Supplementary information

Viral proteins resolve the virus-vector conundrum during hemipteran-mediated transmission by subverting salicylic acid signaling pathway

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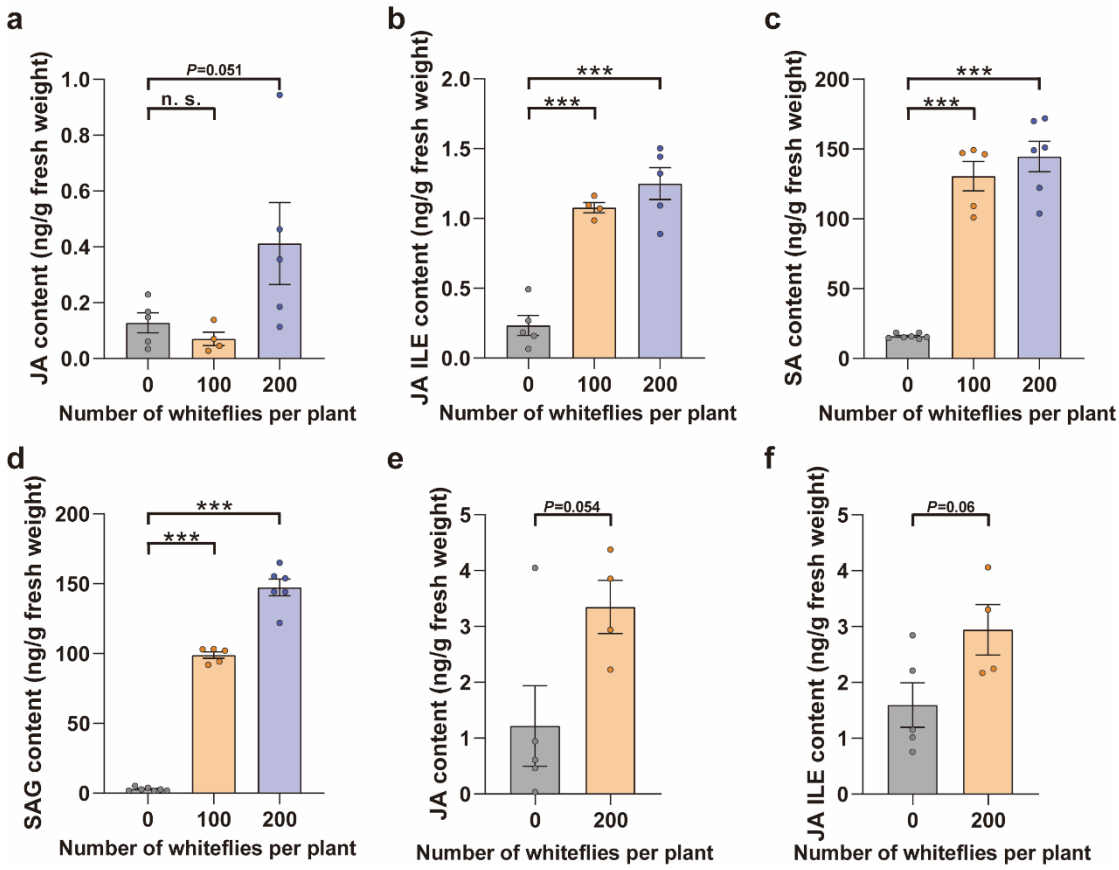
Supplementary Table S1- List of heat shock protein 90 (HSP90) family proteins that were identified in GST-pull down coupled with mass spectrometry
Supplementary Figures 1-15

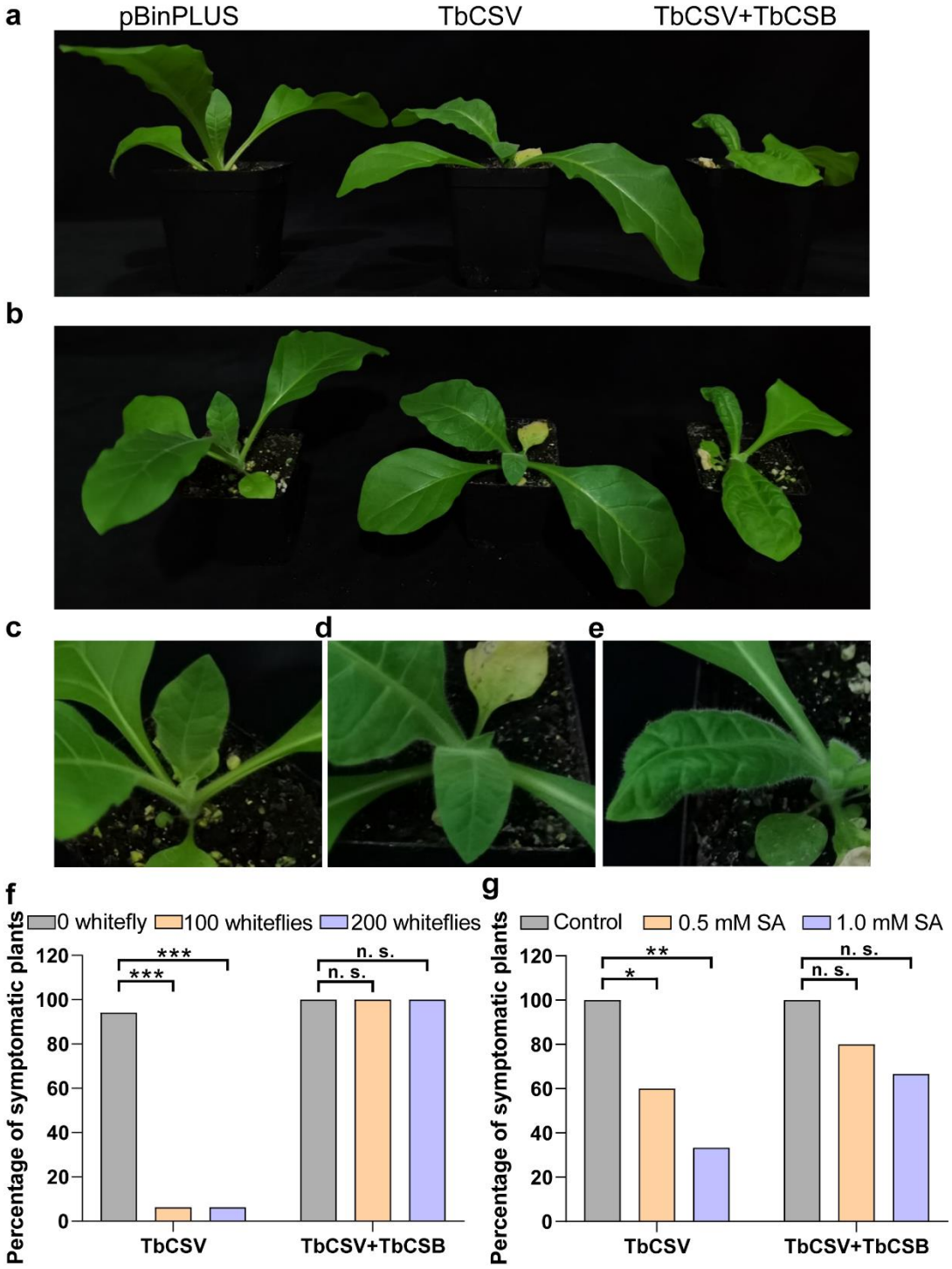
Supplementary Table 1 List of heat shock protein 90 (HSP90) family proteins that were identified in GST-pull down coupled with mass spectrometry. The band designated with an arrow in Fig. S7a were excised and proteins subjected to mass spectrometry analysis. The *N. benthamiana* protein database (https://www.nbenth.com/) was used as the reference database for protein identification.

Protein Name	NbHSP90-2	NbHSP90-3	NbHSP90-6	NbHSP90-10	NbHSP90-11	NbHSP90-12
Accession	NbL11g07290.1	NbL15g18400.1	NbL03g12220.1	NbL04g17030.1	NbL08g13350.1	NbL07g10090.1
Description	Heat shock cognate protein 80-like	Heat shock cognate protein 80	Heat shock protein 82	Heat shock protein 83	Heat shock protein 90-5	Heat shock protein 90-1-like
Score *	256.01	195.53	75.61	50.33	144.71	53.31
Coverage	31.76	31.76	16.07	13.24	33.21	12.71
Unique Peptides	6	5	3	5	12	1
Peptides	20	20	9	8	19	8
PSMs	103	85	38	21	48	19
Amino acids	699	699	703	793	798	826
MW [kDa]	80.2	80.1	80.7	90.1	91.0	94.6
Calculated pI	5.03	5.03	5.10	5.44	5.07	5.12

* Scores were calculated in the software SEQUEST according to the matching between identified ions and amino acid sequence.



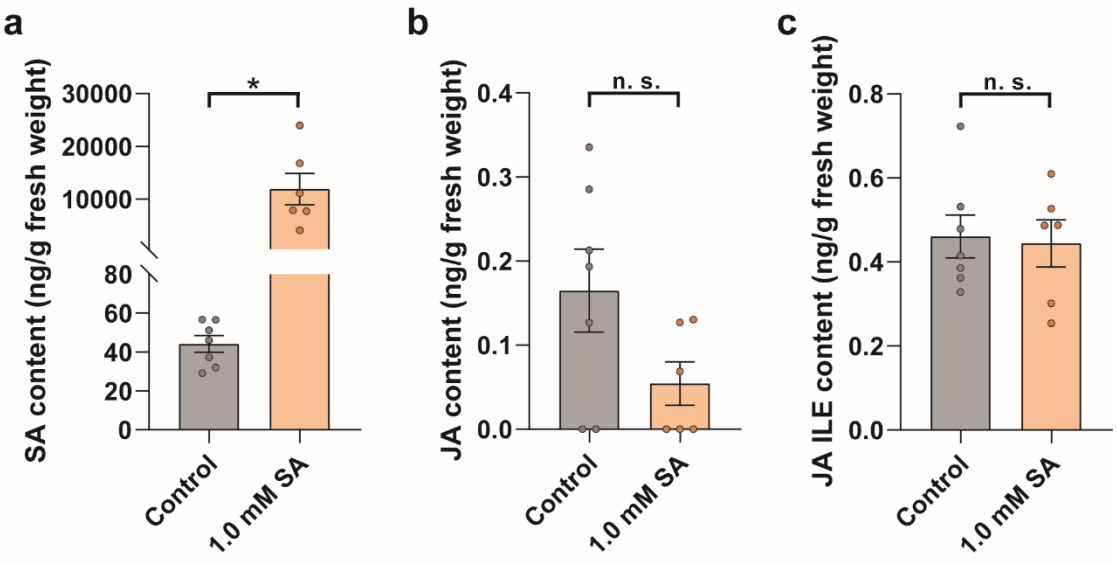
Supplementary Fig. 1 Effect of whitefly infestation on the contents of jasmonic acid (JA), jasmonoyl-isoleucine (JA-Ile), salicylic acid (SA) and SA 2-*O*-*β*-D-glucoside (SAG) in plants. (a and b) JA (a) and JA-Ile (b) content in tobacco plants upon whitefly infestation. (c and d) SA (c) and SAG (d) content in tobacco plants upon whitefly infestation. (e and f) JA (e) and JA-Ile (f) content in *Nicotiana benthamiana* plants upon whitefly infestation. N=4-7 samples (2-3 plants per sample). Data are mean ± SEM. n. s. stands for no significant difference, ***$P < 0.001$ (one-way ANOVA for a-d; two-sided Student's t test for e-f).



Supplementary Fig. 2 Virus infection symptoms in tobacco plants and percentage of symptomatic plants following treatments.

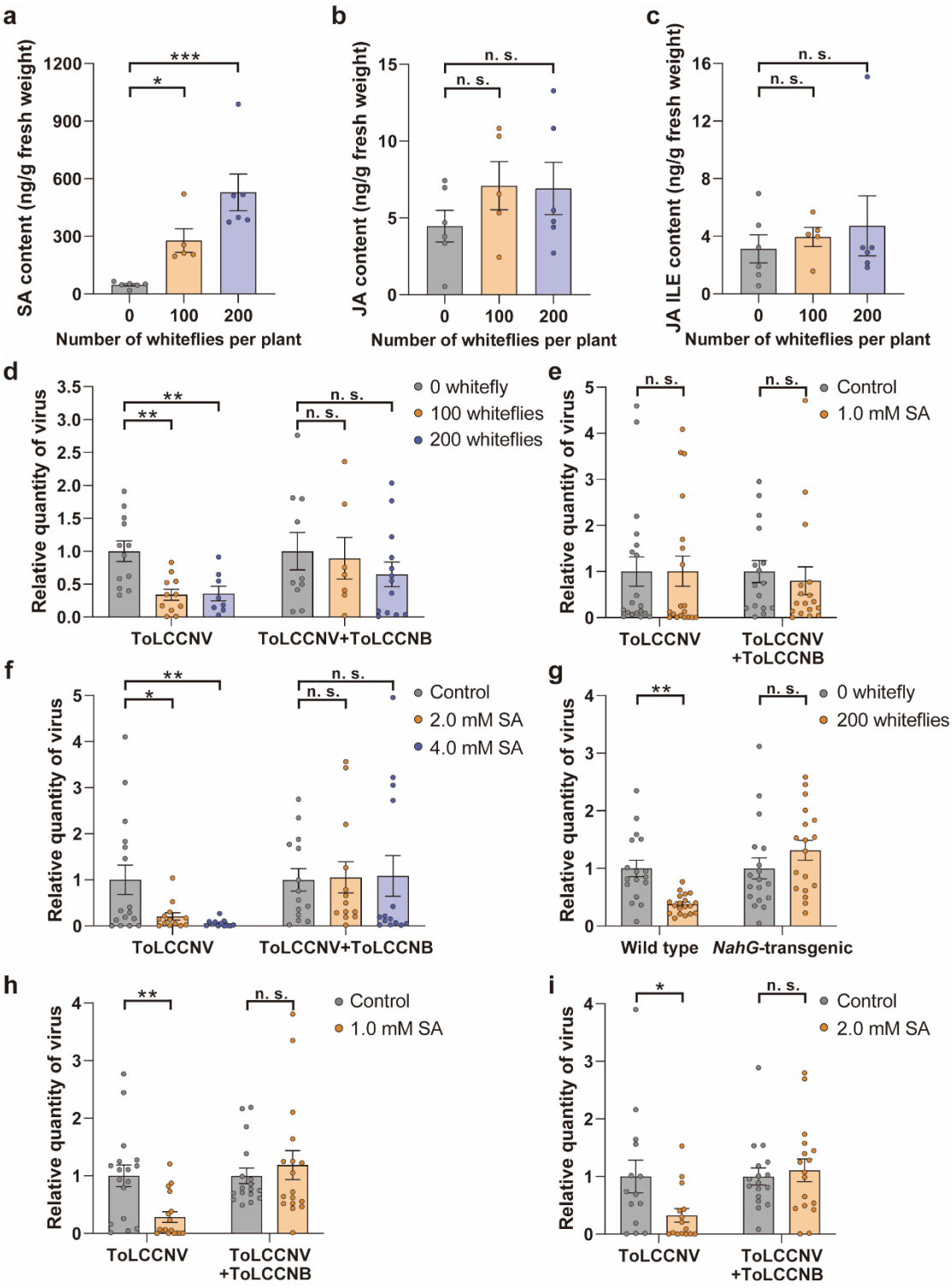
(a-b) Picture of whole tobacco plants, and (c-e) enlarged view of apical leaves. Tobacco plants were inoculated with agrobacteria containing empty vector (pBinPLUS), infectious clones of TbCSV and infectious clones of TbCSV and TbCSB. Pictures were taken at 10 days post inoculation. TbCSV infection resulted in mild downward leaf curling (d) and infection of TbCSV+TbCSB induced severe downward leaf curling and leaf puckering (e). (f and g) Percentage of symptomatic plants in all plants that were first treated with whitefly (f) or SA (g)

and then inoculated with TbCSV or TbCSV+TbCSB. N=15-17 plants for f and g. Data are mean. n. s. stands for no significant difference, *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ (Fisher's exact test of independence).



Supplementary Fig. 3 Effect of SA spray on the content of SA, JA and JA-Ile on and in tobacco leaves.

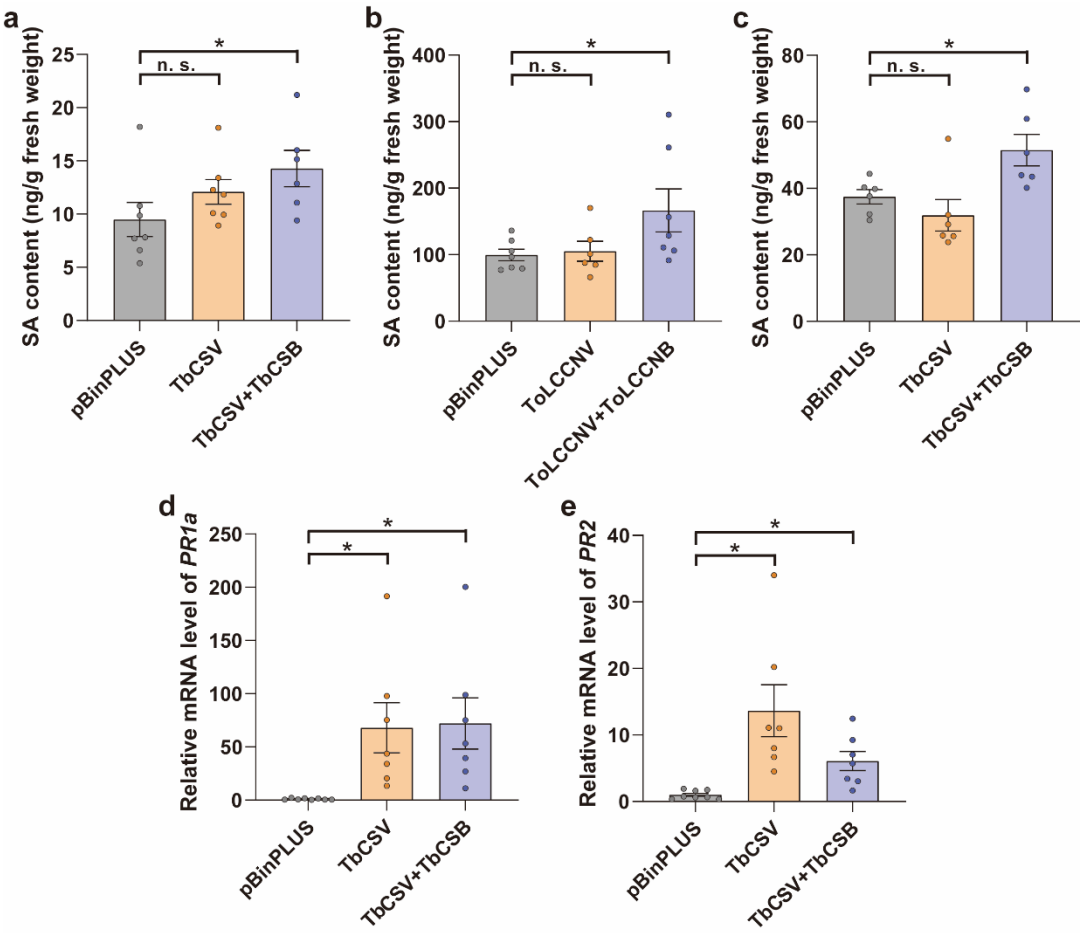
(a) SA content on and in tobacco leaves; (b) JA content in tobacco leaves; (c) JA-Ile content in tobacco leaves. N=6-7 samples (2-3 plants per sample). Data are mean ± SEM. n. s. stands for no significant difference, *$P < 0.05$ (two-sided Student's t test).



Supplementary Fig. 4 Whitefly infestation-induced SA accumulation increased plant resistance to ToLCCNV, but not to the ToLCCNV+ ToLCCNB complex.

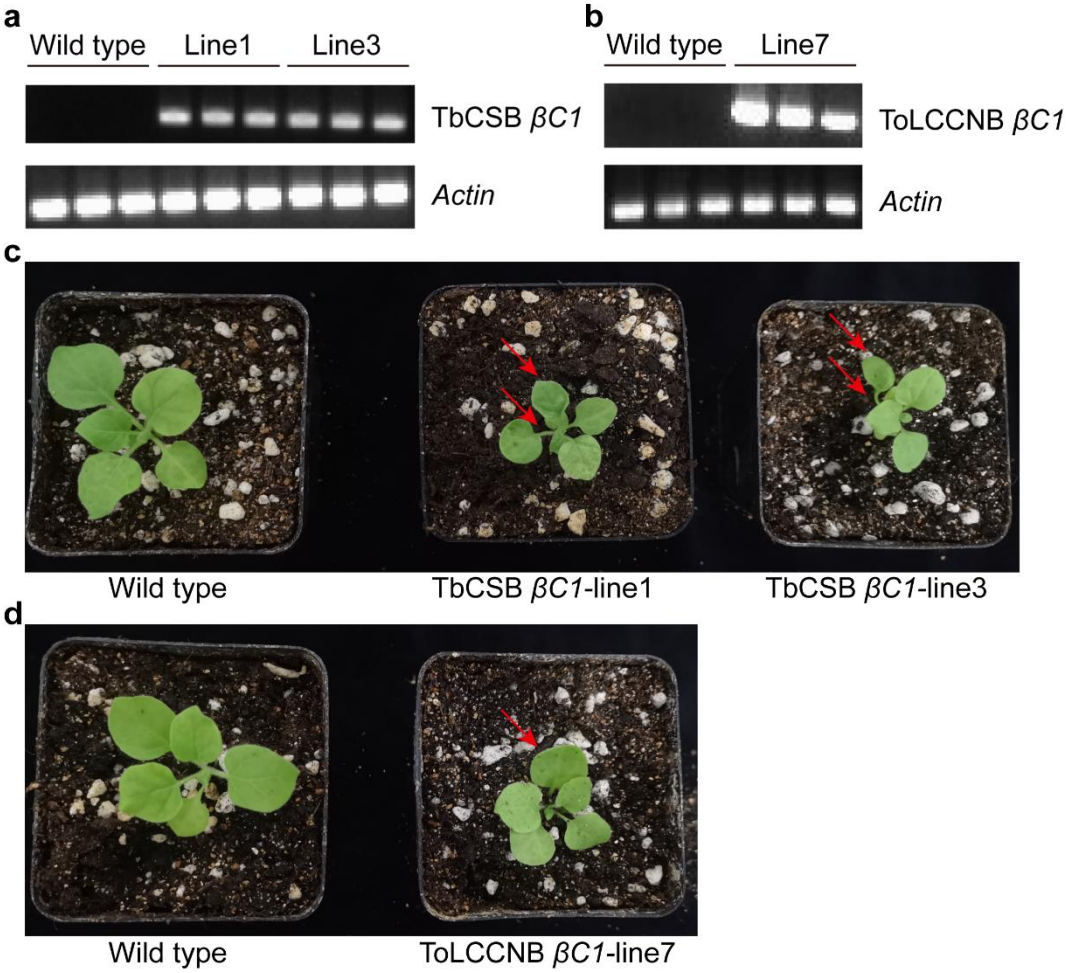
(a-c) SA (a), JA (b) and JA-Ile (c) content in tomato plants upon whitefly infestation; (d) Quantity of ToLCCNV in tomato plants that were first infested by whiteflies and then inoculated with ToLCCNV or ToLCCNV+ToLCCNB; (e and f) Quantity of ToLCCNV in tomato plants that were first sprayed with ethanol (control) or SA and then inoculated with ToLCCNV or ToLCCNV+ToLCCNB; (g) Quantity of ToLCCNV in wild type and *NahG*-transgenic *N.*

benthamiana plants that were first infested by whiteflies and then inoculated with ToLCCNV; (h and i) Quantity of ToLCCNV in *N. benthamiana* plants that were first sprayed with ethanol (control) or SA and then inoculated with ToLCCNV or ToLCCNV+ToLCCNB. N=5-6 samples (2-3 plants per sample) for a-c, 7- 13 plants for d, 12-19 plants for e-i. Data are mean ± SEM. n. s. stands for no significant difference, $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$ (one-way ANOVA for a-d and f; two-sided Student's t test for e, g, h and i).



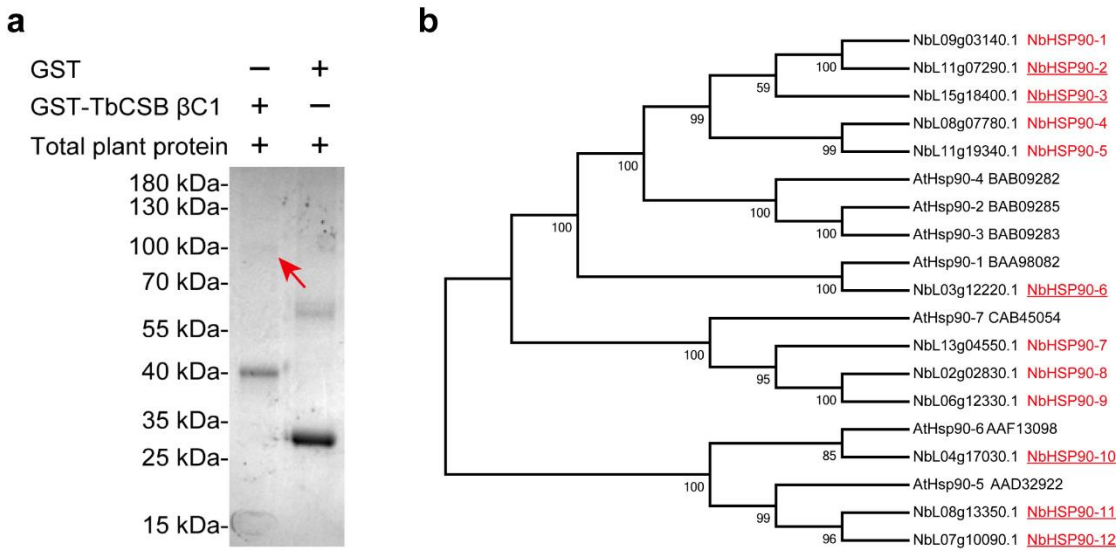
Supplementary Fig. 5 Effect of virus infection on SA content and relative mRNA level of SA-sentinel genes (*PR1a* and *PR2*) in plants.

(a and c) SA content in tobacco (a) and *Nicotiana benthamiana* (c) plants that were inoculated with pBinPLUS, TbCSV, or TbCSV+TbCSB; (b) SA content in tomato plants that were inoculated with pBinPLUS, ToLCCNV, or ToLCCNV+ToLCCNB; (d and e) Relative mRNA level of *PR1a* (d) and *PR2* (e) in *N. benthamiana* plants that were inoculated with pBinPLUS, TbCSV, or TbCSV+TbCSB. Analysis of SA content and gene transcripts was conducted at 10 days post inoculation. N=6-8 samples (2-3 plants per sample). Data are mean ± SEM. n. s. stands for no significant difference, *$P < 0.05$ (one-way ANOVA).



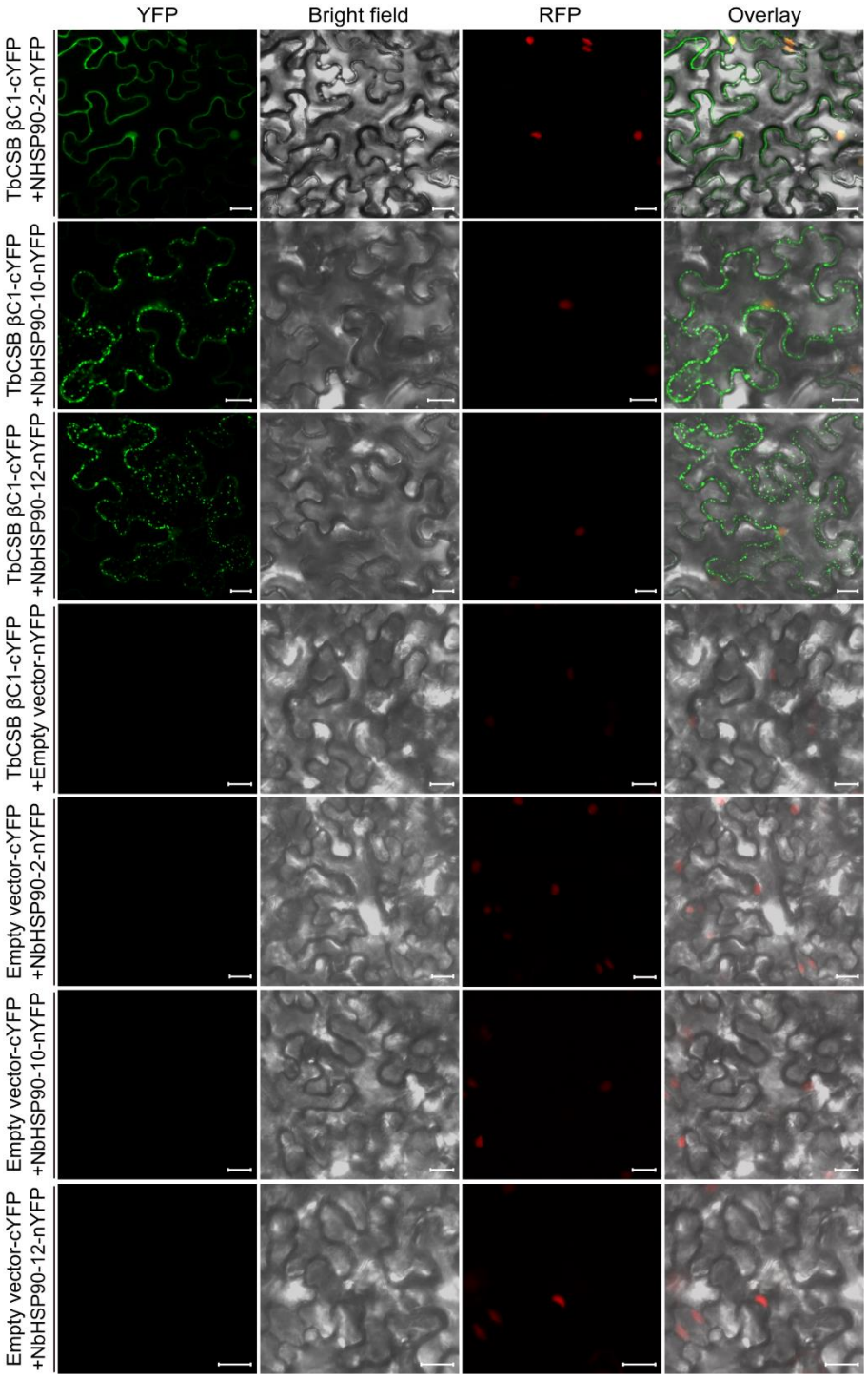
Supplementary Fig. 6 Validation of TbCSB *βC1* and ToLCCNB *βC1* transgenic *N. benthamiana* plants.

(a) PCR amplification of TbCSB *βC1* and *NbActin* in wild type and TbCSB *βC1*-transgenic *N. benthamiana* plants; (b) PCR amplification of ToLCCNB *βC1* and *NbActin* in wild type and ToLCCNB *βC1*-transgenic *N. benthamiana* plants; (c) Picture of wild type and TbCSB *βC1*-transgenic *N. benthamiana* plants. Arrows indicate upward leaf curl and curly shoot; (d) Picture of wild type and ToLCCNB *βC1*-transgenic *N. benthamiana* plants. The arrow indicates upward leaf curl.

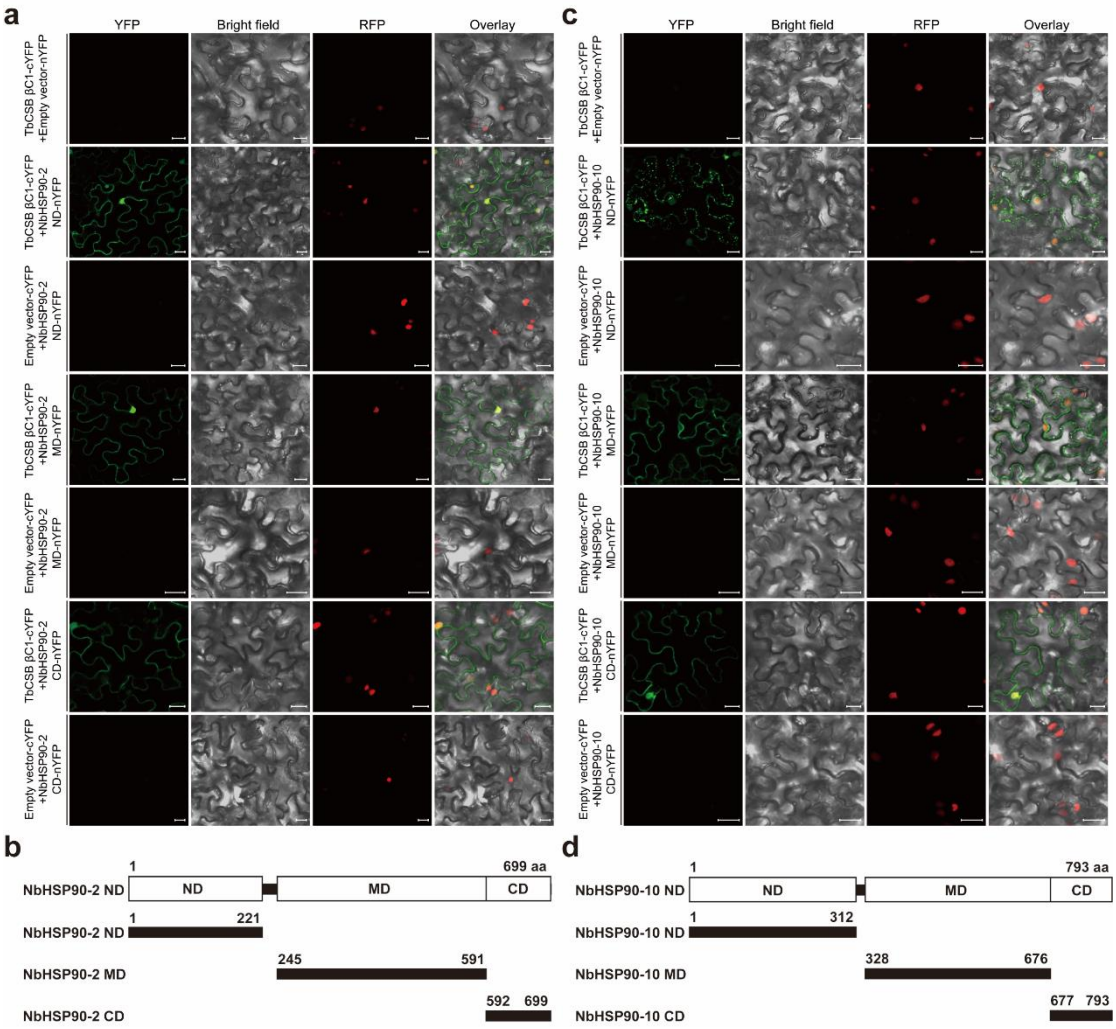


Supplementary Fig. 7 Coomassie blue staining of proteins pulled-down by GST-TbCSB βC1 and phylogenetic analysis of all HSP90 proteins identified in *N. benthamiana* protein database.

(a) The protein band that was specifically pulled-down by GST-TbCSB βC1 (indicated with a red arrow). GST-TbCSB βC1 and GST proteins were expressed in *Escherichia coli* cells, and then incubated with total *N. benthamiana* proteins. GST is around 26 kDa and GST-TbCSB βC1 is around 40 kDa; (b) Phylogenetic tree of all known HSP90 proteins of *Arabidopsis thaliana* and HSP90 proteins identified in *N. benthamiana* protein database (www.nbenth.com). All NbHSP90s name designations are shown (red). NbHSP90s identified by mass spectrometry analysis are underlined. MEGA6 and the incorporated Neighbor-Joining (NJ) algorithm were used for phylogenetic analysis. The reliability of the phylogenetic analysis was examined by percentages obtained through 1000 bootstrap iterations of the datasets. Bootstrap values are shown in the cladogram.

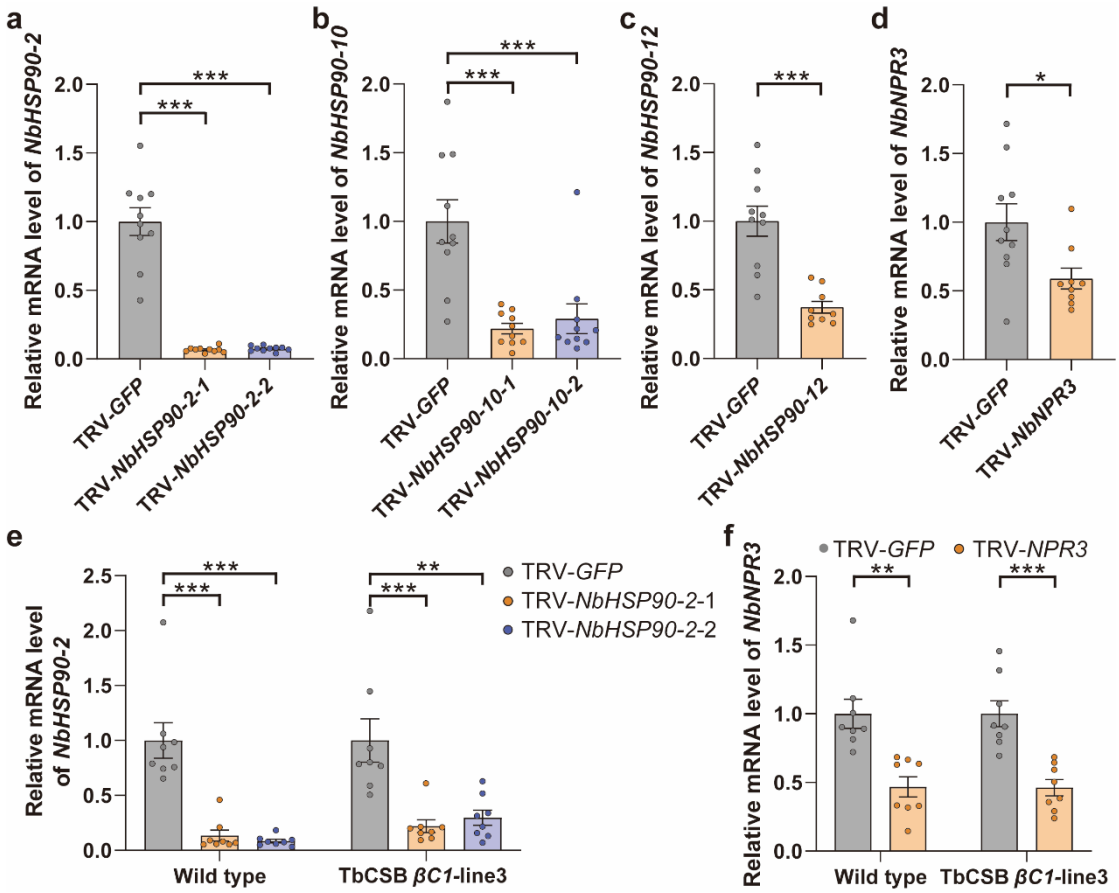


Supplementary Fig. 8 Interactions between TbCSB βC1 and NbHSP90s as revealed by BiFC. TbCSB βC1-cYFP and a NbHSP90s-nYFP were co-expressed in the leaves of H2B-RFP transgenic *N. benthamiana* plants. The pairs of co-expressed proteins tested are indicated on the left. TbCSB βC1-cYFP+nYFP and cYFP+NbHSP90s-nYFP were used as controls. Leaves were observed at 2 days post inoculation. Columns from left to right represent YFP fluorescence, bright field, RFP fluorescence (nuclei), and overlay. Bars represent 20 μm.



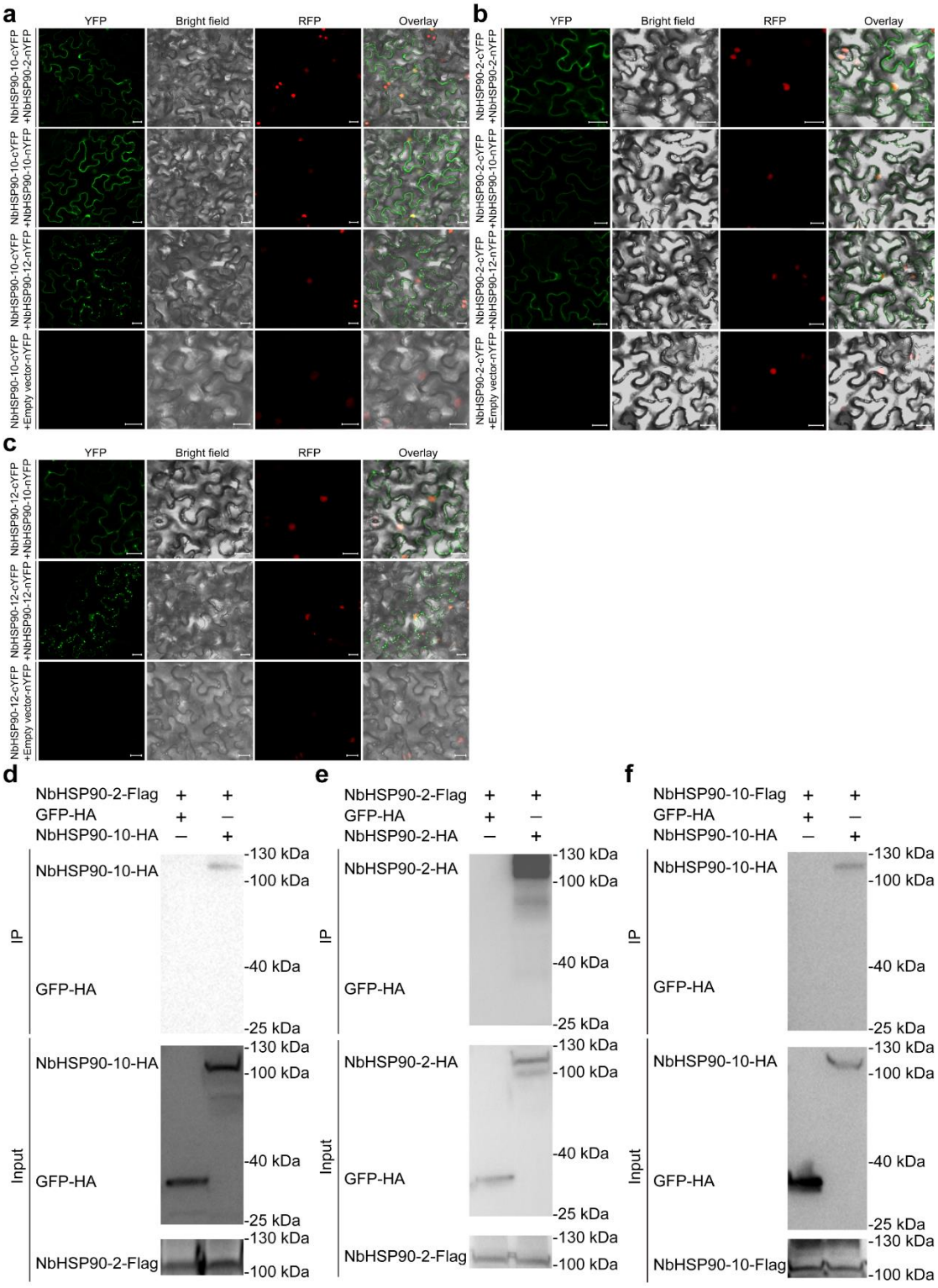
Supplementary Fig. 9 Interactions between TbCSB βC1 and domains of NbHSP90-2 and NbHSP90-10 as revealed by BiFC.

TbCSB βC1-cYFP and a HSP90 domain-nYFP were co-expressed in H2B-RFP transgenic *N. benthamiana* leaves. The pairs of co-expressed proteins are indicated on the left. TbCSB βC1-cYFP+nYFP and cYFP+domain-nYFP were used as controls. Leaves were observed at 2 days post inoculation. Columns from left to right represent YFP fluorescence, bright field, RFP fluorescence (nuclei), and overlay. Bars represent 20 μm.



Supplementary Fig. 10 Verification of VIGS of *NbHSP90*s and *NbNPR3* in wild type and TbCSB-*βC1* transgenic *N. benthamiana* plants.

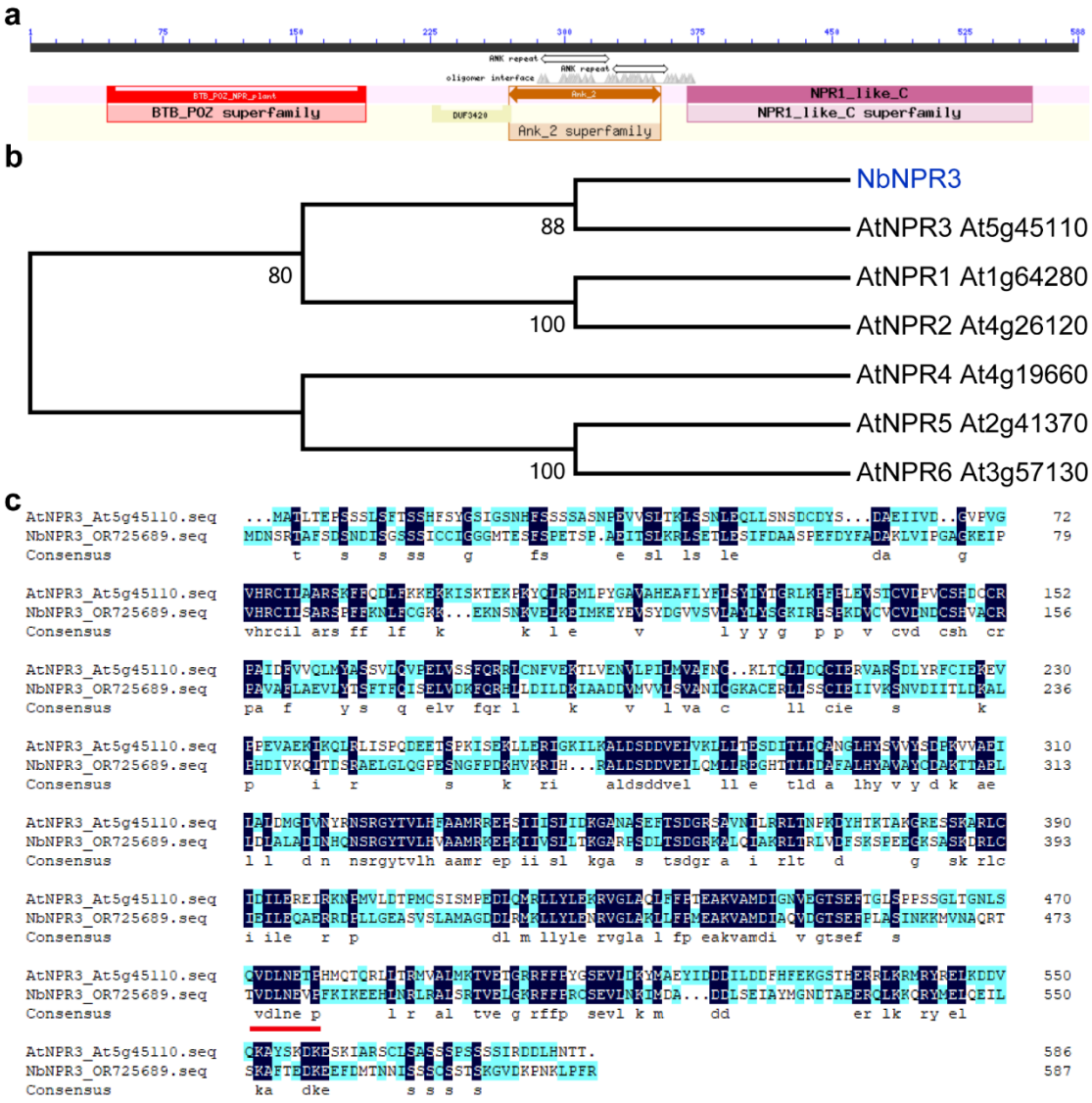
(a) Relative mRNA level of *NbHSP90-2* in wild type *N. benthamiana* plants; (b) Relative mRNA level of *NbHSP90-10* in wild type *N. benthamiana* plant; (c) Relative mRNA level of *NbHSP90-12* in wild type *N. benthamiana* plants; (d) Relative mRNA level of *NbNPR3* in wild type *N. benthamiana* plants; (e and f) Relative mRNA level of *NbHSP90-2* (e) and *NbNPR3* (f) in wild type and TbCSB-*βC1* transgenic *N. benthamiana* plants. Plants were inoculated with pTRV1+pTRV2-target gene or pTRV1+pTRV2-*GFP* (negative control) and leaves were harvested for the analysis of gene expression at 7 days post inoculation. N=8-10 samples (2-3 plants per sample). Data are mean ± SEM. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 (one-way ANOVA for a, b and e; two-sided Student's t test for c, d and f).



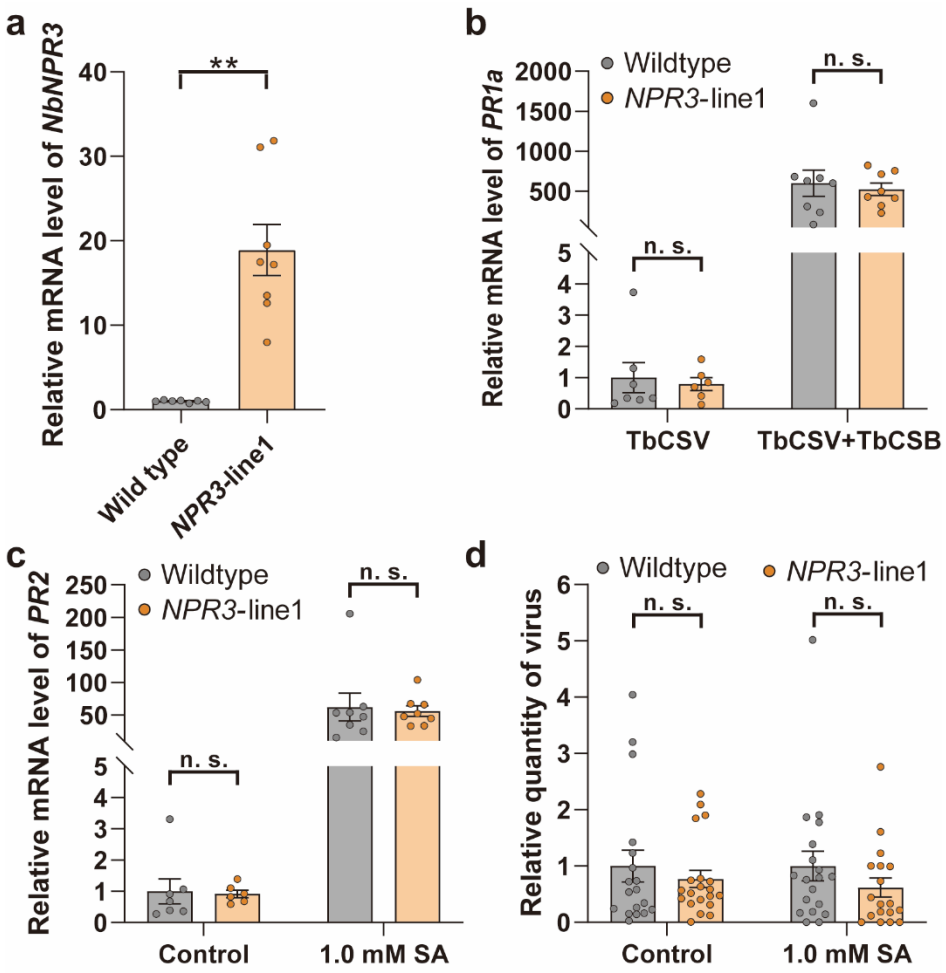
Supplementary Fig. 11 Interactions among NbHSP90s as revealed by BiFC and co-IP.

(a-c) Interactions between NbHSP90s in BiFC assay. NbHSP90s-cYFP and NbHSP90s-nYFP were co-expressed in leaves of H2B-RFP transgenic *N. benthamiana* plants. The pairs tested were: NbHSP90-10+ NbHSP90s (a), NbHSP90-2+ NbHSP90s (b), and NbHSP90-12+NbHSP90s (c). Leaves were observed at 2 days post inoculation. Columns from left to right represent YFP fluorescence, bright field, RFP fluorescence (nuclei) and overlay. Bars represent 20 μm. (d-f)

Interaction between NbHSP90-2 and NbHSP90-10 (d), NbHSP90-2 and NbHSP90-2 (e), NbHSP90-10 and NbHSP90-10 (f) in co-IP assay. NbHSP90-2-Flag (or NbHSP90-10-Flag) and NbHSP90-2-HA (or NbHSP90-10-HA) were co-expressed, and NbHSP90-2-Flag (or NbHSP90-10-Flag)+GFP-HA was used as negative control. Input in leaf extracts were analyzed with anti-HA and anti-Flag antibodies, and then immunoprecipitated with anti-Flag beads.

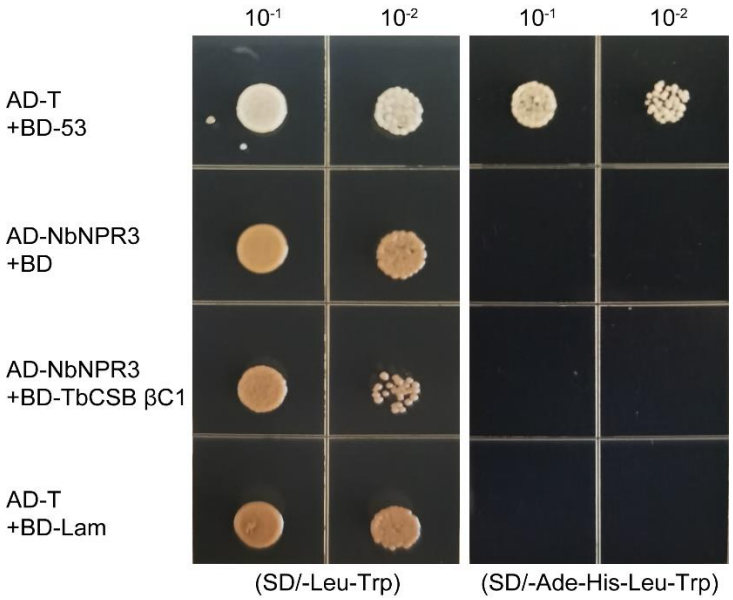


Supplementary Fig. 12 Predicted domains of NbNPR3, phylogenetic analysis of NbNPR3 with NPR proteins from *A. thaliana* and sequence alignment between AtNPR3 and NbNPR3. (a) Domains of NbNPR3 that were predicted in NCBI (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). CDD v3.20-59693 PSSMs database was used. Expect value threshold was 0.01. (b) Phylogenetic tree of all known NPR proteins of *A. thaliana* and NbNPR3. MEGA6 and the incorporated Neighbor-Joining (NJ) algorithm were used. The reliability of the phylogenetic analysis was examined by percentages obtained through 1000 bootstrap iterations of the datasets. Bootstrap values are shown in the cladogram. (c) Sequence alignment between AtNPR3 and NbNPR3. Alignment was performed using DNAMAN (LynnonBiosoft, USA). Dark blue indicates consensus in amino acids between the two sequences, and light blue or white indicate divergence in amino acids. An EAR-like motif in its C-terminus (VDLNEVP) is underlined in red.

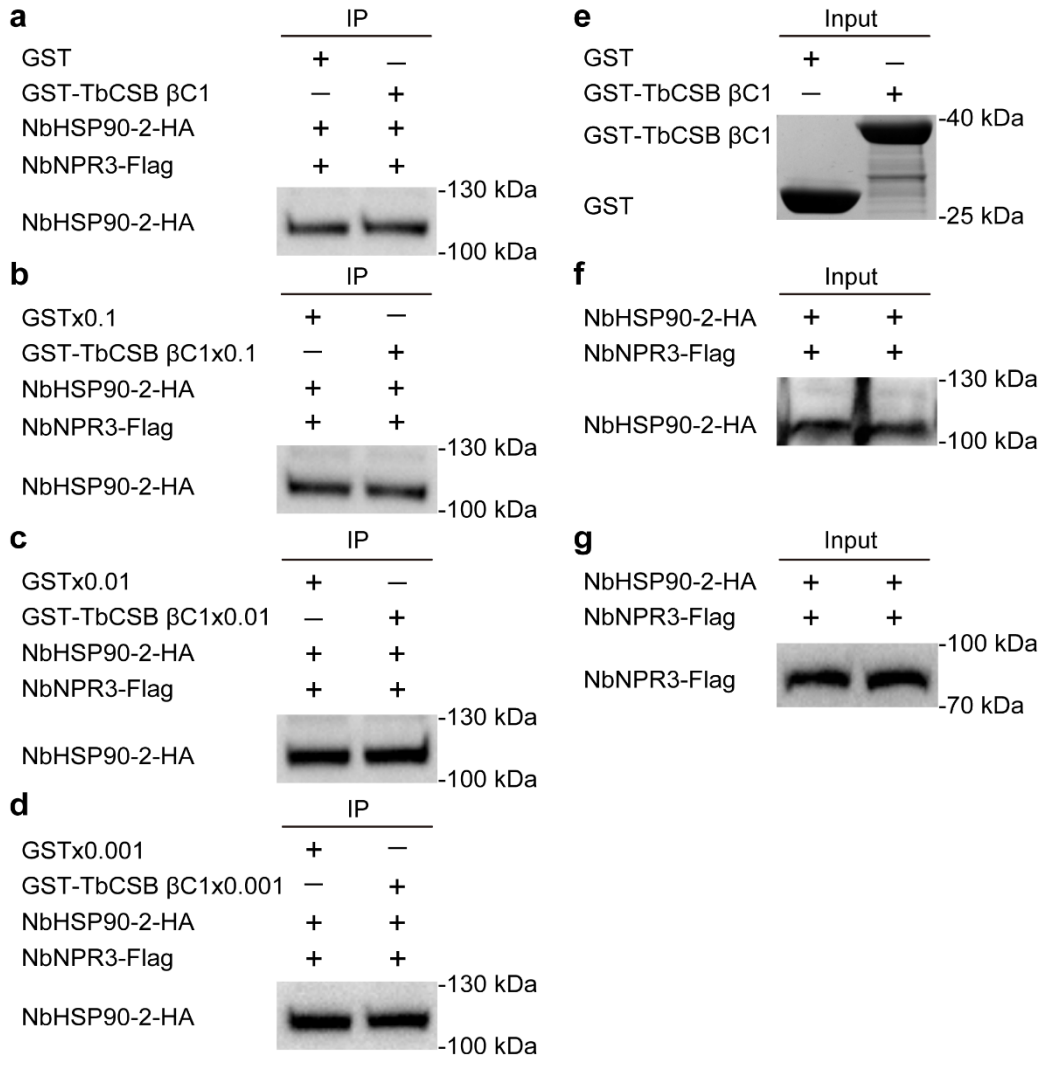


Supplementary Fig. 13 Nb*NPR3* mRNA level in Nb*NPR3*-transgenic *N. benthamiana* plants and effect of the *NbNPR3* transgene on the transcript level of *PR1a* and *PR2* and plant antiviral defenses.

(a) Relative mRNA level of *NbNPR3* in wild type and transgenic *N. benthamiana* plants; (b and c) Relative mRNA level of *PR1a* (b) and *PR2* (c) in wild type and *NbNPR3*-transgenic *N. benthamiana* plants that were sprayed with ethanol (control) or SA; (d) Quantity of TbCSV in wild type and *NbNPR3*-transgenic plants that were first sprayed with ethanol (control) or 1.0 mM SA and then inoculated with TbCSV+TbCSB. N=6-8 samples (2-3 plants per sample) for a-c,18-20 plants for d. Data are mean ± SEM. n. s. stands for no significant difference, $^{**}P < 0.01$ (two-sided Student's t test).

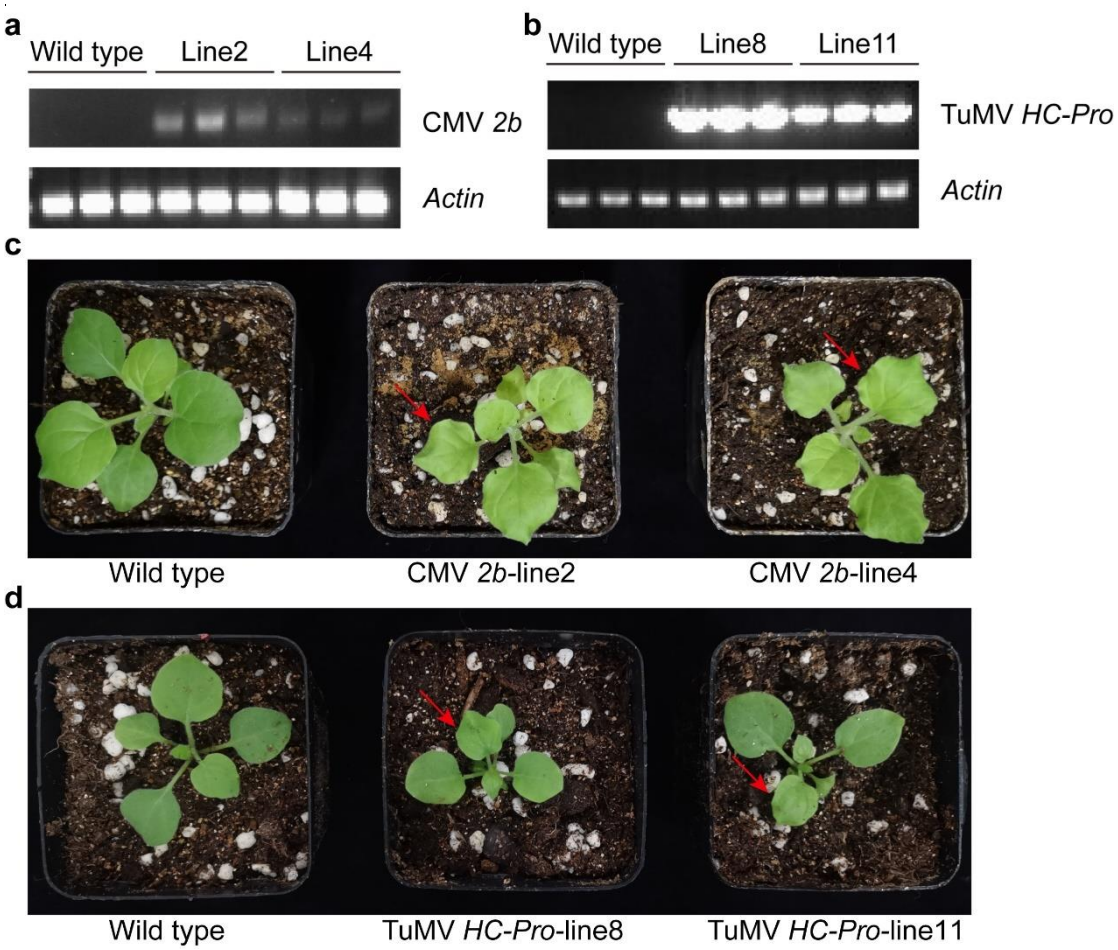


Supplementary Fig. 14 Absence of direct interaction between TbSCB βC1 and NbNPR3.
Yeast strain AH109 transformed with the indicated plasmid combinations was spotted with 10-fold serial dilutions on synthetic dextrose SD/-Leu-Trp and SD/-Ade-His-Leu-Trp medium. AD-T+BD-53 was included as positive control.



Supplementary Fig. 15 Effect of TbCSB βC1 on the interaction between NbHSP90-2 and NbNPR3.

(a-d) Amount of NbHSP90-2-HA immunoprecipitated by NbNPR3-flag when the volume ratio of GST or GST-TbCSB βC1: NbNPR3-Flag+NbHSP90-2-HA was 1 (a), 0.1 (b), 0.01 (c), 0.001 (d); (e) Input of GST and GST-TbCSB βC1 as analyzed by Coomassie blue staining; (f) Input of NbHSP90-2-HA in leaf extracts as analyzed with anti-HA antibodies; (g) Input of NbNPR3-Flag in leaf extracts as analyzed with anti-Flag antibodies. NbNPR3-Flag and NbHSP90-2-HA were co-expressed in plants, and GST or GST-TbCSB βC1 proteins were expressed in *E. coli* cells. NbNPR3-Flag+NbHSP90-2-HA proteins were mixed with various amount of GST or GST-TbCSB βC1 and then subjected to immunoprecipitation.



Supplementary Fig. 16 Validation of CMV *2b* and TuMV *HC-Pro* transgenic *N. benthamiana* plants.

(a) PCR amplification of CMV *2b* and *NbActin* in wild type and CMV *2b*-transgenic *N. benthamiana* plants; (b) PCR amplification of TuMV *HC-Pro* and *NbActin* in wild type and TuMV *HC-Pro*-transgenic *N. benthamiana* plants; (c) Picture of wild type and CMV *2b*-transgenic *N. benthamiana* plants. Arrows indicate downward leaf curl; (d) Picture of wild type and TuMV *HC-Pro*-transgenic *N. benthamiana* plants. Arrows indicate mild downward leaf curl.