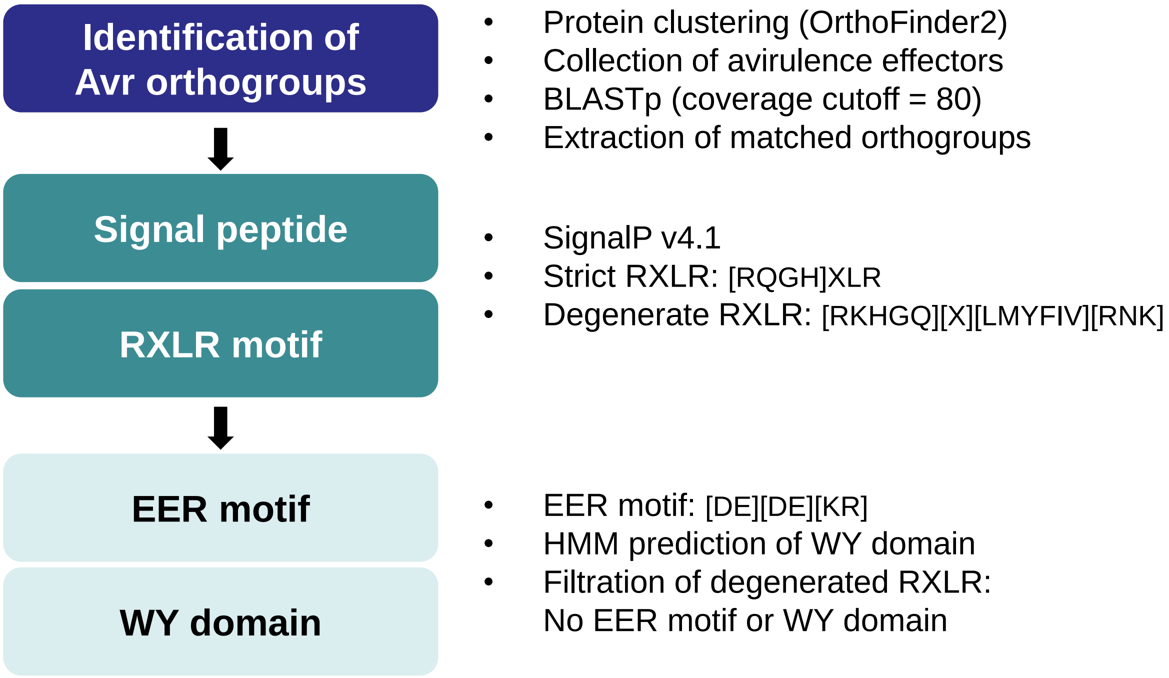
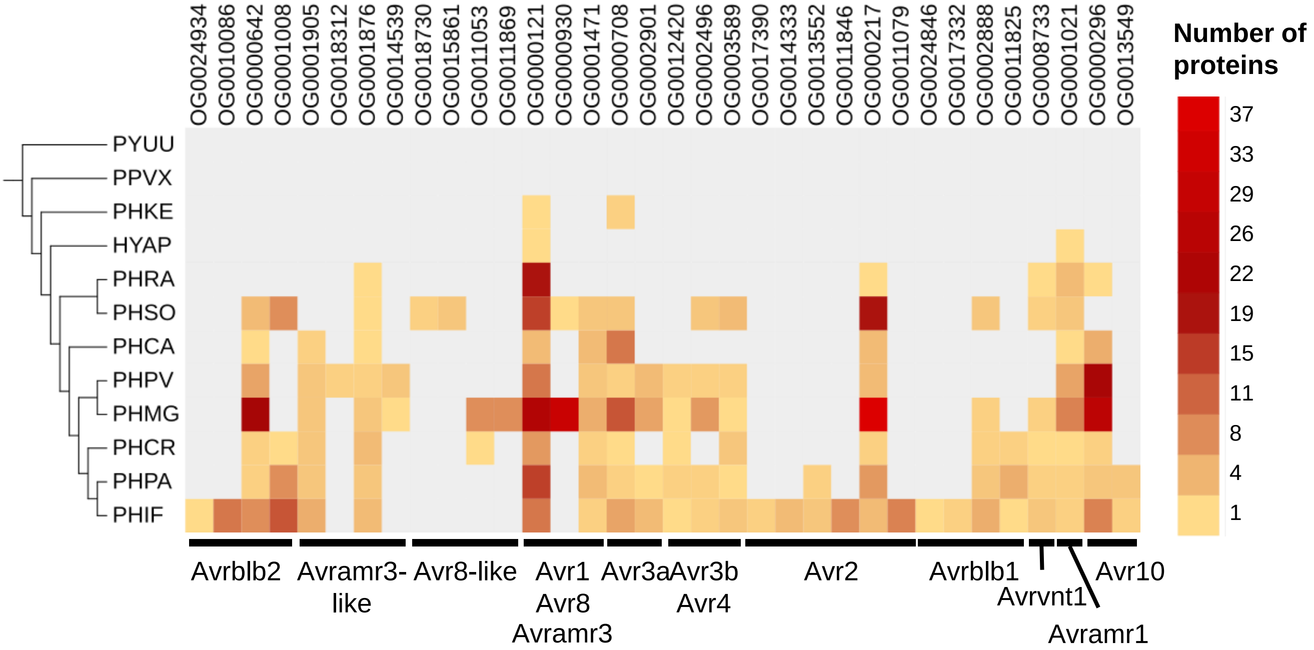


- Protein clustering (OrthoFinder2)
- Collection of avirulence effectors
- BLASTp (coverage cutoff = 80)
- Extraction of matched orthogroups

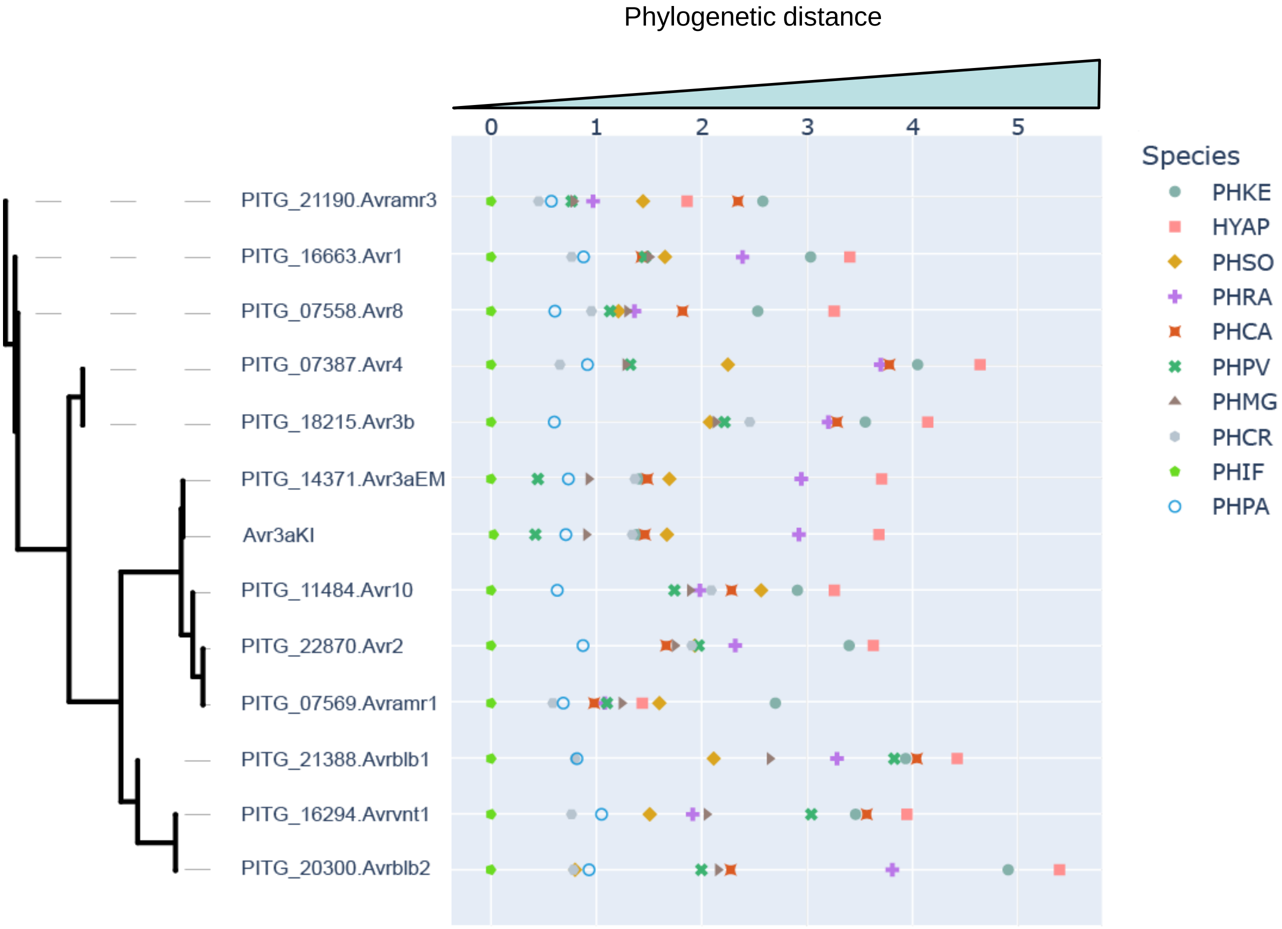
- SignalP v4.1
- Strict RXLR: [RQGH]XLR
- Degenerate RXLR: [RKHGQ][X][LMYFIV][RNK]

- EER motif: [DE][DE][KR]
- HMM prediction of WY domain
- Filtration of degenerated RXLR: No EER motif or WY domain

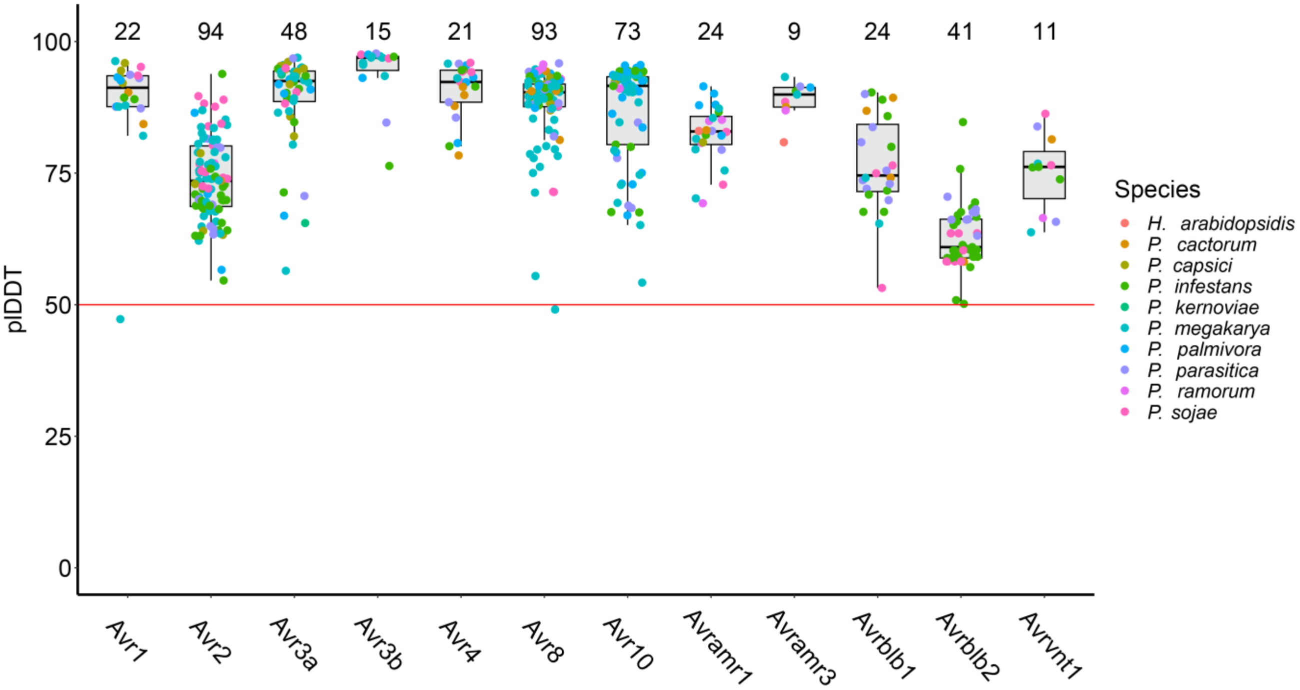
Supplementary Figure 1. Pipeline for identifying and clustering *Phytophthora* effector families with homology to reference avirulence effectors



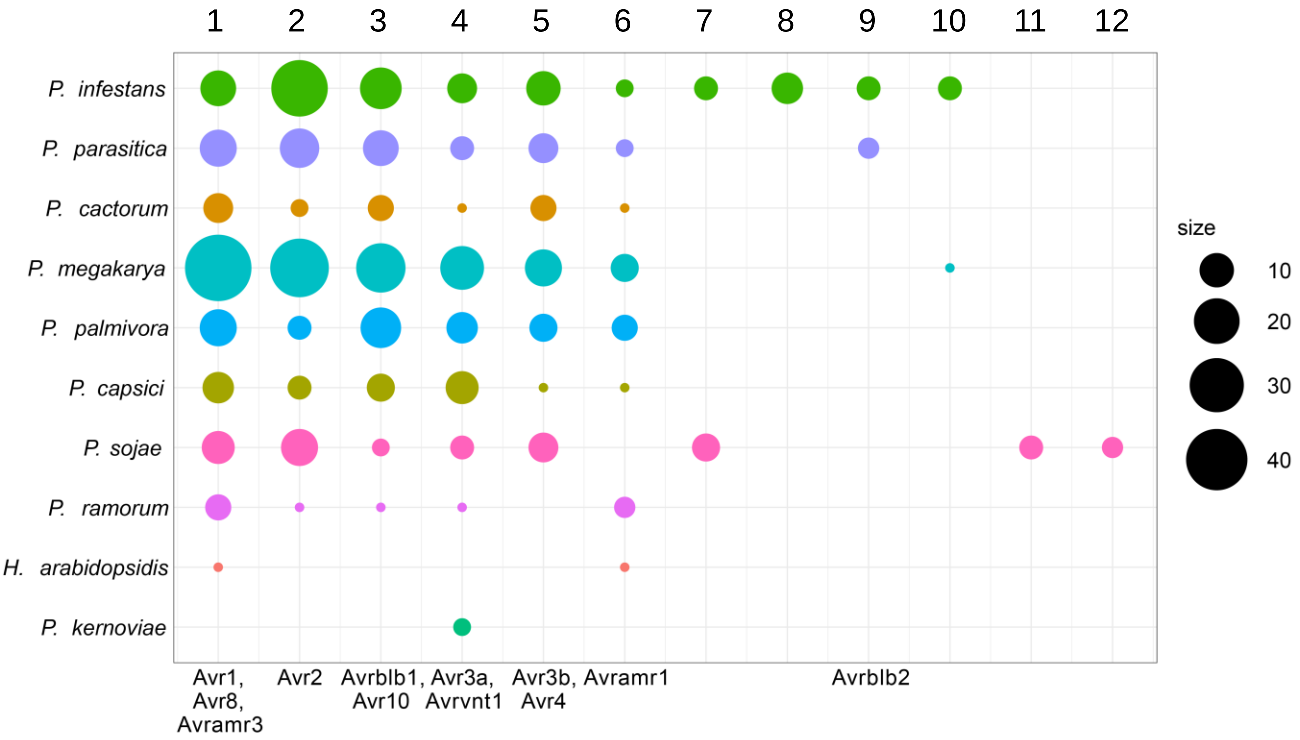
Supplementary Figure 2. Multiple effector families defined by orthogroup clustering are conserved within *Phytophthora* species. For each reference avirulence effector, an orthogroup with an effector with coverage above 80% is marked as the corresponding effector group. Light gray indicates absence of homologous effector. Abbreviations of species names are described in Supplementary Table 2.



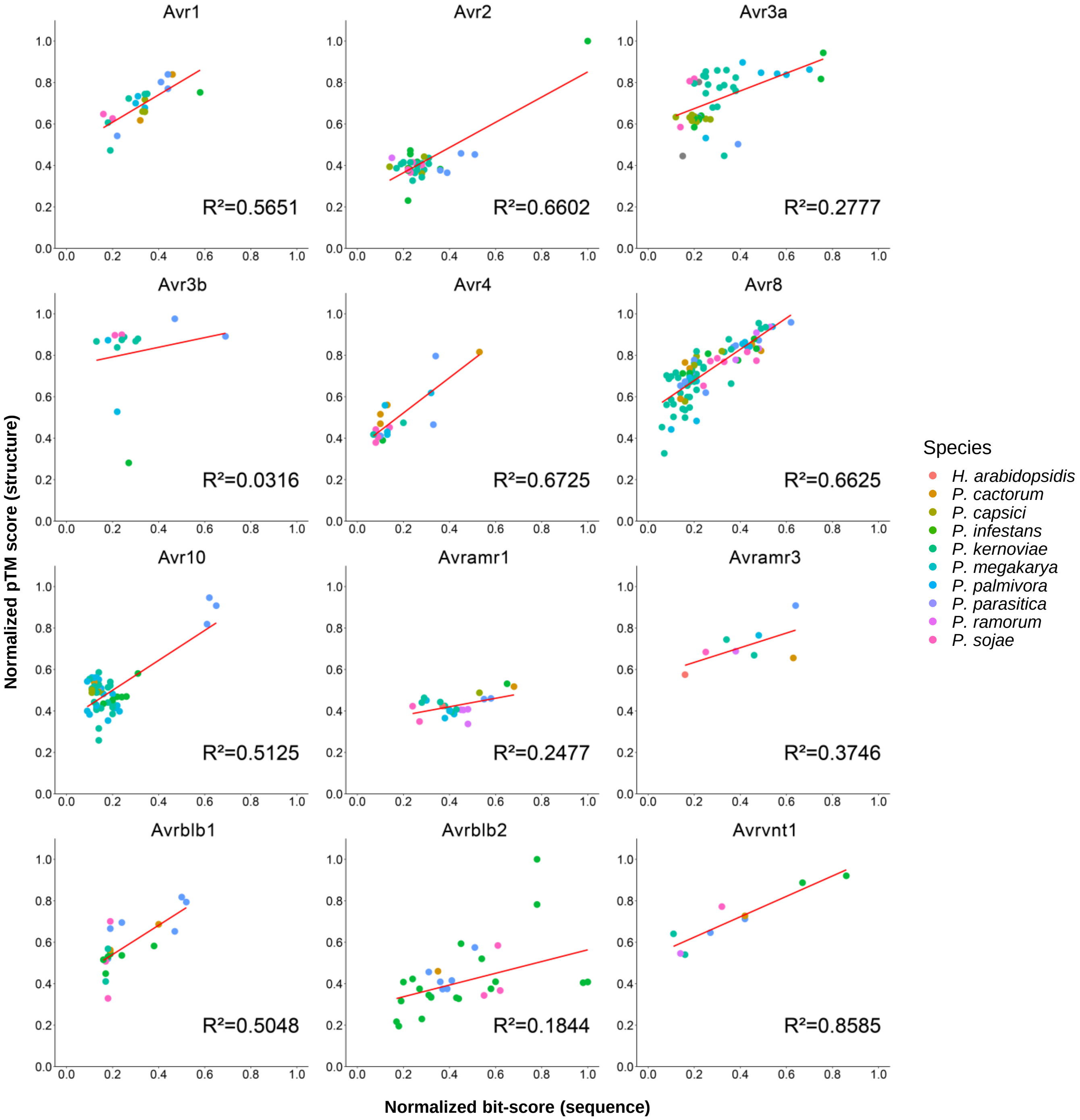
Supplementary Figure 3. Avr1, Avr3a, Avr8, and Avramr1 are conserved within *Phytophthora* species. The phylogenetic (patristic) distance between reference avirulence effector in *P. infestans* and the closest homolog in the other *Phytophthora* species were calculated from the phylogenetic tree in Fig. 1c, as previously described in Harant et al. (2022).



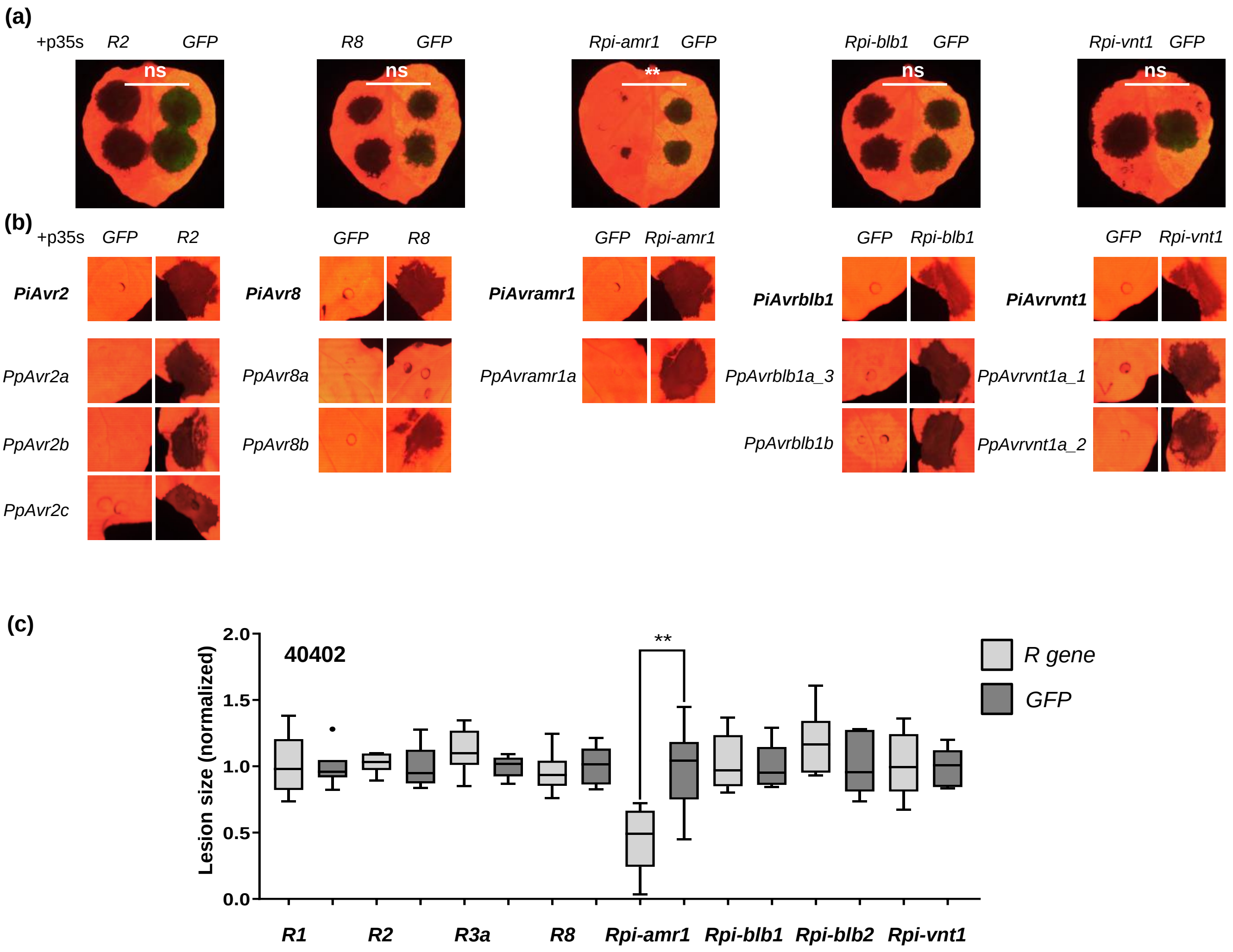
Supplementary Figure 4. Evaluation of structural predictions of effector domain regions. The numbers at the top of the figure show the number of predicted structures for each avirulence effector. The red horizontal line represents the plDDT score cutoff for the effector structure to be used for downstream analysis.



Supplementary Figure 5. Structure-based clustering of avirulence effector homologs. The numbers at the top of the figure represent the cluster number. The reference avirulence effector included in each cluster is indicated at the bottom of the figure.



Supplementary Figure 6. Conservation ratio of protein sequence and structure for each effector homologs. The red line and R^2 value represent the Pearson correlation coefficient between normalized bit-score and normalized pTM score.

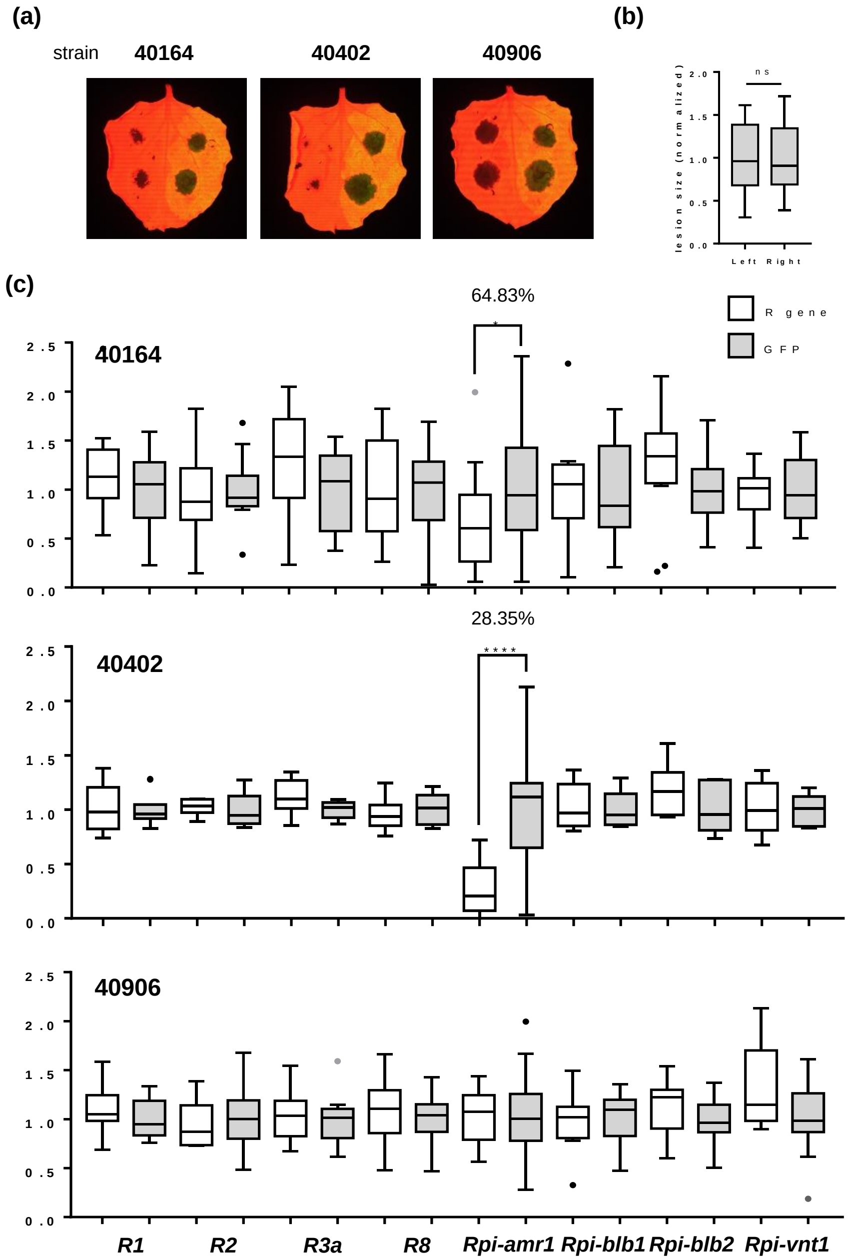


Supplementary Figure 7. *Solanum* NLRs recognizing PiAVR effectors also recognize *P. parasitica* homologs

(a) Representative images of lesion of *P. parasitica* (40402 strain) inoculated on *Solanum* NLR-expressed half compared to the *GFP*-expressed area of *N. benthamiana* leaves (ns: not significant).

(b) Eight *Solanum* NLRs (*R1, R3a,* and *Rpi-blb2* are not presented cause they cannot induced cell death against *PpAVR* candidates) were co-expressed with their putative corresponding effectors of *P. parasitica* in *N. benthamiana* leaves. Representative image of positive cases are presented.

(c) Average lesion size are presented with 10-90 percentile box plot. Total 8 *Solanum* NLRs were tested for resistance assay against *P. parasitica.* Expression of *Rpi-amr1* significantly reduced lesion size compared to GFP-expressed region of *N. benthamiana* leaves. Statistically significances were analyzed with unpaired *t*-test using data collected from 2 independent experiments (**P<0.01).

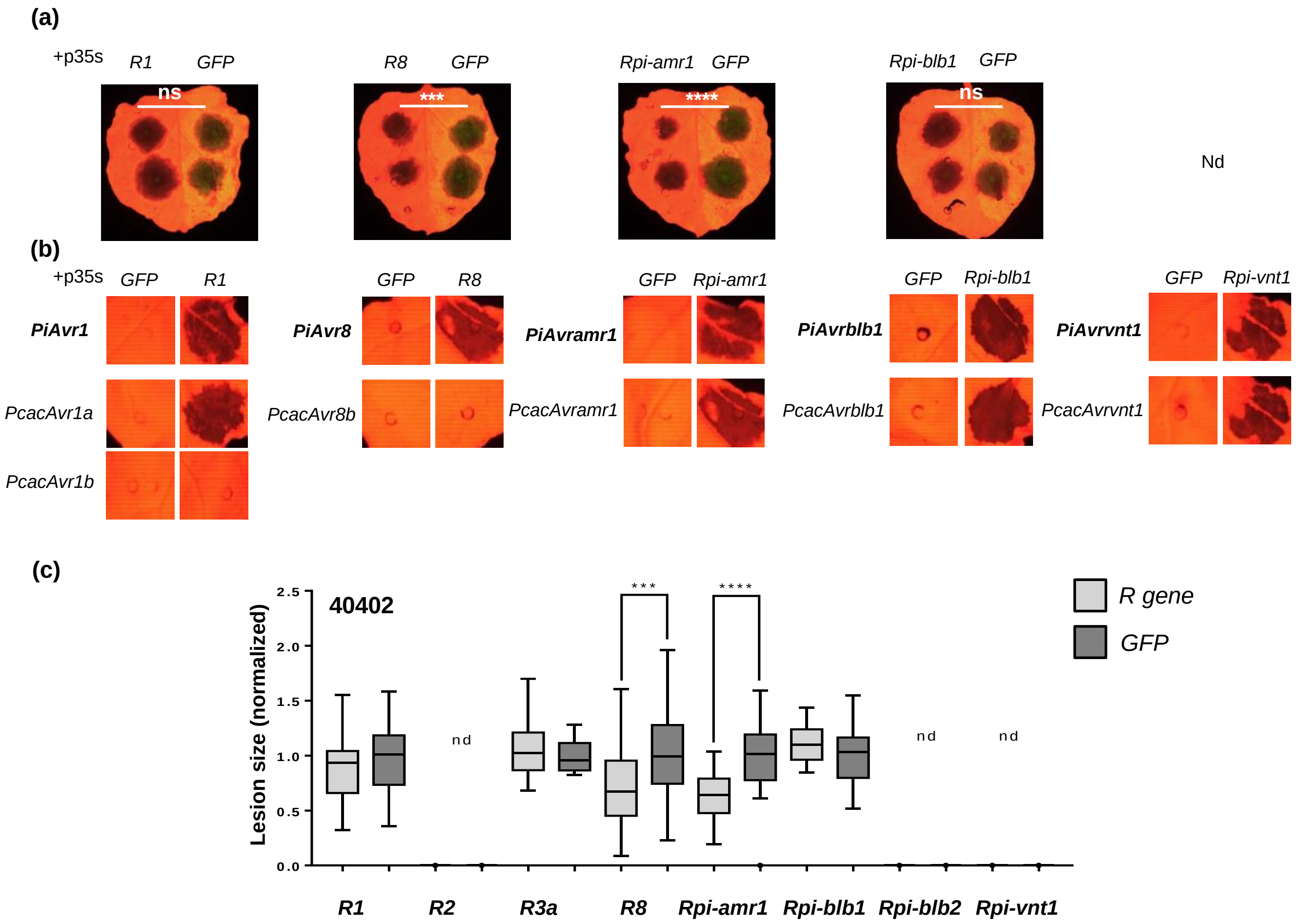


Supplementary Figure 8. *P. parasitica* strains exhibited different phenotypes against *Rpi-amr1* expression

(a) Representative images of lesion of *P. parasitica* (40164, 40402, and 40906 strains) inoculated on *Rpi-amr1*-expressed half compared to the *GFP*-expressed area of *N. benthamiana* leaves.

(b) Average lesion size of *P. parasitica* inoculated on *GFP*-expressed left and right half of *N. benthamiana* leaves were statistically similar (ns: not significant). This result indicate that our inoculation method and environment conditions were even for each cases.

(c) Average lesion size are presented with 10-90 percentile box plot. Total 8 *Solanum* NLRs were tested for resistance assay against *P. parasitica.* Expression of *Rpi-amr1* significantly reduced lesion size of *P. parasitica* 40164 and 40402 strains compared to GFP-expressed region of *N. benthamiana* leaves, but it was not working against 40906 strain. Statistically significances were analyzed with unpaired *t*-test using data collected from 2 independent experiments (*P<0.05; ****P<0.0001).

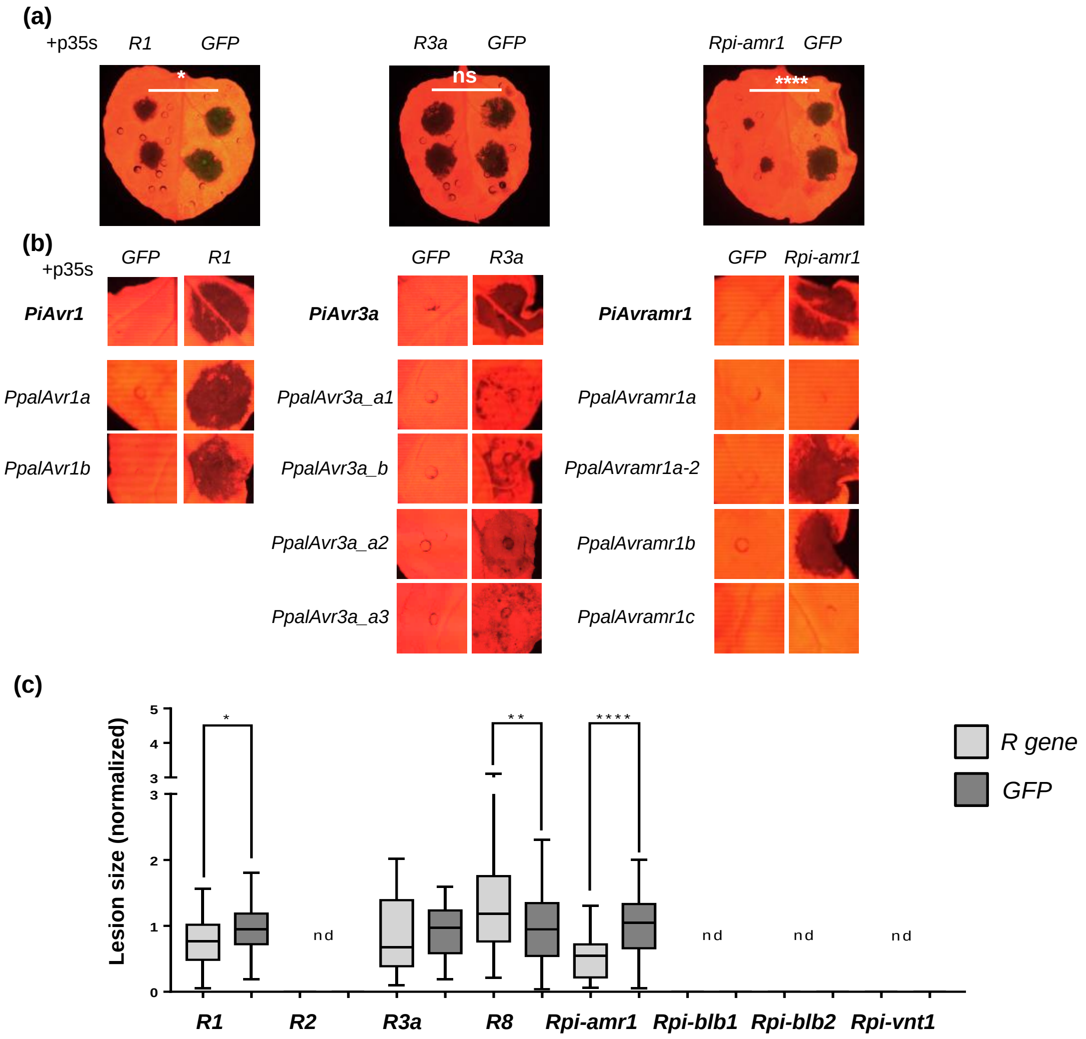


Supplementary Figure 9. *Solanum* NLRs recognizing PiAVR effectors also recognize *P. cactorum* homologs

(a) Representative images of lesion of *P. cactorum* (40166 strain) inoculated on *Solanum* NLR-expressed half compared to the *GFP*-expressed area of *N. benthamiana* leaves (ns: not significant; nd: non-determined).

(b) Six *Solanum* NLRs (*R3a* is not presented cause it cannot induced cell death against *PcacAvr3a* candidates) were co-expressed with their putative corresponding effectors of *P. cactorum* in *N. benthamiana* leaves. Representative image of positive cases are presented.

(c) Average lesion size are presented with 10-90 percentile box plot. Total 5 *Solanum* NLRs were tested for resistance assay against *P. cactorum.* Expression of R8 and *Rpi-amr1* significantly reduced lesion size compared to GFP-expressed region of *N. benthamiana* leaves. Statistically significances were analyzed with unpaired *t*-test using data collected from 2 independent experiments (***P<0.001; ****P<0.0001; nd: non-determined).

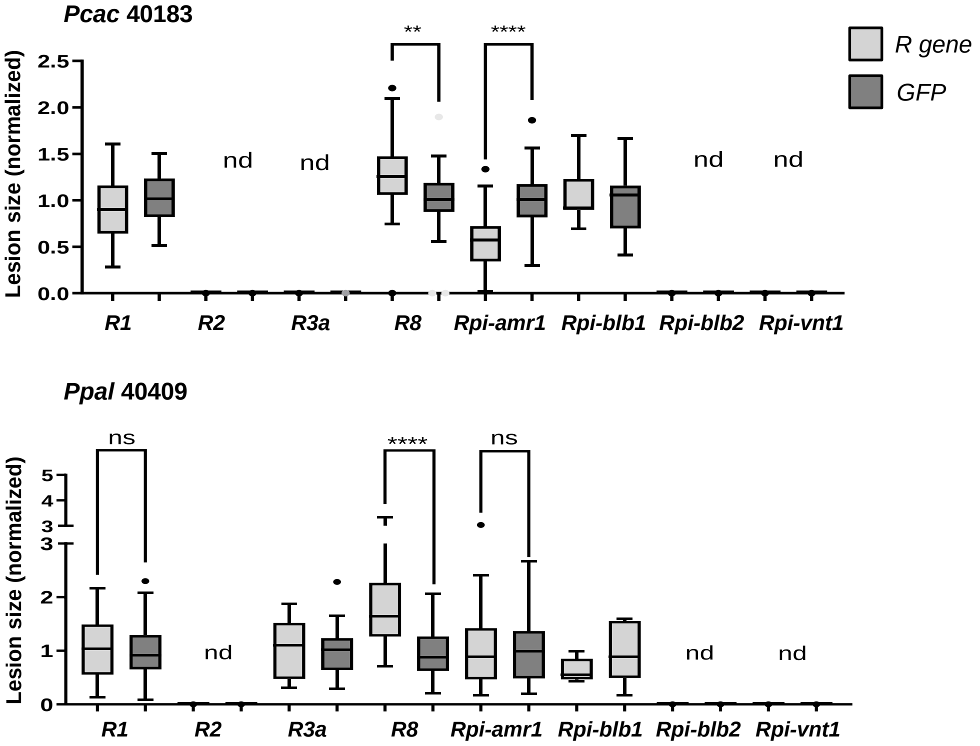


Supplementary Figure 10. *Solanum* NLRs recognizing PiAVR effectors also recognize *P. palmivora* homologs

(a) Representative images of lesion of *P. palmivora* (40410 strain) inoculated on *Solanum* NLR-expressed half compared to the *GFP*-expressed area of *N. benthamiana* leaves (ns: not significant).

(b) Five *Solanum* NLRs (*R8* and *Rpi-vnt1* are not presented cause they cannot induced cell death against *PpalAVR* candidates) were co-expressed with their putative corresponding effectors of *P. palmivora* in *N. benthamiana* leaves. Representative image of positive cases are presented.

(c) Average lesion size are presented with 10-90 percentile box plot. Total 4 *Solanum* NLRs were tested for resistance assay against *P. palmivora.* Expression of R1 and *Rpi-amr1* significantly reduced lesion size compared to GFP-expressed region of *N. benthamiana* leaves. Statistically significances were analyzed with unpaired *t*-test using data collected from 2 independent experiments (*P<0.05; **P<0.01; ****P<0.0001; nd: non-determined).

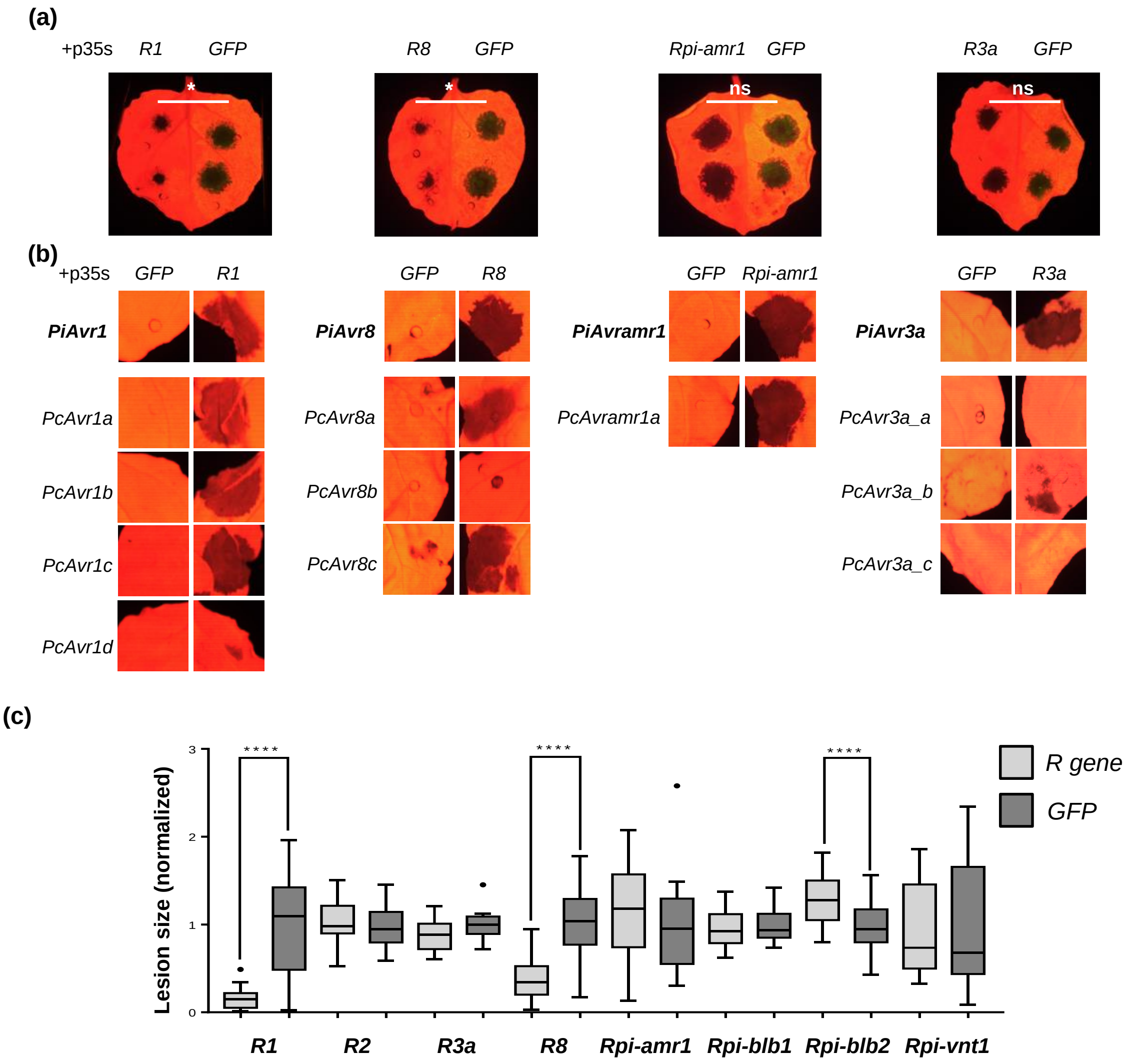


Pcac 40183

Ppal 40409

Supplementary Figure 11. *P. cactorum* and *P. palmivora* strains exhibited different phenotypes against expression of *R1*, *R8* or *Rpi-amr1*.

Average lesion size are presented with 10-90 percentile box plot. Total 4 and 5 *Solanum* NLRs were tested for resistance assay against *P. cactorum* and *P. palmivora,* respectively. Although expression of *R8* significantly reduced lesion size of *P. cactorum* 40166 strain, it was not working against 40183 strain. Similarly, while expression of *R1* or *Rpi-amr1* significantly reduced lesion size of *P. palmivora* 40410 strain, it was not replicated against 40409 strain. Statistically significances were analyzed with unpaired *t*-test using data collected from 2 independent experiments (**P<0.01; **P<0.01; ****P<0.0001; ns: not significant; nd: non-determined).

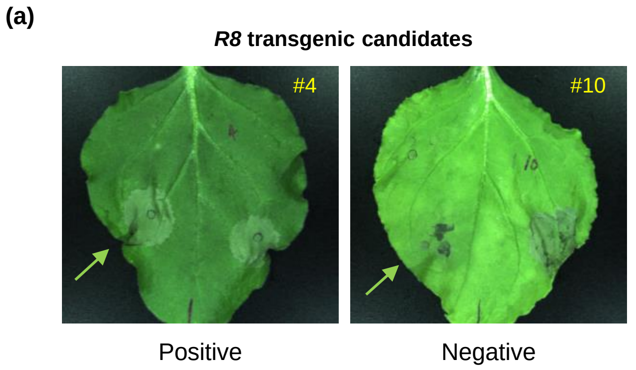


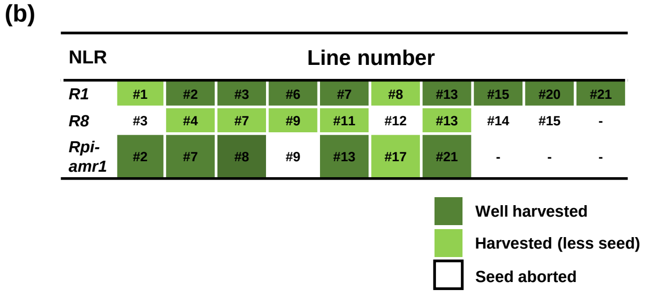
Supplementary Figure 12. *Solanum* NLRs recognizing PiAVR effectors also recognize *P. capsici* homologs

(a) Representative images of lesion of *P. capsici* (40476 strain) inoculated on *Solanum* NLR-expressed half compared to the *GFP*-expressed area of *N. benthamiana* leaves (ns: not significant).

(b) Five *Solanum* NLRs (*R2* is not presented cause it cannot induced cell death against *PcAvr2* candidates) were co-expressed with their putative corresponding effectors of *P. capsici* in *N. benthamiana* leaves. Representative image of positive cases are presented.

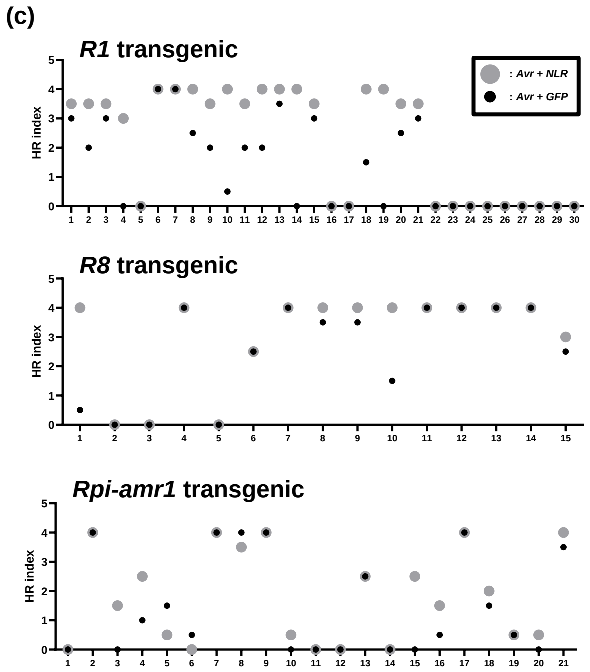
(c) Average lesion size are presented with 10-90 percentile box plot. Total 8 *Solanum* NLRs were tested for resistance assay against *P. capsici.* Expression of R1 and *R8* significantly reduced lesion size compared to GFP-expressed region of *N. benthamiana* leaves. Statistically significances were analyzed with unpaired *t*-test using data collected from 2 independent experiments (****P<0.0001).





NLR	Line number									
R1	#1	#2	#3	#6	#7	#8	#13	#15	#20	#21
R8	#3	#4	#7	#9	#11	#12	#13	#14	#15	-
Rpi-amr1	#2	#7	#8	#9	#13	#17	#21	-	-	-

■ Well harvested
▨ Harvested (less seed)
☐ Seed aborted

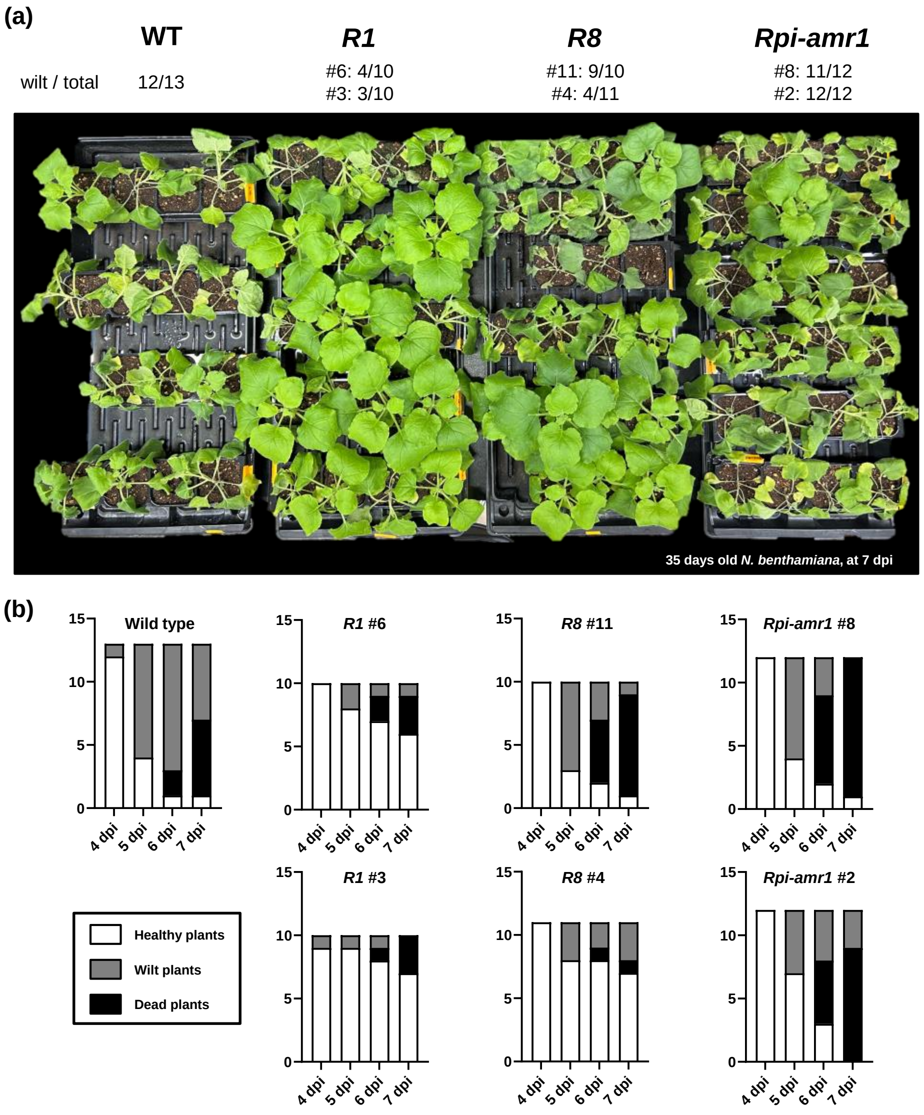


Supplementary Figure 13. HR phenotype screening of T_0 transgenic *N. benthamiana*

(a) Representative images of positive and negative case of HR screening test. Expression of *Avr8* with *GFP* (left, marked with green arrow in picture) through the agroinfiltration triggered clear cell death response in T_0 plant line #4, but not in line #10. *Avr8*/*R8* were co-expressed as a positive control.

(b) Among the 30, 15, and 21 T_0 plant thought to express *R1*, *R8*, and *Rpi-amr1*, respectively, 10, 9, and 7 lines remain after the HR screening step. T_1 seeds were harvested from 6~9 months old *N. benthamiana*.

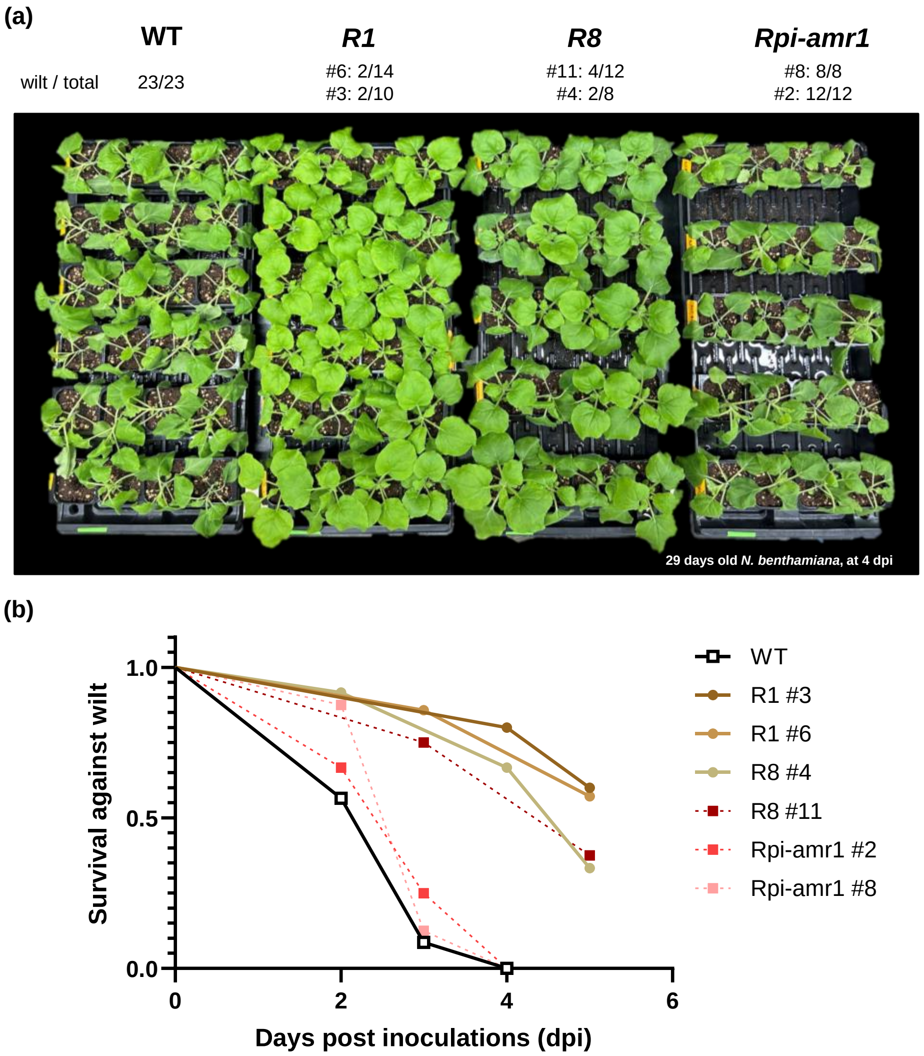
(c) Cell death intensity against corresponding avirulence effector of *P. infestans* in candidate plants thought as expressing *R1*, *R8*, or *Rpi-amr1* is presented as dot blot.



Supplementary Figure 14. T_1 plants expressing *R1* or *R8* were resistant against root infection of *P. capsici*.

(a) Picture of root inoculated plants (wild type, *R1*, *R8*, and *Rpi-amr1* transgenic *N. benthamiana,* 35 days old) at 7 days post inoculation. Each plant is inoculated with zoospores of *P. capsici. R1* (#3 and #6) and *R8* (#4) plants exhibited resistance phenotypes while WT and *Rpi-amr1* transgenic plants were completely wilt and shrivel. The number of wilt/total plants is presented as fraction above each plant line.

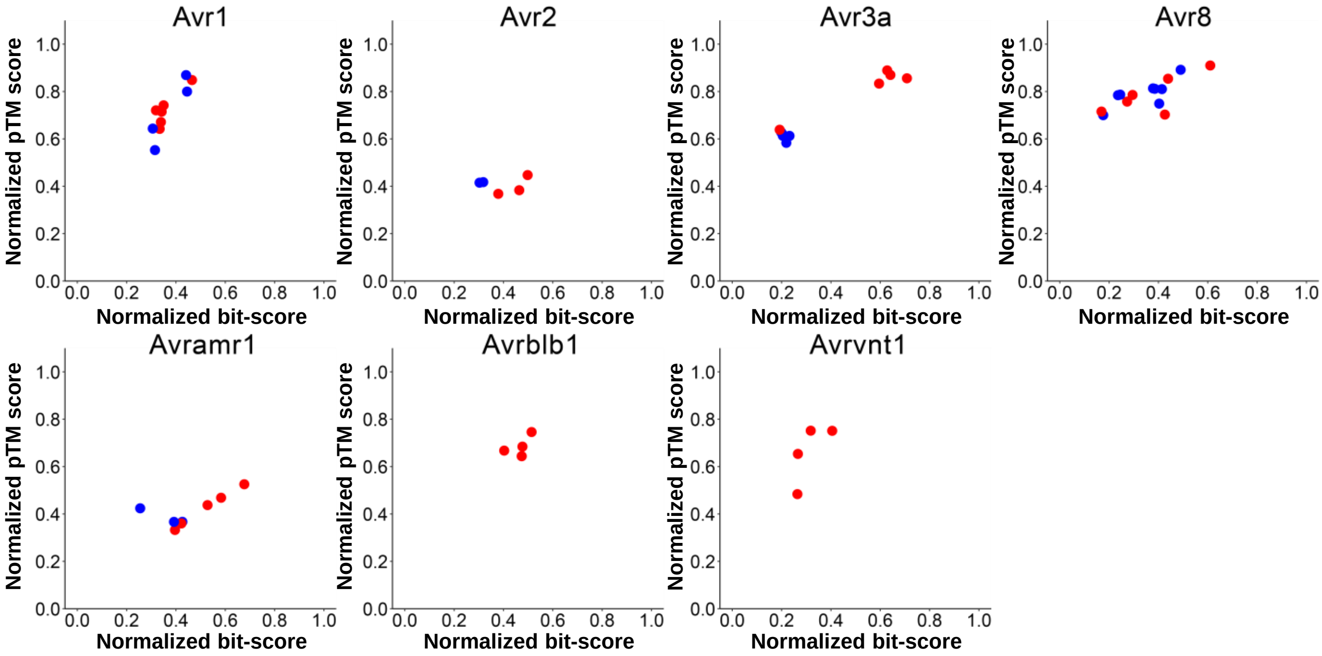
(b) The number of healthy, wilt, and dead plants are presented as bar graph. Approximately 30% of *R1* (#3 and #6) and *R8* (#4) plants were also exhibited wilt phenotypes.



Supplementary Figure 15. T$_1$ plants expressing *R1* or *R8* were resistant against root infection of *P. capsici*.

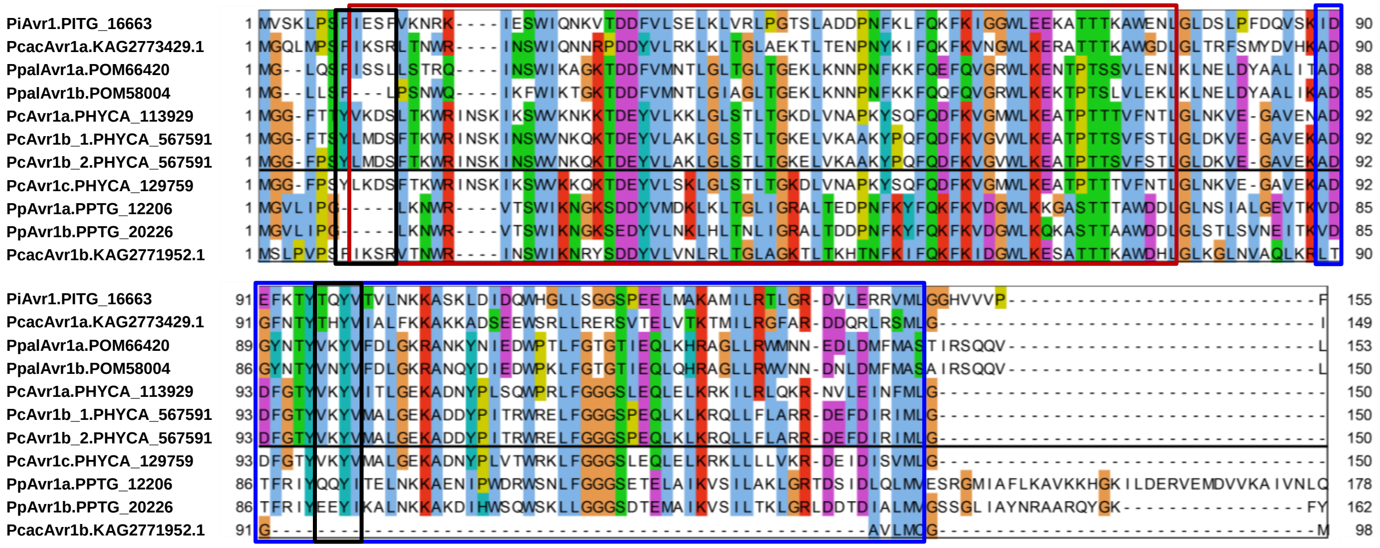
(a) Picture of root inoculated plants (wild type, *R1*, *R8*, and *Rpi-amr1* transgenic *N. benthamiana,* 29 days old) at 4 days post inoculation. Each plant is inoculated with zoospores of *P. capsici. R1* (#3 and #6) and *R8* (#4 and #11) plants exhibited resistance phenotypes while WT and *Rpi-amr1* transgenic plants were completely wilt and shrivel. The number of wilt/total plants is presented as fraction above each plant line.

(b) The ratio of healthy/total plants of each line is presented as line graph. Approximately 30% of *R1* (#3 and #6) and 50% *R8* (#4 and #11) plants were also exhibited wilt phenotypes.

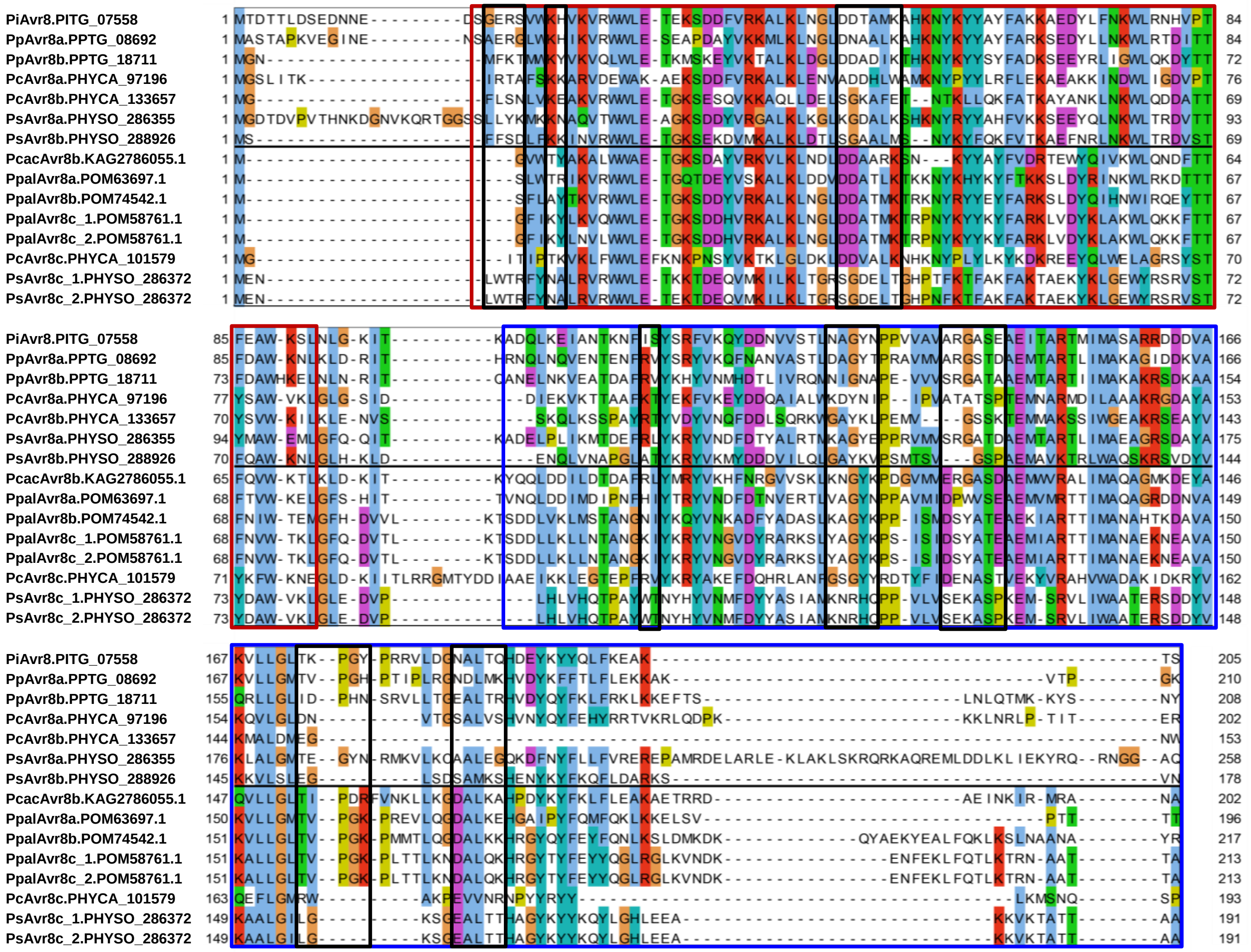


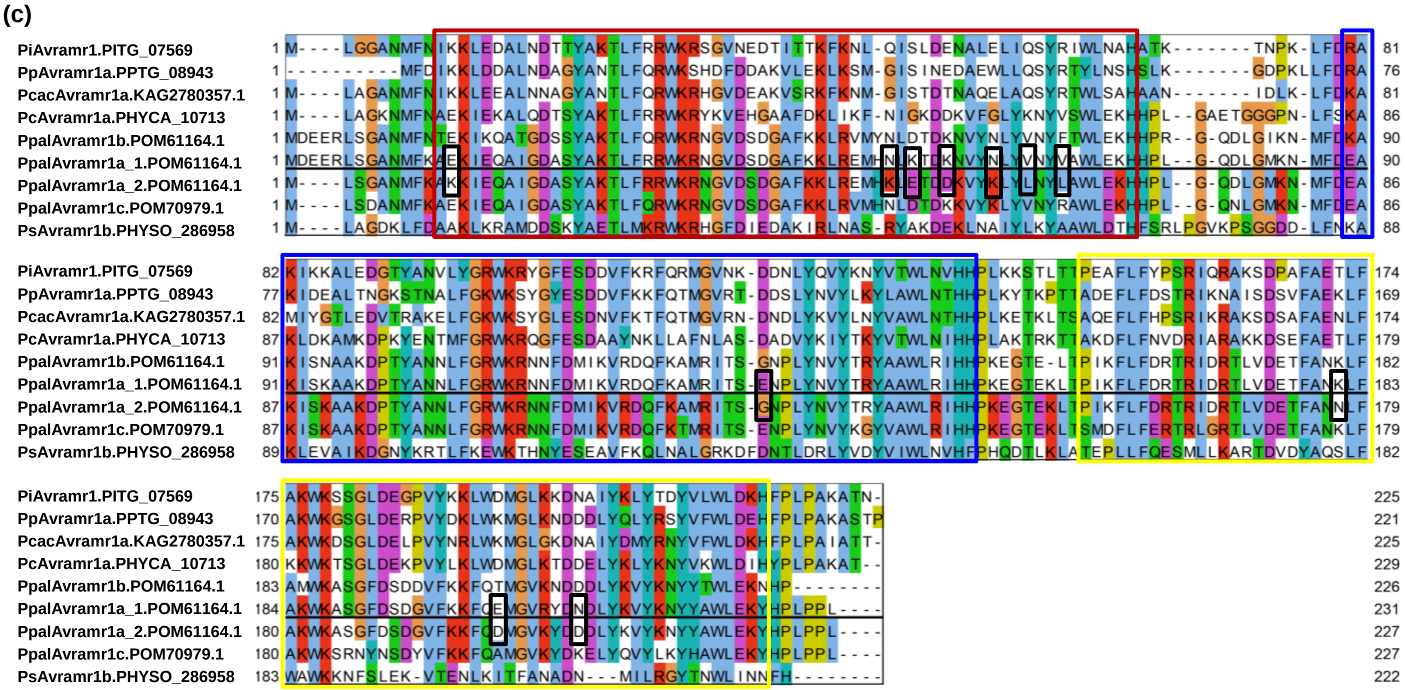
Supplementary Figure 16. Conservation ratio of protein sequence and structure for each cloned effector homologs. Red and blue dots represent HR positive and HR negative, respectively.

(a)

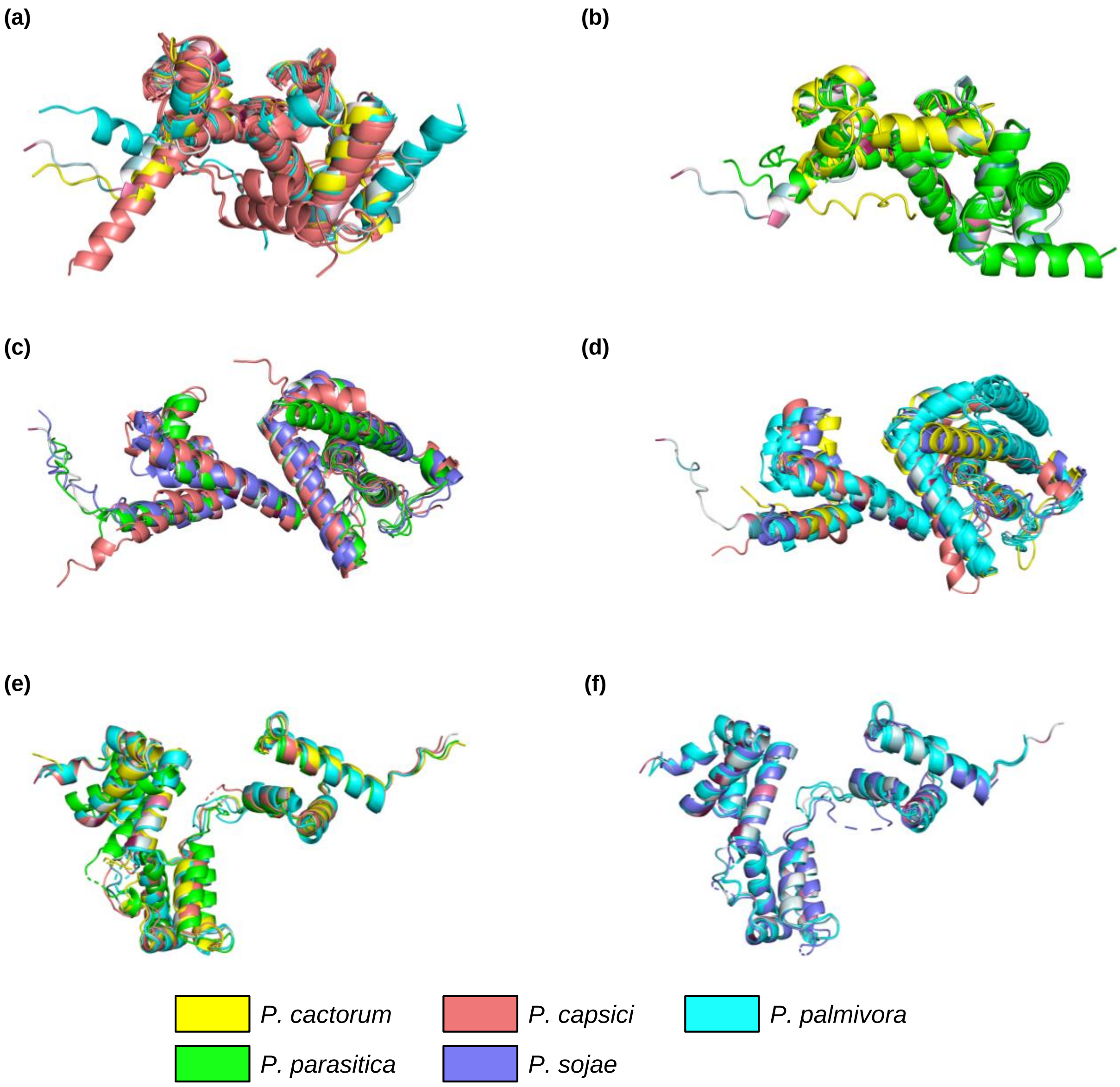

PiAvr1.PITG_16663
PcacAvr1a.KAG2773429.1
PpalAvr1a.POM66420
PpalAvr1b.POM58004
PcAvr1a.PHYCA_113929
PcAvr1b_1.PHYCA_567591
PcAvr1b_2.PHYCA_567591
PcAvr1c.PHYCA_129759
PpAvr1a.PPTG_12206
PpAvr1b.PPTG_20226
PcacAvr1b.KAG2771952.1

(b)


PiAvr8.PITG_07558
PpAvr8a.PPTG_08692
PpAvr8b.PPTG_18711
PcAvr8a.PHYCA_97196
PcAvr8b.PHYCA_133657
PsAvr8a.PHYSO_286355
PsAvr8b.PHYSO_288926
PcacAvr8b.KAG2786055.1
PpalAvr8a.POM63697.1
PpalAvr8b.POM74542.1
PpalAvr8c_1.POM58761.1
PpalAvr8c_2.POM58761.1
PcAvr8c.PHYCA_101579
PsAvr8c_1.PHYSO_286372
PsAvr8c_2.PHYSO_286372



Supplementary Figure 17. Sequence alignments of cloned effector domain for HR-positive and HR-negative effectors in a) Avr1 b) Avr8 c) Avramr1. The top and bottom of the thick solid lines represent HR-positive and HR-negative effectors, respectively. Sequences are colored according to amino acid properties defined in the Clustal X color scheme implemented in Jalview. Red, blue, and yellow boxes indicate each WY domain region, and black boxes indicate sequences that differently conserved between HR positive and negative.



Supplementary Figure 18. Comparison of protein structures of HR-positive and HR-negative effectors. HR positive (a, c, e) and HR negative (b, d, f) effectors are compared against the reference avirulence effectors PiAvr1 (a-b), PiAvr8 (c-d), and PiAvramr1 (e-f), respectively. Effector homologs of one species are shown in the same color.