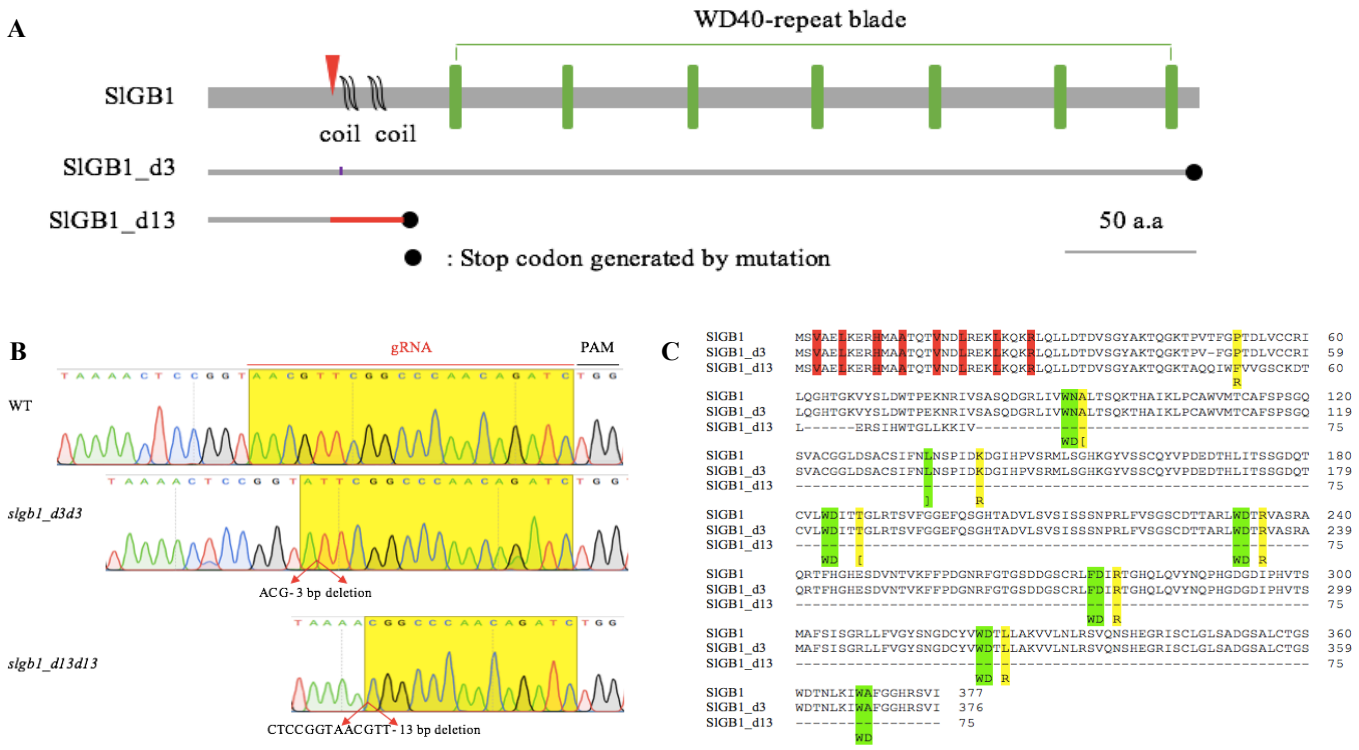

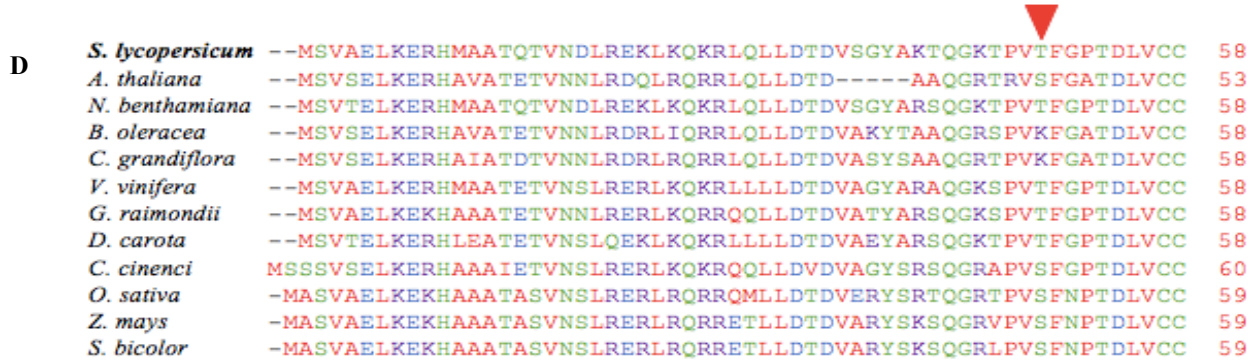

Fig.S1 Characterization of CRISPR/Cas9 *slgb1* mutants in tomato. (A) Schematic diagram of the wild type (WT) and mutated SlGB1 proteins. Upper row, WT SlGB1 protein indicating the position of the mutation (red arrow) upstream of the coil-coiled domain. Middle row, mutated SlGB1_d3 protein encoded by the three bp-deletion mutation. The deletion of three bases results in the omission of a threonine amino acid (a.a) in position 49 indicated by the purple mark. Lower row, mutated SlGB1_d13 protein encoded by the 13 bp-deletion mutation. The deletion of 13 bases produces a frameshift that interrupts the canonical sequence of the protein at position 47 adding an unrelated peptide of 28 a.a before encountering a translational stop codon. In SlGB1_d3 and SlGB1_d13, grey lines indicate native SlGB1 sequences, the red line indicates unrelated peptide. (B) Sanger sequencing of PCR amplicons from WT and two homozygous mutant lines *slgb1_d3d3* and *slgb1_d13d13* show three bases and 13 bases deletions, respectively. (C) Amino acid sequences of WT and mutated SlGB1 proteins. Eight residues important for interaction with Gγ are highlighted in red. WD-40 repeat domains starts and ends are highlighted in green and yellow, respectively. (D) The threonine amino acid in position 49 of the SlGB1 protein indicated by a red arrow is not conserved among plant Gβ subunits.

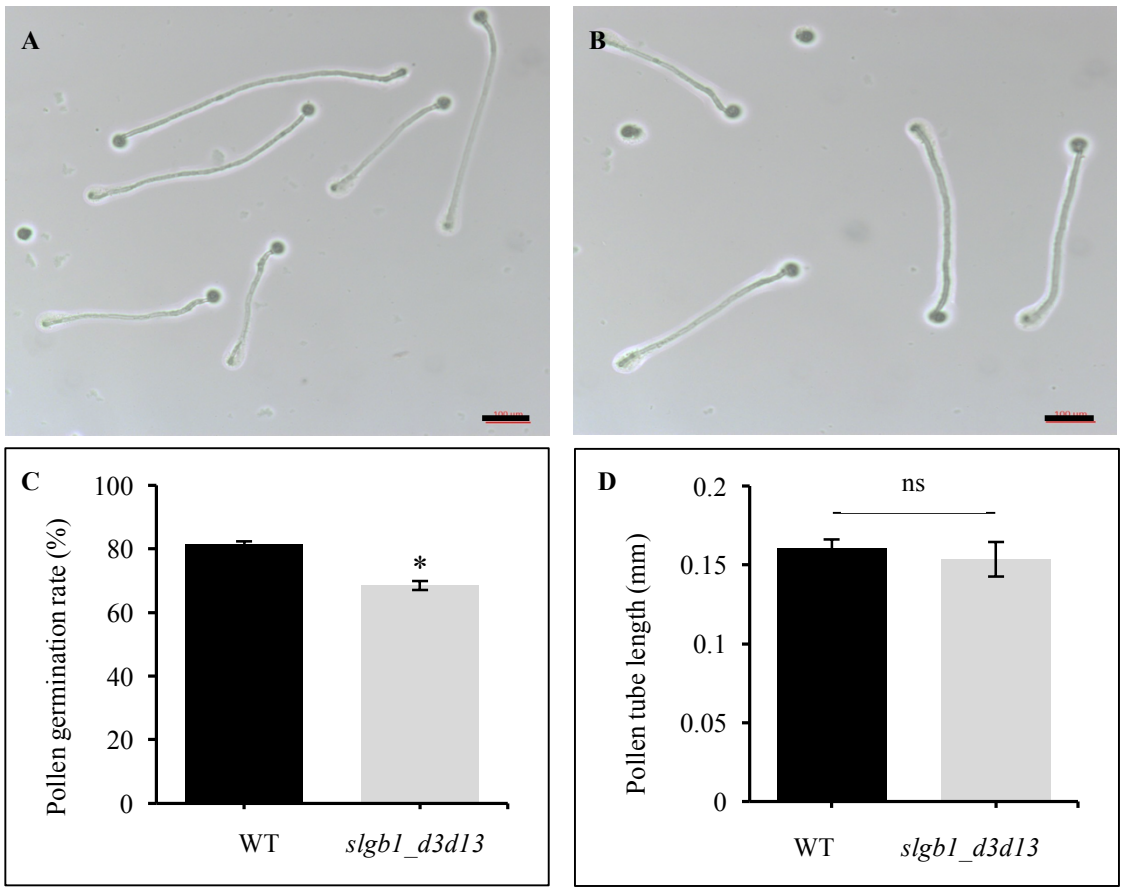

Fig. S2 *In vitro* pollen germination assay. Tomato pollen grains derived from (A) WT and (B) biallelic *slgb1_d3d13* plants were germinated on a solid germination medium. Pictures were taken 24 h after incubation. Scale bars = 100 µm. (C) Pollen germination rate and (D) average pollen tube length from WT and *slgb1_d3d13* plants were measured after incubation for 24 h. In C, bars represent averaged values from three independent experiments with standard errors. Asterisk indicates statistically significant difference evaluated by Student's t-test, * $P < 0.05$. In D, bars represent means ± SEM, $n \geq 65$. "ns" indicates no significant difference ($P > 0.05$) by Student's t-test.

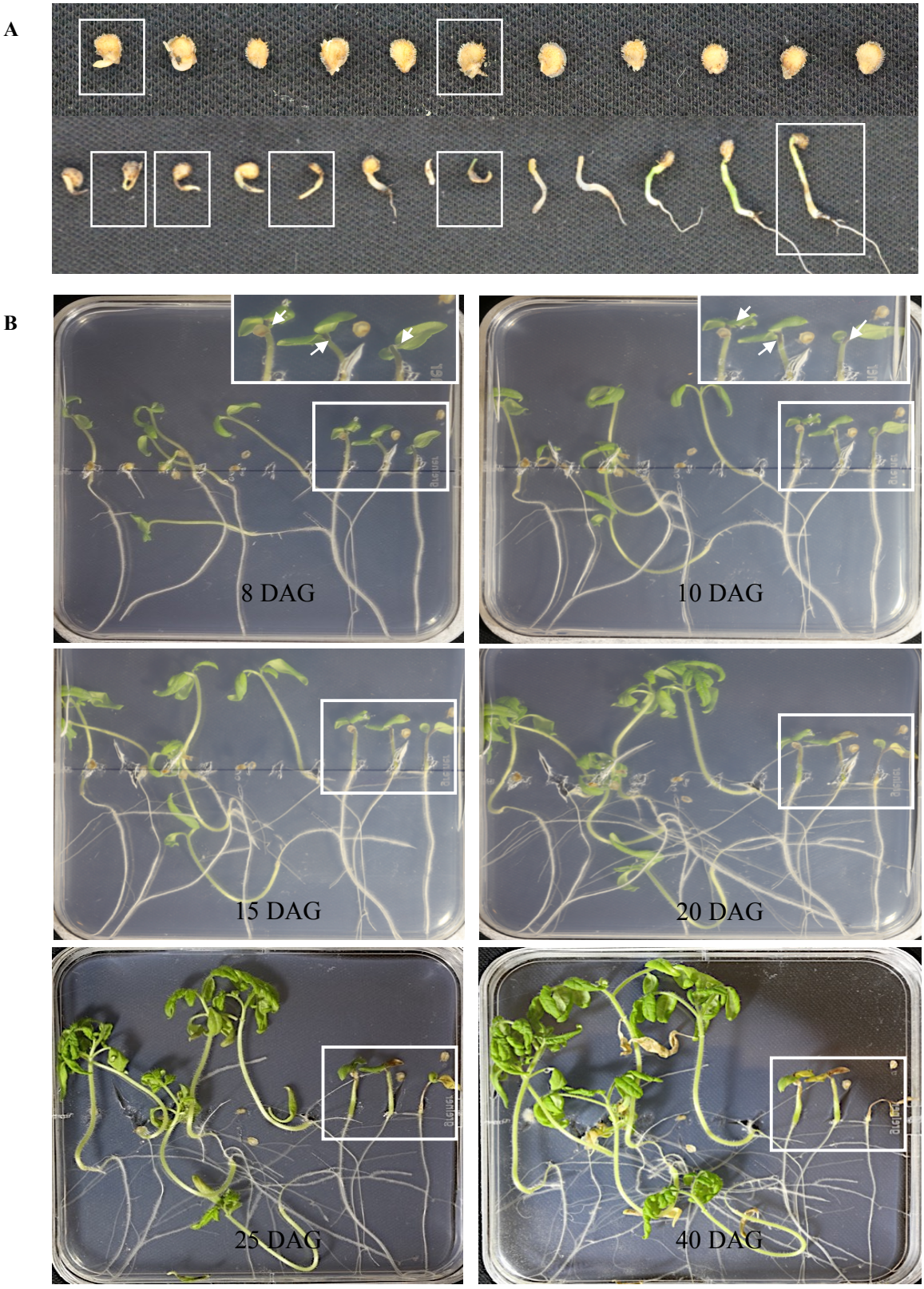

Fig. S3 Homozygous *slgb1_d13d13* mutants exhibit seedling lethality. (A) Non-germinated seeds and defective germination and growth seedlings from self-pollinated heterozygous *slgb1_d13wt* plants two weeks after sowing on soil. (B) Seedlings from self-pollinated *slgb1_d13wt* plants were germinated on MS medium containing 1% (w/v) sucrose. White boxes show homozygous *slgb1_d13d13* seeds/seedlings identified by Sanger sequencing of the targeted genomic region. DAG, days after germination.

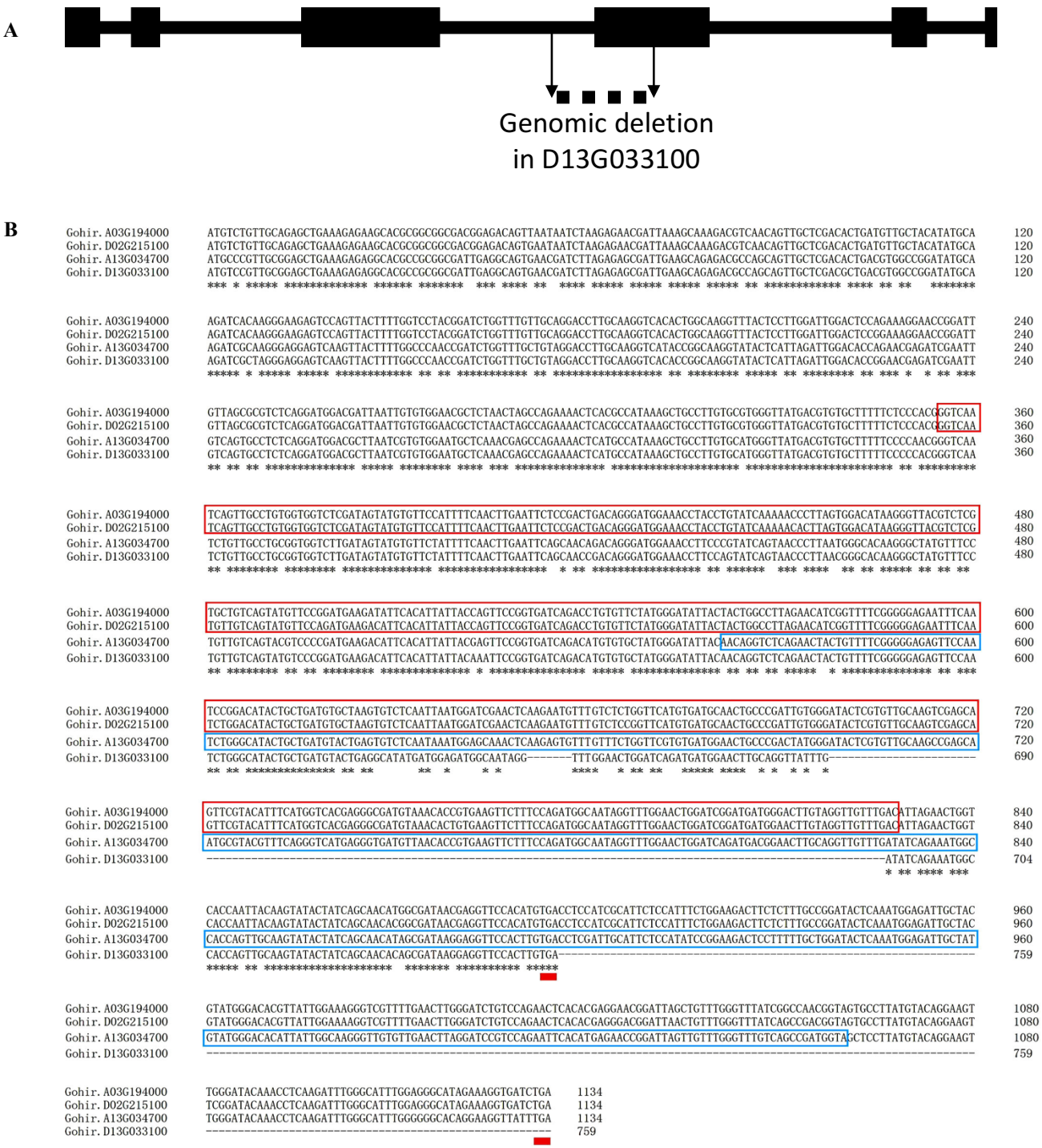

Fig. S4 Genomic structure of cotton *GhGB* genes and cDNA regions used for VIGS constructs. (A) Schematic diagram of the *GhGB* genes showing the position of the genomic deletion in Gohir.D13G033100. Exons are represented as boxes. (B) Sequence alignment of *GhGB1* (Gohir.A03G194000 and Gohir.D02G215100) and *GhGB2* (Gohir.A13G034700 and Gohir.D13G033100) sequences. The target regions of VIGS vectors TRV:β1 and TRV:β2 are indicated with red and blue boxes, respectively. The in frame stop codon in Gohir.D13G033100 is indicated with a red box.

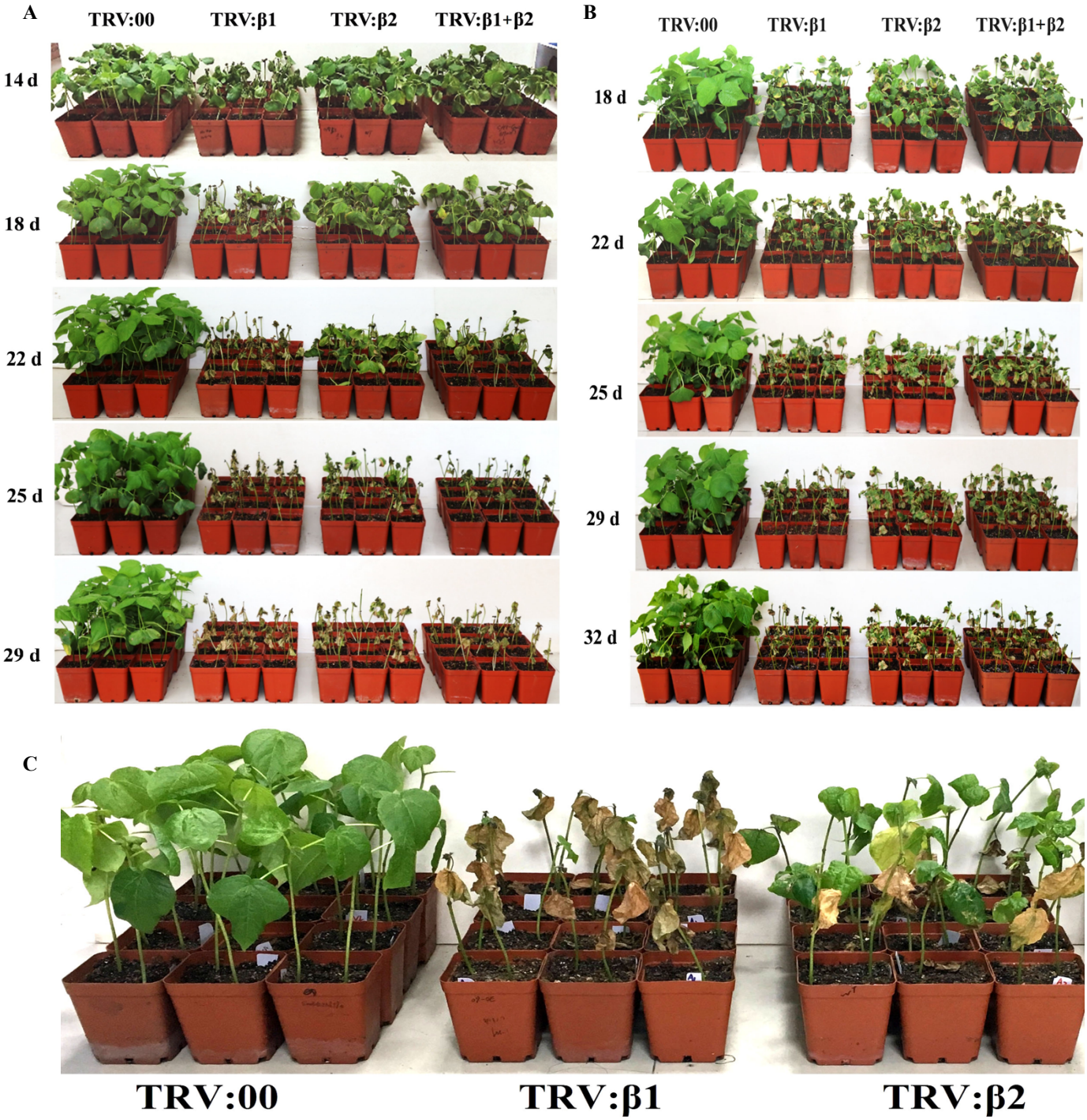

Fig. S5 Silencing of *GhGB1* and *GhGB2* causes plant death in the upland cotton cultivar (A) 'TM1', (B) 'Coker201' and (C) the sea-island (*Gossypium barbadense*) cotton cultivar 'Hai7124'. (A and B) Plants were infiltrated with *A. tumefaciens* strain GV3101 carrying TRV:00, TRV:β1, TRV:β2 or TRV:β1+TRV:β2 vectors. Pictures were taken 14-32 days after infiltration. (C) Plants were infiltrated with *A. tumefaciens* strain GV3101 carrying TRV:00, TRV:β1 or TRV:β2 vectors. Pictures were taken 35 days after infiltration.

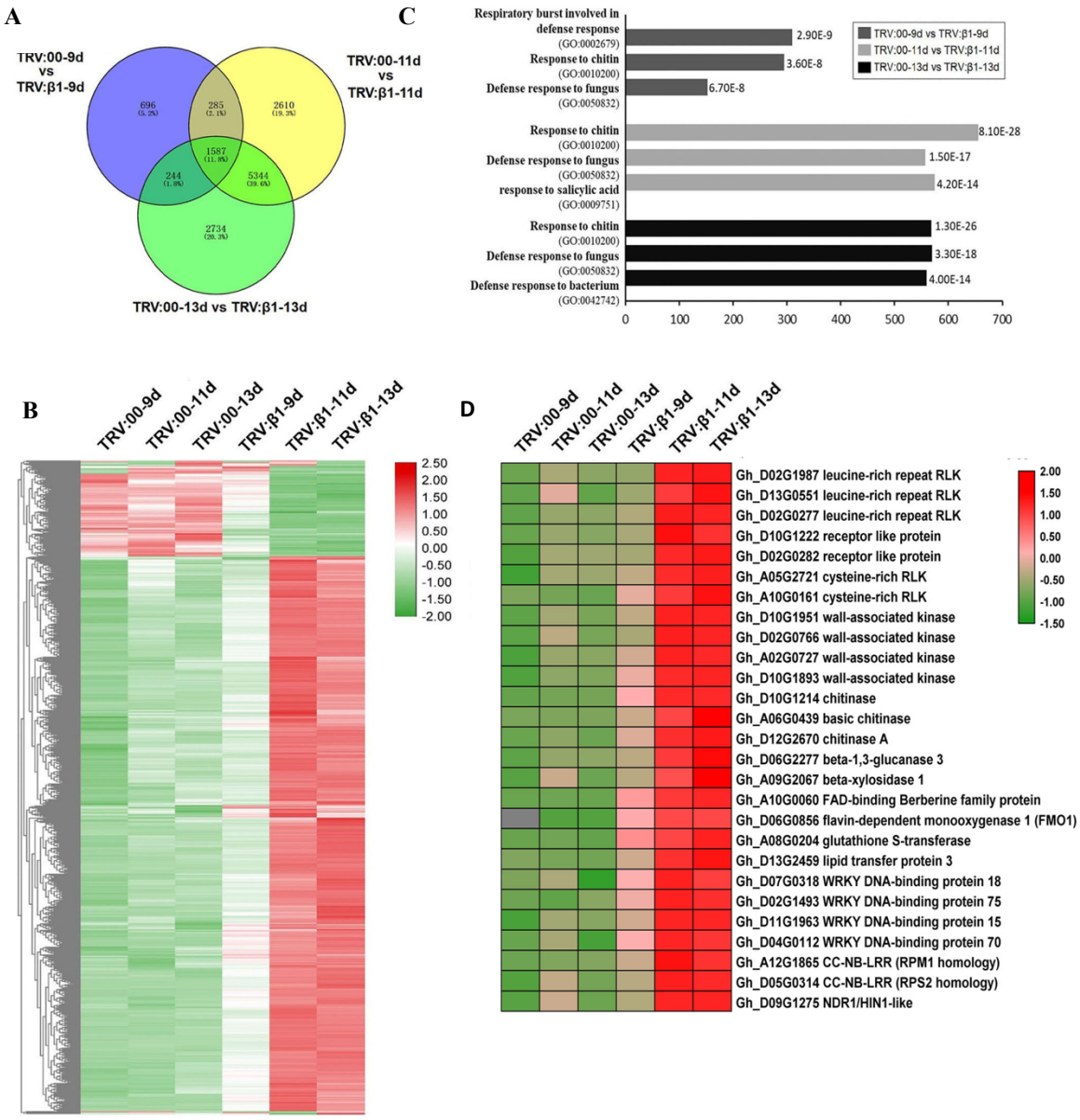

Fig. S6 Transcriptome analysis of Gβ-silenced cotton plants. (A) Venn diagram of differentially expressed gene (DEGs) in the leaves of cotton plants infiltrated with TRV:β1 compared with TRV:00, 9, 11 and 13 d after VIGS infiltration. (B) Heat-map showing the expression changes of all DEGs in both three groups of samples (TRV:β1 and TRV:00; 9 d, 11 d and13 d). (C) The TOP3 enrichment GO groups of the DEGs (TRV:β1 vs TRV:00) in 9 d, 11 d or 13 d samples. The bottom (X-axis) is the gene numbers enriched in each term, and next to the bar (right) is the significance. (D) Heat-map of a part of the upregulated genes in both three groups of samples (TRV:β1 and TRV:00; 9 d, 11 d and 13 d). For (B) and (D), The FPKM value of DEGs are shown by a color gradient from low (green) to high (red). The scale bar stand for the $\log_2$ fold changes in transcription level.

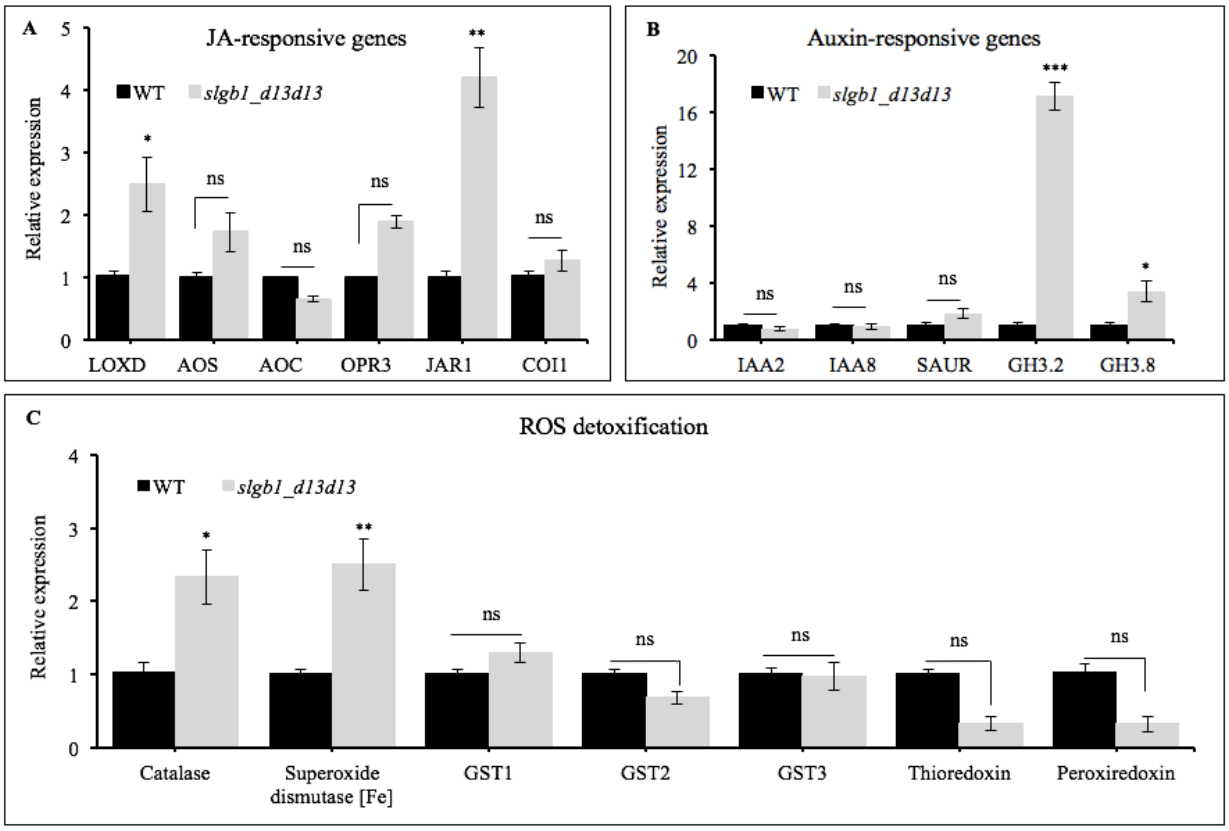

Fig. S7 Expression of key genes involved in Jasmonic acid (JA)-, auxin- and ROS detoxification- signaling pathways is altered in homozygous *slgb1_d13d13* mutants. qRT-PCR was performed to measure relative expression levels of (A) JA-responsive genes, (B) auxin-responsive genes and (C) genes related to ROS detoxification in two-week-old WT and *slgb1_d13d13* tomato seedlings grown on MS medium. Gene expression was normalized to the tomato *Ubiquitin 3 (SlUB3)*. Bars represent means ± SEM, n ≥ 3. Asterisks indicates significant difference evaluated by Student's t-test, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. "ns" indicates no significant difference.

Table S1 Characterization of CRISPR/Cas9-targeted mutations in the T0 generation

T0 line	The sequence of target site	Genotype	Zygosity	Note
# 13	ACTCCGGTAACGTTCGGCCCAACAGATCTGG	WT	Monoallelic	No seeds were obtained
	ACTCCGGTAACGTTCGGCCCAACAAATCTGG	s1		
# 18	ACTCCGGTA---TTCGGCCCAACAGATCTGG	d3	Biallelic	
	A-------------CGGCCCAACAGATCTGG	d13		
# 24	ACTCCGGTA----TCGGCCCAACAGATCTGG	d4	Biallelic	No seeds were obtained
	ACTCCGGTA------GGCCCAACAGATCTGG	d6		

WT, wild type-no mutation was detected at target site; s#, # number of bases substituted at target site; d#, # number of bp deleted at target site. PAM sequence (TGG) is indicated in red and underlined; the 20 bp target sequence is marked in green, the base highlighted yellow means substitution.

Table S2 The differentially expressed genes in both three groups of samples (TRV:00 and TRV:β1; 9 d, 11 d and 13 d after VIGS infiltration) (see separate Excel file)

Table S3 Part of the upregulated genes in both three groups of samples (TRV:00 and TRV:β1; 9 d, 11 d and 13 d after VIGS infiltration) (see separate Excel file)

Table S4 Part of the upregulated genes in both three groups of samples (TRV:00 and TRV:β1; 9 d, 11 d and 13 d after VIGS infiltration) (see separate Excel file)

Table S5 List of primer sequences

Name	Gene ID	Forward primer	Reverse primer	Reference
Primers used for VIGS vector construction in cotton				
GhGB1	Gohir.A03G194000/Gohir.D02G215100	CGACGACAAGACCCTGGTCAATCAGTTGCCTGTGGT	GAGGAGAAGAGCCCTGTCAAACAACCTACAAGTCCCATC	In this study
GhGB2	Gohir.A13G034700	CGACGACAAGACCCTAACTACTGTTTTCGGGGGAGA	GAGGAGAAGAGCCCTTACCATCGGCTGACAAACCC	In this study
GhEDS1	Gh_A12G1816	AGAAGGCCTCCATGGGGATCCTGGAACTAACAGGAATATGGGATG	GAGACGCGTGAGCTCGGTACCTATCCACCATTTGGTAAAGGAGG	In this study
GhEDS5	Gh_A03G1749	AGAAGGCCTCCATGGGGATCCTTCGTTGTGCGACCGAGTAA	GAGACGCGTGAGCTCGGTACCAATAAGCCACCAGTCAAACCAAC	In this study
GhPAD4	Gh_A05G3304	AGAAGGCCTCCATGGGGATCCTAGAAATCTGGTGGCTTTGAGTAAG	GAGACGCGTGAGCTCGGTACCCGAGCAATGGAGAACCGAAT	In this study
GhSAG101	Gh_D07G2218	AGAAGGCCTCCATGGGG	GAGACGCGTGAGCTCGGTA	In this study

		ATCCGGACAGTTAATGTTTACAGCAGGAT	CCTCGTACCAGCATACAACCACC	
GhSID2	Gh_D05G3199	AGAAGGCCTCCATGGGGATCCTGCCCGTAGCAGCAGGATT	AGAAGGCCTCCATGGGGATCCTGCCCGTAGCAGCAGGATT	In this study
GhEIN2	Gh_D09G1403	AGAAGGCCTCCATGGGGATCCTTTAGGAGCCTCAGCGGGA	AGAAGGCCTCCATGGGGATCCTTTAGGAGCCTCAGCGGGA	In this study
GhRbohD	Gh_D05G2471	AGAAGGCCTCCATGGGGATCCTTACTGGGTGACAAGGGAGCA	GAGACGCGTGAGCTCGGTACCGTTAGGCTTGGCGAAGTGAGA	In this study
GhRbohF	Gh_D03G0688	AGAAGGCCTCCATGGGGATCCTGTCCAGTGCCCTGCTGTCT	AGAAGGCCTCCATGGGGATCCTGTCCAGTGCCCTGCTGTCT	In this study
Primers used for qRT-PCR in tomato				
SlUB3	Solyc07g064130.1.1	GCCGACTACAACATCCAGAAGG	TGCAACACAGCGAGCTTAACC	Ricardi et al. (2010)
SlGB1	Solyc01g109560.2.1	TACTCGACACCGATGTTTCTGG	AGACCTTTCCAGTGTGTCCT	In this study
SlPR1b1	Solyc00g174340.1.1	GCCAAGCTATAACTACGCTACCAAC	GCAAGAAATGAACCACCATCC	Song et al. (2010)
SlPR1a2	Solyc09g007020.1.1	CTTGAGGTTCACAACGACGC	CCCGCTCTTGAGTTGGCATA	In this study
SlPR2	Solyc01g060020.2.1	CCAACATTCACATAACAGAGGCT	TAGCGCATTCAAAGCTCCATGA	In this study
SlPR3	Solyc10g055810.1.1	AACTATGGGCCATGTGGAAGA	GGCTTTGGGGATTGAGGAG	Song et al. (2010)
SlPR4	Solyc01g097240.2.1	AGATGCTTGAGGGTGACCAAC	GTTTCCCCTCTGATAGCCCA	In this study
SlPR5	Solyc08g080640.1.1	GCAACAACTGTCCATACACC	AGACTCCACCACAATCACC	Molinari et al. (2014)
SlPAD4	Solyc02g032850.2.1	GAACTCGCAACCTCAACAGC	TCGAGGCACCTCTTTGCTTG	In this study
SlEDS1	Solyc06g071280.2.1	TAGGGACACAGTTTCGCAGG	TGCCCAGAAACAAGACTCGG	In this study
SlNPR1	Solyc07g040690.2.1	GGTCGACAAGTTTCAGAGACACC	TGAGGCAAGGACTTATCAAGGG	In this study
SlETR4	Solyc06g053710.2.1	CGTGAATAGAGCGGTAACAAGTAAG	CAGGGCTAAGAACACCAATACA	Martínez (2018)
SlCTR1	Solyc10g083610.1.1	GCATATCCCCTAGTTGCATCAC	CATGGAAACCAGTTCCTCTTCT	Martínez (2018)
SlCTR2	Solyc01g097980.2.1	TGCAAGCTCAGTCAATAGGAAC	ACCAACATCATCAAACACAGGA	Martínez (2018)
SlEIN2	Solyc09g007870.2.1	CTTGCGCAGATTTGCAGTGA	CCGGTTGCAGTCAGGAAAAC	In this study
SlERF1a	Solyc05g052050.1.1	CTCTAAGCGTCGGATGGTCG	GACGCTGTCTAACGCCTCTA	In this study
SlERF1b	Solyc06g082590.1.1	GCTTTGTCACCCACCTCAGT	CCGTCATAGCAAAATCCGGC	In this study
SlRbohB	Solyc03g117980.2.1	AGGGAATGATAGAGCGTCG	CATCGTCATTGGACTTGGC	Li et al. (2015)
SlRbohD	Solyc06g068680.2.1	CCTCCTACACCACCAAAT	GCCCAGTGCTTCAATCTCT	Li et al.

		C		(2015)
SlRbohF	Solyc07g042460.1.1	CCTTATCTGCACGAGAGGAAAT	CAGCACATTTGTGTCAGATTCC	Li et al. (2015)
SlCAT3	Solyc04g082460.2.1	TGCAGCTCCCAGTTAATGCT	CCGCATGACGACAAGGATCA	In this study
SlSDF	Solyc06g048410.2.1	GGATACACACCACTCCTCACC	TGACTGCTTCCCATGACACC	In this study
SlGST1	Solyc06g009020.2.1	AGTCGTGGCAGAGAACGAAG	CTCCCCGACAAGTAGTGCAA	In this study
SlGST2	Solyc09g074850.2.1	TGATTGGCTTGGGCAGTACC	GTAAAGGAGTCGCCACCCAA	In this study
SlGST3	Solyc06g009040.2.1	TGCTGACAAGGGGAACCAAC	CTTCGTTCTCCGCCACGATT	In this study
SlTHIO (Thioredoxin)	Solyc05g006830.2.1	TCACACCACAAAGCAAGAGGT	TGCAAGGACCACACCATGTA	In this study
SlPERO (Peroxiredoxin)	Solyc03g096040.2.1	TTCACATCGTGGGACCTGAC	GGTTCTCCCGGTTTCCAGTT	In this study
SlLOXD	Solyc03g122340.2.1	GGCTTGCTTTACTCCTGGTC	AAATCAAAGCGCCAGTTCTT	Sun et al. (2017)
SlAOS	Solyc03g120500.2.1	CGATTACCTCCGATTCTGGT	AAATCTTCATCCCACCGAAG	Sun et al. (2017)
SlAOC	Solyc02g085730.2.1	CAGCAGGACTCTGCATTCTG	CGGTGACGGCTAGGTAAGTT	Sun et al. (2017)
SlOPR3	Solyc07g007870.2.1	ATGTTGGTCGTGCATCTCAT	GGTTCCAATTGCTCTTGGTT	Sun et al. (2017)
SlJAR1	Solyc10g011660.2.1	CTAAGCCATTTATAAGAAAGGAGGG	CTGCCATTCAGACCCCATTG	In this study
SlCOI1	Solyc05g052620.2.1	GTGCGGTTACACACAGAGGA	CTGTCAAGCAAAACCAGCCG	In this study
SlIAA2	Solyc06g084070.2.1	TAACAATGATGAACCACCAC	TTTCCTTAAATAAGCCGCAC	Deng et al. (2012)
SlIAA8	Solyc03g120390.2.1	CCTAACAATCTGTAATTCTCAAAGTGAAA	GCATCCAGTCTCCATCTTTATCTTC	Deng et al. (2012)
SlSAUR	Solyc01g110920.2.1	ATGTTGGGGAAAAGCAGAAG	ACCCATCGGATGATTAAAGC	In this study
SlGH3.2	Solyc01g107390.2.1	GTGAACTTTGCACCTATT	AAACACTTCTCCTCCTCT	Liao et al. (2015)
SlGH3.4	Solyc02g092820.2.1	CTCCAGGGTGATTTCTGT	TTCTTTGGTCCACTGTCT	Liao et al. (2015)
Primers used for qRT-PCR in cotton				
GhUBI7	Gh_A12G1102	GAAGGCATTCCACCTGACCAAC	CTTGACCTTCTTCTTCTTGTGCTTG	In this study
GhGB1	Gohir.A03G194000/Gohir.D02G215100	GCCTTGTGCGTGGGTTATGAC	AAACCGATGTTCTAAGGCCAGTAG	In this study
GhGB2	Gohir.A13G034700	CACCAGAACGAGATCGAATTGTC	ATAGCCCTTGTGCCCATTAAGG	In this study
GhPAD4	Gh_A05G3304	GAGTCAACATTAACCGCCATAGAC	GAGTCAACATTAACCGCCATAGAC	In this study
GhEDS1	Gh_A12G1816	GCCGATGCTATTCTTCAACTACT	GATCTCATCCCATATTCCTGTTAGT	In this study
GhEDS5	Gh_A03G1749	TTCGTTGTGCGACCGAGTAA	TTCGTTGTGCGACCGAGTAA	In this study

GhSAG101	Gh_D07G2218	CTCCGATCACGGTGGTTGTAT	GAGGCTCAATAAGCATCTCATACT	In this study
GhSID2	Gh_D05G3199	TGCCCGTAGCAGCAGGATT	AACTGCTGGAGTTGGGTGGAG	In this study
GhAOC4	Gh_A12G1551	GGCATCACGGCTGGACTCT	GCGATGGTGGCACTGGC	In this study
GhEIN2	Gh_D09G1403	TTTTGATCTGGTAGCCCCC	CAATATGAAACCTGCCGCAT	In this study
GhERF1	Gh_A08G1686	CGCAGCGGAGATAAGGGA	CCTAAATCCTCAAACACCACCA	In this study
GhRbohD	Gh_D05G2471	TTACTGGGTGACAAGGGAGCA	TTACTGGGTGACAAGGGAGCA	In this study
GhRbohF	Gh_D03G0688	TGTCCAGTGCCCTGCTGTCT	TTTGAACCAATCAAATGAACCTTG	In this study
GhPR1	Gh_A12G0274	ACCTCAACGCTCACAACACA	GGTCCACTGGAGTGCACAAG	Du et al. (2017)
GhPR4	Gh_D13G1816	CCGCAGAACATAAACTGGGACT	AGCCCTCCATTGCTACATTGAT	In this study
GhPR5	Gh_A12G2071	GGACCATCGATGTGCCTGC	CACCCCAACCTTTGCATTGA	Du et al. (2017)

References

Deng W, Yan F, Liu M, Wang X, Li Z (2012) Down-regulation of *SlIAA15* in tomato altered stem xylem development and production of volatile compounds in leaf exudates. Plant Signaling & Behavior **7**: 911-913

Du X, Wang S, Gao F, Zhang L, Zhao JH, Guo HS, Hua C (2017) Expression of pathogenesis-related genes in cotton roots in response to *Verticillium dahliae* PAMP molecules. Science China Life sciences **60**: 852-860

Li X, Zhang H, Tian L, Huang L, Liu S, Li D, Song F (2015) Tomato SlRbohB, a member of the NADPH oxidase family, is required for disease resistance against *Botrytis cinerea* and tolerance to drought stress. Frontiers in Plant Science **6**: 463

Liao D, Chen X, Chen A, Wang H, Liu J, Liu J, Gu M, Sun S, Xu G (2015) The characterization of six auxin-induced tomato GH3 genes uncovers a member, *SlGH3.4*, strongly responsive to arbuscular mycorrhizal symbiosis. Plant & Cell Physiology **56**: 674-687

Martínez CIM. **2018**. *Ethylene signal transduction during climacteric tomato fruit ripening.* PhD thesis, KU Leuven University, Leuven, Belgium.

Molinari S, Fanelli E, Leonetti P (2014) Expression of tomato salicylic acid (SA)-responsive pathogenesis-related genes in *Mi-1*-mediated and SA-induced resistance to root-knot nematodes. Molecular Plant Pathology **15**: 255-264

Ricardi MM, González RM, Iusem ND (2010) Protocol: fine-tuning of a Chromatin Immunoprecipitation (ChIP) protocol in tomato. Plant Methods **6**: 11

Song YY, Zeng RS, Xu JF, Li J, Shen X, Yihdego WG (2010) Interplant communication of tomato plants through underground common mycorrhizal networks. PLoS One **5**: 1-11

Sun YC, Pan LL, Ying FZ, Li P, Wang XW, Liu SS (2017) Jasmonic acid-related resistance in tomato mediates interactions between whitefly and whitefly-transmitted virus. Scientific Reports **7**: 566