

**Endoplasmic Reticulum Stress Responses Function in the HRT-mediated
Hypersensitive Response in *Nicotiana benthamiana***

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Supporting Information

Table S1 Putative *CaBLP5* homologs identified in *Nicotiana benthamiana*

<i>N. benthamiana</i> homolog*	bp	Annotation	E-value	Identity
<i>BiP5</i> (<i>Nbv3K685813373</i>)	2148	Luminal-binding protein 5 (BiP5), Precursor	0.0	93%
<i>BiP4</i> (<i>Nbv3K645786225</i>)	2004	Nicotiana benthamiana ER luminal-binding protein (BLP4)	0.0	89%
<i>Nbv3K645786225</i>	2126	Luminal-binding protein 4 (BiP4), Precursor	0.0	82%
<i>Nbv3K645789686</i>	2203	Luminal-binding protein 5 (BiP 5), Precursor (similar to)	0.0	81%
<i>Nbv3K585690033</i>	1028	Luminal-binding protein 2 (AtBiP2), Precursor	2E-9	83%
<i>Nbv3K585703505</i>	1945	Heat shock cognate 70 kDa protein (similar to)	2E-13	81%
<i>Nbv3K765636570</i>	998	Heat shock cognate 70 kDa protein 2 (similar..to)	2E-10	82%

**CaBLP5* homologs were isolated using BLAST searches in the *N. benthamiana*_transcriptome_v3_unigenes95 database (<http://benth-web-pro-1.ucc.usyd.edu.au/blast/search.php>), and retrieved six *CaBLP5* homologs, which have less than 10^{-9} e-value and more than 80% identity with positive orientation.

Table S2 List of oligonucleotide primers used.

Primers	Sequence	Utilization
<i>Actin-F</i>	5'-TGGACTCTGGTGATGGTGTC-3'	RT-PCR
<i>Actin-R</i>	5'-CCTCCAATCCAAACTGTGA-3'	RT-PCR
<i>RAR1-F</i>	5'-ATGGAGAGACTTCGTTGCCA-3'	RT-PCR
<i>RAR1-R</i>	5'-TCTAGGACAAGCTTCTTTTCG-3'	RT-PCR
<i>Actin-F</i>	5'-AGAGGCTACTCTTTTACCACCACGG-3'	qRT-PCR
<i>Actin-R</i>	5'-TGAGCTGGTCTTTGCTGTTTCAAGT-3'	qRT-PCR
<i>HRT-F</i>	5'-TGATGGATTTCGCATGGGTCT-3'	qRT-PCR
<i>HRT-R</i>	5'-AGCAGCATCTTCCAACCTCT-3'	qRT-PCR
<i>TCV CP-F</i>	5'-AGCCAAACCTCCGCCCAAC-3'	qRT-PCR
<i>TCV CP-R</i>	5'-CTGATACCATCCGCCACAAAGC-3'	qRT-PCR
<i>HSP90-F</i>	5'-TTGAGACTGCCCTCCTCACC-3'	qRT-PCR
<i>HSP90-R</i>	5'-TCGTCTGTTCGGGAGCTTG-3'	qRT-PCR
<i>SGT1-F</i>	5'-GCCAGAGGAGGTGGTGGTGA-3'	qRT-PCR
<i>SGT1-R</i>	5'-AAGTTTCGTCACCCGGCACA-3'	qRT-PCR
<i>Hin1-F</i>	5'-GCCATGCCGGAATCCAATTT-3'	qRT-PCR
<i>Hin1-R</i>	5'-TTGCAGAGGCAGCCAAAGAGA-3'	qRT-PCR
<i>Hsr203J-F</i>	5'-GCTCCGGCGGGAACATAGTC-3'	qRT-PCR
<i>Hsr203J-R</i>	5'-TCCGATAGGACCGCACGAAA-3'	qRT-PCR
<i>NTCP23-F</i>	5'-AGAGACAGGTTGGGGGCAGC-3'	qRT-PCR
<i>NTCP23-R</i>	5'-CAAGATCCGCACTTGCCCTG-3'	qRT-PCR
<i>PR1a-F</i>	5'-GGGACGACCAGGTAGCAGCC-3'	qRT-PCR
<i>PR1a-R</i>	5'-CATTGACCCACATCTCAACGGC-3'	qRT-PCR
<i>PR2-F</i>	5'-TGTTGCTCCTGCCATGCAAA-3'	qRT-PCR
<i>PR2-R</i>	5'-GGGCGGGTTGGTATTCGCTA-3'	qRT-PCR
<i>PR5-F</i>	5'-GGCATGGCTAAGTCAATCCACC-3'	qRT-PCR
<i>PR5-R</i>	5'-GTCTCCGTCGCCACCAGATG-3'	qRT-PCR
<i>CYP71D20-F</i>	5'-AAGGTCCACCGCACCATGTCCTTAGAG-3'	qRT-PCR
<i>CYP71D20-R</i>	5'-AAGAATTCCTTGCCCCTTGAGTACTTGC-3'	qRT-PCR
<i>WRKY8-F</i>	5'-AACAAATGGTGCCAATAATGC-3'	qRT-PCR
<i>WRKY8-R</i>	5'-TGCATATCCTGAGAAACCATT-3'	qRT-PCR
<i>bZip60-F</i>	5'-CCTGCTTTGGTTCATGGGCATCAT-3'	qRT-PCR
<i>bZip60-R</i>	5'-CACATCACAATTCCTCAATAATG-3'	qRT-PCR
<i>Calreticulin-F</i>	5'-TGCTCGTCGCTGTCTCTCC-3'	qRT-PCR
<i>Calreticulin-R</i>	5'-GCGTCTCCATTCCACTTGCC-3'	qRT-PCR
<i>Beclin1-F</i>	5'-CGTCGTTTTGCCTCCACCAG-3'	qRT-PCR
<i>Beclin1-R</i>	5'-GGAGCATTTTGAGGCCACC-3'	qRT-PCR
<i>p58^{IPK}-F</i>	5'-CTTGCTGTGGAGGAGTACAAAG-3'	qRT-PCR
<i>p58^{IPK}-R</i>	5'-CTCCCTCCCAATCTTCTGTTAG-3'	qRT-PCR
<i>PR4-F</i>	5'-GGTGTGGGTCTACACCAGAATA-3'	qRT-PCR
<i>PR4-R</i>	5'-CAATTCTCACTGTGGTCTGAGC-3'	qRT-PCR
<i>Nbv3K685813373-F (BiP5)</i>	5'-GTTCCAGAGGCAGTGTCTGT-3'	qRT-PCR
<i>Nbv3K685813373-R (BiP5)</i>	5'-TCTCAGGATTAACAGCAGCC-3'	qRT-PCR
<i>Nbv3K645786225-F (BiP4)</i>	5'-TTTCTCCTCCTTCTACCCCTCT-3'	qRT-PCR
<i>Nbv3K645786225-R (BiP4)</i>	5'-AACGATTGCTAGCACCACCA-3'	qRT-PCR
<i>Nbv3K585690033-F</i>	5'-CTTACCCTTCTCTCTTCT-3'	qRT-PCR
<i>Nbv3K585690033-R</i>	5'-CTCGACGTTATTCTCTCT-3'	qRT-PCR
<i>YFP-F</i>	5'-GAAGCAGCACGACTTCTTCA-3'	qRT-PCR
<i>YFP-R</i>	5'-CGGCCATGATATAGACGTTG-3'	qRT-PCR

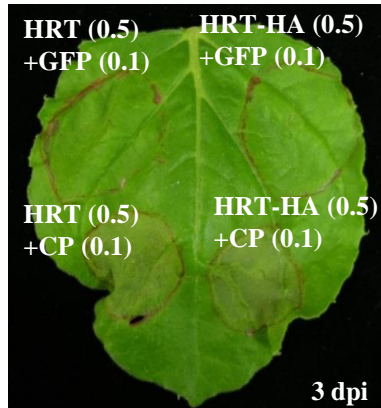


Fig. S1 HA-tagged HRT is functional. Co-expression of *HRT-HA* and *Turnip crinkle virus* (TCV) coat protein (CP) induced the hypersensitive response (HR) in *N. benthamiana*. Numbers indicate concentration (OD_{600}) of *Agrobacterium* cells. The images were photographed 3 days post-infiltration (dpi).

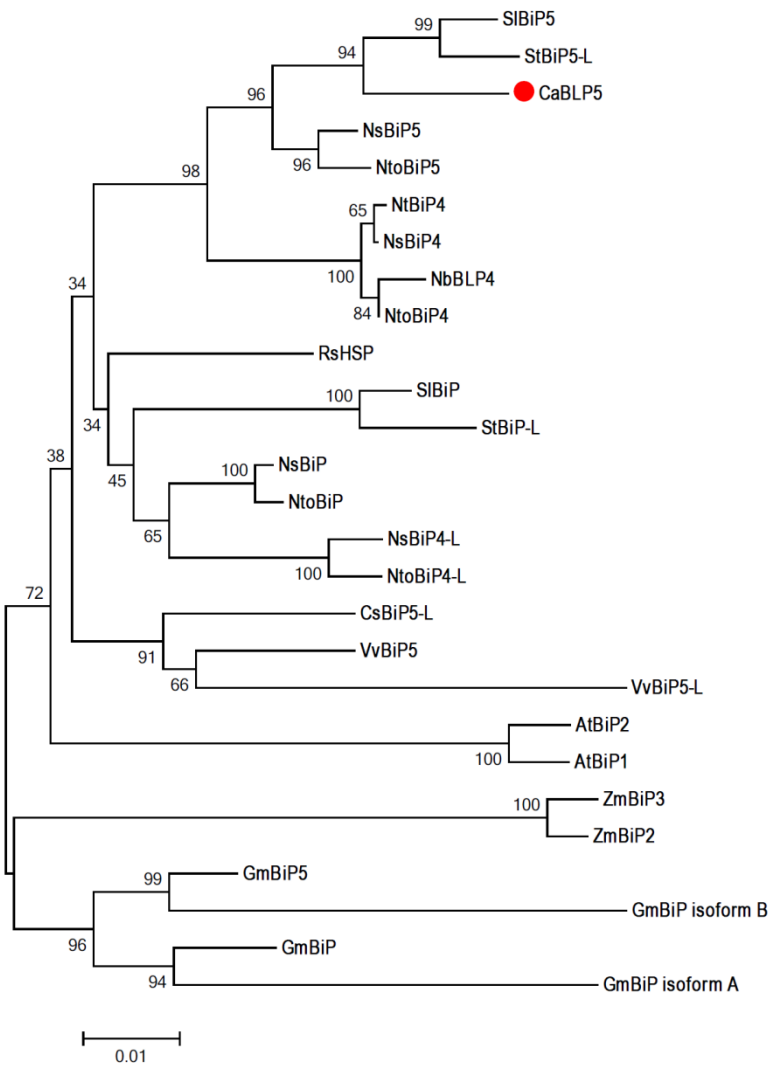


Fig. S2 Phylogenetic analysis of CaBLP5 (GenBank accession No. KC912859) and its homologs. The amino-acid sequences of 26 CaBLP5 homologs were imported into MEGA 6 (Tamura *et al.*, 2013) for multiple sequence alignment with ClustalW (Larkin *et al.*, 2007). Phylogenetic analysis was performed using the neighbor-joining (Saitou and Nei., 1987) and bootstrap methods. The bootstrap consensus tree was inferred from 500 replicates. The scale bar indicates the lengths of the branches (relative evolutionary distance). The protein sequences are deposited in GenBank under the following accession numbers: *Solanum lycopersicum* SIBiP5 (XP_004234985.1) and SIBiP (NP_001234636.1), *Solanum tuberosum* StBiP5-L (XP_006350519.1) and StBiP-L (XP_006343810.1), *Nicotiana benthamiana* NbBLP4 (ACK55195.1), *Zea mays* ZmBiP3 (NP_001105894.1) and ZmBiP2 (NP_001105893.1), *Arabidopsis thaliana* AtBiP2 (NP_851119.1) and AtBiP1 (NP_198206.1), *Nicotiana tabacum* NtBiP4 (Q03684.1), *Cucumis sativus* CsBiP5-L (XP_004143862.1), *Vitis vinifera* VvBiP5 (XP_002263323.1) and VvBiP5-L (XP_002276268.2), *Nicotiana sylvestris* NsBiP5 (XP_009773333.1), NsBiP4 (XP_009788736.1), NsBiP (XP_009802727.1), and NsBiP4-L (XP_009770477.1), *Nicotiana tomentosiformis* NtoBiP5 (XP_009592769.1), NtoBiP4 (XP_009588550.1), NtoBiP (XP_009593820.1), and NtoBiP4-L (XP_009619852.1), *Ricinus communis* RsHSP (XP_002518865.1), *Cucumis sativus* CsBiP5-L (XP_004143862.1), and *Glycine max* GmBiP (XP_003525327.2), GmBiP isoform A (NP_001234941.1), and GmBiP isoform B (NP_001238736.1).

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Nbv3K645789686 -----
Nbv3K685813373 -----
NbBLP4 -----
CaBLP5 -----
Nbv3K585690033 -----

Nbv3K585703505 -----
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Nbv3K645786225 AAAAATTGTACAAAAAGTGTGAAGAATTTGTTATCTGGGTCTTGAATAA----- 108
Nbv3K645789686 -----
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CaBLP5 -----GGAGAAGAGAAAAAGGAAGAAGATATTGTTCG----- 32
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Nbv3K585703505 -----
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Nbv3K645789686 -----
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CaBLP5 -----
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Nbv3K585703505 -----
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Nbv3K645789686 -----
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CaBLP5 -----TTTTGGATCTGCGAGCTATGGCT 55
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Nbv3K645789686 -----
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Nbv3K645789686 -----
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CaBLP5 T-----TTGCATTTTTCGATAGCTAAAAG-AAGAAGCTACCAAGTTAGGAAC 159
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Nbv3K585703505 AGCAATTG--GTAT----TGA

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Nbv3K585703505 TGATCGTGGAGATCA---TAGCGAATGATCAAG---GGAACAGGACGACACCGTCTT 135

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Nbv3K685813373	TGTTTCAGGAAGACAATGGGTCCTGTTAAGAAGGCTATGGAGGATGCTGGGCTACAAAAGA	1245
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Nbv3K765636570	-----	
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Nbv3K645789686	ACCAAATTGATGAGATAGTTCTTGTGGTGGAAAGCACTAGAAATTCAAAAAGTGAACAAC	997
Nbv3K685813373	ACCAGATTGACGAAATTGTATTGGTTGGGGAAGTACCAGGATTCCAAAAAGTTCAACAGC	1305
CaBLP5	ACCAGATCGACGAGATTGTCTTGGTTGGTGGAAAGTACCAGGATTCCAAAAAGTTCAACAGC	1190
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Nbv3K765636570 -----
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Nbv3K645789686 TTGCTTATGGTGCCTGTGTCCAAGTGGTATCTTAAGTGGAGAGGGTGGTGACGAAAATA 1117
Nbv3K685813373 TTGCTTATGGTGCAGCTGTACAAGGAGGAATCTTGAGTGGAGA GGGAGGTGATGAAACCA 1425
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Nbv3K765636570 -----
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CaBLP5 AAGATATCTTCTCCTGGATGTTGCTCCACTGACTCTTGGTATTGAAACTGTTGGAGGAG 1370
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Nbv3K585703505 TAATGACTGTGTTGATACCAAGGAACACAACCTATTTCCACCAAGAAAGAGCAAGTGTCT 1307
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CaBLP5 TGATGACAAAAGTTGATCCCAAGAAACACCGTCATTCTACCAAGAAGTCTCAAGTCTTCA 1430
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Nbv3K685813373 CCACTTACCAGGATCAGCAGACAACAGTAACAATTTTCGGTCTTTGAAGGTGAACGCAGTC 1605
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CaBLP5 **GCTTGAGTCAAGAAGAAATTTGAACGTATGGTGAAGGAGGCTGAGGAGTTTCTGAGGAGG** 1730
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Nbv3K765636570 -----
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Nbv3K645789686 AGAAAGAGGACTACGAGGAGAAGCTGAAAGAGGTCGAGGCAGTATGCAACCCAATCATCA 1777
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Nbv3K765636570 -----
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CaBLP5 CCGCTGTGTATCAGAGGT---CTGGTGGAGCCCCAGGAGGTGCCA-----GTGAGGATT 2021
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Nbv3K765636570 -----
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Nbv3K585703505 -----
Nbv3K765636570 -----
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Nbv3K585690033 -----

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Nbv3K585703505 -----
Nbv3K765636570 -----
Nbv3K645786225 -----
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Nbv3K685813373 -----
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Nbv3K585690033 -----

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Fig. S3 Multiple sequence alignment of the CaBLP5 and six *N. benthamiana* BiP genes with Clustal 2.1. The asterisks at the bottom line of the alignment indicate identical residues in a given sequence position. Within the aligned sequences, the dashes indicate the gaps that were inserted in order to optimize the alignment. The CaBLP5 nucleotide sequences in red color used for *Tobacco rattle virus* (TRV)2-*CaBLP5* construct. Residues underlined red represent gene-specific primer sequences used for detecting each gene expression in *GFP*- or *CaBLP5*-silenced plants. Residues in turquoise indicate stretches of more than 21 nucleotides (Liu *et al.*, 2002) that is identical between TRV2-*CaBLP5* and the corresponding *NbBiP* homologs. Note that TRV2-*CaBLP5* contains a stretch of 21 nucleotides of 100% identity with *Nbv3K645786225* and *Nbv3K645789686* and multiple 21-nucleotide stretches of 100% identity with *Nbv3K685813373*, while no stretches of 21 nucleotides with perfect matches to other *BiP* homologs.

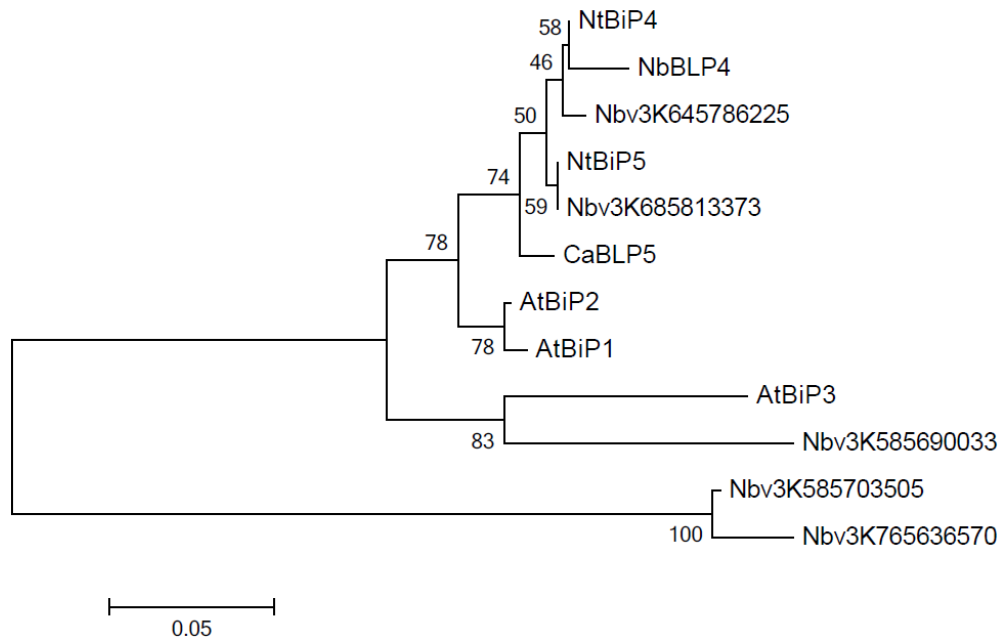


Fig. S4 Phylogenetic analysis of CaBLP5 and its tobacco, and *Arabidopsis* homologs. The amino acid sequences of 11 CaBLP5 homologs were imported into MEGA 6 for multiple sequence alignment with ClustalW. Phylogenetic analysis was performed using the neighbor-joining and bootstrap methods. The bootstrap consensus tree was inferred from 500 replicates. The scale bar indicates the lengths of the branches (relative evolutionary distance). GenBank accession numbers for the homolog protein are mentioned in legend of Fig. S2.

Nbv3K685813373 MAG-AWKRRASLVVFAIVLFGSLFAFSIAKEEATKLGTVIGIDLGGTYSVGVYKNGHVE 59
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 NbBLP4 MAGGAWNRRASLIVFGIVLFGCLFAFSIATEEATKLGTVIGIDLGGTYSVGVYKNGHVE 60
 Nbv3K645786225 MGGGYWRRSSSLVLAIVLFGCLSALSIAATEANKLGTVIGIDLGGTYSVGVYKNGHVE 60

Nbv3K685813373 I IANDQGNRI TPSWVAFTDGERLIGEAANKQA AVNPRTIFDVKRLIGRKFDDKEVQRDK 119
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 NbBLP4 I IANDQGNRI TPSWVAFTDGERLIGEAANKQA AVNPRTIFDVKRLIGRKFDDKEVQRDM 120
 Nbv3K645786225 I IANDQGNRI TPSWVGFDTGERLIGEAANKQA AVNPRTIFDVKRLIGRKFDDKEVQRDM 120

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 Nbv3K645786225 KLVPEYIVNKDGPYIQVKIKDGEV KVFSP EESAMILTKMKETA EAYLGKIKDAVTV 180
 *****: *****: ****. *****: *****: ****. *****

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 NbBLP4 PAYFNDAQRQATKDAGVIAGLNVARI INEPTAAA IAYGLDKKGGEK SILVFDLGGGTFDV 240
 Nbv3K645786225 PAYFNDAQRQATKDAGVIAGLNVARI INEPTAAA IAYGLDKKGGEKNILVFDLGGGTFDV 240
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 NbBLP4 SILTIDNGVFEVLSTNGDTHLGGEDFDQRIMEYFIKLIK KKHGKDISKDNRALGKLRREA 300
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 Nbv3K645786225 ERAKRALSSQHQRVVEIESLFDGVDFSEPLTRARFEELNNDLFRKTMGPVKKAMDDAGLE 360
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 NbBLP4 TKDILLLDVAPLTLGIETVGGVMTKLIPRNTVIPS KKSQVFTTYDQQT TVTIQVFEGE 480
 Nbv3K645786225 TKDILLLDVAPLTLGIETVGGVMTKLIPRNTVIP TKKSQVFTTYDQQT TVTISVYEGE 480
 *****: *****: *****: *****: *****: *****

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 CaBLP5 SMVKDCRLLGKFDLTGIAPAPRGTQIEVTFEVDANGILNVAEDKASGKSEKITITNDK 539
 NbBLP4 SLTKDCRLLGKFDLTGIAPAPRGTQIEVTFEVDANGILNVAEDKASGKSEKITITNDK 540
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 *. *****: *****: *****: *****: *****: *****

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 NbBLP4 GRLSQEEIERMVKEAEFEAEEDKVKKERIDARNSLETYYNMRNQINDKDKLADKLESE 600
 Nbv3K645786225 GRLSQEEIERMVEAEFEAEEDKVKKERIDARNGLETYYNMRNQINDKDKLADKLEVE 600
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 *. *****: *****: *****: *****: *****: *****

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CaBLP5	GRLSQEEIERMVKEAEFFAEEDKKVKERVDARNSLETYYVYNMRNQINDKDKLADKLESDE	599
NbBLP4	GRLSQEEIERMVKEAEFFAEEDKKVKERIDARNSLETYYVYNMRNQINDKDKLADKLESDE	600
Nbv3K645786225	GRLSQEEIERMVREAEFFAEEDKKVKERIDARNGLETYYVYNMKNQINDKDKLADKLEVDE	600
	*****:*****:***.*****:***** **	
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NbBLP4	KEKIETATKEALEWLDDNQSAEKEDYEEKLKEVEAVCNPIITAVYQKSGGAPGGESGASE	660
Nbv3K645786225	KEKIETAVKEALEWLDDNQSAEKEDYEEKLKEVEAVCNPIITAVYQRSGGAPSGS---SA	657
	*****.***:*****:*****:*****.*	
Nbv3K685813373	-----	
CaBLP5	DDDDSHDEL	668
NbBLP4	DDD--HDEL	667
Nbv3K645786225	EEEDGHDEL	666

Fig. S5 Putative amino acid sequence alignment of the CaBLP5 (AGS42239) and its *N. benthamiana* homologs. The amino-acid sequences of three CaBLP5 homologs were aligned with ClustalW. The asterisks at the bottom line of the alignment indicate identical residues in a given sequence position, while single and double dots refer to highly and moderately conserved residues, respectively. Within the aligned sequences, the dashes indicate the gaps that were inserted in order to optimize the alignment.

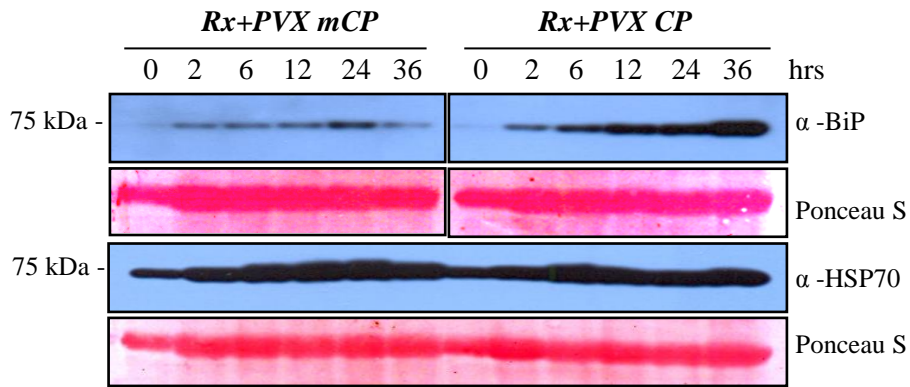


Fig. S6 BiP was promoted at protein level by induction of the Rx-mediated HR. *Rx* and mutant *PVX CP* (*PVX mCP*) or wild type of *PVX CP* (*PVX CP*) were co-expressed in leaves of *N. benthamiana*, respectively. Total protein was extracted from *N. benthamiana* leaves expressing the indicated proteins at the designated time points and analyzed by immunoblotting with anti-BiP or -cytosolic heat shock protein (HSP) 70 antibodies. Ponceau S staining of Rubisco was used for loading control.

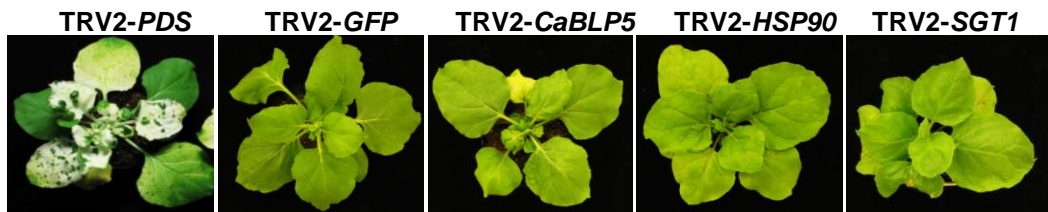


Fig. S7 Morphological phenotype of the *CaBLP5*-VIGS *N. benthamiana* plant. *GFP*- and *PDS*- silenced plants were used as negative and positive reference for the VIGS experiment. The silenced plants were photographed at 21 dpi.

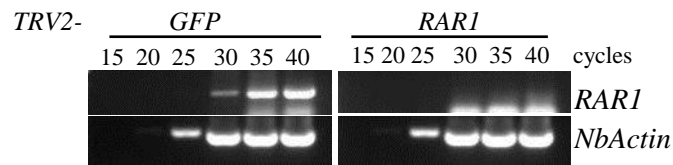
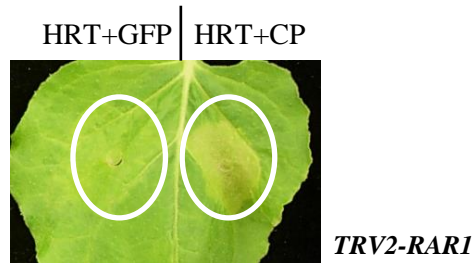
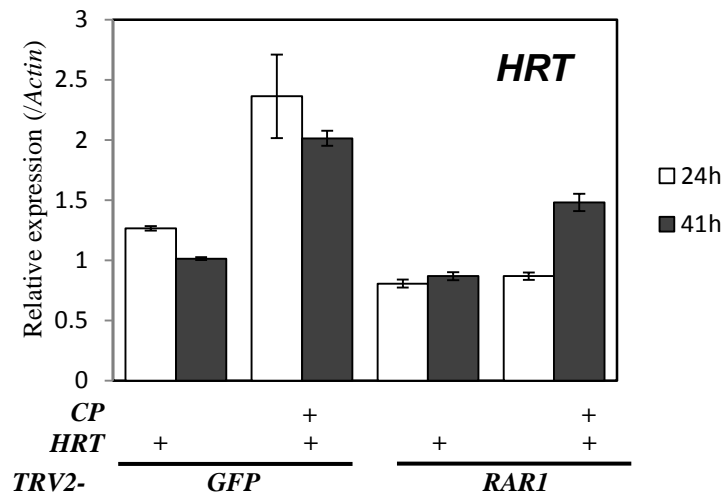
A**B****C**

Fig. S8 Silencing of *RAR1*. (A) Reverse transcription PCR analysis was performed to assess the efficiency of gene silencing. Primer directed to gene specific, or *NbActin*, with use of equal amount of cDNA from silenced plants. Numbers indicate PCR cycles. (B) HRT/CP-mediated HR induction on the leaves of the *RAR1*-silenced plants. (C) Transcriptional levels of the *HRT* in *GFP*- or *RAR1*-silenced plant. The values were normalized to the expression of *NbActin*. Error bars represent SD ($n = 3$). Means with the same letter are not significantly different (t test, P value < 0.001).

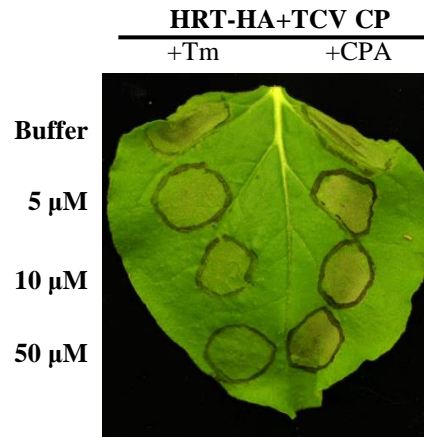


Fig. S9 Effect of Tunicamycin (Tm) on cell death by co-expression of HRT/TCV CP. Endoplasmic reticulum (ER) stress-inducing chemical TM was co-infiltrated as indicated concentrations, and infiltration buffer was used as reference. Pictures were taken 3 dpi.

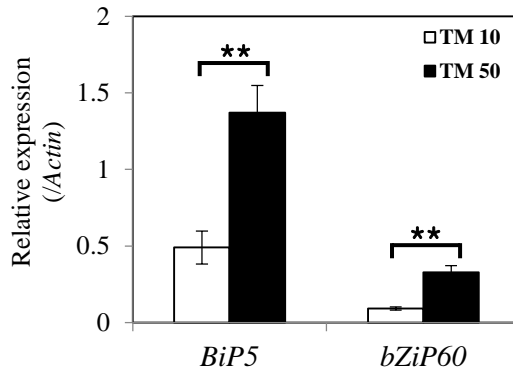
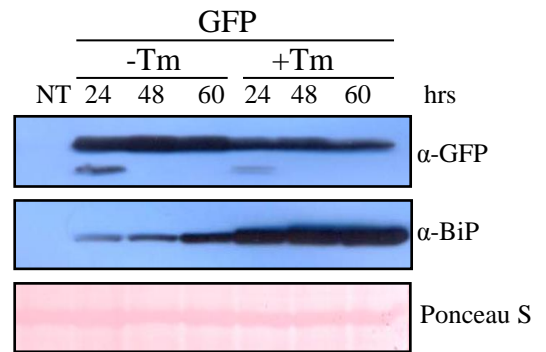
A**B**

Fig. S10 Triggering the unfolded protein response (UPR) pathway by Tm treatment. (A) Expression of *BiP5* and *bZiP60* in TRV2-GFP-infected plants by qRT-PCR. Error bars present standard error of three replicates. Asterisks indicate a significant difference from corresponding control (Student's *t*-test, $**P < 0.005$). Tm 10, 10 μ M Tm; Tm 50, 50 μ M Tm. (B) Effects of Tm treatment on levels of BiP proteins. Total protein was prepared at the indicated time points (hrs). BiP and GFP proteins were detected using anti-BiP and anti-GFP antibodies.

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