The following Supplemental Data Sets were submitted to the Data Dryad Repository and are available at doi:10.5061/dryad.4nt0f:

Supplemental Data Set 1. Computational Analysis of Transcriptome-Wide AS.
Supplemental Data Set 2. Computational Analysis of Transcriptome-Wide Differential Gene Expression.
Supplemental Data Set 3. Splicing Index Analysis.
Supplemental Data Set 4. Analysis of NMD Target Features and Overlap between NMD- and Light-Regulated AS.
Supplemental Data Set 5. Categorization of Light-Regulated and Reference Gene Sets into Functional Subgroups.
Supplemental Data Set 6. Expressed Intergenic Regions.
Supplemental Data Set 7. Computational Analysis of Transcriptome-Wide AS changes in response to red light based on the data from Shikata et al. (2014).

Supplemental Materials in this file:

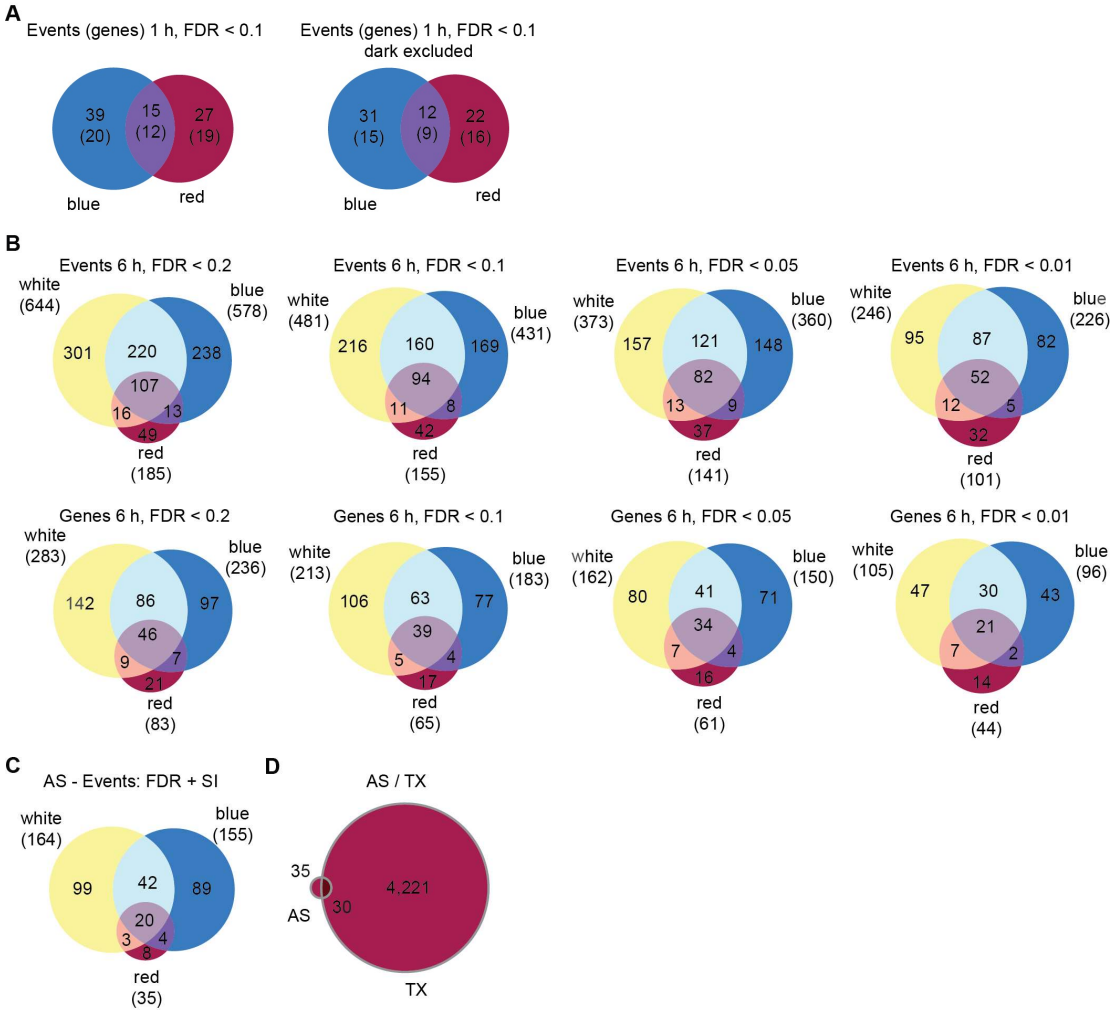
Supplemental Figure 1. Light-Triggered AS Changes Using Different Filter Criteria.

(A) Events (numbers of corresponding genes in parentheses) changing significantly in AS between 0 h and 1 h light (FDR < 0.1). Events changing in opposite directions under the two light conditions were excluded (left and right), as were events changing in the same direction as in light between 0 h and 6 h darkness (right).

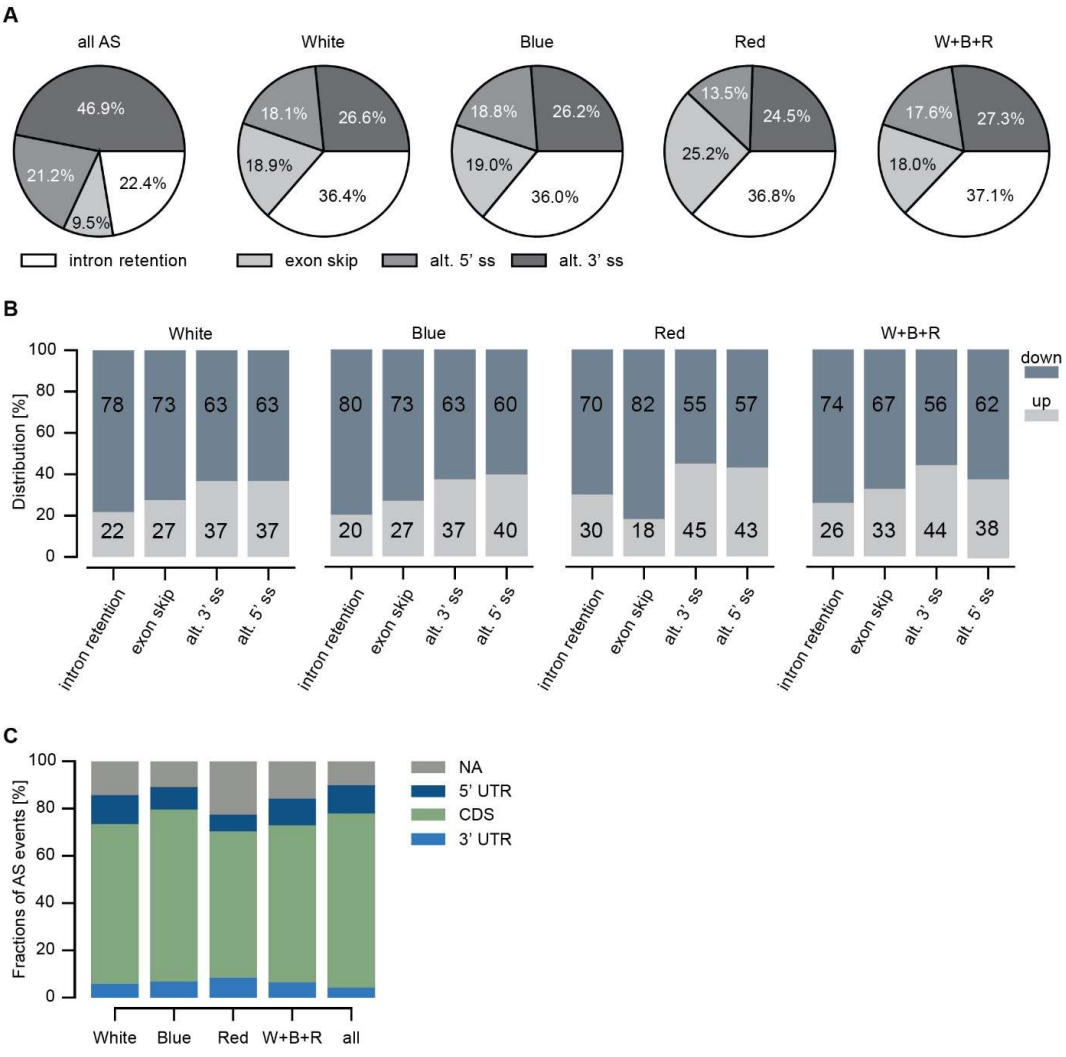
(B) Events and genes changing significantly between 6 h darkness and 6 h light under indicated FDRs. Events changing in opposite directions under two conditions were excluded. For FDR < 0.1 the diagram is taken from Fig. 1A. Total numbers of events/genes changing under each light condition are given in parentheses.

(C) Events changing significantly between 6 h darkness and 6 h light (FDR < 0.1; corresponding to Fig. 1A), and additionally having an SI change > 0.05. Only events with an FDR < 0.1 and SI change > 0.05 in the same color are displayed. For Fig. 1C, all events with an FDR < 0.1 in at least one color have been selected and then displayed only according to an SI change > 0.05. Events changing in opposite directions under two conditions were excluded.

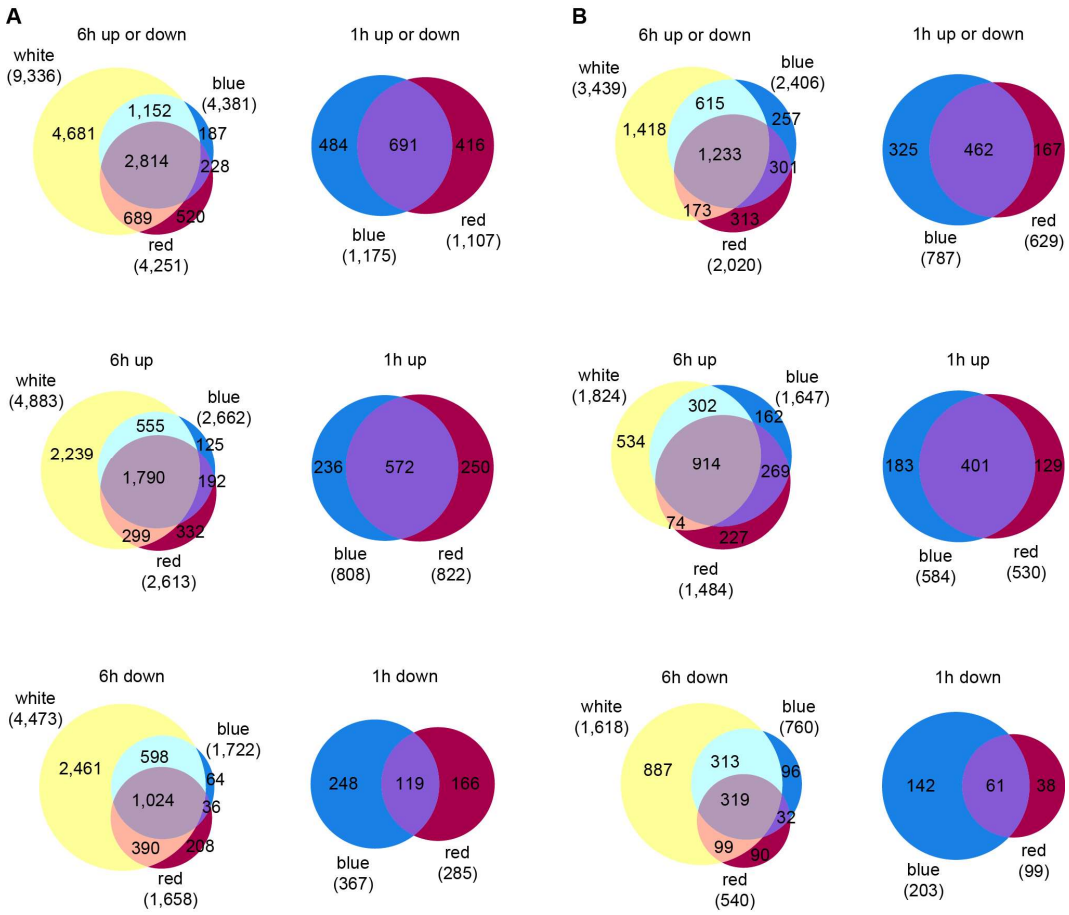
(D) Genes exhibiting changes in AS, TX, or both under red light.



Supplemental Figure 2. Properties of AS Events.
(A) Distribution of AS types in all detected events, and those changing significantly in light qualities as indicated.
(B) Direction of changes in AS. “up” and “down” refer to a relative increase and decrease, respectively, of the longer splicing variant in events exhibiting significant changes in AS. W+B+R shows the distribution in the union of white, blue, and red.
(C) Location of light-dependent AS events within the transcript. AS events were mapped to 5’ UTR, coding sequence (CDS), or 3’ UTR. If the mapping was ambiguous, the event was assigned to NA. All events stem from the comparison 6D vs 6 with FDR < 0.1, or are all detected AS events (all). W+B+R shows the distribution in the union of white, blue, and red.



Supplemental Figure 3. Light-Dependent Changes in Total Transcript Levels.
Differential gene expression (FDR ≤ 0.1) after 6 h or 1 h light exposure for all genes **(A)** and with a 2-fold threshold implemented **(B)**. The 6 h changes are in comparison to 6 h darkness, 1 h changes to 0 h. The total number of genes affected is given in parentheses for each color. For Venn diagrams showing combined up and down changes, genes changing in opposing direction under two conditions were excluded.



Supplemental Figure 4. Sequences of Splicing Variants Identified.

For splicing variants annotated in TAIR10, gene identifiers are provided in parentheses. New splicing variants are described relative to the representative splicing variant. The type and position of the AS event is given. In cases of alt. 3' ss (alternative 3' splice site) and alt. 5' ss (alternative 5' splice site) events, the position is described to be located in an intron for upstream and downstream splice sites, respectively, and in an exon for downstream and upstream splice sites, respectively. Primer binding sites are underlined. Sequences highlighted in yellow are alternatively spliced. If several splice junctions are involved, highlighted sequences alternate between non-italic and italic font.

SR30.1 (AT1G09140.1)

GTCACCTGCTAGATCCATTTCCCCGCGTTCACGGCCCCTTAGTCGTTCTCGCTCGCTATACAGCTCTGTCTCAAG
GTCCCAATCAAGATCAAAATCAAGATCAAGATCAAGATCGAATTCTCCAGTTTCACCTGTGATATCTGGTTGAAA
ATGAAAACTGGCCACTGGCTGTACCCGAATCGTCTCAAGCTTCTCAGGCT

SR30.2 (AT1G09140.2)

GTCACCTGCTAGATCCATTTCCCCGCGTTCACGGCCCCTTAGTCGTTCTCGCTCGCTATACAGCTCTGTCTCAAG
ATCTGGCTCACTGCTACGAGCTGGGGATTGGATCTAGATGGGTCATCTAGATGGATTCTTGGACTGGATTTACAA
AGCTGGATTAGCATGAACTTGAACTTCTGTTTTTACGGTCTGGTCTGGTCTGGTACTCCGCGCGTATCAGCTGTA
GGATCTGATCGCAAAGTTTTGGACTATGATTACTCTGATTCCTCAATATATTTATCTTTTTGACAATAGTGGATT
CTGTGTTGAGTTCTTTTCTAGGACAGCATTTAAGCTCCCGGGACTAGATGGGAGATGGTCAGTAAATTTCTTTGT
TATGCCACACTTACATGGGGTTTTTGGTCTTGCTGCAGGTCCCAATCAAGATCAAAATCAAGATCAAGATCAAGA
TCGAATTCTCCAGTTTCACCTGTGATATCTGGTTGAAAATGAAAACTGGCCACTGGCTGTACCCGAATCGTCTCA
AGCTTCTCAGGCT

SR30.3 cassette exon inclusion intron 10

GTCACCTGCTAGATCCATTTCCCCGCGTTCACGGCCCCTTAGTCGTTCTCGCTCGCTATACAGCTCTGTCTCAAG
ATCTGGCTCACTGCTACGAGCTGGGGATTGGATCTAGATGGGTCATCTAGATGGATTCTTGGACTGGATTTACAA
AGCTGGATTAGCATGAACTTGAACTTCTGTTTTTACGGTCTGGTCTGGTCTGGTACTCCGCGCGTATCAGCTGTC
CCAATCAAGATCAAAATCAAGATCAAGATCAAGATCGAATTCTCCAGTTTCACCTGTGATATCTGGTTGAAAATG
AAAACTGGCCACTGGCTGTACCCGAATCGTCTCAAGCTTCTCAGGCT

U2AF65B.1 (*AT1G60900.1*)

GCGCCTCCTGATATGTTAGCTGCTACTGCTGTTGCTGCAGCAGGCCAGGTTCCTAGTGTGCCAACTACTGCTACT
ATTCCAGGGATGTTCTCAAAC

U2AF65B.3 2 cassette exons included intron 4

GCGCCTCCTGATATGTTAGCTGCTACTGCTGTTGCTGCAGCAGTTTGGAAAGATCATAGTCAATTACACGGTGTG
ACTTTG*TAAGAAGGCTATGTTGGGTTAGTTGCCCCACTGTGCTGACAATGAGCCGACCTCTCTAGCTTTAATTGT*
*ACAAGGAGGAATCAGTGCAACGGTTGTCATGG*GCCAGGTTCCTAGTGTGCCAACTACTGCTACTATTCCAGGGAT
GTTCTCAAAC

U2AF65B.2 retained intron 4

GCGCCTCCTGATATGTTAGCTGCTACTGCTGTTGCTGCAGCAGGTATTTGCTTCATCATCTACTCAGGGTTATTA
TATTTCTTAACTGCTTGTTGTTTTTGATTTTACGTAGTTTGGAAAGATCATAGTCAATTACACGGTGTGACTTTG
GTAAGCTGCAATTTTATTGTTTACTCGTGAACAAAGAGGACTTTGAAGTCTTGCTTTCTTCATAGGATGTCTTGT
TATTTTTGTTTGACTGGTGGTTCTGGAACTGAGAAATTTTTTGTTTGTGTTTTTTTGGTGGTGTTTGTTTGTTTC
TGTGTCTTGTCTTTTTGTAGTAAGAAGGCTATGTTGGGTTAGTTGCCCCACTGTGCTGACAATGAGCCGACCTCT
CTAGCTTTAATTGTACAAGGAGGAATCAGTGCAACGGTTGTCATGGGTATTTTTCATCTTCGACTGGCAACTATA
TGCCTGTTTTCGAGCCTTACAATCTCTGACAACTAAAACGAATATTACATCACAACTTTTCATTTTAATATTCAT
AAGTTGTTGGTATACTTTCTACTTGTGGGTTTTGTAAGTAGCAAGGTGTTATCTGTAGCTTTGTACTTGTTGGTA
GGTCTGCTCCTCCGGGAAACATAATAATGTATCTAACAAACATTTTTATGACGCATACTACTTTACCTAGCAGGC
CAGGTTCCTAGTGTGCCAACTACTGCTACTATTCCAGGGATGTTCTCAAAC

SR34A.1 (AT3G49430.1)

TCCGAGTATTGTTGGCTTCAGACCAAATCTTCTCCGGCGCTGTAGCGTTTCCCCCGTTTCCCCTTTTTAACCTTT
GCAAACAATGAGTGGGCGATTTTCTCGGTCAATCTATGTTGGTAACTTGCCCGGTGACATTAGGGAACATGAGAT

TGAAGATATCTTTTACAAGTATGGCCGCATTGTCGATATTGAATTGAAGGTTCCACCTCGGCCTCCATGTTATTG
CTTTGTTGAGTTTGAGCATTCTCGG

SR34A.2 slightly shorter cassette exon inclusion intron 1 than annotated for *AT3G49430.3*
TCCGAGTATTGTTGGCTTCAGACCAAATCTTCTCCGGCGCTGTAGCGTTTCCCCCGATGGCAAGAGCTTGGAACA
TGAGCATTGCAATGGTTCATAGGGATATAGAGAGGGATTGGGGAGATCAATGTGGATATTGTTTTTATTATTAAT
GGAGATTCATGGGAACGATCTGGAGAAATATATATATTTCAATATTTTAAATAGCTTTTGGAGCATATTGGAGCA
AATAAGCATTTGGAGCTTGTGGAGCACTGCTCTGACCCAGGATTGCTCTCACTTGTTTCCCCTTTTTAACCTTTG
CAAACAATGAGTGGGCGATTTTCTCGGTCAATCTATGTTGGTAACTTGCCCGGTGACATTAGGGAACATGAGATT
GAAGATATCTTTTACAAGTATGGCCGCATTGTCGATATTGAATTGAAGGTTCCACCTCGGCCTCCATGTTATTGC
TTTGTTGAGTTTGAGCATTCTCGG

SR34A.3 alt. 3' ss intron 1
TCCGAGTATTGTTGGCTTCAGACCAAATCTTCTCCGGCGCTGTAGCGTTTCCCCCGATGGCAAGAGCTTGGAACA
TGAGCATTGCAATGGTTCATAGGGATATAGAGAGGGATTGGGGAGATCAATGTGGATATTGTTTTTATTATTAAT
GGAGATTCATGGGAACGATCTGGAGAAATATATATATTTCAATATTTTAAATAGCTTTTGGAGCATATTGGAGCA
AATAAGCATTTGGAGCTTGTGGAGCACTGCTCTGACCCAGGATTGCTCTCACTTGGTTTGCTTCATATATATTTT
GGTCTTACCATGTTTTCTTTTTTCTTTTCTTATCATCGTCTCATTTTGATGCTGGGGCTATGTCGAAAGCTGTTTG
AGTGGAGGTTCTTTGTCTATAGCAGTAAATTTTGATCCTTTATTTATTTCGTTTCCTTTTTATTCTCATGTGCAG
TTTCCCCTTTTTAACCTTTGCAAACAATGAGTGGGCGATTTTCTCGGTCAATCTATGTTGGTAACTTGCCCGGTG
ACATTAGGGAACATGAGATTGAAGATATCTTTTACAAGTATGGCCGCATTGTCGATATTGAATTGAAGGTTCCAC
CTCGGCCTCCATGTTATTGCTTTGTTGAGTTTGAGCATTCTCGG

GRP8.1 (AT4G39260.1)
TGAAGTTGAGTACCGGTGCTTTGTCGGCGGCCTTGCCTGGGCCACCAATGATGAAGATCTTCAAAGGACGTTCTC
ACAGTTCGGCGACGTTATCGATTCTAAGATCATTAACGACCGCGAGAGTGGAAGATCAAGGGGATTCGGATTCGT
CACCTTCAAGGACGAGAAAGCCATGAGGGATGCGATTGAAGA

GRP8.2 retained intron 1
TGAAGTTGAGTACCGGTGCTTTGTCGGCGGCCTTGCCTGGGCCACCAATGATGAAGATCTTCAAAGGACGTTCTC
ACAGTTCGGCGACGTTATCGATTCTAAGGTCTGTTACACGAGAGATCGGTCTCCCGGATCGAGCCGATTCCGATG
ATTCTGATCCTCGACGGATCTGATTCCGATCTGTTTCTCTGTTACTTGATTCGATTACTGTTACTATGTTCTCTC
TCGTTCTTTGTTACTGTTACTTAATTTGTCCCATCGGTACGTTCATCTTCCTGCTTCTATGAGCTCGGAGATCGA
TCGATTTTTGCTTTATATTCATCGCTTTGTTTTATATTCCTTCCACGATTGTTTTTGCTGATGTGTATGATTTTG
TTTGTTTACAGATCATTAACGACCGCGAGAGTGGAAGATCAAGGGGATTCGGATTCGTCACCTTCAAGGACGAGA
AAGCCATGAGGGATGCGATTGAAGA

SUF4.1 (AT1G30970.1)
ACACTAGCGGTCCTTCAATTGGTCCACCTCCTGTAATTGCAAACAAAGCTCCTAGCAATCAGCCTAATGAGGTCT
ATCTTGTATGGGATGATGAAGCGATGTCTATGGAGGAAAGAAGAATGTCCTTACCAAAATACAAAGTGCACGATG
AAACCAGCCAGATGAACTCGATAAATGCAGCGATAGACAGACGA

SUF4.2 retained intron 6
ACACTAGCGGTCCTTCAATTGGTCCACCTCCTGTAATTGCAAACAAAGCTCCTAGCAATCAGCCTAATGAGGTCT
ATCTTGTATGGGATGATGAAGCGATGTCTATGGAGGAAAGAAGAATGTCCTTACCAAAATACAAAGTGCACGATG
AAACCAGCCAGGTAAGTTCTGATTTACCCTTTTTAAAGTTAAGAAGAGTTTGCTTTTGCAAGTCTAACCCATTCG
GTAGGCTTTATTTATACATTTCTGTTCCAGATCCTATTGTTTCTGTGCTAAGAGGTACCACCGTATTATTCAGTT
TATAATTTCTTCCCTTAGAATATGATAAGAAGGACTTAGGCAAGAAACTTGAGCATTTTGAATCAATGTTCGAAA
GGTATCATTTGTAGCTTGCAATAGTAGGTAGTGAGAAACATAGCGTTTGTATCTGTTTGTTTTTCATTATATTTC
CTGTCTATTGTATTGTATTAAAAATGTGGTGATTGGTTCCACAAGATCATACTCTACAGGTTTGGTTTCAAACCC
AGGTCAGCTCTTTTTATATTCTTAACTCTGCAAAGTTTGGTACCTTTGCACAATCTCACTGCCTTGGGGAACCAA
AACAATACGCACTTAACTCTTTCAAAATAAAGCATCCAACTTCTTAACTCAACACCAAGTTTGTTTCTTTTGGTT
TGCAGATGAACTCGATAAATGCAGCGATAGACAGACGA

MYBD.1 (AT1G70000.1)
TCAAACTCCTGATCCCAACCCAACCGATGACGGTGGTTACGCTTCAGACGACGTCGTTCACGCCTCCGGTAGAAA
CCGTGAACGCAAACGAGGAACTCCATGGACAGAGGAAGAACATAG

MYBD.2 retained intron 1
TCAAACTCCTGATCCCAACCCAACCGATGACGGTGGTTACGCTTCAGACGACGTCGTTCACGCCTCCGGTAGAAA
CCGTGAACGCAAACGAGGTTAATAAAATAACCTTTCTTCTCTCTTTCCCAAATCTCATCTCTGTTTTTGTTTCTT
GTTTTTGAAACTTTCTTGCAAATTTGGAATTTAGGAACTCCATGGACAGAGGAAGAACATAG

PPD2.1 (AT4G14720.1)
CGGTGGTTGGGCAAATGACGATATTTTATAGTGGCAAAGTGAATGTATATGATGGAGTACCACCTGAAAAGGCCC
GGTCTATCATGCATTTTGCAGCCAATCCAATTGATTTGCCTGAAAATGGTATTTTTGCTTCTAGTAGAATGATTT
CGAAACCCATGAGTAAAGAGAAGATGGTGGAGCTTCCCCAATATGGACTTGAAAAGGCACCTGCTTCTCGTGATT
CTGATGTTGAGGGTCAGGCGAACAGAAAAGT

PPD2.2 retained intron 3
CGGTGGTTGGGCAAATGACGATATTTTATAGTGGCAAAGTGAATGTATATGATGGAGTACCACCTGAAAAGGCCC
GGTCTATCATGCATTTTGCAGCCAATCCAATTGATTTGCCTGAAAATGGTATTTTTGCTTCTAGTAGAATGATTT
CGAAACCCATGAGTAAAGGTAAGCATTTGCTAAAGTGTCTGATCTGCTTTTCAGTTGAAAGATTGATGATTCAGA
CTTGAAATGAGTTTTTTTTTTAATGTATAATCTAACTTGAAAAAATGACTAGAGCTGCTGCATCTCAAGATGAGT
CTTTCTTTCTTTCTTATGTCAAACACGGCCTTTCATTTTGTCCAATTTTGAAAGGAGGCATTGTTAAATTACCAA
AGAAGCATGTCTAAATGCTGTAATTGCTGCTACCTCTACTAGCTTATGTGTCTACAGATGCCTCGTGCCCCTTTT
TTTGAAATTGTGATTTGATTTTTTCAGAGAAGATGGTGGAGCTTCCCCAATATGGACTTGAAAAGGCACCTGCTT
CTCGTGATTCTGATGTTGAGGGTCAGGCGAACAGAAAAGT

PPL1.1 (AT3G55330.1)
GTGTTGTTGCTCCTTGGATCTCATTGCTTAGTAGAGCTCCATTATCATTTGCTGCAGAAAGCAAAAAAGGATTCC
TTGCTGTCTCTGACAATAAAGATGCTTATGCGTTTCTCTATCCATTTGGTTGGCAGGAAGTTGTGATTGAAGGTC
AAGATAAGGTATACAAAGATGTGATTGAGCCT

PPL1.2 alt. 3'ss exon 3
GTGTTGTTGCTCCTTGGATCTCATTGCTTAGTAGAGCTCCATTATCATAAAGCAAAAAAGGATTCCTTGCTGTCT
CTGACAATAAAGATGCTTATGCGTTTCTCTATCCATTTGGTTGGCAGGAAGTTGTGATTGAAGGTCAAGATAAGG
TATACAAAGATGTGATTGAGCCT

HYP1.1 (AT3G01100.1)
ACTGCCTTTCTTGTTGGTCTACTATTGTCTTGGATATATCATCTACCGCAACCAGCTCCTAAACGTATATGCGGC
CAAGTATGAAACTGGTGGAAAGTTTTGGCCAATAGTTCACAGCTATACTATCTTCTCTTTGGTACTAATGCACAT
TATTGCAGTCGGATTATTCGGGCTTAAAGAGCTTCCAGTGGCATCTTCTTTAACAATTCCCCTTCCGGTTCTCAC
GGTCCTTTTCAGCATTTACTGCCAAAGACGGTTTTTACCAAATTTCAAATCTTATCCTACCCAGTGTCTGGTAAA
CAAAGATAAAGCAGACGAGAGAGAGC

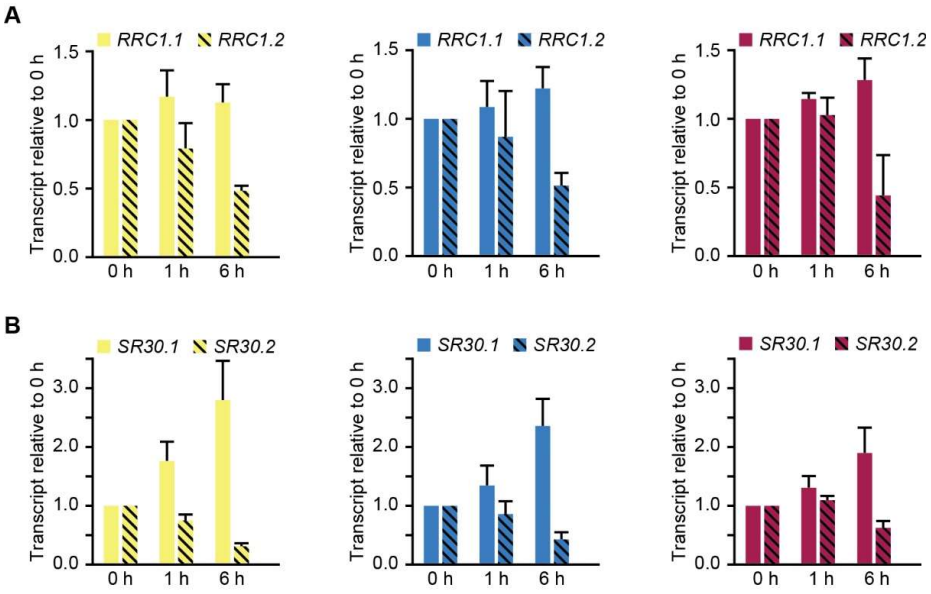
HYP1.2 retained intron 9
ACTGCCTTTCTTGTTGGTCTACTATTGTCTTGGATATATCATCTACCGCAACCAGGTAACGGAACCAATCTCACT
CCTAAGTATAATTCTCTGTTGATCTCTGATAAAATCATATAGGAATCAGCTACACATATTTTCGTTATATGGAAT
CTCACTTTTATCTTCTCTTTATATGTGATCCCTTTTCTAAATTCTCGAATAGCATCTTTAAACCGAAAGTATTTT
TTGGAGCTTCAGTCATGACATAAACGTTACATCAAGAATCCATTTTCTCGTCACATAATTCTTTTTCTTCTTAAT
GAATCCATTGCAACTTGTTGATAGAAATAACATCACCTTTTTGTGCTGCAGCTCCTAAACGTATATGCGGCCAAG
TATGAAACTGGTGGAAAGTTTTGGCCAATAGTTCACAGCTATACTATCTTCTCTTTGGTACTAATGCACATTATT
GCAGTCGGATTATTCGGGCTTAAAGAGCTTCCAGTGGCATCTTCTTTAACAATTCCCCTTCCGGTTCTCACGGTC
CTTTTCAGCATTTACTGCCAAAGACGGTTTTTACCAAATTTCAAATCTTATCCTACCCAGTGTCTGGTAAACAAA
GATAAAGCAGACGAGAGAGAGC

RRC1.1 (AT5G25060.1)
ACTTTTGTTCGAGGTGGGACGATAAATCCTGGTGACAAGCCTAAGGTTGATTCTGAAGGTGAAAAGTCCAAAGAT
GGGGGTTCGGTTTCAAAGAAGGGGAGTAGGTATGTTCCCTCTTTCCTTCCACCA

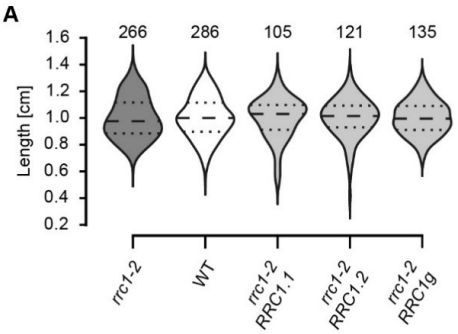
RRC1.2 exon skipping exon 3
ACTTTTGTTCGAGGTGGGACGATAAATCCTGGTGACAAGCCTAAGGTTGATTCTGAAGGTATGTTCCCTCTTTCC
TTCCACCA

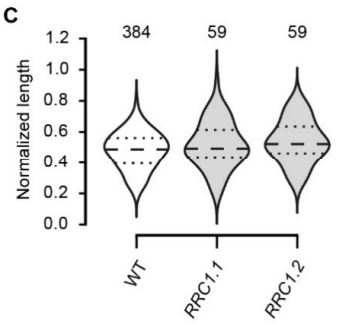
Supplemental Figure 5. Changes in Splicing Variant Levels of *RRC1* and *SR30* in Response to White, Blue, and Red Light.

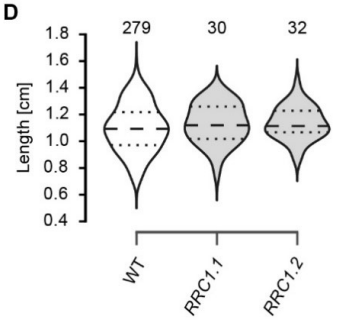
(A, B) Light-dependent AS of *RRC1* **(A)** *and SR30* **(B)** under ~130 µmol m^{-2} s^{-1} white (left), ~6 µmol m^{-2} s^{-1} blue (middle), and ~18 µmol m^{-2} s^{-1} red (right) light. Splicing variants were quantified via RT-qPCR from samples grown in darkness and collected at 0 h or after 1 h or 6 h exposure to light. Bars give average levels of splicing variants relative to total transcripts levels of the corresponding genes with values in darkness set to 1. Error bars are SD, n = 3.

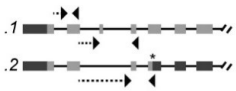


Supplemental Figure 6. Overexpression of *RRC1* Does Not Affect Hypocotyl Length.
(A, D) Hypocotyl length distribution of dark-grown *RRC1* complementation lines (A), and overexpression lines in WT background (D). All constructs are under control of the CaMV 35S promoter. The dashed line represents the median, dotted lines the quartiles. For the complementation constructs, 3 independent lines each have been analyzed in a total of 3 independent experiments, while for the overexpression constructs 1 line each has been analyzed in a single experiment.
(B) Partial gene model of *RRC1* showing the position of RT-qPCR primers. The pair above splicing variant *.1* was used to amplify total *RRC1* transcript. The pairs shown below each variant were specific to the respective variants. Transcript levels were measured using RT-qPCR and normalized to the levels of a control line (pGPTV). Segregation (Segr.) shows percentage of dead seedlings for each line grown on selective medium.
(C) Relative hypocotyl length of *RRC1* overexpression lines in WT background grown in ~10 µmol m^{-2} s^{-1} red light. The length of each hypocotyl was normalized by the mean hypocotyl length in darkness. The dashed line represents the median, dotted lines the quartiles.
(E) p-values for pair-wise comparisons in hypocotyl assays shown in (C) and Fig. 3D.
(F) Immunoblot analysis of *RRC1* complementation lines. Twenty µg total protein extract from dark-grown seedlings were loaded in each lane. The transgene was detected using α-Flag (upper panel). The stained membrane is shown as loading control (lower panel).









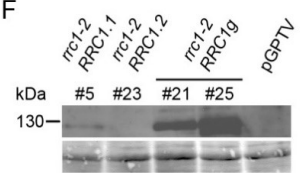
B

Genotype	Line	.1	.2	Total	Segr.
rrc1-2 RRC1g	#4	66.2	31.2	44.1	15%
	#21	23.1	16.2	27.0	23%
	#25	123.2	92.9	167.9	18%
rrc1-2 RRC1.1	#1	12.2	0.7	10.7	14%
	#5	6.9	0.5	5.2	16%
	#34	14.9	1.4	16.5	20%
rrc1-2 RRC1.2	#7	0.6	3.8	1.3	2%
	#10	1.3	8.3	3.4	25%
	#23	1.3	14.8	4.9	17%
RRC1.1	#10	138.5	4.0	99.8	30%
RRC1.2	#17	0.4	194.8	47.4	8%

E

Line 1	Line 2	median test
rrc1-2	WT	6.91×10^{-18}
rrc1-2	*rrc1-2 RRC1.1*	5.36×10^{-06}
rrc1-2	*rrc1-2 RRC1.2*	1.66×10^{-02}
rrc1-2	*rrc1-2 RRC1g*	2.00×10^{-05}
WT	*rrc1-2 RRC1.1*	5.25×10^{-01}
WT	*rrc1-2 RRC1.2*	2.95×10^{-12}
WT	*rrc1-2 RRC1g*	6.79×10^{-04}
rrc1-2 RRC1.1	*rrc1-2 RRC1.2*	8.28×10^{-08}
rrc1-2 RRC1.1	*rrc1-2 RRC1g*	1.58×10^{-01}
rrc1-2 RRC1.2	*rrc1-2 RRC1g*	1.29×10^{-07}
WT	*RRC1.1*	9.85×10^{-01}
WT	*RRC1.2*	4.81×10^{-02}
RRC1.1	*RRC1.2*	4.61×10^{-01}

F



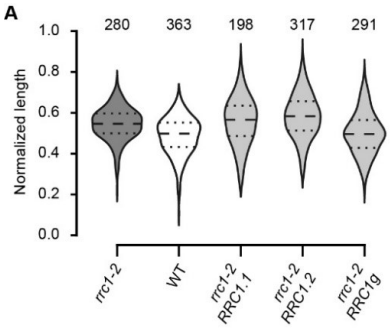
Supplemental Figure 7. Complementation of the *rrc1-2* Mutant Using Constructs under Control of the Endogenous Promoter.

(A) Violin plots depicting relative hypocotyl length of lines grown in red light (~10 µmol m^{-2} s^{-1}). The length of each hypocotyl was normalized by the mean hypocotyl length in darkness. The dashed line represents the median, dotted lines the quartiles. Replicate numbers are provided above the plot. p-values for pair-wise comparisons of hypocotyl lengths using Mood's median test are shown on the right. Data from 3 independent F1 lines per construct, analyzed in 4-5 independent experiments.

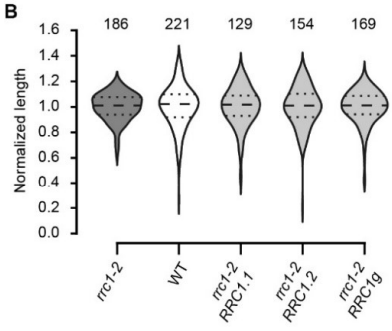
(B) Violin plots showing hypocotyl length distribution of dark-grown seedlings of the different genotypes. The dashed line represents the median, dotted lines the quartiles.

(C) Transcript levels of *RRC1.1* (.1), *RRC1.2* (.2), total *RRC1* (total), and total *RRC1* from the non-mutated allele (WT, specific for WT allele and complementation constructs; does not detect T-DNA allele) in the complementation lines used in the hypocotyl assays. Transcript levels were measured using RT-qPCR and normalized to the levels of a control (mean of WT and empty vector control, pGPTV). Segregation (Segr.) shows percentage of dead seedlings grown on selective medium.

(D) Violin plots depicting relative hypocotyl length of individual lines grown in red light (~10 µmol m^{-2} s^{-1}). This plot is a subset of the data shown in (A), including the *rrc1-2 RRC1.1*#2-12 and the other lines that were grown in parallel. Other details as described in legend to (A).

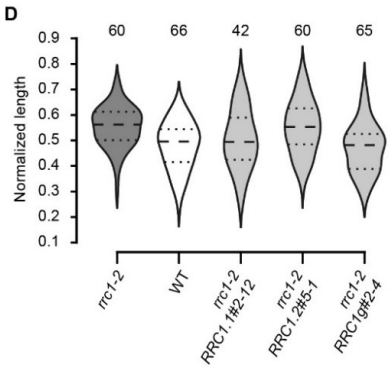


Line 1	Line 2	median test
rrc1-2	WT	1.33×10^{-08}
rrc1-2	*rrc1-2 RRC1.1*	3.07×10^{-01}
rrc1-2	*rrc1-2 RRC1.2*	8.87×10^{-04}
rrc1-2	*rrc1-2 RRC1g*	9.93×10^{-09}
WT	*rrc1-2 RRC1.1*	2.43×10^{-06}
WT	*rrc1-2 RRC1.2*	2.77×10^{-17}
WT	*rrc1-2 RRC1g*	8.75×10^{-01}



C

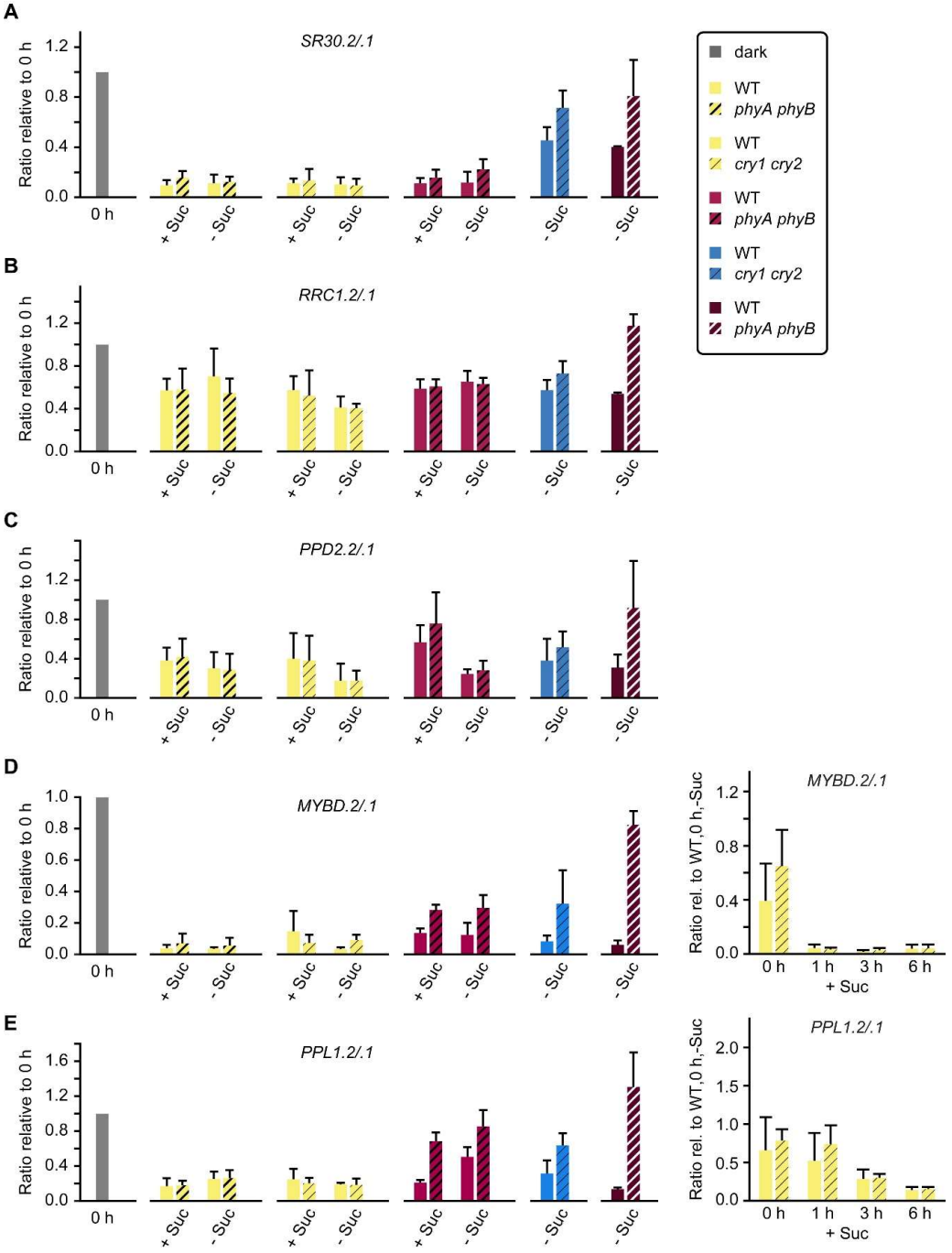
Genotype	Line	.1	.2	total	WT	Segr.
rrc1-2 RRC1g	#1-4	2.3	4.4	2.8	2.1	22%
	#2-2	1.8	2.3	2.1	3.1	8%
	#2-4	4.8	5.2	3.9	3.5	12%
rrc1-2 RRC1.1	#1-3	0.8	0.3	0.8	0.06	21%
	#2-6	1.0	0.5	1.1	0.08	17%
	#2-8	0.8	0.3	0.8	0.05	22%
	#2-12	0.8	0.4	0.8	0.17	15%
rrc1-2 RRC1.2	#3-6	0.7	1.5	0.9	0.18	26%
	#4-4	0.5	3.5	1.0	0.58	9%
	#5-1	0.5	2.3	0.9	0.20	25%



Line 1	Line 2	median test
WT	*rrc1-2*	2.43×10^{-03}
WT	*rrc1-2 RRC1.1#2-12*	1.00
WT	*rrc1-2 RRC1.2#5-1*	4.97×10^{-02}
WT	*rrc1-2 RRC1g#2-4*	3.36×10^{-01}

Supplemental Figure 8. AS Shifts in Response to White Light Are Comparable in Wild Type and Photoreceptor Mutants.

(A-E) AS changes in *SR30* (A), *RRC1* (B), *PPD2* (C), *MYBD* (D), and *PPL1* (E) in WT and photoreceptor mutants upon 6 h exposure to white, red, blue, and far-red light. Further details in legends to Figs. 4 and 5, which are based on the same raw data. Other than in Figs. 4 and 5, AS ratios in this display are normalized to corresponding 0 h samples for each genotype. Panels for *MYBD* and *PPL1* additionally include AS changes of WT and *cry1 cry2* in the presence of sucrose expressed relative to WT at 0 h and grown without sucrose.

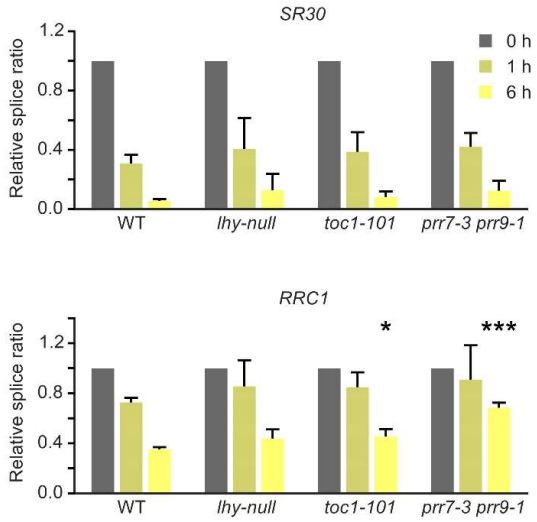


Supplemental Figure 9. Circadian Regulators Do Not Majorly Influence Light-Dependent AS of Select Candidates.

(A) Six-day-old etiolated seedlings were exposed to white light for the indicated periods. Splicing variants were co-amplified and quantified on a Bioanalyzer. Ratios *.2/.1* were calculated and normalized to each 0 h value. p-values are from independent t-tests against the ratio in the WT, * < 0.05, *** < 0.001. n = 4, error bars are SD.

(B) p-values for (A).

A



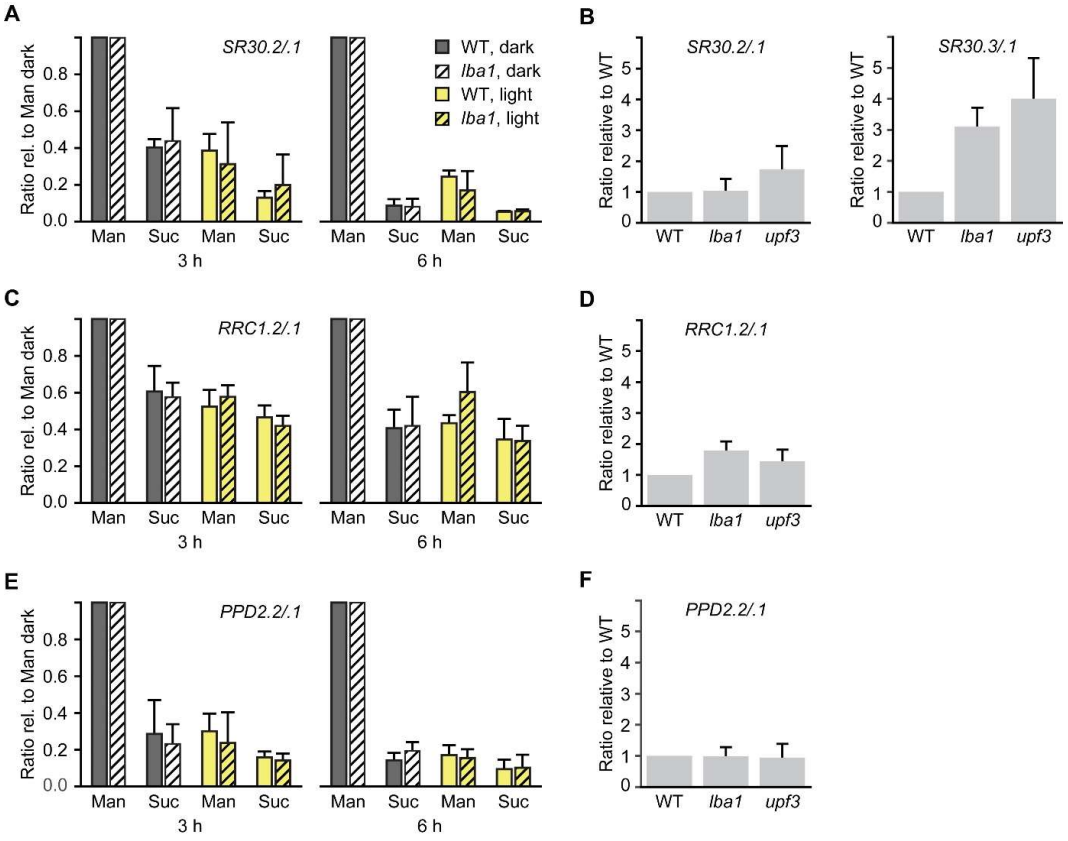
B

Gene	Line	Time	p-value
SR30	*lhy-null*	1 h	0.4056
SR30	*prr7-3 prr9-1*	1 h	0.0883
SR30	*toc1-101*	1 h	0.3170
SR30	*lhy-null*	6 h	0.2629
SR30	*prr7-3 prr9-1*	6 h	0.0927
SR30	*toc1-101*	6 h	0.2277
RRC1	*lhy-null*	1 h	0.2749
RRC1	*prr7-3 prr9-1*	1 h	0.2354
RRC1	*toc1-101*	1 h	0.1005
RRC1	*lhy-null*	6 h	0.0606
RRC1	*prr7-3 prr9-1*	6 h	5.91×10^{-6}
RRC1	*toc1-101*	6 h	0.0192

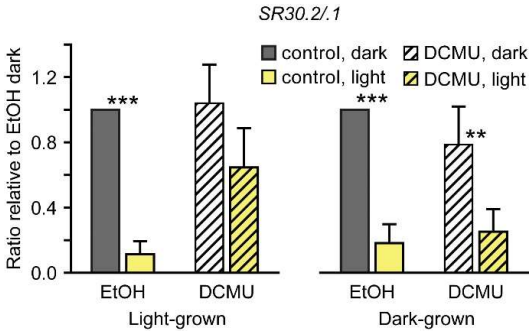
Supplemental Figure 10. Light- and Sucrose-Triggered AS Changes Are Comparable in WT and NMD Mutant Seedlings.

(A, C, E) Seedlings were grown in darkness and incubated in control medium (mannitol, Man) or sucrose (Suc) solutions for 3 h or 6 h in darkness or light. Alternative splice forms were co-amplified and quantified using a Bioanalyzer. Ratios were normalized to the corresponding control samples in darkness. Displayed are mean values (n = 3); error bars are SD.

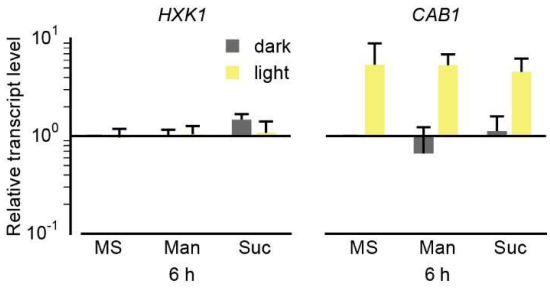
(B, D, F) AS ratios of indicated genes in light-grown WT, *lba1*, and *upf3* seedlings. For *RRC1*, AS variants were co-amplified and quantified using a Bioanalyzer. For the other candidates, levels of the splicing variants were determined using qPCR, followed by ratio calculation. *SR30.3* was included as a control transcript, which contains strong NMD-eliciting features. Displayed are means +SD (n = 3). Note that light-mediated downregulation of the splicing variants with NMD target features might affect their accumulation in light-grown NMD mutants. Accordingly, the ratio *RRC1.2/.1* is less increased in NMD mutants relative to WT in light-grown plants (Supplemental Fig. 10D) compared to etiolated seedlings (Fig. 3C).



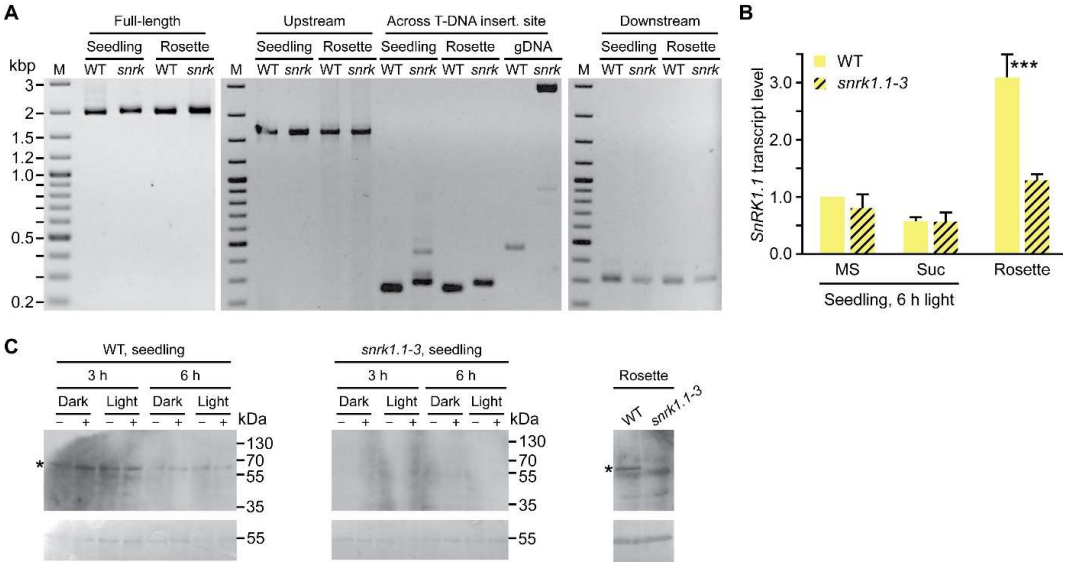
Supplemental Figure 11. DCMU Treatment Reduces Light-Dependent AS Changes in Light- and Dark-Grown *Arabidopsis* Seedlings.
Alternative splicing ratios for *SR30* in light- (left panel) and dark-grown (right panel) seedlings, incubated in darkness or light under control conditions (EtOH) or in the presence of DCMU. Light-grown seedlings were grown for 6 days in light, followed by incubation for 6 h in darkness or light. Alternatively, seedlings were grown for 6 days in darkness, followed by incubation for 6 h in darkness or light. Alternative splice forms were co-amplified and quantified using a Bioanalyzer. Ratios were normalized to the corresponding control samples in darkness. Displayed are mean values (n = 4); error bars are SD. p-values: * < 0.05, ** < 0.01, *** < 0.001, comparing corresponding dark and light samples in an independent t-test, or, if dark sample is set to 1, in a 1-sample t-test.



Supplemental Figure 12. Transcript Levels of *HXK1* and *CAB1* in Response to Sucrose and Light.
Seedlings were grown in darkness and incubated in control medium (MS), mannitol (Man), or sucrose (Suc) solutions for 6 h in darkness or light. Transcript levels were determined using RT-qPCR, and normalized to the control sample in darkness. Displayed are mean values + SD (n = 3) on a log scale. Further details can be found in the legend for Fig. 6C.



Supplemental Figure 13. Analysis of the T-DNA Insertion Mutant *snrk1.1-3*.
(A) PCR amplification of full-length and indicated partial regions of the *SnRK1.1* cDNA from wild-type (WT) and *snrk1.1-3* mutant (*snrk*) samples. cDNAs have been isolated from dark-grown seedlings or rosette leaves; PCR from genomic DNA (gDNA) has been included for the primer pair spanning the T-DNA insertion site. The following primer pairs have been used: 54/55 (full-length cDNA: 1,993 bp in WT), 54/56 (region upstream of T-DNA insertion site; 1,607 bp for cDNA), 57/58 (region spanning the T-DNA insertion site; 247 bp [cDNA] and 457 bp [gDNA] in WT, 274 bp [cDNA] and 2,909 bp [gDNA] in *snrk1.1-3*), and 59/55 (region downstream of T-DNA insertion site; 303 bp for cDNA). The PCR products for the region spanning the T-DNA insertion site have been cloned and sequenced (Supplemental Fig. 14). DNA size marker (M) contains DNA fragments in 100-bp increments, and, in the higher molecular range, as indicated.
(B) *SnRK1.1* transcript level in WT and *snrk1.1-3* mutant samples. RT-qPCR analysis was performed from etiolated seedlings exposed for 6 h to light in control (MS) or sucrose-containing (Suc) medium; additionally, transcript levels were analyzed in leaves from plants at the rosette stage. Data are normalized to WT (MS) and represent mean values +SD (n = 3). A statistically significant difference between WT and *snrk1.1-3* was found in rosette samples (***, p-value < 0.001 based on an independent t-test).
(C) Immunoblot analysis of SnRK1.1 levels in dark-grown WT and *snrk1.1-3* seedlings transferred to medium without (-) or with (+) sucrose and incubated for indicated durations in darkness (dark) or light. Additionally, rosette leaves from plants grown under long-day conditions at the end of the light phase were analyzed. Upper and lower panel show immune signal and part of the amido black-stained membrane as loading control, respectively. A specific SnRK1.1 signal (indicated with asterisk) was only detected in WT samples. Note that all samples have been analyzed on one membrane in the order presented here. Positions of size marker bands are indicated.



Supplemental Figure 14. Genomic, Transcript, and Protein Sequences for the Wild Type *SnRK1.1* and the Mutant *snrk1.1-3* Alleles.

Sequence information for the wild-type allele is based on the annotation available from TAIR10. The T-DNA insertion site and sequence in *snrk1.1-3* have been experimentally determined by sequencing the PCR products from genomic DNA and cDNA (primers 57/58, see also Supplemental Figure 13). 5' UTR, coding sequence, and 3' UTR are highlighted in blue, purple, and pink, respectively. Introns are in italics, and primer binding sites in bold letters.

AT3G01090.1 (main splicing form according to RNA-seq data from this work); genomic sequence of wild-type allele

GATACTTGCT TGCGCCTTAT AGGCTACAGG AGCGAGAGCA ATGAAAGCGA GAGGAAGAAT
CTATCAATAA TTTCTGGGTT TAAGCTGATC TCTGGATCTT CCTCAATTTT ACTTTTTCAG
CTCAGAAAAT TTTGGGACTT TTTTTTGCTG CATTTTTTGG TTCCGAATTT TCTCCTCCGC
CTTTTTTAAT TTTTG*GTAAG TTTCGATATC TCTCAATTTT CTTATTTCGG AAATAAAAAA*
AATTCTCGCC CTTTTGCAAA TCAATGGATT GATCTTGAAA TCGTCATTTC TGAATCGGGT
ACGAGAGATC CAGTAATCCT CTGCGATTGA ATTATTATTA GGGTTCATAT TCCGAATTGG
GGATAGTCTG AAAATTGCTA TTTTAAGCTC TCTTCTTAGT GTTGGTTGGT TTATTCTGGA
TAATAAAAGG GATCCTAATT CTCGCTAAAT TCTCATGTTC AAACGAGTAG ATGAGTTTAA
TTTAGTTTCT TCAACGATCG ATCACAGAAT CTTCAAAAGT GTAAAAATTA CTTGCTTTTT
GTTCCTATTA CTCTTTTGCA GTTTGTTTTG TGTGTTGTGT ACTAGTAGAT CTAATATCTC
*TCTCTCTTTT TTTGTAG*AGA ATGGATGGAT CAGGCACAGG CAGTAGAAGT GGGGTAGAAT
CGATTCTACC AAATTACAAG CTTGGGAGAA CTCTTGGTAT TGGTTCCTTT GGTAGGGTGA
AGATAGCTGA GCATGCATTG ACAGGACATA AGGTTGCTAT CAAGATCCTC AATCGTCGCA
AAATCAAGAA CATGGAGATG GAGGAGAAAG *GTACAAGTTT TGTTTTGTGT ATCCATTCCA*
CCTTAGTTGT GACTTCACGG TACTCTTGGA ATATATAAAT ATATAGCTTC TTTTTCGTCT
*TCCTTCCTTT GAACTTATTT GGTCTTTGAT GCAG*TGAGGA GAGAGATCAA AATCTTGAGA
CTATTTATGC ATCCTCACAT CATCCGTCTC TATGAGGTTA TAGAGACTCC CACAGATATT
TATCTTGTCA TGGAGTATGT GAACTCTGGT GAGCTATTTG ACTATATTGT TGAGAAGGGT
AGATTGCAGG AGGATGAGGC GAGGAACTTT TTTCAGCAG*G TATGTTTTTA TTTTATTTTA*
TCAACATGTA ACTTTCCAAG CAACCATTCG ATTTTATATT ATATTTGAGA GTTAAGAATT
*AGCTACTTGG CTTTGATGTA G*ATAATATCA GGAGTGGAAT ACTGCCATCG AAACATGGTG
GTTCACAGAG ACCTCAAGCC TGAAAACTTG CTTTTGGACT CTAAATGCAA TGTAAAGATT
GCTGATTTTG GCCTGAGCAA CATAATGCGA GATGGTCATT TTTTGAAGAC AAGTTGTGGA
AGTCCAAATT ATGCCGCTCC AGAG*GTAAGT GCTTCAGCCC TCAGGTATCT GAGGAATACT*
*ATAATTAGGC TTGTTTCAAA TGATAGGTTA TAGTCGAGAT CTATAAATTG TTTCAG*GTAA
TTTCGGGCAA GTTATATGCT GGCCCTGAAG TAGATGTCTG GAGCTGTGGT GTGATACTCT
ACGCTCTTCT CTGTGGGACG CTTCCATTTG ATGATGAAAA CATTCCCAAC CTTTTTAAGA
AGATAAAG*GT ACATTCTATT ACTCCTCCTC TTTCGTCTAT TTGAACTAAT GCTTGAATTT*
TTTATTTTAT TTTTCGTATC ACCCTTCGAC TGTAACCTTT TTTTTTTCTC TTTTCGTCCT
*ATAG*GGAGGG ATATACACAT TACCTAGCCA TTTATCTCCT GGTGCTAGAG ATTTGATCCC
CCGGATGCTT GTAGTTGACC CCATGAAACG AGTAACCATC CCTGAGATCC GGCAACACCC
TTGGTTCCAA GCTCATCTTC CGAGGTATTT AGCTGTTCCT CCTCCAGATA CTGTGCAACA
GGCAAAAAAG *GTAAGCCTAA CGTATCTACT CTTATTTTCA TTAGAGAAAT CTCTATTCCC*
*ATTTTTCGAT AAAGGCGTAA AAGCGCTGTC TTCATTTGTA G*ATTGACGAG GAGATTCTCC
AAGAAGTTAT CAATATGGGA TTTGACAGAA ACCACCTCAT CGAATCGCTC CGCAACCGAA
CCCAGAATGA T*GTATGTGTT TCCCACCCAC TTGTTCTTTT GCTGTTTCTT TCATTTCAAA*
GATTAAAAAA AATTCCATCA AAAGATCTTA AATACTGTAT TCTTACCGAC TGTCTTCCAG
GGCACTGTGA CGTACTATCT GATACTGGAC AATCGTTTCC GTGCCTCTAG TGGTTATCTC
GGGGCTGAGT TTCAAGAGAC CATG*GTTAGT CTTTTTTGTC CTTTTCGACT TTAGCAAAAG*
*ATCTTTGACG TAATGCTTCA TTGTTTGATT TAATCAG*GAA GGTACTCCCC GTATGCATCC
AGCAGAAAGC GTTGCTTCAC CTGTTAGCCA TCGGCTTCCA GGACTGATGG AATATCAAGG
AGTTGGCTTG AGATCTCAAT ACCCTGTTGA GAGAAAATGG GCTCTTGGAC TTCAG*GTTTT*
GATATTTTCT GTATTTATGG TATAAAGACT TCAGCTTTTG ATATATATGT GATTAATATT
GTAACAATTT GATCGCACAG TCTCGGGCTC ATCCCCGTGA AATAATGACG GAAGTCCTGA
AAGCCCTGCA AGATTTGAAT GTATGTTGGA AGAAGATAGG GCACTAC**AAC ATGAAGTGCA**
GATGGGTTCC TAACAGCAGC GCAGATGGTA TGCTCAGTAA CTCGATGCAC GATAACAACT
ACTTTGGAGA CGAGTCCAGC ATAATAGAGA ACGAAGCAGC
TGTTAAGTCG CCCAATGTTG TCAAGTTTGA AATTCAG*GTA AATCCTCTTC TCCACTTCAT*
GATACATATC GGTTTCCCGT ACGACATTGT TTCTGATGTG TGATTTCGAG TGAAAGAAAG

CTTTGTGTAT TAGTGTCCAT GGCCATTTTT GTAACTGAAT TAGAACTAGA GTGAATCTTC
ATGAATCTAA CTATCAATCA ATATTTTCTG GGCTTGATGA CGTTTGTTAT TGTTGATGGT
*GAAACAG*TTG TATAAAACTC GGGACGACAA GTATCTACTG GATTTGCAGA GAGTACAAGG
TCCTCAGTTC TTGTTCTTGG **ATCTGTGTGC TGCTTTTCTT GCTC**AGCTCC GAGTCCTCTG
AATACTAAAC CGGACCTCTT CAAGAATAAT CCCCACTTAT TCCTTTTTTT TTTTTTTTCT
TATTCGTCTA AGAAATTATT TAGAAAACGT AACCCTTTTT TTCTTCTTCT CTTTCTTTCT
ATTTCTATAT GCGTGCTCTA CTCAAATACC TCTTTTAACT TAAATGTAAG ACTGAGCAAG
AGTAAAAAAA AGACACTTTG TACATAACTT GTAGTGCATC ATGCCACCAA GATTGATTTT
TGTTTTTGTT TACTCCA

AT3G01090.1, protein sequence (512 amino acids)

```
  1 MDGSGTGSRS GVESILPNYK LGRTLGIGSF GRVKIAEHAL TGHKVAIKIL
 51 NRRKIKNMEM EEKVRREIKI LRLFMHPHII RLYEVIETPT DIYLVMEYVN
101 SGELFDYIVE KGRLQEDEAR NFFQQIISGV EYCHRNMVVH RDLKPENLLL
151 DSKCNVKIAD FGLSNIMRDG HFLKTSCGSP NYAAPEVISG KLYAGPEVDV
201 WSCGVILYAL LCGTLPFDDE NIPNLFKKIK GGIYTLPSHL SPGARDLIPR
251 MLVVDPMKRV TIPEIRQHPW FQAHLPRYLA VPPPDTVQQA KKIDEEILQE
301 VINMGFDRNH LIESLRNRTQ NDGTVTYYLI LDNRFRASSG YLGAEFQETM
351 EGTPRMHPAE SVASPVSHRL PGLMEYQGVG LRSQYPVERK WALGLQSRAH
401 PREIMTEVLK ALQDLNVCWK KIGHYNMKCR WVPNSSADGM LSNSMHDNNY
451 FGDESSIIEN EAAVKSPNVV KFEIQLYKTR DDKYLLDLQR VQGPQFLFLD
501 LCAAFLAQLR VL
```

AT3G01090.1 (main splicing form according to RNA-seq data from this work); genomic sequence of mutant allele (GK 579E09)

Mutant-specific sequence features:
T-DNA insertion sequence
TGGTGAAACAG: WT allele sequence deleted in mutant
AG: novel 3' splice site (part of T-DNA) used in mutant
ATTTCCCGGACATGAAGCCATTTACAA: new exonic sequence in mutant (derived from T-DNA), resulting in an early stop codon (TGA)

GATACTTGCT TGCGCCTTAT AGGCTACAGG AGCGAGAGCA ATGAAAGCGA GAGGAAGAAT
CTATCAATAA TTTCTGGGTT TAAGCTGATC TCTGGATCTT CCTCAATTTT ACTTTTTCAG
CTCAGAAAAT TTTGGGACTT TTTTTTGCTG CATTTTTTGG TTCCGAATTT TCTCCTCCGC
CTTTTTTAAT TTTTG*GTAAG TTTCGATATC TCTCAATTTT CTTATTTCGG AAATAAAAAA*
AATTCTCGCC CTTTTGCAAA TCAATGGATT GATCTTGAAA TCGTCATTTC TGAATCGGGT
ACGAGAGATC CAGTAATCCT CTGCGATTGA ATTATTATTA GGGTTCATAT TCCGAATTGG
GGATAGTCTG AAAATTGCTA TTTTAAGCTC TCTTCTTAGT GTTGGTTGGT TTATTCTGGA
TAATAAAAGG GATCCTAATT CTCGCTAAAT TCTCATGTTC AAACGAGTAG ATGAGTTTAA
TTTAGTTTCT TCAACGATCG ATCACAGAAT CTTCAAAAGT GTAAAAATTA CTTGCTTTTT
GTTCCTATTA CTCTTTTGCA GTTTGTTTTG TGTGTTGTGT ACTAGTAGAT CTAATATCTC
*TCTCTCTTTT TTTGTAG*AGA ATGGATGGAT CAGGCACAGG CAGTAGAAGT GGGGTAGAAT
CGATTCTACC AAATTACAAG CTTGGGAGAA CTCTTGGTAT TGGTTCCTTT GGTAGGGTGA
AGATAGCTGA GCATGCATTG ACAGGACATA AGGTTGCTAT CAAGATCCTC AATCGTCGCA
AAATCAAGAA CATGGAGATG GAGGAGAAAG *GTACAAGTTT TGTTTTGTGT ATCCATTCCA*
CCTTAGTTGT GACTTCACGG TACTCTTGGA ATATATAAAT ATATAGCTTC TTTTTCGTCT
*TCCTTCCTTT GAACTTATTT GGTCTTTGAT GCAG*TGAGGA GAGAGATCAA AATCTTGAGA
CTATTTATGC ATCCTCACAT CATCCGTCTC TATGAGGTTA TAGAGACTCC CACAGATATT
TATCTTGTCA TGGAGTATGT GAACTCTGGT GAGCTATTTG ACTATATTGT TGAGAAGGGT
AGATTGCAGG AGGATGAGGC GAGGAACTTT TTTCAGCAG*G TATGTTTTTA TTTTATTTTA*
TCAACATGTA ACTTTCCAAG CAACCATTCG ATTTTATATT ATATTTGAGA GTTAAGAATT
*AGCTACTTGG CTTTGATGTA G*ATAATATCA GGAGTGGAAT ACTGCCATCG AAACATGGTG
GTTCACAGAG ACCTCAAGCC TGAAAACTTG CTTTTGGACT CTAAATGCAA TGTAAAGATT
GCTGATTTTG GCCTGAGCAA CATAATGCGA GATGGTCATT TTTTGAAGAC AAGTTGTGGA
AGTCCAAATT ATGCCGCTCC AGAG*GTAAGT GCTTCAGCCC TCAGGTATCT GAGGAATACT*
*ATAATTAGGC TTGTTTCAAA TGATAGGTTA TAGTCGAGAT CTATAAATTG TTTCAG*GTAA
TTTCGGGCAA GTTATATGCT GGCCCTGAAG TAGATGTCTG GAGCTGTGGT GTGATACTCT
ACGCTCTTCT CTGTGGGACG CTTCCATTTG ATGATGAAAA CATTCCCAAC CTTTTTAAGA
AGATAAAG*GT ACATTCTATT ACTCCTCCTC TTTCGTCTAT TTGAACTAAT GCTTGAATTT*

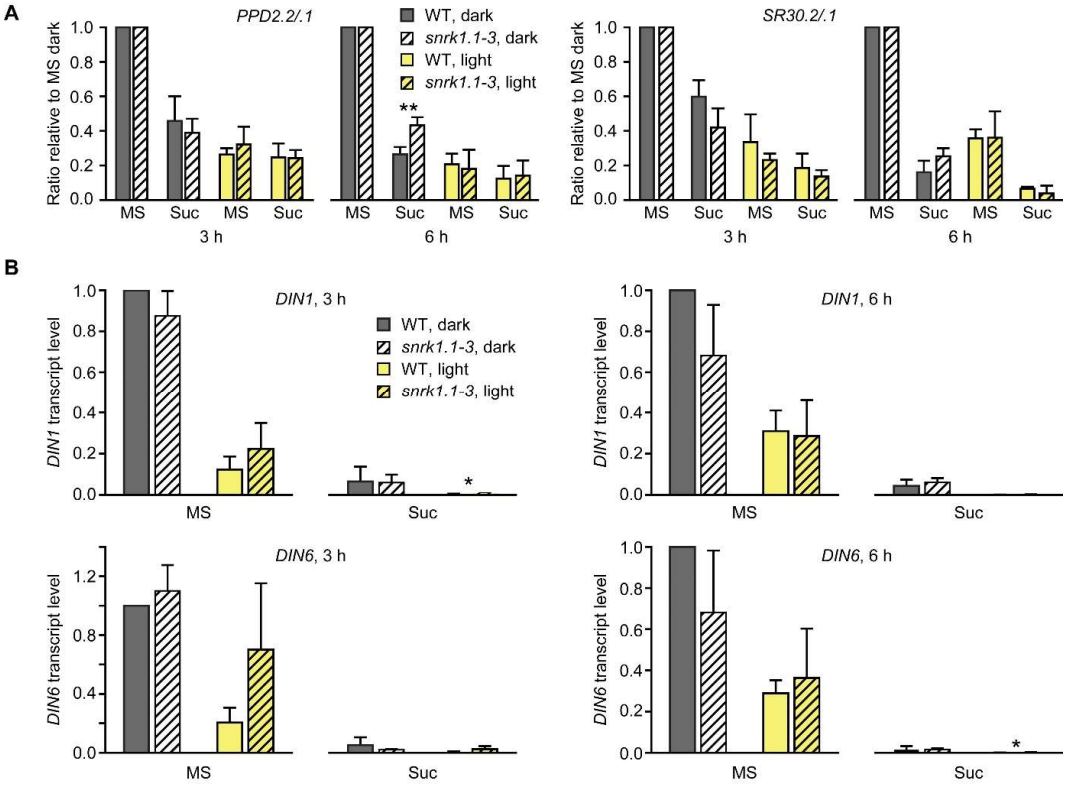
TTTATTTTAT TTTTCGTATC ACCCTTCGAC TGTAACCTTT TTTTTTTCTC TTTTCGTCCT
*ATAG*GGAGGG ATATACACAT TACCTAGCCA TTTATCTCCT GGTGCTAGAG ATTTGATCCC
CCGGATGCTT GTAGTTGACC CCATGAAACG AGTAACCATC CCTGAGATCC GGCAACACCC
TTGGTTCCAA GCTCATCTTC CGAGGTATTT AGCTGTTCCT CCTCCAGATA CTGTGCAACA
GGCAAAAAAG *GTAAGCCTAA CGTATCTACT CTTATTTTCA TTAGAGAAAT CTCTATTCCC*
*ATTTTTCGAT AAAGGCGTAA AAGCGCTGTC TTCATTTGTA G*ATTGACGAG GAGATTCTCC
AAGAAGTTAT CAATATGGGA TTTGACAGAA ACCACCTCAT CGAATCGCTC CGCAACCGAA
CCCAGAATGA T*GTATGTGTT TCCCACCCAC TTGTTCTTTT GCTGTTTCTT TCATTTCAAA*
GATTAAAAAA AATTCCATCA AAAGATCTTA AATACTGTAT TCTTACCGAC TGTCTTCCAG
GGCACTGTGA CGTACTATCT GATACTGGAC AATCGTTTCC GTGCCTCTAG TGGTTATCTC
GGGGCTGAGT TTCAAGAGAC CATG*GTTAGT CTTTTTTGTC CTTTTCGACT TTAGCAAAAG*
*ATCTTTGACG TAATGCTTCA TTGTTTGATT TAATCAG*GAA GGTACTCCCC GTATGCATCC
AGCAGAAAGC GTTGCTTCAC CTGTTAGCCA TCGGCTTCCA GGACTGATGG AATATCAAGG
AGTTGGCTTG AGATCTCAAT ACCCTGTTGA GAGAAAATGG GCTCTTGGAC TTCAG*GTTTT*
GATATTTTCT GTATTTATGG TATAAAGACT TCAGCTTTTG ATATATATGT GATTAATATT
GTAACAATTT GATCGCACAG TCTCGGGCTC ATCCCCGTGA AATAATGACG GAAGTCCTGA
AAGCCCTGCA AGATTTGAAT GTATGTTGGA AGAAGATAGG GCACTAC**AAC ATGAAGTGCA**
GATGGGTTCC TAACAGCAGC GCAGATGGTA TGCTCAGTAA CTCGATGCAC GATAACAACT
ACTTTGGAGA CGAGTCCAGC ATAATAGAGA ACGAAGCAGC
TGTTAAGTCG CCCAATGTTG TCAAGTTTGA AATTCAG*GTA AATCCTCTTC TCCACTTCAT*
GATACATATC GGTTTCCCGT ACGACATTGT TTCTGATGTG TGATTTCGAG TGAAAGAAAG
CTTTGTGTAT TAGTGTCCAT GGCCATTTTT GTAACTGAAT TAGAACTAGA GTGAATCTTC
ATGAATCTAA CTATCAATCA ATATTTTCTG GGCTTGATGA CGTTTGTTAT TGTTGA
ACCTGGATTTTGGTTTTAGGAATTAGAAATTTTATTGATAGAAGTATTTTACAAATACAAATACATACTAAGGGT
TTCTTATATGCTCAACACATGAGCGAAACCCTATAAGAACCCTAATTCCCTTATCTGGGAACTACTCACACATTA
TTATAGAGAGAGATAGATTTGTAGAGAGAGACTGGTGATTTCAGCGGGCATGCCTGTACAGGTCGACCTGCAGCC
AAGCTAGGCATGATCTAACCCTCGGTCTCTGGCGTCGCGACTGCGAAATTTCGCGAGGGTTTCCGAGATGGTGAT
TGCGCTTCGCAGATCTCCAGGCGCGTGGGTGCGGACGTAGTCAGCGCCATTGCCGATCGCGTGAAGTTCCGCCGC
AAGGCTCGCTGGACCCAGATCCTTTACAGGAAGGCCAACGGTGGCGCCCAAGAAGGATTTCCGCGACACCGAGAC
CAATAGCGGAAGCCCCAACGCCGACTTCAGCTTTTGAAGGTTCGACAGCACGTGCAGCGATGTTTCCGGTGCGGG
GCTCAAGAAAAATCCCATCCCCGGATCGAGGATGAGCCGGTCGGCAGCGACCCCGCTCCGTCGCAAGGCGGAAAC
CCGCGCCTCGAAGAACCGCACAATCTCGTCGAGCGCGTCTTCGGGTCGAAGGTGACCGGTGCGGGTGGCGATGCC
ATCCCGCTGCGCTGAGTGCATAACCACCAGCCTGCAGTCCGCCTCAGCAATATCGGGATAGAGCGCAGGGTCAGG
AAATCCTTGGATATCGTTCAGGTAGCCCACGCCGCGCTTGAGCGCATAGCGCTGGGTTTCCGGTTGGAAGCTGTC
GATTGAAACACGGTGCATCTGATCGGACAGGGCGTCTAAGAGCGGCGCAATACGTCTGATCTCATCGGCCGGCGA
TACAGGCCTCGCGTCCGGATGGCTGGCGGCCGGTCCGACATCCACGACGTCTGATCCGACTCGCAGCATTTCGAT
CGCCGCGGTGACAGCGCCGGCGGGGTCTAGCCGCCGGCTCTCATCGAAGAAGGAGTCCTCGGTGAGATTCAGAAT
GCCGAACACCGTCACCATGCACTTTACTCTTCCACCATTGCTTGTAATGGAAGTAATGTCAGTGTTGACCTTCTT
AACTGGGAATCCAGTCATGGATTTGAGGCCGCCGAATGGAGCCACCGCGGCCGATTGCACCGTAGAAGCACGGCT
GACTGTAGTCACAGCTGAAGAGGATATCATAGAAGCCATTTTTCTCACTTCTGTATGAATTGCAAAGCTGGTACG
ACGGATCGATCCACGTGTGGAAGATATGAATTTTTTTGAGAAACTAGATAAGATTAATGAATATCGGTGTTTTGG
TTTTTTCTTGTGGCCGTCTTTGTTTATATTGAGATTTTTCAAATCAGTGCGCAAGACGTGACGTAAGTATCCGAG
TCAGTTTTTATTTTTCTACTAATTTGGTCGTTTATTTCGGCGTGTAGGACATGGCAACCGGGCCTGAATTTCGCG
GGTATTCTGTTTCTATTCCAACTTTTTCTTGATCCGCAGCCATTAACGACTTTTGAATAGATACGCTGACACGCC
AAGCCTCGCTAGTCAAAAGTGTACCAAACAACGCTTTACAGCAAGAACGGAATGCGCGTGACGCTCGCGGTGACG
CCATTTCGCCTTTTCAGAAATGGATAAATAGCCTTGCTTCCTATTATATCTTCCCAAATTACCAATACATTACAC
TAGCATCTGAATTTCATAACCAATCTCGATACACCAAATCGGATCTTATAATTAAATGGCCTTCGCTGCCCATAT
TATTGGTAACTCAACAGCATCAATCACGGGATTTTTCTCGAATTAATTGCGTCGAATCTCAGCATCGAAATATTC
GCCTTTTTCGTCCATTAGACTATCTATTGTGATGGTGGATTTATCACAAATGGGACCCGCCGCCGACAGAGGTGT
GATGTTAGGCCAGGACTTTGAAAATTTGCGCAACTATCGTATAGTGGCCGACAAATTGACGCCGAGTTGACAGAC
TGCCTAGCATTTGAGTGAATTATGTAAGGTAATGGGCTACACTGAATTGGTAGCTCAAACTGTCAGTATTTATGT
ATATGAGTGTATATTTTCGCATAATCTCAGACCAATCTGAAGATGAAATGGGTATCTGGGAATGGCGAAATCAAG
GCATCGATCGTGAAGTTTCTCATCTAAGCCCCCATTTGGACGTGAATGTAGACACGTCGAAATAAAGATTTCCGA
ATTAGAATAATTTGTTTATTGCTTTCGCCTATAAATACGACGGATCGTAATTTGTCGTTTTATCAAAATGTACTT
TCATTTTATAATAACGCTGCGGACATCTACATTTTTGAATTGAAAAAAAATTGGTAATTACTCTTTCTTTTTCTC
*CATATTGACCATCATACTCATTGCTGATCCATGTAG*ATTTCCCGGACATGAAGCCATTTACAA
(deletion: TGGTGAAACAG) TTG TATAAAACTC GGGACGACAA GTATCTACTG
GATTTGCAGA GAGTACAAGG TCCTCAGTTC TTGTTCTTGG **ATCTGTGTGC TGCTTTTCTT**
GCTCAGCTCC GAGTCCTCTG AATACTAAAC CGGACCTCTT CAAGAATAAT CCCCACTTAT
TCCTTTTTTT TTTTTTTTCT TATTCGTCTA AGAAATTATT TAGAAAACGT AACCCTTTTT
TTCTTCTTCT CTTTCTTTCT ATTTCTATAT GCGTGCTCTA CTCAAATACC TCTTTTAACT

```
TAAATGTAAG ACTGAGCAAG AGTAAAAAAA AGACACTTTG TACATAACTT GTAGTGCATC
ATGCCACCAA GATTGATTTT TGTTTTTGTT TACTCCA
```

AT3G01090.1, predicted mutant protein sequence (479 amino acids)
Predicted protein lacks last 37 aa of wild type sequence and has 4 new amino acids at C terminus (ISRT)

```
  1 MDGSGTGSRS GVESILPNYK LGRTLGIGSF GRVKIAEHAL TGHKVAIKIL
 51 NRRKIKNMEM EEKVRREIKI LRLFMHPHII RLYEVIETPT DIYLVMEYVN
101 SGELFDYIVE KGRLQEDEAR NFFQQIISGV EYCHRNMVVH RDLKPENLLL
151 DSKCNVKIAD FGLSNIMRDG HFLKTSCGSP NYAAPEVISG KLYAGPEVDV
201 WSCGVILYAL LCGTLPFDDE NIPNLFKKIK GGIYTLPSHL SPGARDLIPR
251 MLVVDPMKRV TIPEIRQHPW FQAHLPRYLA VPPPDTVQQA KKIDEEILQE
301 VINMGFDRNH LIESLRNRTQ NDGTVTYYLI LDNRFRASSG YLGAEFQETM
351 EGTPRMHPAE SVASPVSHRL PGLMEYQGVG LRSQYPVERK WALGLQSRAH
401 PREIMTEVLK ALQDLNVCWK KIGHYNMKCR WVPNSSADGM LSNSMHDNNY
451 FGDESSIIEN EAAVKSPNVV KFEIQISRT
```

Supplemental Figure 15. AS Patterns and *DIN* Expression in the *snrk1.1-3* Mutant.
(A) WT and *snrk1.1-3* mutant seedlings were grown in darkness and then incubated in control medium (MS) or sucrose (Suc) solution for 3 h or 6 h in darkness or light. AS ratios were determined as described before and are normalized to corresponding MS samples. Displayed are mean values +SD (n = 3). Independent t-test was used to detect significant differences between WT and *snrk1.1-3* (** < 0.01).
(B) *DIN1* and *DIN6* transcript levels in WT and *snrk1.1-3* mutant samples. RT-qPCR analysis was performed from etiolated seedlings exposed for 3 h or 6 h in darkness or light to control (MS) or sucrose-containing (Suc) medium. Data are normalized to WT/MS/dark for each time point, and represent mean values +SD (n = 3). Transcript levels in WT and *snrk1.1-3* were compared for statistically significant differences (*, p-value < 0.05 based on an independent t-test).



Supplemental Table 1. Alignment Statistics for RNA-seq Data.
B = blue light, R = red light, W = white light, R1, 2 = biological replicate,
D = dark, Lane = lane on flow cell, Adapter = number of the multiplexing adapter used.

Library	All reads ($x10^6$)	Unmapped reads ($x10^6$)	Mapped reads ($x10^6$)	Spliced reads ($x10^6$)	Unspliced reads ($x10^6$)	Lane	Adapter
B 0h R1	47.84	4.40	43.44	12.36	31.08	2	012
B 0h R2	46.47	3.87	42.60	12.48	30.11	2	006
B 6hD R1	48.09	3.74	44.35	12.71	31.64	2	019
B 6hD R2	65.36	5.05	60.31	17.74	42.58	2	005
R 0h R1	48.47	4.69	43.78	13.02	30.77	4	012
R 0h R2	79.89	7.83	72.06	21.20	50.86	4	006
R 6hD R1	50.06	5.15	44.91	12.99	31.92	4	019
R 6hD R2	50.05	4.80	45.25	13.09	32.15	4	005
W 0h R1	42.05	4.28	37.77	11.36	26.41	6	012
W 0h R2	85.66	9.02	76.64	22.64	54.00	6	006
W 6hD R1	41.49	4.41	37.08	10.91	26.16	6	019
W 6hD R2	74.55	7.20	67.35	19.83	47.52	6	005
B 1h R1	76.62	6.62	70.00	19.87	50.13	1	012
B 1h R2	148.10	13.73	134.37	38.84	95.53	1	006
B 6h R1	68.61	6.27	62.34	18.55	43.78	3	012
B 6h R2	161.13	15.91	145.22	43.43	101.79	3	006
R 1h R1	92.95	6.56	86.39	25.25	61.13	8	012
R 1h R2	90.51	5.94	84.57	24.79	59.78	8	006
R 6h R1	90.63	7.40	83.23	24.49	58.74	5	012
R 6h R2	90.95	7.15	83.80	24.40	59.40	5	006
W 6h R1	89.97	6.40	83.57	24.87	58.70	7	012
W 6h R2	103.10	6.91	96.19	29.30	66.90	7	006

Supplemental Table 2. Frequencies of AS Types in Different Datasets.
Relative frequencies of AS events (alternative 3' splice site, Alt 3'; intron retention; alternative 5' splice site, Alt 5'; and cassette exon) based on all detected events from this study, a previous study using the same AS analysis pipeline (Rühl et al., 2012), AS events annotated in TAIR10, and an AS analysis in light-grown plants (Mancini et al., 2016). The event distribution in TAIR10 has been determined using ASTALAVISTA (Foissac and Sammeth, 2007).

AS type	All events from this study	Rühl et al., 2012	TAIR10	Mancini et al., 2016
Alt 3'	46.9%	40.6%	42.7%	40.1%
Intron retention	22.4%	29.8%	26.0%	31.3%
Alt 5'	21.2%	21.8%	18.1%	18.1%
Cassette exon	9.5%	7.8%	13.3%	10.5%

Supplemental Table 3. Light-Regulated AS Events of "Exitron" Type.
For the identification of light-regulated exitrons, AS events from the category "intron retention" were filtered using the following criteria: the inner coordinates of the corresponding events fall into a region, which is annotated as coding exon. Table provides the corresponding event IDs, the relevant transcript model from TAIR10, the representative transcript model from TAIR10, information on the splicing of this region in the representative transcript from TAIR10, the prevalent transcript type based on the coverage plots of the RNA-seq data from this study, and information whether this intron is spliced in the prevalent transcript. Note that some events were identified as exitrons based on the annotation of minor AS variants in TAIR10. Exitrons resulting in removal of exonic regions from the coding sequence of an abundant transcript variant are highlighted in bold.

Event ID	Transcript model relevant for exitron definition	Representative transcript model	Intron spliced in representative transcript	Prevalent transcript based on coverage plots	Intron spliced in prevalent transcript
new_alt_region_5228	*AT3G13060.1*	*AT3G13060.2*	yes	*AT3G13060.2*	yes
old_alt_region_1965	*AT3G13060.1*	*AT3G13060.2*	yes	*AT3G13060.2*	yes
new_alt_region_8918	*AT5G01670.2*	*AT5G01670.2*	no	*AT5G01670.1*	yes
new_alt_region_5343	*AT3G16460.1/.2*	*AT3G16460.1*	no	*AT3G16460.1*	no
new_alt_region_4820	*AT2G41840.1*	*AT2G41840.1*	no	*AT2G41840.1*	no
new_alt_region_8208	*AT4G05050.1/.2/.3*	*AT4G05050.1*	no	*AT4G05050.1*	no
new_alt_region_10109	*AT5G60600.3*	*AT5G60600.1*	yes	*AT5G60600.1*	yes
new_alt_region_4365	*AT2G21660.1*	*AT2G21660.1*	no	*AT2G21660.1*	no
old_alt_region_1638	*AT2G21660.1*	*AT2G21660.1*	no	*AT2G21660.1*	no
old_alt_region_1639	*AT2G21660.1*	*AT2G21660.1*	no	*AT2G21660.1*	no

Supplemental Table 4. Genes Underlying Circadian Regulation Are Not Differentially Expressed in Darkness.
Given are the minimum of up- and down-q-values from the analysis of differential gene expression in indicated comparisons for 8 known circadian regulated genes. Background colors indicate light conditions blue (columns 3-5), red (columns 6-8), and white (columns 9-11). Darker shades, darkness controls. q-values < 0.1 are bold.

Gene	Gene ID	0 vs 6D	0 vs 6	6D vs 6	0 vs 6D	0 vs 6	6D vs 6	0 vs 6D	0 vs 6	6D vs 6
CCA1	AT2G46830	1.00	0.21	**0.00**	1.00	**0.00**	**0.00**	1.00	0.91	0.85
LHY	AT1G01060	1.00	**0.00**	**0.00**	1.00	**0.00**	**0.00**	1.00	0.41	0.48
PRR7	AT5G02810	1.00	**0.01**	0.48	1.00	0.32	**0.05**	1.00	0.18	0.26
PRR9	AT2G46790	1.00	**0.01**	0.48	1.00	**0.09**	**0.06**	1.00	0.29	0.25
GI	AT1G22770	1.00	**0.00**	**0.00**	1.00	**0.00**	**0.00**	1.00	**0.00**	**0.00**
TOC1	AT5G61380	1.00	0.49	0.62	1.00	0.76	0.94	1.00	**0.09**	0.78
LUX	AT3G46640	1.00	0.25	**0.10**	1.00	0.62	**0.09**	1.00	**0.00**	**0.00**
ZTL	AT5G57360	1.00	0.12	0.37	1.00	0.98	0.99	1.00	**0.00**	**0.00**

Supplemental Table 5. Statistical Comparison of AS Changes in Response to Light and Sugar. Statistical analysis of data displayed in Fig. 6A comparing either light versus dark samples **(A)** or sugar versus MS control **(B)** treatment. Tests are independent t-tests (indep) when not tested against 1, and 1-sample t-test (1samp) when tested against 1; significance has been defined according to the following p-values: * < 0.05, ** < 0.01, *** < 0.001.

A

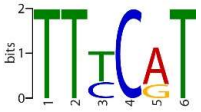
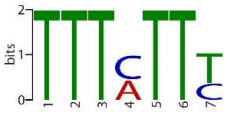
Gene	Treatment	Light	p-value	Signif.	Test
SR30	MS	1 h	0.002775	**	1samp
SR30	MS	6 h	0.015517	*	1samp
SR30	Mannitol	1 h	0.041095	*	indep
SR30	Mannitol	6 h	0.020323	*	indep
SR30	Sucrose	1 h	0.042162	*	indep
SR30	Sucrose	6 h	0.207128	n.s.	indep
RRC1	MS	1 h	0.018431	*	1samp
RRC1	MS	6 h	0.001164	**	1samp
RRC1	Mannitol	1 h	0.016465	*	indep
RRC1	Mannitol	6 h	0.000869	***	indep
RRC1	Sucrose	1 h	0.062169	n.s.	indep
RRC1	Sucrose	6 h	0.203663	n.s.	indep
PPD2	MS	1 h	0.034544	*	1samp
PPD2	MS	6 h	0.000398	***	1samp
PPD2	Mannitol	1 h	0.054986	n.s.	indep
PPD2	Mannitol	6 h	0.000369	***	indep
PPD2	Sucrose	1 h	0.003609	**	indep
PPD2	Sucrose	6 h	0.251424	n.s.	indep
MYBD	MS	6 h	0.000072	***	1samp
MYBD	Mannitol	6 h	0.004927	**	indep
MYBD	Sucrose	6 h	0.012297	*	indep
PPL1	MS	6 h	0.000545	***	1samp
PPL1	Mannitol	6 h	0.003981	**	indep
PPL1	Sucrose	6 h	0.021737	*	indep

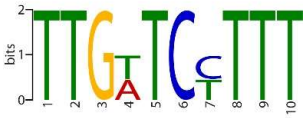
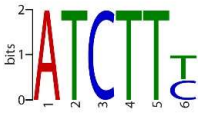
B

Gene	Tested against	Dark /light	Light	p-value	Signif.	Test
SR30	Mannitol	dark	1 h	0.479868	n.s.	1samp
SR30	Sucrose	dark	1 h	0.400477	n.s.	1samp
SR30	Mannitol	light	1 h	0.031387	*	indep
SR30	Sucrose	light	1 h	0.168992	n.s.	indep
SR30	Mannitol	dark	6 h	0.180507	n.s.	1samp
SR30	Sucrose	dark	6 h	0.022653	*	1samp
SR30	Mannitol	light	6 h	0.683503	n.s.	indep
SR30	Sucrose	light	6 h	0.025948	*	indep
RRC1	Mannitol	dark	1 h	0.271816	n.s.	1samp
RRC1	Sucrose	dark	1 h	0.639815	n.s.	1samp
RRC1	Mannitol	light	1 h	0.181789	n.s.	indep
RRC1	Sucrose	light	1 h	0.906444	n.s.	indep
RRC1	Mannitol	dark	6 h	0.202113	n.s.	1samp
RRC1	Sucrose	dark	6 h	0.030863	*	1samp
RRC1	Mannitol	light	6 h	0.808989	n.s.	indep
RRC1	Sucrose	light	6 h	0.008958	**	indep
PPD2	Mannitol	dark	1 h	0.222034	n.s.	1samp
PPD2	Sucrose	dark	1 h	0.206895	n.s.	1samp
PPD2	Mannitol	light	1 h	0.433116	n.s.	indep
PPD2	Sucrose	light	1 h	0.828541	n.s.	indep
PPD2	Mannitol	dark	6 h	0.056459	n.s.	1samp
PPD2	Sucrose	dark	6 h	0.001209	**	1samp
PPD2	Mannitol	light	6 h	0.061499	n.s.	indep
PPD2	Sucrose	light	6 h	0.903986	n.s.	indep
MYBD	Mannitol	dark	6 h	0.759139	n.s.	1samp
MYBD	Sucrose	dark	6 h	0.002682	**	1samp
MYBD	Mannitol	light	6 h	0.538293	n.s.	indep
MYBD	Sucrose	light	6 h	0.554267	n.s.	indep
PPL1	Mannitol	dark	6 h	0.163098	n.s.	1samp
PPL1	Sucrose	dark	6 h	0.009945	**	1samp
PPL1	Mannitol	light	6 h	0.890767	n.s.	indep
PPL1	Sucrose	light	6 h	0.227165	n.s.	indep

Supplemental Table 6. Motifs Enriched in Light-Regulated AS Events.
Sequence logos, set of events analyzed, number of positive hits out of all corresponding instances, number of negative hits in background set, and unerased E-values representing the enrichment in the positive sequences. The analysis was performed for a list of all significant events and separately for each AS event type, as indicated in column “Event set”. Either all significant events with non-identical genomic sequences or one representative event per gene (indicated by “one event/gene”) were considered. Listed are motifs with E-values < 1.0e-05 for all events and E-values < 5.0e-03 for the set reduced to one event per gene. Further details can be found in Supplemental methods.

Logo	Event set	Positives	Negatives	E-value
	all	89/407	58/1000	2.6e-011
	all	221/407	312/1000	8.3e-010
	all	236/407	353/1000	5.4e-009
	all	99/407	84/1000	1.3e-008
	all	146/407	165/1000	6.2e-009
	all	185/407	250/1000	4.1e-008
	all	278/407	470/1000	4.7e-008
	all	220/407	329/1000	1.5e-007

	all	231/407	358/1000	4.2e-007
	all	252/407	423/1000	4.8e-008
	all	103/407	108/1000	4.5e-006
	all	288/407	517/1000	5.2e-006
	all	269/407	475/1000	7.2e-006
	all	178/407	265/1000	5.3e-006
	all	138/407	188/1000	2.8e-007
	intron retention	16/194	0/1000	6.8e-008
	intron retention	18/194	2/1000	2.2e-007
	intron retention	15/194	0/1000	4.5e-007
	intron retention	17/194	2/1000	1.3e-006

	intron retention	17/194	2/1000	1.3e-006
	intron retention	14/194	0/1000	2.9e-006
	intron retention	14/194	0/1000	2.9e-006
	intron retention	14/194	0/1000	2.9e-006
	intron retention	15/194	1/1000	6.1e-006
	intron retention	15/194	1/1000	6.1e-006
	all events (one event/gene)	25/310	9/1000	6.1e-004
	all events (one event/gene)	211/310	501/1000	4.3e-003

Supplemental Table 7. Sequences of DNA Oligonucleotides.

Co-amplification and qPCR primer for validation of light-dependent AS events

Primer	Gene ID	Gene	Fwd/Rev	Sequence	Details
1	AT1G09140	SR30	Fwd	GTCACCTGCTAGATCCATTTCC	
2	AT1G09140	SR30	Rev	AGCCTGAGAAGCTTGAGACG	
3	AT1G60900	U2AF65B	Fwd	GCGCCTCCTGATATGTTAG	
4	AT1G60900	U2AF65B	Rev	GTTTGAGAACATCCCTGG	
5	AT3G49430	SR34A	Fwd	TCCGAGTATTGTTGGCTTCA	
6	AT3G49430	SR34A	Rev	CCGAGAATGCTCAAACTCAA	
7	AT4G39260	GRP8	Fwd	TGAAGTTGAGTACCGGTG	
8	AT4G39260	GRP8	Rev	TCTTCAATCGCATCCCTC	
9	AT1G30970	SUF4	Fwd	ACACTAGCGGTCCTTCAA	
10	AT1G30970	SUF4	Rev	TCGTCTGTCTATCGCTGC	
11	AT1G70000	MYBD	For	TCAAACTCCTGATCCCAACC	
12	AT1G70000	MYBD	Rev	CTATGTTCTTCCTCTGTCCA	
13	AT4G14720	PPD2	Fwd	CGGTGGTTGGGCAAATGA	used for gel picture
14	AT4G14720	PPD2	Rev	ACTTTTCTGTTCGCCTGAC	
15	AT4G14720	PPD2	Fwd	CGGTCTATCATGCATTTTGC	used for quantification
16	AT3G55330	PPL1	Fwd	GTGTTGTTGCTCCTTGGAT	
17	AT3G55330	PPL1	Rev	AGGCTCAATCACATCTTTG	
18	AT3G01100	HYP1	Fwd	ACTGCCTTTCTTGTTGGTC	
19	AT3G01100	HYP1	Rev	GCTCTCTCTCGTCTGCTT	
20	AT5G25060	RRC1	Fwd	ACTTTTGTTCGAGGTGGG	
21	AT5G25060	RRC1	Rev	TGGTGGAAGGAAAGAGGGA	

Primers for cloning *RRC1* complementation constructs

Primer	Gene ID	Gene	Fwd/Rev	Sequence	Details
22	AT5G25060	RRC1	Fwd	*AAAAAGCAGGCT*CTATGAGTTCATTCTCGATCAC	*half attB1*, RRC1
23	AT5G25060	RRC1	Rev	*AGAAAGCTGGGT*GAGTGAGAAATCTTTCAGAAATTGG	*half attB2*, RRC1
24	attB1		Fwd	GGGGACAAGTTTGTACAAAAAAGCAGGCT	Extension att site
25	attB2		Rev	GGGGACCACTTTGTACAAGAAAGCTGGGT	Extension att site
26	AT5G25060	RRC1	Fwd	*CCAAAAGCTTTCTTTGTTTCTCTGTTTACTTTA*	*overhang, HindIII, RRC1*
27	AT5G25060	RRC1	Rev	*CCAATCTAGATGTTTCAGCATCCAACTGCT*	*overhang, XbaI, RRC1*

qPCR primer for *RRC1*, *SR30*, *SnRK1.1, DINs, HXK1, CAB1*

Primer	Gene ID	Gene	Fwd/Rev	Sequence	Details
28	AT5G25060	RRC1	Fwd	CCTAAGGTTGATTCTGAAGGTGA	specific for .1
29	AT5G25060	RRC1	Rev	GTGGTGGTGGAAGGAAAGAG	
30	AT5G25060	RRC1	Fwd	CCTAAGGTTGATTCTGAAGGTATG	specific for .2
31	AT5G25060	RRC1	Rev	CTTTCCCTAGGCCTCTCCTC	
32	AT5G25060	RRC1	Fwd	AAGAAGAAGCTAGGAAGAAGAAAGC	total transcript
33	AT5G25060	RRC1	Rev	TAGTTGCATTATCCCCTTGAAA	
34	AT5G25060	RRC1	Fwd	AATGGACGAGGAACAAAGACAA	total transcript, specific for WT allele
35	AT5G25060	RRC1	Rev	GGGTTTTTCATCCCCTGTTC	
36	AT1G09140	SR30	Fwd	GCAAGAGCAGGAGTGTGTCA	specific for .1
37	AT1G09140	SR30	Rev	TTGATCTTGATTGGGACCTTG	
38	AT1G09140	SR30	Fwd	TCACCTGCTAGATCCATTTCC	specific for .2
39	AT1G09140	SR30	Rev	CCCAGCTCGTAGCAGTGAG	
40	AT1G09140	SR30	Fwd	GCGCGTATCAGCTGTCC	specific for .3
41	AT1G09140	SR30	Rev	CCAGTGGCCAGTTTTCATTT	
42	AT1G09140	SR30	Fwd	TCTAGTGCTTATATACGGGTGAGG	total transcript
43	AT1G09140	SR30	Rev	GCTCCGACTCCTGCTTCTAT	
44	AT3G01090	SnRK1.1	Fwd	TGAGTTTCAAGAGACCATGGAAG	
45	AT3G01090	SnRK1.1	Rev	CCAACTCCTTGATATTCCATCAG	
46	At4g35770	DIN1	Fwd	GAATGAGCTGCCGGTAGAAG	
47	At4g35770	DIN1	Rev	TGATGATTGATACTTGCGTTGAG	
48	At3g47340	DIN6	Fwd	TCTGCGAGACTAACGGTTCC	
49	At3g47340	DIN6	Rev	CATATTGTTGGACCAGCTTGC	
50	At4g29130	HXK1	Fwd	AATGATGGATCAGGCATTGG	
51	At4g29130	HXK1	Rev	GAAAAATGGCGCTCTTTGG	
52	At1g29930	CAB1	Fwd	AGCCATCGTCACTGGTAAGG	
53	At1g29930	CAB1	Rev	TTTCCGGGAACAAAGTTGG	

Analysis of T-DNA insertion in *snrk1.1-3*

Primer	Gene ID	Gene	Fwd/Rev	Sequence	Details
54	AT3G01090	SnRK1.1	Fwd	GATACTTGCTTGCGCCTTAT	Full-length *SnRK1.1* cDNA
55	AT3G01090	SnRK1.1	Rev	TGGAGTAAACAAAAACAAAAATC	
54	AT3G01090	SnRK1.1	Fwd	GATACTTGCTTGCGCCTTAT	Region upstream of T-DNA insertion site
56	AT3G01090	SnRK1.1	Rev	ACAACATTGGGCGACTTAAC	
57	AT3G01090	SnRK1.1	Fwd	AACATGAAGTGCAGATGGGT	Region spanning the T-DNA insertion site
58	AT3G01090	SnRK1.1	Rev	GAGCAAGAAAAGCAGCACACAGAT	
59	AT3G01090	SnRK1.1	Fwd	TCTTGGATCTGTGTGC	Region downstream of T-DNA insertion site
55	AT3G01090	SnRK1.1	Rev	TGGAGTAAACAAAAACAAAAATC	

Supplemental Methods

Computational Parameter Settings

Demultiplexing
Demultiplexing was performed with the FastX-toolkit, using the default parameters.

Alignment
Reads were aligned against the TAIR 10 reference genome in a two-steps approach with PALmapper (Jean et al. 2010). In the first step, an alignment was performed to discover novel splice junctions. For this alignment the following parameters were used:
-M 3 -G 1 -E 4 -l 15 -L 25 -K 8 -C 35 -I 25000 -NI 2 -SA 100 -CT 10 -a -S -seed-hit-cancel-threshold 10000 -report-map-read -report-spliced-read -report-map-region -report-splice-sites 0.9 -filter-max-mismatches 0 -filter-max-gaps 0 -filter -splice-region 5 -qpalma-use-map-max-len 2000 -f bamp -threads 2 -polytrim 40 -qpalma-prb-offset-fix -include-unmapped-reads -min-spliced-segment-len 12 -junction-remapping-coverage 3 -fixtrimleft 4 -fixtrimright 4 -no-gap-end 10 -qpalma-indel-penalty 10

In a second step, the discovered splice junctions from the reads of the initial alignment were used for a sensitive realignment of the reads. For the second alignment the following parameters were used:

-M 4 -G 1 -E 4 -l 15 -L 25 -K 8 -C 35 -I 25000 -NI 2 -SA 100 -CT 50 -a -S -seed-hit-cancel-threshold 10000 -report-map-read -report-spliced-read -report-map-region -report-splice-sites 0.9 -filter-max-mismatches 0 -filter-max-gaps 0 -filter-splice-region 5 -qpalma-use-map-max-len 1000 -f bamn -threads 2 -polytrim 40 -qpalma-prb-offset-fix -min-spliced-segment-len 15 -junction-remapping-coverage 5 -junction-remapping-min-spliced-segment-len 15 -fixtrimleft 4 -fixtrimright 4 -qpalma-indel-penalty 1

Alignment post-processing
The alignments were sorted using Samtools. Subsequently, reads that mapped to multiple locations were removed. Specifically, the best alignment of a read was kept only if the second best alignment had more than 1 mismatch more than the best alignment. Additionally, alignments with more than 2 mismatches were discarded.

Event extraction
Alternative splicing events were extracted with SplAdder (Kahles et al., 2016), as described in Drechsel et al. (2013). We increased the number of detected AS events by including events that were exclusively detected in another, unpublished dataset (events named "old_" in Data Set 1), using the same pipeline as described here. In detail, alternative splicing (AS) information from the 22 library alignments was integrated into a common reference graph using the SplAdder pipeline with confidence level 2 and otherwise default parameters. On this integrated splicing graph we performed AS event calling. This resulted in a total of 53,120 detected and confirmed AS events. In addition to that we also processed a set of 8 libraries that were generated for preliminary analyses. We will denote the 22 and 8 library datasets as ‘new’ and ‘old’ respectively. Based on the eight old libraries, the SplAdder pipeline was run with confidence level 3 (to only keep events of highest confidence) to generate a common splicing graph representation. From this common graph, we called and confirmed a total of 13,784 AS events.
The two AS event lists generated from the old and new libraries, respectively, were then united into a common list of AS events. In this merged list 3,150 events were only found in the old analysis graph. These events have the prefix ‘old_’. All other events have the prefix ‘new_’.
We then used SplAdder to also quantify the splicing graphs generated from the old dataset with the alignments of the new libraries. Due to the graphs being generated from different data source the re-quantification resulted in a number of 14,412 AS events to be called and confirmed on the old graph using the new library data. Not all of the above 3,150 events could be confirmed using the new data. In this case, we give NA as quantification values.

‘old’ read data set:
1 lba1_0h
2 WT_0h
3 WT_1h
4 WT_6h
5 cry1cry2_0h

6	cry1cry2_1h
7	cry1cry2_6h
8	lba1_6h

'new' read data set:

1	B0_R1	12	R1_R2
2	B0_R2	13	R6D_R1
3	B1_R1	14	R6D_R2
4	B1_R2	15	R6_R1
5	B6D_R1	16	R6_R2
6	B6D_R2	17	W0_R1
7	B6_R1	18	W0_R2
8	B6_R2	19	W6D_R1
9	R0_R1	20	W6D_R2
10	R0_R2	21	W6_R1
11	R1_R1	22	W6_R2

Read counting and testing for differential events
For read counting and differential testing of events, we used scripts implemented in the differential testing toolbox rDiff (Drewe et al., 2013), as described in Drechsel et al. (2013).

Test for differential gene expression
The differential gene expression was analyzed using the DESeq (Anders and Huber, 2010) package for the R Bioconductor Suite, as described in Drechsel et al. (2013).

False discovery rate estimation
The false discovery rate was estimated as described by Benjamini and Hochberg (1995).

Calling expressed genes
We determined the expressed genes as described in Gan et al. (2011).

Motif analysis
To determine sequence elements that are overrepresented in the differentially regulated events, we used the following procedure. First, we extracted for all events the corresponding genomic sequences. Next, we randomly selected 1000 of the events that were not differentially regulated. To avoid a confounding effect of gene expression levels on the analysis, the selection was performed such that the genes containing the differentially regulated events and the selected events had the same minimal and maximal gene expressions. To avoid a bias in the motif analysis by overlapping events, from all events with an identical sequence one representative was chosen. For a subset, one representative event per gene for the differentially expressed events was chosen. Finally, we applied the motif finding software DREME (Bailey, 2011) using the sequences of the differential events as foreground set and the 1000 randomly chosen non-differential events as a background set. For DREME (v.4.11.1) we used the following parameters: -mink 6 -norc -maxk 12 -g 1000. Analyses were performed either separately or combined for the four AS types (intron retention, exon skip, alt. 3' ss, alt 5'ss).

References

Anders, S., and Huber, W. (2010). Differential expression analysis for sequence count data. Genome biology 11, R106.

Bailey, T.L. (2011). DREME: motif discovery in transcription factor ChIP-seq data. Bioinformatics 27, 1653-1659.

Benjamini, Y., and Hochberg, Y. (1995). Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society. Series B (Methodological) 57, 289-300.

Drechsel, G., Kahles, A., Kesarwani, A.K., Stauffer, E., Behr, J., Drewe, P., Rätsch, G., and Wachter, A. (2013). Nonsense-mediated decay of alternative precursor mRNA splicing variants is a major determinant of the Arabidopsis steady state transcriptome. Plant Cell 25, 3726-3742.

Drewe, P., Stegle, O., Hartmann, L., Kahles, A., Bohnert, R., Wachter, A., Borgwardt, K., and Rätsch, G. (2013). Accurate detection of differential RNA processing. Nucleic acids research 41, 5189-5198.

Foissac, S., and Sammeth, M. (2007). ASTALAVISTA: dynamic and flexible analysis of alternative splicing events in custom gene datasets. Nucleic acids research 35, W297-299.

Gan, X., Stegle, O., Behr, J., Steffen, J.G., Drewe, P., Hildebrand, K.L., Lyngsoe, R., Schultheiss, S.J., Osborne, E.J., Sreedharan, V.T., Kahles, A., Bohnert, R., Jean, G., Derwent, P., Kersey, P., Belfield, E.J., Harberd, N.P., Kemen, E., Toomajian, C., Kover, P.X., Clark, R.M., Rätsch, G., and Mott, R. (2011). Multiple reference genomes and transcriptomes for Arabidopsis thaliana. Nature 477, 419-423.

Jean, G., Kahles, A., Sreedharan, V.T., De Bona, F., and Rätsch, G. (2010). RNA-Seq read alignments with PALMapper. Current protocols in bioinformatics / editoral board, Andreas D. Baxevanis ... [et al.] Chapter 11, Unit 11 16.

Kahles, A., Ong, C.S., Zhong, Y., and Rätsch, G. (2016). SplAdder: Identification, quantification and testing of alternative splicing events from RNA-Seq data. Bioinformatics 32, 1840-1847.

Mancini, E., Sanchez, S.E., Romanowski, A., Schlaen, R.G., Sanchez-Lamas, M., Cerdan, P.D., and Yanovsky, M.J. (2016). Acute Effects of Light on Alternative Splicing in Light-Grown Plants. Photochemistry and photobiology 92, 126-133.

Rühl, C., Stauffer, E., Kahles, A., Wagner, G., Drechsel, G., Rätsch, G., and Wachter, A. (2012). Polypyrimidine tract binding protein homologs from Arabidopsis are key regulators of alternative splicing with implications in fundamental developmental processes. Plant Cell 24, 4360-4375.