**Supplementary material** 

The cotton MYB108 forms a positive feedback regulation loop with CML11 and participates in defense response against *Verticillium dahliae* infection

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**Supplementary Fig. S1.** Multiple sequence alignments of *GhMYB108* and potential off-target *MYB* genes.

Sequence used for *GhMYB108* VIGS construct was marked with single line. CotAD\_39363, CotAD\_06007, CotAD\_23926, CotAD\_43649, CotAD\_64321, CotAD\_20091: potential off-target *MYB* genes.



**Supplementary Fig. S2.** Expression pattern of *GhMYB* genes upon *V. dahliae* infection in cotton plant.

Expression of *GhMYB108*, *GhMYB4*, *GhMYB9*, *GhMYB10*, *GhMYB36*, and *GhMYB38* genes in cotton roots in response to *V. dahliae* infection. Error bars represent the SD of three biological replicates. Asterisks indicate statistically significant differences, as determined by Student's *t*-test (\*P < 0.05).



**Supplementary Fig. S3.** Photobleaching phenotype in *GhCLA1*-silenced cotton plants.

The *GhCLA1* gene was used as a positive control with a photobleaching phenotype after VIGS in cotton. Plants infiltrated with *Agrobacterium* containing empty vector (*TRV:00*) or pTRV2-GhCLA1 (*TRV2:GhCLA1*) showed different phenotypes in leaves. Photo was taken 14 days after VIGS. The inset indicated the expression levels of *GhCLA1* in control and *GhCLA1*-silenced cotton plants analyzed by RT-PCR. *Histone3* was used as an internal control.





represent the SD of three biological replicates.



**Supplementary Fig. S5.** Disease symptoms of *GhMYB108*-overexpressing Arabidopsis plants inoculated with *B. cinerea* or *Pst* DC3000.

(A) Photographs of leaves 3 days post inoculation with *B. cinerea* between WT (wild type) and *GhMYB108* transgenic Arabidopsis lines (7-4, 35-3 and 39-2). (B) Lesion size measured at 3 days after *B. cinerea* inoculation. Error bars represent the SD (n = 25) of three biological replicates. Asterisks indicate statistically significant differences, as determined by Student's *t*-test (\*P < 0.05). (C) Symptoms of WT and *GhMYB108* transgenic Arabidopsis lines infected with *Pst* DC3000. Photos were taken 3 days post inoculation. (D) Growth of *Pst* DC3000 in infected leaves of WT and *GhMYB108* transgenic plants. Error bars represent the SD (n = 20) of three biological replicates.



**Supplementary Fig. S6.** The  $Ca^{2+}$ -dependent mobility shift assay of GhCML11. GhCML11 proteins were separated on a 15% SDS-PAGE gel in the presence of  $Ca^{2+}$  or EGTA in sample buffers. Protein bands were visualized with Coomassie brilliant blue staining. M: molecular weight marker (kDa).



**Supplementary Fig. S7.** Expression of *PDF1.2*, *PR4*, and *PR5* genes in *GhMYB108*-silenced and *GhMYB108*-overexpressing plants and binding of GhMYB108 to their promoter sequences.

(A) Expression levels of *GhPDF1.2*, *GhPR4*, and *GhPR5* in control (*TRV:00*) and GhMYB108-silenced (*TRV:GhMYB108*) cotton plants. (B) Expression levels of *AtPDF1.2*, *AtPR4*, and *AtPR5* in WT (wild-type) and *GhMYB108* transgenic Arabidopsis plants (7-4, 35-3, and 39-2). Asterisks indicate statistically significant differences, as determined by Student's *t*-test (\*P < 0.05). (C) Binding of GhMYB108 to the promoter sequences of *GhPDF1.2*, *GhPR4*, and *GhPR5*. EMSA analysis was performed with the purified recombinant GhMYB108 proteins and DNA sequences containing the promoter region of *GhPDF1.2*, *GhPR4*, and *GhPR5* genes, respectively. Purified proteins were incubated with labeled DNA probes from corresponding genes in the absence or presence of 100-fold molar excess of unlabelled probes. (D) The promoter sequences used in EMSA. Numbers indicate positions relative to the translation start site of the corresponding gene. The underlined sequence indicates the core motif of MYB binding site.



**Supplementary Fig. S8.** Verification of transcriptomic data in *GhMYB108*-silenced cotton plants.

The down-regulated defense-related genes from the transcriptomic data were verified by qRT-PCR. *TRV:00*: control plants; *TRV:GhMYB108*: *GhMYB108*-silenced plants. Error bars represent the SD of three biological replicates. Asterisks indicate significant differences, as determined by Student's *t*-test (\*P < 0.05).



**Supplementary Fig. S9.** Expression levels of defense-related genes in *GhMYB108* transgenic Arabidopsis plants.

qRT-PCR analysis of expression levels of defense-related genes in *GhMYB108* transgenic Arabidopsis plants. WT: wild-type plants; 7-4, 35-3, 39-2: *GhMYB108* transgenic lines. Error bars represent the SD of three biological replicates. Asterisks indicate statistically significant differences, as determined by Student's *t*-test (\*P < 0.05).



**Supplementary Fig. S10.** Transient expression analysis of GhCML11-enhanced transcriptional activation activity of GhMYB108.

(A and D) Expression levels of *GhMYB108* and *GhCML11* in tobacco leaves transformed with indicated constructs in (B) and (E). Different letters indicate significant differences at P < 0.01 (Student's *t*-test,  $n \ge 15$ , 3 biological repeats). (B and E) Luminescence signal on *N. benthamiana* leaves. Luminescence imaging was performed 48 h after co-infiltrated with *Agrobacterium* strains containing indicated constructs on the left panel. (C and F) Quantitative analysis of luminescence intensity in (B) and (E). Different letters indicate significant differences at P < 0.05 (Student's *t*-test, n = 30, 3 biological repeats).



Supplementary Fig. S11. Effects of  $Ca^{2+}$  on the expression of *GhMYB108* and *GhCML11*.

(A and B) qRT-PCR analysis of expression levels of *GhMYB108* and *GhCML11* after treatment of cotton roots with different concentrations of CaCl<sub>2</sub>. (C and D) qRT-PCR analysis of expression levels of *GhMYB108* and *GhCML11* after treatment with LaCl<sub>3</sub> before and after *V. dahliae* inoculation. LaCl<sub>3</sub>, Ca<sup>2+</sup> influx blocker. Error bars represent the SD of three biological replicates. Asterisks indicate statistically significant differences, as determined by Student's *t*-test (\**P* < 0.05).



Supplementary Fig. S12. Expression pattern of *GhCML11* in cotton plants.

(A) qRT-PCR analysis of *GhCML11* gene expression in root (R), stem (S), leaf (L), and flower (F) of cotton plants. Error bars represent the SD of three biological replicates. (B) Accumulation of *GhCML11* transcripts in cotton roots in response to *V. dahliae* infection. Error bars represent the SD of three biological replicates. Different letters indicate significant differences, as determined by Student's *t*-test (P < 0.05).



**Supplementary Fig. S13.** Increased susceptibility of *GhCML11*-silenced cotton plants to *V. dahliae*.

(A) Analysis of *GhCML11* expression levels. Total RNAs were extracted from leaves of cotton plants at 14 days post agroinfiltration and the expression level of *GhCML11* in VIGS plants was compared with that of control plants (*TRV:00*). Error bars represent the SD of three biological replicates. Asterisks indicate statistically significant differences, as determined by Student's *t*-test (\**P* < 0.05). (B) Disease symptom of control (*TRV:00*) and *GhCML11*-silenced (*TRV:GhCML11*) plants infected by *V. dahliae*. (C) Rate of diseased plants and disease index of the control and *GhCML11*-silenced plants. Error bars represent the SD of three biological replicates ( $n \ge 30$ ). Asterisks indicate statistically significant differences, as determined by Student's *t*-test (\**P* < 0.05).

Supplementally Table S1. Finners used in this su
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Primer name	Sequence (5' to 3')	
GhMYB4-qRT-F	AAGCGAGGAGAGAGAGAGAGCCTGGTG	
GhMYB4-qRT-R	TTCGGTGAAAATGTTTTCTTCAATC	
GhMYB9-qRT-F	ATTACCAGCAAACCCAACCAGAGTC	
GhMYB9-qRT-R	ATTCCAAGATACCAGATTTCAAGCC	
GhMYB10-qRT-F	TGATTCGTCACCCTCACCGGATCAG	
GhMYB10-qRT-R	AGAAGAAGAGCAGAATTATTGATGG	
GhMYB36-qRT-F	TCCTATGCAGCAGCACTTTCCGTTG	
GhMYB36-qRT-R	CAGTACTGATATTCGTATCATGGCC	
GhMYB38-qRT-F	ACGGGGAGTACAGTCATGGTACCGA	
GhMYB38-qRT-R	TAATACAACAAAGTGCGAACTTTGG	
GhMYB108-qRT-F	ACGTCATCAGACTCCCTTGACACCC	
GhMYB108-qRT-R	ACCAACAAACCATTGCCATGCTCCT	
GhMYB108-pBI121-F	CGGCTCTAGAATGGATGTTTACAAG	
GhMYB108-pBI121-R	CCAGAGCTCGATCCCATCACGAAGC	
GhMYB108-5-race-R	TCAACTAAACGGGGGGATCCAAACGTAACGC	
GhMYB108-3-race-F	TCAACCTTCTACCACCACCAAAACCACCTA	
GhMYB108-mbp-F	CTAGTCGACATGGATGTTTACAAGAGAGG	
GhMYB108-mbp-R	GACAAGCTTTTAGATCCCATCACGAAGC	
GhMYB108-gfp-F	CTAGTCGACATGGATGTTTACAAGAGAGG	
GhMYB108-gfp-R	CTTGGTACCGATCCCATCACGAAGCTGC	
GhMYB108∆C-gfp-F	ACTCGTCGACATGGATGTTTACAAGAGAG	
GhMYB108∆C-gfp-R	CTACAAGCTTTTAAACTAAACGGGGGGATC	
GhMYB108∆N-gfp-F	ACTCGTCGACATGAACGGATCCGTGCTTC	
GhMYB108∆N-gfp-R	CATCAAGCTTTTAGATCCCATCACGAAGC	
GhMYB108-vigs-F	CGACGAATTCTTGATTCAGCAAC	
GhMYB108-vigs -R	ACTAGGTACCTTCATCTCTGCCC	
GhMYB108-dlr-F	CGGCTCTAGAATGGATGTTTACAAGAGAGG	
GhMYB108-dlr-R	CGATGTCGACGATCCCATCACGAAGCTGC	
GhMYB108-BD-F	CGACCATATGATGGATGTTTACAAGAGAG	
GhMYB108-BD-R	CTCAGTCGACTTAGATCCCATCACGAAGC	
GhMYB108-AD-F	CGACCATATGATGGATGTTTACAAGAGAG	
GhMYB108-AD-R	CTTCGGATCCTTAGATCCCATCACGAAGC	
GhCML11-AD-F	CGACCATATGATGGGTGATATACTAACTC	
GhCML11-AD-R	CATAGGATCCTCATCCAACGGTCGTCATC	
GhCML11-BD-F	CGACCATATGATGGGTGATATACTAACTC	
GhCML11-BD-R	CTACGTCGACTCATCCAACGGTCGTCATC	
GhMYB108-NLuc-F	CCGAGGTACCATGGATGTTTACAAGAGAG	
GhMYB108-NLuc-R	CATGGTCGACGATCCCATCACGAAGCTGC	
GhMYB108-pro-R1	GTGGATATTGACATGCGCCTTAAGC	
GhMYB108-pro-R2	CATCCATTTCTCAACTTAAAACAGG	
ITS1-F	AAAGTTTTAATGGTTCGCTAAGA	
ST-VE1-R	CTTGGTCATTTAGAGGAAGTAA	

GhCML11-CLuc-R GhCML11-mCherry-F GhCML11-mCherry-R GhCML11-gst-F GhCML11-gst-R GhCML11-vigs-F GhCML11-vigs-R GhCML11-gRT-F GhCML11-qRT-R GhCML11pro-pGWB435-F GhCML11pro-pGWB435-R GhPDF1.2pro-pGWB435-F GhPDF1.2pro-pGWB435-R GhPR4pro-pGWB435-F GhPR4pro-pGWB435-R GhPR5pro-pGWB435-F GhPR5pro-pGWB435-R GhPR4-qRT-F GhPR4-qRT-R GhPR5-qRT-F GhPR5-gRT-R GhPDF1.2-qRT-F GhPDF1.2-qRT-R Histone3-F Histone3-R AtPR4-qRT-F AtPR4-qRT-R AtPR5-gRT-F AtPR5-qRT-R AtPDF1.2-qRT-F AtPDF1.2-qRT-R AtEF-1*α*-F AtEF-1a -R GhEHD2-qRT-F GhEHD2-qRT-R GhPBP1-qRT-F GhPBP1-qRT-R GhNRT1.2-gRT-F GhNRT1.2-qRT-R GhRBOHF-qRT-F GhRBOHF-qRT-R GhIQD1-gRT-F GhIQD1-qRT-R GhIQD14-qRT-F

CATGGTCGACTCATCCAACGGTCGTCATC CGACGGATCCATGGGTGATATACTAACTC CATCGTCGACTCCAACGGTCGTCATCATT CATAGGATCCATGGCGGACCAGCTTACCG CGCCGTCGACTTAAAGAAAAATGCATTTACG CTACGGATCCCCAAGGTACCAAATTGA CATAGGTACCACTTTGGGAAGTTTAAG ATTTCCTTGCATTGAAACTCC GTTTTGTTTGTTTGGTGAGC TCCAGCTCACCAAAACAAACAAAAA CGAGGGTCTCAAGAAGGTACAGCCT ATTCCTCGAAAAATAATAATCTAGAAAGA TAAATATTATTATAACCGAGCGAATGATTGAAA TTTGGGGGGTCCAAAATGAAGCCACC TTCAGTTTTGTGTGTAATTTTCATGCT ΑΤCΑΑΑΤΑΤΑΑΤCCTTΑΤΤΑΑΤΑΑΤΤCΑΑΤ GGTTGGTTTTTACTTGGTGATTTGTTCATA GCTACCTACCATTACTACAACCCTG TTGCTGCACTGATCCACAATTCTTA AGCCGCCTCAGCGTTTATTTTAAA TACGAGCCATGGCTGTGCCAGCAG CTGTGGTAGCGGATGGTGATAAG GTGCAGACGCATTTGCGAAGGAA GCCAAGCGTGTCACAATTATGC ACATCACATTGAACCTACCACTACC GAAGAACACAAGAACAAATGCTGCA GTAGACCGATCGATATTGACCTCAA GCTACGCTTATGACGACGAAACGAG AAATCAGCTGAGTGTAACAACTGAC GGAGCCAAACATGGATCATGCAACT TGTAACAACAACGGGAAAATAAACA TTGGCGGCACCCTTAGCTGGATCA ATGCCCCAGGACATCGTGATTTCAT GGCAAAGCTAAAGCTCAAC CTGGGATTTCATATCCTAAC TTGGAAACCTCAACTCACC TGATGGAGGAATCGCAGAC ACCTTACCCGTGACTTTCC GTGCATCGCCTTCCTCGTA AGCAGCAATGAATCAACCA GTGCATCGCCTTCCTCGTA ATACAATGCCGACTTCAGA GTGGATACAGTGGCCGAGA AAGGCATGGAGAAACCAAT

GhIQD14-qRT-R AAGCATCTTTATGAGGCAC GhIQD31-qRT-F TGATTTGGCTGTGGCTCCTC GhIQD31-qRT-R CGCCGATTGTTCTTGCTTTAT GhCLA1-vigs-F GCTCGAATTCCACAACATCGATGAT GhCLA1-vigs-R CCGAGAGCTCATGATGAGTAGATTGCACAA GhCIPK6-qRT-F AAAACCCATCTTTACTCCAT GhCIPK6-qRT-R TGATCTGCTCCATCATCCC CotAD 39363-qRT-F CGGGACTCAAGTTTCACCAAT CotAD 39363- qRT -R CATCACCATCACCTACCCAAT CotAD 06007- gRT -F CAATAATGGGTTCCAGGTTAC CotAD 06007- gRT -R ACATGGGATAATTGTTGGTCT CotAD 23926- qRT -F TACCCGAATAGCTTACAGAAC CotAD 23926- qRT -R CATATCATCCTTGTCATCATAAA CotAD 43649- qRT -F CTTCGTCGGAAACCCAAGTCA AAGCTGCTGTTGTAAGAACCAA CotAD 43649- gRT -R CotAD\_64321- qRT -F CTTCGTCGGAAATCCAAGTCA CotAD 64321- qRT -R AAGCTGCTGTTGTAAGAACCA CotAD 20091- gRT -F TCATAACCCTAATCCAAACTG CotAD 20091- gRT -R TTGCTGCTGTAAGAAGTAAA D4-qRT-F CACAACCATCTTCCTCCTT D4-qRT-R GTAGCCATCTGTTCCACCA D5-qRT-F AGTCAGCCTAACAATACAATG D5-qRT-R AGACAGTAATCCGTCCAAA D6-qRT-F TATGGGAAGAAATGGGTGAA D6-qRT-R GCGAGATAGTAACGGTAAAA D7-qRT-F CCAGAGGCCATTGCACAAA D7-qRT-R TGCTCCCACCAGGAACCAG D8-qRT-F TTGACCCTGAGTATTTCCA D8-qRT-R GCCATTGCCGTTACCATTT D9-qRT-F GTGGCTAATCATCACTGCA D9-qRT-R GTGGGAATAGACCCGACAG D10-qRT-F GCTAATCCAGGTGGCTCTG D10-qRT-R TGGGCTTGGTTGAACTCTT D11-qRT-F AGCACTTGATTGAACCCTC D11-qRT-R ATCCCATGTCGATGAACTA D12-qRT-F CCGAGACGACGACACCCAT D12-qRT-R CGAACCGATCCAACAAGCT D13-qRT-F GTAGGCTGGCGGAACTAAC D13-qRT-R CATAAAGGCGAGACGAAGG ATGTGCTATTGGACGTTTC D14-qRT-F D14-qRT-R ATGCTCTTTCTTTAGGGTT D15-qRT-F CAGGCAAATGAACAGATGA D15-qRT-R TGCTACAGCCAGGTAAAGA D16-qRT-F GTTTATGTTTGGGGCTGCTA

D16-qRT-R	GAAATGGTGCCTGTGAGAT
D17-qRT-F	AATACCTCCCATAATCACCA
D17-qRT-R	ATTGGCTCTTTCCCACCTA
D18-qRT-F	TTTGATCCCTCAAGATTTG
D18-qRT-R	GCTGTTACTACCCACCATT
D19-qRT-F	CACTTCACTTATTTCGTCTACC
D19-qRT-R	ATTCTACTCTTGCCCACCA
D20-qRT-F	CTGGAAAGACTACCAAACC
D20-qRT-R	CGATGTATCACCAAATGCT
D21-qRT-F	CCCTCACATTCACAAGTCT
D21-qRT-R	ATCCCTACCCTAAACAAAG
D22-qRT-F	TCATCGGTTATCCTTTCTC
D22-qRT-R	AGCAGCCATTTCAGGTGTC
D23-qRT-F	TGCGAGATGATACTAACGG
AtWRKY18-qRT-F	TAGCGACATACGAAGGGAC
AtWRKY18-qRT-R	ATAGCAGCAGCAAGAGCAG
AtWRKY33-qRT-F	TGCTATTGCTGGTCACTCC
AtWRKY33-qRT-R	AGGTCTCCTCGTTTGGTTC
AtWRKY50-qRT-F	GTATGGGAAGAAGATGGTG
AtWRKY50-qRT-R	CTTGAGTGATTGTGGGAAC
AtbHLH87-qRT-F	ACTTCTGTGACAAGGGTGG
AtbHLH87-qRT-R	GCAATTATCTTACGCTATCT
AtWAK2-qRT-F	CCGACAATGACCGAAGTAG
AtWAK2-qRT-R	GTTTCTGAGGGATAACGAC
AtFLS2-qRT-F	AACGACCCTTTAGGAGTATTA
AtFLS2-qRT-R	TATGGCTGGAGACAGAACA
AtBAK1-qRT-F	CTGGGTGAAAGGGTTGTTA
AtBAK1-qRT-R	GTTGGGTAGTTGAAATCTTGTCT
AtLYK4-qRT-F	GAGTTGCGGTTCTTGAGCT
AtLYK4-qRT-R	ACAGATGGACGCGAGTTAA
AtANP3-qRT-F	AGATTAACTCTAGTATCCGTAGC
AtANP3-qRT-R	GACCCTGAGTCTTCTCCTT
AtMKK4-qRT-F	GTCGCCGTCCTGATCTTAC
AtMKK4-qRT-R	ATACCGTTCCACCTGCTCC
AtMKK6-qRT-F	CTTGGCAGCAATAGTAGAG
AtMKK6-qRT-R	AACAGGTGGTTCCAGAGTG
AtAHK4-qRT-F	GATGGACGGATTTGAAGCA
AtAHK4-qRT-R	TACGACGAAGGTGAGATAGGA
AtRLP12-qRT-F	CTCCCTCCTTGTTCACTCT
AtRLP12-qRT-R	TGACTCCATTCCAAAGACA
AtCYP82G1-qRT-F	GCCTCTATTCGGACACCTC
AtCYP82G1-qRT-R	TGCTGTAGCCAAGTCGTTG
AtCYP707A1-qRT-F	TTCATCATCTCACCACCAA
AtCYP707A1-qRT-R	AAGGCAATTCTGTCATTCTAC

AtRGA2-qRT-F	TGCTATTGCTGGTCACTCC
AtRGA2-qRT-R	GAGGAGCATTCAGCTACGA
AtRPP13-qRT-F	GGATAGTAGAAGAAGGGTCG
AtRPP13-qRT-R	AAATTGAACATCGGGAATG
AtH2A1-qRT-F	AGTTGGCGATTAGAGGAGA
AtH2A1-qRT-R	GGATAAAGGAAAATGAGTC
AtSOT17-qRT-F	ACTGGCTTTCTTCTTCAA
AtSOT17-qRT-R	GTTCACCACATATTCCTCC
AtPUB23-qRT-F	AAGCGATAGAGCGGTTAGG
AtPUB23-qRT-R	TCAGCAGGGATATGCAAGA

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Custom ID	Gene ID	Ratio	Annotation
D1	Unigene24024	-3.219552634	Defensin-like protein 16 (PDF1.2)
D2	Unigene39636	-2.144169535	Pathogenesis-related protein PR-4A
D3	Unigene24561	-1.109232546	Pathogenesis-related protein 5
D4	CL11789	-1.392639921	WRKY transcription factor 18
D5	CL5469	-1.103135385	Probable WRKY transcription factor 33
D6	Unigene14461	-1.22437439	Probable WRKY transcription factor 50
D7	CL4272	-1.496137059	Transcription factor bHLH87
D8	CL3156	-2.951792885	Wall-associated receptor kinase 2
D9	CL5714	-1.660371486	LRR receptor-like serine/threonine-protein kinase FLS2
D10	Unigene13686	-1.236825379	BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1(BAK1)
D11	Unigene11352	-1.017808689	LysM domain receptor-like kinase 4
D12	CL10768	-1.206741829	Mitogen-activated protein kinase kinase kinase 3
D13	CL6810	-1.25759391	Mitogen-activated protein kinase kinase 4
D14	Unigene3343	-1.257265737	Mitogen-activated protein kinase kinase 6
D15	Unigene42675	-1.363014048	Histidine kinase 4
D16	CL2475	-6.764145213	Receptor-like protein 12
D17	CL9316	-2.240225367	Cytochrome P450 82G1
D18	CL4312	-2.189863033	Abscisic acid 8'-hydroxylase1 (CYP707A1)
D19	Unigene27837	-2.533284106	Disease resistance protein RGA2
D20	Unigene11468	-1.469416175	Disease resistance protein RPP13
D21	Unigene14161	-1.807599783	Histone H2A variant 1
D22	Unigene15182	-1.668735646	Cytosolic sulfotransferase 17
D23	CL1628	-1.009230557	E3 ubiquitin-protein ligase PUB23

**Supplementary Table S3.** Defense-related genes down-regulated in *GhMYB108*-silenced cotton plants.