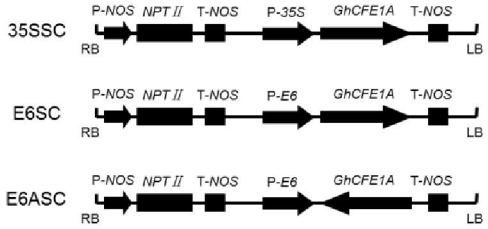
GhCFE1A, a dynamic linker between ER network and actin cytoskeleton, plays an important role in cotton fiber cell initiation and elongation

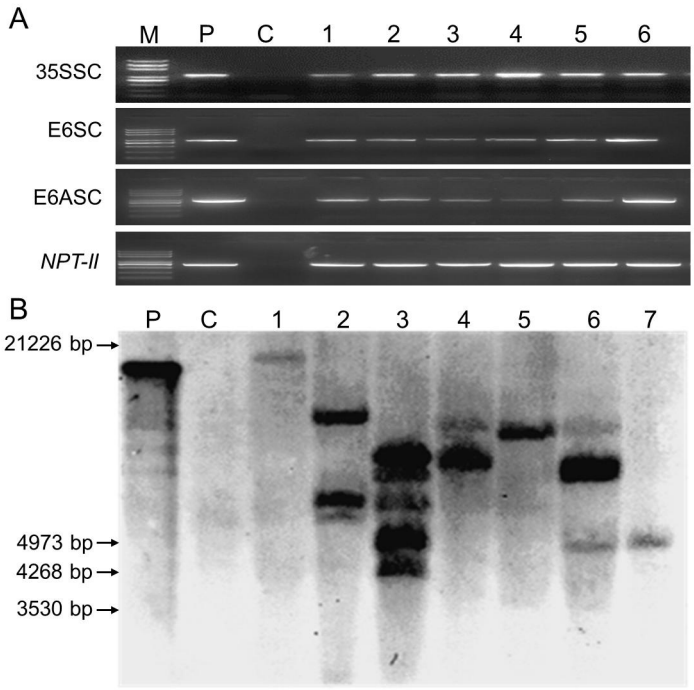
Fenni Lv[1], Haihai Wang[1], Xinyu Wang[2], Libo Han[3], Yinping Ma[3], Sen Wang[1], Zhidi Feng[3], Xiaowei Niu[1], Caiping Cai[1], Zhaosheng Kong[3], Tianzhen Zhang[1], Wangzhen Guo[1, *]

Supplementary data



Supplementary Fig. S1. Constructions of sense and antisense expression vectors of *GhCFE1A*.

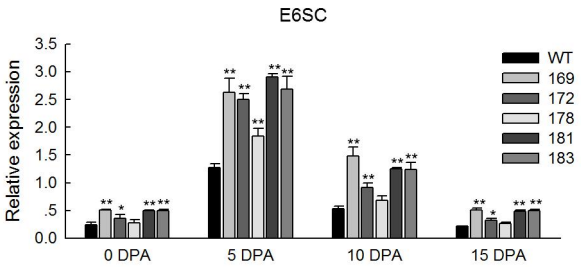
35SSC, *35S*-sense-*GhCFE1A* expression vector; E6SC, *E6*-sense-*GhCFE1A* expression vector; E6ASC, *E6*-antisense-*GhCFE1A* expression vector; RB, right border repeat; P-*NOS*, nopaline synthase gene promoter; *NPTII*, neomycin phosphortransferase II gene; P-*35S*, the cauliflower mosaic virus (CaMV) 35S promoter; P-*E6*, *GhE6* promoter; *GhCFE1A*: cotton fiber expressed protein gene; LB: left border repeat.



Supplementary Fig. S2. Molecular confirmation of the independent transgenic lines.

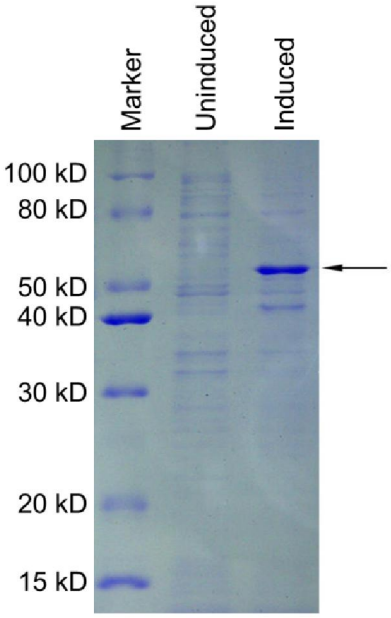
(A) PCR analysis of transgenic plants to determine the *NPTII*, *35S*-sense-*GhCFE1A* (35SSC), *E6*-sense-*GhCFE1A* (E6SC) and *E6*-antisense-*GhCFE1A* (E6ASC). M, molecular weight marker; P, positive control; C, non-transformed plant; 1-6, transgenic plants.

(B) Southern bolt analysis of independent T3 transgenic lines. Genomic DNA was digested with *Eco*R I and hybridized with a 0.75-kb fragment of the *NPTII*.



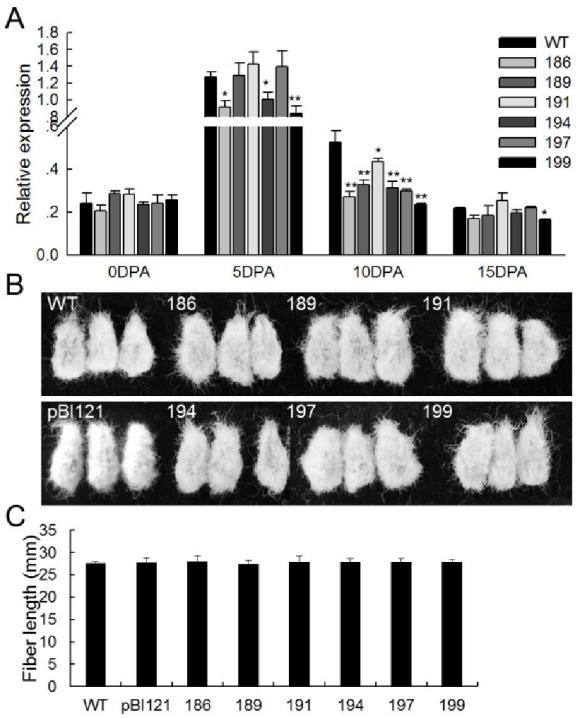
Supplementary Fig. S3. *GhCFE1* expression in ovules and developing fibers of E6SC transgenic cotton.

Quantitative RT-PCR (qRT-PCR) measurements of *GhCFE1* transcripts in 0 DPA ovules and 5 - 15 DPA fibers of the wild-type (WT) and E6SC transgenic (lines 169, 172, 178, 181, and 183) plants. Error bars represent standard deviation of triplicate experiments, and *His3* was used as an internal control (*P < 0.05, **P < 0.01, by Student's *t*-test).



Supplementary Fig. S4. Sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) analysis of GhCFE1A recombination protein in *Escherichia coli*.

The strain of *E. coli* carried a plasmid of pET-30a (+)-*GhCFE1A*, with the *GhCFE1A* gene placed under the control of the isopropylthio-*β*-galactoside (IPTG) -inducible promoter. Induced by IPTG for 4 hours, a new protein band indicated by the black arrow, GhCFE1A recombination protein, appeared on the SDS–PAGE gel.

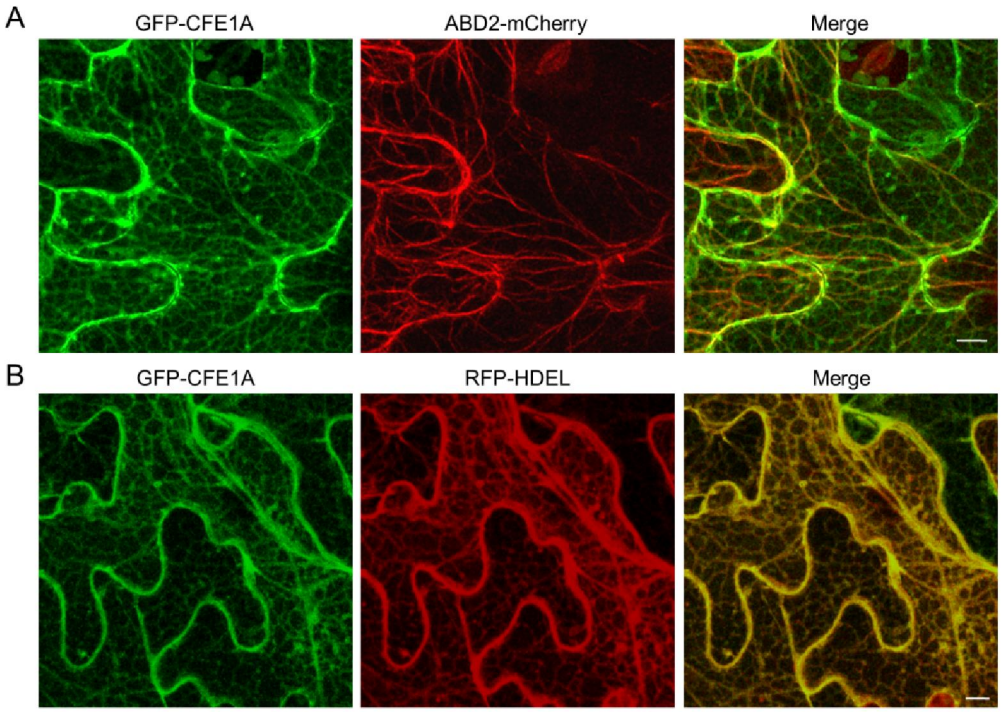


Supplementary Fig. S5. Inhibition of *GhCFE1* in cotton exhibits no obvious phenotype changes.

(A) qRT-PCR analysis of *GhCFE1* transcripts in 0 DPA ovules, 5 – 15 DPA fibers of wild-type (WT) and *GhCFE1*-knockdown transgenic cotton lines (186, 189, 191, 194, 197, and 199). Error bars represent standard deviation of triplicate experiments, and *His3* was used as an internal control (*P < 0.05, **P < 0.01, by Student's *t*-test).

(B) Unaltered phenotype of fuzz fiber initials in *GhCFE1*-knockdown T3 transgenic cotton lines (186, 189, 191, 194, 197, and 199), comparing with the wild-type and null control (pBI121) plants. The number of the fiber initials counted based on the SEM images existed no difference either and listed in Supplementary table S2.

(C) Average fiber length from wild-type, pBI121 control and *GhCFE1*-knockdown transgenic plants (lines 186, 189, 191, 194, 197, and 199). Error bars indicate standard deviation of triplicate experiments.



Supplementary Fig. S6. GhCFE1A locates on cortical endoplasmic reticulum (ER) and colocalizes with actin bundles.

(A) GFP-CFE1A co-expressed with an actin filaments marker, ABD2-mCherry in tobacco (*Nicotiana benthamiana*) leaf epidermal cells. Scale bar = 10 μm.

(B) GFP-CFE1A co-expressed with an ER luminal marker, RFP-HDEL in tobacco leaf epidermal cells. Scale bar = 10 μm.

Supplementary Table S1. Oligonucleotides used in this study.

Primer Name	Sequences(5'-3')	Underlined	Destination
CFE1- full-F	CCCCTTACCTTCATAGAGAG		*CFE1* gene cloning
CFE1- full-R	TGAGGGTTATGGCAGAAGAT		
GhCFE1D-SNP-F	TGTGGAGTATGATACAAAGAGCGGGTGGTA		molecular mapping
GhCFE1D-SNP-R	TTTGTTAAAGAGATTCGATACAGAGGCCGAA		
GhCFE1-qRT-F	GACGCTGGAAAACACTTGGAAA		qRT-PCR
GhCFE1-qRT-R	GACTCAGCGACGGTTCTTTTCT		
His3-qRT-F	GGTGGTGTGAAGAAGCCTCAT		qRT-PCR
His3-qRT-R	AATTTCACGAACAAGCCTCTGGAA		
GhCFE1-RT-F	TGTGGCGGTTAAAGATG		RT-PCR and southern
GhCFE1-RT-R	GACTCAGCGACGGTTC		blot probe
EF-1αF	AGACCACCAAGTACTACTGCAC		RT-PCR
EF-1αR	CCACCAATCTTGTACACATCC		
GhCFE1A pET-30a/pGEX-6P-1-F	GCGGATCCCTTAAAATCTCTGTTCCATTGGTT	*Bam*HI	Heterologous expression of *GhCFE1A*
GhCFE1A	CGCTCGAGCTAACTTCCACGGTCAACC	*Xho*I	

pET-30a/pGEX-6P-1-R			
OE-*GhCFE1A*-F	GCCGGATCCACCCCTTACCTTCATAGAG	*Bam*HI	Overexpression
OE-*GhCFE1A*-R	TCCGAGCTCGCAGAAGATATTGGTCTAT	*Sac*I	Vector
Knockdown-*GhCFE1A*-F	TCCGAGCTCACCCCTTACCTTCATAGAG	*Sac*I	Suppression Vector
Knockdown-*GhCFE1A*-R	CGCGGATCCGCAGAAGATATTGGTCTAT	*Bam*HI	
35SSC-F	TGCCATCATTGCGATAAA		*35S*-sence*GhCFE1A*
35SSC-R	CTGACTCAGCGACGGTTC		detection
E6SC-F	CCATTCACCACTTGCTCCT		*E6*-sence*GhCFE1A*
E6SC-R	GGCATTGATTTCCCTTCC		detection
E6ASC-F	TATTGGTGTAATGTGAAGGGAC		*E6*-antisence*GhCFE1*
E6ASC-R	CAAAGAGCGGGTGGGACT		*A* detection
NPTII-F	GAGGCTATTCGGCTATGACTG		*NPTII* detection
NPTII-R	TAGAAGGCGATGCGCTGCGA		
GhCFE1A BKT7-F	GGAATTCCATATGCTTAAAATCTCTGTTCC	*Nde* I	
GhCFE1A BKT7-R	AACTGCAGGACTTCCACGGTCAACCATTT	*Pst* I	bait vector
BD-ΔC-F	GGAATTCCATATGCTTAAAATCTCTGTTCC	*Nde* I	construction
BD-ΔC-R	AACTGCAGGCAGCGACGGTTCTTTTCTCA	*Pst* I	

BD-ΔN-F	GGAATTCCATATGAACCAAAACAACGGCGAG	*Nde* I	
BD-ΔN-R	AACTGCAGGACTTCCACGGTCAACCATTT	*Pst* I	
GhACT2-F	CTCTGAAGCTCCTCTTGGTTCT		*GhACT2*gene cloning
GhACT2-R	GGTGATACGGTGAAAGGCTAA		
*GhACTa*SNP-F	ATTTACTCAGTATTGTAAAAGATGGCCGACGG		*GhACTa*gene cloning
*GhACTa*SNP -R	CCCCCACATAAACCAGACTTTTGAGTTGAC		
*GhACTb*SNP-F	ATTTACTCAGTATTGTAAAAGATGGCCGACGG		*GhACTb*gene cloning
*GhACTb*SNP-R	ATGCAAAATGTAGATCACCATCAAAAGCAAGTAC		
*GhACT4*SNP-F	TTGTAAAAGATGGCCGATGCTGAGGATA		*GhACT4*gene cloning
*GhACT4*SNP-R	CAGAAAATGTAACTCACCATTAAATGACTTGTCAAT		
*GhACT*ADT7-F1	GGAATTCCATATGGCCGACGGTGAGGAT	*Nde* I	pGADT7-GhACT vector construction
*GhACT*ADT7-F2	GAATTCCATATGGCCGATGCTGAGGAT	*Nde* I	
*GhACT*ADT7-R	CGGGATCCCGAAGCACTTCCTGTGGAC	*Bam*H I	
CFE1A-GFP-F	caccATGGCGACGGCGAGTACTTGGATAT		CFE1A-GFP vector construction
CFE1A-GFP-R	tgctcctgctccACTTCCACGGTCAACCATT		
GFP-CFE1A-F	caccATGGCGACGGCGAGTACTTGGATAT		GFP-CFE1A vector construction
GFP-CFE1A-R	CTAACTTCCACGGTCAACCATTTCCATGT		
MYB2-F	TTGCTTTAGTTTGGATTGGGTG		qRT-PCR
MYB2-R	CCAAGAAACATTTGGTTATAGCC		

MYB25-F	ATGGCAAGGTGTCGTCTG	qRT-PCR
MYB25-R	GGCAATGTTGTGGTGTTCTC	
MYB109-F	GGAGGAAGACAAGTTACTCATTGA	qRT-PCR
MYB109-R	CATCCACCTTAGCCGACAA	
TTG3-F	TCCGCTTCATCCTCTTCT	qRT-PCR
TTG3-R	ACAGTGACAGGTTCAATGG	
HOX1-F	ACACCTTGAGTGCCAGAA	qRT-PCR
HOX1-R	ATGTTGTAATGTTGCCATCCA	

Supplementary Table S2. Statistics of lint and fuzz physical parameters of 35SSC, E6SC, and E6ASC transgenic lines.

Lines	Vector	Lint index/g	Fuzz index/g	NLFI	NFFI
W0		5.60±0.29	1.45±0.09	219.33±23.32	14.45±5.92
155	35SSC	4.79±0.62*	0.99±0.15*	161.50±19.80**	7.05±2.09**
158	35SSC	5.08±0.11	1.10±0.13	170.53±23.30**	8.45±3.28**
159	35SSC	4.55±0.27*	0.98±0.04*	153.11±19.37**	6.42±2.32**
164	35SSC	4.68±0.27*	1.08±0.10	167.60±23.05**	7.95±3.56**
169	E6SC	5.89±0.38	1.23±0.05	194.33±21.38*	10.42±2.99*
172	E6SC	5.69±0.69	1.26±0.02	209.70±19.39	10.74±2.94*
178	E6SC	5.91±0.14	1.19±0.01	212.65±19.92	12.50±4.41
181	E6SC	5.80±0.40	1.13±0.33	189.67±21.06*	11.07±6.36*
183	E6SC	5.66±0.41	1.31±0.08	197.50±23.57*	10.95±3.29*
186	E6ASC	6.41±0.35	1.63±0.08	223.59±26.38	16.50±5.49
189	E6ASC	5.80±0.64	1.51±0.11	213.76±21.43	14.65±4.37
191	E6ASC	5.62±0.53	1.59±0.03	219.05±26.33	14.70±4.22
194	E6ASC	6.55±0.13	1.69±0.08	226.21±23.00	16.64±6.44
197	E6ASC	5.98±0.97	1.49±0.22	218.26±28.97	15.35±5.64
199	E6ASC	6.85±0.73	1.70±0.18	227.65±37.99	16.79±5.09

Fiber samples were harvested from field-grown T3 transgenic cotton plants and wild-type cotton plants for measurement. Values of lint index and fuzz index are mean ± standard deviation of assays for samples of three individual repeats from each line. The number of lint fiber and fuzz fiber initials (NLFI and NFFI) was counted from scanning electron microscope (SEM) images (250 μm × 170 μm) of the central portion of ovules at 0 and 4 days post-anthesis (DPA). The values were averaged over 20 images of 10 selected ovules. Asterisks indicate statistically significant differences between transgenic lines and wild-type plants, as determined by the Student's *t* test (*P < 0.05; **P < 0.01). WT, wild type; 35SSC, *35S*-sense-*GhCFE1A* expression vector; E6SC, *E6*-sense-*GhCFE1A* expression vector; E6ASC, *E6*-antisense-*GhCFE1A* expression vector.

Supplementary Table S3. Comparison of fiber quality parameters between transgenic lines and null control or wild-type plants.

Lines	Vector	Length(mm)	Strength ($cN \cdot tex^{-1}$)	Micronaire
WT		27.58±0.43	29.42±1.94	5.69±0.60
pBI121	pBI121	27.67±1.13	28.23±0.40	5.23±0.12
155	35SSC	26.47±0.37**	28.65±2.51	5.58±0.34
158	35SSC	26.28±1.31**	29.46±4.07	5.89±0.50
159	35SSC	24.90±0.90**	28.07±2.90	5.58±1.00
164	35SSC	26.49±0.59**	28.45±1.91	5.60±0.52
169	E6SC	26.84±0.80*	28.82±2.57	5.67±0.65
172	E6SC	27.04±1.50	29.22±2.40	5.66±0.23
178	E6SC	27.64±1.13	29.17±2.88	5.77±0.38
181	E6SC	26.65±0.51*	28.84±1.87	5.86±0.62
183	E6SC	26.70±0.60*	27.87±1.58	6.05±0.73
186	E6ASC	28.06±1.23	30.82±2.42	5.86±0.70
189	E6ASC	27.51±0.72	28.91±2.38	5.35±0.43
191	E6ASC	27.84±1.43	28.32±2.46	5.37±0.44
194	E6ASC	27.86±0.80	29.69±2.68	5.56±0.66
197	E6ASC	27.88±0.79	29.09±1.50	5.76±0.57
199	E6ASC	27.94±0.37	28.79±2.73	5.89±0.52

Fiber samples were harvested from field-grown T3 transgenic cotton, wild-type and null control plants for measurement. Values are mean ± standard deviation of assays for samples of three individual repeats from each line. WT, wild type; pBI121, empty expression vector; 35SSC, *35S*-sense-*GhCFE1A* expression vector; E6SC, *E6*-sense-*GhCFE1A* expression vector; E6ASC, *E6*-antisense-*GhCFE1A* expression vector. Asterisks indicate statistically significant differences between transgenic lines and null control or wild-type plants, as determined by the Student's *t*-test (*$P < 0.05$; **$P < 0.01$).

Supplementary Table S4. Clones identified by yeast two-hybrid library screening.

Clone	Length	Conserved domains	Homologous protein	Accession number	E-Value	Homology[a]
1	234 aa	ACTIN	actin [*Gossypium hirsutum*]	AAP73449	1e-167	Id=99%; Po=99%
2	144 aa	DAP-epimerase	Diaminopimelate epimerase, putative [*Ricinus communis*]	XP_002533003	6e-85	Id=84%; Po=93%
3	104 aa	cyanin_plasto	plastocyanin precursor [*Hordeum vulgare*]	CAA68696	1e-68	Id=99%; Po=100%
4	102 aa	No putative conserved domains have been detected	hypothetical protein OsI_22798 [*Oryza sativa Indica Group*]	EEC80521	0.016	Id=54%; Po=57%
5	83 aa	No putative conserved domains have been detected	None			
6	69 aa	No putative conserved domains have been detected	None			