

**Supplemental Figure S1. Characterization of** *PpERF98-1* and 2 genes in *Prunus persica.* (A) Differentially expressed *PpERFs* in *Lasiodiplodia theobromae*-induced peach gummosis. Red and black dots represent upregulated and down-regulated *PpERF* genes, respectively. (B) Phylogenetic analysis of PpERF98-1 and 2 with their orthologs from *Arabidopsis thaliana*, *Camelina sativa*, *Citrus sinensis*, *Oryza sativa*, *Hevea brasiliensis*, *Malus domestica*, *P. dulcis*, *P. mume*, *P. persica*, *Rosa chinensis*, *Solanum lycopersicum*, and *Theobroma cacao*. All protein sequences were retrieved from the GenBank of NCBI. The predicted protein sequences were clustered using ClustalX and MEGA7. Phylogenetic relationships were calculated with maximum-likelihood principle, and bootstrap values within 1000 replicates were determined. Scale bar gives the number of substitutions per cite. Peach ERF98-1 and 2 are noted with red square and rhombus, respectively, and Arabidopsis ERF98 is marked with a red triangle.



Supplemental Figure S2. Transcriptional response of *PpERF98-1* and 2 to hormonal treatment of peach shoots. (A-C) Transcript levels of *PpERF98-1* in peach shoots after treatment with 500  $\mu$ M salicylic acid (SA), 100  $\mu$ M methyl jasmonate (MeJA) and 10  $\mu$ L L<sup>-1</sup> gaseous ethylene (ET). (D-F) Relative transcripts of *PpERF98-2* after SA, MeJA and ET treatments. Gene transcripts were normalized on *PpTEF2* and are displayed as fold changes over values at time 0 h (which are therefore set to 1). Data are means  $\pm$  SD of three biological replicates and four analytical replicates. Different letters on top of bars indicate statistical significance at *P* < 0.05 based on Duncan's post hoc test.



Supplemental Figure S3. Relative transcripts of *PpERF98-1* and 2 in the inoculated peach shoots pretreated with ethylene inhibitors. Transcript levels of *PpERF98-1* (A) and 2 (B) in the ethylene (ET) inhibitors-pretreated shoots at different hours post *L. theobromae* inoculation (hpi). Mock: the peach shoots were pretreated with ddH<sub>2</sub>O and inoculated with sterile PDA plugs; AVG/1-MCP+*L. theobormae*: the peach shoots were pretreated with the ET biosynthetic inhibitor AVG (aminoethoxyvinylglycine, 15 ng mL<sup>-1</sup>) or signaling inhibitor 1-MCP (1-methylcyclopropene, 0.1 mg mL<sup>-1</sup>) for 24 h before *L. theobromae* inoculation. Relative transcript levels of the tested genes were normalized on the reference gene *PpTEF2*, and are displayed in relation to the transcript levels in mock samples at time 0 (which was therefore set to 1). Different letters show statistical significance of different treatments at the same time point and *P* < 0.05 based on Duncan's post hoc test. Error bars represent are means  $\pm$ 

SD of three independent replicates, and each in four analytical replicates.



**Supplemental Figure S4. Amino acid alignment of PpERF98-1 and 2 and their homologs from other plant species.** Deep blue shaded amino acids are the most conserved, followed by red, and light blue as least conserved. The conserved AP domain and residues EDLL are highlighted in red and green boxes, respectively. Numbers by the sequences represent the amino acid position in the full-length protein sequence.





PNRSV-PpPDS



Supplemental Figure S5. Phenotype of two-week-old *PpPDS*-silenced peach seedlings created by virus-induced gene silencing (VIGS) and molecular characterization of the transgenic peach plants and tomato lines. (A and B) The VIGS system was implemented using a *Prunus* necrotic ringspot virus (PNRSV)-based vector. The phenotype of peach seedlings infiltrated with *Agrobacterium tumefaciens* GV3101 harboring the PNRSV vector (control; A), or PRNSV-*PpPDS* (phytoene desaturase; B). Top views of seedlings are shown on the left image of each panel, and single leaves on the right. (C and D) RT-qPCR quantification of *PpERF98-1* and 2 transcript levels in peach plants transiently silenced in either gene by VIGS. The control was agroinfiltrated with empty PRNSV vector, and independent VIGS plants are numbered. (E and F) Transcript levels of *PpERF98-1* and 2 in tomato wild type (WT) and transgenic lines overexpressing either gene. Gene transcripts were normalized over the reference genes *PpTEF2* and *SlActin* in peach and tomato, respectively, and are displayed as transcripts fold change over control (WT) samples (which are therefore set to 1). Data are means  $\pm$  SD of three biological and four analytical replicates. Asterisks or different letters on top of bars indicate statistical significance between transgenic plants and corresponding controls at *P* < 0.05 based on Duncan's post hoc test.



Supplemental Figure S6. Constitutive heterologous overexpression of *PpERF98-1/2* increases the susceptibility

of tomato plants to *Botrytis cinerea*. (A and B) Leaf symptom and lesion areas of the tomato lines overexpressing *PpERF98-1/2* and WT (wild type) infected with *Botrytis cinerea* (*Bc*) at 48 and 72 h. Bar represents 1 cm. (C and D) Transcripts of *pathogenesis-related* (*PR*) genes [*SlPR1*, *SlPR2*, *SlPR5*, *SlPRNP24* (*Prion protein 24*), *SlPR4* and *SlCHI* (*Chitinase*)] in the infected plants overexpressing *PpERF98-1/2*. Data are the means  $\pm$  SD of three independent replicates, with four analytical replicates for transcript quantification. Asterisks on top of bars indicate statistical significance at *P* < 0.05 based on Student's *t* test, while ns indicates no significance.



Supplemental Figure S7. Relative transcript levels in peach seedlings transiently overexpressing *PpERF1-1* and 2. Transcript abundance of *PpERF1-1* (A) and 2 (B) was analyzed in the peach seedlings transformed with *PpERF1*-overexpression (OE) or the empty (Empty) vector after *L. theobromae* inoculation at different hours. Data are means  $\pm$  SD of three independent replicates, each in four analytical replicates for transcript quantification. Asterisks on top of paired bars indicate statistical significance between transgenic and control plants at the same timepoint and *P* < 0.05 based on Student's *t* test, while ns indicates no significance.



**Supplemental Figure S8. Binding test of PpERF98-1/2 with the promoters of** *Pathogenesis-related* **genes (pro***PpRs***).** (A) Diagram of fragments acting as reporters in the luciferase (LUC) activity assay. Numbers above the green bold lines and below the lower slim fragments indicate nucleotide position at the 5'-3' end of each fragment relative to the translation start-site (ATG). The slim fragments were used for reporter constructs. Red pentagrams stand for the putative binding sites of PpERF98-1/2 as identified by the MEME software, while red and green triangles indicate the ERF (ethylene response factor) binding sequences TTTGAATT/ATTTCAAA and CCGAC, respectively, in the promoters of the tested genes. (B) Transient expression assays showing that both PpERF98-1 and 2 could activate the transcripts of *PpPR3*, *PpPR4* and *PpCHI*. Imaging of tobacco leaves 48 h after infiltration shows that the co-infiltration with reporter and effector pairs triggers luminescence emission.





Gene 1: PpERF1-1Gene 1: PpERF1-2Gene 1: PpERF1-1Gene 1: PpERF1-2Gene 2: PpERF1-2Gene 2: PpERF1-2Gene 2: PpERF1-2Gene 2: PpERF1-1

Supplemental Figure S9. PpERF1-1 and 2 cannot form hetero- and homo-dimers with each other. (A) BiFC assay of PpERF1-1 and 2 in tobacco leaf cells. No signal was detected when PpERF1-1/2-nYFP and PpERF1-1/2-cYFP were co-expressed. Scale bar =  $20 \mu m$ . (B) Interactions between PpERF1-1 and -2, and PpERF1 with itself as determined by LCI. Combinations of nLUC or cLUC with the corresponding PpERF1-1/2 constructs were used as negative controls.



Supplemental Figure S10. Contents of JA and SA in the *PpERF98-1/2*overexpressing tomato lines inoculated with *L. theobromae* at 0 and 24 hpi. Quantification of JA (A) and SA (B) in tomato plants. JA: jasmonate acid; SA: salicylic acid; FW: fresh weight. Data are means  $\pm$  SD of three biological replicates. Different letters on top of bars indicate statistical significance within the same timepoint based on Duncan's post hoc test at *P* < 0.05.



Supplemental Figure S11. Transcripts of *PpERF1-1/2* and SA-related genes are affected by *PpERF98-1/2*. (A-E) Relative transcript levels of *PpERF1-1*, *PpERF1-2*, *PpICS2*, *PpNPR1* and *PpTGA3* in the *PpERF98-1*-silenced peach seedlings and control (PNRSV) at 0, 24 and 48 hours post *L. theobromae* inoculation (hpi). (F-J): Transcript levels of *PpERF1-1*, *PpERF1-2*, *PpICS2*, *PpNPR1* and *PpTGA3* in peach seedlings transiently overexpressing *PpERF1-1* and control (Empty) inoculated with *L. theobromae*. Gene transcripts were normalized on the reference gene *PpTEF2* and are displayed as fold change over the mock-transformed, infected controls at time 0 hpi (which are therefore set to 1). Data are means  $\pm$  SD of three independent biological and four analytical replicates. Different letters represent statistically significant differences between treatments at the same timepoint and *P* < 0.05 based on Duncan's post hoc test.



Supplemental Figure S12. Transcriptional response of *PpERF1-1*, *PpERF1-2* and SA-related genes to *L*. *theobromae* invasion in peach leaves transiently modified in *PpERF98-2* expression. Time-course transcripts of *PpERF1-1*, *PpERF1-2*, *PpICS2*, *PpNPR1* and *PpTGA3* after *L*. *theobromae* inoculation in peach seedlings silenced for *PpERF98-2* (A-E) or overexpressing it (F-J). *PpERF98-2* was silenced by PNRSV-mediated VIGS or overexpressed under the CaMV-35S promoter, while the controls were mock-transformed with empty vectors. Gene transcripts were normalized on the reference gene *PpTEF2*, and are displayed as fold changes over the control samples at time 0 hpi (which are therefore set to 1). Data are means  $\pm$  SD of three biological and four analytical replicates. Different letters on top of bars indicate statistical significance between transgenic and corresponding control plants at the same timepoint and *P* < 0.05 based on Duncan's post hoc test.

Gene	Cis-element	Sequence	Site	Predicted function
	DRE	CCGAC	-940	Ethylene response factor binding site
	ERE	ATTTTAAA		Ethylene-responsive element
	GCC-box	GCCGCC		Ethylene response factor binding site
PpERF98-1	ABRE	ACGTG	-861	Abscisic acid-responsive element
	CGTCA-motif	CGTCA	-203, -895	Jamonic acid-responsive element
	TGA-element	AACGAC	-810, -818	Auxin-responsive element
	W-box	TTGACC	-256, -1795	WRKY transcription factor binding site
PpERF98-2	DRE	CCGAC		Ethylene response factor binding site
	ERE	ATTTTAAA	-69, -1182, -1890, -1896	Ethylene-responsive element
	GCC-box	GCCGCC	-628	Ethylene response factor binding site
	ABRE	ACGTG	-189, -1993	Abscisic acid-responsive element
	CGTCA-motif	CGTCA		Jamonic acid-responsive element
	TGA-element	AACGAC	-36, -1056,	Auxin-responsive element
	W-box	TTGACC	-195	WRKY transcription factor binding site

 Table S1. Cis-acting regulatory elements present in the promoter regions of PpERF98-1/2

Note: Numbers indicate the positions of the nucleotides at the 5'-3' end of each fragment relative to the translation start-site in reporters.

## Table S2. Primer sequences used in this study

Purpose	Gene name	Gene ID	Primer-Forword (5'-3')	Primer-Reverse (5'—3')	
Gene cloning	PpERF98-1 CDS	Prupe.8G224700	ATGCACTATATATCTTGCATGTCACAAG	CTAATGGGTTGGTTTCCCCTGTCTA	
	PpERF98-2 CDS	Prupe.1G037800	ATGGAAGACCCTCGTAGAGGAAAGG	TCACTTCCGTTTGTTTTTCTCCTCT	
	PpERF1-1 CDS	Prupe.6G348700	ATGGATTCTTCATTCTTCCAAAACC	CTAGGTCATCGCAGATATGCGGAGA	
	PpERF1-2 CDS	Prupe.8G224600	ATGGAGATGGAATCTGCTAATTTCT	CTAATAGCTTTGTTCCAAAAGTTCC	
	PpPDS CDS	Prupe.1G174100	ATGTCTCAGTGGGCTTGTGTCTC	TCACCGAAGGCTTGCCTCA	
Vector construction for dual-luciferase assay	pGreenII 62-SK-PpERF98-1		cgctctagaactagtggatccATGCACTATATATCTTGCATGTCACAAG	atcgaattcctgcagcccgggCTAATGGGTTGGTTTCCCCTGTCTA	
	pGreenII 62-SK-PpERF98-2		cgctctagaactagtggatccATGGAAGACCCTCGTAGAGGAAAGG	atcgaattcetgcagcceggg TCACTTCCGTTTGTTTTTCTCCTCT	
	pGreenII 0800-proPpMYC2 pGreenII 0800-proPpERF1-1		ctatagggcgaattgggtaccCTGTCCTAACGTTTTAGGTGAC	cgctctagaactagtggatccCATCCAAAATACAAATCATTATCAC	
	F1 pGreenII 0800-proPpERF1-1		ctatagggcgaattgggtaccTGATATAGATGGGACTTTTATTTGGTC	cgctctagaactagtggatccTCGTGCGAACGTCATGTATTTC	
	F2 pGreenII 0800-proPpERF1-1		ctatagggcgaattgggtaccTAATAACCTCCGTACGTCCGTATAAG	cgctctagaactagtggatccACCCTTTGCAGAGGCTGTCTATT	
	F3 pGreenII 0800-proPpERF1-1		ctatagggcgaattgggtaccATGTAGCATGCACGAACTTTGACT	cgctctagaactagtggatccCAACTGATATTGCAGCATGTGTTTA	
	F4 pGreenII 0800-proPpERF1-1		ctatagggcgaattgggtaccGCTACTAGCTATGCGATGACGTTG	cgctctagaactagtggatccTATGGCTGAGCCTCCTTCTCC	
	F5 pGreenII 0800 proPpEPE1 2		ctatagggcgaattgggtaccGCCATGAAAACCCTCAACTCAA	cgctctagaactagtggatccAGTTTGAATAATTTGGAAATGGAATTT	
	F1 F1 F1 F1		ctatagggcgaattgggtaccTACATCAATGTTTCATCTTTAAGG	cgctctagaactagtggatccGGCGTTAGTTGGATTGTCTCAAG	
	F2 F2 F2 F2 F2 F2		ctatagggcgaattgggtaccTCGTTGTTAGGTTTGCTGATAGGC	cgctctagaactagtggatccGTTGCCGTGGCATCATGG	
	F3		ctatagggcgaattgggtaccTGTAATATGTAATTTTGGTCAAATCCC	cgctctagaactagtggatccGCAGCTGAAAGCTACGATTTGTG	
	pGreenII0800-proPpPR4		GGGGTACC GGATCTTGGGGGAAGGGAGGAGCTGT	CCGCTCGAG TCGATGTTTGATGTACATTGTTTTG	
	pGreenII 0800-proPpCHI		GGGGTACC TGCCGGCTGCAATCGGTCAGAGGAC	CCGCTCGAG GTCTGTTTGGGGGTTCTTGTTTTGGT	
	pGreenII 0800-proPpPR3		GGGGTACC AATCAAATTCAAAATTTAAAAGCTT	CCGCTCGAG TTTTCTGTAGGGATATGTATGGGTA	
	pGreenII 0800-proPpMYC2		GGGGTACC CTGTCCTAACGTTTTAGGTGAC	CCGCTCGAG CATCCAAAATACAAATCATTATCAC	
Vector construction for yeast one-hybrid (Y1H) assay	pGADT7-PpERF98-1		ggccagtgaattccacccgggATGCACTATATATCTTGCATGTCACAAG	cagetegagetegatggateeCTAATGGGTTGGTTTCCCCTGTCTA	
	pGADT7-PpERF98-2		ggccagtgaattccacccgggATGGAAGACCCTCGTAGAGGAAAGG	cagetegagetegatggateeTCACTTCCGTTTGTTTTTCTCCTCT	
	pAbAi-proPpERF1-1 F1		cttgaattcgagctcggtaccCAAAAGGAACATCTAAAAAAATTGAATT	atacagagcacatgcctcgagGAAAGAAGAGGGGTTGAATTTGAAA	
	pAbAi-proPpERF1-1 F2		cttgaattcgagctcggtaccGCATACACATTGACTGTTAAACAAAATAT	atacagagcacatgcctcgagCAACTGATATTGCAGCATGTGTTTA	
	pAbAi-proPpERF1-2		cttgaattcgagctcggtaccAACTGTTTATTCAATTCGTTATGCCA	atacagagcacatgcctcgagGTGCAGGTTAATTGTTCTTTCTATATTG	
Specific primers for quantitative reverse- transcriptase PCR	PpERF1-1	Prupe.6G348700	ACCACCATCACAGTCAACCC	CTGACACCGTTTCGAGTGGA	
	PpERF1-2	Prupe.8G224600	GGAGATGCTGCTCTATGATGC	TAGGACTTTTCCGTTGTGGGT	
	PpERF98-1	Prupe.8G224700	TGATCAGCACTTCATCCTCTTCA	CTCCTCCAAAACCATGTTGTCC	
	PpERF98-2	Prupe.1G037800	AAATGTGCCAAGCCACAGAC	GAAGGGAAGCGAAGAAGTGC	
	PpICS2	Prupe.5G187000	TCCAAGTCCAGCAGTTTGTG	TCTCTCCTCCAAACCAA	
	PpNPR1	Prupe.4G107800	TGCTGCCTTATGGCGATGTT	TTAAGAAGGCGTCGCTGGAA	
	PpTGA3	Prupe.1G508100	CTCTCACGCAGGGAATGGAG	CGCAGCCGGTGAAAGTATTC	
	РрМҮС2	Prupe.5G035400	GCTCGACGGAGCTCATCTAC	ATGTTCACCGGGTCCTTGAC	
	PpPR1	Prupe.8G153800	TGACAAGGTGTGTGGGGCATT	CGGATCATAGTTGCACCCGA	

	PpPR2	Prupe.1G122900	AAGTTTGAGGCAGCCAGTGA	GGGTTCTCCAAGAGGTAGGC
	PpPR3	Prupe.1G205600	ACGGCTTAAACTCGCCAGAA	TGATGCGGGCTTGAACTAGG
	РрСНІ	Prupe.2G305200	CGGTGCAGGAAGCTACTCTC	CCATCCAAAACTGCGTCACC
	PpPR4	Prupe.6G141100	GGTGACAAACACGGGCACAGGAG	AAGAAGCGATCCCACTTTGAACT
	PpTEF2	Prupe.4G138700	AGCAAGTCACCCAACAAGCATA	CCAACCAAACTCTTCAGCCAAT
	LtTUBULIN	HQ660474	AATCGGTGCTGCTTTCTGG	TTGTTGGACGCCTCGTTG
	SlPR1a	NM_001247199.2	GATGTGGGACGATGAGAAGCAATG	GTTGCATCGAACCCTAGCACAACCT
	SIPR2	XM_015209904.2	CAGATTTCACTTCCGTATGCTCTT	CCATCCACTCTCTGACACAACAAT
	SIPRNP24	XM_015227829.1	GAGGGGAACTAAGATGGCACGTAT	CTCCACCACAATCACCAGTCTGAC
	SIPR5	NM_001330783.1	AACTGCCCCTACACCGTTTG	GCCCAAAACCACCAACTCTG
	SICHI	NM_001247475.2	TTTTTCGGTCAAACATCTCACG	ATTATCCTGTTCTGTCATCC
	SIPR3	LOC101251136	TGCCCAAACCTCCCATGAAA	AAAAGGTCCACTCCGATGGC
	SlPR4	LOC101243897	TGGTCCTGCTGGAAAAGACAT	TAGCTCGAATCGTTGCCCC
	SlActin	NM_001330119.1	ATGGCAGACGGAGAGGATATTCA	GCCTTTGCAATCCACATCTGCTG
Vector construction for luciferase	771- PpERF98-1-nLUC		acgggggacgagctcggtacc ATGCACTATATATCTTGCATGTCACAA	cgcgtacgagatctggtcgacATGGGTTGGTTTCCCCTG
	772-cLUC-PpERF98-1		tacgcgtcccggggggggtacc ATGCACTATATATCTTGCATGTCACAA	cgcgtacgagatctggtcgac CTAATGGGTTGGTTTCCCCTG
	771-PpERF98-2-nLUC		acgggggacgagetcggtace ATGGAAGACCCTCGTAGAGGAAA	cgcgtacgagatctggtcgac CTTCCGTTTGTTTTTCTCCT
	772-cLUC-PpERF98-2		tacgcgtcccggggggggtacc ATGGAAGACCCTCGTAGAGGAAA	cgcgtacgagatctggtcgac TCACTTCCGTTTGTTTTTCTCCT
complementation	771-PpERF1-1 -nLUC		acgggggacgagctcggtacc ATGGATTCTTCATTCTTCCAAAACC	cgcgtacgagatctggtcgac GGTCATCGCAGATATGCGGA
imaging	771-cLUC-PpERF1-2		tacgcgtcccggggggggtacc ATGGATTCTTCATTCTTCCAAAACC	cgcgtacgagatctggtcgac CCTAGGTCATCGCAGATATGCGG
	771-ERF1-2-nLUC		acgggggacgagctcggtacc ATGGAGATGGAATCTGCTAATTTCT	cgcgtacgagatctggtcgac ATAGCTTTGTTCCAAAAGTTCCTC
	772- cLUC-ERF1-2		tacgcgtcccggggggggtacc ATGGAGATGGAATCTGCTAATTTCT	cgcgtacgagatctggtcgac CTAATAGCTTTGTTCCAAAAGTTCCTC
Vector construction for overexpression and luciferase complementation imaging	attB1-PpERF98-1		aaaaagcaggetee ATGCACTATATATCTTGCATGTCACAAG	agaaagctgggtt CTAATGGGTTGGTTTCCCCTGTCTA
	attB1-PpERF98-2		aaaaagcaggetee ATGGAAGACCCTCGTAGAGGAAAGG	agaaagctgggtt TCACTTCCGTTTGTTTTTTCTCCTCT
	attB1-PpERF1-1		aaaaagcaggetee ATGGATTCTTCATTCTTCCAAAAACC	agaaagctgggtt CTAGGTCATCGCAGATATGCGGAGA
	attB1-PpERF1-2		aaaaagcaggetee ATGGAGATGGAATCTGCTAATTTCT	agaaagctgggtt CTAATAGCTTTGTTCCAAAAGTTCC
	Adapter attB1		GGGGACAAGTTTGTACAAAAAAGCAGGCT	
	Adapter attB2		GGGGACCACTTTGTACAAGAAAGCTGGGT	
Vector construction for virus-induced gene silencing	PNRSV-PpERF98-1		GCTCTAGA ATGTCACAAGCACAACCCATATT	GCTCTAGA ACCTTAGTCTGCTCCTTCTGC
	PNRSV-PpERF98-2		GCTCTAGA ATGGAAGACCCTCGTAGAGG	GCTCTAGA CCCATGGCCTCCTTCGGA
	PNRSV-PpPDS		GCTCTAGA AGAAAGCTGAAGAACACAT	GCTCTAGA GATTGTAATATTCCTTACAT

Note: Letters in lowercase and pink font indicate adapter sequence and restriction endonuclease recognition sequence.