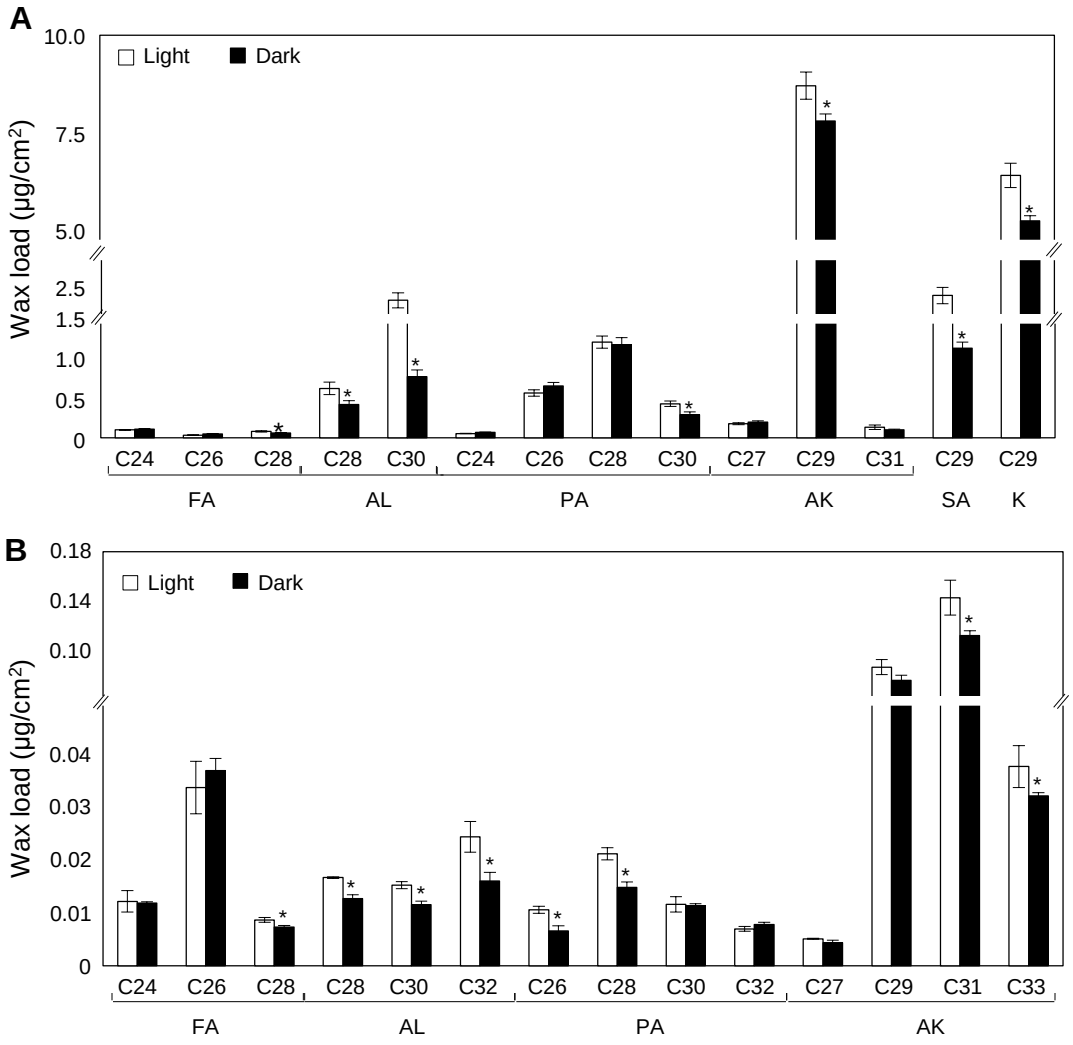
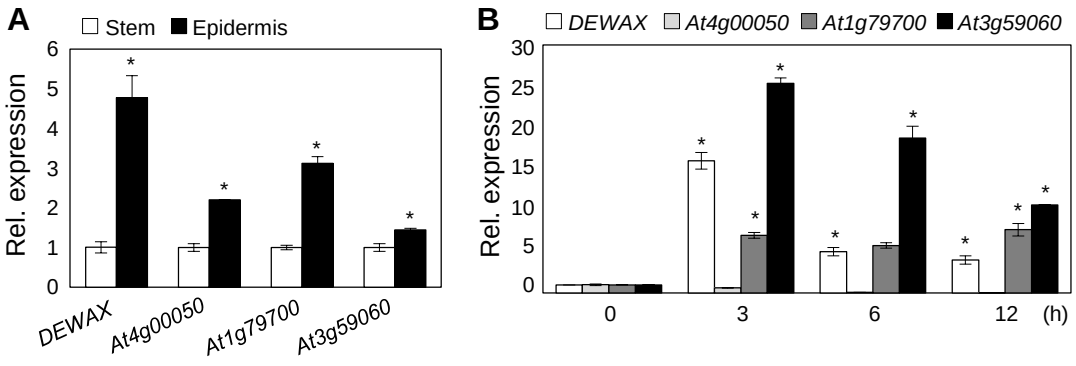
Supplemental Figure 1



Supplemental Figure 1. Cuticular Wax Amounts and Composition from Stems and Leaves of *Arabidopsis* under Long-Day and Dark Conditions.

Cuticular wax amounts and composition from stems (**A**) and leaves (**B**) of 3- and 5-week-old *Arabidopsis* wild type Plants, which were Grown under long-day conditions (16 h light/ 8 h dark, Control) and in the dark for 6 days (dark) were analyzed by gas chromatography (GC) and GC-mass spectrophotometry. Data were statistically analyzed using Student's *t*-test (* $P < 0.01$). Error bars indicate ± SE from triplicate experiments. FA, fatty acids; AL, aldehydes; PA, primary alcohols; AK, alkanes; SA, secondary alcohols; K, Ketone.

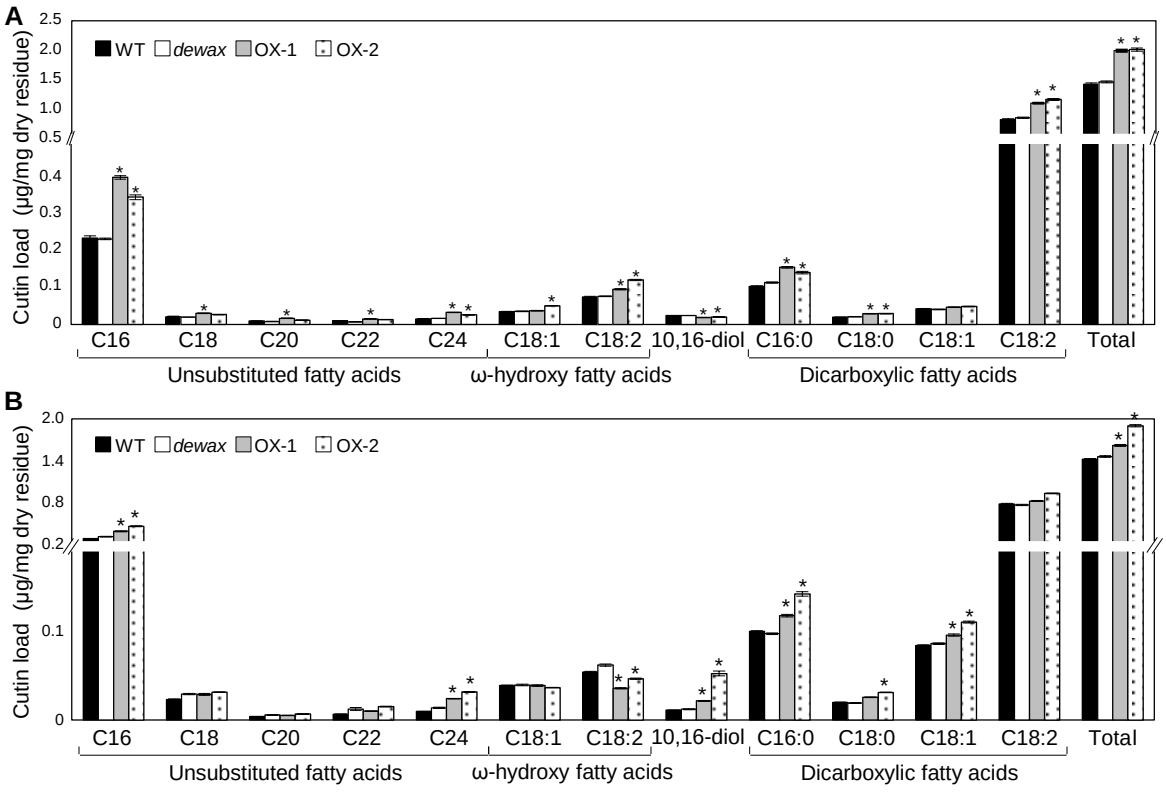
Supplemental Figure 2



Supplemental Figure 2. qRT-PCR Analysis of Four Genes Encoding Transcription Factors.

(A) qRT-PCR analysis of four genes in *Arabidopsis* stems and stem epidermal peels. Total RNAs were extracted from stems and stem epidermal peels of 5-week-old plants and subjected to qRT-PCR analysis. Data were statistically analyzed using Student's *t*-test (* $P < 0.01$). Error bars indicate ± SD from triplicate experiments.
(B) qRT-PCR analysis of four genes in *Arabidopsis* stems after dark treatment. Five-week-old wild type plants grown under long-day conditions were transferred to dark conditions and stems were harvested 0, 3, 6, and 12 h after dark treatment. Total RNAs were extracted from each sample and subjected to qRT-PCR analysis. Data were statistically analyzed using Student's *t*-test (* $P < 0.01$). Error bars indicate ± SD from triplicate experiments.

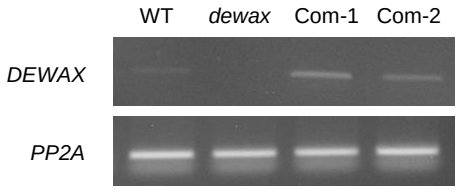
Supplemental Figure 3



Supplemental Figure 3. Cutin Monomer Amounts and Composition in Stems and Leaves of Wild Type, *dewax*, and *DEWAX* Overexpression Lines.

Cutin monomer amounts and composition in stems (**A**) and leaves (**B**) of wild type, *dewax*, and *DEWAX* overexpression lines. Stems and leaves of 5-week-old wild type (WT), *dewax*, and overexpressing *DEWAX* lines (OX-1 and OX-2) were lyophilized, delipidated, and hydrolyzed, and then lipid-soluble extracts were analyzed using GC and GC-mass spectrometry. Data were statistically analyzed using Student's *t*-test (* $P < 0.01$). Error bars indicate $\pm$ SE from triplicate experiments.

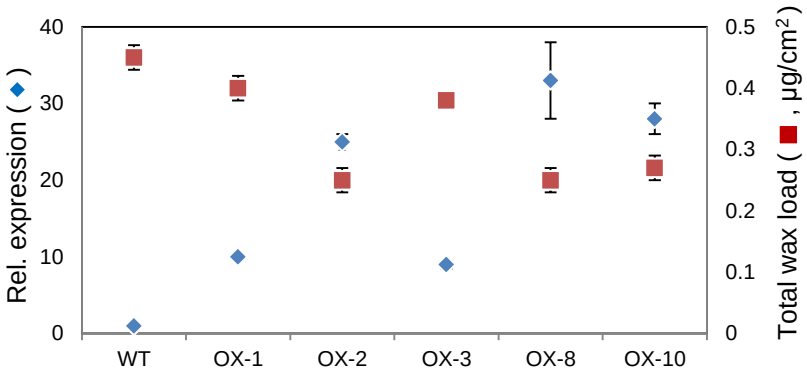
Supplemental Figure 4



Supplemental Figure 4. Accumulation of *DEWAX* Transcripts in Leaves of Wild Type, *dewax*, and Complementation lines of *dewax*.

Total RNAs were extracted from leaves of wild type (WT), *dewa*x, and complementation lines (Com-1 and Com-2) of *dewax* and subjected to reverse-transcription-PCR (RT-PCR) analysis. The similar results were obtained from triplicate experiments. *PP2A* was used to determine the quantity and quality of the cDNAs.

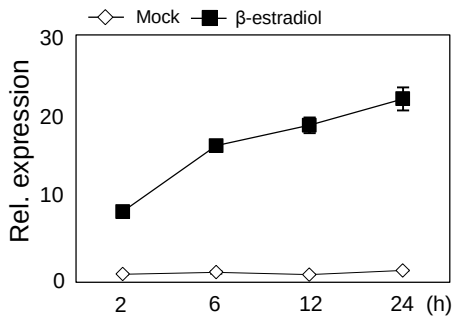
Supplemental Figure 5



Supplemental Figure 5. *DEWAX* Transcript Accumulation and Total Wax Amounts in Leaves of Wild Type and *DEWAX* Overexpression Lines.

Total RNAs were extracted from leaves of wild type (WT) and *DEWAX* overexpression lines (OX-1, OX-2, OX-3, OX-4 and OX-5) and subjected to quantitative reverse-transcription-PCR (qRT-PCR) analysis. Error bars indicate ± SE from triplicate experiments. Cuticular waxes were extracted from leaves of 3-week-old *Arabidopsis* wild type and *DEWAX* Overexpression Lines with chloroform and analyzed by gas chromatography (GC) and GC-mass spectrophotometry. Error bars indicate ± SE from triplicate experiments.

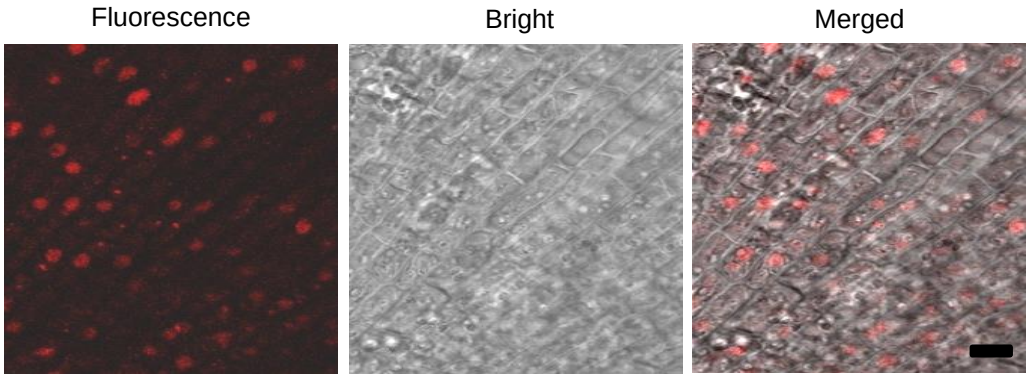
Supplemental Figure 6



Supplemental Figure 6. Expression of *DEWAX* Transcripts in Leaves of Transgenic *Arabidopsis* expressing *DEWAX* under the Control of a β-Estradiol Inducible Promoter after β-Estradiol Treatment.

Three-week-old transgenic plants expressing *DEWAX* under the control of a β-estradiol inducible promoter were sprayed with 10 μM β-estradiol or ethanol (mock). Total RNA was isolated from leaves, which were harvested 2, 6, 12, and 24 h after β-estradiol treatment, and subjected to qRT-PCR analysis. Data were statistically analyzed using Student's *t*-test (**P* <0.01). Error bars indicate ± SD of the mean of triplicate experiments.

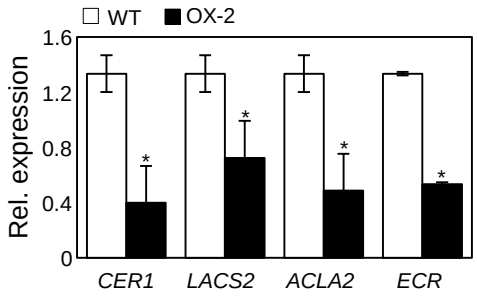
Supplemental Figure 7



Supplemental Figure 7. Subcellular Localization of the *DEWAX* Gene in Transgenic Plant Root.

The 35S:DEWAX:mRFP constructs were introduced into *Arabidopsis* plants. Fluorescent signals in a transgenic plant root were visualized under a confocal laser scanning microscope. Bar is 50 μm.

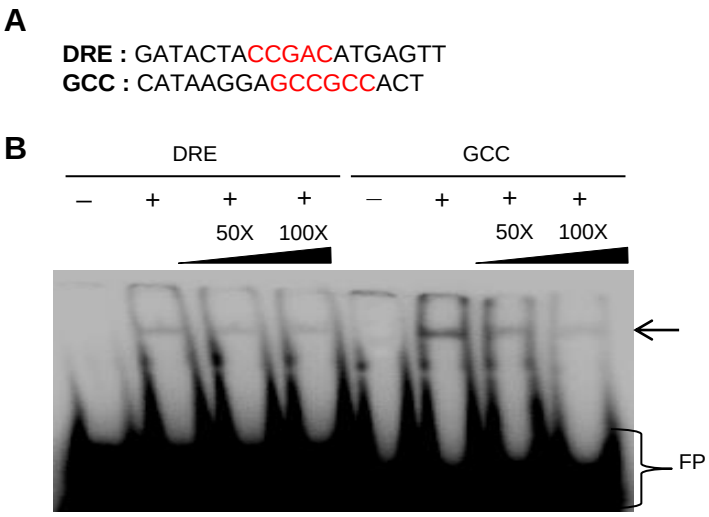
Supplemental Figure 8



Supplemental Figure 8. Expression of *CER1, LACS2, ACLA2,* and *ECR* in the Leaves of the Wild Type and *DEWAX* Overexpression Line.

Total RNAs were isolated from leaves of the wild type and *DEWAX* overexpression line (OX-2), and subjected to qRT-PCR analysis. Data were statistically analyzed using Student's *t*-test (**P* <0.01). Error bars indicate ± SD of the mean of triplicate experiments.

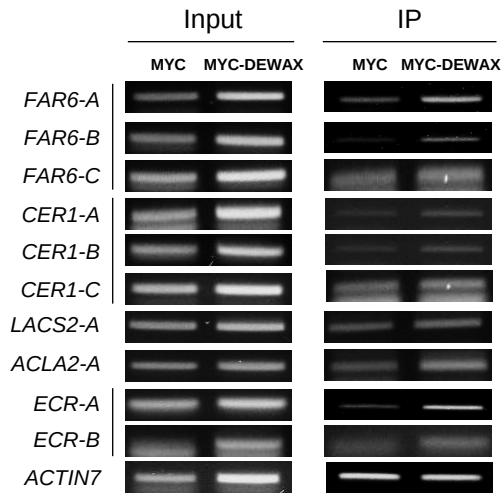
Supplemental Figure 9



Supplemental Figure 9. Electrophoretic Mobility Shift Assay of DEWAX to DRE and GCC Motifs.

(A) Nucleotide sequences of the DRE and GCC Motifs. Core binding sequences are shown in red.
(B) Dose-dependent binding of MBP-DEWAX to the DRE and GCC motifs. The fusion protein, maltose binding protein (MBP):DEWAX (5 µg) was purified from *Escherichia coli* and incubated with ^{32}P-labeled DRE- and GCC motifs with or without competitor DNA fragments. The reaction mixtures were electrophoresed on 10% native PAGE gels, dried, and scanned using a phosphorimager. The DNA-protein binding complexes are indicated by an arrow. -, Absence of MBP:DEWAX; +, Presence of MBP:DEWAX; FP, Free probe.

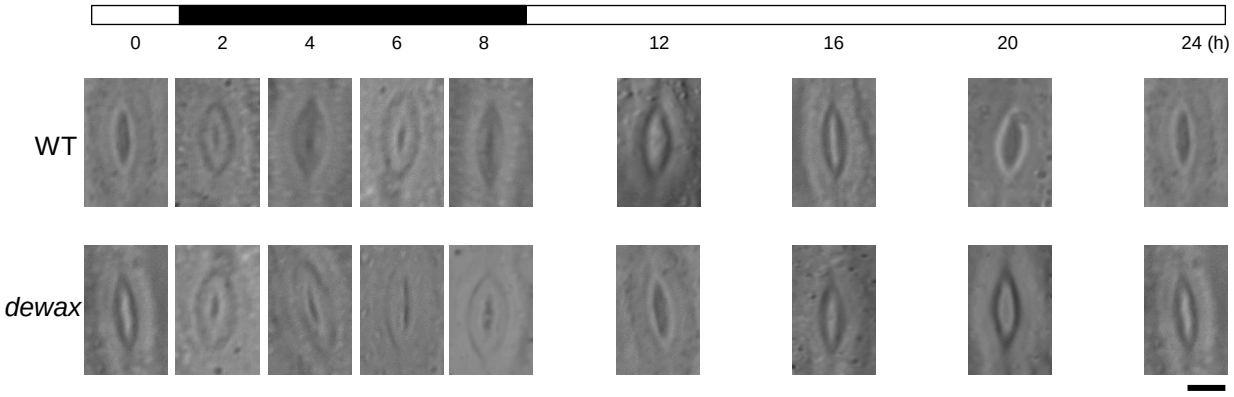
Supplemental Figure 10



Supplemental Figure 10. Chromatin Immunoprecipitation (ChIP) Assay in 35S:MYC and 35S:MYC-DEWAX Transgenic Plants.

Total protein extracts from 35S:MYC and 35S:MYC-DEWAX transgenic plants grown on MS-agar plates for 2 weeks were immunoprecipitated with an anti-MYC antibody. Fragmented genomic DNA was eluted from the protein-DNA complexes and subjected to PCR analysis. The *ACTIN7* gene was used to determine the quantity and quality of the cDNAs. Input, Total protein extracts; IP, Immunoprecipitated protein extracts.

Supplemental Figure 11



Supplemental Figure 11. Stomatal Apertures in Wild Type and *dewax* during Daily Light and Dark Cycle.

DIC images of stem epidermal peels of 4-week-old wild type and *dewax*, which were harvested 2 h, 4 h, 6 h, and 8 h after the lights were turned off, and 4 h, 8 h, 12 h, and 16 h after the lights came on, were observed under the DM2500 microscope. Black box; dark period, White box; light period. Bar indicates 20 μm.

Supplemental Table 1. List of Genes that Are Involved in Wax Biosynthesis and Accumulation and that Are Down-Regulated in Stems of the *DEWAX* Overexpression Line (OX-2) Relative to the Wild Type.

		Expression level			
Locus	FC	WT	OX-2	Gene	Annotation
At3g56700	-14.1	817	57	FAR6	Fatty acyl-CoA reductase
At4g22490	-2.4	130	53	LTP6	Lipid transfer protein
At1g02205	-2.3	5795	2518	CER1	Alkane-forming enzyme
At1g49430	-1.8	294	162	LACS2	Long-chain acyl-CoA synthetase
At1g60810	-1.8	2675	1469	ACLA2	ATP citrate lyase A subunit
At3g55360	-1.5	3101	2124	ECR	Enoyl-CoA reductase

Supplemental Table 2. Nucleotide Sequences of Promoter Regions of Wax Biosynthetic Genes regulated by DEWAX

Gene	Region	Position	Nucleotide Sequences of Promoter Regions
FAR6	A	-1693 ~ -1467	cttcctgcttcacattcctgccatgtgcagaaattggtggcagggatatgtgttcaactttctctgctacact aattgagtgatgatggaggattgtatgaaacagaagaagctccgtaagggaagctaactcctgataactt ctgctctgataagaaacaaacaaaaaaagtatagagtgctacgcagagaacaaatgagtgaaggatttg aattagttgcctcg
	B	-846 ~ -702	gtagggtacacattaaacgttattgaatatttagcccaaaacacagaagttcgttcaccttagctttgatta gtttgattgcatgtaatatggtctggtcattacaagaaaagcttcgtgaatcacaatcgaccaacgtgtac
	C	-218 ~ -83	ccacgaggccaaaacacagcttcactaggtccacatgtgatccgcgacctaaccacattaattcctaact aataactttctctatatatacactcccactaaagcccaaaaaagaccacttcaaagcttcc
CER1	A	-915 ~ -755	cccttgttacccttggtgccacgtgcttcatatatgttagaaagggcaaaaaaattcgtcgtatgatatgctt agttaaattttataaaactcaataaaaatcttcagaaacagtgctatgatcattacatcttaactaagtgatat atatctgcgtgcc
	B	-412 ~ -298	gcatcaatacctaacacatgcccaacttggttcattagtattctttcattggtaaaataccccttacctttcaata atatccagaaataaatatatgaagccatccatcaaccgg
	C	-317 ~ -152	caagccatccatcaaccggtgcatttcctcaaggcatggatatgatatcagaacatcgatgaaggtggg agggggtaattagctgagtgtcataaatgaggatccatgtggagatcatcgaatggtagtagtacatgttt ggtcttagctggcccaccacaagg
LACS2	A	-1595 ~ -1321	ctttgtagtttaaccgtgtaaaaacttttacctataaaaaagaaaaaaaaataatagggataagttttttttat atatacccataataattaaaacatcgccgaaaattaaattgtTaaggctggggaagaattattgttcacgc caacaatcacgttatggttcaaatttggatattttccttataaaaagttaaagaaaaagcatgttttgtaaatt taattcatagaaaatgtctgaaattttccattatttataaagattatagattc
ACLA2	A	-1055 ~ -897	gatcactagttcgttaccaataagcagaatcccttatagctgaggtctgtccacttcattacagcctatgga ccaatgcagaacttttgaagcgagccattatagccatgcttgtttcctagataagcctcctttattagaggt atatatgcgttac
ECR	A	-826 ~ -733	cacactacttccatgtggttaaggccgggatcatttattatttgcatcttttatacgacaatgacaaggatt cgttctcattactccatccc
	B	-291 ~ -153	gtaattactaatggtggatccttaaaacaaagccataatttgcaattgcaacaaatgtttttgtttggagtaa aaaccaaaaaaaaaatgatggggagcaactaaaaatgtaaaatggtcccaatctaatcgcataag

*GCC motifs are shown in red.

Supplemental Table 3. Primers Used in This Study.

Reaction	Primer name	Sequence information
Mutant isolation	DEWAX F1	5'-cttattctcctgtcttgtc
	DEWAX R1	5'-tctaacaggttcttggctc
	LBa1	5'-tggttcacgtagtgggccatcg
Promoter analysis	DEWAX PF1	5'-ggtcgacgtcaacgtacgatgacgttttc
	DEWAX PR1	5'-ccccgggcctcaaaagtctccctttcttcc
Overexpression and Subcellular localization	DEWAX cNDA F1	5'-gagctcatggagacttttgaggaaag
	DEWAX cDNA R1	5'-cccggggtttgatgacgatgatgaag
qRT-PCR of wax biosynthetic genes	FAR6 real F1	5'-ggaggaggattgagcacgaag
	FAR6 real R1	5'-gtaatgctcccagtcaatgcc
	LTP6 real F1	5'-cactgccctcaatggcctc
	LTP6 real R1	5'-ggacactggaaaccgatagg
	ACLA2 real F1	5'-caatggcataatccgagctc
	ACLA2 real R1	5'-cctccttgcatatacctgtc
	LACS1 real F1	5'-ccggttcaaccaatcattgc
	LACS1 real R1	5'-cacacacatcatcgcaacac
	KCS1 real F1	5'-ggcttataccgaagctaagg
	KCS1 real R1	5'-ccggttcaaccaatcattgc
	CER1 real F1	5'-catattgcacgccttagaag
	CER1 real R2	5'-ttaatgatgtggaaggaggagagg
	CER2 real F1	5'-agatagattcggttggcgag
	CER2 real R1	5'-gtttcgccgcggatattcag
	CER3 real F1	5'-gtgatctagcagctatgaag
	CER3 real R1	5'-gatacggtcaacatcaatgg
	CER4 real F1	5'-atctctatcagccttacctc
	CER4 real R1	5'-gcagcccaataacatgtgtg
	ECR real F1	5'-tcaacatcgctactcagacc
	ECR real R2	5'-ggaatggaggaagtatcacccatc
	KCS2 real F1	5'-cgctaaacagcttcttcag
	KCS2 real R1	5'-gagttggtatttggagcgg
	KCS6 real F1	5'-gtgaagccctcaaggcaaac
	KCS6 real R1	5'-cgaaggccagcttgaaatcc
	WBC11 real F1	5'-gttcccaacttcctcatggg

	WBC11 real R2	5'-cttgaaagcactcccttggctg
	KCR1 real F1	5'-actctgtttatgctggtgc
	KCR1 real R1	5'-tgcaactaagaaggatgctc
	LACS2 real F1	5'-ccctcattgctcagatatgggtc
	LACS2 real R1	5'-tgcggtagagttaagctcatccaag
Transcription activation assay	CER1 pro F2	5'-ggtcgaccatacatctatgtcccctttc
	CER1 pro R2	5'-aactagttataccgtcgaatgtaatatg
	FAR6 pro F2	5'-ggtcgacgcttcacattcctgccatgtg
	FAR6 pro R1	5'-aactagtggaagcttaatttgaagtggtc
	LTP6 pro F1	5'-ggtcgacgaatcacgttggagtttac
	LTP6 pro R1	5'-aactagtggatgctttcgatgatgttg
	LACS2 pro F2	5'-ggtgaggtctatcacaatc
	LACS2 pro R1	5'-aactagtggaggaatgaagaagataagg
	ACLA2 pro F2	5'-gttgggagttgaaacgtttg
	ACLA2 pro R1	5'-aactagtggattcgagacctcttgattc
	ECR pro F1	5'-ggacctcactatgtcgacacacaacatcaaac
	ECR pro R1	5'-tacgtcgacggtgcttaagcggagcaaac
ChIP assay	DEWAX myc F1	5'-ccccgggaaatggagacttttgaggaaagc
	DEWAX myc R1	5'-ccccgggttagtttgatgacgatgatgaag
	FAR6 CHIP-AF1	5'-cttcctgcttcacattcctg
	FAR6 CHIP AR1	5'-cgaggcaactaattcaaatcc
	FAR6 CHIP BF2	5'-gtagggtacacattaaacg
	FAR6 CHIP BR2	5'-gtacacgttggtcgattg
	FAR6 CHIP CF3	5'-ccacgaggccaaaacacag
	FAR6 CHIP CR3	5'-ggaagcttaatttgaagtggtc
	CER1 CHIP AF1	5'-ccctttgttacccttggtgcc
	CER1 CHIP AR1	5'-ggcacgcagatatatatcacttag
	CER1 CHIP BF2	5'-gcatcaatacctaacacatgc
	CER1 CHIP BR2	5'-cggttgatggatggcttc
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	ECR CHIP AR1	5'-gggatggagtaatgagaac
	ECR CHIP BF2	5'-gtaattactaatggtggatcc
	ECR CHIP BR2	5'-cttatgcgattagattgggac
	LCAS2 CHIP AF1	5'-ctttgtagtttaaccgtg

	LACS2 CHIP AR1	5'-cataacgtgattgttggc
	ACLA2 CHIP AF1	5'-gatcactagttcgttacc
	ACLA2 CHIP AR1	5'-gtaacgcatatatacctc
EMSA	DRE cis-element F1	5'-gatactaccgacatgagtt
	GCC cis-element F1	5'-cataaggagccgccact
	DRE cis-element R1	5'-aactcatgtcggtagtatc
	GCC cis-element R1	5'-agtggcggctccttatg