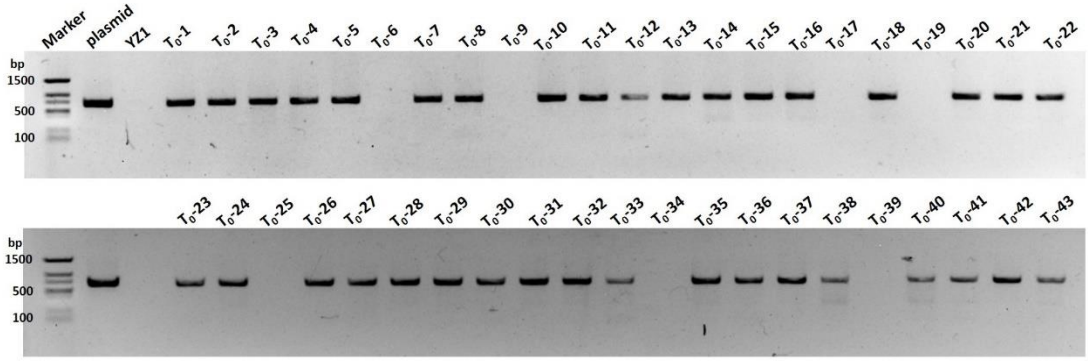
Supplementary Material

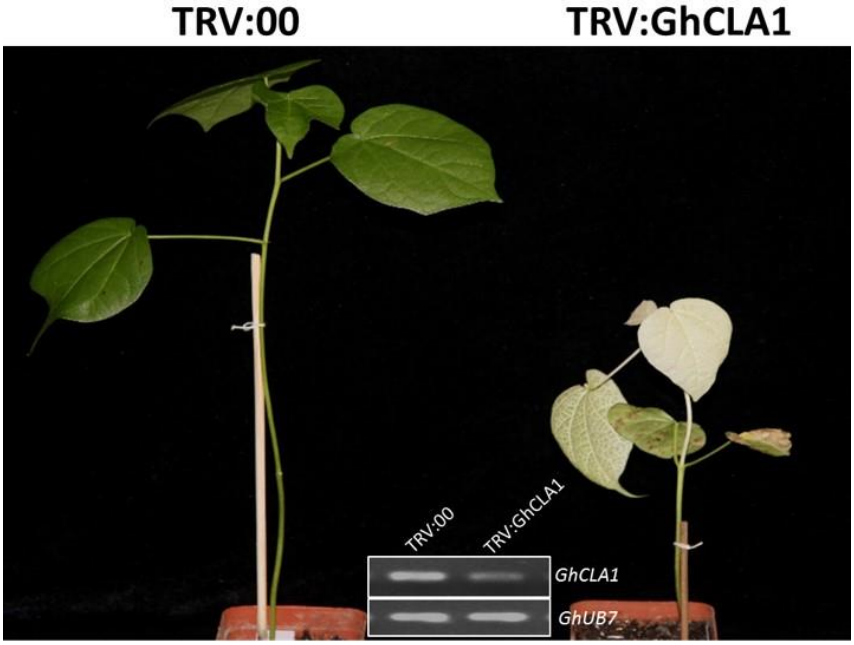
Supplementary Table S1 List of primers used in this study.

Primer ID	primer sequence (5'-3')	Description
GhCLA1-gRT1+	TATGCTCGCGGAATGATCAGGTTTTAGAGCTAGAAAT	For *sgRNA1-GhCLA1* cloning
GhCLA1-U6-29T1-	CTGATCATTCCGCGAGCATACAATCTCTTAGTCGACT	
GhCLA1-gRT2+	CATGCAAAGGGCATATGACCGTTTTAGAGCTAGAAAT	For *sgRNA2-GhCLA1* cloning
GhCLA1-U3bT2-	GGTCATATGCCCTTTGCATGTGACCAATGTTGCTCC	
GhCLA1-gRT3+	CTGCAGGCTTGGCCTGTGAGTTTTAGAGCTAGAAAT	For *sgRNA3-GhCLA1* cloning
GhCLA1-U6-29T3-	TCACAGGCCAAGCCTGCAGCAATCTCTTAGTCGACT	
GhEF1-gRT1+	GGCTTGTCTGAAGGCCTCTGTTTTAGAGCTAGAAAT	For *sgRNA1-GhEF1* cloning
GhEF1-U3bT1-	AGAGGCCTTCAGACAAGCCTGACCAATGTTGCTCC	
GhPDS-gRT1+	AAGCGAGAGATGTTCTAGGGTTTTAGAGCTAGAAAT	For *sgRNA1-GhPDS* cloning
GhPDS-U6-29T1-	CCTAGAACATCTCTCGCTTCAATCTCTTAGTCGACT	
GhPDS-gRT2+	TGGATGGAAACCCTCCCGAGGTTTTAGAGCTAGAAAT	For *sgRNA2-GhPDS* cloning
GhPDS-U6-29T2-	CTCGGGAGGGTTTCCATCCACAATCTCTTAGTCGACT	
GhPDS-gRT3+	ATCACTGGGGGGTGAGGTCGTTTTAGAGCTAGAAAT	For *sgRNA3-GhPDS* cloning
GhPDS-U3b-T3-	GACCTCACCCCCCAGTGATTGACCAATGTTGCTCC	
GhPDS-F1	TGCATGATCCATCACTCAAGTTT	For mutant detection of *GhPDS*
GhPDS-R1	GAACGAAAGGCCCTTTCTTTC	

GhPDS-F2	ATTCATTTCGTGTTCATTCATTTGTAT	For mutant detection of *GhPDS*
GhPDS-R2	GAAAGTTCAACATCCATCAGCTAT	
GhCLA1-F1	GGATCTGAAAGGTGAAAGGAATC	For mutant detection of *GhCLA1*
GhCLA1-R1	TACCGTGATACTTGTCAGCAGCT	
GhCLA1-F2	TTTACTGGTGCCTCGATATCTGA	For mutant detection of *GhCLA1*
GhCLA1-R2	CATCGTGTACGACCTGTTGCAG	
GhCLA1-F3	CACGGTAACATACAGAATAAGCC	For mutant detection of *GhCLA1*
GhCLA1-R3	ACGTCATGTACGACCTGTTGC	
GhEF1-F	TGGTATCACCATTGATATTGCCT	For mutant detection of *GhEF1*
GhEF1-R	ATGACCTGAGAAGTGAAGTTTGC	
NPT Ⅱ.-F	GGCACAACAGACAATCGGC	For mutant detection of *NPT Ⅱ*
NPT Ⅱ-R	CGTAAAGCACGAGGAAGCG	
Cas9-RT-F	TCAACGTACATATCCCTACCG	For RT-PCR analysis
Cas9-RT-R	AGGCTCAAGACTTACGCTCAT	

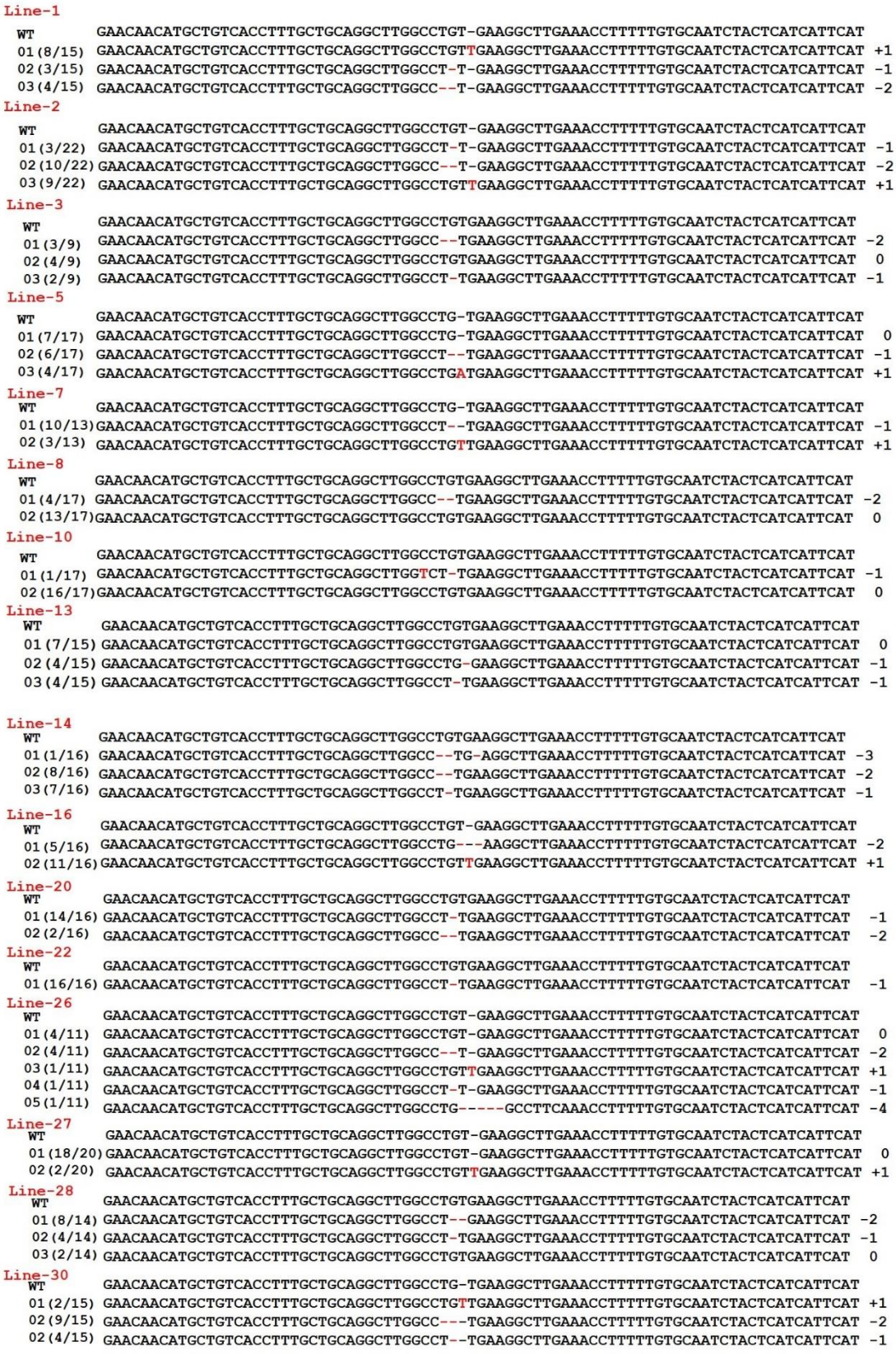


Supplementary Figure S1 PCR analysis of genomic DNA (43 transgenic lines) to detect the plant-selectable marker gene *NPT Ⅱ*. Lanes: Marker, molecular weight markers; Plasmid, positive control vector pYLCRISPR/Cas9-N; YZ1, genomic DNA of wild type plant; T_0-T_{43}, independent transformed kanamycin-resistant calluses lines.



Supplementary Figure S2 Silencing of *GhCLA1* in cotton by tobacco rattle virus (TRV)-mediated virus-induced gene silencing (VIGS). Ten-day-old seedlings were used for infiltration with *A. tumefaciens* carrying *TRV:CLA1* vector. The photobleaching phenotype was appeared after two weeks inoculation. RT-PCR analysis indicated the expression of *CLA1* was reduced after inoculation.

Supplementary Note S1 Sequencing information of *GhCLA1* transgenic lines using CRISPR/Cas9 induced stable transformation.



```
Line-31
   WT        GAACAACATGCTGTCACCTTTGCTGCAGGCTTGGCCTGTGAAGGCTTGAAACCTTTTTGTGCAATCTACTCATCATTCAT
   01(5/16)  GAACAACATGCTGTCACCTTTGCTGCAGGCTTGGCCT-TGAAGGCTTGAAACCTTTTTGTGCAATCTACTCATCATTCAT -1
   02(11/16) GAACAACATGCTGTCACCTTTGCTGCAGGCTTGGCCTGTGAAGGCTTGAAACCTTTTTGTGCAATCTACTCATCATTCAT  0
Line-35
   WT        GAACAACATGCTGTCACCTTTGCTGCAGGCTTGGCCTG-TGAAGGCTTGAAACCTTTTTGTGCAATCTACTCATCATTCAT
   01(8/11)  GAACAACATGCTGTCACCTTTGCTGCAGGCTTGGCCT--TGAAGGCTTGAAACCTTTTTGTGCAATCTACTCATCATTCAT -1
   02(1/11)  GAACAACATGCTGTCACCTTTGCTGCAGGCT----------------------------------ACTCATCATTCAT -36
   03(2/11)  GAACAACATGCTGTCACCTTTGCTGCAGGCTTGGCCTGATGAAGGCTTGAAACCTTTTTGTGCAATCTACTCATCATTCAT +1
Line-38
   WT        GAACAACATGCTGTCACCTTTGCTGCAGGCTTGGCCTGT-GAAGGCTTGAAACCTTTTTGTGCAATCTACTCATCATTCAT
   01(9/13)  GAACAACATGCTGTCACCTTTGCTGCAGGCTTGGCCTGTTGAAGGCTTGAAACCTTTTTGTGCAATCTACTCATCATTCAT +1
   02(3/13)  GAACAACATGCTGTCACCTTTGCTGCAGGCTTGGCC--T-GAAGGCTTGAAACCTTTTTGTGCAATCTACTCATCATTCAT -2
   02(1/13)  GAACAACATGCTGTCACCTTTGCTGCAGGCTTGGCCT-T-GAAGGCTTGAAACCTTTTTGTGCAATCTACTCATCATTCAT -1
Line-40
   WT        GAACAACATGCTGTCACCTTTGCTGCAGGCTTGGCCTG-TGAAGGCTTGAAACCTTTTTGTGCAATCTACTCATCATTCAT
   01(6/10)  GAACAACATGCTGTCACCTTTGCTGCAGGCTTGGCCTGTTGAAGGCTTGAAACCTTTTTGTGCAATCTACTCATCATTCAT +1
   02(3/10)  GAACAACATGCTGTCACCTTTGCTGCAGGCTTGGCCT--TGAAGGCTTGAAACCTTTTTGTGCAATCTACTCATCATTCAT -1
   02(1/10)  GAACAACATGCTGTCACCTTTGCTGCAGGCTTGGCC---TGAAGGCTTGAAACCTTTTTGTGCAATCTACTCATCATTCAT -2
Line-41
   WT        GAACAACATGCTGTCACCTTTGCTGCAGGCTTGGCCTGTGAAGGCTTGAAACCTTTTTGTGCAATCTACTCATCATTCAT
   01(17/17) GAACAACATGCTGTCACCTTTGCTGCAGGCTTGGCCT-TGAAGGCTTGAAACCTTTTTGTGCAATCTACTCATCATTCAT -1
Line-42
   WT        GAACAACATGCTGTCACCTTTGCTGCAGGCTTGGCCTGTGAAGGCTTGAAACCTTTTTGTGCAATCTACTCATCATTCAT
   01(12/16) GAACAACATGCTGTCACCTTTGCTGCAGGCTTGGCCT-TGAAGGCTTGAAACCTTTTTGTGCAATCTACTCATCATTCAT -1
   02(4/16)  GAACAACATGCTGTCACCTTTGCTGCAGGCTTGGCC--TGAAGGCTTGAAACCTTTTTGTGCAATCTACTCATCATTCAT -2
Line-43
   WT        GAACAACATGCTGTCACCTTTGCTGCAGGCTTGGCCTGTGAAGGCTTGAAACCTTTTTGTGCAATCTACTCATCATTCAT
   01(2/17)  GAACAACATGCTGTCACCTTTGCTGCAGGCTTGGCCT-TGAAGGCTTGAAACCTTTTTGTGCAATCTACTCATCATTCAT -1
   02(4/17)  GAACAACATGCTGTCACCTTTGCTGCAGGCTTGGCCTGTGAAGGCTTGAAACCTTTTTGTGCAATCTACTCATCATTCAT  0
   03(11/17) GAACAACATGCTGTCACCTTTGCTGCAGGCTTGGCCT--GAAGGCTTGAAACCTTTTTGTGCAATCTACTCATCATTCAT -2
```