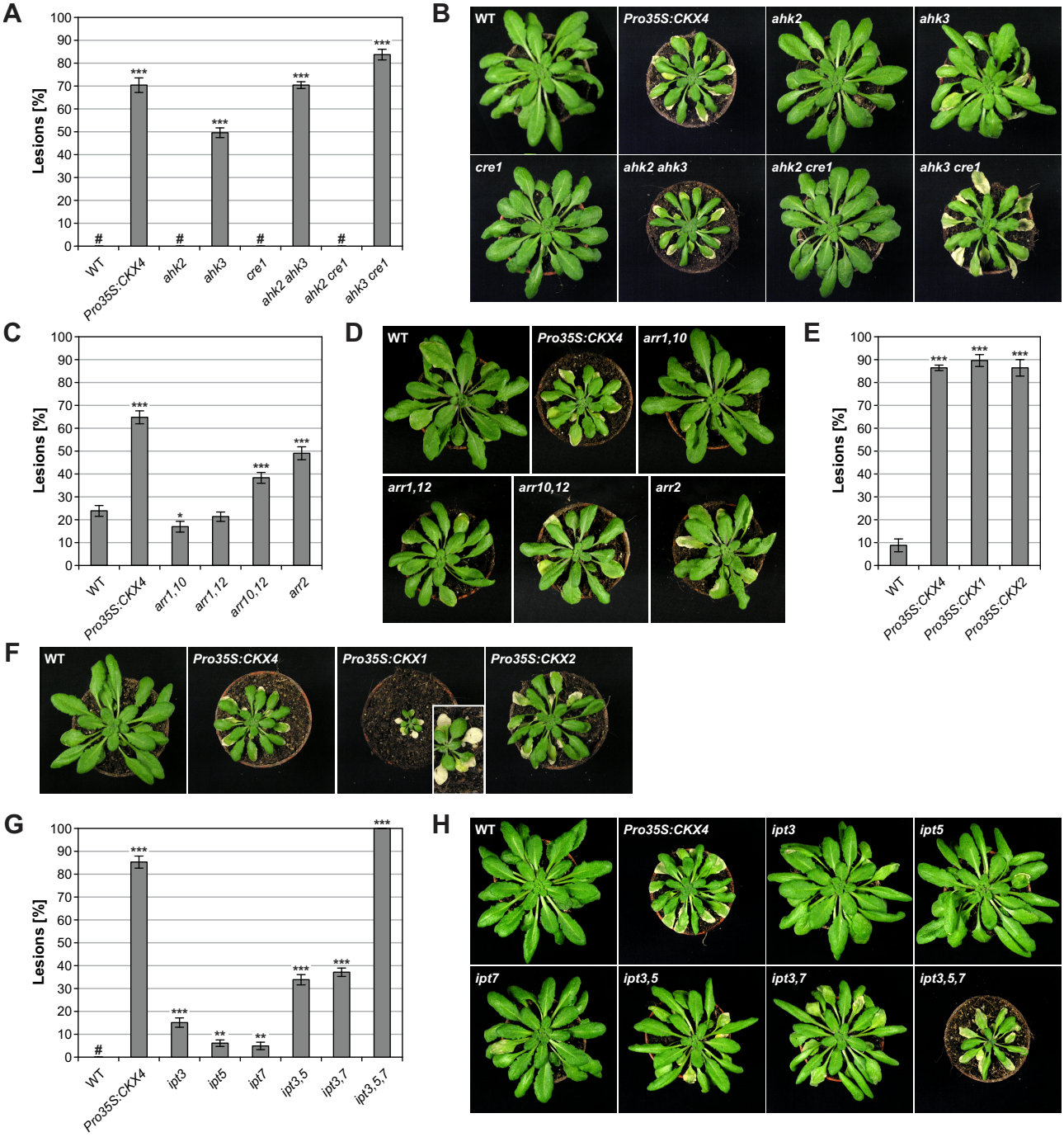
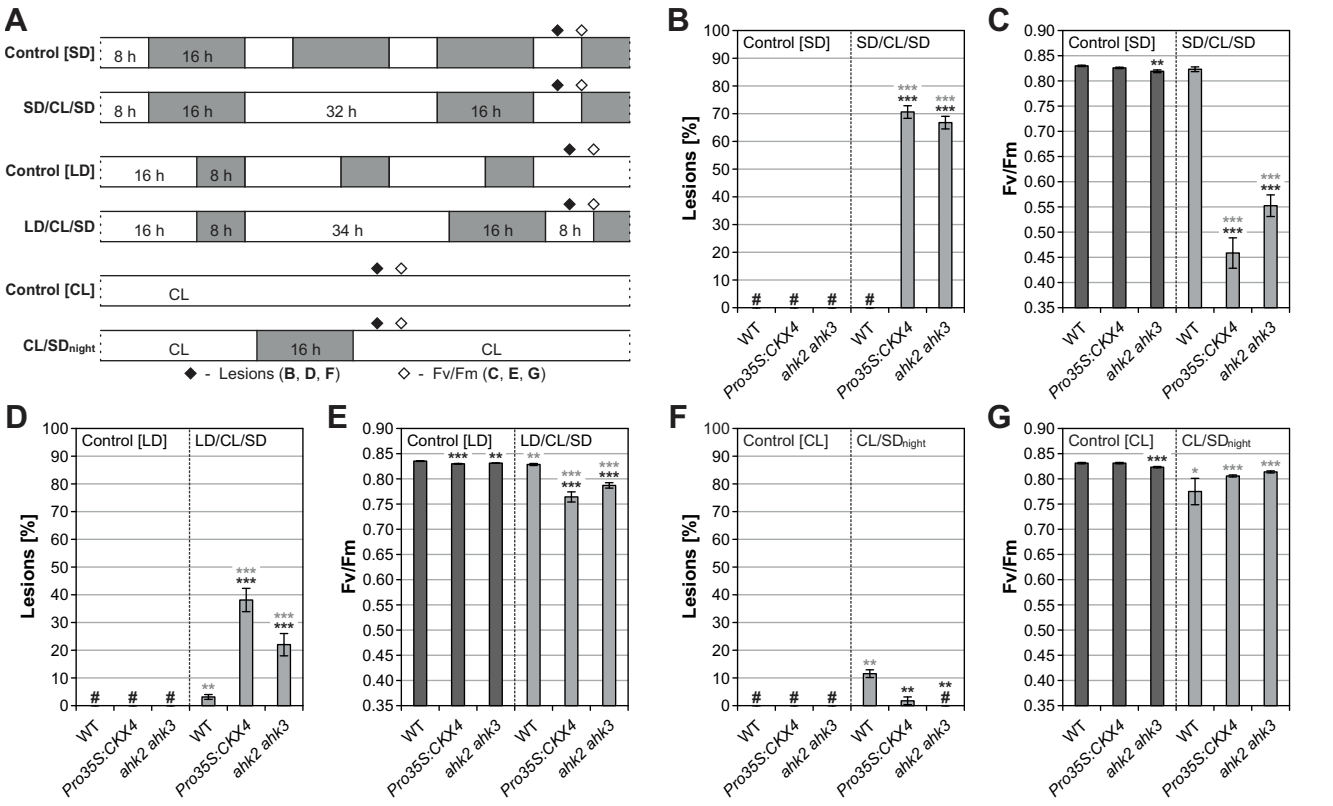


Supplemental Figure 1. Defective Cytokinin Signaling and a Reduced Cytokinin Content Lead to Cell Death in Response to CL Treatment.

(A), **(C)**, **(E)**, and **(G)** Percentage of mature leaves with lesions in cytokinin receptor mutants (**[A]**, *n* = 10), B-type *arr* mutants (**[C]**, *n* = 16), *CKX* overexpressing transgenic plants (**[E]**, *n* = 8-12), and *ipt* mutants (**[G]**, *n* = 14) 1 d after CL treatment. #, not detected.

(B), **(D)**, **(F)**, and **(H)** Representative phenotypes 2 to 3 d after CL treatment, showing lesions in plants with defective cytokinin signaling or reduced cytokinin levels in comparison with wild-type. *Pro35S:CKX4* transgenic plants served as a positive control.

Data are mean values ± SE. Asterisks indicate significant differences from wild type (black). * P < 0.05; ** P < 0.01; *** P < 0.001 (*t* test).

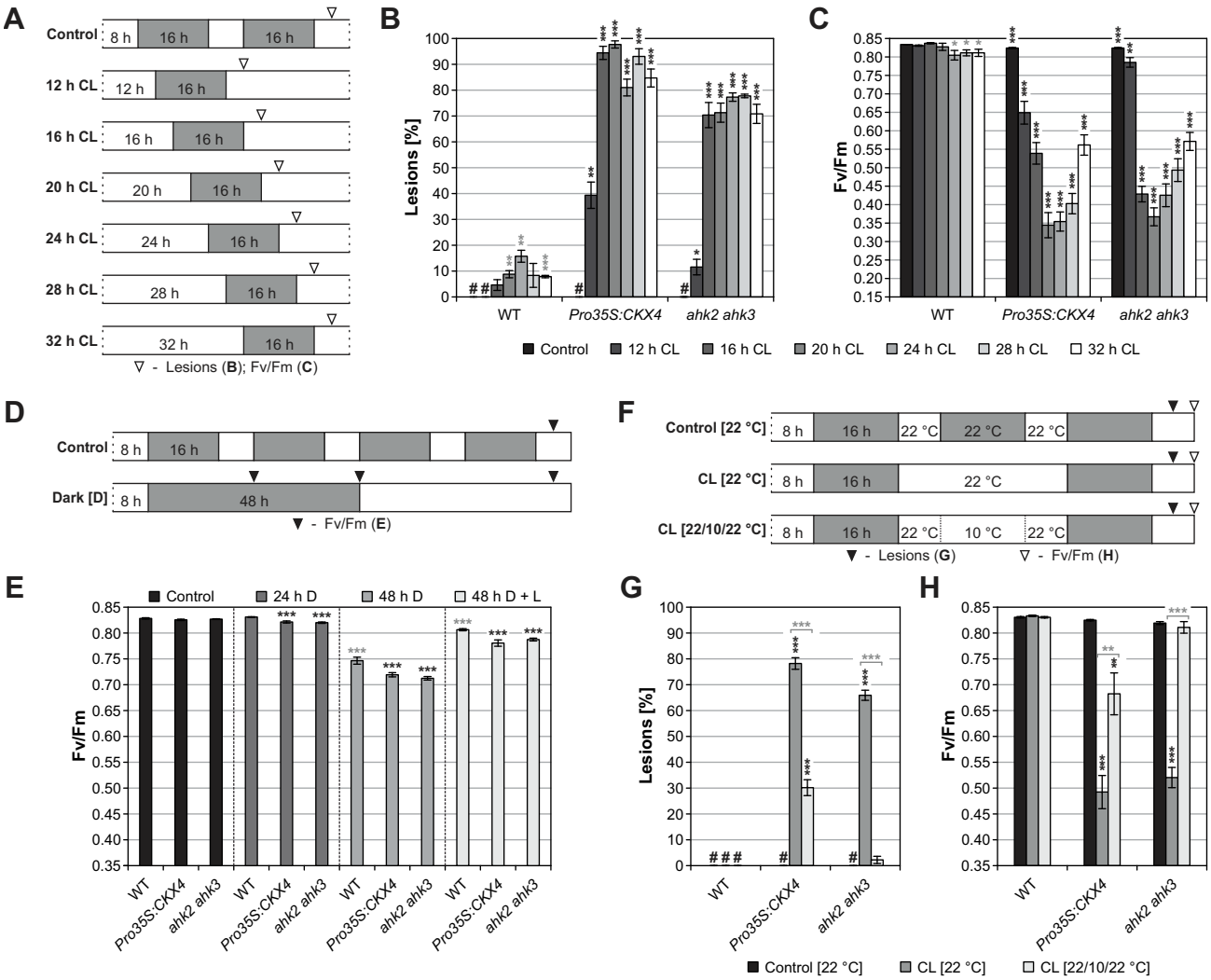


Supplemental Figure 2. The Entrainment Strongly Influences the Severity of Cell Death Following Changes in the Light-Dark Regime.

(A) Experimental scheme for **(B)** to **(G)**. White, light period; gray, dark period; SD, short day; CL, continuous light; LD, long day. Time of sampling is indicated by rhombs.

(B) to **(G)** Analysis of the response to changes in the light-dark regimes after different entrainment regimes. Percentage of mature leaves with lesions (**[B]**, *n* = 10; **[D]**, *n* = 10; **[F]**, *n* = 7; #, not detected) and stress-induced decrease in F_v/F_m (**[C]**, *n* = 12; **[E]**, *n* = 15 **[G]**, *n* = 20), measured at time points indicated in **(A)**. Consequences of SD (~5 weeks, **[B]** and **[C]**), LD (~4 weeks, **[D]** and **[E]**), and CL entrainment (~3 weeks, **[F]** and **[G]**).

Data are mean values ± SE. Asterisks indicate significant differences from respective wild types (black) and corresponding controls (gray). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (*t* test).



Supplemental Figure 3. The Cell Death Phenotype Depends on an Extended Light Period and can be Alleviated by Temperature Cycles.

(A) Experimental scheme for **(B)** and **(C)** to analyze the influence of prolonged light treatments of varying length. Plants were SD-entrained for 6 weeks. Controls remained under SD conditions. White, light period; gray, dark period; SD, short day; CL, continuous light. Time of sampling is indicated by triangles.

(B) and **(C)** Percentage of mature leaves with lesions (**[B]**, $n = 5$; #, not detected) and stress-induced decrease in F_v/F_m (**[C]**, $n = 12$), measured at time points indicated in **(A)**.

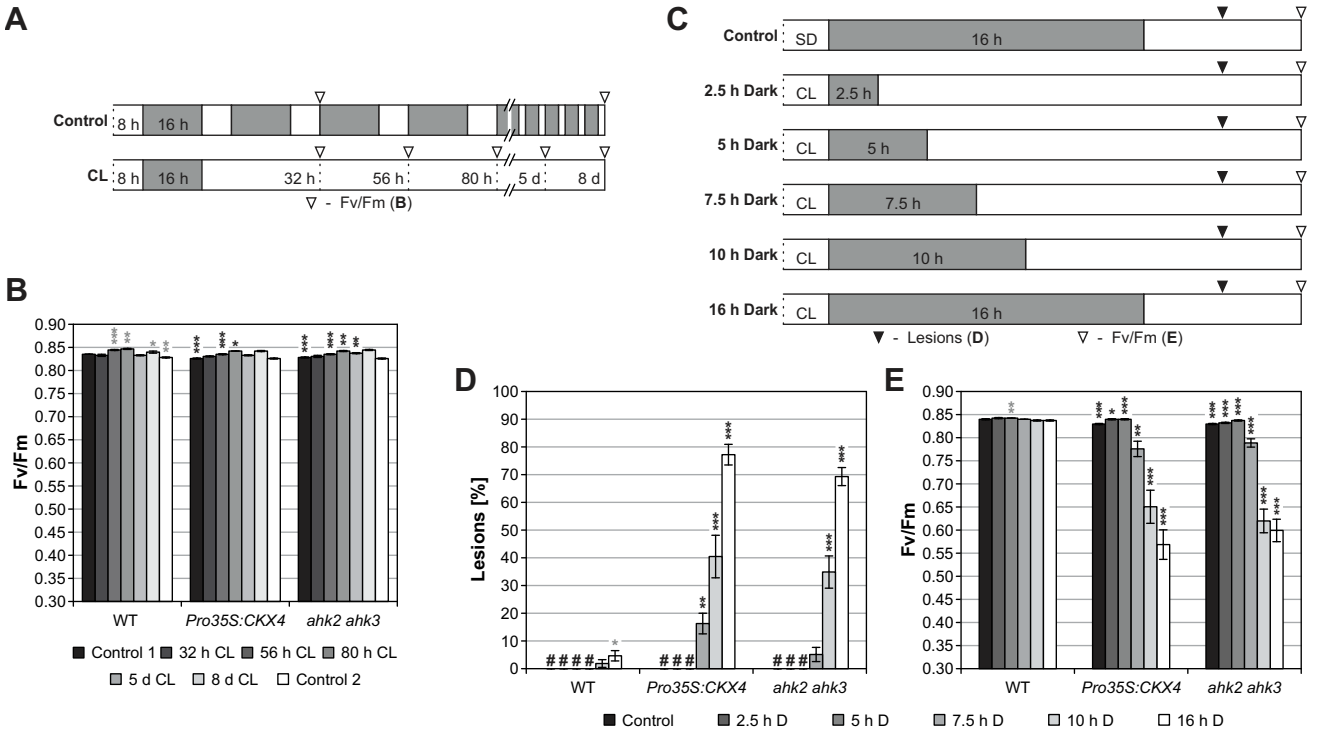
(D) Experimental scheme for **(E)** to examine the influence of a prolonged dark period. Plants were SD-entrained for 5 weeks. Time of sampling is indicated by triangles.

(E) F_v/F_m after prolonged dark periods (D) and after relaxation (L, light) from 48 h of darkness ($n = 12$).

(F) Experimental scheme for **(G)** and **(H)** to test the influence of a temperature cycle. Plants were SD-entrained for 6 weeks. Time of sampling is indicated by triangles.

(G) and **(H)** Percentage of mature leaves with lesions (**[G]**, $n = 10$; #, not detected) and stress-induced decrease in F_v/F_m (**[H]**, $n = 12$), measured at time points indicated in **(F)**.

Data are mean values ± SE. Asterisks indicate significant differences from the respective wild types (black), the corresponding controls (gray in **[B]**, **[C]**, and **[E]**, for wild type only), and between the two CL treatments (gray in **[G]** and **[H]**). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (*t* test).



Supplemental Figure 4. A Dark Period Following Extended Light Treatment Is Required to Induce the Cell Death Phenotype.

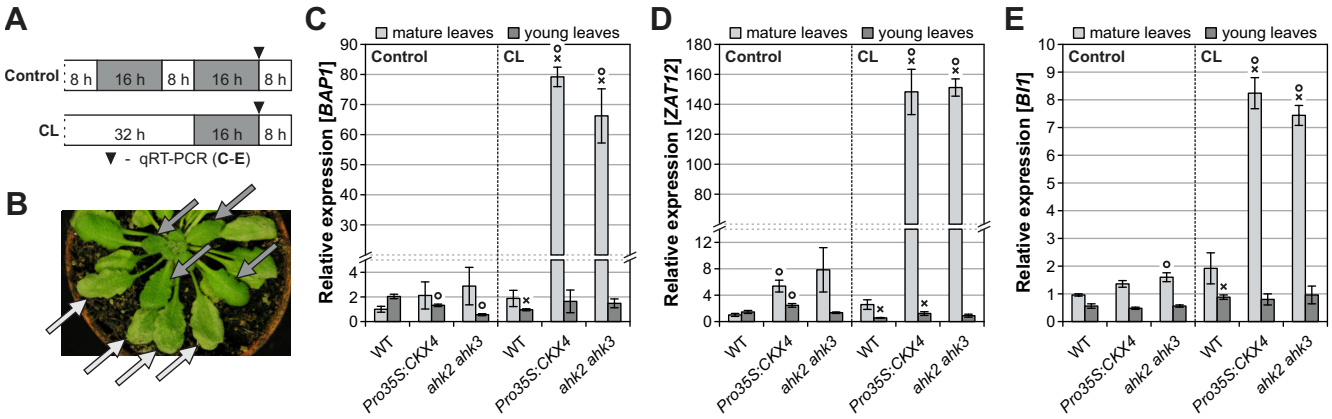
(A) Experimental scheme for **(B)** to test the influence of continuous light treatment without a following dark period. Plants were SD-entrained for 5 weeks. White, light period; gray, dark period; CL, continuous light; SD, short day. Time of sampling is indicated by triangles.

(B) Stress-induced decrease in F_v/F_m ($n = 12$), measured at time points indicated in **(A)**.

(C) Experimental scheme for **(D)** and **(E)** to examine the influence of dark periods of different length following standard CL treatment. Plants were SD-entrained for 5 weeks. Time of sampling is indicated by triangles.

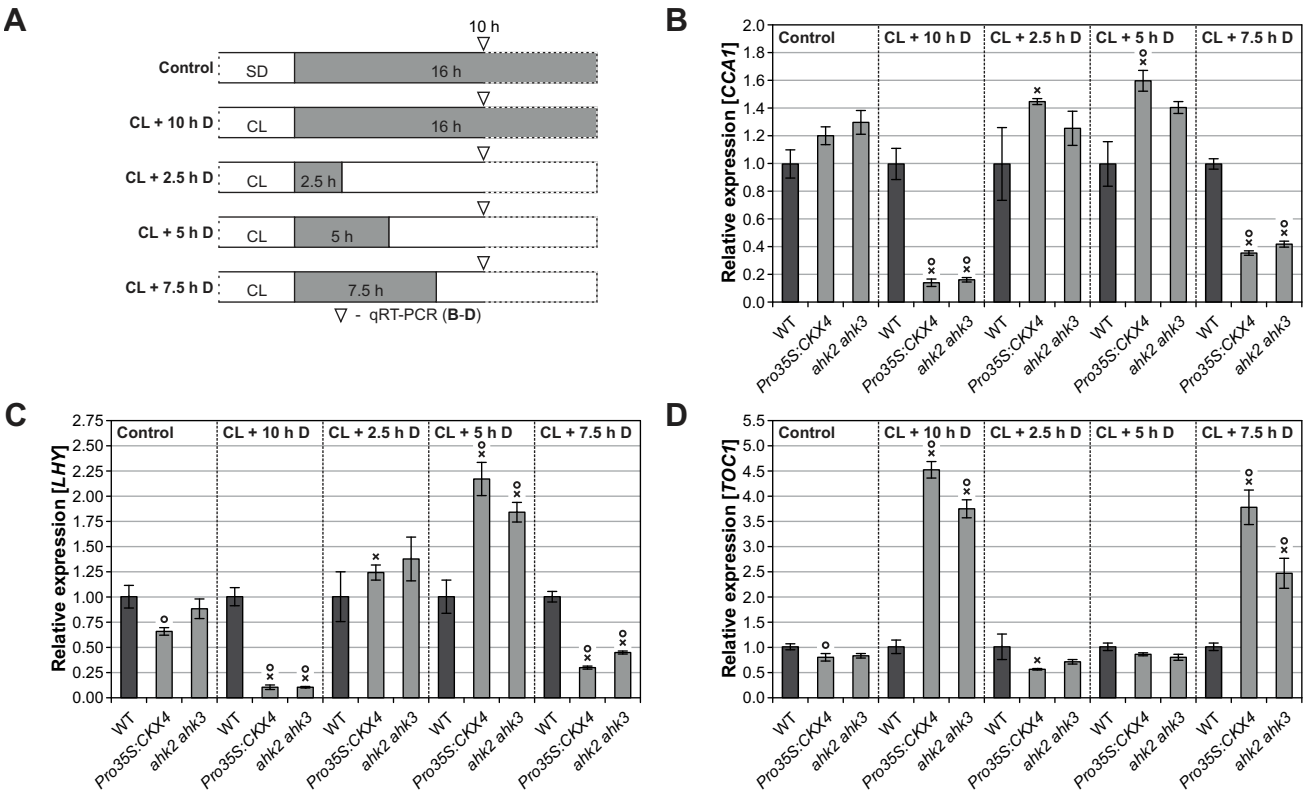
(D) and **(E)** Percentage of mature leaves with lesions (**[D]**, $n = 10$; #, not detected) and stress-induced decrease in F_v/F_m (**[E]**, $n = 14$) measured at time points indicated in **(C)**.

Data are mean values ± SE. Asterisks indicate significant differences from the respective wild types (black) and the corresponding controls (gray, for wild type only). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (*t* test).



Supplemental Figure 5. Induction of Stress- and Cell Death-Associated Genes Only Occurs in Mature Leaves, Initiating Cell Death.

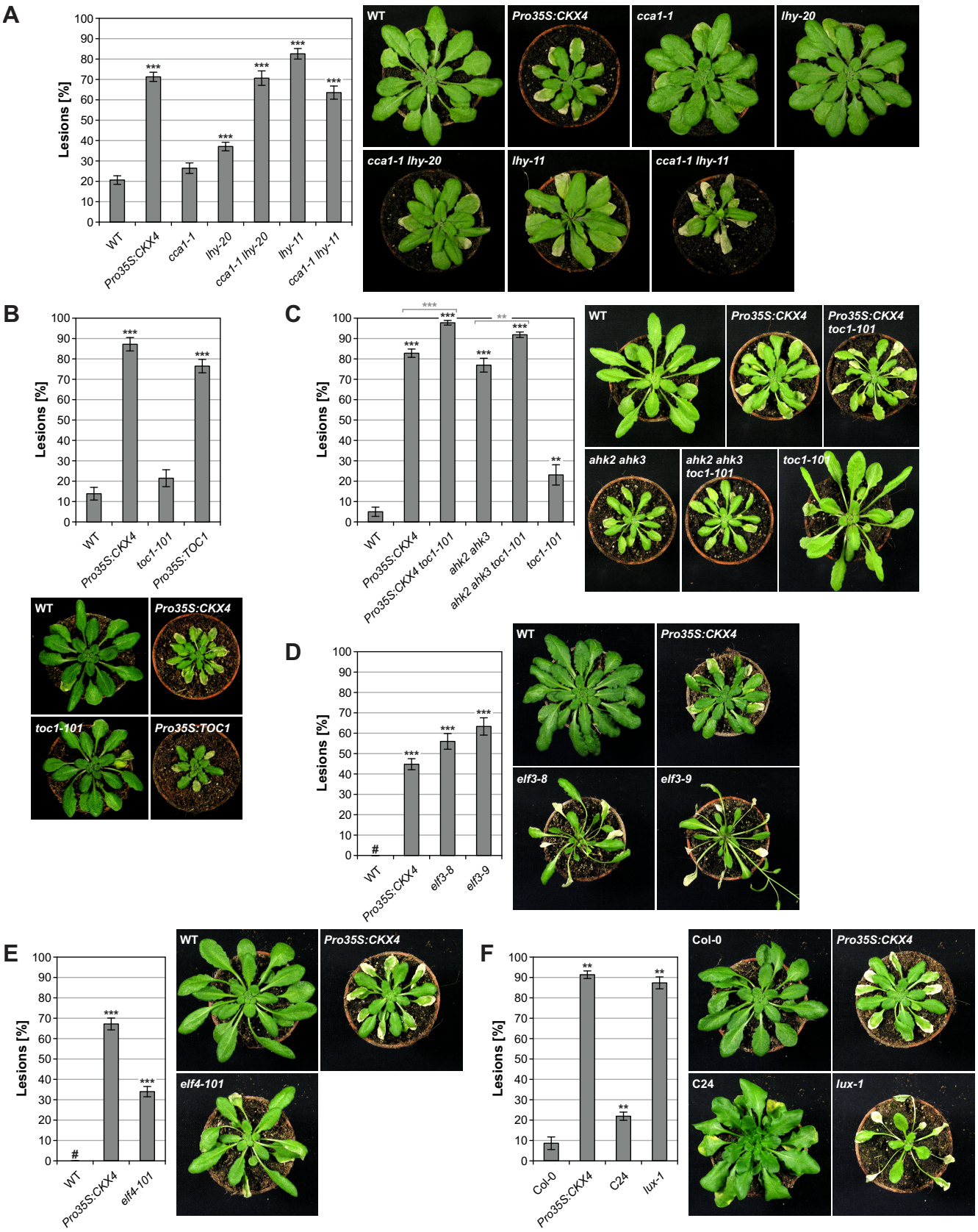
(A) Experimental scheme for **(C)** to **(E)**. Prior to the experiment plants were SD-entrained for 6 weeks. Leaf samples were collected at the indicated time point (arrow head). White, light period; gray, dark period; SD, short day; CL, continuous light.
(B) Cell death phenotype of a *Pro35S:CKX4* plant following CL treatment at the time point indicated in **(A)**. Arrows point to affected mature leaves (light gray) and to unaffected young leaves (dark gray), corresponding to the sampled material in **(C)** to **(E)**.
(C) to **(E)** Transcript levels in mature and young leaves at the end of a 16-h dark period following CL treatment. The expression levels of oxidative stress marker genes *BAP1* **(C)** and *ZAT12* **(D)** and cell death marker gene *BI1* **(E)** are shown. Expression levels were normalized to the mature leaves wild-type control, which was set to 1. Data are mean values of four biological replicates ± SE. Symbols indicate significant differences ($P < 0.05$; *t* test) from the corresponding control (X) and the respective wild type (O).



Supplemental Figure 6. Earlier Onset of Light, Following Short Nights after CL Treatment, Resets Circadian Clock Gene Expression, Coinciding with the Lack of Cell Death in Cytokinin-Deficient Plants.

(A) Experimental scheme for **(B)** to **(D)**. Prior to the experiment, plants were SD-entrained for 6 weeks. Leaf samples were collected at the indicated time point (arrow head). White, light period; gray, dark period (D); CL, continuous light.

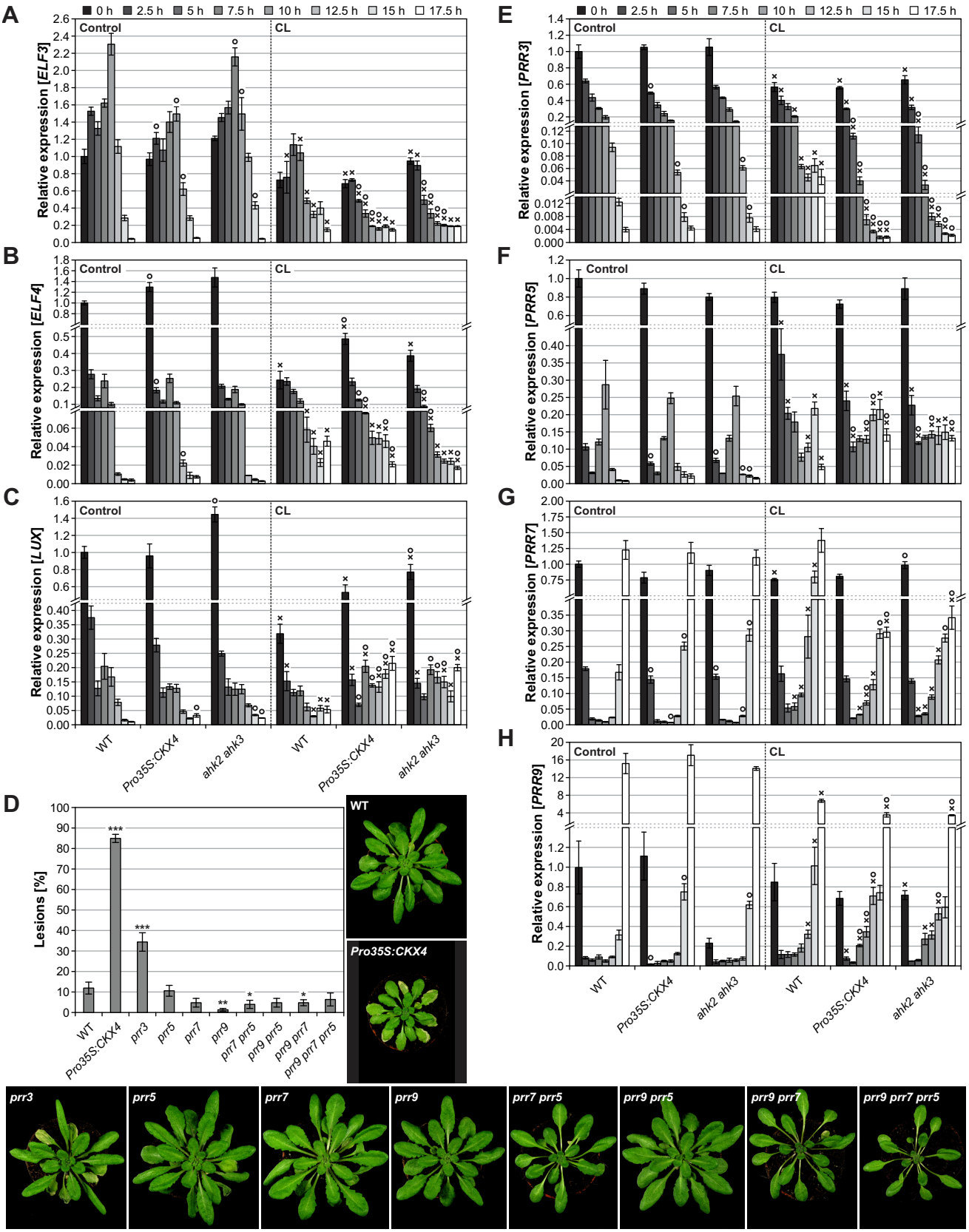
(B) to **(D)** Transcript levels of clock genes *CCA1* **(B)**, *LHY* **(C)**, and *TOC1* **(D)** at the time point indicated in **(A)**. For plant phenotypes following different night lengths see Figure 4 and Supplemental Figure 4C to 4E. Expression levels were normalized to the corresponding wild type (dark gray columns) which were set to 1. Data are mean values of four biological replicates ± SE. Symbols indicate significant differences ($P < 0.05$; *t* test) from the corresponding control (X) and the respective wild type (O).



Supplemental Figure 7. Clock Mutants Are Also Sensitive to Circadian Stress.

Supplemental Figure 7. Clock Mutants Are Also Sensitive to Circadian Stress.

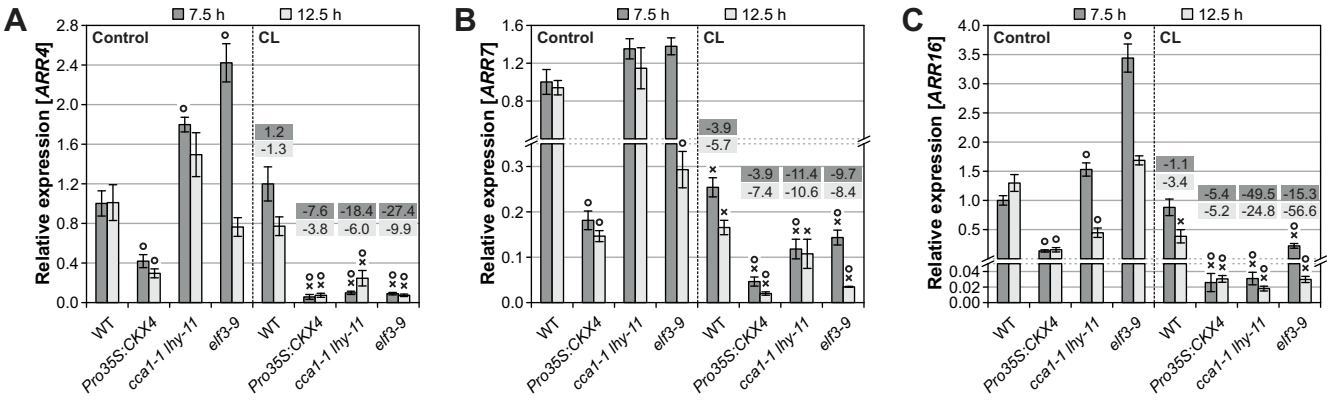
(A) to **(F)** Percentage of mature leaves with lesions in plants with *CCA1*/*LHY* loss-of-function **(A),** *TOC1* loss of function and overexpression **(B)**, *TOC1* loss of function in the *Pro35S:CKX4* and *ahk2 ahk3* background, respectively **(C)** as well as in *elf3* **(D)**, *elf4* **(E)**, and *lux* mutants (**[F]**, corresponding wild type of *lux-1* is C24) 1 d after CL treatment. Plants were SD-entrained for 5 to 6 weeks. For each experimental setup representative phenotypes 2 to 3 days after CL treatment are shown. Data are mean values ± SE (n = 10-15). Asterisks indicate significant differences from wild type (Col-0, black) and between cytokinin-deficient plants in the wild-type or *toc1-101* background (gray, in **[C]** only). ** $P < 0.01$; *** $P < 0.001$ (t test).



Supplemental Figure 8. Kinetics of Evening Complex and Pseudo-Response Regulator Gene Expression After CL Treatment and CL Responses of Different *prr* Mutants.

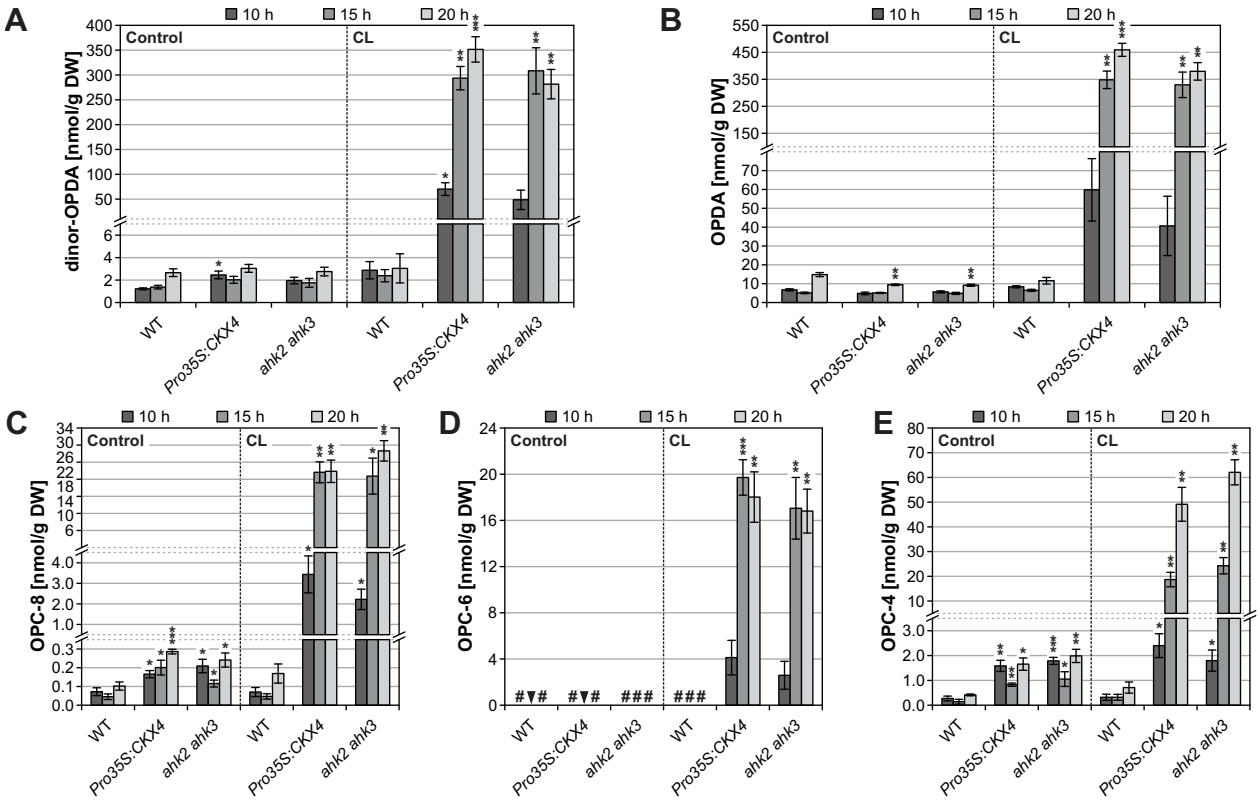
Supplemental Figure 8. Kinetics of Evening Complex and Pseudo-Response Regulator Gene Expression after CL Treatment and CL Responses of Different *prr* Mutants.

(A) to **(C)** Transcript abundances of *ELF3* **(A)**, *ELF4* **(B)**, and *LUX* **(C)** at different time points during the dark period following CL treatment (four biological replicates). The experimental scheme is shown in Figure 5B. Expression levels were normalized to the 0 h wild-type control, which was set to 1.
(D) Percentage of mature leaves with lesions in *prr* single, double, and triple mutant plants 1 d after CL treatment (n = 11). Plants were 6 weeks old. Representative phenotypes 2 d after CL treatment are shown.
(E) to **(H)** Transcript abundances of *PRR3* **(E)**, *PRR5* **(F)**, *PRR7* **(G)**, and *PRR9* **(H)**. The experimental design is the same as in **(A)** to **(C)**.
Data are mean values ± SE. Symbols in **(A)** to **(C)** and **(E)** to **(H)** indicate significant differences ($P < 0.05$; *t* test) from the corresponding control (X) and the respective wild type (O). Asterisks in **(D)** indicate significant differences from wild type. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (*t* test). CL, continuous light.



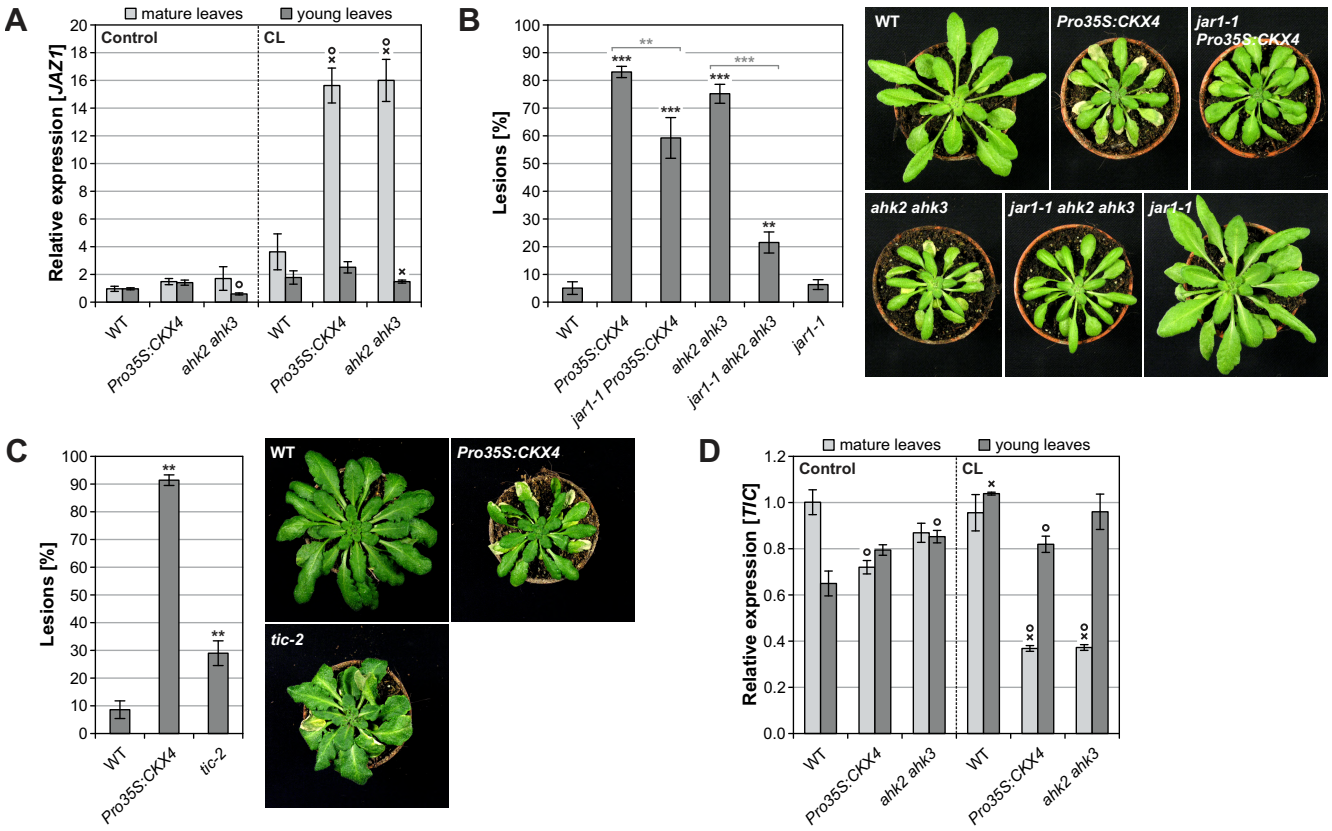
Supplemental Figure 9. A-Type *ARR* Gene Expression Is Decreased in *Pro35S:CKX4* Plants as well as in Clock Mutants in Response to Circadian Stress.

(A) to **(C)** Transcript levels of A-type *ARR* genes *ARR4* **(A)**, *ARR7* **(B)**, and *ARR16* **(C)** during the dark period at 7.5 and 12.5 h under control conditions (left) and after CL treatment (right), respectively. Fold changes of relative expression levels (CL conditions compared with respective control conditions) are displayed in the graphs. The experimental design corresponds to the one shown in Figure 5B. Expression levels were normalized to the 7.5 h wild-type control, which was set to 1. Data are mean values of four biological replicates ± SE. Symbols indicate significant differences ($P < 0.05$; *t* test) from the corresponding control (X) and the respective wild type (O).



Supplemental Figure 10. Jasmonic Acid Precursor Levels in Response to CL Treatment.

(A) and **(E)** JA precursor contents measured at late time points following CL treatment as described in Figure 10A. OPDA, oxo-phytodienoic acid; OPC, oxo-pentenyl-cyclopentane; #, not detected; triangles, < 0.01 nmol/g DW. Data are mean values ± SE (n = 4). Asterisks indicate significant differences from the respective wild types (black) and the corresponding controls (gray, for wild type only). * P < 0.05; ** P < 0.01; *** P < 0.001 (t test).



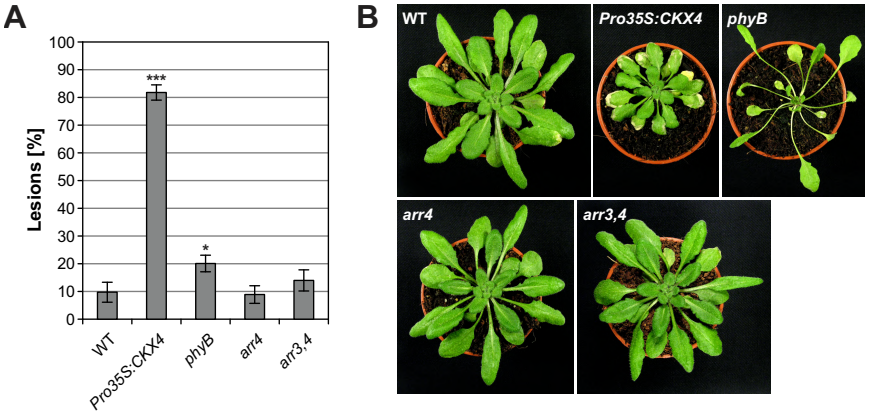
Supplemental Figure 11. Circadian Stress-Induced Cell Death in Cytokinin-Deficient Plants Is Linked to an Activated Jasmonic Acid Pathway.

(A) Transcript levels of the *JAZ1* gene in mature and young leaves at the end of a 16-h dark period following CL treatment (four biological replicates). The experimental scheme is shown in Supplemental Figure 5A. Expression levels were normalized to the mature leaves wild-type control, which was set to 1.

(B) and **(C)** Percentage of mature leaves with lesions in cytokinin-deficient plants in wild-type and *jar1-1* background (**[B]**, $n = 10$) and *Pro35S:CKX4* plants in comparison with *tic-2* plants (**[C]**, $n = 10$) 1 d after CL treatment. Plants were 5 to 6 weeks old. For each experimental setup representative pictures of the plant phenotypes 2 to 3 d after CL treatment are shown.

(D) Transcript levels of the *TIC* gene in mature and young leaves. The experimental design is the same as in **(A)**.

Data are mean values ± SE. Symbols in **(A)** and **(D)** indicate significant differences ($P < 0.05$; *t* test) from the corresponding control (X) and the respective wild type (O). Asterisks in **(B)** and **(C)** indicate significant differences from wild type (black) and between cytokinin-deficient plants in the wild-type or *jar1-1* background (gray, in **[B]** only). ** $P < 0.01$; *** $P < 0.001$ (*t* test).



Supplemental Figure 12. PHYB, ARR3, and ARR4 Have No Major Role under Circadian Stress.

(A) Percentage of mature leaves with lesions in *phyB*, *arr4*, and *arr3,4* mutants compared with *Pro35S:CKX4* plants 1 d after CL treatment. Plants were 6 weeks old. Data are mean values ± SE (*n* = 10). Asterisks indicate significant differences from wild type. * $P < 0.05$; *** $P < 0.001$ (t test).
(B) Representative pictures of the plant phenotypes 2 d after CL treatment.

Supplemental Table 1. Primers Used in this Study.

Gene	Accession Number	Forward Primer	Reverse Primer	Efficiency [%]
ARR4	AT1G10470	CCGTTGACTATCTCGCCT	CGACGTCAACACGTCATC	83.4
ARR7	AT1G19050	CTTGGAACCAATCTGCTCTC	ATCATCGACGGCAAGAAC	90.8
ARR9	AT3G57040	GATAGAGCACGTCCTAGATTCG	CTGCATTCCCTACTGAAACC	86.4
ARR16	AT2G40670	TCAGGAGGTTCTTGTTCGTCTT	AACCCAAATACTCCAATGC	114.0
BAP1	AT3G61190	CCAGAGATTACGGCGCGTGTT	TACAGACCCCAAACCGGAACTCC	99.0
BFN1	AT1G11190	GCCGGACCAGCACATGTAGT	TAAGAGCAGGCTTGGTCGGGA	89.5
BI1	AT5G47120	GCTCTTGTGGCGTCTGCCTT	AAGGGGCCAACAGAAGCACC	91.3
CAB2	AT1G29920	AGAGGCCGAGGACTTGCTTTAC	GCCAATCTTCCGTTCTTGAGC	93.4
CAT2	AT4G35090	CCGCCTGCTGTCTGTTCT	AATCGTTCTTGCCTCTCTGGT	89.6
CCA1	AT2G46830	AGCAACGTGAAAGGTGGACTGAG	GCGCTTGACCCATAGCTACACC	88.8
COI1	AT2G39940	ACAAGGAATGGAGGACGAAG	GGCGGAAGTCACAGAGGT	92.6
ELF3	AT2G25930	AACAGCAACAGCCAACAAAG	GTCACTCCTCCCCCATCTCT	94.4
ELF4	AT2G40080	AGGCAGAGCAGGGAGAGG	GGTGATTGTCGTTGACTTGTTG	93.2
FER1	AT5G01600	CAACGGTGACCACACGCCTT	ACGAGAGTGCGTTTGAGGCC	86.8
JAZ1	AT1G19180	CCCAACACCATTGACAGAAC	CTAAACCGAGCCACGACA	88.0
LHY	AT1G01060	CAACGAAACAGGTAAGTGGCGACA	TGCGTGGAAATGCCAAGGGT	87.5
LOX3	AT1G17420	ACGTTGTCGTACTGGTCGCC	GTCTCGTGGCACATACATAGGTAATG	88.7
LOX4	AT1G72520	AAGGTCTCCCTGCTGATCTCAT	AAGCCCATGTGGTTGTGTTG	94.4
LUX	AT3G46640	GCTCATCATCTTCACAAACCA	CTTCGTCGCTTGGTAATCC	87.0
MCP2D	AT1G79340	AACCCGCTATGCAGACACACG	CAGTTGGTTTCCCCGCTGGA	96.1
MYC2	AT1G32640	CAGAGAAACTCCAAATCAAGAACC	CAACGCCGACATCAACCT	87.0
OPR3	AT2G06050	GAGACATGACGGCGGCACAA	AATACTCTGCCAACGCCGCG	90.8
PP2AA2	AT3G25800	CCATTAGATCTTGTCTCTCTGCT	GACAAAACCCGTACCGAG	89.1
PRR3	AT5G60100	TGGGAGTAGTGGTGGTTTGAG	ATTGATTTGAAGGCGAGGTG	96.0
PRR5	AT5G24470	GAATGAAGCGAAAGGACAGA	GATTGGACTTGACGAACGAA	89.3
PRR7	AT5G02810	CATCGTTTCAGCCTTTACCC	CATTCCTCCAGCATTCATACC	91.0
PRR9	AT2G46790	TATGGGGGAGATTGTGGTTT	GGCAGTGATGATTTGACGAG	88.2
SAG12	AT5G45890	TCTGGTGTGTTCACTGGAGAGT	ATCCGTTAGTAGATTCGCCGTA	101.0
SAND	AT2G28390	CAGATTCGAGGTCTTCTCCT	GTGTGGCTACCATCAGAGACT	76.2
TIC	AT3G22380	TATGACGACGGAGGTGTAGG	ATTGTTGTGGCTGTGATGGT	94.5
TOC1	AT5G61380	TTGGTCACCGGCAGGAAATCC	ACTGACCCTTAACGCGGGGT	107.0
UBC10	AT5G53300	CCATGGGCTAAATGGAAA	TTCATTTGGTCCTGTCTTCAG	89.4
ZAT12	AT5G59820	CGCTTTGTCGTCTGGATTG	AGCAGCCCCACTCTCGTT	88.6

Supplemental Table 2. List of Accession Numbers.

Gene	Accession Number		Gene	Accession Number
AHK2	AT5G35750		*IPT3*	AT3G63110
AHK3	AT1G27320		*IPT5*	AT5G19040
AHK4/CRE1	AT2G01830		*IPT7*	AT3G23630
ARR1	AT3G16857		*JAR1*	AT2G46370
ARR2	AT4G16110		*JAZ1*	AT1G19180
ARR4	AT1G10470		*LHY*	AT1G01060
ARR7	AT1G19050		*LOX3*	AT1G17420
ARR9	AT3G57040		*LOX4*	AT1G72520
ARR10	AT4G31920		*LUX*	AT3G46640
ARR12	AT2G25180		*MCP2D*	AT1G79340
ARR16	AT2G40670		*MYC2*	AT1G32640
BAP1	AT3G61190		*OPR3*	AT2G06050
BFN1	AT1G11190		*PP2AA2*	AT3G25800
BI1	AT5G47120		*PRR3*	AT5G60100
CAB2	AT1G29920		*PRR5*	AT5G24470
CAT2	AT4G35090		*PRR7*	AT5G02810
CCA1	AT2G46830		*PRR9*	AT2G46790
CKX1	AT2G41510		*SAG12*	AT5G45890
CKX2	AT2G19500		*SAND*	AT2G28390
CKX4	AT4G29740		*TIC*	AT3G22380
COI1	AT2G39940		*TOC1*	AT5G61380
ELF3	AT2G25930		*UBC10*	AT5G53300
ELF4	AT2G40080		*ZAT12*	AT5G59820
FER1	AT5G01600			