1 Tables

2 Supplementary Data Table 1. Primers used in this study.

		Gene	F primer	R primer
		CaKAN3		GGGGACCACTTTGTACAAGAAAGCTGGGTC
		Cultaity	TTCATGATGGAATATTTCTCAAT	TTAACAATAGAAATATGAAG
		CaKAN3-GFP	GGGGACAAGTTTGTACAAAAAAGCAGGC	GGGGACCACTTTGTACAAGAAAGCTGGGTC
			TTCATGATGGAATATTTCTCAAT	ACAATAGAAATATGAAG
		CaKAN3-VIGS	GGGGACAAGTTTGTACAAAAAAGCAGGC	GGGGACCACTTTGTACAAGAAAGCTGGGTC
			TTC AAGGGAGAACAATATTTGGC	TTTCTCACTAACCAAAGGAC
		CaHSF8-VIGS	GGGGACAAGTTTGTACAAAAAAGCAGGC	GGGGACCACTTTGTACAAGAAAGCTGGGTC
			TTC TGAGTAGCTCAAATGCGCCA	TCTGTCCATGAGCAGGCTTG
study		CaKAN4-GFP	GGGGACAAGTTTGTACAAAAAAGCAGGC	GGGGACCACTTTGTACAAGAAAGCTGGGTC
HSF8			TTCATGATGAAAAAAATATTCAT	GGGAAAAGTTTCTTCAAAAC
l Cal		CaHSF8	GGGGACAAGTTTGTACAAAAAAGCAGGC	GGGGACCACTTTGTACAAGAAAGCTGGGTC
V3 and			TTCATGGGTTCTGCTTCAATGGA	TCATACTTTTTTACTGTTTG
aKAl		CaHSF8-GFP	GGGGACAAGTTTGTACAAAAAAGCAGGC	GGGGACCACTTTGTACAAGAAAGCTGGGTC
for Co			TTCATGGGTTCTGCTTCAATGGA	TACTTTTTTACTGTTTG
nsed		CaHSF8-D1	GGGGACAAGTTTGTACAAAAAAGCAGGC	GGGGACCACTTTGTACAAGAAAGCTGGGTC
imers			TTCATGGGTTCTGCTTCAATGGA	TCAGCGCCTACTAATATTTCTAA
Pri		CaHSF8-D2	GGGGACAAGTTTGTACAAAAAAGCAGGC	GGGGACCACTTTGTACAAGAAAGCTGGGTC
			TTCATGGGTTCTGCTTCAATGGA	TCACAGTAAACTTTCAGGACTGT
		CaHSF8-D3	GGGGACAAGTTTGTACAAAAAAGCAGGC	GGGGACCACTTTGTACAAGAAAGCTGGGTC
			TTCATGGGTTCTGCTTCAATGGA	GTTTTGAAGAAACTTTTCCC
		CaKAN3 ^{myb}	GGGGACAAGTTTGTACAAAAAAGCAGGC	GGGGACCACTTTGTACAAGAAAGCTGGGTC
			TTCATGATGGAATATTTCTCAAT	TTATCTGTACATCTGGAGATGGC
		CaHSFA1-GFP	GGGGACAAGTTTGTACAAAAAAGCAGGC	GGGGACCACTTTGTACAAGAAAGCTGGGTC
			TTCATGCCTTTACACTTCCCTCT	GTTTTGAAGAAACTTTTCCC
-b		<i>CaKAN3-</i> qPCR	AATCATCAGTTCCTCGTCTT	GTCAATCCCTTTACTTCCATC
ters used for pepper PCR analysis	sis	<i>CaHSF8</i> -qPCR	TTGAAGAAGAGGTTGAGAGG	TCGCTGCTCCATAATATGAA
	t analy	<i>CaR1B23-</i> qPCR	TGGCCTGGACACCACAATTT	TTGTTTCCGGAAGACCCTCG
	PCR	<i>CaR1B11-</i> qPCR	GTACTACCCCGTTGAGCCAC	AGGTGTAGCTCTTGTTCCAACT
Prin		<i>CaR1A</i> -qPCR	AGCTACGACATGTGGACACC	TCTGCTCTGCTGCTGAACAA

	CaR1B12-qPCR	CCTAGACGTGACTTCCGAGC	TCTTCCCAACAATCGCAGCT
	<i>CaR1A6-</i> qPCR	TGTGCATGGGTCACTGTCTC	TCCCAAGCCTCAGTACTCCA
	<i>CaR1B16-</i> qPCR	ATGACACGTCGAGCCCAAAT	ACTACCACCAGCAACCATGG
	<i>CaHSP17.4B-</i> qPCR	TCAGCTTCATCAGATGCACCT	TCGTGGCAAGCAATTCACTT
	CaHSP70-qPCR	GTGCAACTTCCGGTGGAATG	GGACAGTAGGGGTGAGGGAA
	<i>CaHSP18.2-</i> qPCR	TGTGCAGTGACCCTTCAGAA	CGACGATATTCCCCACAAAGC
	<i>CaHSP70-15-</i> qPCR	TGCTGAGCCAATGGAGATGG	TGTTGACTGCTGCTGTAGGT
	CaPRP1-qPCR1	ACCAAACTCGGATATTCCAACT	GAGGAATCCTCGGAACCAAGT
	CaMgst3-qPCR ¹	TCCAGCTTTTCGCACTCTCT	CGAGATCTCGCCACCCAATT
	<i>CaACTIN</i> -qPCR ²	AGGGATGGGTCAAAAGGATGC	GAGACAACACCGCCTGAATAGC
	<i>NbR1B23-</i> qPCR	GATGATGCTTGCTCATCGCT	ACGGCAGGTGACAGCTAAAA
	<i>NbR1B11-</i> qPCR	CCCTTCATGTTGGGGGGACTC	TATTGTGTGCCAGGCCACAT
	<i>NbR1B12-</i> qPCR	GAACTCCTACTCCCGCGAAG	CCGGCCATTCCCATAATCGA
	<i>NbR1A6-</i> qPCR	AACGACGGGCTAATGGAAGG	GAAAGCATAGCTCGCCGTTG
	<i>NbR1B16-</i> qPCR	ATGCCCGAGACGCTAACAAA	CCTTGAGGGTCGGAAACTCC
	<i>NbEF-1a-</i> qPCR ²	TGCTGCTGTAACAAGATGGATGC	GAGATGGGGACAAAGGGGATT
	pCaR1B23-P1	AGTCCGGTGCACTAAAGCTC	CCACAAGGGAGAAGGAAAATCC
	pCaR1B23-P2	TCCCTGATATCCCTGCATGAC	CGGATTGTCACGGATGATGC
	pCaR1B23-P3	CCTTTGCTCTCTCCTTGCAGA	TGCGAAGGTAAGAGCTTGGA
	pCaR1B23-HSE	AGAAGGCATCATCCGTGACA	AAGTGGTAGGTGCGTGATGA
imers used for ChIP-PCK	pCaR1B11-P1	CCGAACAGTCACTCCTTTTCC	CCATTCACGTGGGGGGATTGT
	pCaR1B11-P2	GGCTATTGTAACACCCCGCA	TGCCCAACTAGATTCACACACT
	pCaR1B11-P3	TGATGAACTTGGGGGGCTTACT	TGTTGGAGCAAAATGGGCAT
	pCaR1B11-HSE	CCGTCATTTCTAGAAGTAGATGTTCC	TGATCTCGAATCATGCCCAACT
2	pCaR1A-P1	AGGTTGAACTGAATATTCTTTGTCGA	TCACTGCTATGGAATCTTTCTCA
	pCaR1A-P2	ATGAAATTGGGCTACACCGT	ACCCTCTAATGATTGTGCGAA
	pCaR1A-P3	GGGTAATGCGCTCCCTAACA	CAAGGATTGTGGGGGGAGTAGT
	pCaR1A-HSE	CTCCCCCACAATCCTTGTTGA	ACCTTAATATGGCAGTGAGGACT

pCaR1B12-P1	AGTTGGATATATAGTTTCCATCAGCA	TGGTGTCAAGTTTAGGGTCGT
pCaR1B12-P2	ACCTTTGATTTTGCAACTTCATGC	ACCTCTCTCCAAGTCAGCCT
pCaR1B12-P3	TGTGCACTGTCAAAGTTTAAGGT	TGTGAACTCTTCAAACCCCTT
pCaR1B12-P4	AAAGGGGTTTGAAGAGTTCACA	TTAGTTTTGTGCTTTGCAACGA
pCaR1B12-P5	TCTTCCCAACAATCGCAGCT	ACGTGACTTCCGAGCAGTAG
pCaR1B12-HSE	AGTTGGATATATAGTTTCCATCAGCA	TGGTGTCAAGTTTAGGGTCGT
pCaR1A6-P1	TGTCATCTTTCATTTAGACTTTGTTGA	AGTTCATGGTCAAGTCATCCGT
pCaR1A6-P2	TGTGCTTTGTTCCGGGTAATG	ACAAGCAATGCAAGACAGTGT
pCaR1A6-P3	AGCAGGTTCTCGAGTATTCCA	CCTTCCTGTTGATCTAGCTGAGG
pCaR1A6-HSE	GGGGGAAATGAAATTCCTAACCT	CATTACCCGGAACAAAGCACA
pCaR1B16-P1	TCTCAATTCTCACCACCACCA	ACTGCACCTTAGGGGATCTTG
pCaR1B16-P2	TGAAAACAATAACAAGATCCGACCT	AGCTGAGTTCTGCTTTGGACA
pCaR1B16-P3	ACAGCAATTACACTCAACTATCCA	CCTCATTGGTTGCTTGTGTTGT
pCaR1B16-P4	CACAAGCAACCAATGAGGAAA	TCGGTTTTCGTTTGAAAGCACA
pCaR1B16-P5	TGTGCTTTCAAACGAAAACCGA	TGGCTGAAATTCTCATGCTTTCC
pCaR1B16-HSE	TGAAAACAATAACAAGATCCGACCT	AGCTGAGTTCTGCTTTGGACA
pCaMgst3-P1	AAGCTTCCGTCGAAGTTTGC	TCCGTTGACATTGTCCCCTT
pCaMgst3-P2	TCGTTCTTGATAGCAGAACCA	GCGAAACGAATAAATGTTTGTCGA
pCaMgst3-P3	TCCCGTTCTTCATTAAGTTAGTCT	ACGAGTTAAGGACCCGTTTGG
pCaMgst3-P4	TCCAGCTTTTCGCACTCTCT	CGAGATCTCGCCACCCAATT
pCaPRP1-P5	TTGTCGTGTTTGATCCTATC	ACTACCGTAGTGGGAAATTTA
pCaPRP1-P6	AGTCGAAAATTATTTTCCGACAAGC	GACGGCCAATGTAGTCGGAA
pCaPRP1-P7	CCCTTTTAACGACGTGGCAC	TGAACAAATATGACCTCCGATTTTGT
pCaHSP17.4B ^{AACAA}	CACAATTCGTGCCTACTTTAAGT	AGTTCATCCCACTTACCGGG
pCaHSP17.4B-HSE	TGCTTCATGTCCCAATGCGA	CGGCAAAAAGGGTTGAGCAA
рСаНSP70 ^{алсла}	TCGAAATAACATTATGTGAAGGGTT	TCTTCACATTTTTAATTCGCAGTGT
pCaHSP70-HSE	TCTTGCAGTCTGCTTGGTCA	TGCATGTGATGTGTACTGAGA
pCaHSP18.2 ^{AACAA}	ACAAAGCCAAGACCCACAAC	AGTGCATGCAGATCAGCTGT
pCaHSP18.2-HSE	GTTTCACCGGGATTCGCTTT	GTTGTGGGTCTTGGCTTTGT
1		

рСаНSP70-15 ^{ласла}	GCTATAAGGAGCCAAACACAGC	ACATTATCCTGTTTACCAGAGGGG
pCaHSP70-15-HSE	TTTTCGGTCTGGCAATTCGC	CGCTATCCTTTTTCTTTTTCTGTGC
pNbR1B23-P1	ACGATTTTGCTTTTAAAATTAAGCTGT	ATTGCAGCAACCCAAAGCTC
pNbR1B23-P2	AGGAATCTCGTGCCACATGC	CGAAGGAGAGATGATCTAGTTACCC
pNbR1B23-P3	TGATGCTGCTCTTTGTCCTT	AGTTATCAGCTATCATTAACCACTCA
pNbR1B23-P4	CGTCTGCAACAACTTGGCAA	TGAGGCTCTGCAAGTAGAGA
pNbR1B23-PH1	CAGTGGCTCTCCTTCTTCCC	TGGTTAGAAGACATTCTTTTCTGAGA
pNbR1B11-P1	TTCCGAATTGCTCCGGCTAA	TATCCTCCCCAGACCTCACG
pNbR1B11-P2	TGTTGTTTGACCATCGGCTT	ACCTCAACCATAACTCACGAGG
pNbR1B11-P3	TGACAGAATGAGTTCCAAACCTG	TCATTTCGGATTATGGAGAAGTCA
pNbR1B11-P4	GTCTGTTTCCTTTCACTTGACGG	CCAGGGGTCTAGGAGGCTAA
pNbR1B11-PH2	TCAGTAATTTCGGTTAAGTTGAGAAGT	ATTTTGATATTCGCGCGGGC
pNbR1B12-P1	TGGCACACAATCTCACTTTCTG	ATCCGGGTATAGGTCAGGGG
pNbR1B12-P2	TGCGGGAATCTTATTACCCCC	AAAAATACATGTGGGGGCGCG
pNbR1B12-P3	CGGCGACAGATTTTTATCCCG	TTTGCTCTTCCTCCCACACA
pNbR1B12-P4	GCTAAGGGTAAGGTCTGCGG	CTGTTCCACAACAATAGCAGCA
pNbR1B12-PH3	TGTGTGGGAGGAAGAGCAAA	AACCTTCCCACGCCGAAAAA
pNbR1A6-P1	GGAGCCCCTCATATCGAAGC	CCATGATATCCGGTTGGGCA
pNbR1A6-P2	AGTGCAAGCTCGGAACTGAA	GCGAGTTGGCATCGATTGTG
pNbR1A6-P3	CGGGTGGAATCGAGGGAAAA	TGATGCGCTCGGACGTAAAG
pNbR1A6-PH4	TAATATCCGTGCCCAACCGG	TGGTATGCGTGTTACGATGTG
pNbR1B16-P1	TGTAATGCTTGATGACAACGAACA	GGGTAAAGTAGGATCACTTTCTGC
pNbR1B16-P2	TTGACCAACAAAGAGCTGGA	TGCTAAAGTCACAGTAGTTTTGAAA
pNbR1B16-P3	ATCAGAGGCAGGATTTCCGC	GCTTAGTGGTCAAGGGGTTCA
pNbR1B16-P4	TGATCATGGACCTCAGCAAGT	GGCAATAAGGGTGTGAAGCC
pNbR1B16-PH5	GCATAGACAGGTCTGCAAGT	TCCTAGTTCATGCACCAAAACA
WT ^{CaR1B23AACAA} probe	CTCTTTGAAACAAGAAGGGA	TCCCTTCTTGTTTCAAAGAG
M ^{CaR1B23AACAA} probe	CTCTTTGAAAAAAGAAGGGA	TCCCTTCTTTTTTCAAAGAG
WT ^{CaR1B11AACAA} probe	CGGGCTAGAACAAAACCGTC	GACGGTTTTGTTCTAGCCCG

M ^{CaRIBIIAACAA} probe	CGGGCTAGAAAAAACCGTC	GACGGTTTTTTTCTAGCCCG
WT ^{CaR1AAACAA} probe	TGCGCTCCCTAACAAAGGAG	CTCCTTTGTTAGGGAGCGCA
M ^{CaR1AAACAA} probe	TGCGCTCCCTAAAAAGGAG	CTCCTTTTTTAGGGAGCGCA
WT ^{CaR1B12AACAA} probe	GAAAGAAATAACAAGAGTTA	TAACTCTTGTTATTTCTTTC
MC ^{aR1B12AACAA} probe	GAAAGAAATAAAAAGAGTTA	ТААСТСТТТТТАТТТСТТТС
WT ^{CaR1A-6AACAA} probe	CTTTTTGGAACAACTCCAGT	ACTGGAGTTGTTCCAAAAAG
M ^{CaR1A-6AACAA} probe	CTTTTTGGAAAAACTCCAGT	ACTGGAGTTTTTCCAAAAAG
WT ^{CaR1B16AACAA} probe	TGAAAACAATAACAAGATCC	GGATCTTGTTATTGTTTTCA
M ^{CaR1B16AACAA} probe	TGAAAAAAATAAAAAGATCC	GGATCTTTTTATTTTTTTCA
WT ^{CaR1B23HSE} probe	CAATCCGTTCTAGAAAGTGA	TCACTTTCTAGAACGGATTG
M ^{CaR1B23HSE} probe	CAATCCGAAAAAAAAAGTGA	TCACTTTTTTTTCGGATTG
WT ^{CaR1B11HSE} probe	CGTCATTTCTAGAAGTAGAT	ATCTACTTCTAGAAATGACG
M ^{CaR1B11HSE} probe	CGTCATAAAAAAAAGTAGAT	ATCTACTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
WT ^{CaR1AHSE} probe	ATTACTTTCTAAGATTTATG	CATAAATCTTAGAAAGTAAT
M ^{CaR1AHSE} probe	АТТАСТАААААААТТТАТG	CATAAATTTTTTTAGTAAT
WT ^{CaR1B12HSE} probe	CAAAGTTTCTAGAAAGAAAT	ATTTCTTTCTAGAAACTTTG
M ^{CaR1B12HSE} probe	CAAAGTAAAAAAAAAGAAAT	ATTTCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
WT ^{CaR1A-6HSE} probe	AATGTTTTTTTCTAGAATTAC	GTAATTCTAGAAAAAACATT
M ^{CaR1A-6HSE} probe	AATGTTTTAAAAAAAATTAC	GTAATTTTTTTTAAAACATT
WT ^{CaR1B16HSE} probe	GACCTTTCTAGAAAATCACT	AGTGATTTTCTAGAAAGGTC
M ^{CaR1B16HSE} probe	GACCTAAAAAAAAAATCACT	AGTGATTTTTTTTTAGGTC
3x AACAA probe	AACAA AACAA AACAA	TTGTT TTGTT TTGTT
3x AACAAm probe	ААААА ААААА ААААА	TTTTT TTTTT TTTTT
3x HSE probe	ААТТСТАБААААААТТСТАБААААААТТС	TTTTCTAGAATT TTTTCTAGAATT
	TAGAAAA	TTTTCTAGAATT
3x HSEm probe	AATTCTTTCTAA AATTCTTTCTAA	TTAGAAAGAATT TTAGAAAGAATT
	ААТТСТТТСТАА	TTAGAAAGAATT

	G		
	CaR1B23-VIGS	GGGGACAAGTTTGTACAAAAAAGCAGGC	GGGGACCACITIGTACAAGAAAGCIGGGIC
		TTC TGTGGGAAGCTGATGTGTGG	TCTTCTCCATCTCGGCCAGA
	CaR1B11-VIGS	GGGGACAAGTTTGTACAAAAAAGCAGGC	GGGGACCACTTTGTACAAGAAAGCTGGGTC
		TTC ACCACTTCCCTCCAACGTTG	TCCTCGCTCCGATCATTTGG
	CaR1A-VIGS	GGGGACAAGTTTGTACAAAAAAGCAGGC	GGGGACCACTTTGTACAAGAAAGCTGGGTC
		TTC AGCTACGACATGTGGACACC	CAGGCTCGAAGGGAAGTGAA
	CaR1B12-VIGS	GGGGACAAGTTTGTACAAAAAAGCAGGC	GGGGACCACTTTGTACAAGAAAGCTGGGTC
/sis		TTC GTCCTTTGGAGCTACGCAGT	CGGCACAAACTCCTCAGCTA
naly	CaR1A6-VIGS	GGGGACAAGTTTGTACAAAAAAGCAGGC	GGGGACCACTTTGTACAAGAAAGCTGGGTC
VIGS a		TTC GGCATGCAGCTGACCAAAAT	TCCCAAGCCTCAGTACTCCA
Rs	CaR1B16-VIGS	GGGGACAAGTTTGTACAAAAAAGCAGGC	GGGGACCACTTTGTACAAGAAAGCTGGGTC
for NL		TTC GGACGCATTCCTCGCAAATC	AACGTCCCATTTTCCCTGCT
ied 1	NbR1B23-VIGS	GGGGACAAGTTTGTACAAAAAAGCAGGC	GGGGACCACTTTGTACAAGAAAGCTGGGTC
mers us		TTCCCTGCCCCAGTACGTTGATT	ACGGCAGGTGACAGCTAAAA
Pri	NbR1B11-VIGS	GGGGACAAGTTTGTACAAAAAAGCAGGC	GGGGACCACTTTGTACAAGAAAGCTGGGTC
		TTCCAAGTCAGCAGAGGCCGTTA	CGCCCTGCTAGCAATCCATA
	NbR1B12-VIGS	GGGGACAAGTTTGTACAAAAAAGCAGGC	GGGGACCACTTTGTACAAGAAAGCTGGGTC
		TTCGAACTCCTACTCCCGCGAAG	TCAGCCATGCTATGACCACC
	NbR1A6-VIGS	GGGGACAAGTTTGTACAAAAAAGCAGGC	GGGGACCACTTTGTACAAGAAAGCTGGGTC
		TTCGAAGATATGGTTGCGCTGGC	CACTAGTGACGGCTGCAGAA
	NbR1B16-VIGS	GGGGACAAGTTTGTACAAAAAAGCAGGC	GGGGACCACTTTGTACAAGAAAGCTGGGTC
		TTCGGACGCATTCCTCGCAAATC	CCTTGAGGGTCGGAAACTCC
	pCaR1B23-LUC	GTCGACGGTATCGATAAGCTTAGCTAACG	CAGGAATTCGATATCAAGCTTCACGGCTGTG
		AATGCACGACTT	GATCTCCGAG
	pCaR1B11-LUC	GTCGACGGTATCGATAAGCTTATTCATAT	CAGGAATTCGATATCAAGCTTCCATCAGAAT
nalysis		AAAGGACCTCAA	TGTCATCACT
ter a	pCaR1A-LUC	GTCGACGGTATCGATAAGCTTAGTGGATG	CAGGAATTCGATATCAAGCTTAGAATCGGG
promot		AGTTTGGGGTGG	AGATCACTACC
for]	pCaR1B12-LUC	GTCGACGGTATCGATAAGCTTTGAGTTGG	CAGGAATTCGATATCAAGCTTACGTGACTTC
s used		ATATATAGTTTC	CGAGCAGTAG
mer	pCaR1A6-LUC	GTCGACGGTATCGATAAGCTTTTCAAGTA	CAGGAATTCGATATCAAGCTTCACCTCCTTC
Pri		TGGTGCCCCATG	CTGAGATAAC
	pCaR1B16-LUC	GTCGACGGTATCGATAAGCTTTCTCAATT	CAGGAATTCGATATCAAGCTTGTTCACTGAA
		CTCACCACCACC	TTTGACGTAT

pCaR1B23-GW	GGGGACAAGTTTGTACAAAAAAGCAGGC	GGGGACCACTTTGTACAAGAAAGCTGGGTC
	TTCAGCTAACGAATGCACGACTT	CACGGCTGTGGATCTCCGAG
pCaR1B11-GW	GGGGACAAGTTTGTACAAAAAAGCAGGC	GGGGACCACTTTGTACAAGAAAGCTGGGTC
	TTCATTCATATAAAGGACCTCAA	CCATCAGAATTGTCATCACT
pCaR1A-GW	GGGGACAAGTTTGTACAAAAAAGCAGGC	GGGGACCACTTTGTACAAGAAAGCTGGGTC
	TTCAGTGGATGAGTTTGGGGGTGG	AGAATCGGGAGATCACTACC
pCaR1B12-GW	GGGGACAAGTTTGTACAAAAAAGCAGGC	GGGGACCACTTTGTACAAGAAAGCTGGGTC
	TTCTGAGTTGGATATATAGTTTC	ACGTGACTTCCGAGCAGTAG
pCaR1A6-GW	GGGGACAAGTTTGTACAAAAAAGCAGGC	GGGGACCACTTTGTACAAGAAAGCTGGGTC
	TTCTTCAAGTATGGTGCCCCATG	CACCTCCTTCCTGAGATAAC
pCaR1B16-GW	GGGGACAAGTTTGTACAAAAAAGCAGGC	GGGGACCACTTTGTACAAGAAAGCTGGGTC
	TTCTCTCAATTCTCACCACCACC	GTTCACTGAATTTGACGTAT

5 Supplementary Data Table S2. Grading standards for evaluation of disease resistance of

6	pepper plants to <i>R. solanacearum</i> by root irrigation ³

Score	Condition
0	Pepper plant is normal and asymptomatic.
1	Plant has slight withering, the basal one or two leaves are withered, but the apical region of the plant is normal.
2	In addition to the top leaves, one or two leaves are withered, but the apical region of the plant is normal.
3	Two-thirds of the leaves of the pepper plant are withered, while the top of the plant is normal.
4	The whole plant is withered or dead.





10 Supplementary Data Fig.1|CaKAN3 sequence analysis and phylogenetic tree of CaKAN3

- 11 and the KANADI members in pepper, potato, tomato and Arabidopsis. a, The hidden
- 12 Markov model (HMM) profile of the GARP domain (Pfam: PF16731) (http://pfam.xfam.org/)

- 13 was used as a BLAST query against the pepper genome database PGP
- 14 (http://peppergenome.snu.ac.kr/) and the KANADI proteins in Arabidopsis, Solanum
- 15 lycopersicum and Solanum tuberosum from UniProt (https://www.uniprot.org/). Motifs were
- 16 identified by MEME tools in KANADIs. In total, 3 motifs were identified, and only the GARP
- 17 domain was identified in CaKAN3. **b**,Phylogenetic tree of KANADI members in pepper, potato,
- 18 tomato and Arabidopsis; the phylogenetic tree was constructed with the Tree Browser from
- 19 Solgenomics (https://solgenomics.net/tools/tree_browser).



22 Supplementary Data Fig.2| Response of CaKAN3 to Ralstonia solanacearum infection under

23 room temperature (RSRT), high-temperature and high-humidity (HTHH) stress and

24 Ralstonia solanacearum infection under high temperature and high humidity (RSHT). a,

25 Cis-elements in the promoter region of CaKAN3 by plant care

26 (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/). **b**, Transcript levels of *CaKAN3* in

27 the roots of pepper plants challenged with RSRT, HTHH or RSHT, its transcript levels under

28 RTHH (room temperature and high humidity) at 1 hpt were set to 1, and the data are shown as

29 the means \pm standard errors of four replicates. Different uppercase letters above the bars indicate

30 significant differences between means (P < 0.01) by Fisher's protected LSD test. Source data are

31 provided as a Source Data file.

32



36 Supplementary Data Fig.3|Nuclear Localization of CaKAN3 in N. benthamiana Epidermal

- 37 **Cells.** *N. benthamiana* leaves were infiltrated with Agrobacterium GV3101 cells containing
- 38 *35Spro:CaKAN3-GFP* (using *35Spro:GFP* as a control). NbH2B (histone H2B)-RFP was used
- 39 to indicate the nucleus. Subcellular localization of the CaKAN3-GFP fusion protein or control
- 40 GFP was captured on a fluorescence confocal microscope at 24 hpi. Fluorescence images (left),
- 41 bright-field images (middle), and the corresponding overlay images (right) of representative cells
- 42 expressing GFP or CaKAN3-GFP fusion protein are shown, $Bars = 50 \mu m$. The experiment was
- 43 carried out thrice with similar results.





46 Supplementary Data Fig.4|The effect of CaKAN3ectopicoverexpression on the response of

- 47 Nicotiana benthamiana plants to Ralstonia solanacearum infection under room temperature
- 48 (RSRT), hightemperature and high humidity (HTHH) or *Ralstonia solanacearum* infection
- 49 under hightemperature and highhumidity (RSHT). a, Confirmation of *CaKAN3*
- 50 overexpression by RT–qPCR and immunoblot analysis with antibody of GFP in the two T₂
- 51 transgenic *N. benthamiana* lines. α-GFP: anti body of GFP. **b**, The *CaKAN3*-overexpressing *N*.
- 52 *benthamiana* plants displayed lower level of disease index and higher level of resistance to
- 53 RSHT but not to RSRT compared to the wild-type control plants. The experiment was carried
- 54 out twice with similar results. **c**, *R. solanacearum*-inoculated, *CaKAN3*-overexpressing *N*.
- 55 *benthamiana* plants showed higher pathogen growth than the wild type (shown as colony-
- 56 forming units [cfu]) upon RSHT but not upon RSRT. Data are shown as the means ±standard
- 57 errors of four replicates. Asterisks above the bars indicate significant differences between means
- 58 (P < 0.01) by Fisher's protected t test. The center line represents the median value and the
- 59 boundaries indicate the 25th percentile (upper) and the 75th percentile (lower). Whiskers extend
- 60 to the largest and smallest value. In **b**, and **c**, the experiments were carried out three with similar
- 61 results. In **a**, to **c**, source data are provided as a Source Data file.



- 64 Supplementary Data Fig.5|The success of transient overexpression of CaHSFA1,CaHSF8,
- 65 CaKAN3 and CaKAN4 in *N. benthamiana* plants. The proteins were isolated from leaves of
- 66 *N. benthamiana* plants agroinfiltrated with GV3101 cells at 48 hpi. The experiment was carried
- 67 out once.





71 Supplementary Data Fig.6|Transcript expression level analysis and subcellular localization

72 **analysis of CaHSF8. a**, Transcript levels of *CaHSF8* in the roots of pepper plants challenged

73 with RSRT, HTHH or RSHT. The transcript levels of *CaHSF8* under RTHH at 1 hpt were set to

1, and the data are shown as the means \pm standard errors of four replicates. Different uppercase

75 letters above the bars indicate significant differences between means (P < 0.01) by Fisher's

76 protected LSD test. Source data are provided as a Source Data file. **b**, Subcellular localization of

77 CaHSF8-GFP in epidermal cells of *N. benthamiana* leaves. NbH2B (histone H2B)-RFP was used

to indicate the nucleus. Red and green fluorescence, visible light and merged images were taken

79 on a confocal microscope. Bars = $50 \ \mu m$.



80

81 Supplementary Data Fig.7|The silencing of CaHSF8 significantly reduced pepper immunity

against RSHT. a, The success of *CaHSF8* silencing by virus-induced gene silencing (VIGS)
 was determined by measuring the transcript levels of *CaHSF8* in RTHH-, RSRT-, HTHH- and

84 RSHT-challenged TRV:*CaHSF8* pepper plants at 24 hours post-treatment (hpt). The transcript

KSH1-chanengeu TKV. Cansro pepper plants at 24 hours post-treatment (hpt). The transci

85 levels of *CaHSF8* in TRV:00 pepper plants under RTHH were set to 1. **b**, Effect of *CaHSF8*

- silencing on the response of pepper plants to RSRT and RSHT treatment at 3 and 12 dpt,
- 87 respectively. **c**, and **d**, The growth of *R*. solanacearum in *R*. solanacearum-inoculated CaHSF8-
- 88 silencing plants under RSHH or upon HTHH, shown as colony-forming units (cfu). Data were
- shown as the means \pm standard errors of eight replicates. Asterisks above the bars indicate
- 90 significant differences between means (P < 0.01), as calculated with a t test. The center line
- represents the median value and the boundaries indicate the 25th percentile (upper) and the 75th
 percentile (lower). Whiskers extend to the largest and smallest value. e, Decreased flg22-induced
- 92 percentine (lower). Whiskers extend to the largest and smallest value. e, Decreased fig22-induced
- 93 H_2O_2 production in *CaHSF8*-silencingpepper plants upon HTHH. The results shown were
- 94 representative of two independent experiments. Data were shown as the means \pm standard errors

95 of six replicates. In **b**, and **c**, the experiments were carried out three with similar results. In **a**, **c**, 96



d, and **e**, source data are provided as a Source Data file.





benthamiana immunity against RSHT. a, and b, Confirmation of *CaHSF8* overexpression by 99

100 fluorescence detection, RT-qPCR and immunoblot analysis with antibody of GFP in the two T₂

101 transgenic *N. benthamiana* lines. α-GFP: antibody of GFP. **c**, The *CaHSF8*-overexpressing *N*.

benthamiana plants displayed lower level of disease index and higher level of resistance upon 102

103 RSHT but not upon RSRT than the wild-type control plants. The experiment was carried out

twice with similar results. d, R. solanacearum-inoculated CaHSF8-overexpressing N. 104

105 benthamiana plants showed higher pathogen growth than the wild type (shown as colony-

106 forming units [cfu]) upon RSHT but not upon RSRT. Data were shown as the means ±standard

107 errors of four replicates. The center line represents the median value and the boundaries indicate

108 the 25th percentile (upper) and the 75th percentile (lower). Whiskers extend to the largest and

- 109 smallest value. In **a** and **d**, the asterisk above the bars indicates significant differences between
- 110 means (P < 0.01) by Fisher's protected t test. In **a**, **c**, and **d**, source data are provided as a Source
- 111 Data file.
- 112



114 Supplementary Data Fig.9/The expression of CaHSF8 and CaKAN3 and their functions in immunity of pepper inbred lines against RSHT. a. The relative transcript level of CaKAN3 115 116 and CaHSF8 in pepper inbred lines with different level of resistance to RSHT in pepper plants 117 upon RTHH, RSRT, HTHH or RSHT. Data are shown as the means ± standard errors of three 118 replicates. The transcript levels of 101-1-c-2-3/RTHH were set to 1. b, The silencing efficiency 119 of CaKAN3 and CaHSF8 in different pepper inbred lines by RT-qPCR. The transcript levels of 120 *CaKAN3* and *CaHSF8* in TRV::00 pepper plants under RTHH were set to 1. Data are shown as 121 the means \pm standard errors of four replicates. Asterisks above the bars indicate significant 122 differences between means (P < 0.01) by Fisher's protected t test. c, The silencing of CaKAN3 or 123 *CaHSF8* significantly reduced pepper immunity against RSHT in pepper lines with higher level 124 of RSHT resistance but not in lines with lower level of RSHT resistance. d, The silencing of 125 *CaKAN3* or *CaHSF*8 significantly increased dynamic disease index from 0 to 12 dpt in pepper lines with high level of RSHT resistance but not in lines with lower level of RSHT resistance. 126 127 Data were shown as the means \pm standard errors of twelve replicates. e, The silencing of CaHSF8or CaKAN3supported an enhanced level of bacterial growth at 48 hpi by leaf inoculation 128 in pepper lines with high level of RSHT resistance but not in lines with lower level of RSHT 129 130 resistance. f, The success of transient overexpression of CaHSF8-HA or CaKAN3-HA by 131 immune blotting using antibody of HA in pepper line GZN13-36 and 203 that are susceptible to 132 RSHT. The experiment was carried out once. g, The transient overexpression of CaKAN3 but 133 not CaHSF8 significantly repressed the bacterial growth in the RSHT susceptible line GZN13-36

- and 203. **h**, The transient overexpression of CaKAN3 significantly repressed the proliferation of
- 135 RS by activating HR response in leaves of RSHT susceptible line GZN13-36 and 203 at 48 hpi,
- and conferred enhanced RSHT resistance at 6 dpi, but the transient overexpression of *CaHSF8*
- 137 did not make any difference. In **a**, **e**, and **g**, Different uppercase letters above the bars indicate
- 138 significant differences between means (P < 0.01) by Fisher's protected LSD test. In **c**, **d**, and **h**,
- 139 the experiments were carried out three with similar results. In **e** and **g**, the center line represents
- the median value and the boundaries indicate the 25th percentile (upper) and the 75th percentile
 (lower). Whiskers extend to the largest and smallest value. In **a**, **b**, **d**, **e**, and **g**, source data are
- 142 provided as a Source Data file.



144

145 Supplementary Data Fig.10|The effect of *CaKAN3* or *CaHSF8* silencing on the response of

- 146 pepper plants to inoculation of different *Ralstonia solanacearum* strains(FJ1470,GMI1000)
- and to *Pst DC3000*. a, The silencing of *CaKAN3* or *CaHSF8* significantly increased
 susceptibility of pepper plants to inoculation of both FJ1470 and GMI1000 or *Pst DC3000* under
- 148 Susceptionity of pepper plants to inoculation of both FJ1470 and GW11000 of *Pst DC5000* under 149 HTHH, but did not affect the response of pepper plant to the inoculation of *Pst DC3000* under
- 150 RTHH. **b**, The silencing of *CaKAN3* and *CaHSF8* significantly increased the dynamic disease
- index of RSHT challenged pepper plants from 0 to 12 dpt. Data were shown as the means \pm
- standard errors of twelve replicates. \mathbf{c} , The silencing of *CaKAN3* or *CaHSF8* promoted the
- *Ralstonia solanacearum* or *Pst DC3000* growth displayed by cfu(clone forming units) in the
- leaves of pepper plants inoculated using GMI1000,FJ1470 at 48 hpt or *Pst DC3000* at 2 and
- 155 4dpt.Different upper ase letters above the bars indicate significant differences between means
- (P < 0.01) by Fisher's protected LSD test. The center line represents the median value and the
- boundaries indicate the 25th percentile (upper) and the 75th percentile (lower). Whiskers extend
- to the largest and smallest value. In **a**, and **b**, the experiments were carried out three with similar
- results. In **b**, and **c**, source data are provided as a Source Data file.
- 160



162 Supplementary Data Fig.11| The possible CaKAN3- or CaHSF8-bound cis-elements were

163 determined by ChIP-seq and the transcriptional activity of CaKAN3 or CaHSF8. a, The

- peak measured by ChIP-seq and its matched cis-element by HOMER motif search. b, The 164 binding of CaKAN3-GST or CaHSF8-GST to the 3×AACAA or 3×HSE cis-element fragment 165 by electrophoretic mobility shift assay in vitro. The experiment was carried out once. c, Activity 166 of GUS driven by synthetic promoters containing AACAA or HSE in leaves of transient 167 overexpression of CaKAN3 or CaHSF8 or control pepper plants at 48 hpt. Data represent the 168 169 mean \pm SD of twelve biological replicates. ifferent uppercase letters above the bars indicate 170 significant differences between means (P < 0.01) by Fisher's protected LSD test. Source data are provided as a Source Data file. The center line represents the median value and the boundaries 171 172 indicate the 25th percentile (upper) and the 75th percentile (lower). Whiskers extend to the 173 largest and smallest value. d, Transcriptional activity of CaKAN3 or CaHSF8 by nutrition 174 disfigurement assay in GAL4-based yeast one-hybrid system. In c, and d, the experiments were
- 175 carried out twice with similar results.





178 Supplementary Data Fig.12|Bindings of CaKAN3 to the promoters of *CaR1B23*, *CaR1B12*,

179 *CaR1A*, *CaR1B11*, *CaR1A6* and *CaR1B16* by ChIP-qPCR. a, Schematic diagram of the cis-

- 180 element HSE (red squares) and AACAA (blue squares) in the promoters of *CaR1B23*, *CaR1B12*,
- 181 *CaR1A*, *CaR1B11*, *CaR1A6* and *CaR1B16*. P1-P22 were fragments for designing the primer
- pairs used to amplify the target DNA fragments in the promoters. **b**, to **g**, The bindings of
- 183 CaKAN3 to the different cis-elements by ChIP-qPCR,GV3101 cells containing 35S:CaKAN3-
- 184 *HA* were infiltrated into pepper leaves, which were harvested at 48 hpi for ChIP–qPCR analysis
- using specific primer pairs; IP: IgG was used as the control. The enrichment levels of the tested
- 186 genes were compared with those in the control, and the relative enrichment of IgG was set to a 187 value of 1 after normalization by input. Data represent the mean \pm SD of three biological
- replicates. Different capital letters above the bars indicate significant differences (P < 0.01) by
- Fisher's protected t test. In **b**, to **g**, source data are provided as a Source Data file.
- 190



192 Supplementary Data Fig.13|Neither CaKAN3 nor CaHSF8 bound the promoters of

193 *CaMgst3* and *CaPRP1*. a, The distribution of the fragments in the promoter of CaMgst3 or

194 CaPRP1 corresponding to primers used in ChIP-qPCR to assay the enrichment of CaKAN3 or

195 CaHSF8 in the promoters of *CaMgst3* and *CaPRP1*. **b**, The enrichment of CaKAN3 in the

promoter of CaMgst3 or CaPRP1 by ChIP-qPCR. **c**, The enrichment of CaHSF8 in the promoter

of *CaMgst3* and *CaPRP1* by ChIP-qPCR. b, and c, GV3101 cells containing *35S:CaKAN3-HA* or *35S:CaHSF8-HA* were infiltrated into pepper leaves, which were harvested at 48 hpi for

198 Of 555. Cansport A were initiated into pepper leaves, which were harvested at 48 hpr for 199 ChIP-qPCR analysis using specific primer pairs; IP: IgG was used as the control. The enrichment

200 levels of the tested genes were compared with those in the control, and the relative enrichment of

anti-HA was set to a value of 1 after normalization by input. Data represent the mean \pm SD of

202 three biological replicates. In **b**, to **c**, source data are provided as a Source Data file.



- 205 Supplementary Data Fig.14|The targeting of NLR genes in N. benthamiana by CaKAN3 or
- 206 **CaHSF8**. **a**, Phylogenetic analysis of the five tested NLRs with its orthologs in *N*. *benthamiana*.
- 207 b, The distribution of CaKAN3 and CaHSF8 responsive cis-elements and the fragments
- 208 corresponding to primers used in ChIP-qPCR or ChIP-PCR assay in the promoters of NLR genes
- 209 in *N. benthamiana*. **c**, The result of ChIP-PCR showed that both CaKAN3 and CaHSF8 bound
- 210 the promoters of all of the five tested NLR genes. The experiment was carried out once. **d**, The
- 211 data from ChIP-qPCR showed that both CaKAN3 and CaHSF8 bound the promoters of all of the
- 212 five tested NLR genes. The enrichment levels of the tested genes were compared with those in
- 213 the control, and the relative enrichment of IP: Anti-GFP/CaKAN3-GFP or IP: Anti-
- 214 GFP/CaHSF8-GFP was set to a value of 1 after normalization by input. Data represent the mean
- \pm SD of three biological replicates. Asterisks above the bars indicate significant differences
- between means (P < 0.01) by Fisher's protected t test. **e**, The data from RT-qPCR showed that the
- 217 overexpression of both CaKAN3 and CaHSF8 significantly upregulated the five tested NLR
- 218 genes in *N. benthamiana* plants challenged with HTHH at 1 hpt. Data represent the mean \pm SD
- of four biological replicates. The transcript levels of WT were set to 1, *NbActin* was used as an internal control, and different capital letters above the bars indicate significant differences (P <
- (1 < 0.01) by Fisher's protected LSD test. In **d**, to **e**, source data are provided as a Source Data file.









- 228 treatment with different temperatures. a, CaR1B23, CaR1B12, CaR1A, CaR1B11, CaR1A6,
- 229 *CaR1B16* and *CaHSF8* were upregulated at 1 hpt of high temperature treatment. **b**, The
- 230 transcript levels of CaR1B23, CaR1B12, CaR1A, CaR1B11, CaR1A6 and CaR1B16 upon high
- temperature (37 °C, 90% humidity) treatment at 0, 1, 6, and 24 hpt by GUS activity assay. Data 231
- 232 represent the mean \pm SD of four biological replicates. Different capital letters above the bars
- 233 indicate significant differences ($P \le 0.01$) by Fisher's protected LSD test. c, The transcript levels
- 234 of CaR1B23, CaR1B12, CaR1A, CaR1B11, CaR1A6 and CaR1B16 upon high temperature
- 235 treatment (37 °C, 90% humidity) at 0, 1, 3, 6, 12, and 24 hpt by a LUC assay. d, *CaR1B23*,
- 236 CaR1B12, CaR1A, CaR1B11, CaR1A6, CaR1B16 and CaHSF8 were upregulated by different
- 237 high temperatures (from 31 to 37 °C, 90% humidity) treatment at 1 hpt. In **a**, and **d**, means (n =
- 238 4) of fold induction compared to 0 hpt treatment shown as log2 were used to construct the
- 239 heatmap using TBtools. e, The transcripti levels of CaR1B23, CaR1B12, CaR1A, CaR1B11,
- 240 *CaR1A6* and *CaR1B16* upon high temperature(25, 28, 31, 34, 37, 45 °C, 90% humidity) at 1 hpt
- 241 by LUC assay. In **a**, **b**, and **d**, source data are provided as a Source Data file.





245 The success of NLR silencing by virus-induced gene silencing (VIGS) by measuring the

246 transcript levels of NLRs in RSRT-, HTHH- or RSHT-challenged TRV:NLR pepper plants at 1 247 hour post-treatment (hpt). b, Effect of NLR silencing on the response of pepper plants to RSRT 248 and RSHT treatment at 3 and 9 dpt. c, The disease indices of CaKAN3-silenced pepper plants 249 challenged with RSRT or RSHT from 0 to 12 dpt. The experiment was carried out twice with 250 similar results. Data are shown as the means \pm standard errors of 24 replicates. Asterisks above 251 the bars indicate significant differences between means (P < 0.01), as calculated with a t test. **d**, 252 The growth of R. solanacearum in R. solanacearum-inoculated NLR-silenced plants at room 253 temperature or under HTHH, shown as colony-forming units (cfu). Data are shown as the means 254 \pm standard errors of eight replicates. Asterisks above the bars indicate significant differences 255 between means (P < 0.01), as calculated with a t test. The center line represents the median value 256 and the boundaries indicate the 25th percentile (upper) and the 75th percentile (lower). Whiskers 257 extend to the largest and smallest value. e, Relative transcript levels of *CaMgst3* and *CaPRP1* in TRV:00 and TRV:NLRs pepper plants challenged by RSHT. f. The result of BiFC showed that 258 259 CaR1B-11 interacted with CaR1B23, CaR1A-6, CaR1B12, CaR1A, CaR1B16 in the epidermal cells of *N. benthamiana* leaves by agroinfiltration based transient overexpression at 48 hpi. The 260 261 experiment was carried out once. g, CaR1B-11 interacted with CaR1B23, CaR1A-6, CaR1B12, CaR1A, CaR1B16 by MST, all of the proteins were expressed in and isolated from E.coli strain 262 263 BL21 and the isolated CaR1B-11 was labeled with red fluorescence using a kit provided by manufactory (Monolith NT.115). In a, and e, The transcript levels of TRV:00 were set to 1, 264 CaActin was used as an internal control, and asterisks above the bars indicate significant 265 differences between means (P < 0.01), as calculated with Fisher's protected t test. In **b**, and **c**, the 266 267 experiments were carried out three with similar results. In **a**, **c**, **d**, and **e**, source data are provided 268 as a Source Data file.

269



271

272 Supplementary Data Fig.17|The effect of NLRs silencing on the response of N. benthamiana 273 plant to RSHT. a, The confirmation of NLR gene silencing by RT-qPCR, the result showed the 274 all of the NLR genes were successfully silenced by VIGS. Data represent the mean \pm SD of four 275 biological replicates. The transcript levels of TRV::00 plants were set to 1, NbActin was used as 276 an internal control, and asterisks above the bars indicated significant differences between means 277 (P<0.01) by Fisher's protected t test. **b**, The silencing of NbR1B23, NbR1B11 or NbR1B12 278 significantly increased *N. benthamiana* susceptibility to RSHT, and the silencing of the other two 279 NLR genes did not produced any phenotypic effect. c, The silencing of NbR1B23, NbR1B11 or 280 NbR1B12 significantly increased the dynamic disease index of N. benthamiana plants challenged 281 with RSHT from 0 to 12 dpt, the silencing of other two NLR genes did not affect the dynamic 282 disease index. Data represent the mean \pm SD of twelve biological replicates. **d**, The silencing of NbR1B23, NbRIB11 or NbR1B12 significantly promoted the bacterial growth in leaves of N. 283 284 benthamiana plants challenged with RSHT at 72 and 96 hpt. Different capital letters above the 285 bars indicate significant differences (P < 0.01) by Fisher's protected LSD test. The center line 286 represents the median value and the boundaries indicate the 25th percentile (upper) and the 75th percentile (lower). Whiskers extend to the largest and smallest value. In b, and c, the 287 288 experiments were carried out twice with similar results. In a, c, and d, source data are provided 289 as a Source Data file.

292



293 Supplementary Data Fig.18|Regulation of the transcription of the six tested NLR genes by

CaKAN3 and CaHSF8. a, The effect of CaKAN3 or CaHSF8 transient overexpression on the
 transcript levels of the six tested NLR genes in pepper leaves treated with condition of 37 °C and
 90% humidity at 0 and 1 hpt by RT-qPCR. b, Relative transcript levels of NLRs in TRV:00 and

- 297 TRV:*CaKAN3* or TRV:*CaHSF8* pepper plants challenged by condition of 37 °C and 90%
- humidity at 0 and 1 hpt. In **a** and **b**, the transcript levels of TRV:00/0 hpt were set to 1,and
- 299 *CaActin* was used as an internal control. Data are shown as the means ± standard errors of four
- 300 replicates. Asterisks above the bars indicate significant differences between means (P < 0.01), as
- 301 calculated with a t test. In **a**, and **b**, source data are provided as a Source Data file.



303

304 Supplementary Data Fig.19|CaHSF8 acted positively in pepper thermotolerance. a,

305 CaHSF8 overexpressing N. benthamiana plants displayed enhanced thermotolerance compared

to the wild type control plants. The experiments were carried out twice with similar results. **b**,

307 and **c**, *CaHSF8* overexpressing *N*. *benthamiana* plants showed higher Fv/Fm or Δ F/Fm upon

308 ETHH (42 °C, 90% humidity) treatment. **d**, *CaHSF8* overexpressing *N. benthamiana* plants

- 309 showed lower level H_2O_2 accumulation displayed by DAB staining upon ETHH (42 °C, 90%)
- humidity) compared to the wild type control plants. **e**, and **f**, *CaHSF8*-silencing pepper plants
- showed lower Fv/Fm or Δ F/Fm upon ETHH (42 °C,90% humidity) compared to the wild type
- 312 control plants. **g**, *CaHSF8*-silenced pepper plants accumulated higher level of H_2O_2
- accumulation displayed by DAB staining upon ETHH (42 °C, 90% humidity) treatment. In **a**, **c**,
- and **f**, source data are provided as a Source Data file.
- 315



317 Supplementary Data Fig.20|Direct regulation of a subset of HSP genes by CaHSF8 alone in

318 pepper plants upon ETHH. a, *CaHSF8* silencing pepper plants displayed reduced

- thermotolerance upon ETHH (42 °C, 90% humidity). **b**, Mortality of pepper plants upon ETHH
- 320 from 0 to 12 dpt (days post-treatment). c, CaHSF8 overexpressing N. benthamiana plants
- 321 displayed increased thermotolerance upon ETHH compared to the wild type control plants. **d**,

- 322 Mortality of the two CaHSF8 overexpressing N. benthamiana lines, CaHSF8#1 and CaHSF8#2
- 323 upon ETHH, 12 plants of each line were calculated. **e**, Integrative Genomics Viewer (IGV)
- 324 images of ChIP-seq showed that the promoters of CaHSP17.4B, CaHSP70, CaHSP18.2 and
- 325 *CaHSP70-5* were targeted by CaHSF8. **f**, The data from ChIP-qPCR showed that *CaHSP17.4B*,
- 326 CaHSP70, CaHSP18.2 and CaHSP70 were directly targeted by CaHSF8. The relative
- 327 enrichment of Anti-HA/CaHSF8-HA was set to a value of 1 after normalization by input. Data
- 328 are shown as the means \pm standard errors of three replicates. Asterisks above the bars indicate
- 329 significant differences between means (P < 0.01), as calculated with a t test. **g**, *CaHSP17.4B*,
- 330 *CaHSP70*, *CaHSP18.2* and *CaHSP70-5* are upregulated by the transient overexpression of
- 331 *CaHSF8* in pepper leaves. Data are shown as the means \pm standard errors of four replicates.
- Asterisks above the bars indicate significant differences between means (P < 0.01), as calculated
- with a t test. **h**, The silencing of *CaKAN3* did not reduce the targeting of *CaHSP17.4B*,
- 334 *CaHSP70*, *CaHSP18.2* and *CaHSP70-5* by CaHSF8. The relative enrichment of Anti-
- HA/TRV::00 was set to a value of 1 after normalization by input. Data are shown as the means \pm
- 336 standard errors of three replicates. Asterisks above the bars indicate significant differences
- between means (P < 0.01), as calculated with a t test. In **a**, to **d**, the experiments were carried out
- twice with similar results. In **b**, **d**, **f**, **g**, and **h**, source data are provided as a Source Data file.



- 341 Supplementary Data Fig.21| The negative controls in Fig. 5a and the success confirmation
- 342 of transient overexpression in Fig. 5b. a, The negative controls in BiFC assay in Fig. 5a. b,
- 343 The success of transient overexpression of CaHSFA1-GLUC, CaHSF8-CLUC, CaKAN3-NLUC
- 344 and CaKAN4-NLUC in pepper leaves by immunoblotting using antibody of NLUC or CLUC in
- 345 Fig. 5b. The experiment was carried out once.

346



350 Supplementary Data Fig.22|Transient overexpression of CaHSF8 clearly upregulated the

four HSP genes in pepper plants challenged HTHH or RSHT at 1 and 6 hpt. The effect of

352 transient overexpression of CaHSF8 on the transcript levels of HSPs in pepper leaves upon

353 HTHH or RSHT at 1 and 6 hpt by RT-qPCR. The transcript levels of 35S:00 plants upon HTHH

at 1 hpt were set to 1, and *CaActin* was used as an internal control. Data are shown as the means \pm

355 standard errors of four replicates. Asterisks above the bars indicate significant differences

between means (P < 0.01), as calculated with a t test. Source data are provided as a Source Data file.

348



359 Supplementary Data Fig.23|Enrichment of CaKAN3 and CaHSF8 to the promoters of NLR

360 and HSP genes upon RTHH and HTHH at 1 and 6 hpt or at 1 hpt upon different

361 temperature (28 °C, 37 °C, and 45 °C, 90% humidity). a, The enrichment of CaHSF8 in the 362 promoters of the 6 NLR genes and 4 HSP genes, the chromatins used for ChIP-qPCR were

isolated from CaHSF8 transiently overexpressing pepper plants challenged with RTHH (28 °C,

90% humidity) and HTHH (37 °C, 90% humidity) at 1 and 6 hpt, the relative enrichment in 364 pepper plants upon RTHH at 1 hpt was set to a value of 1 after normalization by input. Asterisks 365 366 above the bars indicate significant differences between means (P < 0.01), as calculated with a t test. b, The enrichment of CaHSF8 in the promoters of the 6 tested NLR genes and 4 tested HSP 367 genes, the chromatins used for ChIP-qPCR were isolated from leaves of pepper plants upon 368 RTHH, HTHH or ETHH (45 °C, 90% humidity) at 1 hpt, the relative enrichment of CaHSF8 in 369 370 the promoters upon RTHH at 1 hpt was set to a value of 1 after normalization by input. c, The 371 enrichment of CaKAN3 in the promoters of the 6 NLR genes, the chromatins used for ChIPqPCR were isolated from CaKAN3 transiently overexpressing pepper plants challenged with 372 373 RTHH or HTHH at 1 and 6 hpt. The relative enrichment of CaKAN3 to the six tested promoters 374 upon RTHH at 1 hpt was set to a value of 1 after normalization by input. **d**, The enrichment of 375 CaKAN3 in the promoters of the 6 tested NLR genes, the chromatins used for ChIP-qPCR were 376 isolated from leaves of pepper plants upon RTHH or HTHH at 1 hpt, the relative enrichment of KAN3 to the 6 tested NLR gene upon RTHH at 1 hpt was set to a value of 1 after normalization 377 by input. In **a** to **d**, data were shown as the means \pm standard errors of three replicates; different 378 379 capital letters above the bars indicate significant differences (P < 0.01) by Fisher's protected LSD 380 test. In **a**, and **b**, the experiments were carried out twice with similar results. In **a**, to **d**, source 381 data are provided as a Source Data file.

384 **References**

- Yang, S. *et al.* Solanaceous plants switch to cytokinin-mediated immunity against Ralstonia
 solanacearum under high temperature and high humidity. *Plant, cell & environment*45,
 459-478, doi:10.1111/pce.14222 (2022).
- Dang, F. *et al.* CaWRKY40, a WRKY protein of pepper, plays an important role in the
 regulation of tolerance to heat stress and resistance to Ralstonia solanacearum infection.
 *Plant, cell & environment*36, 757-774, doi:10.1111/pce.12011 (2013).
- 391 3 Shen, L. *et al.* Pepper CabZIP63 acts as a positive regulator during Ralstonia solanacearum
 392 or high temperature-high humidity challenge in a positive feedback loop with CaWRKY40.
 393 *Journal of experimental botany*67, 2439-2451, doi:10.1093/jxb/erw069 (2016).
- 394