Identification of Avr orthogroups

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

Signal peptide

RXLR motif

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

EER motif

WY domain

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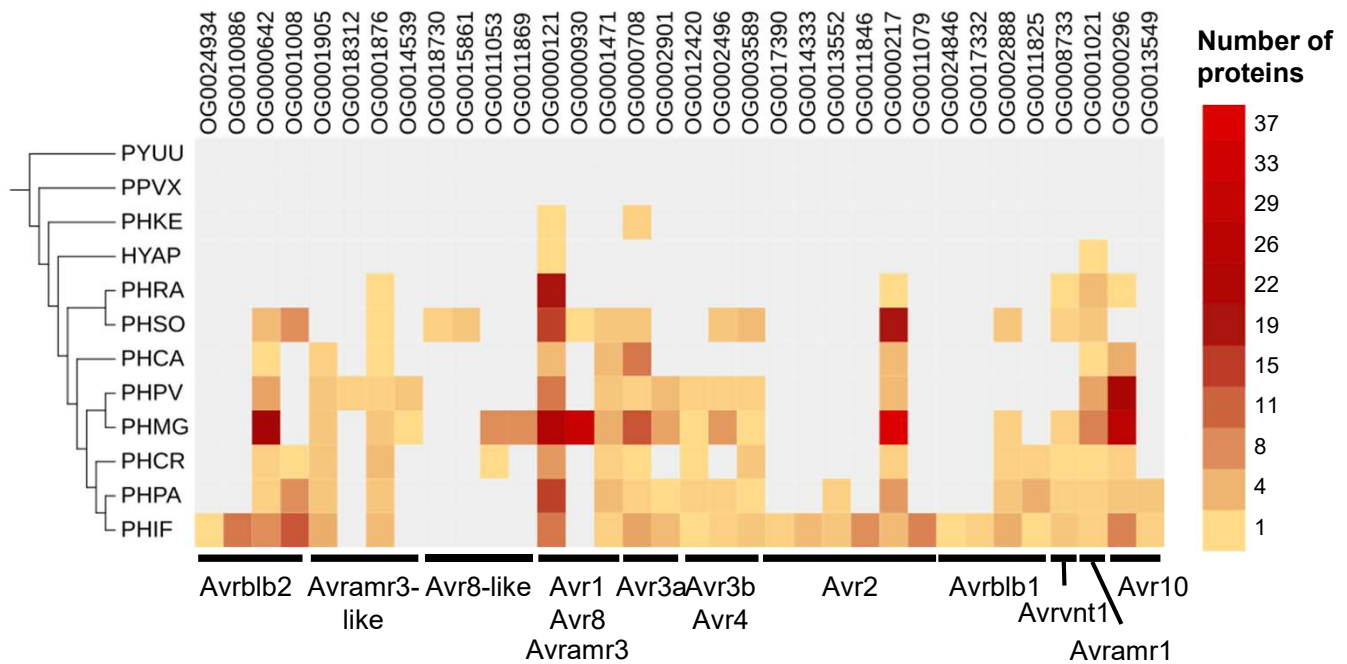- Filtration of degenerated RXLR: No EER motif or WY domain

**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.
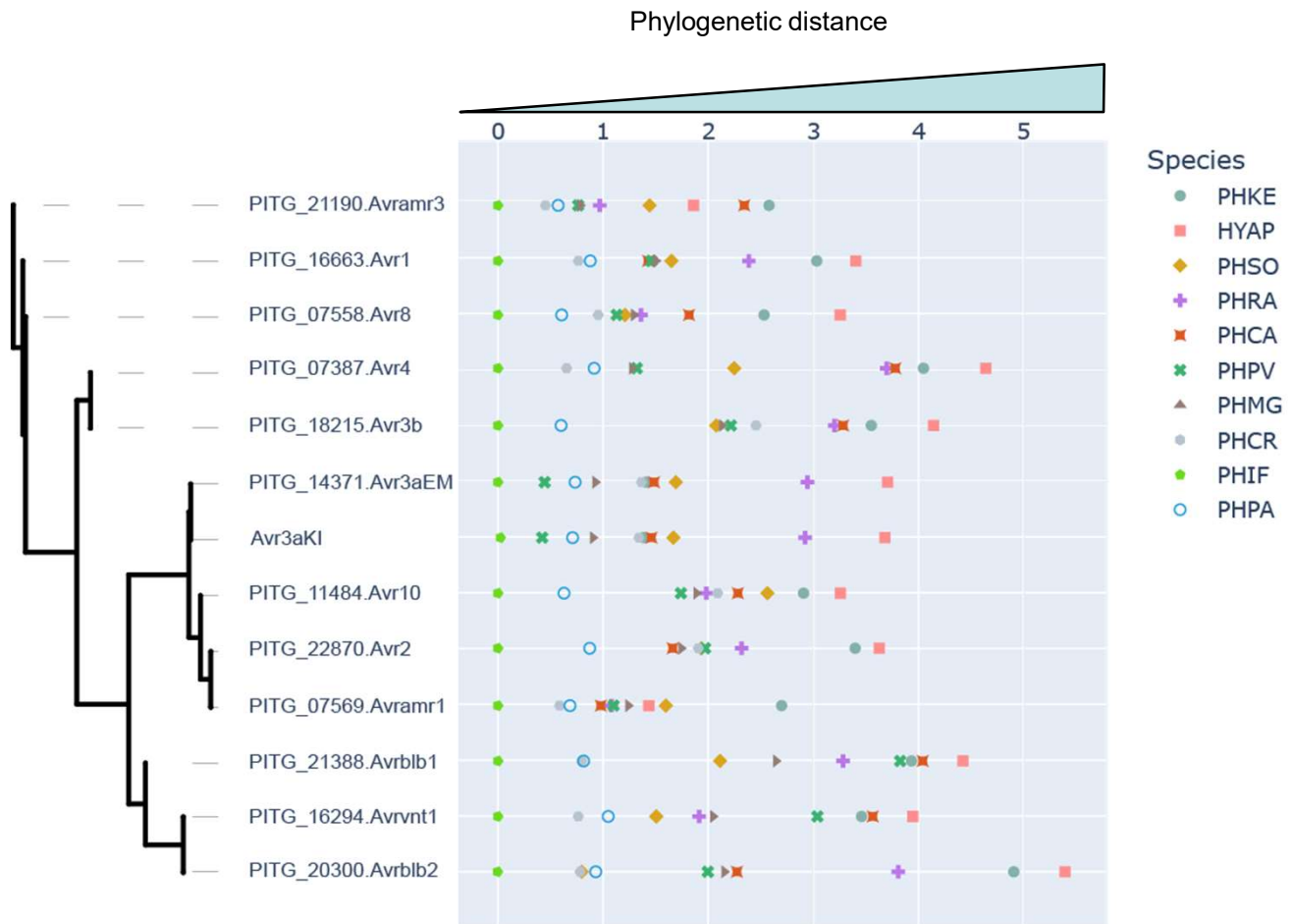
Supplementary Figure 3. *Avr1*, *Avr3a*, *Avr8*, *Avramr1*, and *Avramr3* are conserved within *Phytophthora* species. The phylogenetic (patristic) distance between reference avirulence effector in *P. infestans* and the closest homolog in the other *Phytophthora* species were calculated from the phylogenetic tree in Fig. 1c, as previously described in Harant et al. (2022).
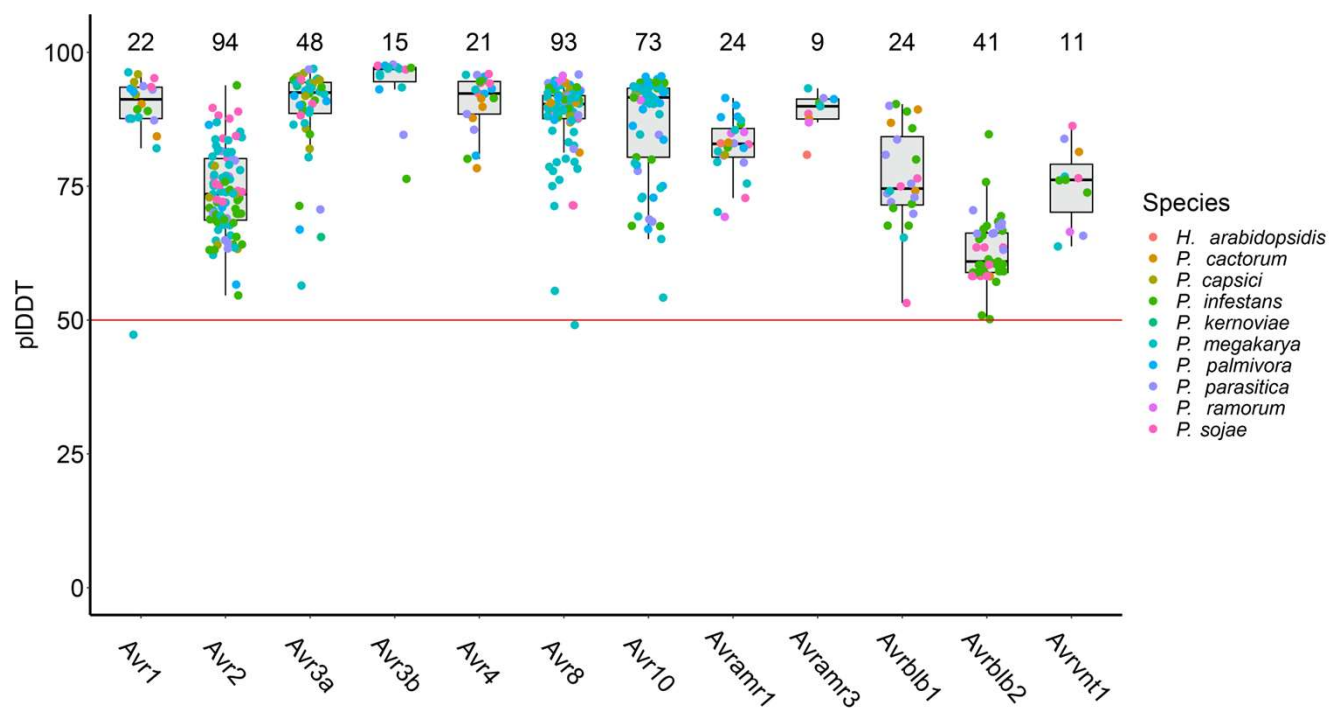
Supplementary Figure 4. Evaluation of structural predictions of effector domain regions. The numbers at the top of the figure show the number of predicted structures for each avirulence effector. The red horizontal line represents the pIDDT score cutoff for the effector structure to be used for downstream analysis.
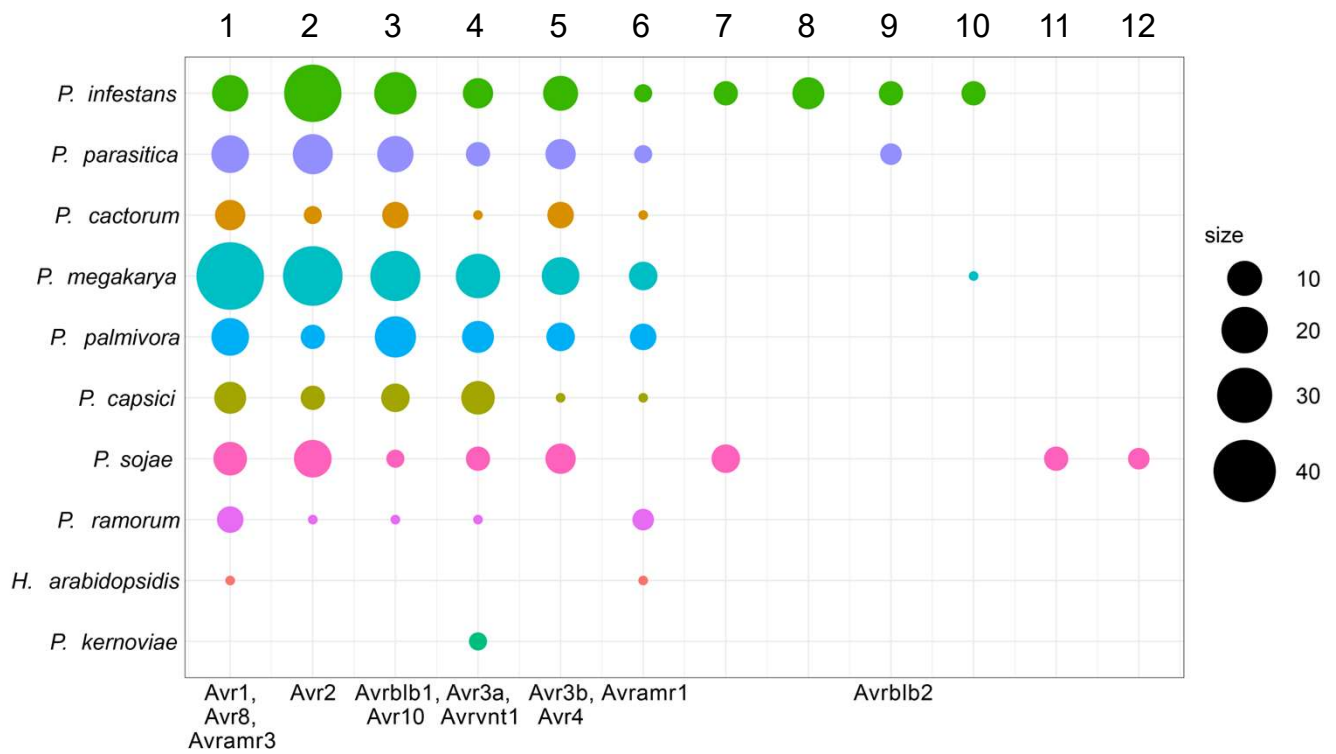
Supplementary Figure 5. Structure-based clustering of avirulence effector homologs. The numbers at the top of the figure represent the cluster number. The reference avirulence effector included in each cluster is indicated at the bottom of the figure.
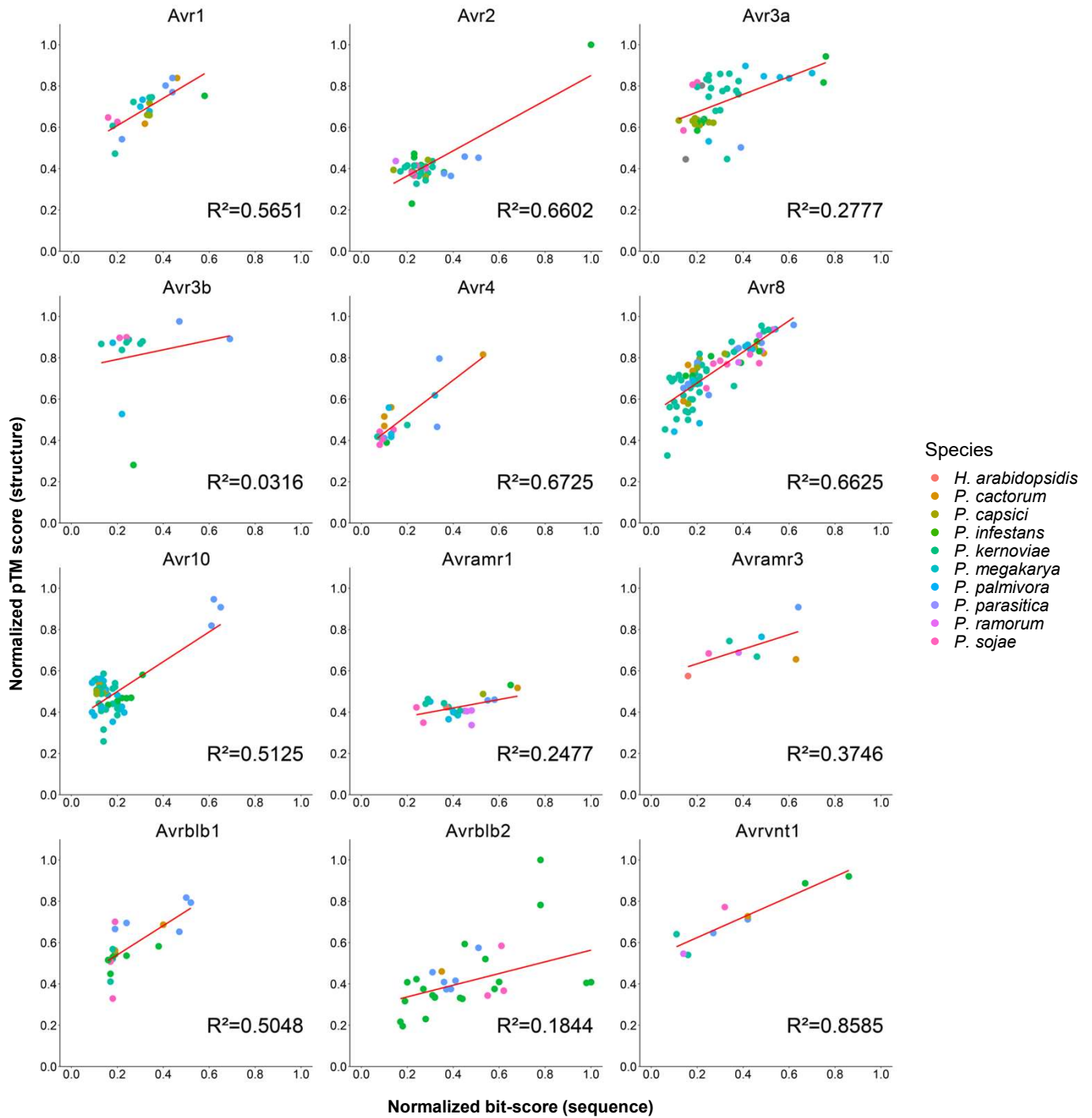
Supplementary Figure 6. Conservation ratio of protein sequence and structure for each effector homologs. The red line and R^2 value represent the Pearson correlation coefficient between normalized bit-score and normalized pTM score.
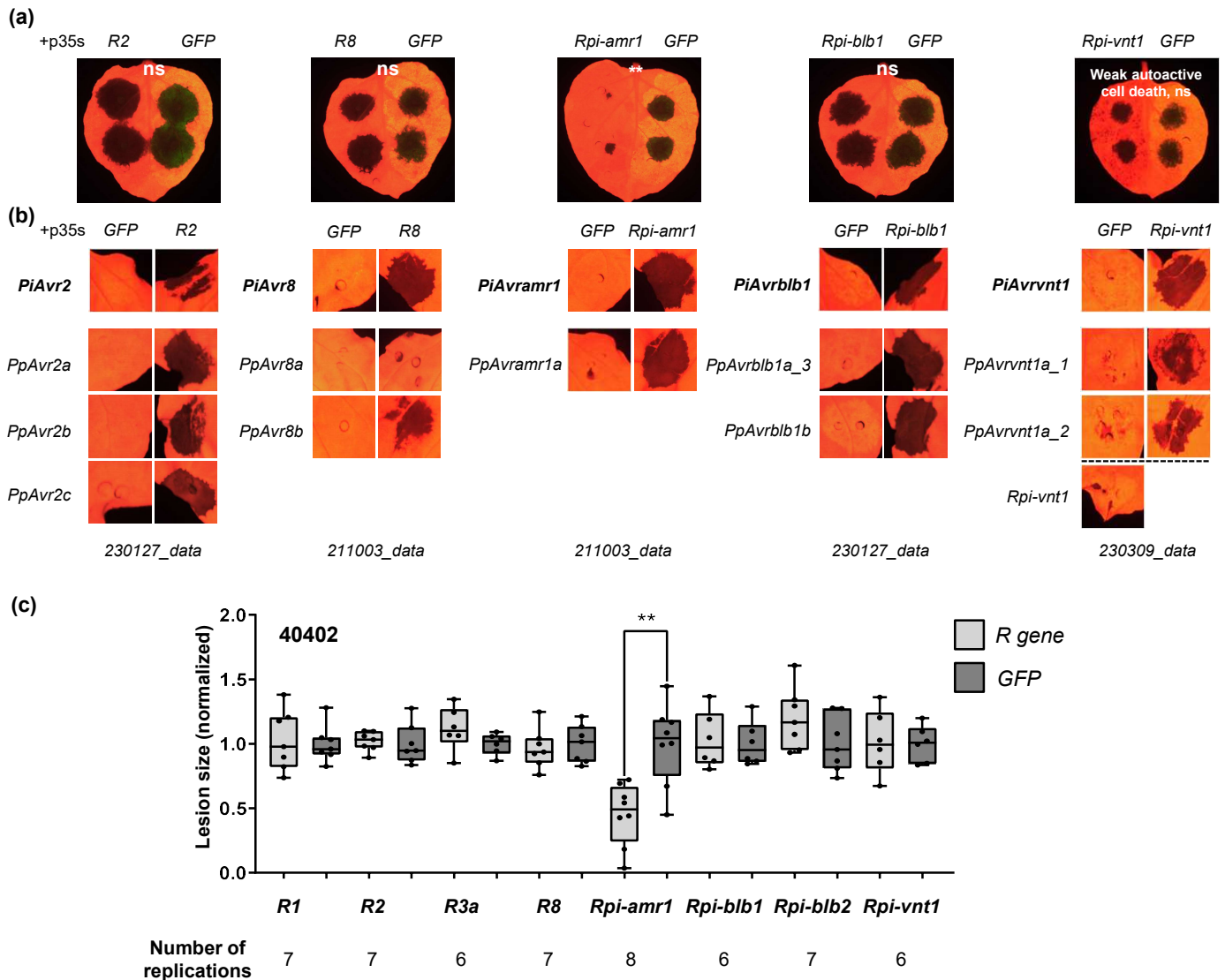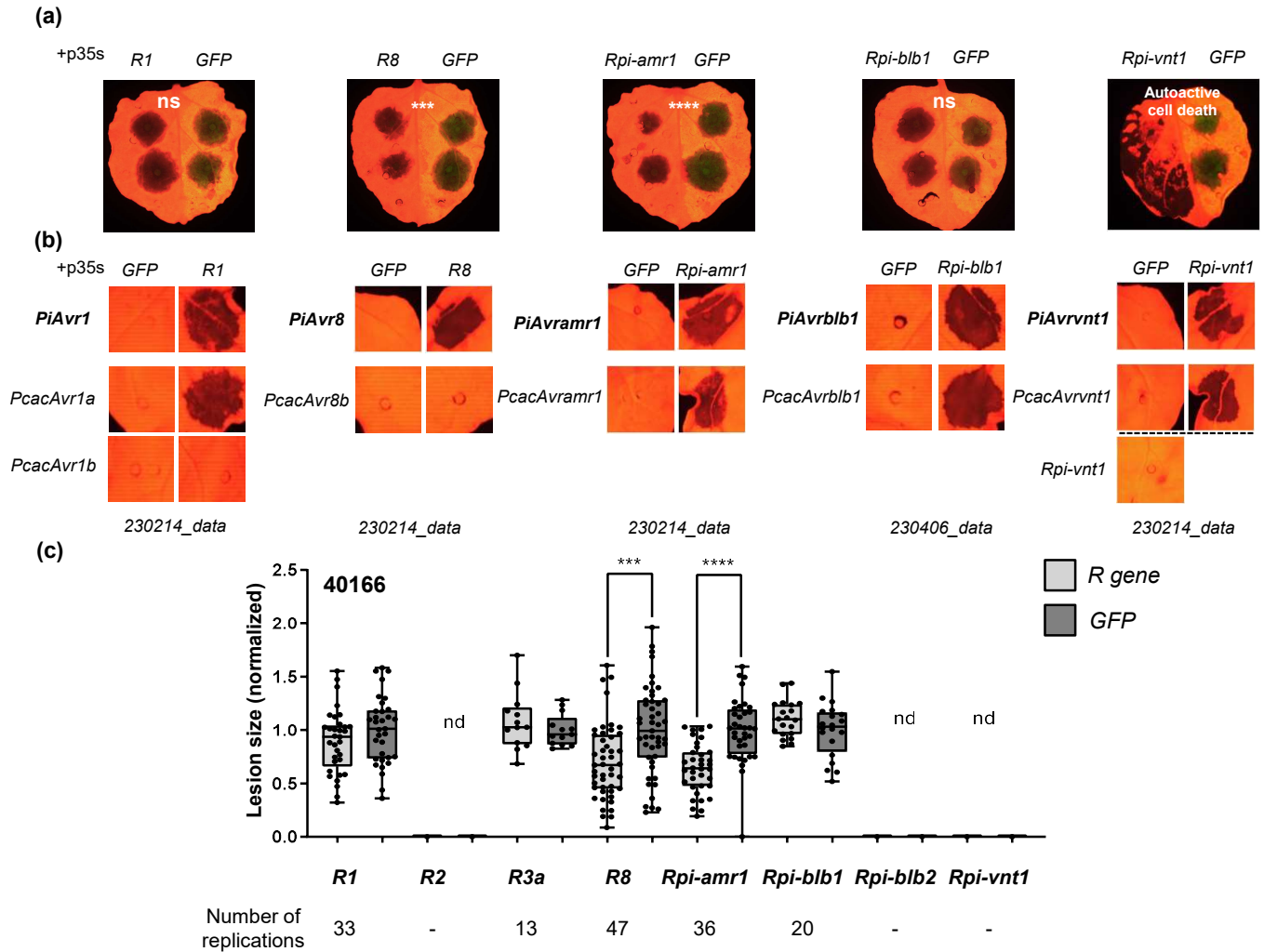
Supplementary Figure 7. *Solanum* NLRs recognizing PiAVR effectors also recognize *P. parasitica* homologs

(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). Expression of *Rpi-vnt1* induced weak cell death phenotype at 2 dpi on the infiltrated region of leaves (black spots). **(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 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 (**P<0.01).
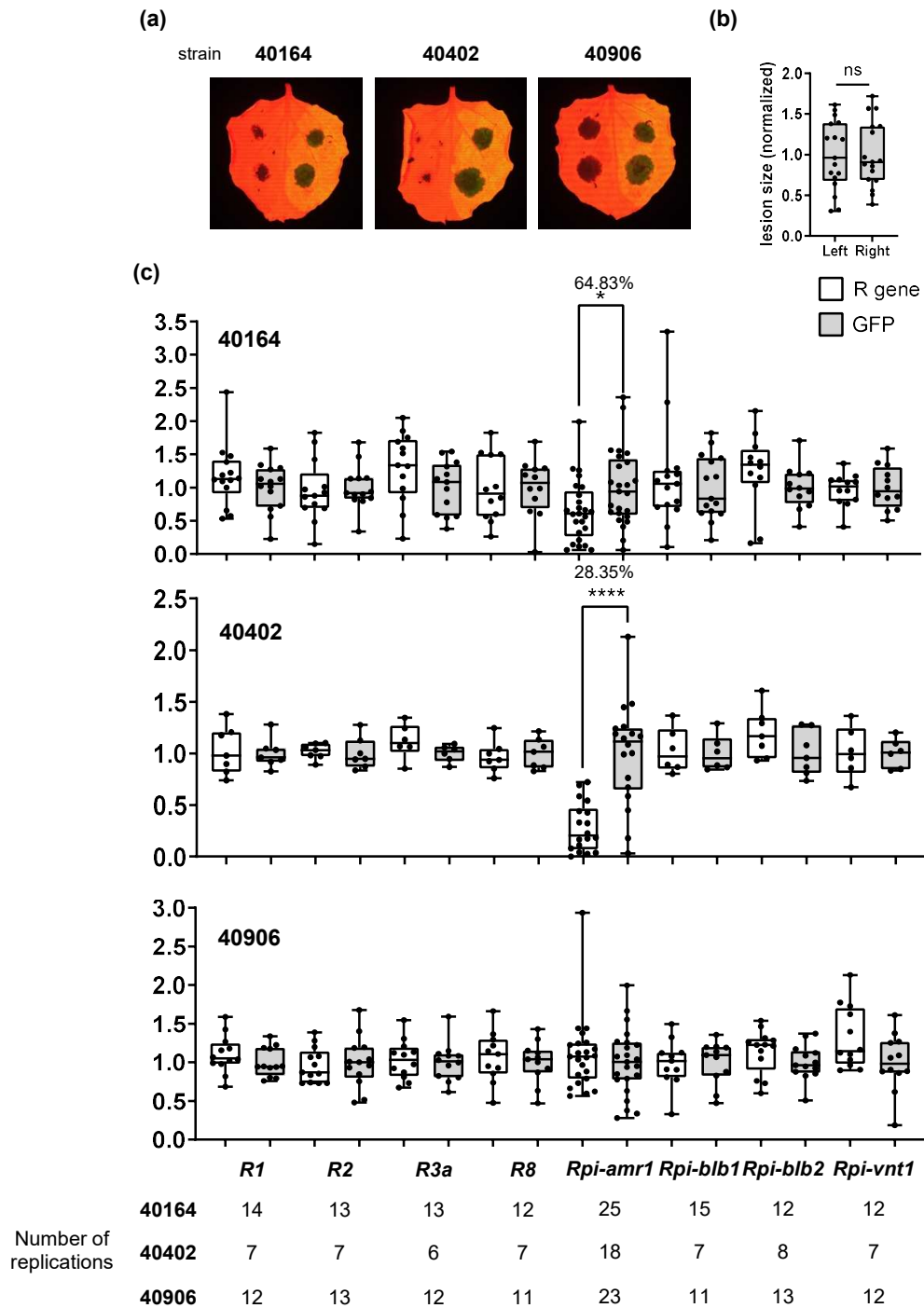
Supplementary Figure 8. *P. parasitica* strains exhibited different phenotypes against *Rpi-amr1* expression

(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 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 (*P<0.05; ****P<0.0001).
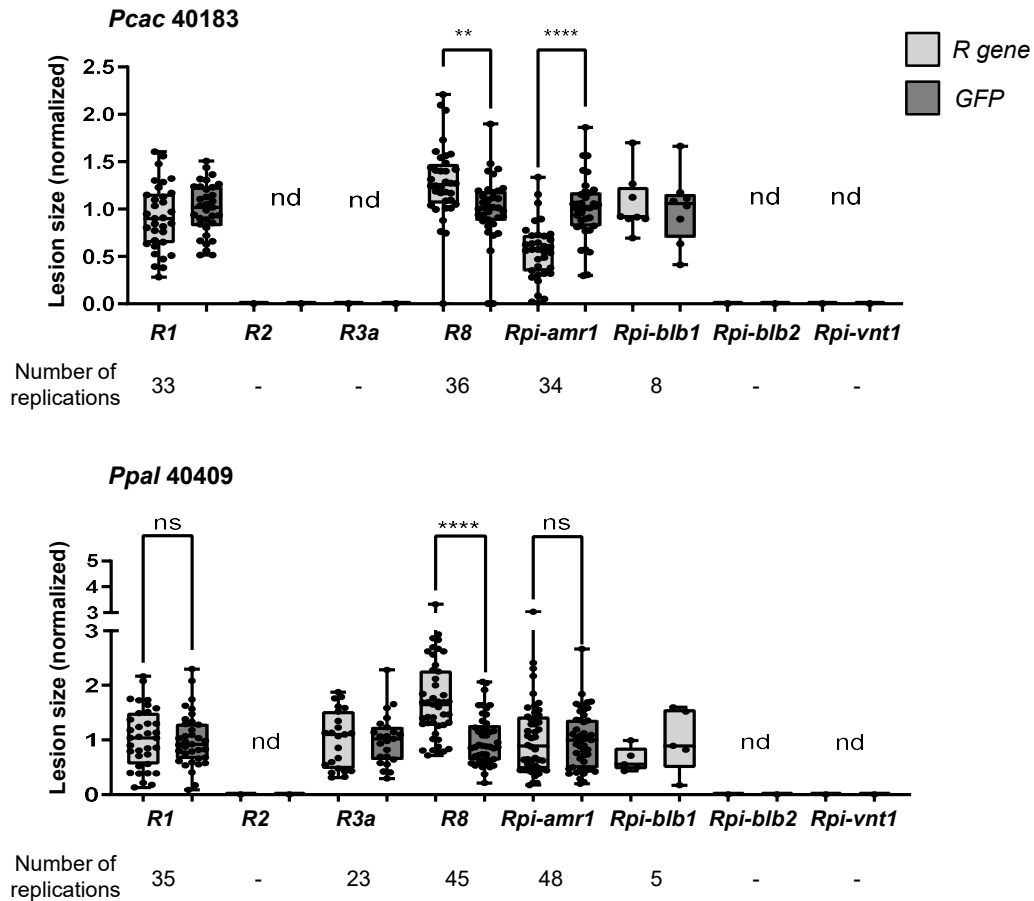
Supplementary Figure 9. *Solanum* NLRs recognizing PiAVR effectors also recognize *P. cactorum* homologs

(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). Expression of *Rpi-vnt1* induced intensive cell death phenotype at 2.5 dpi on the infiltrated region of leaves (left half of leaf in the picture). **(b)** Five *Solanum* NLRs 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 box plot. Total 5 *Solanum* NLRs (*Rpi-vnt1* was excluded due to auto-active cell death phenotype started from 2 dpi) 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 (***P<0.001; ****P<0.0001; nd: non-determined).
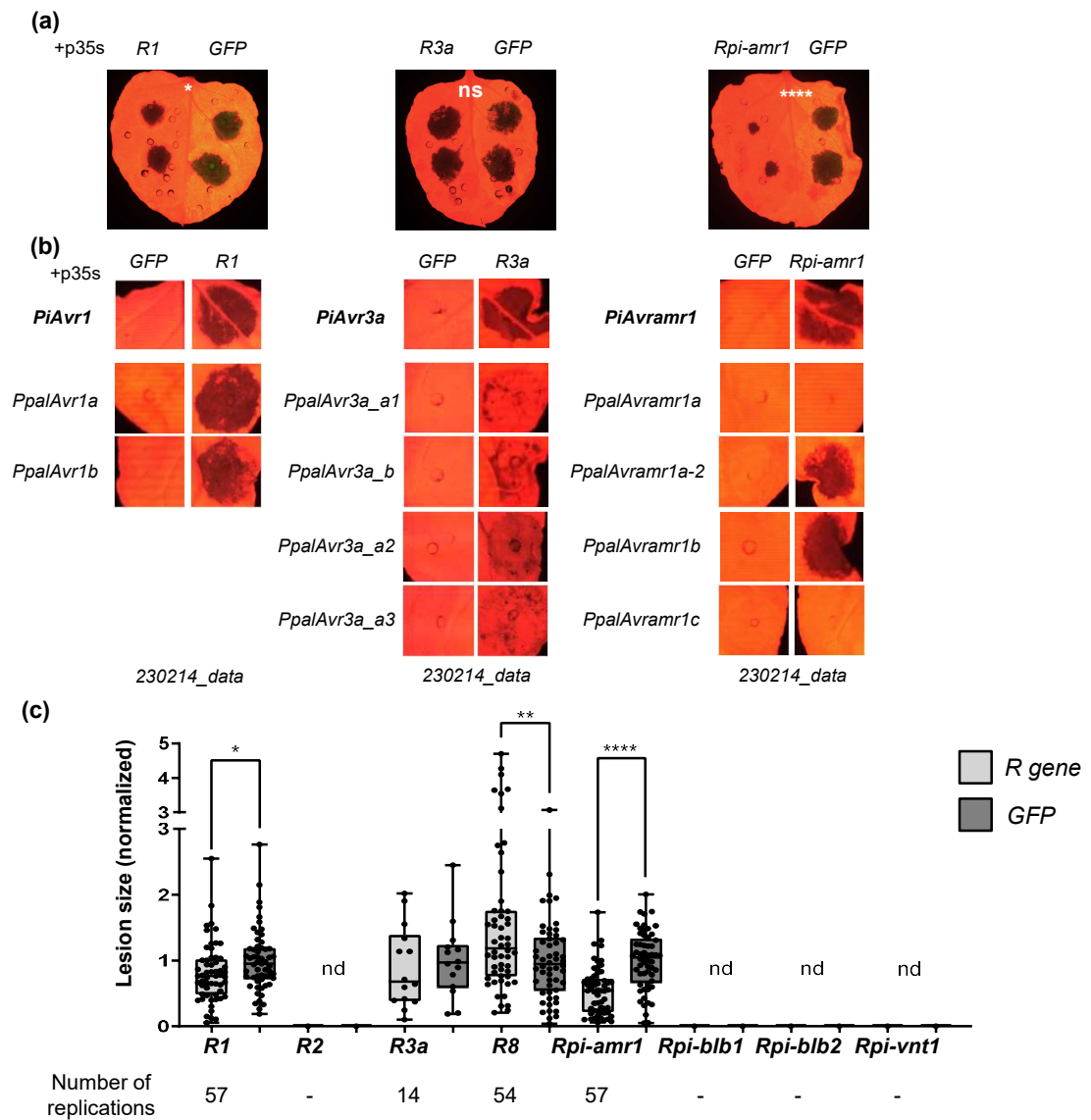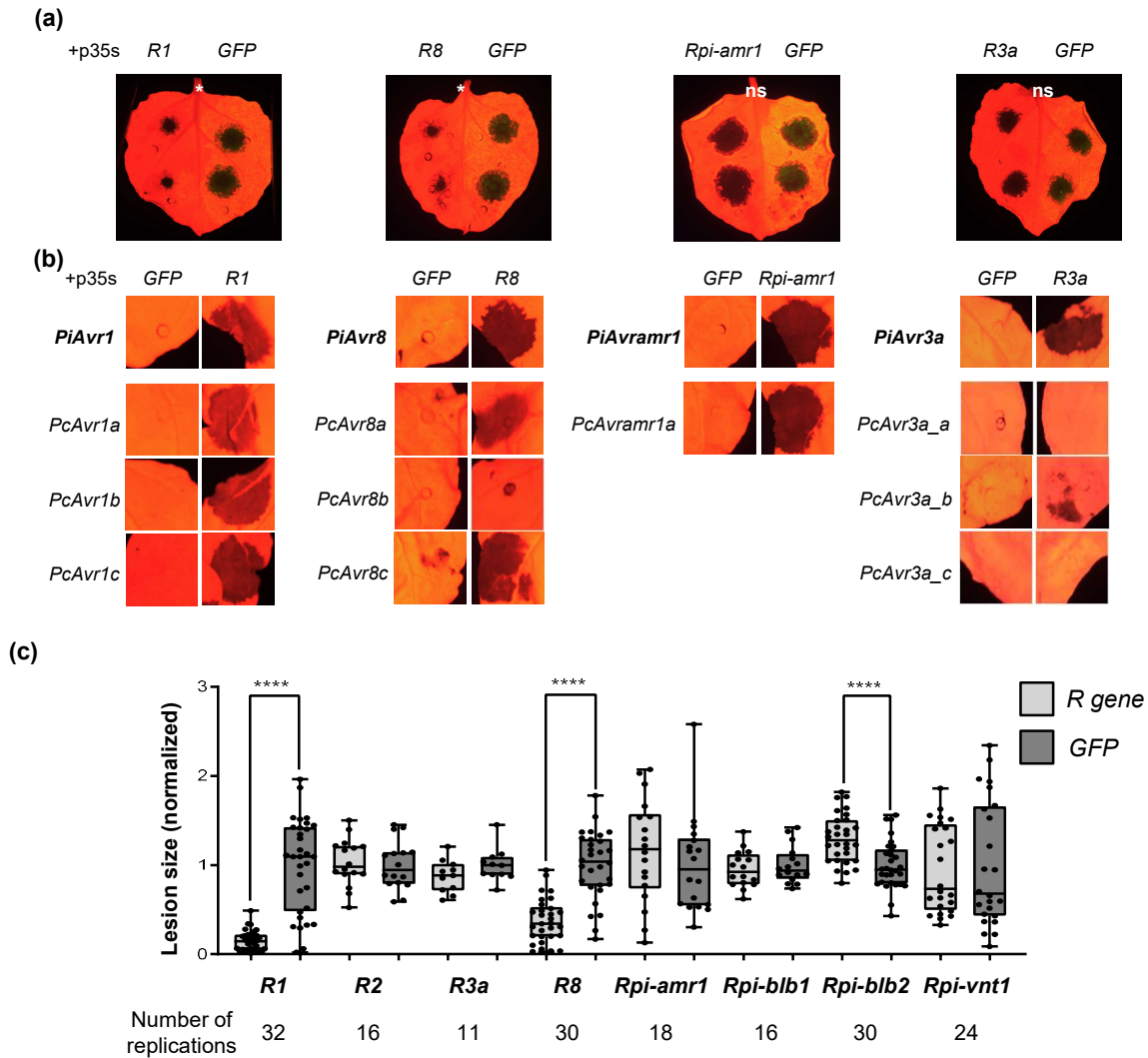
Supplementary Figure 10. *Solanum* NLRs recognizing PiAVR effectors also recognize *P. palmivora* homologs

(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)** Four *Solanum* NLRs (*R8* is not presented cause it 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 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 (*P<0.05; **P<0.01; ****P<0.0001; nd: non-determined).

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* significantly reduced lesion size of *P. palmivora* 40410 strain, it was not replicated against 40409 strain. Statistically significances were analyzed with unpaired *t*-test (**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 (****$P < 0.0001$).
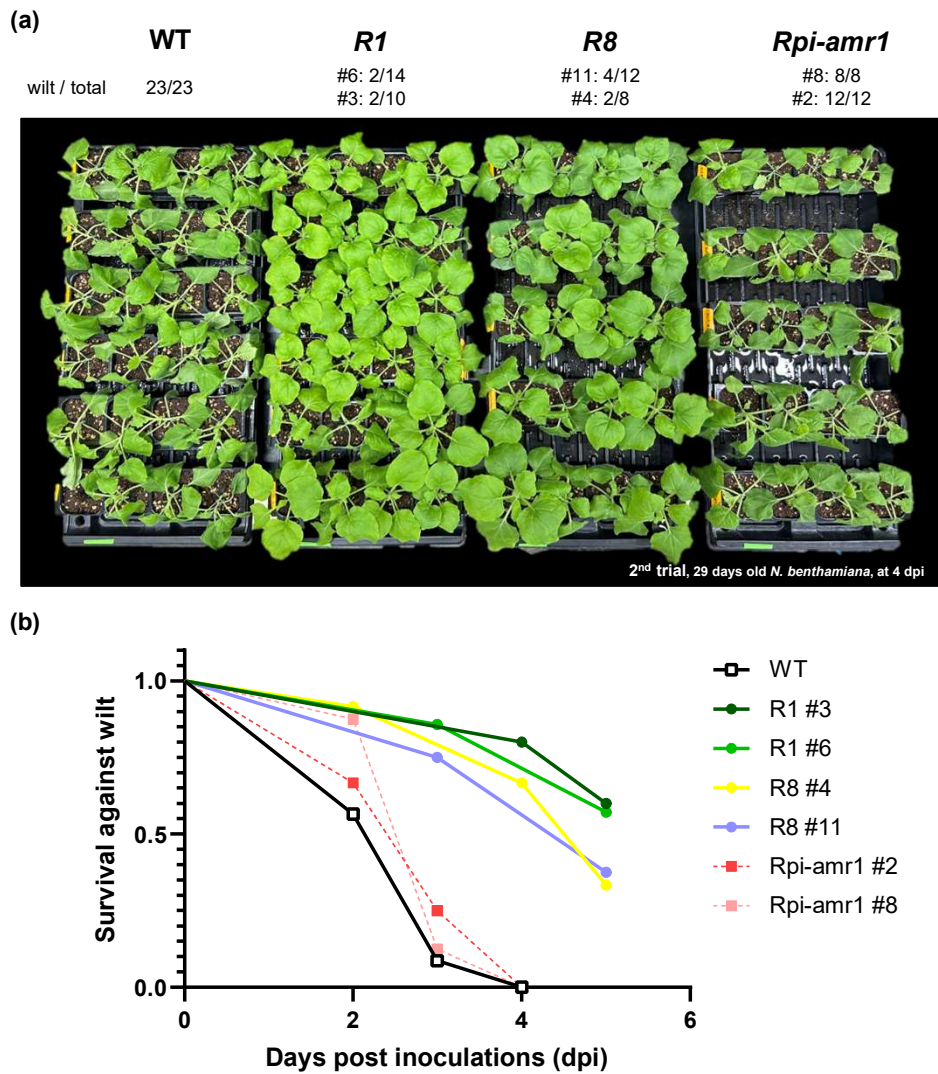
(a)

R8 transgenic candidates

#4

#10

Positive

Negative

(b)

R1 transgenic

R8 transgenic

Rpi-amr1 transgenic

: Avr + NLR
: Avr + GFP

(c)

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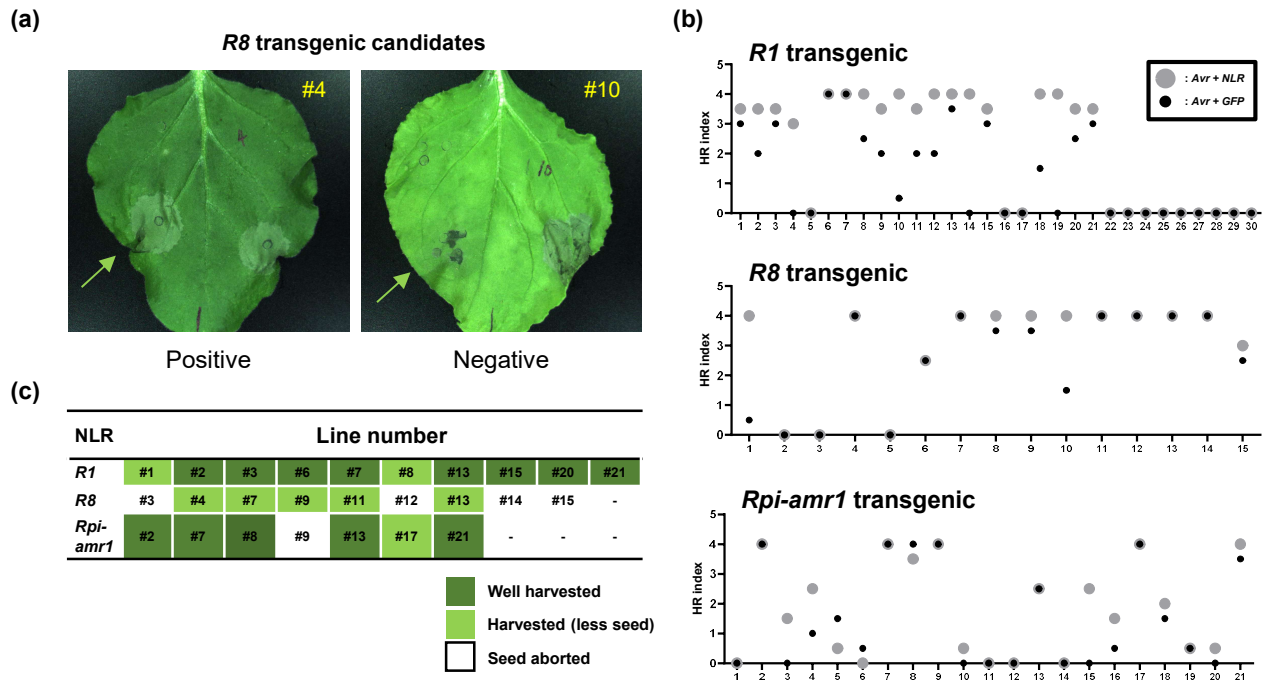
Supplementary Figure 13. HR phenotype screening of T_0 transgenic *N. benthamiana*

(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.
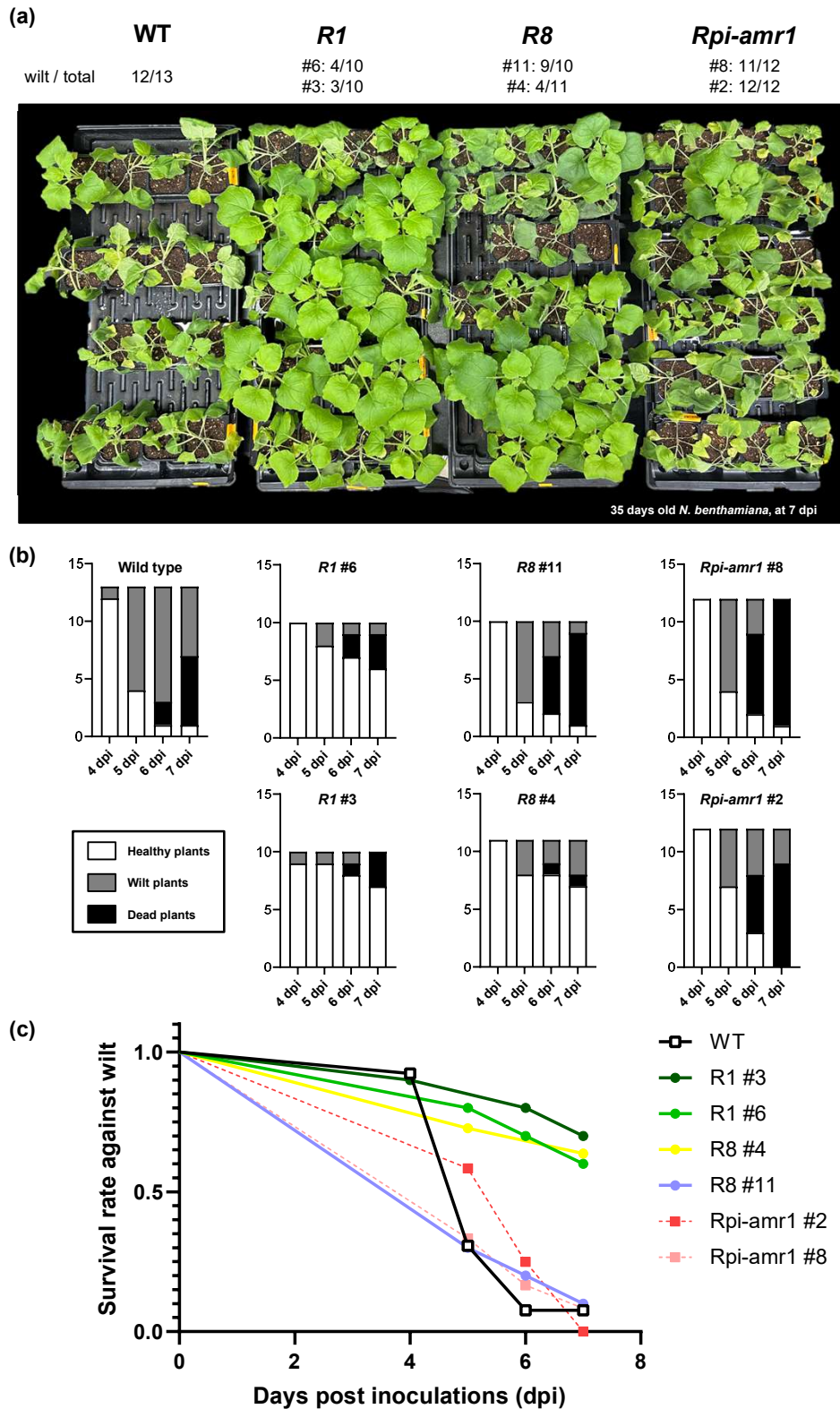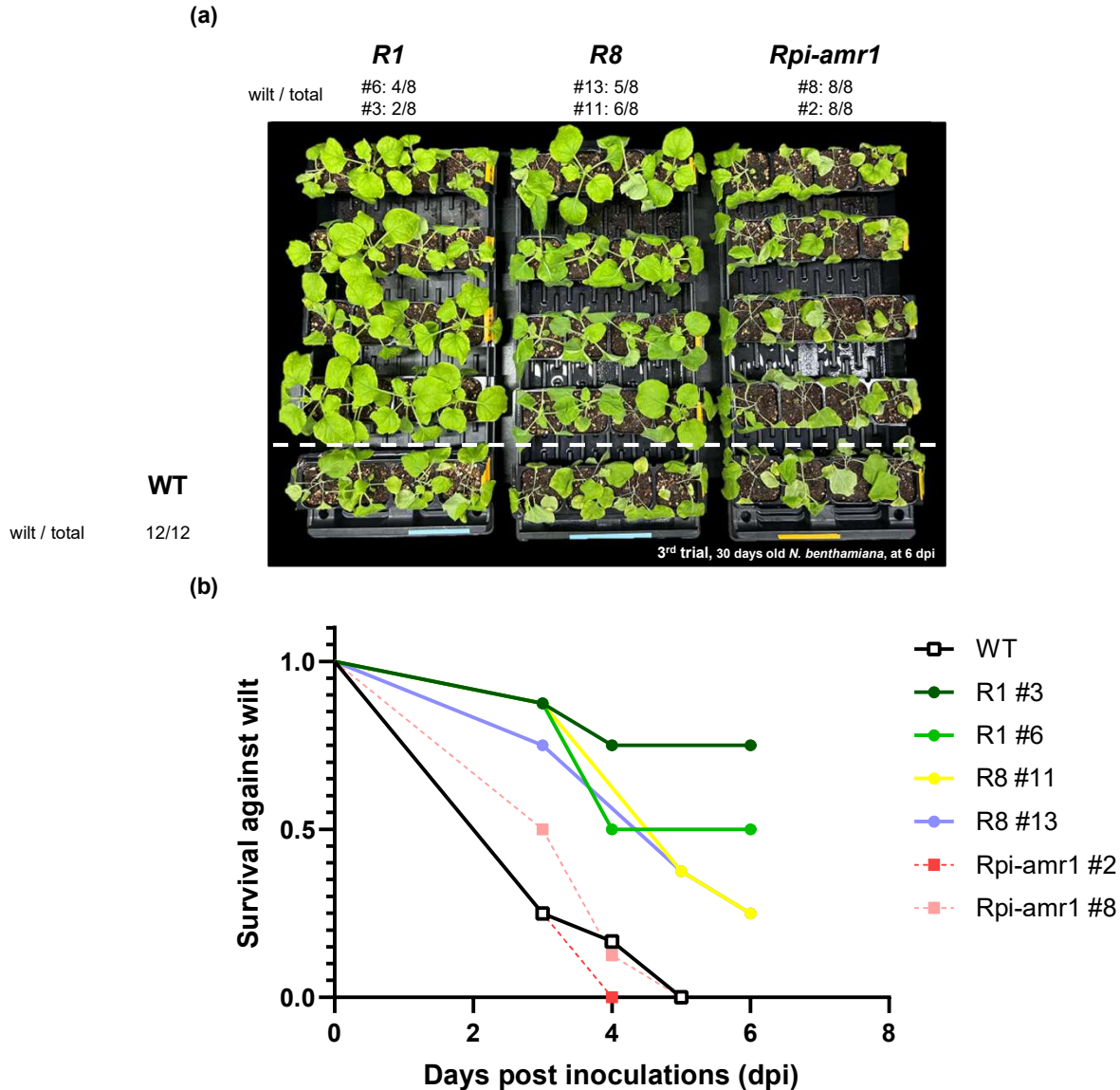
Supplementary Figure 14. T_1 plants expressing *R1* or *R8* were resistant against root infection of *P. capsici* (1st trial).

(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 due to heterogeneity of T_1 plants. **(c)** The ratio of healthy/total plants of each line is presented as line graph. Approximately 30% of *R1* (#3 and #6) and *R8* (#4) plants were also exhibited wilt phenotypes due to heterogeneity of T_1 plants.

(a)

	WT	R1	R8	Rpi-amr1
wilt / total	23/23	#6: 2/14 #3: 2/10	#11: 4/12 #4: 2/8	#8: 8/8 #2: 12/12

2nd trial, 29 days old *N. benthamiana*, at 4 dpi

(b)

Supplementary Figure 15. T_1 plants expressing *R1* or *R8* were resistant against root infection of *P. capsici* (2nd trial).

(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.

(a)

Supplementary Figure 16. T_1 plants expressing *R1* or *R8* were resistant against root infection of *P. capsici* (3rd trial).

(a) Picture of root inoculated plants (wild type, *R1*, *R8*, and *Rpi-amr1* transgenic *N. benthamiana,* 30 days old) at 6 days post inoculation. Each plant is inoculated with zoospores of *P. capsici*. *R1* (#3 and #6) and *R8* (#11 and #13) plants exhibited resistance phenotypes (*R8* exhibited relatively less intensive resistance) 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 70% *R8* (#11 and #13) plants were also exhibited wilt phenotypes.
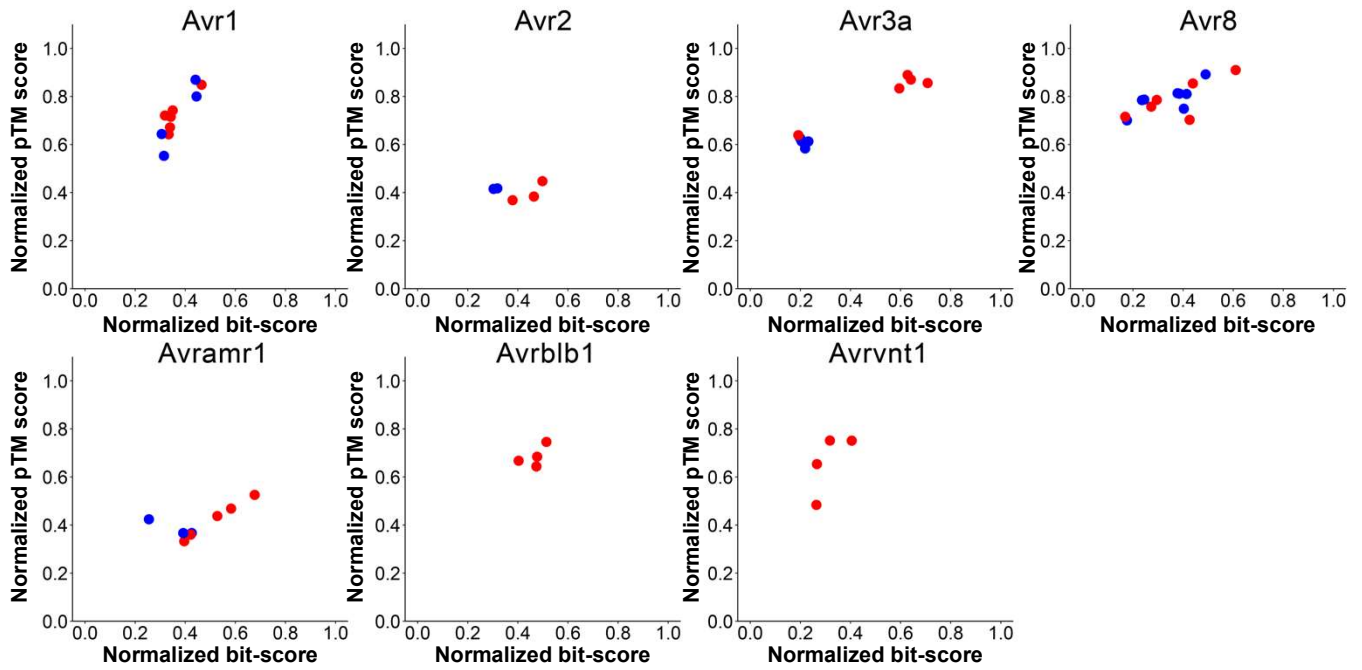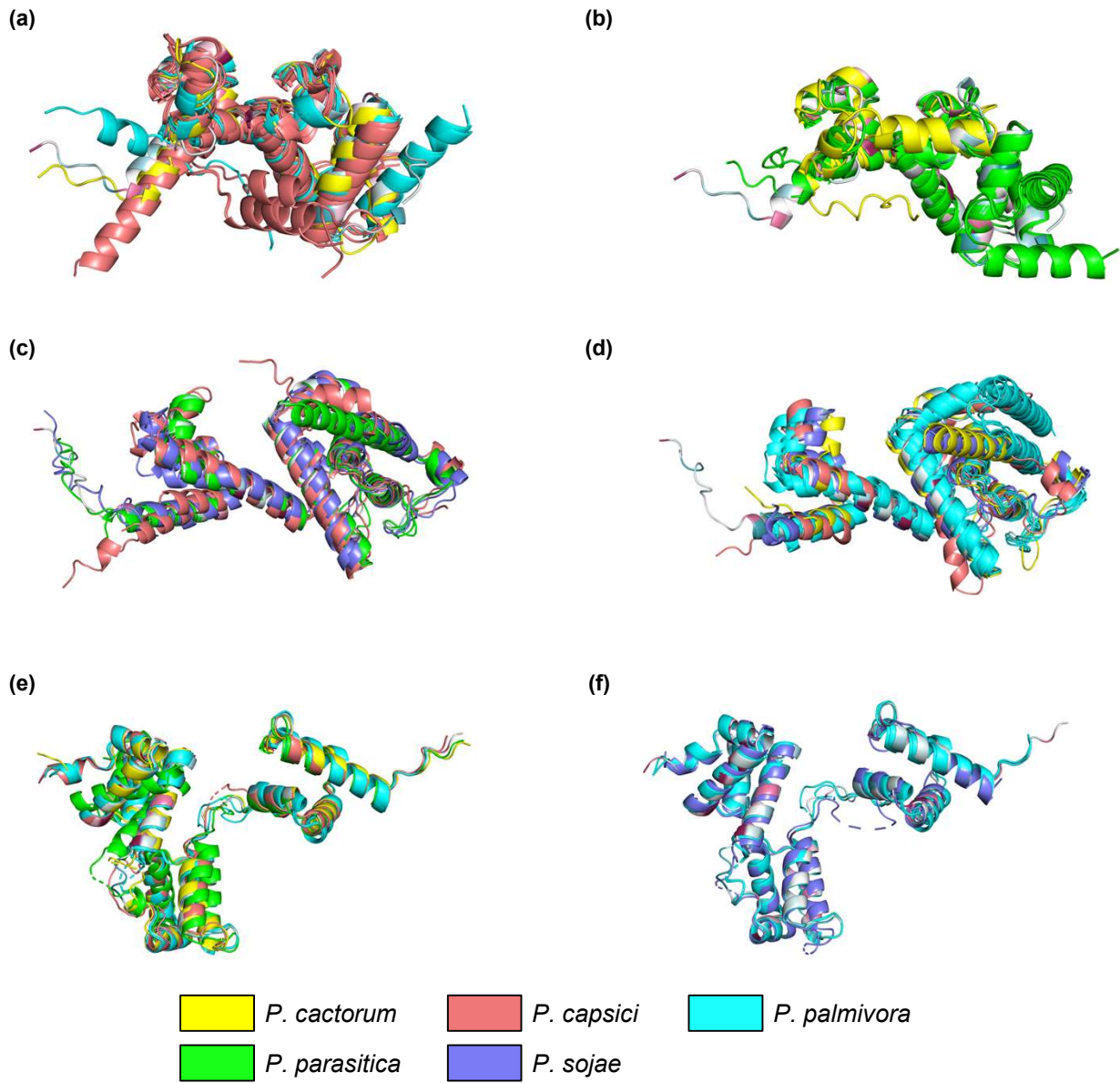
Supplementary Figure 17. Conservation ratio of protein sequence and structure for each cloned effector homologs. Red and blue dots represent HR-positive and HR-negative, respectively.

(a)

(b)

(c)

(d)

(e)

(f)

🟨 *P. cactorum*	🟥 *P. capsici*	🟦 *P. palmivora*
🟩 *P. parasitica*	🟪 *P. sojae*	

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.

(a)

(b)

(c)
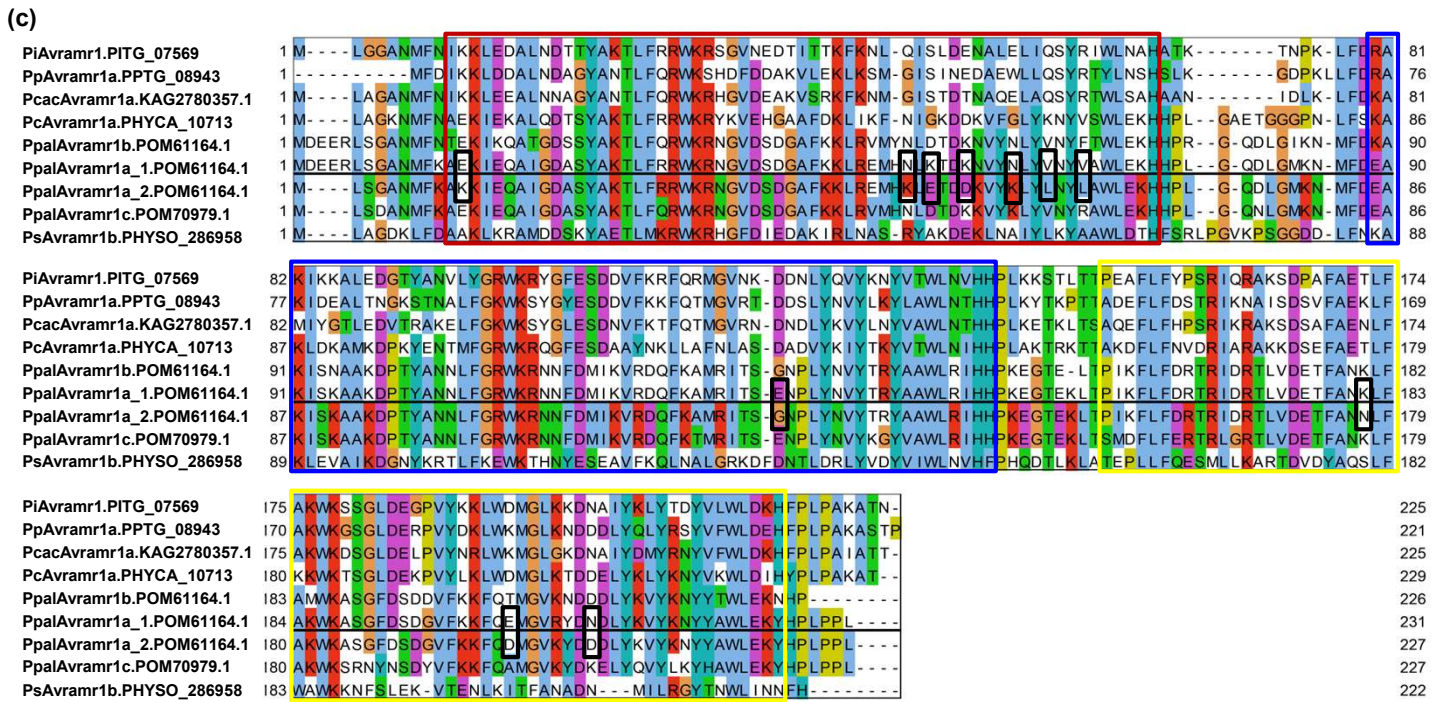
Supplementary Figure 19. Sequence alignments of cloned effector domain for HR-positive and HR-negative effectors in a) Avr1 b) Avr8 c) Avramr1. The top and bottom of the thick solid lines represent HR-positive and HR-negative effectors, respectively. Sequences are colored according to amino acid properties 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 Table 1. List of avirulence effectors used in this study

Name	PITG locus_tag	UniprotID (Swiss-Prot)
Avr1	PITG_16663	sp_D0NVB5_AVR1_PHYIT
Avr2	PITG_22870	
Avr3aEM	PITG_14371	sp_D0NPN8_A3AEM_PHYIT
Avr3aKI	PITG_14371	sp_E2DWQ7_A3AKI_PHYIN
Avr3b	PITG_18215	sp_D0NXM3_SFI7_PHYIT
Avr4	PITG_07387	sp_B1NNT7_AVR4_PHYIT
Avr8	PITG_07558	
Avr10	PITG_11484	sp_D0NIW0_AVR10_PHYIT
Avramr1	PITG_07569	
Avramr3	PITG_21190	
Avrblb1	PITG_21388	sp_D0P3S7_ABLB1_PHYIT
Avrblb2	PITG_04085	
Avrblb2	PITG_04086	
Avrblb2	PITG_04090	
Avrblb2	PITG_18683	sp_D0NZB7_BLB2B_PHYIT
Avrblb2	PITG_20300	sp_D0P1A8_ABLB2_PHYIT
Avrblb2	PITG_20301	
Avrblb2	PITG_20303	sp_D0P1B2_SFI8_PHYIT
Avrvnt1	PITG_16294	sp_D0NTY1_AVNT1_PHYIT

Supplementary Table 2. The number of homologous effectors in selected species

Species	Prefix	Reported host plants	Homolgous RXLR effectors	Database	Download URL
Phytophthora infestans	PHIF	Potato, tomato	124	OGOB	https://ogob.ie/v1/gob/data.html
Phytophthora parasitica	PHPA	Potato, egg plant, pepper, tobacco	70	OGOB	https://ogob.ie/v1/gob/data.html
Phytophthora cactorum	PHCR	Strawberry	35	NCBI	https://www.ncbi.nlm.nih.gov/datasets/genome/GCA_016864655.1/
Phytophthora megakarya	PHMG	Cocoa	208	NCBI	https://www.ncbi.nlm.nih.gov/datasets/genome/GCA_002215365.1/
Phytophthora palmivora	PHPV	Phalaenopsis	72	NCBI	https://www.ncbi.nlm.nih.gov/datasets/genome/GCA_002911725.1/
Phytophthora capsici	PHCA	Pepper, pumpkin, cucumber	32	OGOB	https://ogob.ie/v1/gob/data.html
Phytophthora sojae	PHSO	Soybean	74	OGOB	https://ogob.ie/v1/gob/data.html
Phytophthora ramorum	PHRA	Oak tree	27	OGOB	https://ogob.ie/v1/gob/data.html
Hyaloperonospora arabidopsidis	HYAP	Arabidopsis	2	OGOB	https://ogob.ie/v1/gob/data.html
Phytophthora kernoviae	PHKE	Beech tree, Azalea	3	OGOB	https://ogob.ie/v1/gob/data.html
Phytopythium vexans	PPVX	Citrus	0	OGOB	https://ogob.ie/v1/gob/data.html
Pythium ultimum	PYUU	Corn, potato	0	OGOB	https://ogob.ie/v1/gob/data.html

Supplementary Table 3. *Phytophthora* strains used in the study

Species	Strain number	Host plant species	Reference[a]
Phytophthora infestans	T30-4	Potato	(Haas *et al., Nature* 2009)
Phytophthora parasitica	40164	Potato	KACC
Phytophthora parasitica	40402	Egg plant	KACC
Phytophthora parasitica	40906	Tobacco	KACC
Phytophthora cactorum	40166	Apple tree	KACC
Phytophthora cactorum	40183	Strawberry	KACC
Phytophthora palmivora	40409	Cymbidium	KACC
Phytophthora palmivora	40410	Fig tree	KACC
Phytophthora capsici	40476	Chili pepper	KACC
Phytophthora sojae	40412	Soy bean	KACC
Phytophthora sojae	40468	Soy bean	KACC
Phytophthora sojae	48989	Soy bean	KACC

a) KACC: Korean Agricultural Culture Collection, https://genebank.rda.go.kr/eng/uat/uia/actionMain.do

Supplementary Table 4. *Solanum* NLRs used for the functional validations

Gene	Promoter/terminator	Reference*	*Solanum* species
R1	p35s / tNOS	35	S. demissum
R2	p35s / tNOS	36	S. demissum
R3a	Native elements	37	S. demissum
R8	Native elements	38	S. demissum
Rpi-amr1	p35s / tNOS	39	S. americanum
Rpi-blb1	p35s / tNOS	40	S. bulbocastanum
Rpi-blb2	Native elements	41	S. bulbocastanum
Rpi-vnt1	p35s / tNOS	42	S. venturii

*reference in manuscript