et al. 2017) found to be significantly induced in at least one chloroplast $^1O_2$ accumulating mutant; *fc2* (Woodson et al. 2015), *flu* (op den Camp et al. 2003), and *ch1* (Ramel et al. 2013). Shown are enriched GO terms from shared **A)** up-regulated or **B)** down-regulated genes. GO terms were identified using GO::TermFinder (https://go.princeton.edu/cgi-bin/GOTermFinder) (Boyle et al. 2004) and selected based on a p-value ≤ 0.01. Qualifying GO terms were exported to REVIGO ("small" option for filtering was applied) to create the scatterplot (http://revigo.irb.hr) (Supek et al. 2011), which shows the GO cluster representatives (i.e., remaining GO terms after removal of redundancies) as circles in a grid representing semantic similarities. The closer the circles are to each other, the more related the GO terms are. The size

of the circle indicates the frequency of the GO term in the underlying GO annotation database. Aa such, larger circles represent more general terms. The color of the circles represents the $\log_{10}$ (p-value) of the GO term.

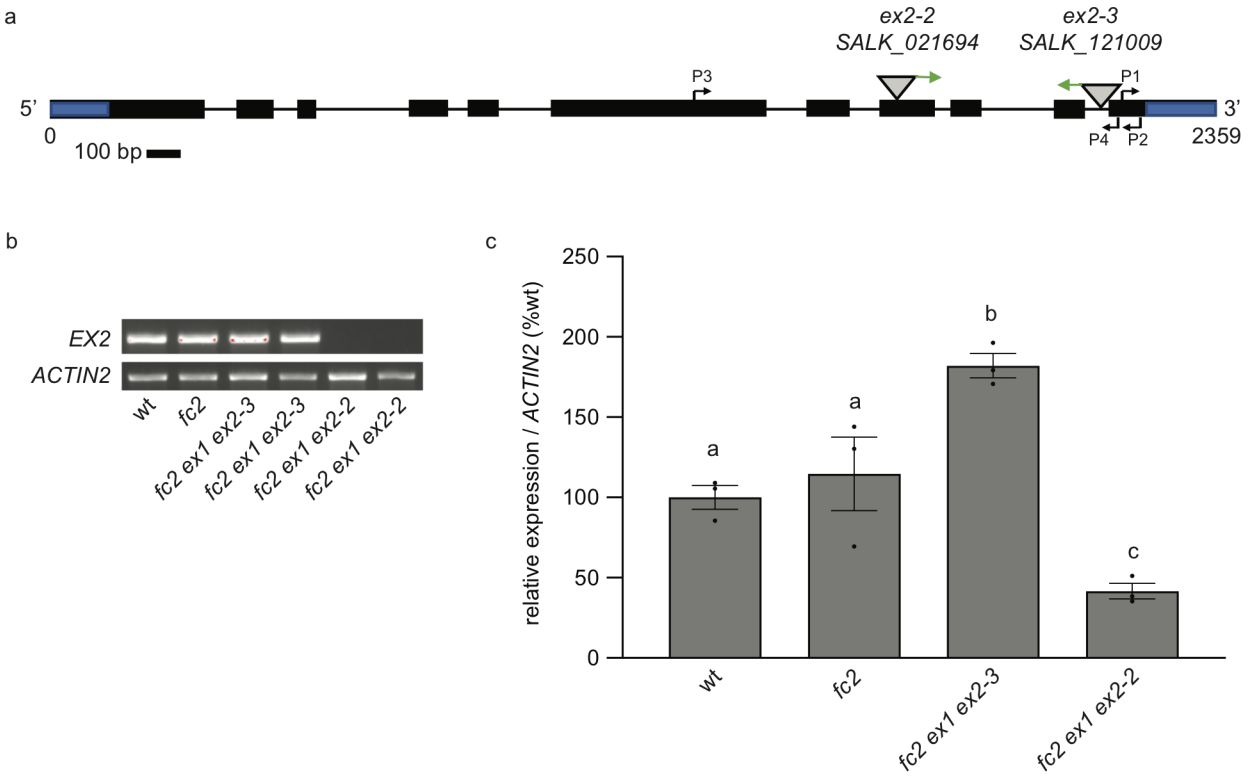
Figure S5. Effects of ex1 and ex2 mutations on cell death in the fc2 mutant background.



The effects of the *ex1* and *ex2* mutations on the *fc2* cell death phenotype were assessed. **A)** Shown are six-day-old seedlings grown under constant light (24h) or diurnal cycling light (6h light / 18h dark) conditions. **B)** Representative images of the same seedlings stained with trypan blue. The dark blue color indicates cell death. **C)** Shown are mean intensities of trypan blue signal (+/- SE,

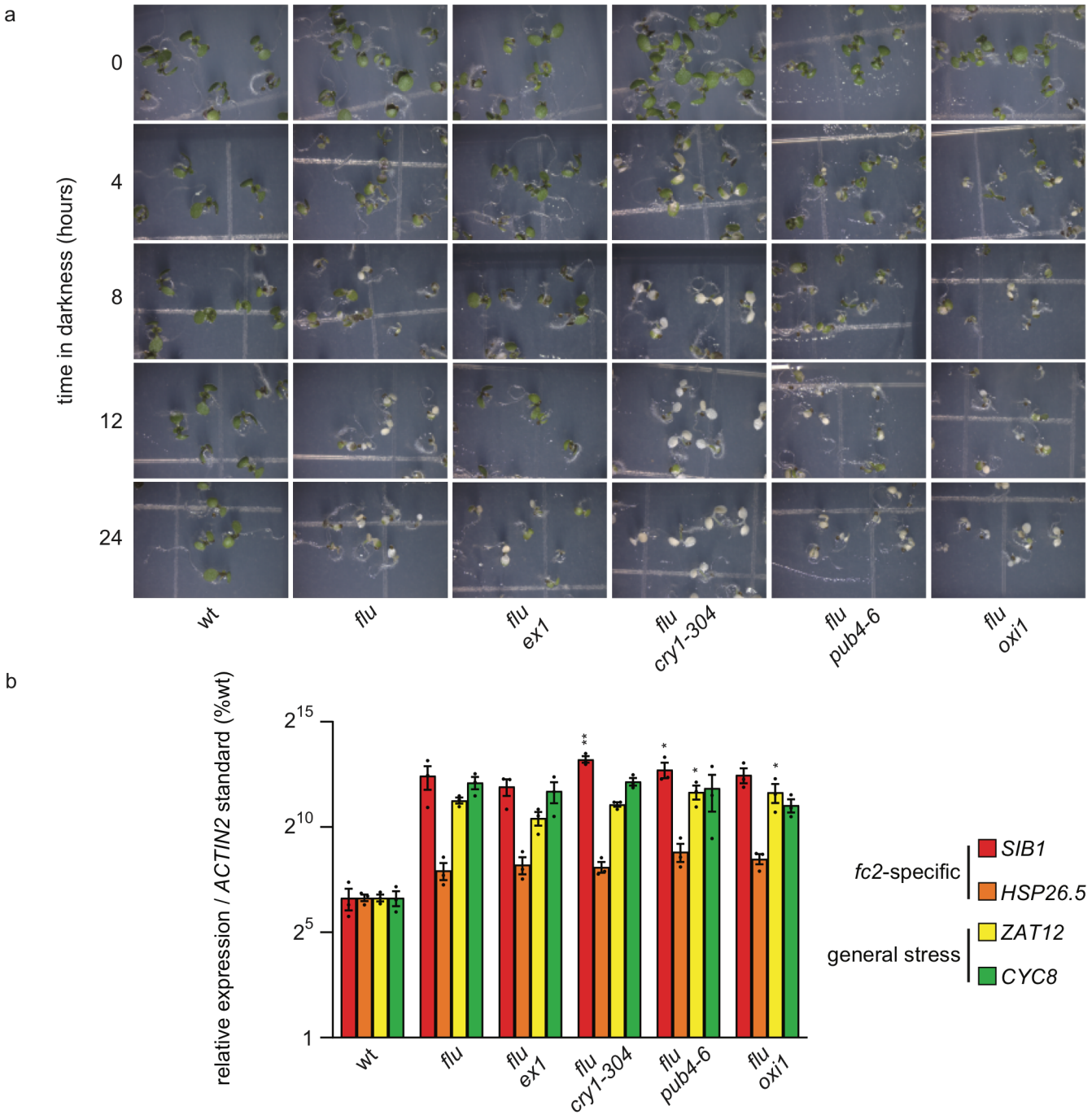
n ≥ 10 seedlings) from **B**. Statistical analyses were performed using a one-way ANOVA followed by a Tukey HSD test. Statistical significance in respect to *fc2* is indicated as follows: n.s. = p-value ≥ 0.05, *** = p-value ≤ 0.001. Closed circles represent individual data points.

Figure S6. EX2 expression levels in ex2 T-DNA mutants.



The effects of two *ex2* T-DNA mutant alleles were assessed. **A)** Gene diagram of *EX2* (*At1g27510*). Triangles represent T-DNA insertions with the right borders denoted by green arrows. The primers P1 and P2 are WLO1688/1689 used for RT-qPCR (panel **C** in this figure). The primers P3 and P4 are WLO1642/1643 used for semi-RT-qPCR (panel **B** in this figure, primer sequences in Table S2). The 5' and 3' untranslated regions are marked by a blue rectangle in the first and last exons respectively. Scale bar = 100bp. **B)** Shown is a semi-RT-qPCR analysis of cDNA generated from RNA extracted from five-day old seedlings grown in 24h constant light. PCR was repeated for 30 cycles. **C)** Shown is RT-qPCR analysis of cDNA generated from RNA extracted from four-day-old seedlings grown under 6h light / 18h dark cycling diurnal conditions (+/- SE, n = 3 seedling pools). Statistical analyses were performed using a one-way ANOVA followed by a Tukey HSD test. Different letters above bars indicate significant differences within data sets (p-value $\leq$ 0.05). Closed circles represent individual data points.

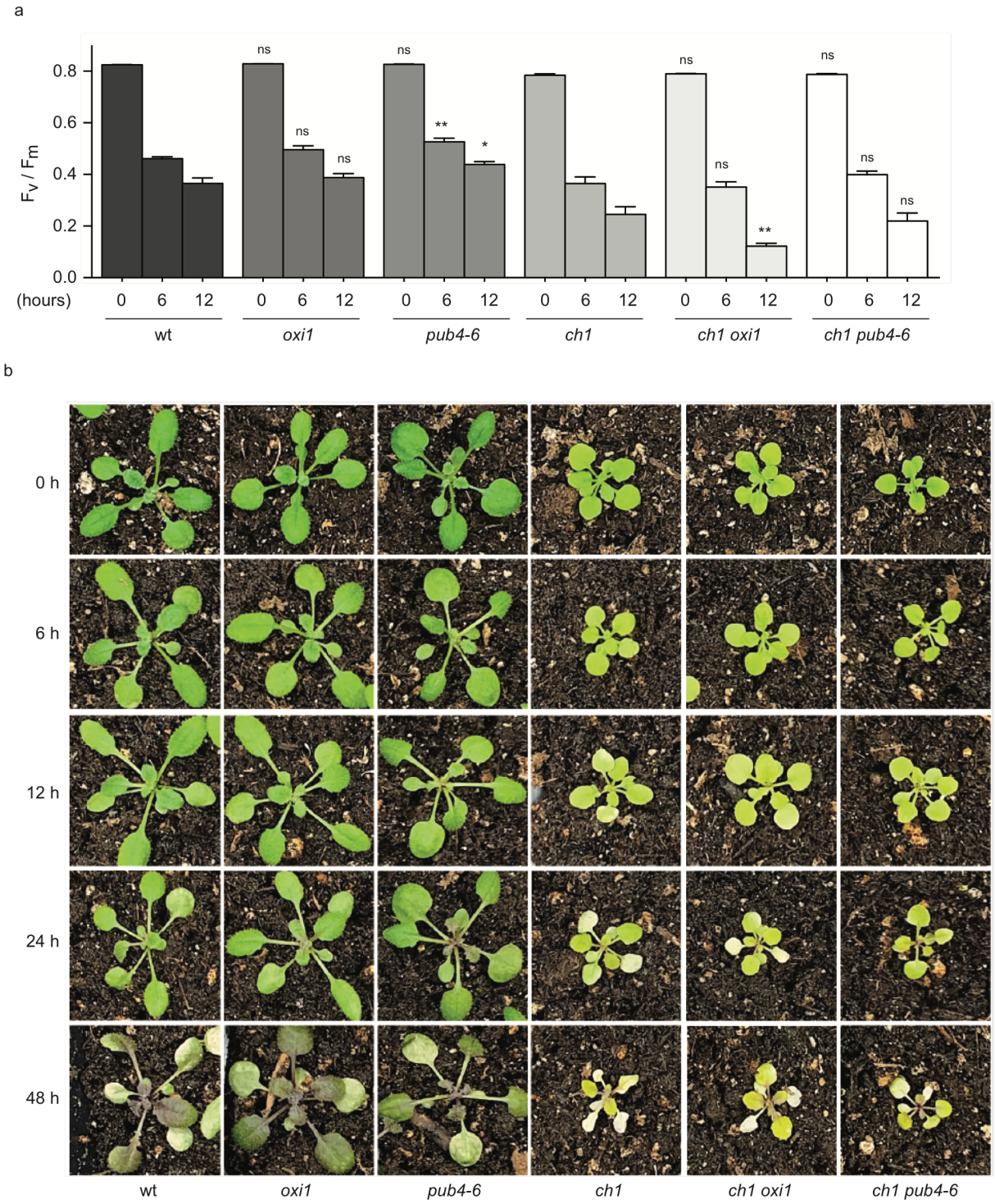
Figure S7. Analysis of extended dark periods on retrograde signaling and the severity of cell death in flu mutant seedlings.



The extent of cotyledon bleaching in the *flu* mutant caused by increasing dark periods was tested. **A)** Shown are seedlings grown for five days in constant light, shifted to dark under the indicated amount of time (0-24 hours), and then shifted back to light for 36h (seven days total). Bleaching

of cotyledons indicates cell death caused by $^1O_2$ signaling. The row for the 12h dark incubation was used in Fig. 4a. **B)** RT-qPCR of stress gene markers (from *fc2* mutants; *SIB1* and *HSP26.5* (Woodson et al. 2015)) and general stress (*ZAT12* and *CYC8* (Baruah et al. 2009)) of five-day old seedlings grown under 24h constant light then dark-incubated for 12 hours, harvested one hour after re-exposure to light. Shown are mean expression values (+/- SE, n = 3 biological replicates). Statistical analyses were performed using a one-way ANOVA followed by a Tukey HSD test. Statistical significance in respect to wt is indicated as follows: * = p-value ≤ 0.05, ** = p-value ≤ 0.01. Closed circles represent individual data points.

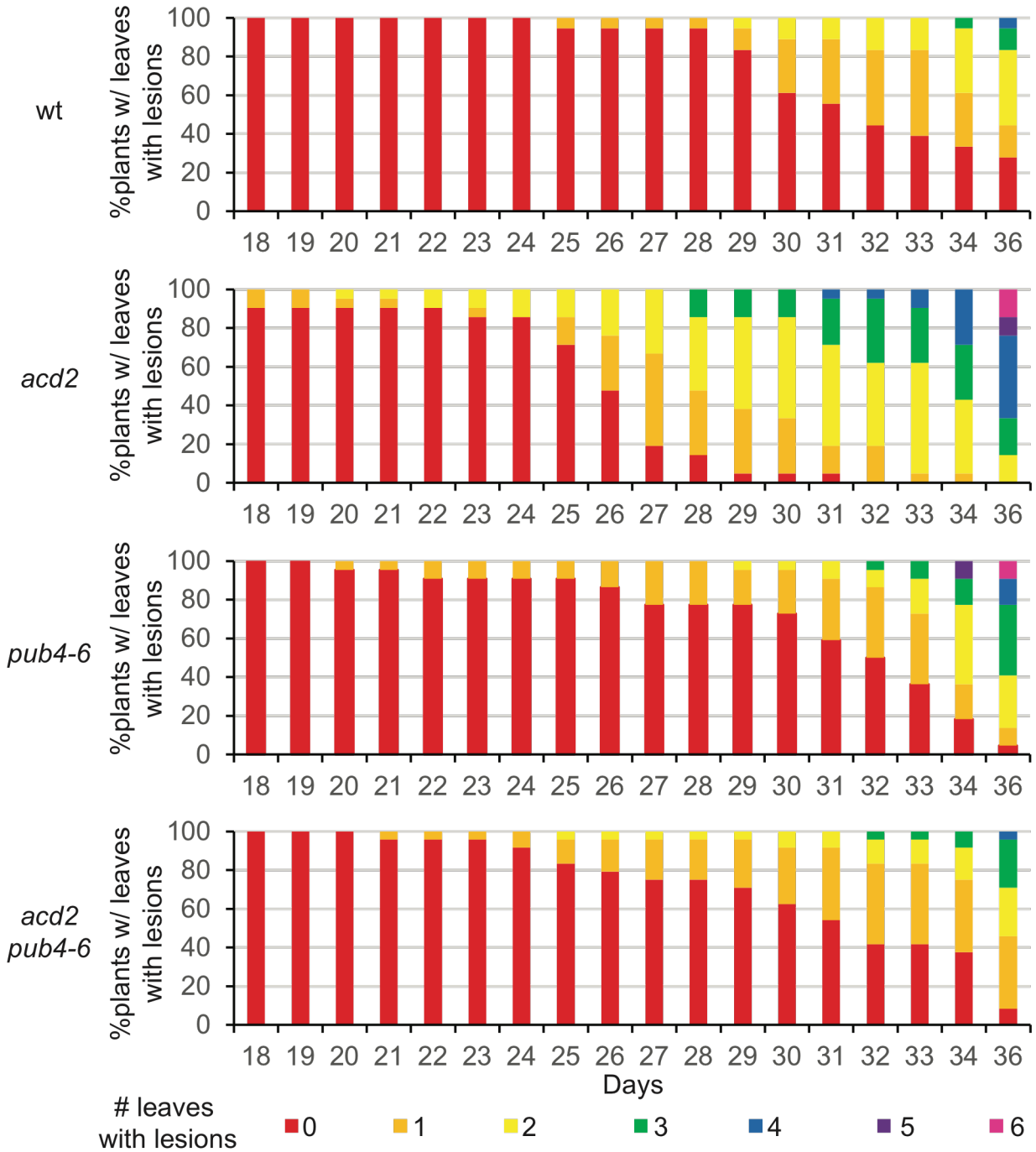
Figure S8. Effect of singlet oxygen signaling mutations on EL-induced phenotypes.



The effect of the *oxi1* and *pub4-6* mutations were tested in adult plants treated with excess light (EL). **A)** Time course analysis of maximum PSII quantum efficiency (F_v/F_m) in these plants during

the initial 12 hours of EL (1,300 μmol sec^{-1} m^{-2}) and 10 degrees C. **B)** Shown are representative images of EL-treated plants at the indicated times.

Figure S9. The *pub4-6* mutation slows the progression of spontaneous cell death in the *acd2* mutant.



The *acd2* spontaneous cell death and lesion phenotypes were assessed. Shown are the percentage of plants containing different numbers of leaves with lesions from 18 to 36 days old ($n \geq 18$ plants). Any type of discoloring was considered a lesion.

Table S1. Mutant lines used in study

Mutant	Gene Name	Gene #	DNA/Protein Change	Notes	Ref.
acd2-2	*ACCELERATED CELL DEATH 2 ARABIDOPSIS THALIANA RED CHLOROPHYLL CATABOLITE REDUCTASE*	*At4g37000*	G to A substitution in the intronic splice site	Generated by ethyl methanesulfonate treatment	(Mach et al. 2001)
ch1-1	*CHLORINA 1*; *CHLOROPHYLL A OXYGENASE*	*At1g44446*	Deletion	Generated by X-ray Diffraction	(Havaux et al. 2007)
cry1-304	*CRYPTOCHROME 1*	*At4g08920*	Deletion	Generated by neutron mutagenesis	(Bruggemann et al. 1996)
ex1	*EXECUTER 1*	*At4g33630*	SALK_002088 T-DNA in 2nd exon		(Lee et al. 2007)
ex2-2	*EXECUTER 2*	*At1g27510*	SALK_021694 T-DNA in 8th exon		(Uberegui et al. 2015)
ex2-3	*EXECUTER 2*	*At1g27510*	SALK_121009 T-DNA in 10th intron		This Study
fc2-1	*PLASTID FERROCHELATASE 2*	*At2g30390*	GABI_766H08 T-DNA in 5'UTR	Sulfadiazine Resistant	(Woodson et al. 2011)
flu-1	*FLUORESCENT IN BLUE LIGHT*	*At3g14110*	C-terminal amino acid substitution (A262V)	Generated by ethyl methanesulfonate treatment	(Meskauskiene et al. 2001)
oxi1-1	*OXIDATIVE SIGNAL-INDUCIBLE 1*	*At3g25250*	GABI_355H08 T-DNA in 2nd exon		(Camehl et al. 2011)
pub4-6	*PLANT U-BOX 4*	*At2g23140*	U-box domain amino acid substitution (G255R)	Generated by ethyl methanesulfonate treatment	(Woodson et al. 2015)

Table S2. Primers used for RT-qPCR and genotyping

Gene	Primer orientation / name	Sequence
RT-qPCR primer pairs		
ACTIN2 / At3g18780	For. / JP199	GCACTTGCACCAAGCAGCAT
	Rev. / JP200	CCTTTCAGGTGGTGCAACGAC
SIB1 / At3g56710	For. / JP589	CAACCGGAGCCCATCTATT
	Rev. / JP590	GGAGAAAGGTTGTGGTCGTC
HSP26.5 / At1g52560	For. / JP585	CGAGCTTATCGTTGCCTGAT
	Rev. / JP586	CTCCGCCTTAATGTCCTCAA
BAP1 / At3g61190	For. / JP338	ATTGATGGATACGGTGGCCG
	Rev. / JP339	CAGACCCCAAACCGGAACTC
Atpase / At3g28580	For. / JP336	GAAGATCGGAAAAGCGTGGAA
	Rev. / JP337	CCGGGTGGTCCAAACAAAAG
ZAT12 / At5g59820	For. / JP344	GCGTTGGTTACACGCGCTT
	Rev. / JP345	CTTCAACGTAGTCACCGTGGG
CYC8 / At4g37370	For. / JP1130	AATGGGCATTGTCGAACGTG
	Rev. / JP1131	TCGCCTTGTTCAATACATCCG
NOD1 / At5g64870	For. / JP340	GCTGATGCTGCCTTCTATTCAA
	Rev. / JP341	TGCGACAAGTCCCTCTGCA
EX2 / At1g27510	For. / WLO1688	CATATGTGAAGGGCGCAGATC
	Rev. / WLO1689	CAGACGGTTGAAAAGGACCAAG
Semi-qPCR primer pairs		
ACTIN2 / At3g18780	For. /WLO1401	GGCTGAGGCTGATGATATTC
	Rev. /WLO1402	TCTGTGAACGATTCCTGGAC
EX2 / At1g27510	For. /WLO1642	CGTGTGTCGGCAAATATAATGGA
	Rev. /WLO1643	TCTGCGCCCTTCACATATGG
Genotyping primer pairs		

SALK T-DNA Left Border	LB1.3	ATTTTGCCGATTTCGGAAC
GABI-KAT Left Border	Gabi-KAT 03144	ATATTGACCATCATACTCATTGC
acd2-2 dCAPS genotyping: *Pst*I digestion, wt = 124 bp, mutant = 149 bp	For. / JP1144	GAGAATCTTAAAGTTTGTTTTGTTCTGCA
	Rev. / JP1145	TCGACCACAAAGTTTGGAGCT
cry1-304	For. / WLO1451	ATGTCTGGTTCTGTATCTGGTTGTGGTTC
	Rev. / WLO1452	ATAGTTCTCATCCACAGCCCAAG
ex1-1 (*SALK_002088*)	For. / JP415	TCTGACACTTGATGGGAAAGG
	Rev. / JP416	TAAGCTGCGACTCCTTTTCTG
ex2-2 (*SALK_021694*)	For. / JP1140	CACTAAGCTTGTCATCGGAGG
	Rev. / JP1141	AAATGTCAATGTGGCTGGAAC
ex2-3 (*SALK_121009*)	For. / JP1142	TCCCTATTGATTTGCAGAAGC
	Rev. / JP1143	ATTGTTTCCAGAGGAATTGGG
fc2-1 (*GabiKat_766H08*)	For. / JP283	GAGCAACGCCAAACATAGAAG
	Rev. / JP284	TCAAAGGCAATGAATGTTTCC
flu-1 dCAPS genotyping: *HpyI*88I digestion, wt = 127 bp, mutant = 102 bp	For. / JP1138	AGCTTTGGAACTTGCCCAGA
	Rev. / JP1139	TAGAACCATGGAGTGATACTGTCTG
oxi1-1 (*Gabi_355H08*)	For. / JP1291	CCTTTCCAAACAAAGCAAGTG
	Rev. / JP1292	AAGAAACGTCTCTTCCGCTTC
pub4-6 dCAPS genotyping: *Hpa*II digestion, wt = 97 bp, mutant = 121 bp	For. / JP742	TATTAGAGTAGTGTGAGTCAGG
	Rev. / JP743	GATCCAGTGATTGTGTCATCC

Table S16. Conditions for previously published transcript profiling experiments

Genotype	RNA Extraction	Expression Analysis	Data Sets Selected	Growing Conditions	Age	Ref.
fc2	RNeasy Plant Mini Kit (Qiagen)	Affymetrix GeneChip Arabidopsis ATH1 Genome Array.	2 hr timepoint was used. *fc2* was compared to wt grown in the same conditions.	First grown in the dark for 4 days and then exposed to 2 hours of white light (120 µmol photons m^{-2} s^{-1}).	4-day old etiolated seedlings	(Woodson et al. 2015)
flu	Not listed	Affymetrix GeneChip Arabidopsis ATH1 Genome Array.	1 hr timepoint was used. *flu* following dark/light shift was compared to wt treated similarly	First grown in continuous white light (80-100 photons µmol m^{-2} s^{-1}). Incubated in dark for 8 hours and then exposed again to white light for 1 hr	Rosette stage adult plants	(op den Camp et al. 2003)
ch1	TRIzol extraction (Invitrogen) and Message Amp aRNA kit (Ambion)	CATMAv5 Complete Arabidopsis Transcriptome MicroArray.	Excess light treated *ch1* was compared to untreated *ch1*.	First grown in 8h white light (180 µmol photons m^{-2} s^{-1}) / 16 h dark photoperiod. Then exposed to 2 days of 1,000 µmol photons m^{-2} s^{-1} (same photoperiod)	5-8 week old adult plants	(Ramel et al. 2013)

β-cc-treated wild type	TRIzol extraction (Invitrogen) and Message Amp aRNA kit (Ambion)	CATMAv5 Complete Arabidopsis Transcriptome MicroArray.	β-cc-treated wt was compared to untreated wt (mock treatment of distilled water).	First grown under a 16 hr white light (100 µmol photons m^{-2} s^{-1}) / 8h dark photoperiod. Then exposed to 50 µL of β-CC for 4 hr in an airtight box (60 µmol m^{-2} s^{-1})	4 week old adult plants	(Ramel et al. 2012)
flu RNAseq	RNeasy Plant Mini Kit (Qiagen) and NEBNext Ultra Directional RNA Library Prep Kit for Illumina	Libraries constructed using NEBNext Ultra Directional RNA Library Prep Kit for Illumina. Sequenced on an Illumina HiSeq 2500 platform to generate 100 bp paired-end reads. False discovery rate of 0.05 and 1 transcript/million minimum.	Light-exposed (both 30 and 60 minute time points) *flu* plants were compared to *flu* plants without reillumination.	First grown under continuous white light (40 µmol photons m^{-2} s^{-1}) for 5 days and the incubated in the dark for 4 hrs. Next, seedlings were re-illuminated with white light for 30 and 60 min (both timepoints used in analysis)	5-day old seedlings	(Dogra et al. 2017)

Table S17. Common genes differentially expressed between fc2, flu, and ch1 data sets

Genotype	Differentially expressed genes in data set (#) (total / unique)	Gene Overlap with *fc2* (# / %)	Gene Overlap with *flu* (# / %)	Gene Overlap with *ch1* (# / %)	Overlap among all 3 Genotypes (# / %)
Up-regulated Genes					
fc2	185 / 100	-	72/38.9	38/20.5	25/13.5
flu	588 / 352	72/12.2	-	189/32.1	25/4.3
ch1	520 / 318	38/7.3	189/36.3	-	25/4.8
Down-regulated Genes					
fc2	241 / 194	-	20/8.3	38/15.8	11/4.6
flu	77 / 41	20/26.0	-	27/35.1	11/14.3
ch1	370 / 316	38/10.3	27/7.3	-	11/3.0
Up- and Down-regulated Genes					
fc2	426 / 294	-	92/21.6	76/17.8	36/8.5
flu	665 / 393	92/13.8	-	206/31.0	36/5.4
ch1	890 / 634	76/8.5	206/23.1	-	36/4.0

Table S18. Common genes differentially expressed by treatment with β-cyclocitral.

Genotype	# of differentially expressed genes in common	# of differentially expressed genes overall	% Overlap
Up-regulated			
fc2	23	185	12.4
flu	75	588	12.8
ch1	127	520	24.4
Down-regulated			
fc2	4	241	1.7
flu	6	77	7.8
ch1	34	370	9.2
Up- and Down-regulated			
fc2	34	426	8.0
flu	83	665	12.5
ch1	166	890	18.7

Table S19. Early Singlet Oxygen Response Genes (*ESORGs*) induced in mutants

Genotype	# up-regulated ESORGs	# up-regulated genes	% Overlap
fc2	34	185	8.0
flu	78	588	11.7
ch1	43	520	4.8

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