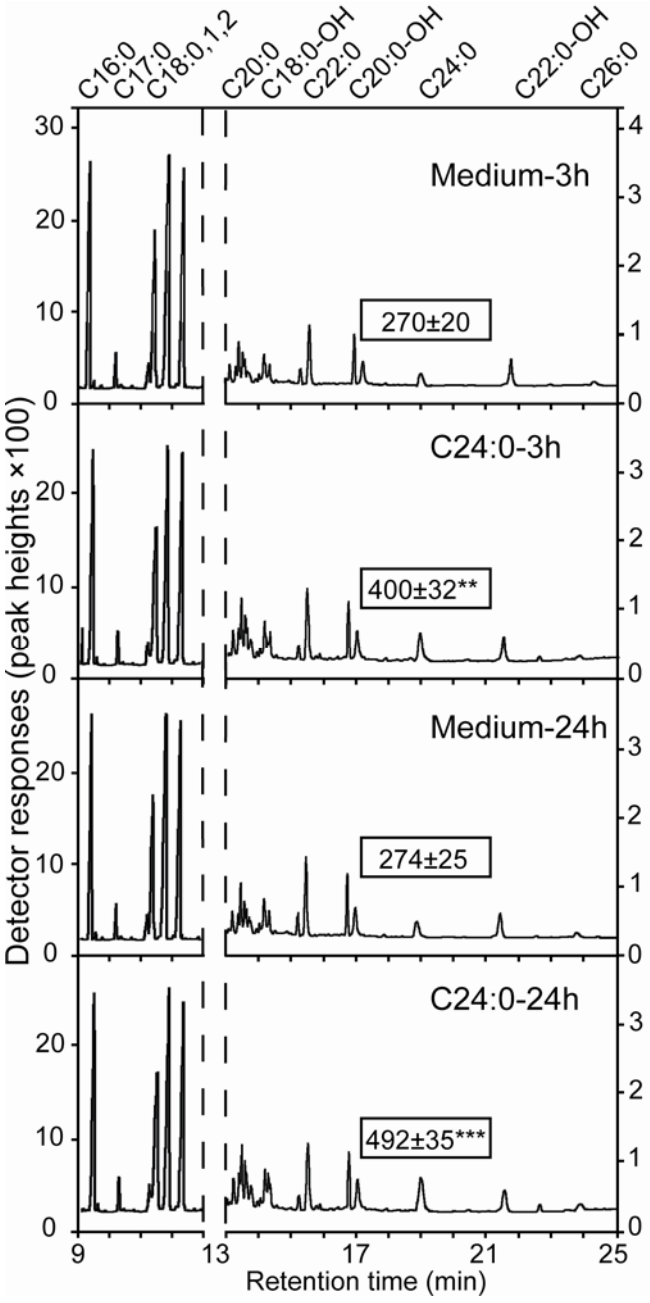
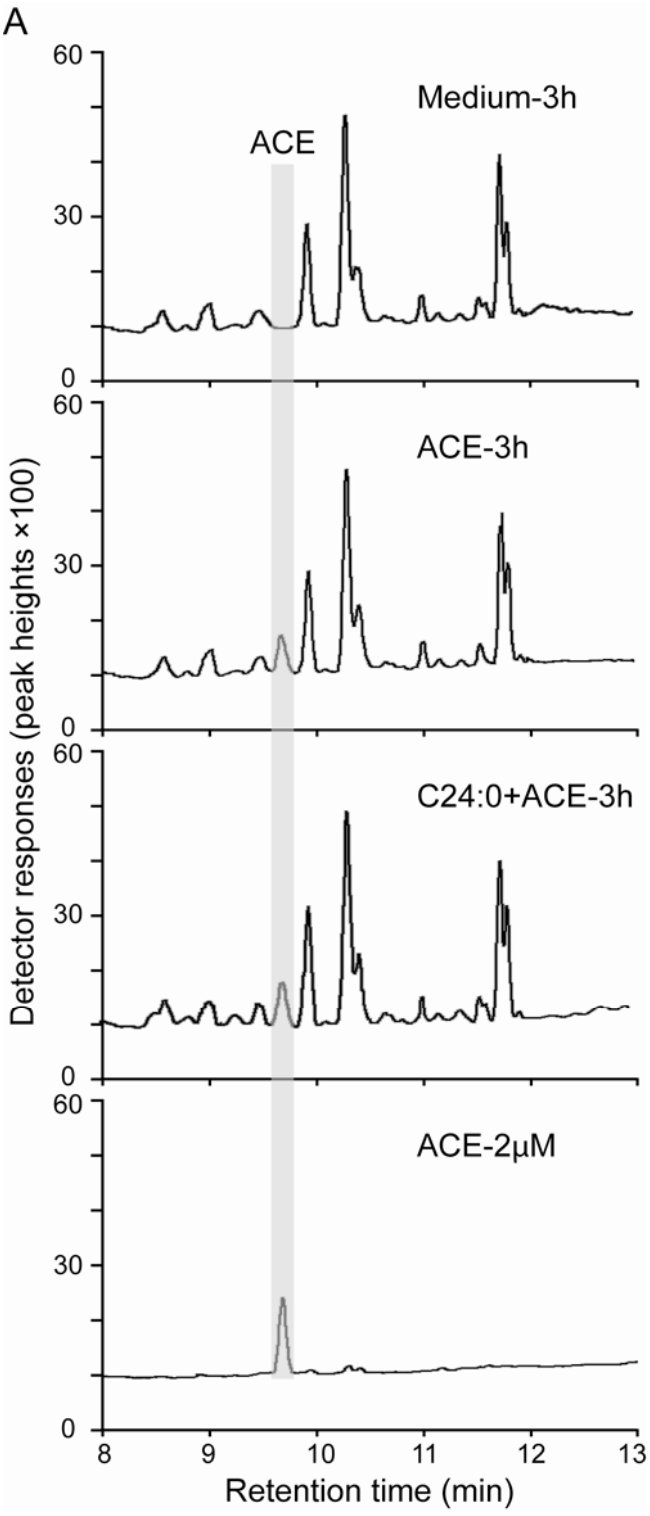
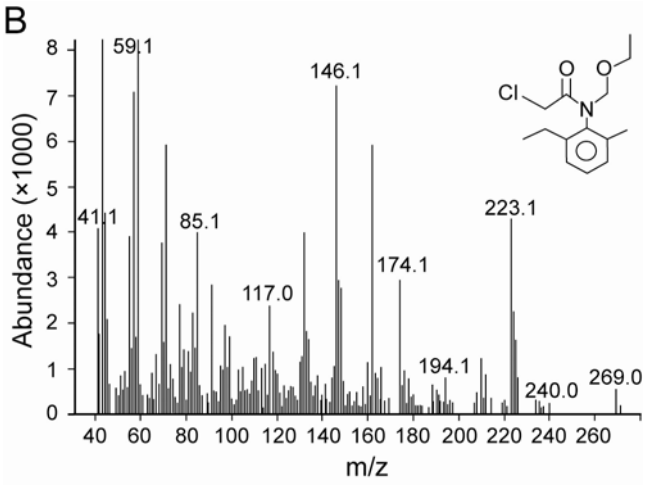
Supplemental Online. Qin et al. 2007. Saturated Very-Long-Chain Fatty Acids Promote Cotton Fiber and Arabidopsis Cell Elongation by Activating Ethylene Biosynthesis.



Supplemental Figure 1. Cotton ovules took up C24:0 from the culture medium. Numbers in small rectangles represented mean ± SE of integrated peak areas obtained from triplicate GC runs using samples of independent ovule cultures. Different response scales are used to show peaks that appeared before 13 min (primarily LCFAs) and those that appeared after 13 min (VLCFAs) from the original GC traces. **, ***, significant at the level of $p = 0.01$ or 0.001, respectively, comparing to ovules that did not receive C24:0 (Medium-3h or Medium-24h) for the same period.

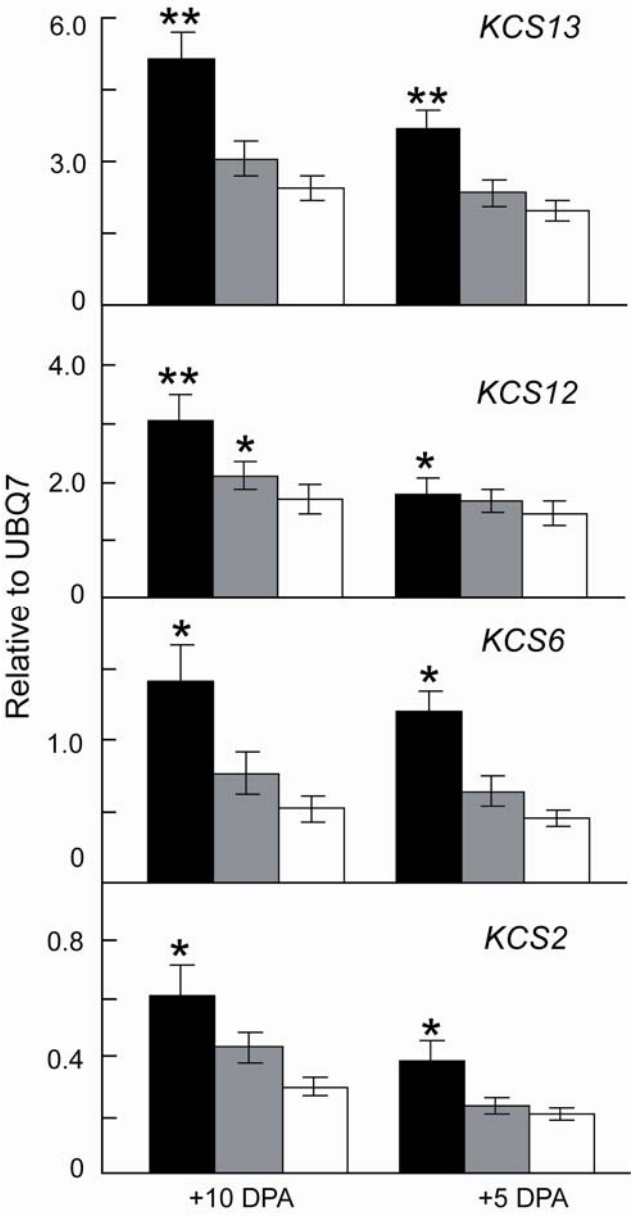

A
Medium-3h
ACE
ACE-3h
C24:0+ACE-3h
ACE-2μM
Detector responses (peak heights ×100)
Retention time (min)
0
30
60
8
9
10
11
12
13



Supplemental Figure 2. ACE was actively transported into cotton ovules in 3 h after it was added to the media and C24:0 treatment did not affect the rate of ACE taken up.

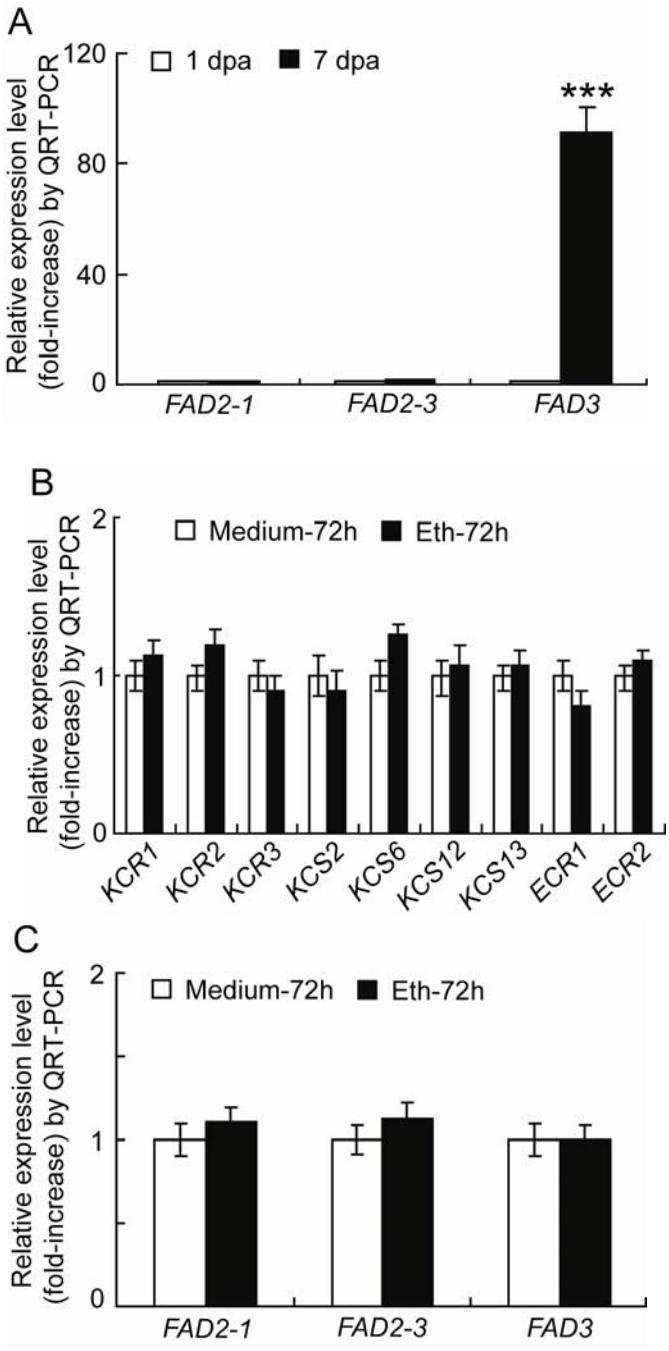
(A) GC analysis of the levels of ACE present in cotton ovules after various treatments. ACE peaks were shaded to facilitate visual identification.

(B) Mass spectrometry identification of ACE present in cotton ovules.



Supplemental Figure 3. Abundance of cotton *KCS* transcripts in long-, intermediate-, and short-fiber cotton germplasms.

Significance values were obtained by comparing to that of the short fiber germplasm. Long-fiber, ■; Intermediate-fiber, ■; Short-fiber, □.



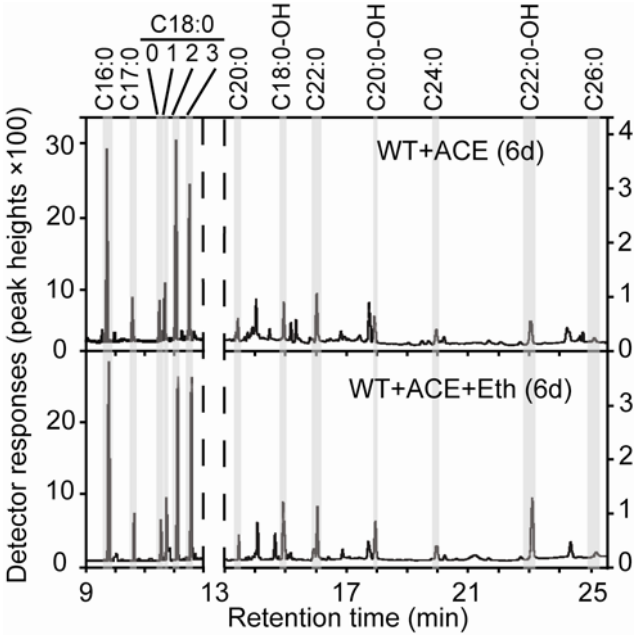
Supplemental Figure 4. QRT-PCR quantification of cotton genes encoding enzymes involved in C18:3 and VLCFA biosynthesis.

(A) *FAD3* (Genbank accession no. AI726347), but not *FAD2*-1 (Genbank accession no. X87016) and *FAD2*-3 (Genbank accession no. AF331163), is developmentally upregulated during fiber elongation.

(B) All known VLCFA biosynthesis genes were not induced by ethylene treatment for as long as 72 h.

(C) Both *FAD2* and *FAD3* are not activated by ethylene treatment for as long as 72 h. All QRT-PCR were performed in triplicate using RNA templates prepared from independent cotton samples. For each respective gene, the transcript level obtained from 1 dpa ovules or from ovules of Medium-72 h were set to 1 arbitrary for

comparisons in (A), (B) or (C). PCR data for *FAD2-2* was not included in the figure because it was not successfully amplified.

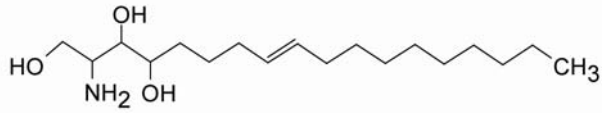


Supplemental Figure 5. Ovules treated with ethylene and ACE simultaneously for 6 d contained similar amounts of VLCFAs when compared with those that received ACE only.

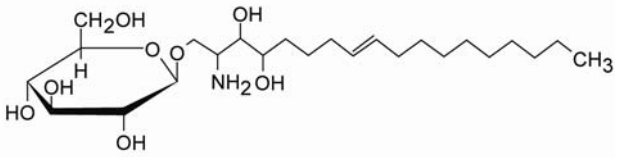
Total fatty acids were extracted from three independent cultures and triplicate samples from each culture were analyzed on GC with only one representative trace for each treatment shown. Because a new DB-225MS column was used for this experiment, the retention times of a few VLCFA peaks were slightly different from those reported in figures 2 and 3.

A

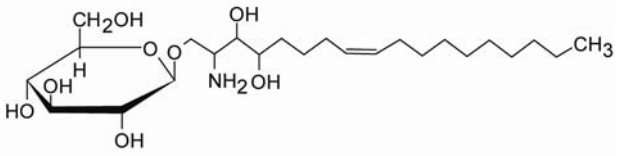
t18:1(8*E*)



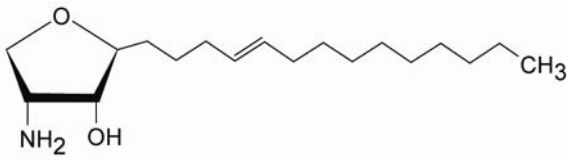
t18:1(8*E*)-glucose



t18:1(8*Z*)-glucose

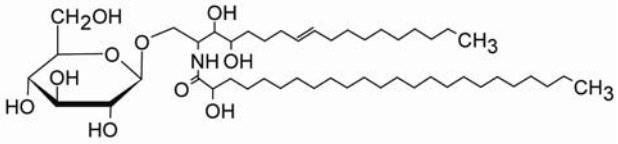


1,4-anhydro- t18:1(8*E*)

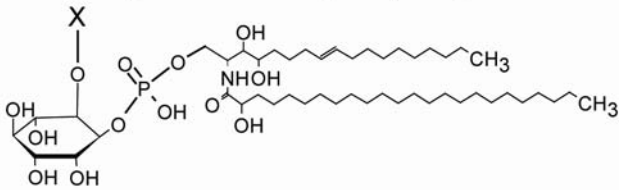


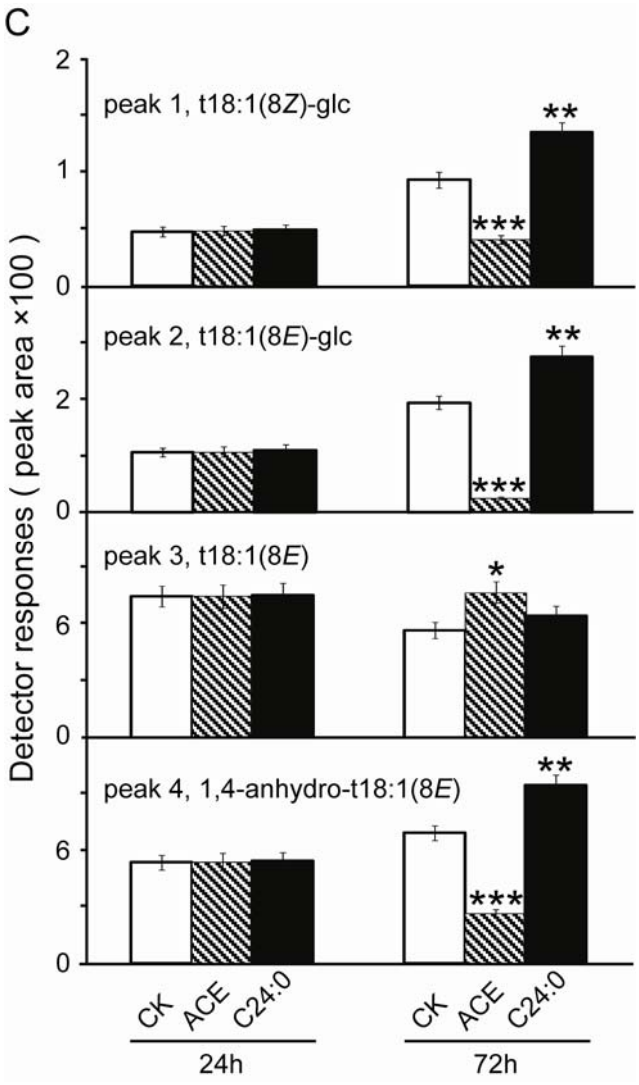
B

Glucosylceramide



Glycosylated inositolphosphorylceramide





Supplemental Figure 6. Schematic drawing and quantification of major LCBs from cotton fiber cells.

(A) Molecular structures of the identified long-chain bases, t18:1(8*Z*)-glc, t18:1(8*E*)-glc, t18:1(8*E*), and 1,4-anhydro- t18:1(8*E*).

(B) Molecular structures of two main classes of sphingolipid, glucosylceramide and glycosylated inositolphosphorylceramide. Each sphingolipid molecule has three components, the long-chain base (t18:1(8*E*) is shown), the fatty acid moiety (hydroxylated C24:0 is shown) and a head-group. "X" on the lower molecule represents various oligosaccharides.

(C) Quantification of t18:1(8*Z*)-glc, t18:1(8*E*)-glc, t18:1(8*E*), and 1,4-anhydro-t18:1(8*E*) (corresponding to peaks 1-4 in Fig. 5D, respectively), prepared from 1 dpa wild-type ovules cultured in the medium (CK), in presence of 2 μM ACE or 5 μM C24:0 for 24h and 72h. Data was obtained from LC/MS analysis using triplicate samples extracted from independent ovule cultures. Statistical significances were determined using one-way ANOVA software combined with Tukey's test. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

Qin et al., Suppl. Table 1. Primers used for QRT-PCR analyses.

Genes	Sequences	Annealing temperature (℃)	Fragment length (bp)
*ACS6**	5'-TCAAGTCGGTATCGGTTCG	55.4	335
	5'-CCCAACTAACTCCATACCC		
ACS7	5’-GAGGATGTTCTGGCTGCTGCTA	58.0	218
	5’-CCAACAGTAGAAACCCCCACTG		
ACO1	5’-CGCCACTTGCCTGAATCTAAC	56.0	257
	5’-TGTGAGCCCTGAGTCCCTTG		
ACO2	5’-TGAGGAGAGAGGAGCCACC	54.1	485
	5’-CCCTTAGCCCCTTGATTAGC		
ACO3	5’-CACCAATGGCAAGTACAAAAGT	57.6	375
	5’-GCAAACAACACACATCTACGA		
ACO4	5’-GCTGCTGGACTTGTTCTGTGAG	58.1	334
	5’-GGCAATCACCCTATGCTCCAC		
KCS2	5'-GAAGCCAGGAAGGAGGCCGAGACA	63.1	413
	5'-TATTTGGAGCGGTGGCGATCAGATG		
KCS6	5'-AAGGGAGCTTCTCAAGGACCAATC	59.0	315
	5'-TCCTAAATCCCTTACAACCTCCCAC		
KCS12	5'-CGTTCTCTCCCAACTATCTCGCCCTT	64.0	378
	5'-GTGATCAGCACCATGGGTACGGAGG		
KCS13	5’-GTCGTTCCGAGAGGTGGCGTG	62.4	493
	5’-ACCCTGTCCCCTTTTTTCATCCTCC		
KCR1	5’-CACTTTGGGTTCTTTATCACTCTT	58.0	315
	5’-TTTATCTTCTTCACGCCTTCAT		
KCR2	5’- TATCCTTTCTCACCCGCTCC	60.0	401
	5’- CACCACTTCCTC CTCCACCTC		
KCR3	5’-GTTTGCGACCAACCATTTAG	56.0	300

	5’-ATTGCTCCAGGATGAAGTGA		
ECR1	5’-TGGGAGCGGAGGCTATCAAA	60.0	293
	5’-GTCCATCGAGTAGGAAGTTTTACCC		
ECR2	5’-GGTTCGGGTTTGGTATAGTTTG	58.0	362
	5’-AAGTACGATGCTCATCGATCC		
FAD2-1	5’-TGTTTCATCAACCTGGCGTTAAACTG-3’	62.0	368
	5’-GACCCCACTCGTGTGCGATGAC-3’		
FAD2-2	5’-CAGCGAGGAAGCCACGAAGATAATAG-3’	63.0	306
	5’-AGAGAGCCTGAGGAAGGTTAGGGAAG-3’		
FAD2-3	5’-CCACACTGTTTCCAACGCTCACTTATC-3’	63.0	277
	5’-CGGGACAAGAAGGGATGAATGGAG -3’		
FAD3	5’-TACCTTCACCACCACGGATACGAG-3’	62.0	327
	5’-TACTATCTCTCCGCTGTTGCTCACG-3’		
PSS	5’-GCTCCTTCTACGGTCCTTACTCG	58.1	193
	5’- ATGCGACAATCAGCACCATAGGA		
PSD1	5’- CCCTGTCTGTGGCGAGGTAGTTG	59.2	334
	5’- CCTTTATCCATAAGGCCAAGTCC		
PSD2	5’-TTTGGAAAGGTGGCATTTGTTG	58.0	233
	5’- AACTCCCAGCGTCATTCCAACA		
PECT1	5’- AAGGGACCTGGACCAAATTCTCG	59.5	339
	5’-GTTTCACCCAGGCAACAAAGAAT		
PECT2	5’-CTCCCTGATGGAAGTGATGCTTA	59.1	260
	5’-AGGTCCCTTGCCATTTGAGAATT		
PECT3	5’-ATGGTCGCATTGGCTACATTGGT	58.3	230
	5’- AAGGTCCACCGATGATAACTTCA		
LCB1	5’-ACAGAACAAGCGGGCTAAGAAA	59.2	164
	5’- CCCGCAGTATTCAGTGAGACCC		
LCB2	5’- GCTCCAGATGGTTGGTTTGATGT	61.0	300
	5’- TTGTCACATAGCCCATGCCGAAG		

PEAMT	5’- GCATTCTCCGTTATGAGCGTGTC	58.3	229
	5’- AGTAGCTCGTTCAAGAGCAAAGG		
PGS	5’-GTCCGCTGTTAGGGAATGGGC	58.0	288
	5’-CGCAGCCATTGCAATCCAGCAGG		
PIS	5’-GTGGATGGTTGGTGTGCTCGCA	58.3	228
	5’-GTCTCTCACATCTTTATGACTAGCC		
UBQ7	5’-GAAGGCATTCCACCTGACCAAC	57.2	198
	5'-CTTGACCTTCTTCTTCTTGTGCTTG		
At*ACO1*	5'-TTGAGTGAAGGCAAAACCTCAGATG	59.2	312
	5'-ATCCGTATGTTCTCTCAGCCCTCTC		
At*ACO2*	5'-AAGGACCATTACAAGACATGCCAAG	58.6	428
	5'-TGGAGACCACTGACCTTGTCGTC		
At*ACO3*	5'-CATACGGTAAGTCCATCGGGAAAAG	58.3	453
	5'-CCCAAGACCGACGACAAAAGAAC		
At*ACO4*	5'-GTCTCTAATATCTCCGATGTCCCTG	58.6	324
	5'-GACCCACTCGCCGTCTTTAAG		
At*ACO5*	5’-AACCTGATCTAACCATCGGCACA	56.2	412
	5’-CTTGTAATGCGACAGATACGATG		
At*ACO6*	5’-CGGCAGAGTATGTGCTTAGGGTC	60.3	236
	5’-CGTTCTTTAACCTCCCGTTGGTC		
At*UBQ5*	5’-GGTGCTAAGAAGAGGAAGAAT	58.0	237
	5’-CTCCTTCTTTCTGGTAAACGT		

* All genes without prefixes in this table are cotton genes. *ACS*, 1-aminocyclopropane-1-carboxylate synthase; *ACO*, 1-aminocyclopropane-1-carboxylate oxidase; *KCS*, 3-ketoacyl-CoA synthase; *KCR*，3-ketoacyl-CoA reductase; *ECR*, *trans*-2-enoyl-CoA reductase; *FAD2*, Δ^{12}-desaturase; *FAD3*, Δ^{15}-desaturase; *PSS*, phosphatidyl serine synthase; *PSD*, phosphatidylserine decarboxylase; *PECT*, phosphorylethanolamine cytidyltransferase; *LCB*, serine palmitoyltransferase; *PEAMT*, phosphor-ethanolamine N-methyltransferase; *PGS*, phosphatidylglycerol phosphate synthase; *PIS*, phosphatidyl-inositol synthase; *UBQ*, ubiquitin extension protein. At *ACO1*, At2g19590; At *ACO2*, At1g63280; At *ACO3*, At 3g47920; At *ACO4*, At 1g05010; At *ACO*5, At 1g04350; At *ACO6*, At 5g63600.

Qin et al., Suppl. Table 2. Quantitative analysis of fatty acid composition from wild-type yeast and *elo2Δelo3Δ* double-deletion mutant cells genetically complemented with different cotton *KCS*s as reported in Figure 2.

Yeast strain	LCFA(μg/g fresh weight)				
	C16:0	C16:1	C18:0	C18:1	C18:0-OH
W1536 5B	360.0±31.2	855.3±75.3	227.2±20.0	824.9±70.8	8.4±0.7
elo2Δelo3Δ+KCS12	305.8±26.0	444.3±40.3	234.1±20.0	632.0±57.1	8.1±0.7
elo2Δelo3Δ+KCS6	483.2±41.0	770.9±71.0	268.7±20.9	693.7±60.9	8.7±0.7
elo2Δelo3Δ+KCS13	340.6±30.0	972.2±90.9	221.6±18.9	528.5±49.0	7.2±0.6
elo2Δelo3Δ+KCS2	250.9±20.0	681.4±61.2	248.3±20.9	787.8±75.2	7.7±0.6

Yeast strain	VLCFA(μg/g fresh weight)					
	C20:0 & C20:1	C22:0 & C22:1	C24:0 & C24:1	C26:0	C20:0-OH	C22:0-OH
W1536 5B	8.9±0.9	8.1±0.6	5.7±0.5	33.1±2.9	3.2±0.3	1.4±0.1
elo2Δelo3Δ+KCS12	11.7±0.9*	6.6±0.5	15.3±1.3**	43.1±3.6**	3.9±0.3	1.2±0.1
elo2Δelo3Δ+KCS6	10.9±0.8*	11.9±1.1*	11.7±0.9*	23.3±2.0	7.1±0.6**	3.3±0.2*
elo2Δelo3Δ+KCS13	18.1±1.5**	125.5±10.0***	10.4±0.9*	2.7±0.2	3.2±0.3	12.7±1.0***
elo2Δelo3Δ+KCS13	23.6±1.8***	5.8±0.5	1.7±0.1	0	3.9±0.3	0

*, **, ***, Higher than that of the W1536 5B cells at the level of $p = 0.05$, 0.01 or 0.001, respectively.

Qin et al., Suppl. Table 3. Quantitative analysis of fatty acid composition from wild-type cotton fiber cells, wild-type or *fl* mutant ovule cells and from ACE-treated wild-type cotton ovules as reported in Figure 3.

Cotton tissue	LCFA(μg/g fresh weight)					
	C16:0	C18:0	C18:1	C18: 2	C18:3	C18:0-OH
FL-1dpa-ovule	443.1±40.1	43.6±4.0	160.3±12.1	645.7±50.0	339.8±30.0	7.3±0.7
FL-7dpa-ovule	424.9±38.0	43.9±3.9	156.2±10.5	639.8±50.1	327.8±28.8	7.5±0.7
ACE-1d	453.9±41.0	44.2±4.0	159.2±11.0	643.1±51.0	330.7±30.0	7.3±0.7
ACE-6d	465.5±40.6	43.8±4.0	159.1±11.6	624.1±49.9	338.3±30.0	7.4±0.7
WT-1dpa-ovule	454.6±40.0	44.2±4.1	292.2±25.1**	633.4±49.9	449.7±39.9*	7.5±0.7
WT-7dpa-ovule	447.1±40.8	43.8±3.9	158.1±11.2	610.4±48.8	431.4±39.2*	10.8±0.9*
WT-7dpa-fiber	400.9±38.0	43.5±4.0	102.9±9.7*	214.1±19.0***	600.3±56.3**	22.2±2.0***
Cotton Tissue	VCLFA(μg/g fresh weight)					
	C20:0	C22:0	C24:0	C26:0	C20:0-OH	C22:0-OH
FL-1dpa-ovule	6.3±0.6	12.8±1.0	6.6±0.6	2.9±0.2	10.9±1.0	12.4±1.1
FL-7dpa-ovule	6.4±0.6	12.9±1.0	6.5±0.6	2.9±0.3	11.1±0.8	12.3±1.0
ACE-1d	6.2±0.6	12.7±0.9	6.3±0.6	2.8±0.2	11.1±1.0	12.3±1.0
ACE-6d	6.2±0.5	12.6±0.9	6.5±0.6	2.9±0.2	10.9±0.9	12.2±1.0
WT-1dpa-ovule	7.3±0.7	20.5±1.8**	10.1±0.8*	3.4±0.3*	13.7±1.0	14.1±1.3
WT-7dpa-ovule	10.1±0.9*	21.6±1.8**	10.6±0.9*	3.8±0.3*	14.8±1.3*	19.2±1.6**
WT-7dpa-fiber	30.1±2.5***	69.8±5.8***	27.3±2.2***	9.4±8.0***	49.2±4.0***	37.4±3.1***

*, **, ***, Higher than that of the FL-1dpa-ovule at the level of $p = 0.05$, 0.01 or 0.001, respectively.