

SUPPORTING INFORMATION

THE FUSED LEAVES1-*ADHERENT1* REGULATORY MODULE IS REQUIRED FOR MAIZE CUTICLE DEVELOPMENT AND ORGAN SEPARATION

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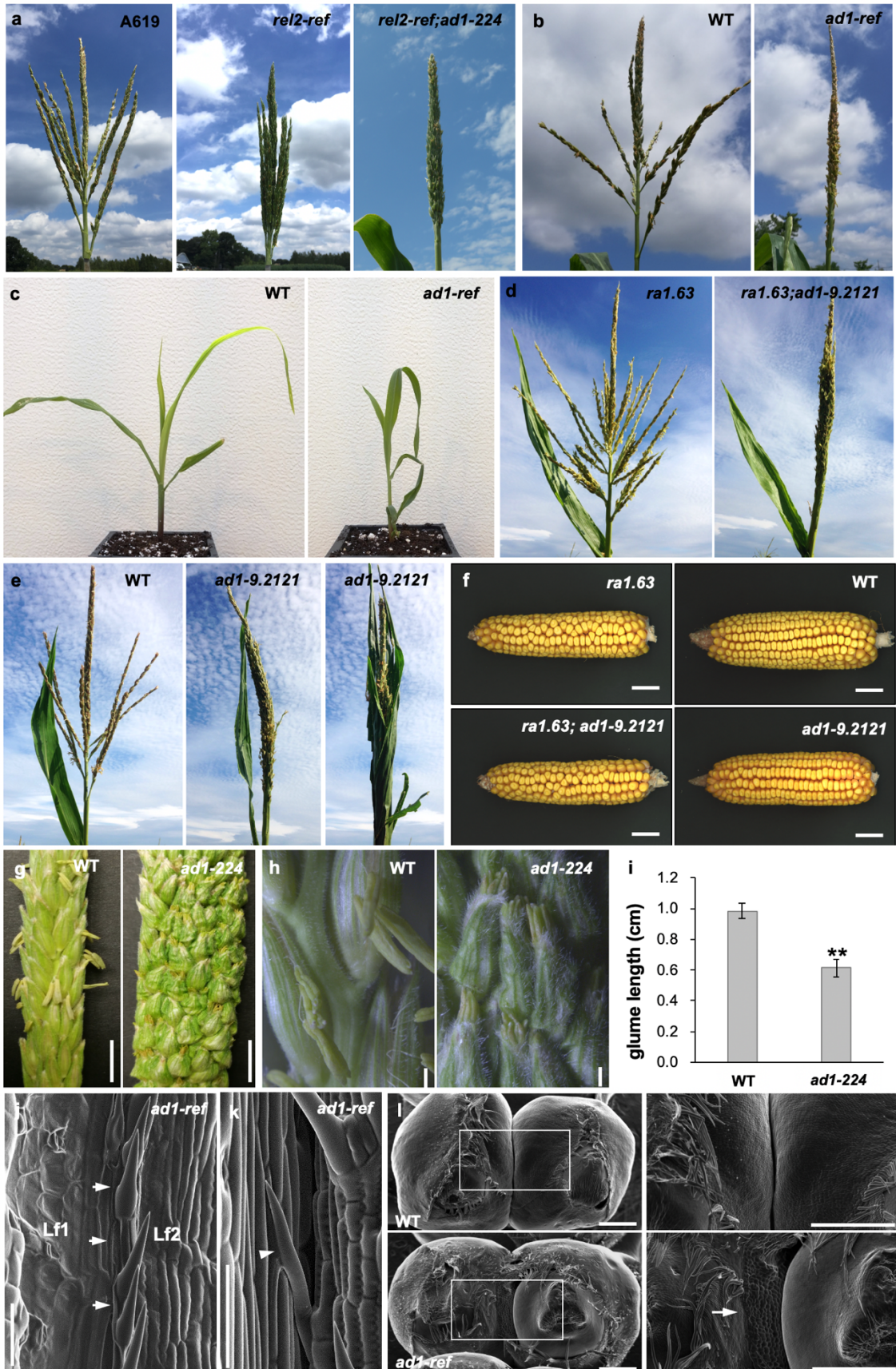


Figure S1. Phenotypic characterization of *ad1* alleles.

(a) The tassel phenotype of A619, *rel2-ref* and *rel2-ref;ad1-224* original M2 family. (b) The tassel phenotype of wild type sibling and *ad1-ref* mutants. (c) The seedling phenotype of wild type sibling and *ad1-ref* mutants. (d) The original *ad1-9.2121* allele in *ra1-63.3359* Mo17 background. (e) The *ad1-9.2121* allele in BC3(B73). (f) Mature ear phenotype of *ad1-9.2121* mutants. No visible defects is observed. (g) Tassel spikelets of wild type and *ad1-224* mutants. Scale bars, 1 cm. (h) Images of glumes in wild type and *ad1-224* mutant tassels. Scale bars, 0.1 cm. (i) Quantification of the glume length of wild type and *ad1-224* mutants ($n \geq 20$). Error bars show SD; $**p < 0.001$. (j) SEM image showing the second leaf (Lf2) fused to the surface of first leaf (Lf1) along the edge in *ad1-224* mutants. (k) SEM showing a macrohair fused to epidermal cells in *ad1-224* mutants. Scale bars in J and K, 100 μ m. (l) SEM images of immature ears in wild type and *ad1-ref*. The glume of adjacent spikelets are fused in *ad1-ref* mutants. Right image is higher magnification of the area framed in left panel. Scale bars, 500 μ m. Arrows point to regions of fusion events.

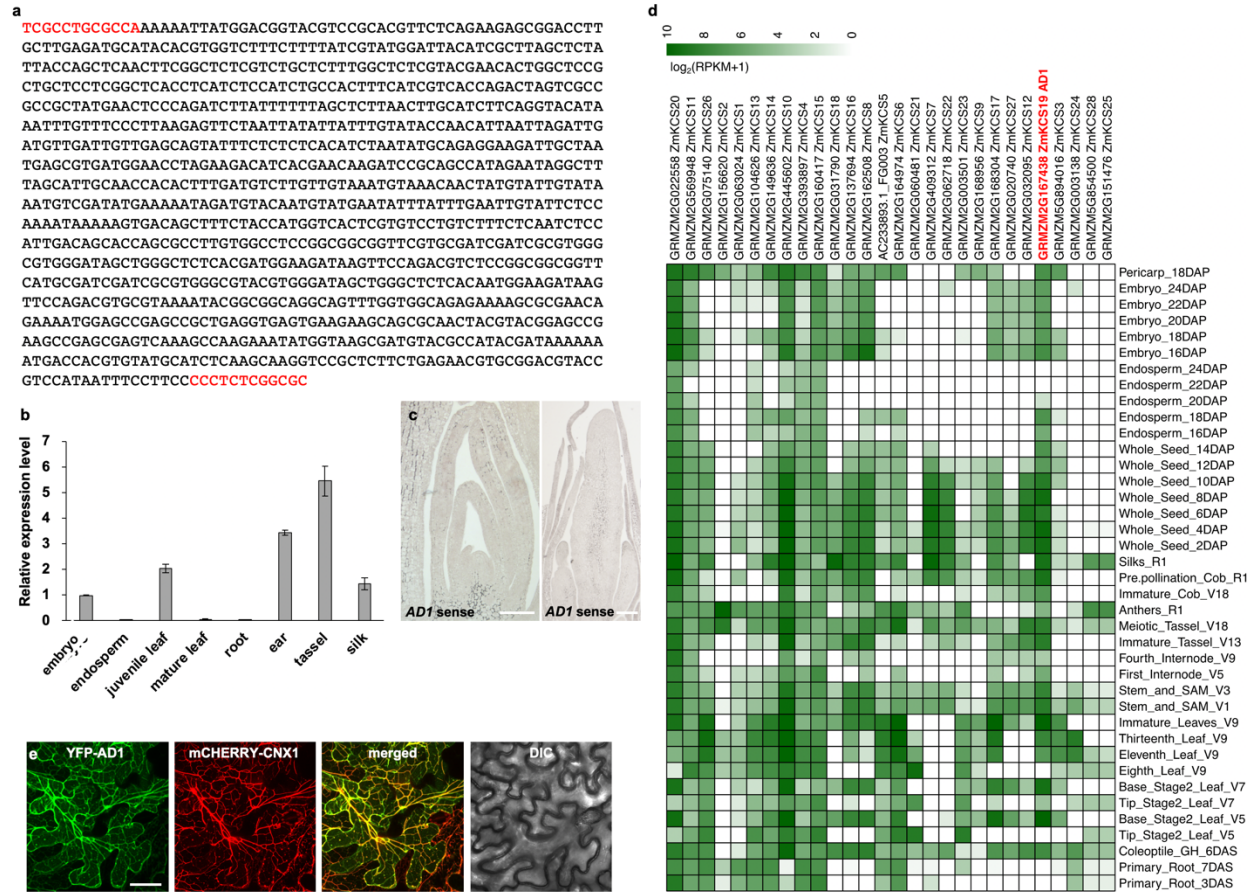


Figure S2. Expression analysis of the *KCS* gene family in maize.

(a) Sequence of the insertion in the *ad1-ref* allele at position +947. In red, partial coding sequence. (b) Quantitative RT-PCR of *AD1* in different maize tissues. The y axis shows the fold change relative to embryo expression levels. Error bars show SD, n=3. (c) *In situ* hybridizations of *AD1* sense control probe in embryo (left panel) and tassel (right panel). Scale bar, 200 μ m. (d) RNA-seq expression levels of *KCS* genes in different tissues, from Stelplflug et al. 2016. (e) Confocal images of YFP-*AD1* shows co-localization with the ER marker mCHERRY-CNX1 in *N. benthamiana* leaf epidermal cells. Scale bars, 50 μ m.

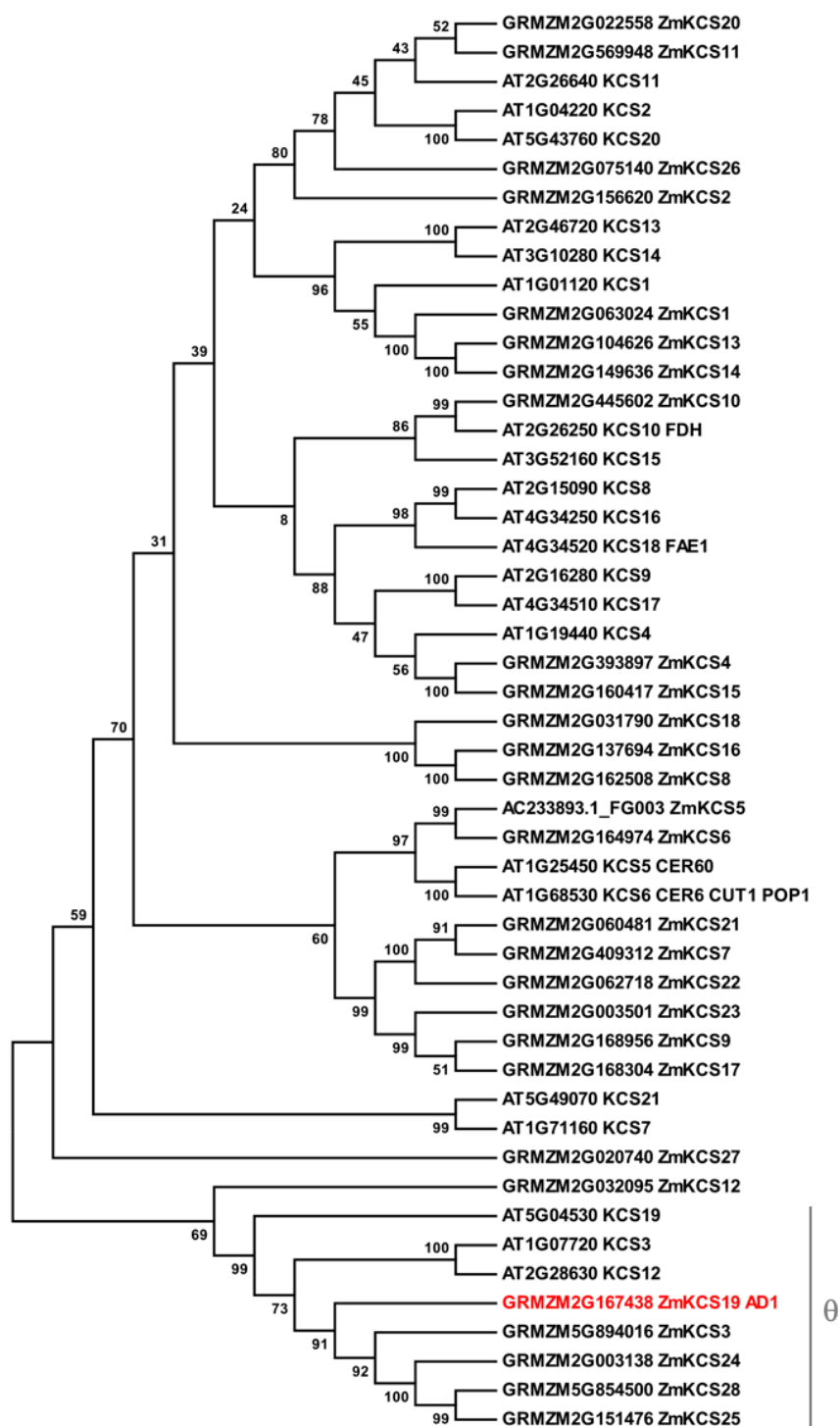


Figure S3. Neighbor-joining phylogenetic tree of maize (GRMZM) and Arabidopsis (AT) KCS family proteins. Numbers represent reliability levels based on 1000 bootstrap replications.

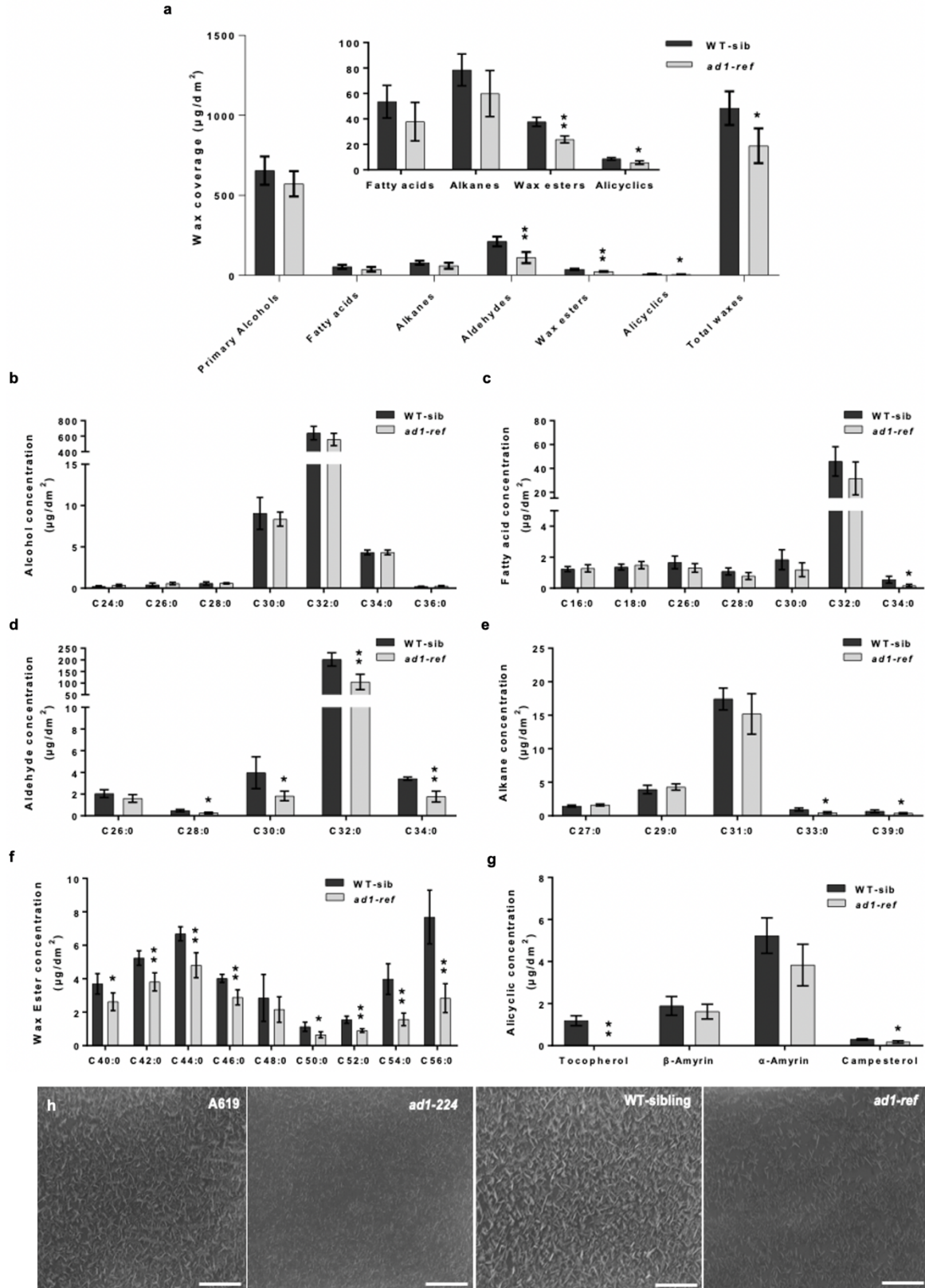


Figure S4. Cuticular wax analysis in *ad1-ref* mutants.

(a) Total wax coverage and amount of each wax class in *ad1-ref* mutants and wild type leaves. The inset shows the less abundant wax classes at a different scale to more clearly visualize significant differences. (b-g) Concentration of individual components in each wax class. primary alcohol (b), fatty acid (c), aldehyde (d), alkane (e), wax ester (f) and alicyclic (g). Means of 4 replicates and SD are reported. * $p < 0.05$; ** $p < 0.01$, Student's test. (h) SEM images of epicuticular wax crystals on the third leaves in A619, *ad1-224*, WT-sibling and *ad1-ref* samples used for the analysis. Scale bars, 5 μ m.

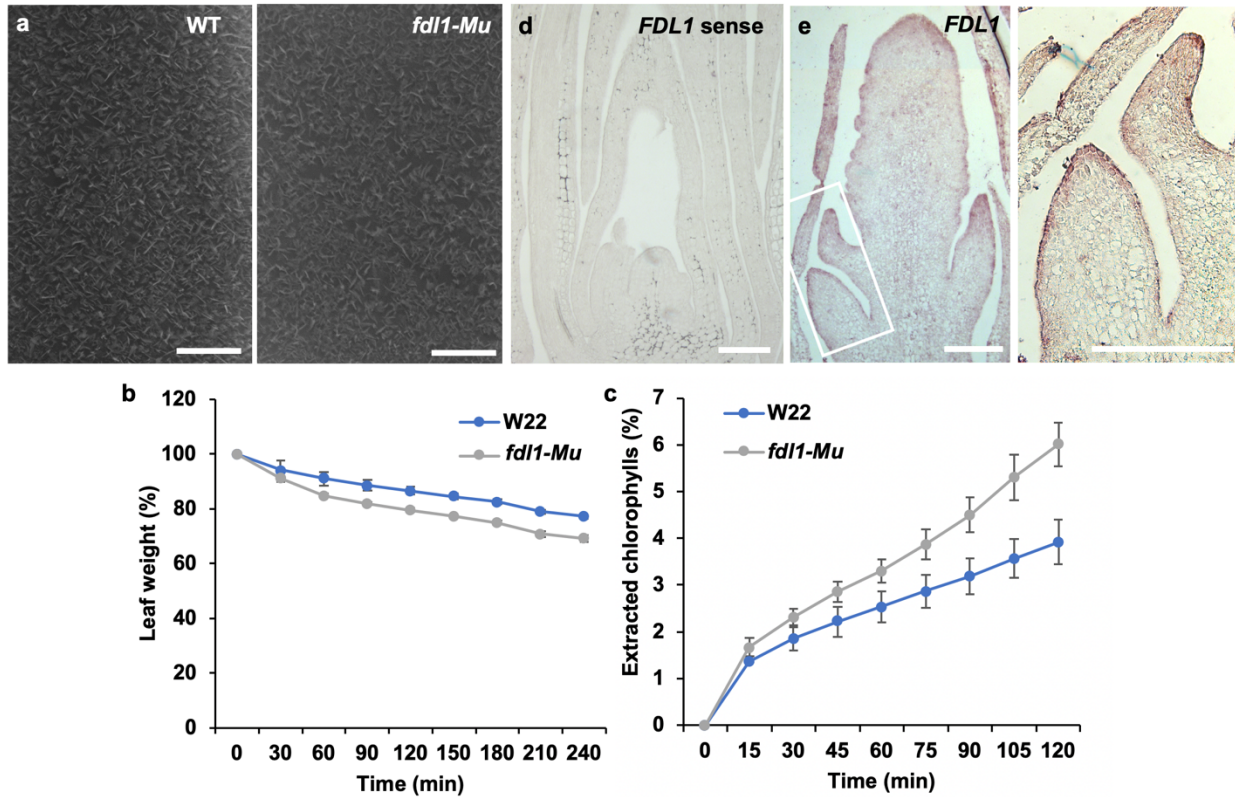


Figure S5. Analysis of *FDL1* function.

(a) SEM images of epicuticular wax crystals on the third leaves in wild type and *fdl1-Mu*. Scale bars, 5 μ m. (b) Water loss assays. Error bars show SD, n=3. (c) Chlorophyll leaching assays. Error bars show SD, n=3. (d,e) mRNA *in situ* hybridizations using *FDL1* sense control (d) and antisense probes (e). Expression pattern in embryo (d) and tassel (e); the right image is a higher magnification of the area framed in the left panel. Scale bars, 200 μ m.

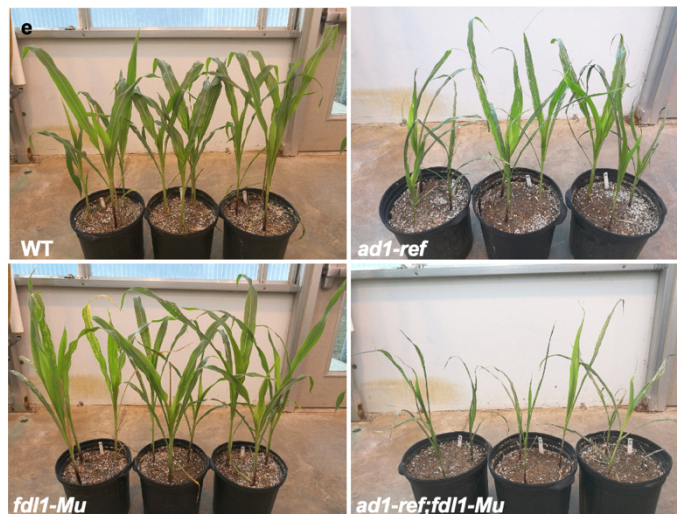
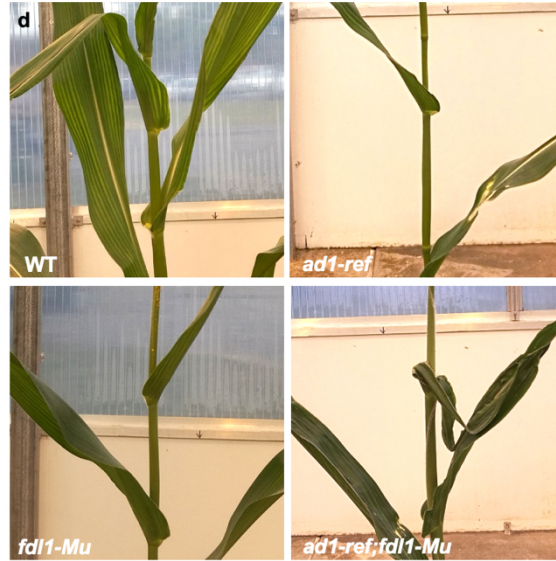
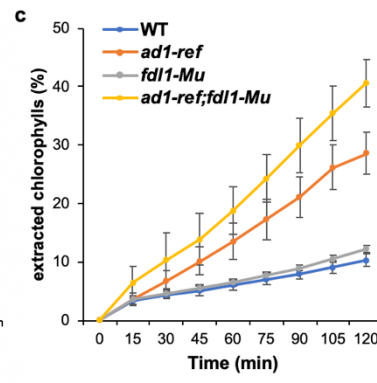
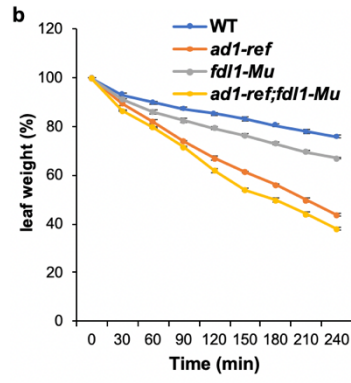
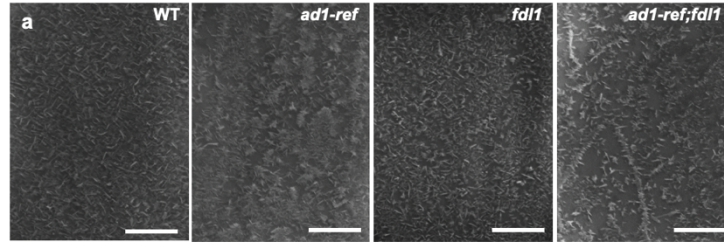


Figure S6. Double *ad1-ref;fdl1-Mu* mutant analysis.

(a) SEM images of epicuticular wax crystals on the third leaves in wild type, *ad1-ref*, *fdl1-Mu* and *ad1-ref;fdl1-Mu*. Scale bars, 5 μ m. (b) Water loss assays of dark-acclimated plants. Error bars show SD, n=3. (c) Chlorophyll leaching assays. Extracted chlorophyll contents at individual time points were expressed as percentages of that at 24 h after initial immersion. Error bars show SD, n=3. (d) Mature leaf fusion defects in single and double *ad1 fdl1* mutant adult plants. (e) Phenotype of single and double mutant plants exposed to drought stress.

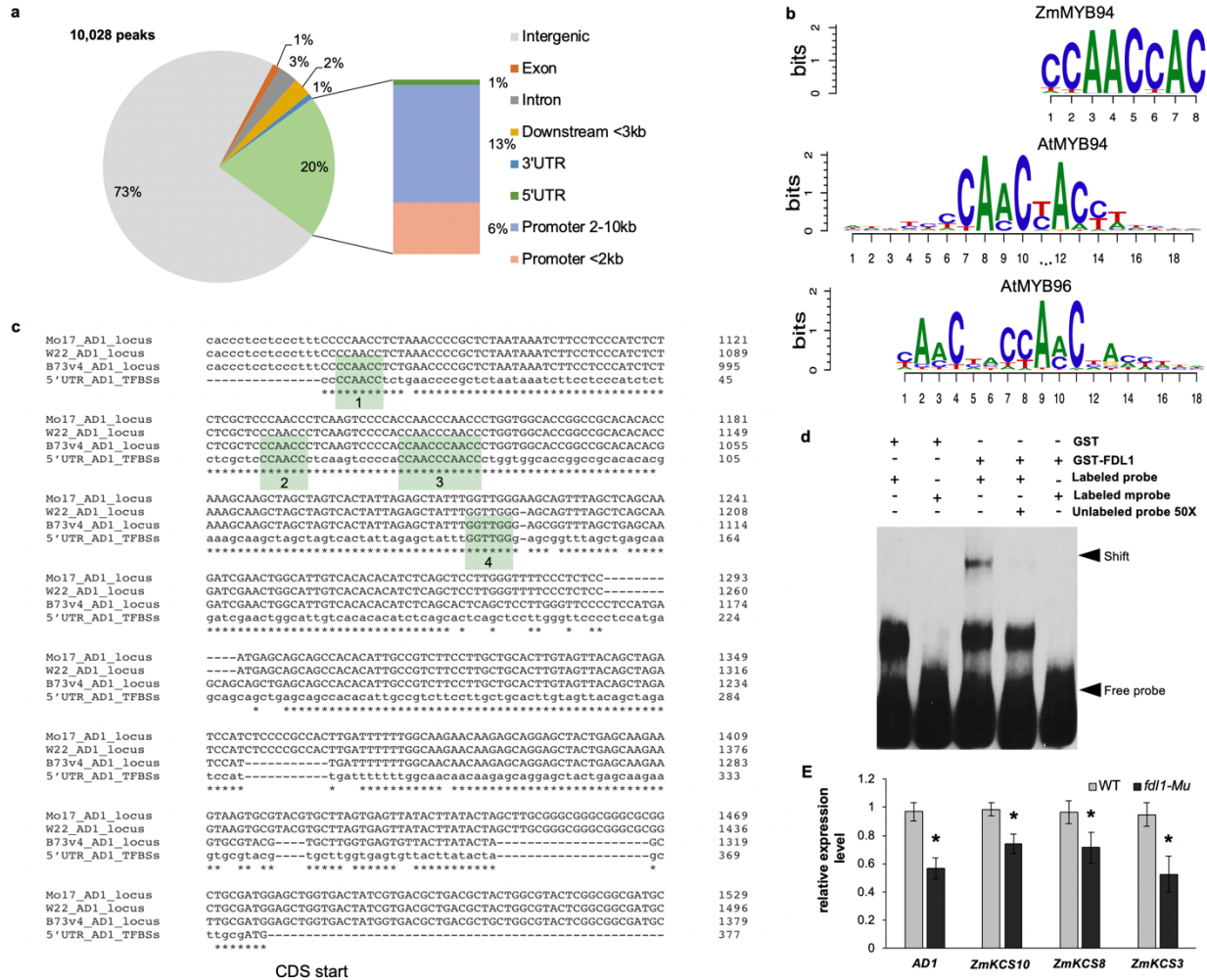
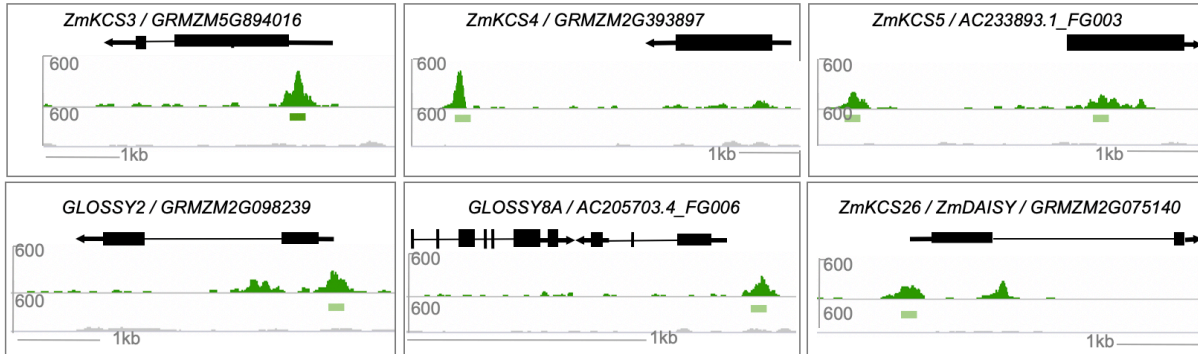


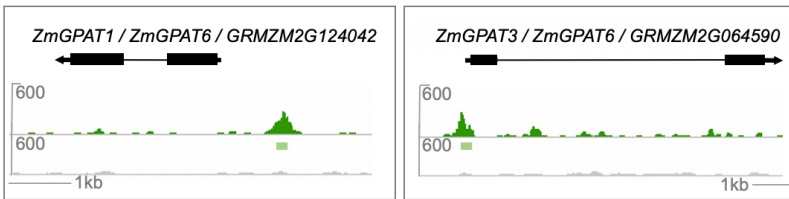
Figure S7. FDL1 DNA binding analysis.

(a) Distribution of FDL1 peaks within gene features. (b) Comparison of DAP-seq identified motifs for ZmMYB94 and AtMYB94 and AtMYB96 (O'Malley et al., 2015). ZmMYB94 and AtMYB94 show highly similar motifs with minor nucleotide variants. (c) Alignment of *AD1* 5'UTR region from maize reference genome inbred line B73 and two additional inbred lines, Mo17 and W22. Region containing CCAACC motifs (highlighted in green; numbered) are conserved across inbreds. (d) EMSA showing GST-FDL1 directly binds to the promoter of *AD1*. Mutation of three of the four CCAACC motifs shown in (c) abolishes the interaction. Competition with unlabeled probe shows specificity of interaction. (e) Comparison of *AD1* and selected KCS genes expression levels between wild type and *fd1-1-Mu*. The maize *UBIQUITIN* gene was used as reference. Values are means \pm SD (n=3). * $p < 0.001$, Student's two-tail t-test.

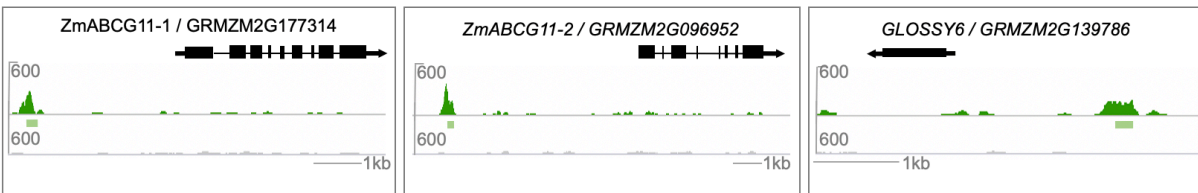
a CUTICULAR WAX BIOSYNTHESIS RELATED GENES



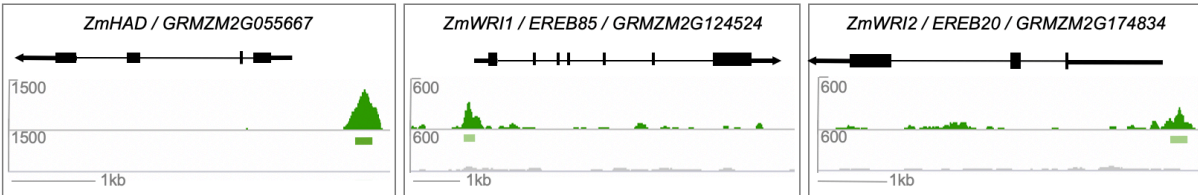
b CUTIN BIOSYNTHESIS RELATED GENES



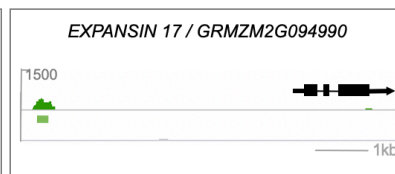
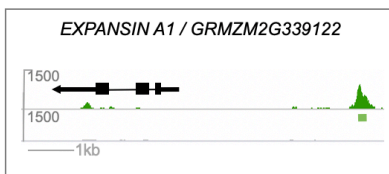
c CUTICLE COMPONENT TRANSPORT



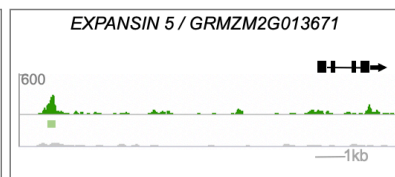
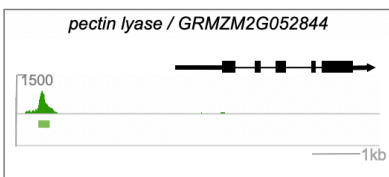
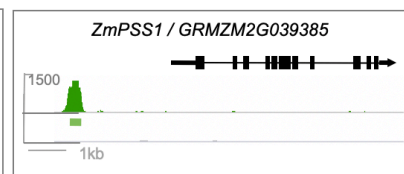
d FATTY ACID RELATED



e CELL WALL MODIFYING ENZYMES



f PHOSPHOLIPID SYNTHESIS



g DROUGHT/DESSICATION TOLERANCE

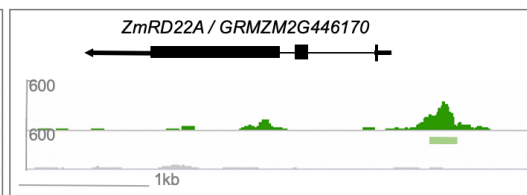
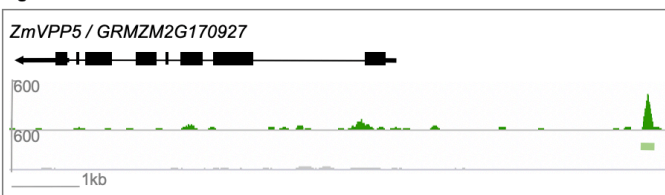


Figure S8. Genome browser screenshots of FDL1 binding.

(a) Genome browser screenshots showing location of FDL1 binding events in several *KCS* and other cuticular wax related biosynthesis genes. Screenshots showing FDL1 binding in cutin biosynthesis related genes (b), cuticular transporters (c), genes related to fatty acid biosynthesis and regulation (d), genes encoding cell wall modifying enzymes (e), a gene regulating phospholipid biosynthesis (f), and genes involved in drought or desiccation tolerance (g). In all panels, 200bp peak regions are shown as green intensity bars under the green FDL1 binding peaks. A negative control sample (affinity tag only) is shown as a gray colored peak track under the green FDL1 peak track.

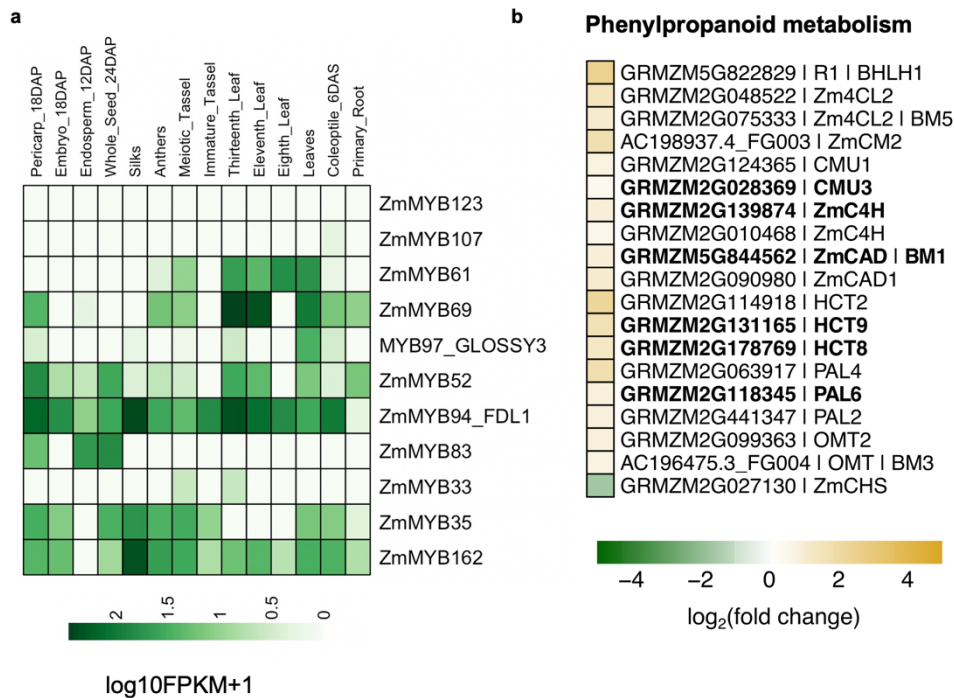


Figure S9. Analysis of *FDL1* expression and binding targets.

(a) *FDL1/ZmMYB94* and closely related *MYB* transcription factors are expressed in many tissues throughout development. *ZmMYB162* is directly bound by *FDL1* and up-regulated in *fdl1-Mu* coleoptiles. *ZmMYB83* and *ZmMYB123* are directly bound by *FDL1* in DAP-seq. Tissue specific RNA-seq data from Stelpflug et al. (b) Differentially expressed genes in *fdl1-Mu* mutant coleoptiles related to phenylpropanoid metabolism. Genes that were directly bound by *FDL1* in DAP-seq are shown in bold.

PRIMERS	SEQUENCE 5'-3'	PURPOSE
<i>ad1-224</i>	For: TGTCAGTCATGGATGCTGGATTACG Rev: CATGAAGGTGCGCTTGTGCCCCGGTA	dCAPS KpnI - genotyping
<i>ad1-ref</i>	For: TGTCAGTCATGGATGCTGGATTACG Rev: CTCAGCTAGCATTGTTGTGCTCACC	genotyping
<i>fdl1-Mu</i>	For: GAGTACACGAGCAATCCGCAA Rev: CGCCAGGTTGATGTCCGTCT	genotyping
<i>UBI-RT</i>	For: GAGTGCCCCAACGCCGAGTG Rev: CTACGCCTGCTGGTTGTAGACGTA	Real-time PCR
<i>AD1-RT</i>	For: TCTCCAACCCATTCATGGACAA Rev: TTGAGACGTTGAGTTGAGCAGCT	Real-time PCR
<i>ZmKCS10-RT</i>	For: ATGGACGCCTAGCACGTACCT Rev: GGTGAAGTGACGACTGCACT	Real-time PCR
<i>ZmKCS8-RT</i>	For: GAGACCACCTACAAGTTCGCTGA Rev: GCTGATGATGAGAAATCAATCATGT	Real-time PCR
<i>ZmKCS3-RT</i>	For: GGAGGACTGCATCGACCAGTA Rev: TCGTTAACCCATCCGAACCTGT	Real-time PCR
<i>AD1-antisense-Probe</i>	For: GAATTCGGCCGTCAAGGCCACCCTAGCTGCTCAACTCAACGT Rev: AGTCGACGGCCCATGAGGCCAGGACGACACTGACTGATTGACT	<i>In situ</i>
<i>FDL1-antisense-Probe</i>	For: GAATTCGGCCGTCAAGGCCAGTGTCCAATCGATCAAGCTCAA Rev: AGTCGACGGCCCATGAGGCCACTTGACACAAGGGGATCTACGTA	<i>In situ</i>
<i>AD1-sense-Probe</i>	For: AGTGAATTCGGTAATACGACTCACTATAGGGCTCCCTAGCTGCTCAACTCAAC Rev: AGTCGACGGCCCATGAGGCCAGGACGACACTGACTGATTGACT	<i>In situ</i>
<i>FDL1-sense-Probe</i>	For: AGTGAATTCGGTAATACGACTCACTATAGGGCTGTGTCCAATCGATCAAGCTC Rev: AGTCGACGGCCCATGAGGCCACTTGACACAAGGGGATCTACGTA	<i>In situ</i>
<i>AD1-EMSA-Probe</i>	For: GACCCATGTCATGATGTGGTAT Rev: CCAAATAGCTCTAATAGTACTA	EMSAs
<i>AD1-Pro-mut1</i>	For: TTCAAGTCCCCATTTTTTTTTTTTGGTGGCACCGGCCGCACAC Rev: AAAAAAATGGGACTTGAAAAAAGCGAGAGAGATGGGAGG	EMSAs
<i>AD1-Pro-mut2</i>	For: CCCTTTTTTTTTTCTGAACCCCGCTCTAATAAATCTTCT Rev: GGTTTCAGAAAAAAAAAAGGGAGGAGGGTGGAGGGTGGTT	EMSAs
<i>pGreenII-AD1Pro</i>	For: TCGAGGTCGACGGTATCGATCCACACAATCGAGCACGACAT Rev: CGGCCGCTCTAGAAGTGTGCGCAAGCTAGTATAAGTAACA	Transient Transcription Assay
<i>pRI101-MYB94</i>	For: AAGTTCTTCACTGTTGATACATGGTGAGGCCGCGTGCTG Rev: AGTTGTTGATTGAGAATTCGCTAGAAGGGGTAATCCATGG	Transient Transcription Assay

Table S1. List of primers used in this study.

ZmName	geneID	peak location	peak distance from TSS	Peak Signal Value	log2FC in fdl1-Mu coleoptiles	Arabidopsis homolog	description
Cuticular wax and fatty acid elongation							
<i>ADHERENT1, KCS19</i>	GRMZM2G167438	Promoter (<=1kb)	0	33	#N/A	<i>KCS12, KCS19</i>	3-ketoacyl-CoA synthase
<i>ZmKCS3</i>	GRMZM5G894016	5' UTR	643	47	#N/A	<i>KCS12, KCS3</i>	3-ketoacyl-CoA synthase
<i>ZmKCS4</i>	GRMZM2G393897	Downstream (2-3kb)	5130	44	#N/A	<i>KCS4</i>	3-ketoacyl-CoA synthase
<i>ZmKCS5</i>	AC233893.1_FG003	Promoter (2-3kb)	-2617	28	#N/A	<i>CER6, CUT1, G2, KCS6, POP1</i>	3-ketoacyl-CoA synthase
		Exon	330	33.2			
<i>ZmKCS26</i>	GRMZM2G075140	Promoter (<=1kb)	0	30	-0.554471	<i>KCS2</i>	3-ketoacyl-CoA synthase
<i>GLOSSY8A</i>	AC205703.4_FG006	Promoter (<=1kb)	-627	40	#N/A	<i>ATKCR1, KCR1, YBR159</i>	beta-ketoacyl reductase
<i>GLOSSY26</i>	GRMZM2G481843	Promoter (<=1kb)	0	35.8	-0.396167	<i>CER10, ECR, TSC13</i>	3-oxo-5-alpha-steroid 4-dehydrogenase family protein
<i>GLOSSY2</i>	GRMZM2G098239	Promoter (<=1kb)	0	34	#N/A	<i>CER2, VC-2, VC2</i>	HXXXD-type acyl-transferase family protein
<i>ZmCER3</i>	GRMZM2G029912	Intron	214	35	-0.524724	<i>CER3, FLP1, WAX2, YRE</i>	Fatty acid hydroxylase superfamily
<i>GLOSSY6</i>	GRMZM2G139786	Promoter (1-2kb)	-1851	39	-0.518466	<i>AT1G09310</i>	Protein of unknown function, DUF538
Cutin							
<i>ZmBDG</i>	GRMZM2G030636	5' UTR	122	44	-1.12516	<i>BDG1, BODYGUARD</i>	alpha/beta-Hydrolases superfamily protein
		Promoter (<=1kb)	0	97			Zm
<i>ZmGPAT3</i>	GRMZM2G064590	Promoter (<=1kb)	0	37	#N/A	<i>ATGPAT5, ATGPAT6</i>	glycerol-3-phosphate acyltransferase
<i>ZmGPAT1</i>	GRMZM2G124042	Promoter (<=1kb)	-805	35	#N/A	<i>GPAT6</i>	glycerol-3-phosphate acyltransferase 6
Wax and cutin transport							
<i>ABCG11-2</i>	GRMZM2G177314	Promoter (2-3kb)	-2639	31	-0.432011	<i>ABCG11, COF1, DSO, WBC11</i>	white-brown complex homolog protein 11
<i>ABCG11-5</i>	GRMZM2G096952	Promoter (6-7kb)	-6563	50	#N/A	<i>ABCG11, COF1, DSO, WBC11</i>	white-brown complex homolog protein 11
Fatty Acid synthesis							
<i>ZmHAD</i>	GRMZM2G055667	Promoter (<=1kb)	-773	176	-0.354572	<i>HAD</i>	Thioesterase superfamily protein
TFs related to fatty acid/cuticle formation							
<i>EREB85, WR11</i>	GRMZM2G124524	Promoter (<=1kb)	0	36	0.626041	<i>AIL5, CHO1, EMK, ASML1, ATWR11</i>	AINTEGUMENTA-like 5, EREB TF
<i>EREB20, WR12</i>	GRMZM2G174834	Promoter (<=1kb)	-115	31	#N/A	<i>AIL5, CHO1, EMK, ASML1, ATWR11</i>	AINTEGUMENTA-like 5, EREB TF
<i>EREB143</i>	GRMZM2G141219	Promoter (<=1kb)	-110	24.6	#N/A	<i>ASML1, ATWR11, ADAP</i>	ARIA-interacting double AP2 domain protein, EREB TF
<i>ZmNFXL2</i>	GRMZM2G139369	Promoter (1-2kb)	-1881	68	#N/A	<i>ATNFXL2</i>	sequence-specific DNA binding transcription factor
Phospholipid Synthesis							
<i>ZmPSS1</i>	GRMZM2G039385	Promoter (2-3kb)	-2572	141.2	#N/A	<i>AT1G71150</i>	phosphatidyl serine synthase family protein
Vesicle trafficking and secretory pathway							
<i>SEC14</i>	GRMZM2G704053*	Promoter (3-4kb)	-3358	31	0.435357	<i>AT1G75170</i>	Sec14p-like phosphatidylinositol transfer family protein
<i>ZmSYP121</i>	GRMZM2G414540	Promoter (<=1kb)	-20	74	NA	<i>PEN1, SYP121, AT3G11820</i>	syntaxin of plants 121
Signaling							
<i>ZmGSO1</i>	GRMZM2G313643	Promoter (<=1kb)	0	32	0.540328	<i>GSO1, SGN3, AT4G20140</i>	LRR receptor-like serine/threonine-protein kinase GSO1
<i>ZmGSO1</i>	GRMZM2G172429	NA	NA	NA	-0.92111	<i>GSO1, SGN3, AT4G20140</i>	LRR receptor-like serine/threonine-protein kinase GSO1

Table S2. List of genes involved in cuticle formation directly bound by FDL1 in DAP-seq.

REFERENCES

O'Malley RC, Huang SSC, Song L, Lewsey MG, Bartlett A, Nery JR, Galli M, Gallavotti A, Ecker JR. 2016. Cistrome and Epicistrome Features Shape the Regulatory DNA Landscape. *Cell* **165**(5): 1280-1292.

Stelpflug SC, Sekhon RS, Vaillancourt B, Hirsch CN, Buell CR, de Leon N, Kaeppler SM. 2016. An Expanded Maize Gene Expression Atlas based on RNA Sequencing and its Use to Explore Root Development. *Plant Genome* **9**(1); doi.org/10.3835/plantgenome2015.04.0025.