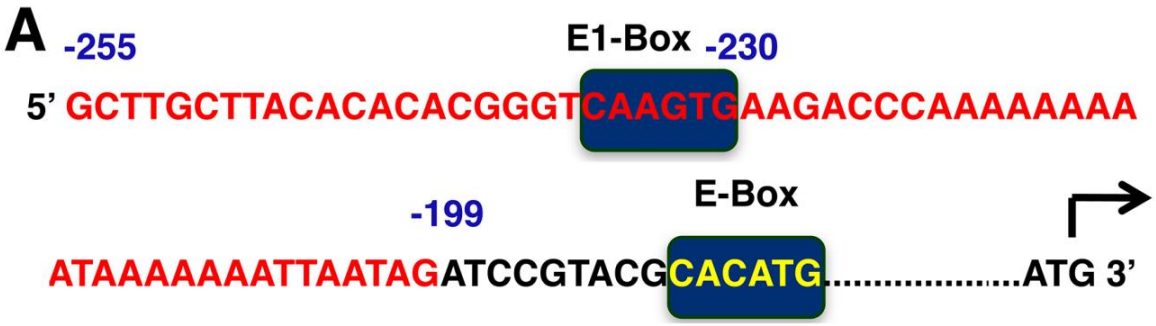
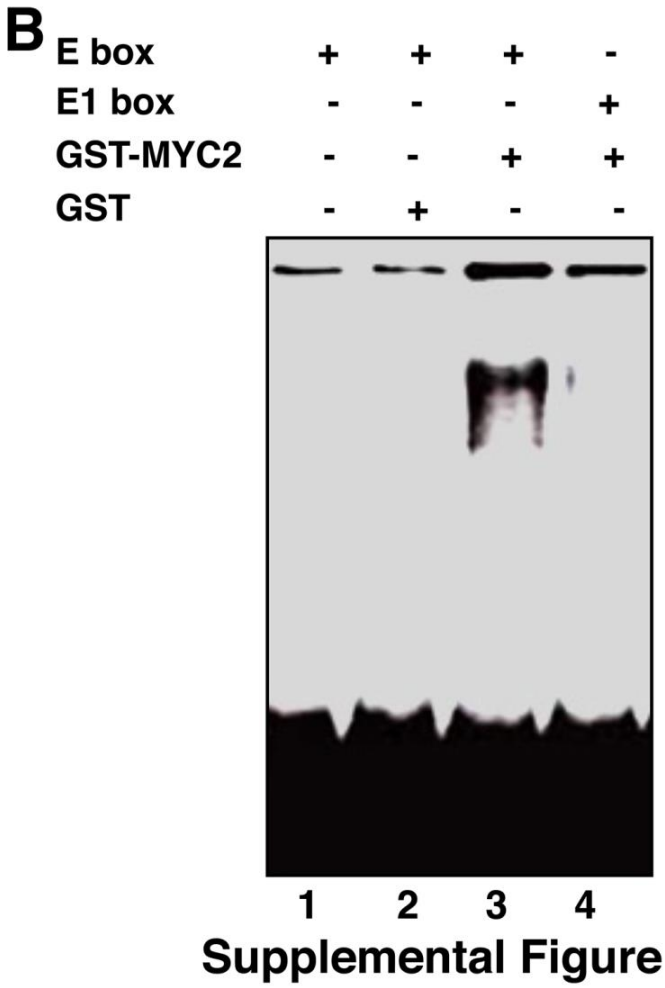
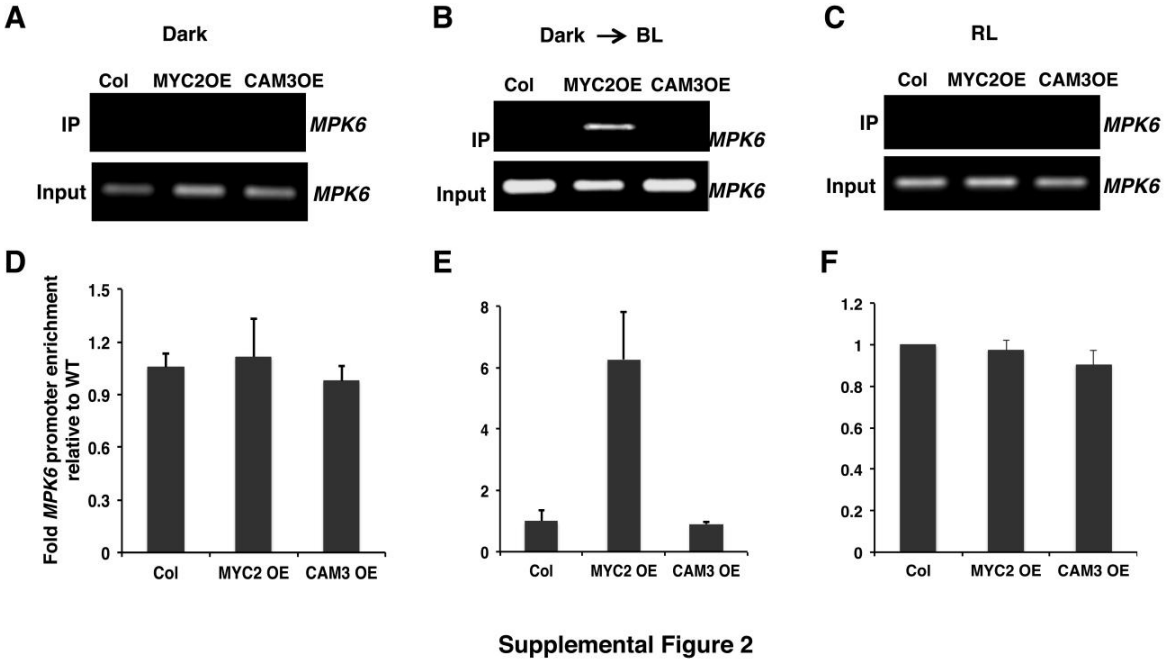
Supplemental Data





Supplemental Figure 1

Supplemental Figure 1. MYC2 Binds to the E-box but not the E1-box of the *MPK6* Promoter.
(A) E1-box and E-box (wild type) containing *MPK6* promoter fragment. The region shown in red denotes the 57-bp fragment used for the gel shift assay. The blue boxes denote the position of the E1-box and E-box, the numbers in blue denote the position of base pairs. The text in yellow is the E-box nearest to the start site. The arrow denotes transcriptional start site.
(B) MYC2 does not bind to the E1 box that is 230 bp upstream of the ATG start codon. Approximately 500 ng of recombinant protein was added (lanes 3 and 4) to radioactively labelled 78-bp fragment of the *MPK6* E-box and 57-bp fragment of the *MPK6* E1-box containing promoter, respectively. No protein was added in lane 1, 500 ng of GST protein was added in lane 2. The plus and minus signs indicate the presence or absence of competitors, GST-MYC2 or GST.

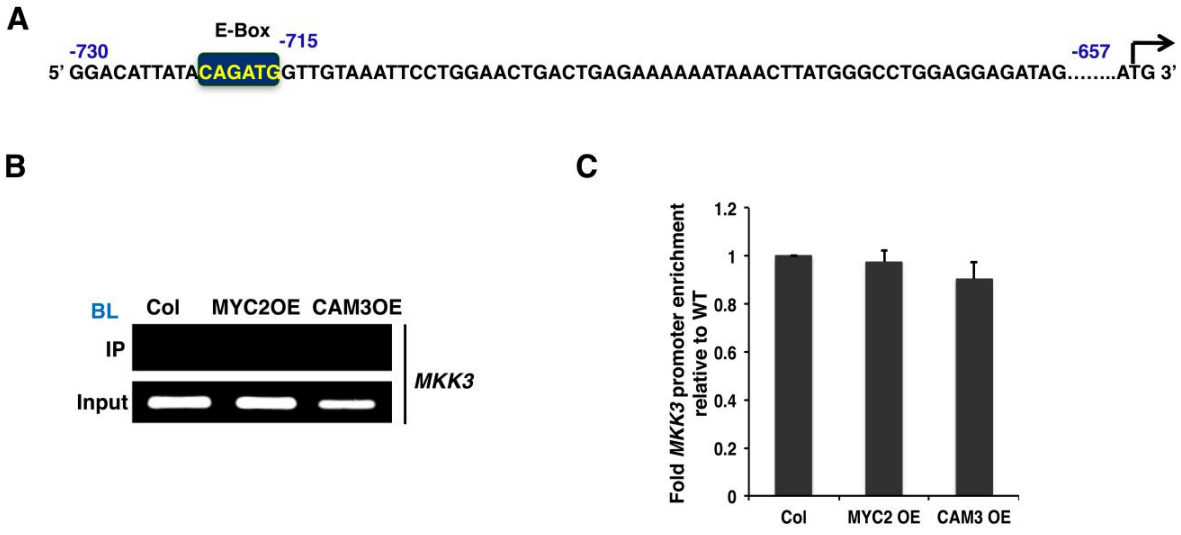


Supplemental Figure 2

Supplemental Figure 2. MYC2 Binds to the E-box of the *MPK6* Promoter in Blue Light.

(A) to (C) Chromatin immunoprecipitation (ChIP) assays of *MPK6* promoter from Col, MYC2 overexpresser transgenic seedlings (MYC2-cMycOE) and Cam3OE, using cMyc antibody and 10 DAG seedlings grown in constant dark, dark-to-BL (four days grown in BL, followed by six days in dark, exposed to 10 min BL: 30 µmol $m^{-2}s^{-1}$), and constant RL (60 µmol $m^{-2}s^{-1}$), respectively. The gel picture shows the results of PCR amplification of *MPK6* promoter fragment in immunoprecipitate (upper panel in each case) and input (lower panel in each case).

(D) to (F) Results of real time qPCR are presented as fold enrichment relative to WT (Col). ChIP values were first normalized by the respective input values and then fold enrichment relative to wild-type was calculated. Error bars represent SE (n=3).



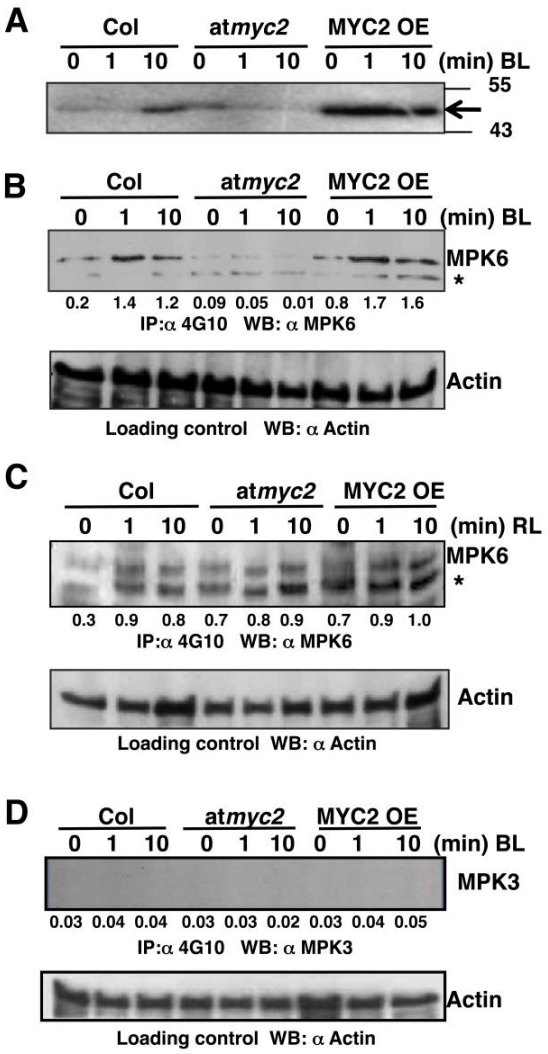
Supplemental Figure 3

Supplemental Figure 3. MYC2 Does Not Bind to the E-box of the *MKK3* Promoter.

(A) E-box containing the *MKK3* promoter fragment (74 bp) used for the gel shift assays.

(B) Chromatin immunoprecipitation (ChIP) assays of the *MKK3* promoter from MYC2 overexpresser transgenic seedlings (MYC2-cMycOE), 10 DAG in constant blue light (30 µmol m^{-2} s^{-1}) using antibodies to cMyc. The gel picture shows the results of PCR amplification of the *MKK3* promoter fragment in immunoprecipitate and input.

(C) Results of real time qPCR are presented as fold enrichment relative to WT (Col). ChIP values were first normalized by the respective input values and then fold enrichment relative to wild-type was calculated. Error bars represent SE (n=3).



Supplemental Figure 4

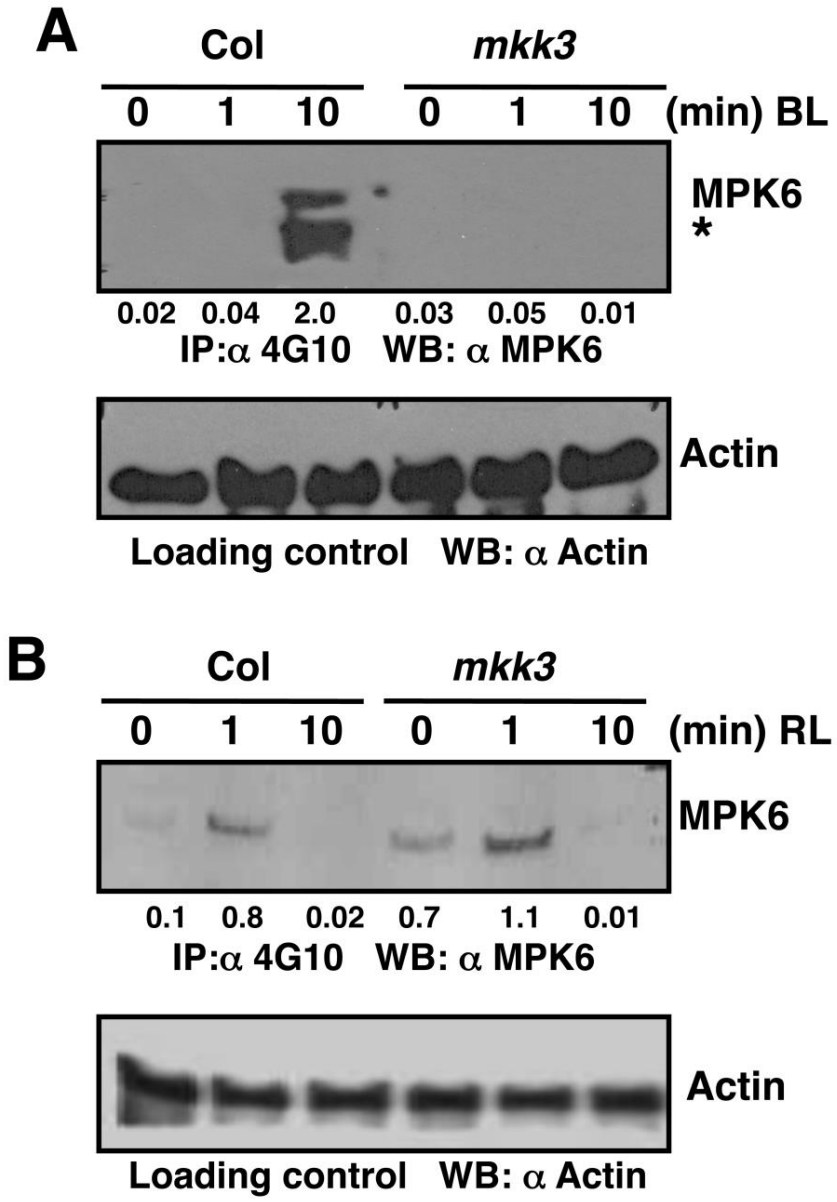
Supplemental Figure 4. Activation of MPK6 in Blue Light is MYC2 Dependent.

(A) In-gel kinase activity assays using 200 µg of total protein from dark grown Col, *atmyc2* and MYC2 OE lines exposed to BL (30 µmol m^{-2} s^{-1}) for 0, 1, 10 min. The MAP kinase activity was analyzed using MBP (Myelin Basic Protein) as a substrate. The arrow corresponds to the activated MAP kinases in BL.

(B) Immunoprecipitation assays using 200 µg of total protein from dark grown Col, *atmyc2* and MYC2 OE lines exposed to BL (30 µmol m^{-2} s^{-1}) for 0, 1, 10 min. The MPK6 activation was analyzed by immunoprecipitation with anti-phosphotyrosine (α-4G10) followed by Immunoblot analysis with anti-MPK6 antibody. Lower panel shows Immunoblot with anti-Actin using 50 µg of total protein as loading control. Asterisk indicates cross-reacting bands in each case. Values below panel indicate quantification of each band with respect to actin, the loading control.

(C) Immunoprecipitation assay of dark grown Col, *atmyc2* and MYC2 OE lines exposed to RL (60 µmol m^{-2} s^{-1}) for 0, 1, 10 mins. The specific MPK6 activation was analyzed by immunoprecipitation with anti-phosphotyrosine (α-4G10) followed by western analysis with anti-MPK6 antibody. Lower panel shows Immunoblot with anti-Actin using 50 µg of total protein as loading control. Asterisk indicates cross-reacting bands in each case. Values below panel indicate quantification of each band with respect to actin, the loading control.

(D) MPK3 is not activated in BL. Immunoblot probed with anti-MPK3 antibody acts as the negative control. Lower panel shows western analysis with anti-actin using 50 µg of total protein as loading control.

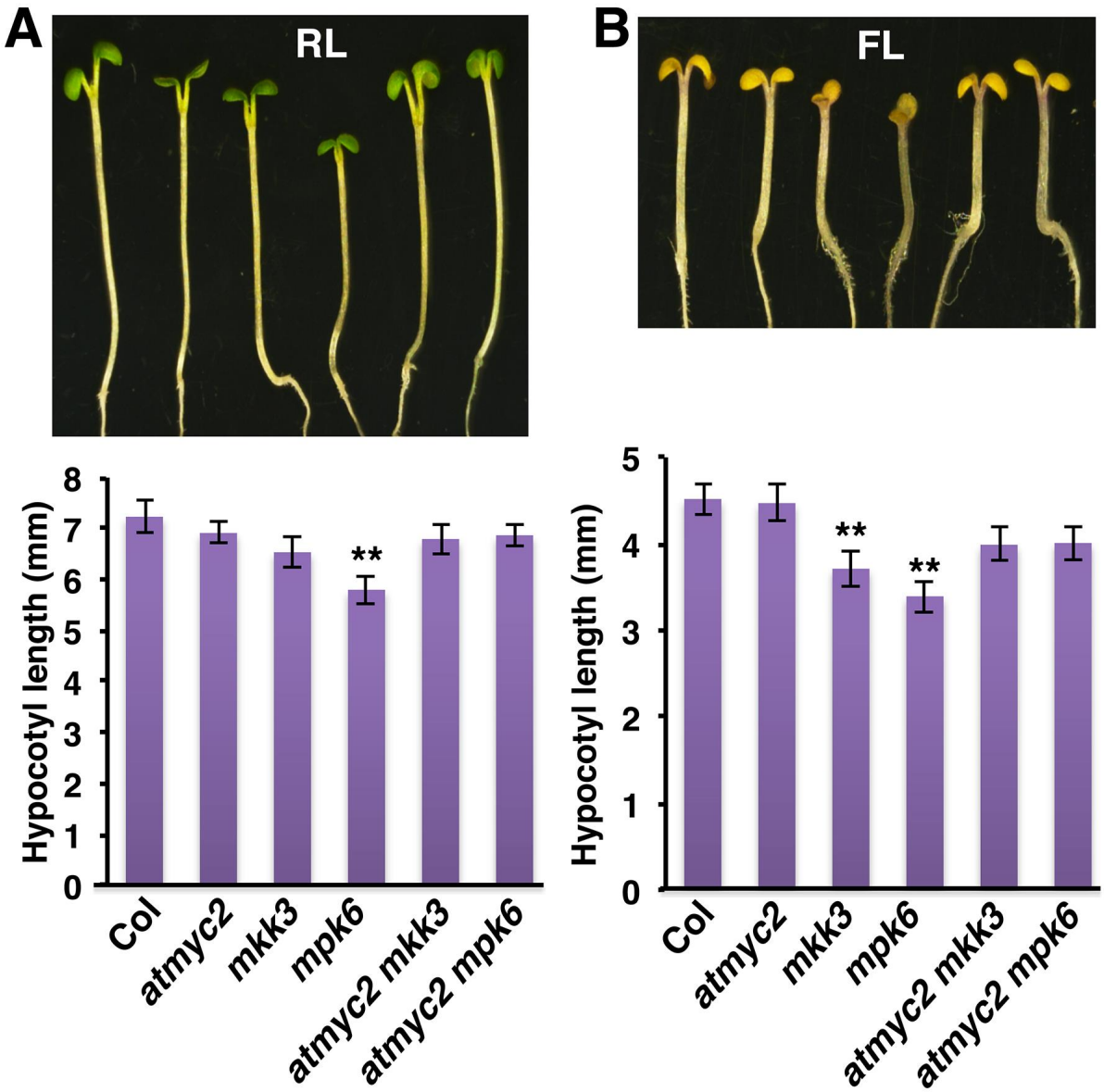


Supplemental Figure 5

Supplemental Figure 5. MKK3 activates MPK6 in Blue Light.

(A) Activation of MPK6 by MKK3 in BL. Immunoprecipitation assays of dark grown Col, *mkk3* mutant lines exposed to BL (30 µmol m^{-2} s^{-1}) for 0, 1, 10 min. Experimental details are similar to those used in Supplemental Figure 4B. Values below the panel indicate quantification of each band with respect to actin, the loading control.

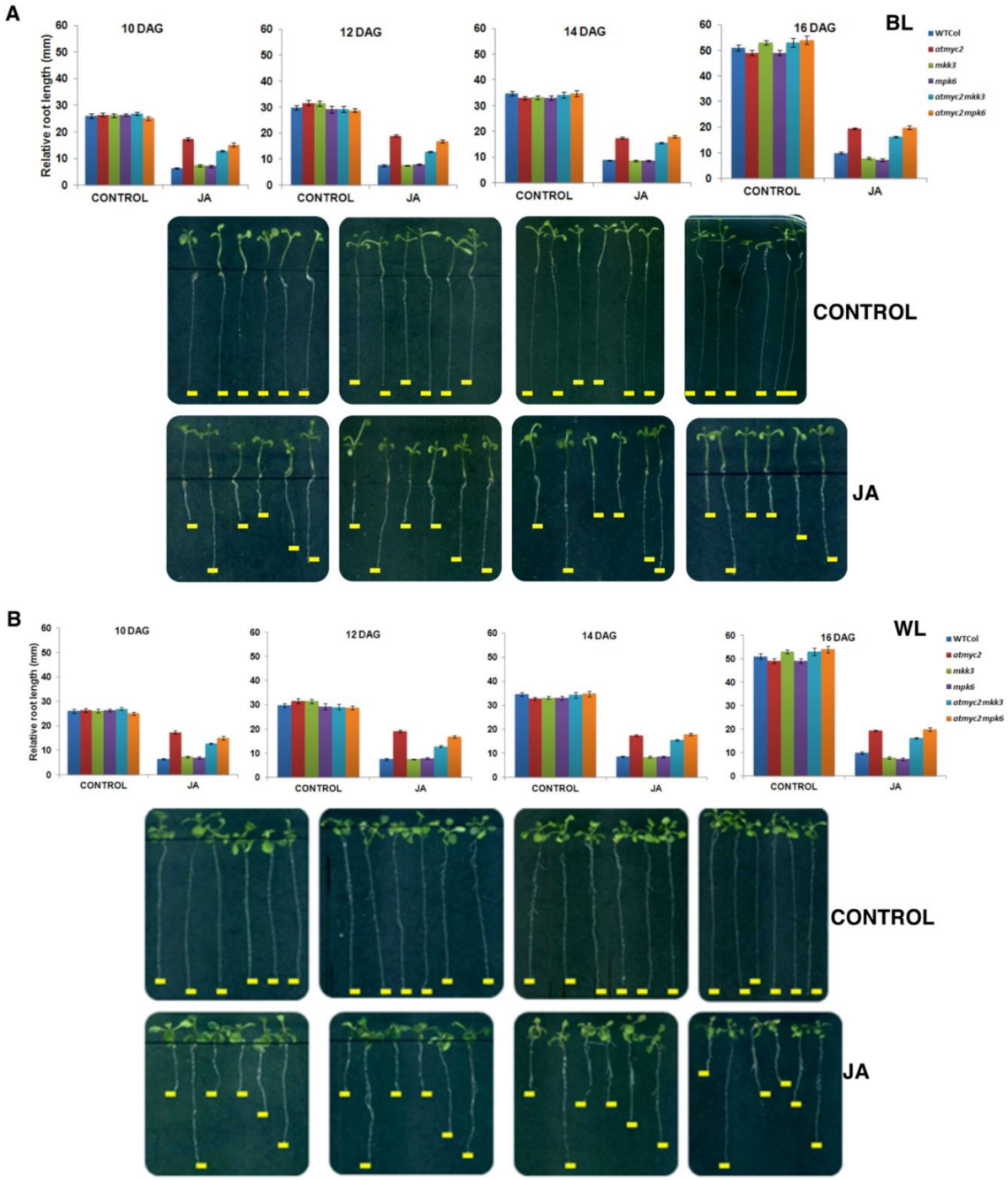
(B) MKK3-dependent activation of MPK6 is not RL specific. Immunoprecipitation assay of dark-grown Col, *mkk3* lines exposed to RL (60 µmol m^{-2} s^{-1}) for 0, 1, 10 min. Experimental details are similar to those Supplemental Figure 4C. Values below the panel indicate quantification of each band with respect to actin, the loading control.



Supplemental Figure 6

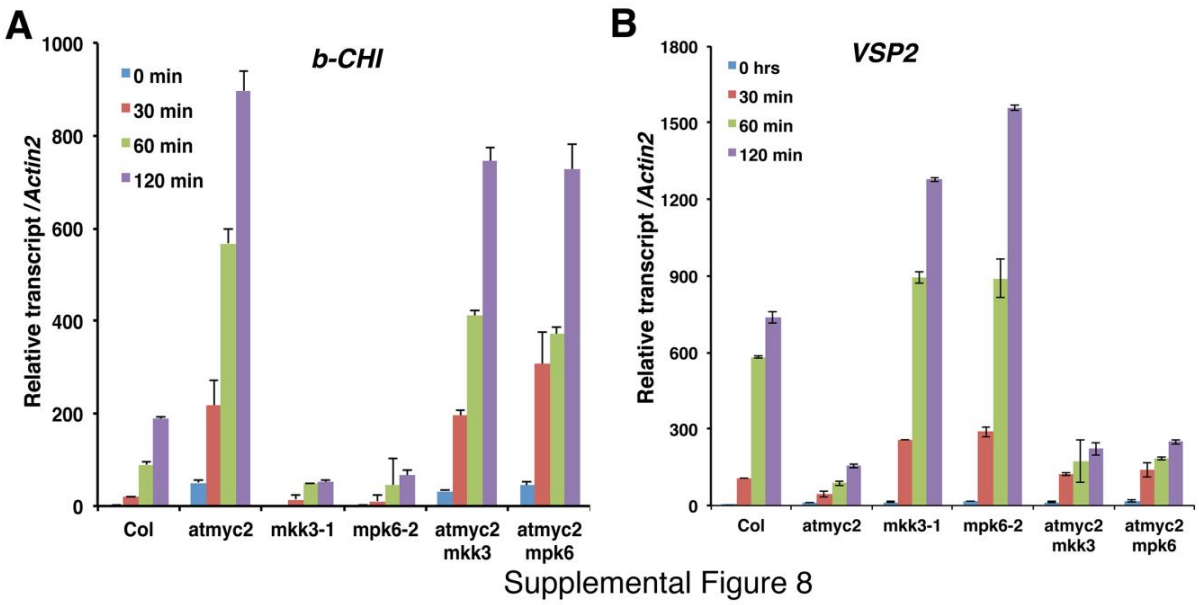
Supplemental Figure 6. Genetic Interactions of *atmyc2* with *mpk6* and *mkk3* in Red light and Far-red Light.

(A) and (B) Hypocotyl length phenotype of 6-day-old wild type and different mutant seedlings grown in constant red light (RL: 90 µmol m^{-2} s^{-1}) and constant far red light (FL: 60 µmol m^{-2} s^{-1}) respectively. Lower panels shows the quantification of hypocotyl length of seedlings. Error bars represent SD (n ≥ 30). Asterisks indicate genotypes that differs significantly (Student's t-test, ** P ≤ 0.01) from wild type (Col).



Supplemental Figure 7. JA Responses in *atmyc2 mkk3* and *atmyc2 mpk6* mutants.

(A) Quantification of root length of 10, 12, 14 and 16 DAG constant BL grown (30 µmol m^{-2} s^{-1}) seedlings in the absence (control) and presence of 20 µM JA respectively. Error bars represent SD (n ≥ 20). Lower panels show the photographs of root length. Representative seedlings of WT Col, *atmyc2, mkk3, mpk6, atmyc2 mkk3* and *atmyc2 mpk6* are shown from left to right respectively. **(B)** Quantification of root length of 10, 12, 14 and 16 DAG constant WL grown (100 µmol m^{-2} s^{-1}) seedlings in the absence (control) and presence of 20 µM JA respectively. Error bars represent SD (n ≥ 20). Lower panels show the photographs of root length. Representative seedlings of WT Col, *atmyc2, mkk3, mpk6, atmyc2 mkk3* and *atmyc2 mpk6* are shown from left to right respectively. 20 seedlings was taken in each case at each time point for quantification.



Supplemental Figure 8

Supplemental Figure 8. Expression of JA Marker Genes in Various Mutant Backgrounds.

(A) and (B) Real-time PCR analysis of JA marker genes, *b-CHI* and VSP2 transcript levels, respectively, from 6-day-old constant BL (30 µmol m^{-2} s^{-1}) grown wild type and various mutant seedlings treated with 50 µM JA for 0, 30, 60 and 120 min. *Actin2* was used as control. Error bars represents S.D. n=3 independent experiments with similar results.

Supplemental Table 1: List of primers used in this study

Primer Name	Primer Sequence	Purpose
β- *actin2* FP β- *actin2* RP	5’ GCCATCCAAGCTGTTCTCTC 3’ 5’ GCTCGTAGTCAACAGCAACAA 3’	qPCR
MPK6 FP *MPK6 RP*	5’ TACCGGTCATGGACGGTGGTTCAG 3’ 5’ GCGTTAGCAATTTACGAGAGCGTTG 3’	qPCR
MKK3 FP *MKK3 RP*	5’ CATCTTCCACATCTCAAGGAGACAC 3’ 5’ ACCCGCATTTCGTGAGAAGCACATTG 3’	qPCR
MPK3 FP *MPK3 RP*	5’ CAAAGCAAC CACTGAATCTCGAC 3’ 5’ GTCTTCTTAGTGGTGGTGGAAC 3’	qPCR
CAB1 FP *CAB1 RP*	5’ GTTAACAACAACGCATGGGC 3’ 5’ CCTCTCACACTCACGAAGCA 3’	qPCR
RBCS1A FP *RBCS1A RP*	5’ TCGGATTCTCAACTGTCTGATG 3’ 5’ ATTTGTAGCCGCATTGTCCT 3’	qPCR
CHS FP *CHS RP*	5’ CTGTCCTCCGTATCGCTAAGGATC 3’ 5’ ACGTGTCGCCCTCATCTTCTCTTC 3’	qPCR
VSP2 FP *VSP2 RP*	5’ GGCCTTGCATCTTTACCAAAAC 3’ 5’ GTAGTAGAGTGGATTTGGGAGC 3’	qPCR
b-CHI FP *b-CHI RP*	5’ GCAATAGGAGTTGACTTACTCAAC 3’ 5’ CTAAATAGCAGCTTCGAGGAG 3’	qPCR
MPK6 PRO FP MPK6 PRO RP	5’ GCTCTAGAGATCCGTACGCACATGACTTTCAC 3' 5’ CCCAAGCTTGGAGAAATTGATATTCGTTGG 3'	Gel shift and CHIP
MPK6 mE FP MPK6 mE RP	5’ GCTCTAGAGATCCGTACGACCACAACTTTCAC 3' 5’ CCCAAGCTTGGAGAAATTGATATTCGTTGG 3'	Gel shift
MPK6 E1 FP MPK6 E1 RP	5’ GCTCTAGAGCTTGCTTACACACACG 3’ 5’ CCCAAGCTTCTATTAATTTTTTTATTTTTTTTTGGG 3’	Gel shift
MKK3 PRO FP MKK3 PRO RP	5' GCTCTAGAGGACATTATACAGATGGTTG 3' 5' CCCAAGCTTCTATCTCCTCCAGGCCCATAAG 3'	CHIP
MYC2M1F MYC2M1R	5’ GACGGAGATCGAGTGCGCCGCCG 3’ 5’ CGGCGGCGCACTCGATCACCGTC 3’	Kinase assay
MYC2M2F MYC2M2R	5’ CCGATTGGAGCACCTGGATCTAACG 3’ 5’ CGTTAGATCCAGGTGCTCCAATCGG 3’	Kinase assay
MYC3M2F MYC3M2R	5’ CTGGACCGGCTCCGAGTCCGGTTC 3’ 5’ GAACCGGACTCGGAGCCGGATCCAG 3’	Kinase assay
MYC4M2F MYC4M2R	5’ GGATCCGACTCCGGCTCCGGTTC 3’ 5’ GAACCGGAGCCGGAGTCGGATCC 3	Kinase assay
GST-MYC2 FP GST-MYC2 RP	5’ TGGCCTCCGGCGTCGACGA CAACC 3’ 5’ ATAAGAATGCGGCCGCATATCAATATATACAAGTTTACTC 3’	Pull down and Kinase assay
MPK6-His FP MPK6-His RP	5’CGGGATCCTTCCATTTCCCTCTTCTCG 3’ 5’ACGCGTCGACTTGCTGATATTCTGGATTG 3’	Pull down assay
MYC2-BiFC-FP MYC2-BiFC-RP	5’ GACTAGTATGACTGATTACCGGCTAC 3’ 5’ CCATCGATACCGATTTTTGAAATC 3’	BiFC assay