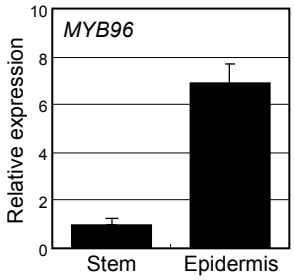
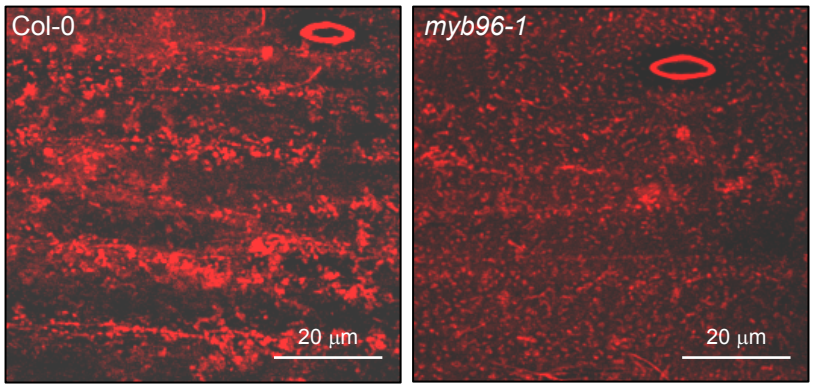
Supplemental Figures



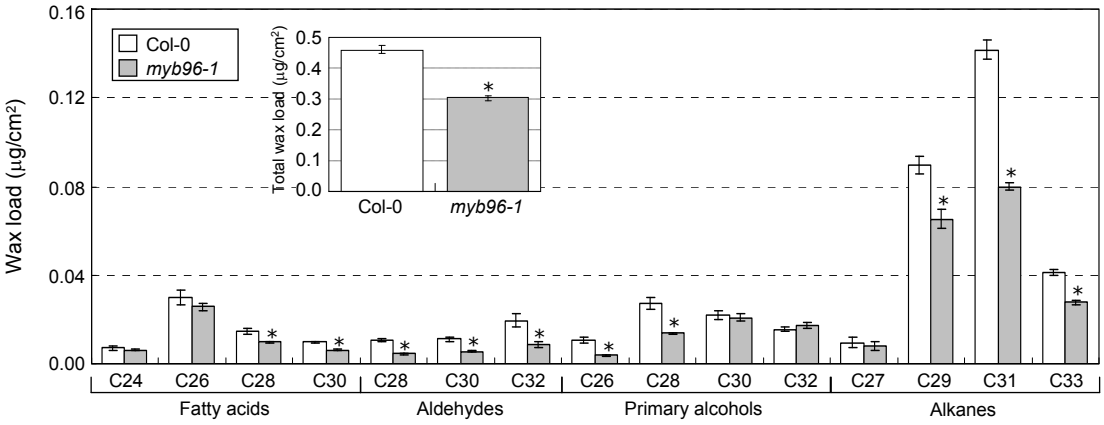
Supplemental Figure 1. Expression of *MYB96* in epidermal cells of stems
Arabidopsis plants were grown in soil for 5 weeks before harvesting plant materials. Total RNAs were extracted from the inflorescence stems and stem epidermal peels as described previously (Suh et al., 2005). The *Actin7* (At5g09810) gene was used as internal control. Transcript levels were examined by qRT-PCR. Biological triplicates were averaged. Bars indicate standard error of the mean.

Suh, M.C., Samuels, A.L., Jetter, R., Kunst, L., Pollard, M., Ohlrogge, J., and Beisson, F. (2005). Cuticular lipid composition, surface structure, and gene expression in *Arabidopsis* stem epidermis. Plant Physiol. **139:** 1649-1665.

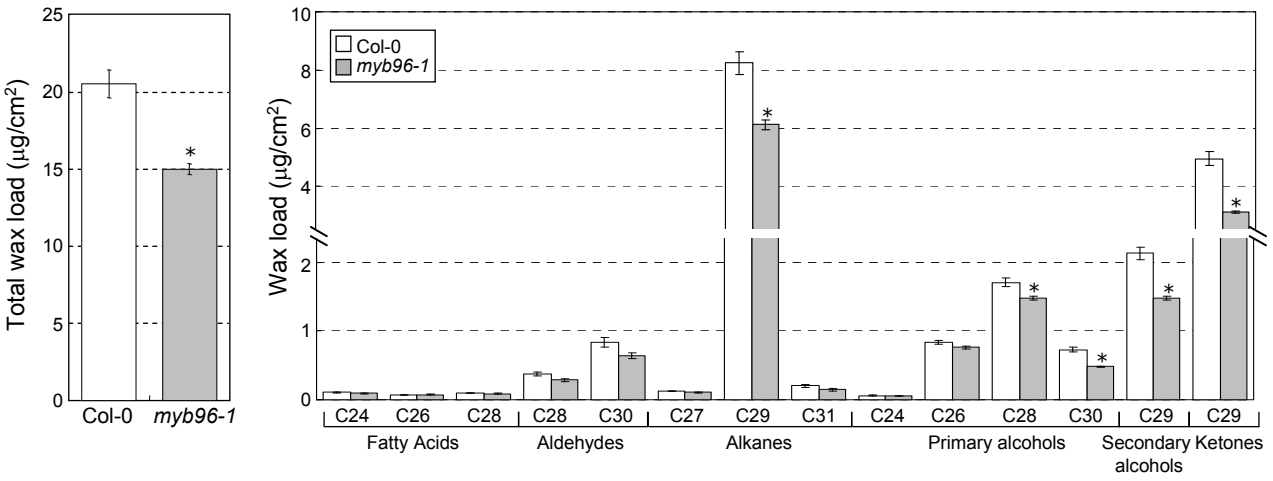


Supplemental Figure 2. Epicuticular wax deposition in *myb96-1* stems
Four-week-old plants grown in soil were used. Epicuticular wax crystals on the inflorescence stem segments from tip to 2 cm were visualized by Nile red staining as described previously (Pighin et al., 2004).

Pighin, J.A., Zheng, H., Balakshin, L.J., Goodman, I.P., Western, T.L., Jetter, R., Kunst, L., and Samuels, A.L. (2004). Plant cuticular lipid export requires an ABC transporter. Science **306:** 702-704.

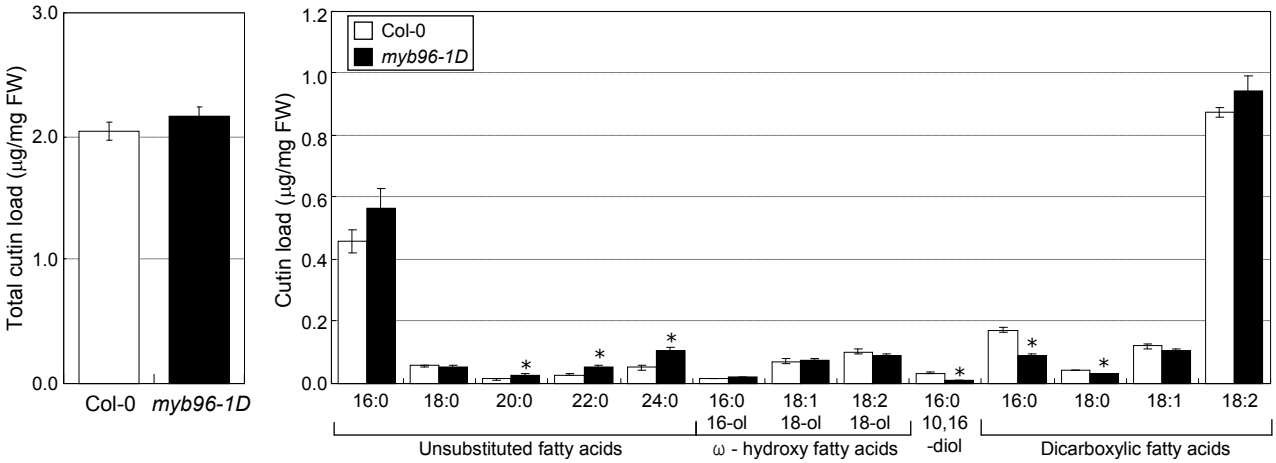


Supplemental Figure 3. Cuticular wax composition in *myb96-1* leaves
Rosette leaves of 4-week-old plants grown in soil were used for analysis of cuticular wax composition and loads. Biological triplicates were averaged and statistically treated using a student *t*-test (*P<0.01). Bars indicate standard error of the mean. The wax loads were expressed in μg/cm^2 .



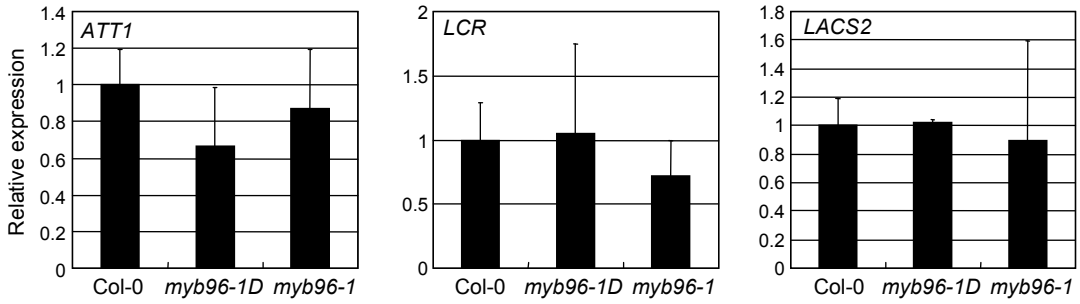
Supplemental Figure 4. Cuticular wax composition in *myb96-1* stems

Inflorescence stems of 5-week-old plants grown in soil were used for analysis of cuticular wax composition and loads. Biological triplicates were averaged and statistically treated using a student *t*-test (*P<0.01). Bars indicate standard error of the mean. Wax loads were expressed in μg/cm^2.

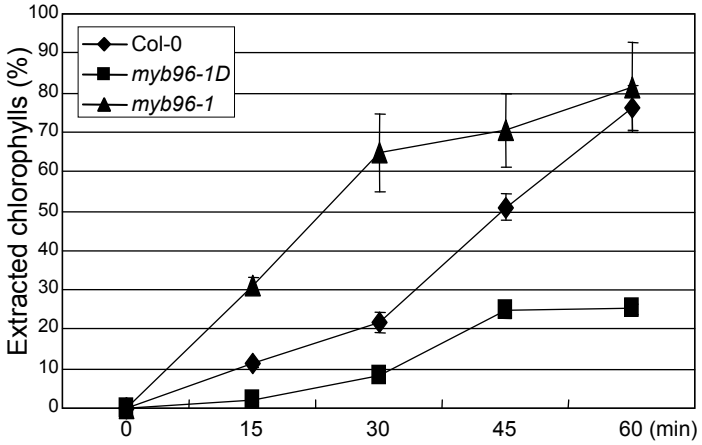


Supplemental Figure 5. Cutin biosynthesis in *myb96-1D* leaves
Rosette leaves of 4-week-old plants grown in soil were used for measurements of composition and contents of cutin polyester monomers as described previously (Li-Beisson et al., 2010). Biological triplicates were averaged and statistically treated using a student *t*-test (*P<0.01). Bars indicate standard error of the mean.

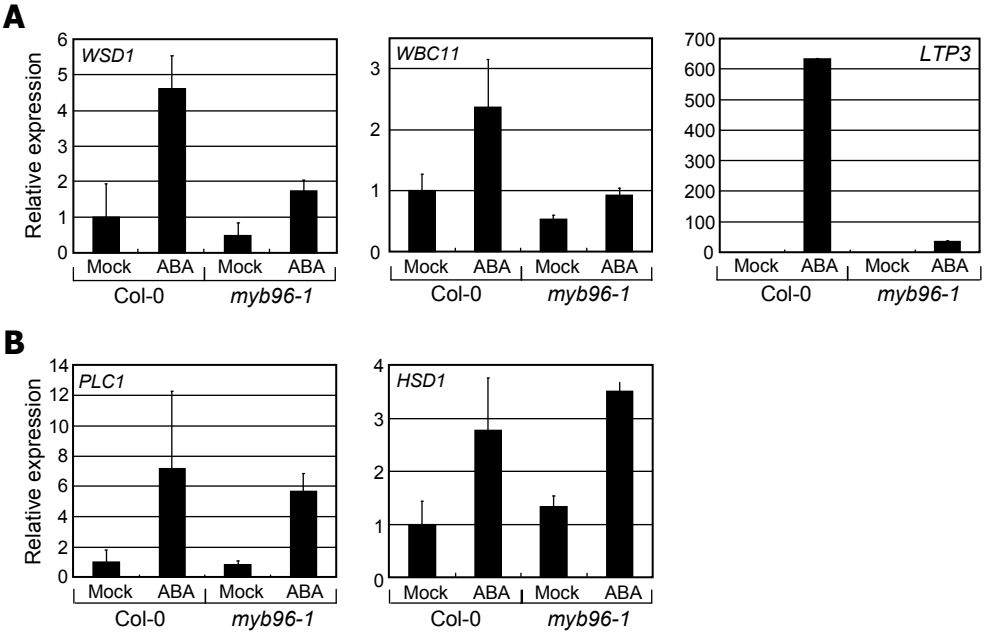
Li-Beisson, Y., Shorrosh, B., Beisson, F., X. Andersson, M., Arondel, V., Bates, P., Baud, S., Bird, D., DeBono, A., Durrett, T., Franke, R., Graham, I., Katayama, K., Kelly, A., Larson, T., Markham, J. Miquel, M., Molina, I., Nishida, I., Rowland, O. Samuels, L., Schmid, K., Wada, H., Welti, R., Xu, C., Zallot, R., and Ohlrogge, J. (2010). Acyl-Lipid Metabolism. In the Arabidopsis Book, Rockville, MD: American Society of Plant Biologist.



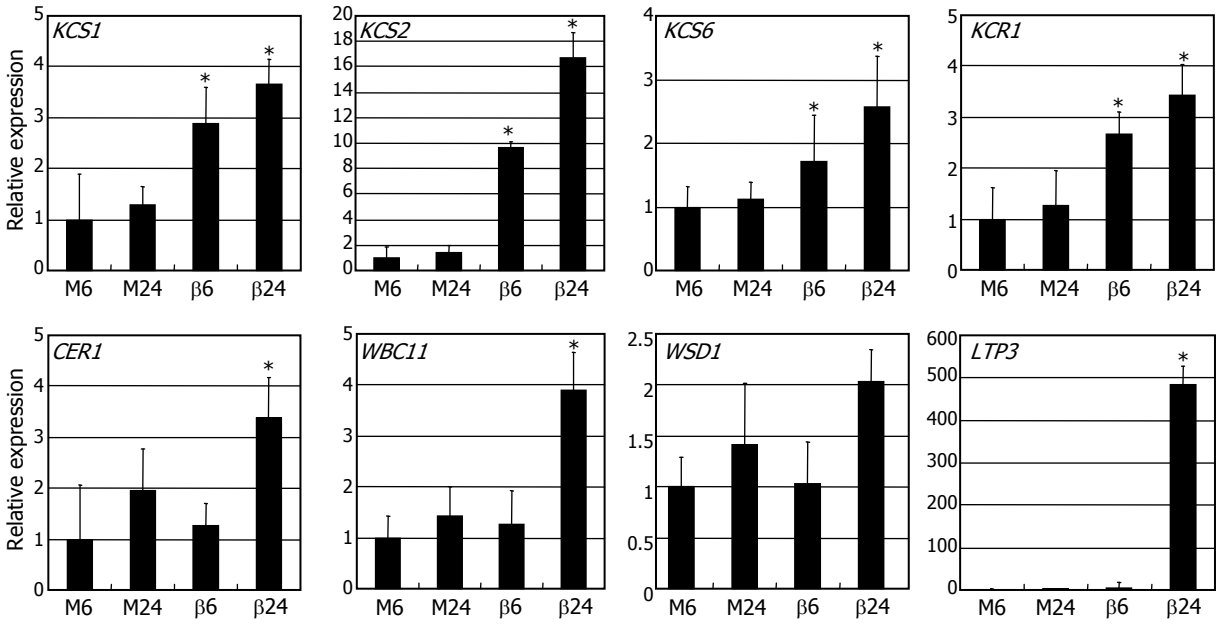
Supplemental Figure 6. Expression of cutin biosynthetic genes in *myb96-1D* and *myb96-1*
Two-week-old whole plants grown on MS-agar plates were used for extraction of total RNAs. Transcript levels were examined by qRT-PCR. Biological triplicates were averaged. Bars indicate standard error of the mean.



Supplemental Figure 7. Rate of chlorophyll leaching in *myb96-1D* and *myb96-1* leaves
Rosette leaves of 2-week-old plants grown on MS-agar plates were incubated on ice for 30 min and subsequently soaked in 80 % ethanol for the indicated time periods. The extracted chlorophyll contents at individual time points were expressed as percentages of extracted chlorophyll contents at 24 h after initial immersion. Three measurements were averaged at each time points. Bars indicate standard error of the mean.



Supplemental Figure 8. MYB96 regulation of wax biosynthetic genes in response to ABA
Two-week-old plants grown on MS-agar plates were transferred to MS liquid cultures supplemented with 20 μM ABA (6 h) before harvesting whole plants for extraction of total RNAs. Transcript levels were examined by qRT-PCR. Biological triplicates were averaged. Bars indicate standard error of the mean. Note that ABA regulation of wax biosynthetic genes depends on MYB96 **(A)**. Genes encoding lipid metabolic enzymes are also induced by ABA. However, the inductive effects of ABA are largely independent of MYB96 **(B)**.

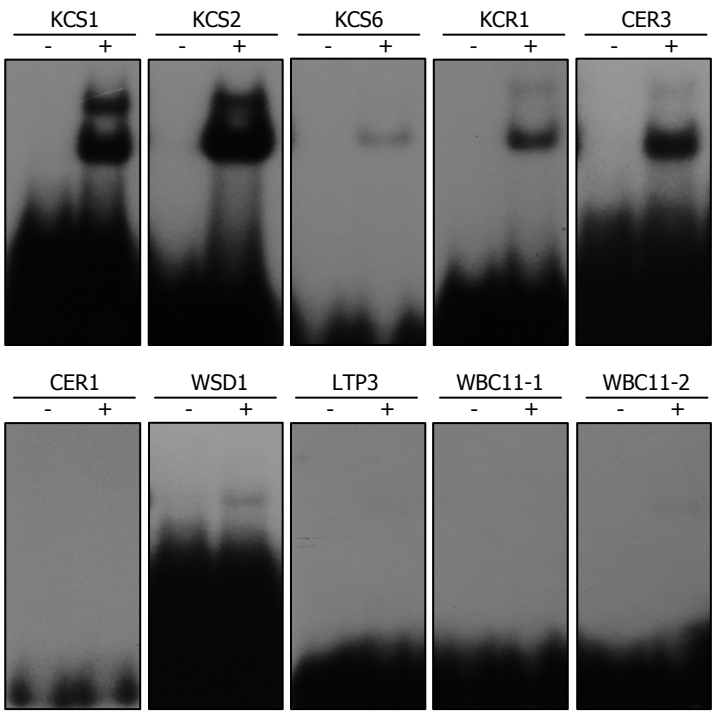


Supplemental Figure 9. Effects of *MYB96* induction on wax biosynthetic gene expression
Two-week-old transgenic plants expressing the *pER8-MYB96* gene fusion under the control of a β-estradiol-inducible promoter were incubated in MS liquid cultures supplemented with 10 μM β-estradiol (β). Whole plants were harvested at the indicated time points (h) after β-estradiol application for total RNA extraction. Transcript levels were examined by qRT-PCR. Biological triplicates were averaged. Statistical significance of the measurements was determined using a student *t*-test by comparing with M6 measurements (*t*-test, *P<0.01). Bars indicate standard error of the mean. M, mock.

A

KCS1	-1031	AA***TAACTA***GAAAAATACTCGAAAAC***TAACTA***GA
KCS2	-2091	GTCCC***TAACTA***C***TAACTA***TATTC
KCS6	-1548	AAGAT***TAACTG***TATTC
KCR1	-1936	AGTTG***CAGTTA***AGA***TAACTG***CAGAT
CER1	-797	ATGCT***TAGTTA***AATTT
CER3	-986	AGTG***TAACCA***TCGTTGCTT***CAACTG***TAAA
WSD1	-761	TC***TGGTTT***GTCCTTAATTTTAAATATGT***AAACCA***CT
LTP3	-930	GGCCA***CAGTTA***ATTAA
WBC11-1	-1240	AAAAA***TGGTTA***CCGAG
WBC11-2	-873	TGAAT***TGGTTA***GAAAT

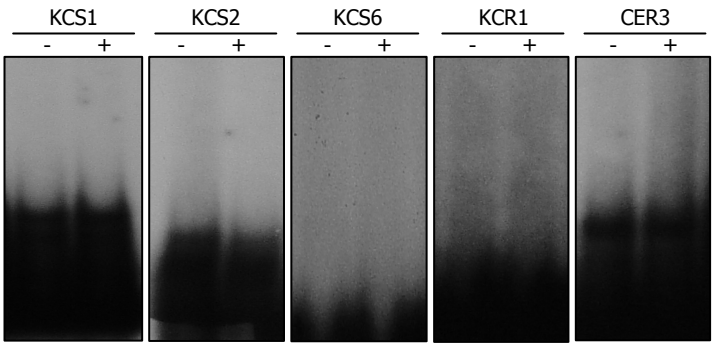
B



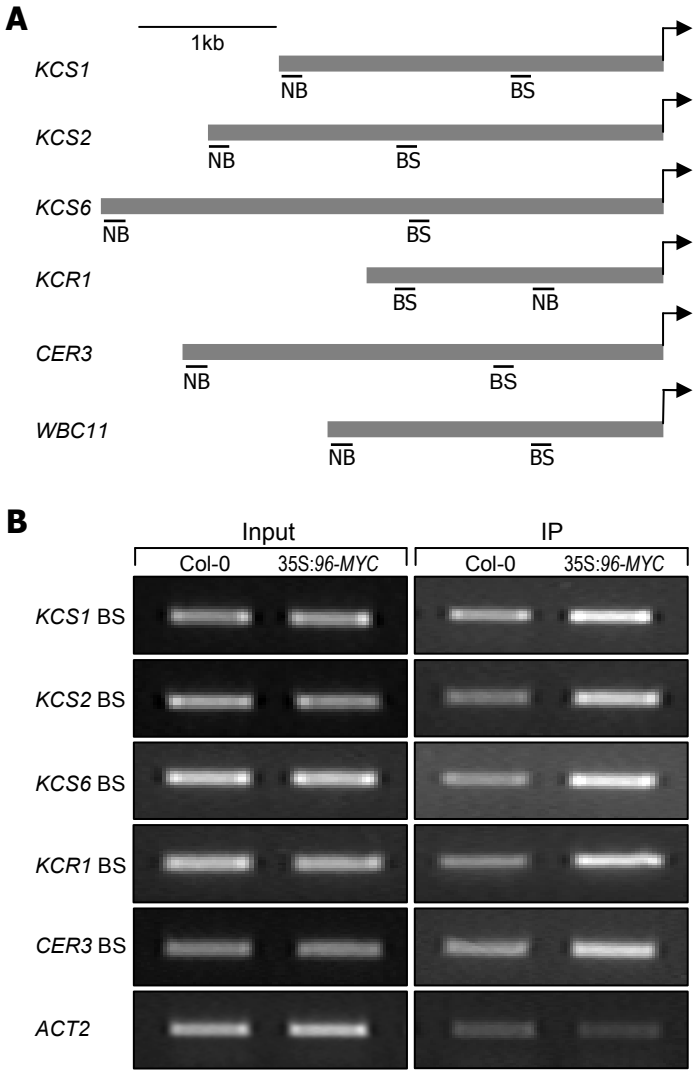
Supplemental Figure 10. Binding of MYB96 to the consensus sequences in the promoters of wax biosynthetic genes *in vitro*

(A) Putative MYB-binding consensus sequences in the gene promoters. Core binding sequences are marked by bold, italic characters.

(B) *in vitro* binding of MYB96 to the consensus sequences. The (-) lanes are controls without adding a recombinant maltose-binding protein (MBP)-MYB96 protein.



Supplemental Figure 11. *in vitro* binding of MBP alone to the consensus sequences
The (-) lanes are controls without adding MBP proteins.



Supplemental Figure 12. Direct binding of MYB96 to the promoters of wax biosynthetic genes *in vivo*

(A) Representation of the wax biosynthetic gene promoters. Black lines indicate the sequence regions used for ChIP assays. NB, non-binding sequence; BS, binding sequence.

(B) ChIP assays with an anti-MYC antibody. The 35S:*96-MYC* transgenic plants grown on MS-agar plates for 3 weeks were used. The *ACT2* gene was used as negative control.

Supplemental Table 1. Primers used in qRT-PCR and subcloning

Primer	Usage	Sequence
MYB96-F	qRT-PCR	5′-TGCAGTCTCGGAAGAAGGTG
MYB96-R	qRT-PCR	5′-CATCTCGTGGCTTTGCTCAT
KCS1-F	qRT-PCR	5′-TCTCCTGCTACAAACCGGAA
KCS1-R	qRT-PCR	5′-CCGTGTCATCGGTGAATGAT
KCS2-F	qRT-PCR	5′-CAACCTCGCTTTCCAACAAA
KCS2-R	qRT-PCR	5′-TCCGGTTTTCTCAAGCACTG
KCS6-F	qRT-PCR	5′-TATTGTCGCCGTTGAGCTTC
KCS6-R	qRT-PCR	5′-TTCCATGAAAGTTGCGAAGG
KCR1-F	qRT-PCR	5′-CAAATCACAACCCACTTGGC
KCR1-R	qRT-PCR	5′-AGGTTTTTGGATGGTCGGAG
CER1-F	qRT-PCR	5′-AGGTCGACAGGGAGACCAAC
CER1-R	qRT-PCR	5′-ATAAGCGCTGCCATCAACAC
CER3/WAX2-F	qRT-PCR	5′-GGAAACGCAACGTTATTGGA
CER3/WAX2-R	qRT-PCR	5′-AGCGTAACCGTAGATCGCAC
LTP3-F	qRT-PCR	5′-TGGCTCCATGTGCAACCTAT
LTP3-R	qRT-PCR	5′-GGACTGGATGCATCTGCAAG
PLC1-F	qRT-PCR	5′-GACATGTTTTCCGTTGTCGG
PLC1-R	qRT-PCR	5′-AAGACAAACCGCATGAGCAG
WBC11-F	qRT-PCR	5′-CCTGCGTTAAGGAACCCTTC
WBC11-R	qRT-PCR	5′-GGATCATCGCTTGCTTCAAA
HSD1-F	qRT-PCR	5′-GTGCTTCCTCCGGTATAGGC
HSD1-R	qRT-PCR	5′-GGAGACATCAGCATGAACGG
WSD1-F	qRT-PCR	5′-GCTTGGTGGTTGTTTGTTGG
WSD1-R	qRT-PCR	5′-TCGGGTTACCCATAAGAGGG
ATT1-F	qRT-PCR	5′-AATTCAAACCGGAGAGGTGG
ATT1-R	qRT-PCR	5′-AGATCCTAGGTCCGGCGTTA
LCR-F	qRT-PCR	5′-TAAAGGTCCAACGTGGCAAG
LCR-R	qRT-PCR	5′-CTTACGCTGAAAAAGCCACG
LACS2-F	qRT-PCR	5′-GTTGATCCGGGACACTGTTG
LACS2-R	qRT-PCR	5′-ACGTAGGTGATCCCTTGGCT
eIF4a-F	qRT-PCR	5′-TGACCACACAGTCTCTGCAA
eIF4a-R	qRT-PCR	5′-ACCAGGGAGACTTGTTGGAC
ACT2-F	qRT-PCR	5′-CCATCCTCCGTCTTGACCTT
ACT2-R	qRT-PCR	5′-ACTTGCCCATCGGGTAATTC
MYB96-F	Subcloning	5′-AAAAAGCAGGCTCGATGGGAAGACCACCTTGC
MYB96-R	Subcloning	5′-AGAAAGCTGGGTTCTAGAACATCCCTTCTTGTCCT
MYB96(pER8)-F	Subcloning	5′-GGCTCGAGATGGGAAGACCACCTTGC
MYB96(pER8)-R	Subcloning	5′-CCTTAATTAACTAGAACATCCCTTCTTGTCC
MYB96(MBP)-F	Subcloning	5′-AAAAAGCAGGCTCGATGGGAAGACCACCTTGC
MYB96(MBP)-R	Subcloning	5′-AGAAAGCTGGGTTTCATTCCCATTGACCTTTGTTAGTG
MYB96(MYC)-F	Subcloning	5′-AACTCGAGATGGGAAGACCACCTTGC
MYB96(MYC)-R	Subcloning	5′-CCGGCGCGCCGAACATCCCTTCTTGTCC

qRT-PCR primers were designed using the Primer Express Software installed into the Applied Biosystems 7500 Real-Time PCR System. The sizes of PCR products ranged from 80 to 150 nucleotides in length. F, forward primer; R, reverse primer.

Supplemental Table 2. Primers used in ChIP assays

Primer	Sequence
KCS1(BS)-F	5′-ATTTTGGACACCTTGGCACA
KCS1(BS)-R	5′-TGGGCCATGGTAAATTGATG
KCS1(NB)-F	5′-GCCGTTGAAGAACAAAAGCA
KCS1(NB)-R	5′-CCAACCCGAGTTTTGAGGAT
KCS2(BS)-F	5′-ATGCTCGCTTTGATTGATGC
KCS2(BS)-R	5′-ACACAAATCCGAATAGCCGA
KCS2(NB)-F	5′-CCACTCTCGAGCCATCAAAG
KCS2(NB)-R	5′-CCAGTCTCCAGATTGCATGG
KCS6(BS)-F	5′-TGATTTTCCCTCATGCTTGC
KCS6(BS)-R	5′-TGGGCCCAATTGTGTCATT
KCS6(NB)-F	5′-GTCGTAACAACCCGCAAAAA
KCS6(NB)-R	5′-TCGAGATGTGCAAGCATCAA
KCR1(BS)-F	5′-CCAAATGTGCAGGTTGCTCT
KCR1(BS)-R	5′-ACCATGCAAGTGATCTCCCA
KCR1(NB)-F	5′-TGGACATAACAGGCCAAGGA
KCR1(NB)-R	5′-TGCCTTTGAGCTGCTTGAAC
CER3(BS)-F	5′-TGAAGAAGCAAGCCACCAAG
CER3(BS)-R	5′-ACCCAATTGATGCAATGGAC
CER3(NB)-F	5′-AACCATTGTTAGATCAAATGCG
CER3(NB)-R	5′-TCTCTTTCGTCACACCGTGAT

BS, DNA fragments containing MYB96-binding sequences; NB, non-binding DNA fragments. F, forward primer; R, reverse primer.