

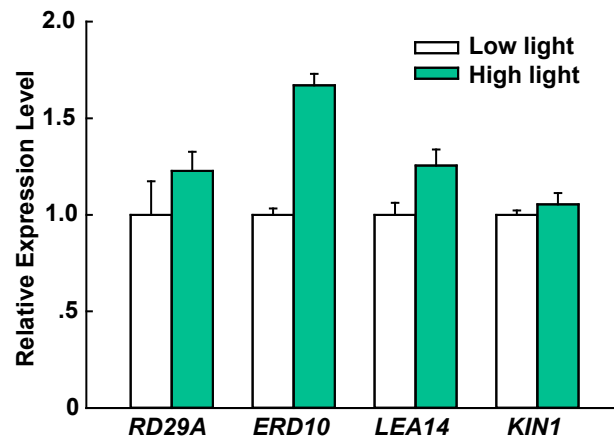
**Supplemental Figure 1. Characterization of the *myb75-c* Mutants Generated Using the CRISPR-Cas9 System.**

(A) Schematic illustrating the two sgRNA:Cas9 targets (red) and corresponding PAMs (blue) of *MYB75* gene. The *NcoI* and *AgeI* sites are underlined. PAM: protospacer-adjacent motif sequence.

(B) Sequencing of the sgRNA:Cas9-induced *MYB75* mutations in two independent transgenic lines of Arabidopsis.

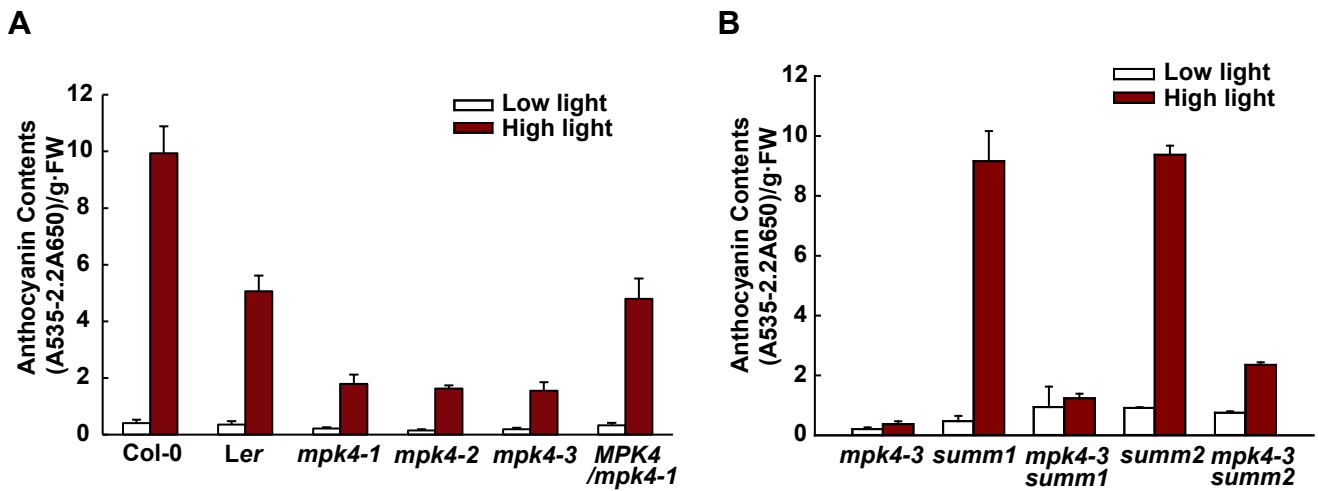
(C) Twelve-day-old Arabidopsis seedlings of wild type (WT), *myb75-c*, and *myb75-c1* grown on plates under low light and moderate high light (high light). Bars = 0.5 cm.

(D) Anthocyanin contents of the seedlings in (C). FW, fresh weight. Error bars represent SD of three replicates (as described in Figure 2B).



**Supplemental Figure 2. Expression Levels of General Stress Genes under Low Light and Moderate High Light Conditions.**

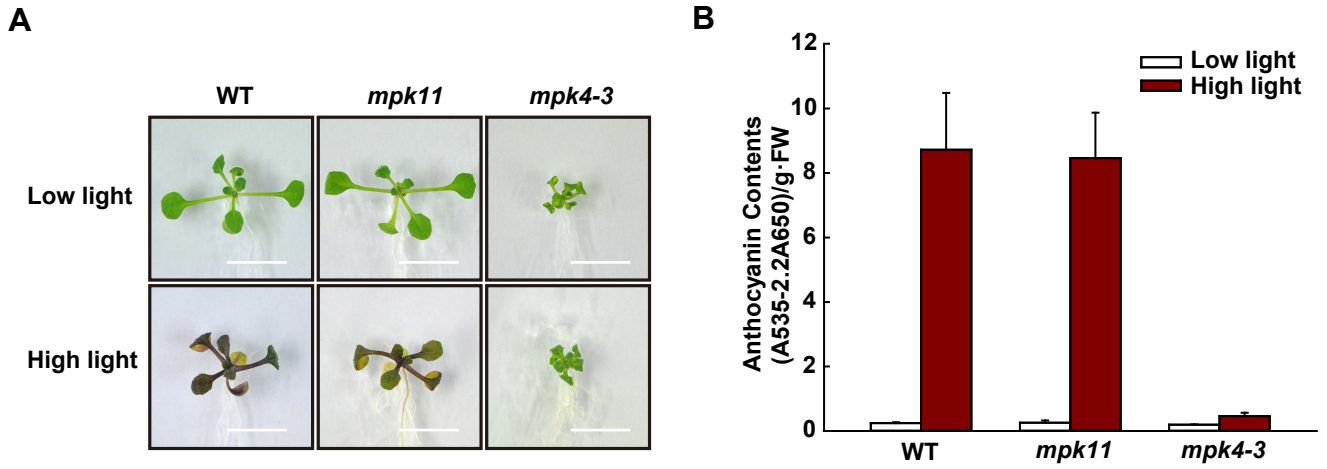
Quantitative real-time PCR analysis showing the expression of general stress responsive genes, *RD29A*, *ERD10*, *LEA14*, and *KIN1* in Arabidopsis under low light ( $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and moderate high light ( $175 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) condition. Results were normalized to *ACTIN8* and expression levels of the genes under low light condition were set at one unit. Error bars indicate SD of three replicates (as described in Figure 2B).



**Supplemental Figure 3. Mutations of *MPK4* Lead to Compromised Anthocyanin Accumulation.**

**(A)** Anthocyanin contents of twelve-day-old seedlings of Col-0, *Ler*, *mpk4-1* (*Ler*), *mpk4-2* (Col), *mpk4-3* (Col), and the complemented line of *mpk4-1* grown on plates under low light and moderate high light (high light). FW, fresh weight. Error bars represent SD of three replicates (as described in Figure 2B).

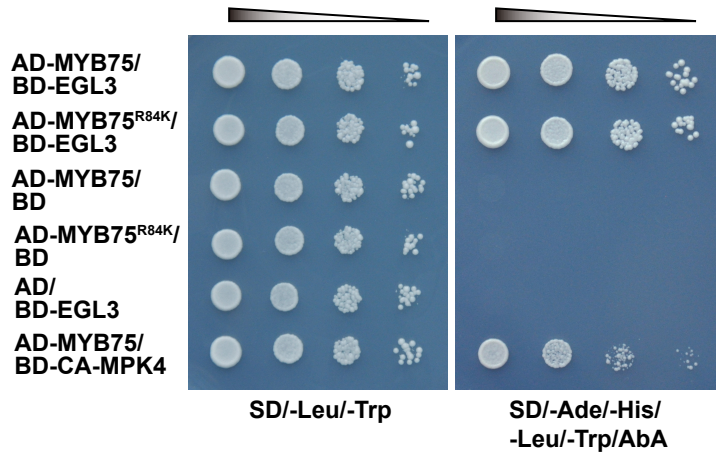
**(B)** Anthocyanin contents of twelve-day-old seedlings of *mpk4-3*, *summ1*, *mpk4-3 summ1*, *summ2*, and *mpk4-3 summ2* grown on plates under low light and moderate high light (high light). FW, fresh weight. Error bars represent SD of three replicates (as described in Figure 2B).



**Supplemental Figure 4. MPK11 Is Not Involved in High Light-Induced Anthocyanin Accumulation.**

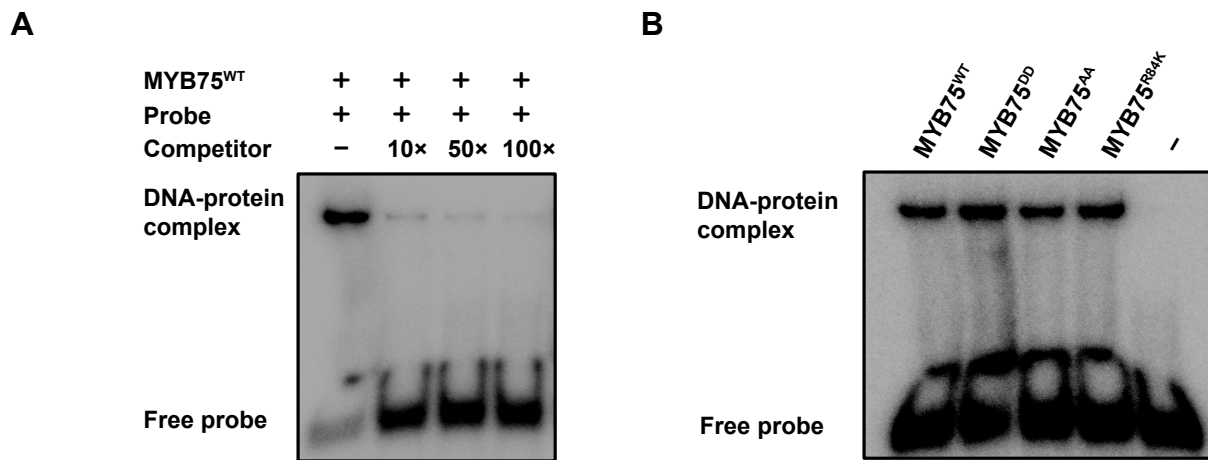
**(A)** Twelve-day-old seedlings of wild type (Col-0), *mpk11*, and *mpk4-3* grown on plates under low light or moderate high light (high light). Bars = 1 cm.

**(B)** Anthocyanin contents of the seedlings in **(A)**. FW, fresh weight. Error bars represent SD of three replicates (as described in Figure 2B).



**Supplemental Figure 5. R84K Mutation of MYB75 Does Not Change Its Interaction with EGL3 in Yeast.**

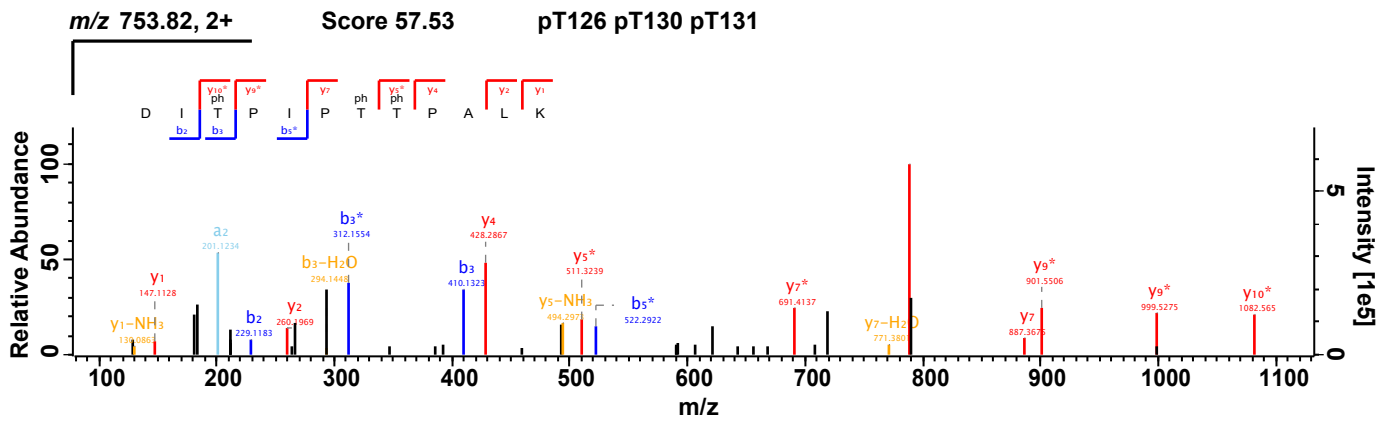
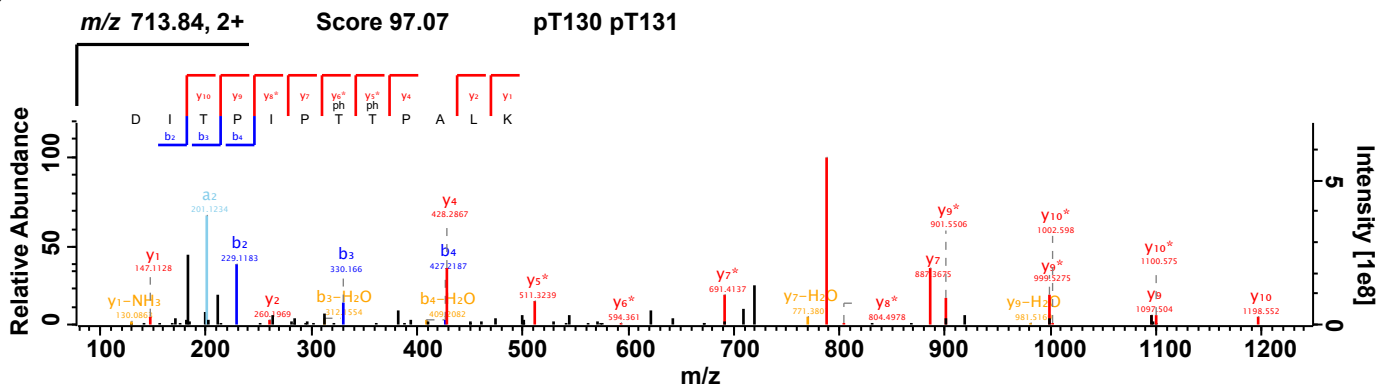
MYB75<sup>R84K</sup> interacts with EGL3 in the yeast two-hybrid system. Serial dilutions of transformed yeast cells were spotted on the indicated amino acid dropout agar plates. Co-transformation of MYB75 and CA-MPK4 was used as a positive control. AD, GAL4 activation domain; BD, GAL4 DNA binding domain. SD, synthetically defined medium; AbA, Aureobasidin A.



**Supplemental Figure 6. Specific Mutations of MYB75 Do Not Affect Its DNA Binding Activity.**

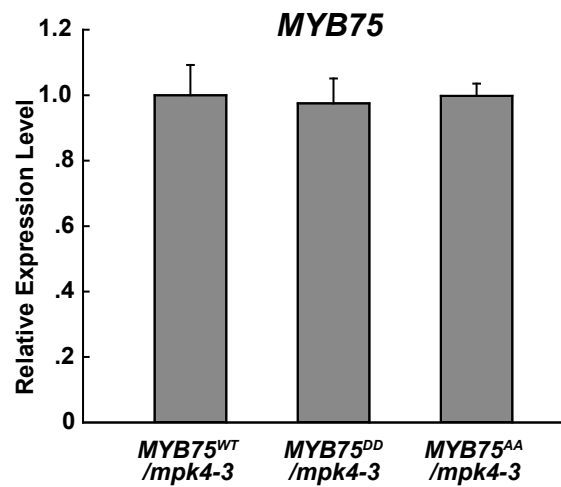
**(A)** EMSA showing that MYB75<sup>WT</sup> specifically binds to a probe containing MYB75/PAP1 *cis*-regulatory element (PCE) from the promoter of the *DFR* gene. Freshly prepared recombinant MYB75<sup>WT</sup> was incubated with labeled DNA probe containing PCE for 30 min. The indicated amounts of unlabeled probe were used in competition assay.

**(B)** MYB75 variants display similar DNA binding activities. The DNA binding activities of the MYB75 variants were determined by EMSAs as in **(A)**.

**A****B****Supplemental Figure 7. LC-MS/MS Analysis of *in vitro* Phosphorylation of MYB75.**

**(A)** LC-MS/MS analysis showing that MYB75 Thr126, T130, and Thr131 are phosphorylated. The sequence of a doubly-charged peptide ion at *m/z* 753.82, score 57.53, matches DlpTPIpTpTPALK of MYB75. “b” and “y” denote peptide fragment ions retaining charges at the N and C terminus, respectively. The subscript numbers indicate their positions in the identified peptide. pT indicates phosphorylated Thr.

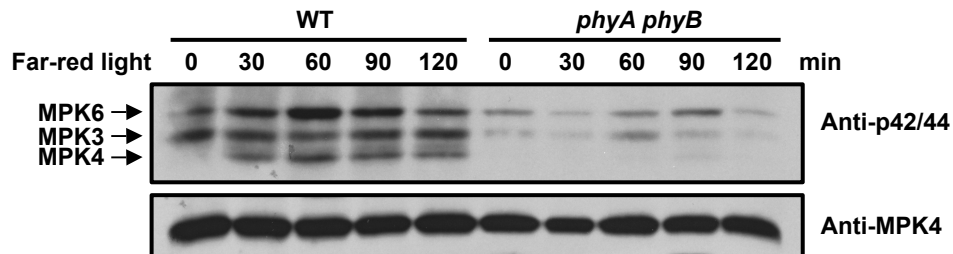
**(B)** LC-MS/MS analysis showing that MYB75 Thr130 and Thr131 are phosphorylated. The sequence of the doubly-charged peptide ion at *m/z* 713.84, score 97.07, matches DITPIpTpTPALK of MYB75. “b” and “y” denote peptide fragment ions retaining charges at the N and C terminus, respectively. The subscript numbers indicate their positions in the identified peptide. pT indicates phosphorylated Thr.



**Supplemental Figure 8. Expression Levels of *MYB75* Variant Transgenes in the *mpk4-3* Mutant.**

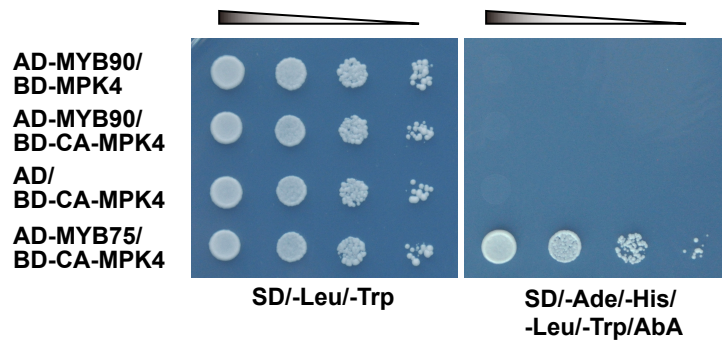
Quantitative real-time PCR analysis showing comparable *MYB75* expression levels in *35S:MYB75<sup>WT</sup>*, *35S:MYB75<sup>DD</sup>*, and *35S:MYB75<sup>AA</sup>* transgenic seedlings in the *mpk4-3* background. Primers were designed to detect both transgenic and endogenous *MYB75* transcripts. Results were normalized to *ACTIN8* and expression levels of the genes in the *35S:MYB75<sup>WT</sup>/mpk4-3* transgenic seedlings were set at one unit. Error bars indicate SD of three replicates (as described in Figure 2B).





**Supplemental Figure 9. Far-Red Light Does Not Activate MAPKs in the *phyA phyB* Mutant.**

Four-day-old dark-grown wild-type (WT) and *phyA phyB* seedlings were exposed to far-red light ( $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) for the indicated times. MAPK activity was analyzed by immunoblotting with Phospho-p44/42 MAPK (Erk1/2) antibody (top panel), and the level of MPK4 was determined by immunoblotting with anti-MPK4 antibody (bottom panel).



**Supplemental Figure 10. MPK4 Does Not Interact with MYB90/PAP2 in Yeast.**

MPK4 does not interact with MYB90/PAP2 in the yeast two-hybrid system. Serial dilutions of transformed yeast cells were spotted on the indicated amino acid dropout agar plates. Co-transformation of MYB75 and CA-MPK4 was used as a positive control. AD, GAL4 activation domain; BD, GAL4 DNA binding domain. SD, synthetically defined medium; AbA, Aureobasidin A.

**Supplemental Table 1. Phosphopeptides Identified in MYB75 by Mass Spectrometric Analysis**

Peptide <sup>a</sup>	Phosphosites	Phospho (STY) Probabilities <sup>b</sup>	PEP <sup>c</sup>	Score <sup>d</sup>	Freq. <sup>e</sup>
124-DI <sub>p</sub> TPIPTTPALK-135	T126	DIT(1)PIPTTPALK	1.40E-07	69.258	2
124-DITPIPP <sub>p</sub> TPALK-135	T130	DITPIPT(0.892)T(0.108)PALK	4.77E-07	77.185	1
124-DITPIPT <sub>p</sub> TPALK-135	T131	DITPIPT(0.082)T(0.918)PALK	4.75E-159	144.88	59
124-DI <sub>p</sub> TPIPT <sub>p</sub> TPALK-135	T126,T131	DIT(1)PIPT(0.031)T(0.969)PALK	6.82E-23	106.6	11
124-DITPIPP <sub>p</sub> T <sub>p</sub> TPALK-135	T130,T131	DIT(0.011)PIPT(0.992)T(0.997)PALK	5.53E-11	97.073	9
124-DI <sub>p</sub> TPIPP <sub>p</sub> T <sub>p</sub> TPALK-135	T126,T130,T131	DIT(1)PIPT(1)T(1)PALK	2.26E-06	57.525	2

<sup>a</sup>The positions of the peptides in the MYB75 sequence are indicated by the numbers before and after the peptides. pT denotes phosphorylated residues.

<sup>b</sup>Sequence representation of the peptide including PTM positioning probabilities for Phospho (STY).

<sup>c</sup>Posterior Error Probability of the identification. This value essentially operates as a p-value, where smaller is more significant.

<sup>d</sup>Andromeda score for the best associated MS/MS spectrum.

<sup>e</sup>The number of peptide matches to the same peptide sequence with the same modifications.

**Supplemental Table 2. Sequences of the Primers Used in This Work.****Cloning and mutation primers**

Gene	Forward Primer	Reverse Primer
<i>MPK4</i>	GGAATTCATGTCGGCGGAGAGTT GTTTC	CGGGATCCCCTAGAGTCTTGAGG ATTGAAC
<i>MPK4<sup>AEF</sup></i>	CTGACTTTATGGCTGAATTTGTT GTTACAC	TCTCGGATTTGGTCTCTCGCAAG
<i>CA-MPK4</i> ( <i>MPK4<sup>D198G/E202A</sup></i> )	CGAGACTGGCTTTATGACTGCAT ATGTTG	GATTGGTCTCTCGCAAGCCC
<i>MKK1<sup>DD</sup></i>	AAGCGAAAGTAGTCTTGCTAATG ATTTTCGTGGGCAC	GTCAAGATCTTGCTGACACCAAA G
<i>MYB75 genomic</i>	TAGGTACCATACTTTTACAATT TGTTTATATATTTTACG	ACCTGTCGACATCAAATTTACA GTCTCTCCATC
<i>MYB75 CDS</i>	ACGGATCCATGGAGGGTTCGTCC AAAG	TAAATGCGGCGCTAATCAAATT TCACAGTCTCTC
<i>MYB75<sup>R84K</sup></i>	CTAGGGAATAAGTGGTCTTTAAT TGCTG	AAGCCTATGAAGGCGAAGAAGAA G
<i>MYB75<sup>W85A</sup></i>	CTAGGGAATAGGGCTTCTTTAAT TGCTG	AAGCCTATGAAGGCGAAGAAGAA G
<i>MYB75<sup>T126A</sup></i>	GAGAGACATTGCTCCCATTCTTA C	TTTTTCATCTTTATCTTACAACA CGGTTTCATG
<i>MYB75<sup>T131A</sup></i>	CATTCTACAGCAACCGGCACTAA AAAAC	GGCGTAATGTCTCTCTTTTTTCAT CTTTATC
<i>MYB75<sup>AA</sup></i> ( <i>MYB75<sup>T126A/T131A</sup></i> )	GAGAGACATTGCTCCCATTCTTA CAGCAACCGGCACTAAAAAAC	TTTTTCATCTTTATCTTACAACA CGGTTTCATGTTTC
<i>MYB75<sup>DD</sup></i> ( <i>MYB75<sup>T126D/T131D</sup></i> )	GAGAGACATTGATCCCATTCTTA CAGATCCCGGCACTAAAAAAC	TTTTTCATCTTTATCTTACAACA CGGTTTCATGTTTC
<i>MYB75ΔN1</i>	GGAATTCATGCACCAAGTTCCTG TAAGAGCTG	CGGGATCCCTAATCAAATTTTCAC AGTCTCTCCATC
<i>MYB75ΔN2</i>	GGAATTCATGAGCTCTGATGAAG TCGATCTTCTTC	CGGGATCCCTAATCAAATTTTCAC AGTCTCTCCATC
<i>MYB75ΔN3</i>	GGAATTCATGGTCAAGAATTACT GGAACACTCATCTG	CGGGATCCCTAATCAAATTTTCAC AGTCTCTCCATC
<i>MYB75ΔN4</i>	GGAATTCATGCGCCTTCATAGGC TTCTAGGG	CGGGATCCCTAATCAAATTTTCAC AGTCTCTCCATC
<i>MYB75ΔN5</i>	GGAATTCATGAGGTGGTCTTTAA TTGCTGGAAG	CGGGATCCCTAATCAAATTTTCAC AGTCTCTCCATC
<i>MYB75ΔN6</i>	GGAATTCATGTTACCTGGTCGGA CCGCAAATG	CGGGATCCCTAATCAAATTTTCAC AGTCTCTCCATC
<i>MYB75ΔN7</i>	GGAATTCATGTCTTTAATTGCTG AAGATTACCTG	CGGGATCCCTAATCAAATTTTCAC AGTCTCTCCATC

<i>MYB75ΔN8</i>	GGAATTCATGATTGCTGGAAGAT TACCTGGTCG	CGGGATCCCTAATCAAATTTAC AGTCTCTCCATC
<i>MYB75ΔN9</i>	GGAATTCATGGGAAGATTACCTG GTCGGAC	CGGGATCCCTAATCAAATTTAC AGTCTCTCCATC
<i>MYB75ΔC1</i>	GGAATTCATGGAGGGTTCGTCCA AAGG	CGGGATCCGCTTTCTCTAGGAA TTTCTCTAAC
<i>MYB75ΔC2</i>	GGAATTCATGGAGGGTTCGTCCA AAGG	CGGGATCCCATGGAGGATTAACG TCAACTTTTG
<i>MYB75ΔC3</i>	GGAATTCATGGAGGGTTCGTCCA AAGG	CGGGATCCGTGCCGGTGTGTAG GAATG

Note: The restriction enzyme sites are underlined. For point-mutation primers, the mutation sites are in bold.

### RT-qPCR primers

Gene	Forward Primer	Reverse Primer
<i>MYB75</i>	AGATAAGAAGAAAGACCAACTAGTG	CCAAGGTGTCCCCCTTTTC
<i>MYB75-EGFP</i>	TTCTGAAGCGACGACAACAG	GTCCAGCTCGACCAGGATG
<i>DFR</i>	TGGTGTCGGTCCATTCAT	GAGAGAGCGCGGTGATAAGG
<i>LDOX</i>	TCCGGGTTTGCAGCTTTTC	ATCAGGAACACATTTTGCAGTGA
<i>UF3GT</i>	TGGAGGTGGCGGTTGAA	CTTTGCCGCGAGAACCA
<i>RD29A</i>	ACGTCGAGACCCCGATAAC	CAATCTCCGGTACTCCTCCA
<i>ERD10</i>	CTCTGAACCAGAGTCGTTTGTG	TTGTCGAGGAGACTTGGCTTG
<i>LEA14</i>	GGACTTCGTGGCGGATAAAC	GCCAAGTACTCAACTGAGTCAC
<i>KIN1</i>	ATCTCTTCTCATCATCACTAACC	AACATTGCTCTTCTCCTCAG
<i>ACTIN8</i>	TCAGCACTTCCAGCAGATG	CTGTGGACAATGCCTGGAC