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Supplemental Information

A *Phytophthora* Effector Suppresses Trans-Kingdom RNAi to Promote Disease Susceptibility

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Figure S1

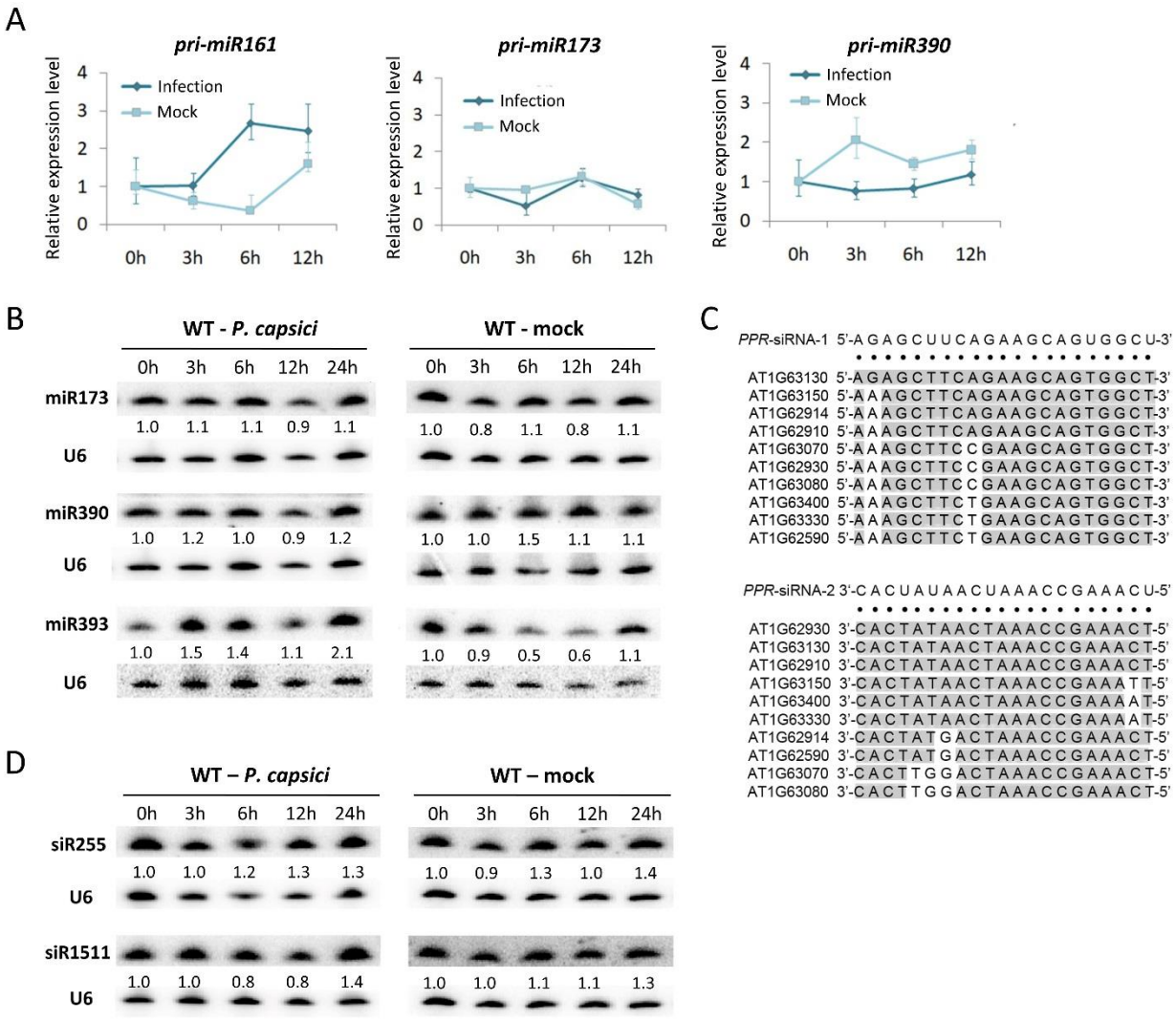


Figure S1. Accumulation of secondary siRNA-triggering miRNAs and tasiRNA in *Arabidopsis* during *P. capsici* infection, related to Figure 2.

(A) Quantitative RT-PCR showing transcript abundances of *pri-miRNA*s in wild-type *Arabidopsis* Col-0 during *P. capsici* infection at 0, 3, 6 and 12 hours post inoculation (hpi). Values are mean±SD. Water was used as a mock treatment.

(B) Northern blotting showing the abundances of miR173, miR390 and miR393 during *P. capsici* infection in wild-type (WT) *Arabidopsis* plants. Numbers represent relative signal intensities. U6 was used as a loading control.

(C) Sequence alignment of two *PPR*-siRNAs. *PPR*-siRNA-1 and *PPR*-siRNA-2 were used as representatives to show abundance changes in *Arabidopsis*. Similar sequences could be produced from multiple *PPR* loci.

(D) Northern blotting showing the abundances of two tasiRNAs (siR255 and siR1511) during *P. capsici* infection (0-24 hours post inoculation). The production of these tasiRNAs is triggered by miR173. Water was used as a mock treatment. U6 served as a loading control. Numbers represent relative signal intensities.

Figure S2

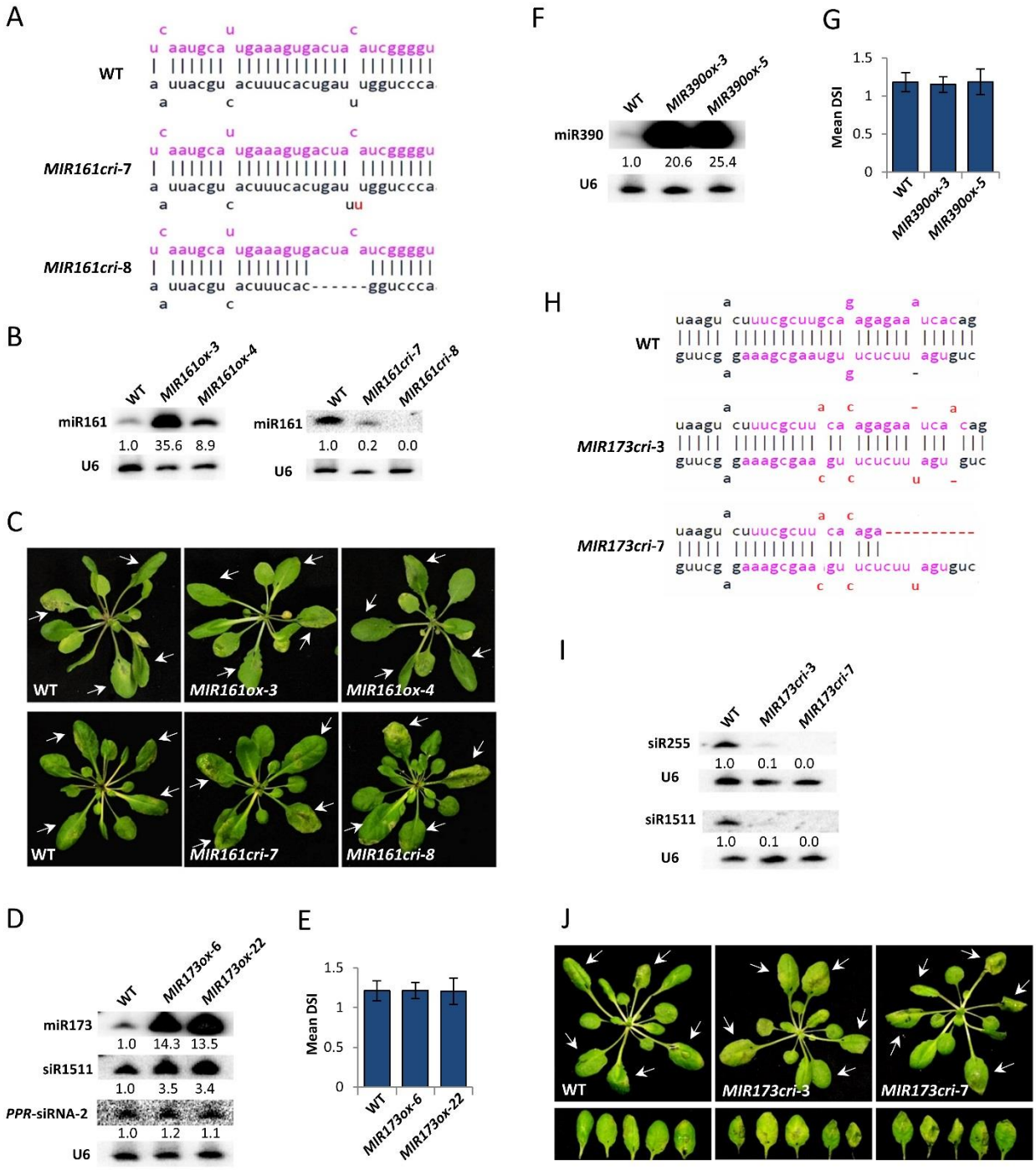


Figure S2. Disease susceptibility of miR161 and miR173 mutants, related to Figure 2.

(A) Sequences in the foldback region of *pri-miR161* in wild-type (WT) and *MIR161cri* lines. The sequences in purple correspond to miR161. *MIR161cri*-7 has one nucleotide insertion (in

red), which reduces, but not abolishes miR161 production. *MIR161cri*-8 has a deletion of six nucleotides, abolishing miR161 production.

(B) Northern blotting showing the abundance of miR161 in wild-type *Arabidopsis* (WT), *MIR161* over-expression lines (*MIR161ox*), and crispr mutants (*MIR161cri*) lines. Numbers represent relative signal intensities. U6 was used as a loading control.

(C) Disease symptoms of WT, *MIR161ox* and *MIR161cri* plants after inoculated with *P. capsici* zoospore suspension. Photos were taken at 3 dpi. Arrows indicate inoculated leaves.

(D) Northern blotting showing the increased accumulation of miR173 and tasiRNAs (represented by siR1511) in *MIR173* over-expression lines (*MIR173ox*). However, the abundance of *PPR*-derived siRNAs (represented by *PPR*-siRNA-2) was not increased. Numbers represent relative signal intensities. U6 was used as a loading control.

(E) *MIR173ox* plants exhibited similar susceptibility to wild-type (WT) when inoculated with *P. capsici* zoospore suspension. DSI was determined at 3 dpi. Values are mean±SEM of three biological replicates.

(F) Northern blotting showing the increased accumulation of miR390 in *MIR390* over-expression (*MIR390ox*) lines.

(G) *MIR390ox* plants exhibited similar susceptibility to wild-type (WT) when inoculated with *P. capsici* zoospore suspension. DSI was determined at 3 dpi. Values are mean±SEM of three biological replicates.

(H) Sequences in the foldback region of *MIR173* in wild-type and *MIR173cri* lines. miR173 can be generated from both purple regions. Mutated nucleotides in *MIR173cri*-3 are highlighted in red. In addition to mutations, *MIR173cri*-7 also have a deletion of ten nucleotides, leading to abolishment of miR173 production.

(I) Northern blotting showing the diminished accumulation of tasiRNAs (represented by siR255 and siR1511) in *MIR173cri* mutants. Numbers represent relative signal intensities. U6 was used as a loading control.

(J) Symptoms of *MIR173cri* mutants after *P. capsici* infection. Photos were taken at 3 dpi. Arrows indicate inoculated leaves.

Figure S3

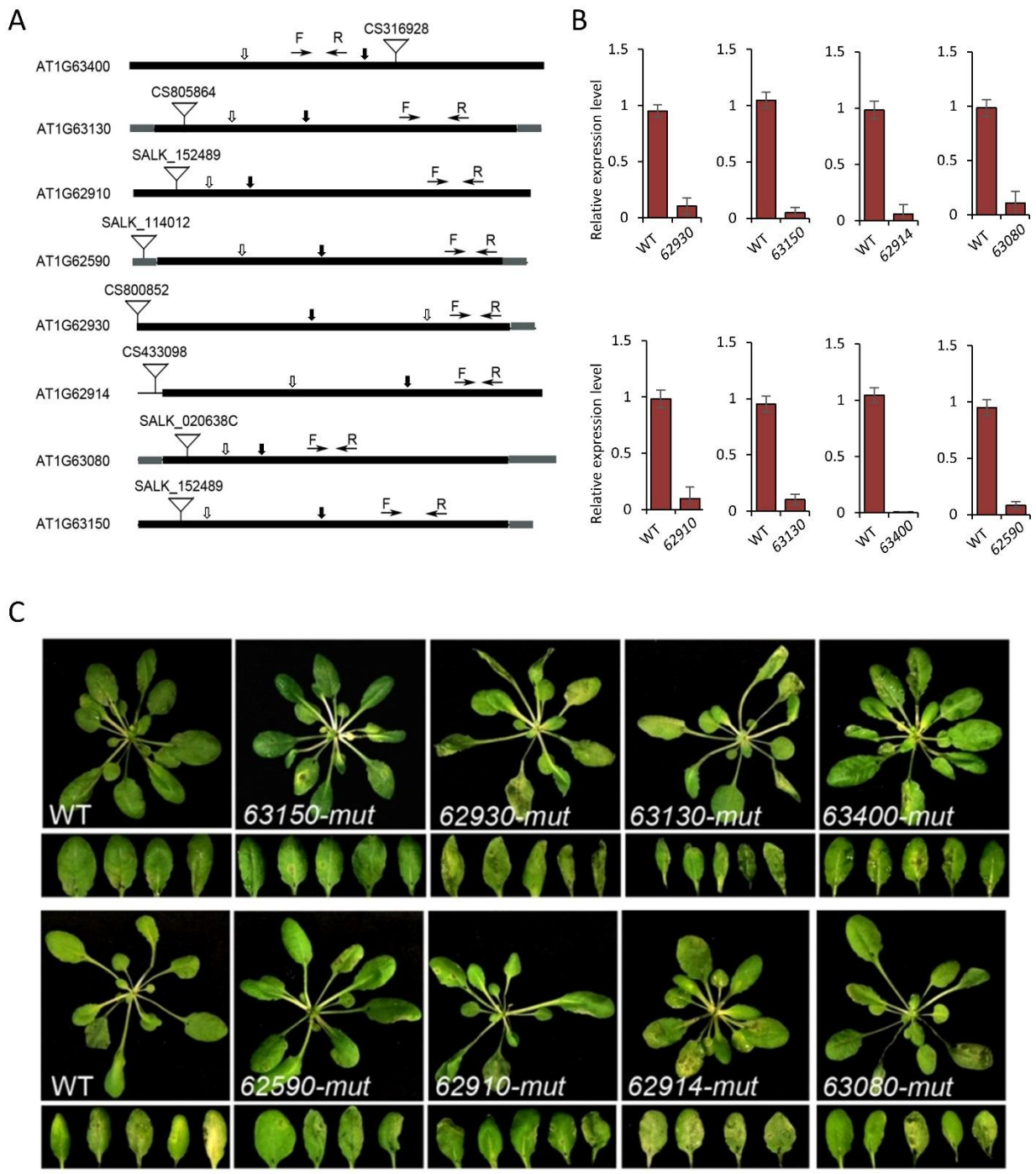


Figure S3. Characterization of the T-DNA insertion mutants of eight *PPR* genes that can generate secondary siRNAs, related to Figure 2.

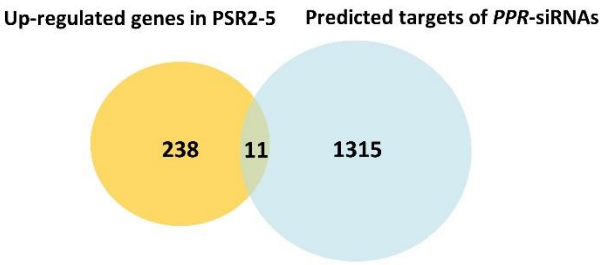
(A) Schematic maps of the T-DNA insertion site in each mutant and the locations of the primers used for quantitative RT-PCR. Black arrows label the predicted target sites of miR161. White arrows label the predicted target sites of tasiRNAs.

(B) Quantitative RT-PCR measuring the transcript levels of the corresponding *PPR* gene in each mutant. Values are mean±SD of at least three replicates.

(C) Disease symptoms of the mutants after *P. capsici* inoculation. Photos were taken at 3 dpi. Inoculated leaves were removed from the plants at 3 dpi to better show the symptoms.

Figure S4

A



B

At4g31240	protein kinase C-like zinc finger protein
At1g63240	hypothetical protein
At2g21880	Ras-related GTP-binding protein
At2g22830	squalene monooxygenase
At5g57130	heat shock protein-related
At4g03230	S-locus lectin protein kinase family protein
At5g07610	F-box family protein
At2g42320	nucleolar protein gar2-related
At2g22800	HAT9__homeobox-leucine zipper protein 9
At1g12775	pentatricopeptide (PPR) repeat-containing protein
At4g19890	Pentatricopeptide repeat (PPR-like) superfamily protein

C

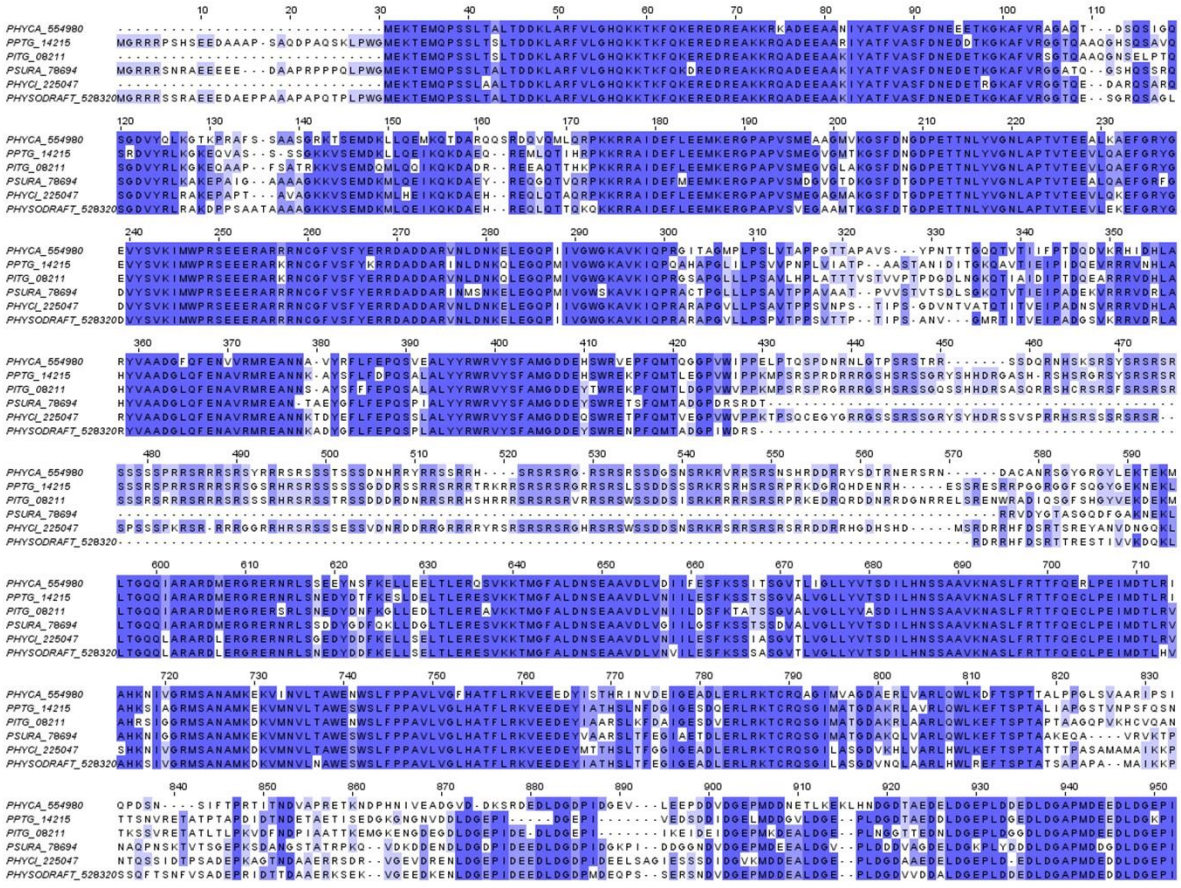


Figure S4. Potential targets of *PPR*-derived siRNAs in *Arabidopsis* and *Phytophthora*, related to Figure 3.

(A) Venn diagram showing a minimal overlap between genes up-regulated in PSR2-5 plants, compared to wild-type *Arabidopsis* Col-0, and the genes predicted to contain target site(s) of *PPR*-derived siRNAs.

(B) A list showing the 11 genes that are up-regulated in PSR2-5 and are also potential targets of *PPR*-derived siRNAs. Only two of the 11 genes contain PPR domains in their deduced amino acid sequences. At1g12775 can also produce siRNAs, but it is not in the gene cluster that produces the large majority of *PPR*-derived siRNAs.

(C) Amino acid sequence alignment of homologs of *Phyca_554980* encoded protein in *Phytophthora* species *P. capsici*, *P. parasitica*, *P. infestans*, *P. ramorum*, *P. cinnamomi*, and *P. sojae* (from the top to the bottom in the alignment). The protein sequences were identified using FungiDB (http://fungidb.org/fungidb/). Highlighted areas represent highly conserved sequences.

Figure S5

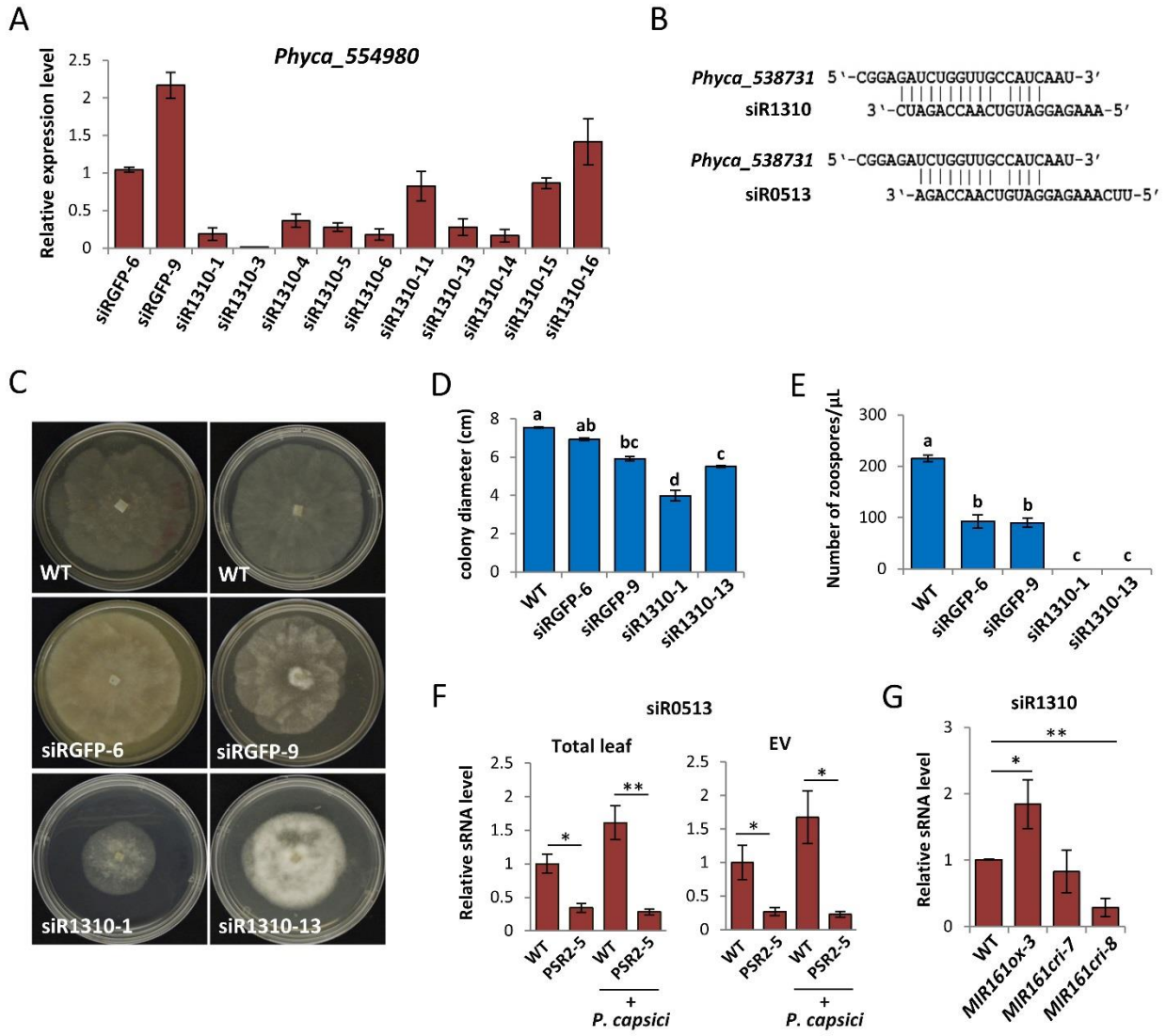


Figure S5. Target gene silencing and developmental defects of *P. capsici* transformants carrying siR1310, related to Figure 3.

(A) Quantitative RT-PCR of the predicted target gene *Phyca_554980* in ten independent transformants of *P. capsici* that potentially carry siR1310. Transformants potentially carrying siRGFP were used as controls. Values are mean±SEM.

(B) Insufficient base pairing of the *PPR*-derived siR1310 and siR0513 with their predicted off-target *Phyca_538731* of *P. capsici*.

(C) Mycelium growth of two siR1310 transformants with largely reduced transcript levels of the target gene *Phyca_554980*. *P. capsici* strains were grown on V8 plates. Pictures were taken

after 5 days of growth. Transformants carrying siRGFP and wild-type (WT) strain were used as controls.

(D) Mycelium growth rate (shown as colony diameters) of *P. capsici* strains. Values are mean±SEM. Different letters indicate values that are statistically different ($p < 0.05$).

(E) Number of zoospores produced by *P. capsici* strains. Sporangia growing in sterilized water were stressed to release zoospores by illumination for 12 hours and then chilling at 4°C for 1 hour. Zoospores were counted from four randomly selected fields of view under a microscope for each strain using a hemocytometer. Values are mean±SEM. Different letters indicate values that are statistically different ($p < 0.05$).

(F) Quantitative RT-PCR showing the level of siR0513 in WT and PSR2-5 plants in EVs isolated from leaves tissues. Values are mean±SEM. * and ** label results that are statistically different with the WT value at $p \leq 0.05$ or $p \leq 0.01$.

(G) Stem loop qPCR showing the level of siR1310 in *MIR161ox* and *MIR161cri* mutant plants compared to wild-type (WT) *Arabidopsis*. Values are mean±SEM. * and ** label results that are statistically different with the WT value at $p \leq 0.05$ or $p \leq 0.01$.

Figure S6

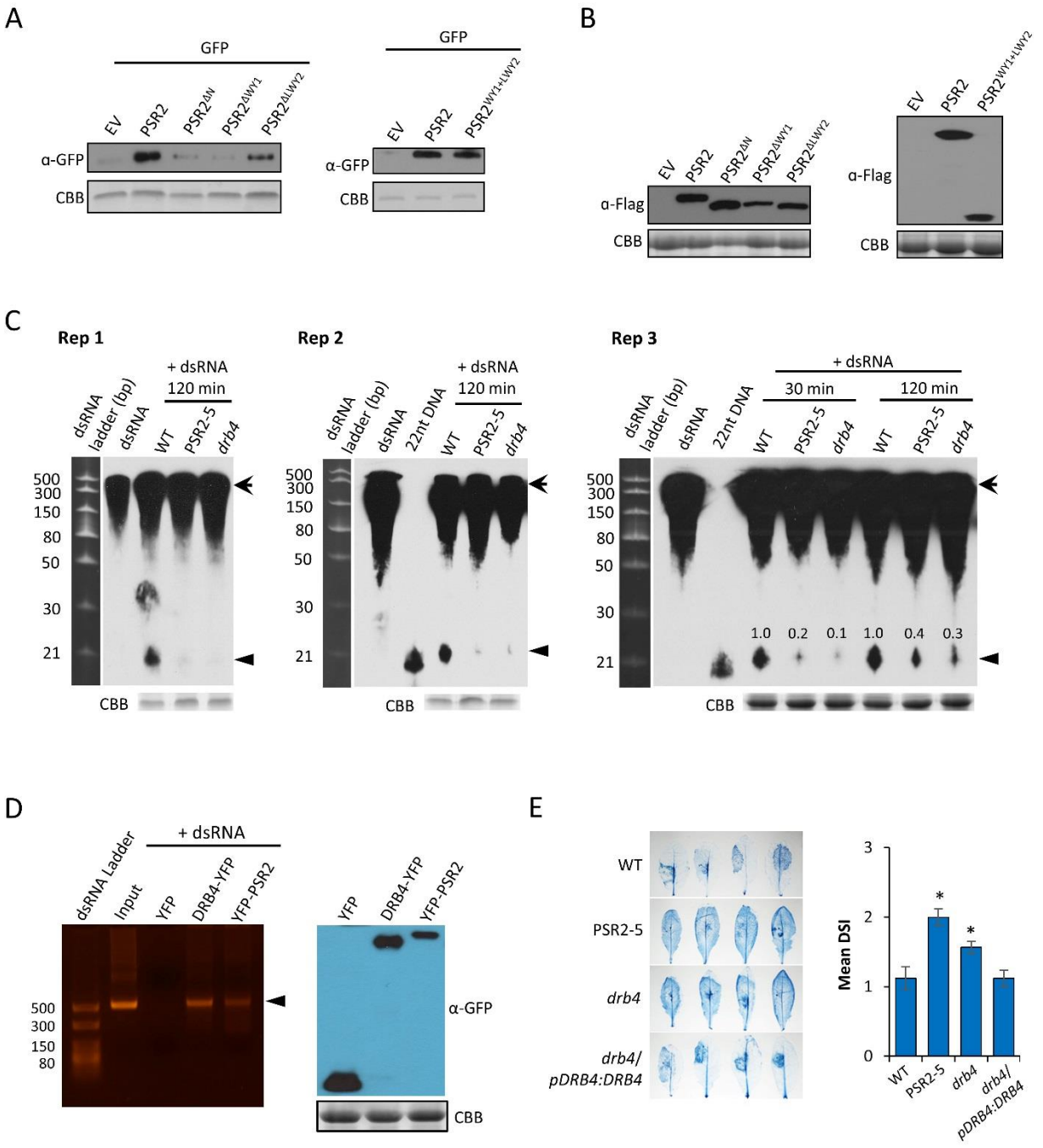


Figure S6. The RNAi suppression activity of PSR2 depends on its association with DRB4, related to Figure 5 and 6.

(A) Western blotting showing the abundance of GFP proteins in *N. benthamiana* 16c leaves expressing wild-type or truncated PSR2. Total proteins were extracted from leaves infiltrated

with *Agrobacterium* carrying *35S-GFP* and another strain carrying either the pEG100 empty vector (EV) or various truncates of PSR2-Flag at 5 dpi. Coomassie Brilliant blue staining (CBB) was used as loading control.

(B) Western blotting showing the protein levels of PSR2 or its derivatives in the infiltrated leaves. Total proteins were extracted from infiltrated leaves at 2 days after *Agro*-infiltration and the proteins were detected by anti-Flag antibody. Coomassie Brilliant blue staining (CBB) was used as loading control.

(C) Reduced dsRNA cleavage in *PSR2*-expressing *Arabidopsis* and a *drb4* mutant plants. In vitro synthesized 510-bp dsRNAs were labeled with biotin, and then incubated with crude protein extracts for 30 or 120 minutes at 23°C. The cleavage products were analyzed on 15% denaturing PAGE containing 8 M urea. A 22-nt DNA end-labeled with ^{32}P and a dsRNA ladder stained with SybrGold were used as size markers to indicate the position of sRNA products. Numbers below the gel represent the relative abundances of the sRNAs with the level in wild-type (WT) plants set to 1. The crude extracts were analyzed on SDS-PAGE and stained with Coomassie Brilliant Blue (CBB) as a loading control. The arrow labels the 510-bp dsRNAs and the arrowhead labels sRNA products. Three biological replicates including a time-course analysis are presented.

(D) Double-stranded RNA binding assay of DRB4 and PSR2. YFP-PSR2 and DRB4-YFP were expressed in *N. benthamiana* through *Agrobacterium*-mediated transient expression. Total proteins were extracted from the infiltrated leaves with treatment of RNase III and the immune complexes were pulled-down using anti-GFP magnetic beads. The immuno-precipitated proteins were then incubated with in vitro synthesized dsRNA (510-bp in length). dsRNA enriched on the beads was extracted using Trizol/chloroform and then analyzed on 2% agarose gel along with Ethidium bromide staining. YFP was used as a negative control. The Arrowhead shows the position of the 510-bp dsRNA. Protein levels of immuno-precipitated YFP, PSR2 and DRB4 were confirmed by western blotting using anti-GFP antibody.

(E) Detached leaves from four-week-old plants of wild-type (WT), PSR2-5, *drb4* and a complementation line of *drb4* (*DRB4* expressed under its native promoter) were inoculated with zoospore suspensions of *P. capsici* strain LT263. Inoculated leaves were stained with trypan blue to visualize the lesions at 3 dpi. Disease severity index (DSI) was also determined. Values are mean $\pm$ SD. * labels results that are statistically different at $p \leq 0.05$.