Supplementary Information

Oomycete Pathogens Encode RNA Silencing Suppressors

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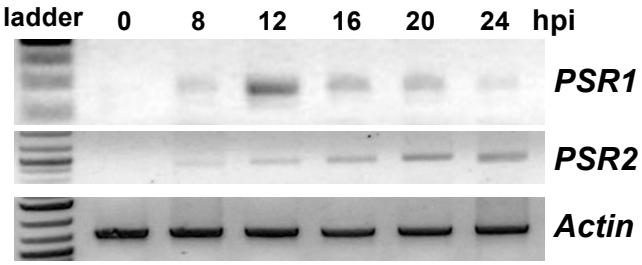
Supplementary Figures



Supplementary Figure 1. Expression of *PSR1* and *PSR2* genes upon *Phytophthora sojae* infection of soybean roots. Transcript levels of *PSR1* and *PSR2* were determined by semi-quantitative RT-PCR during a time course (0-24 hours post infection) using total RNAs extracted from roots infected with *P. sojae* strain P6497. Soybean actin gene was used as an internal control.

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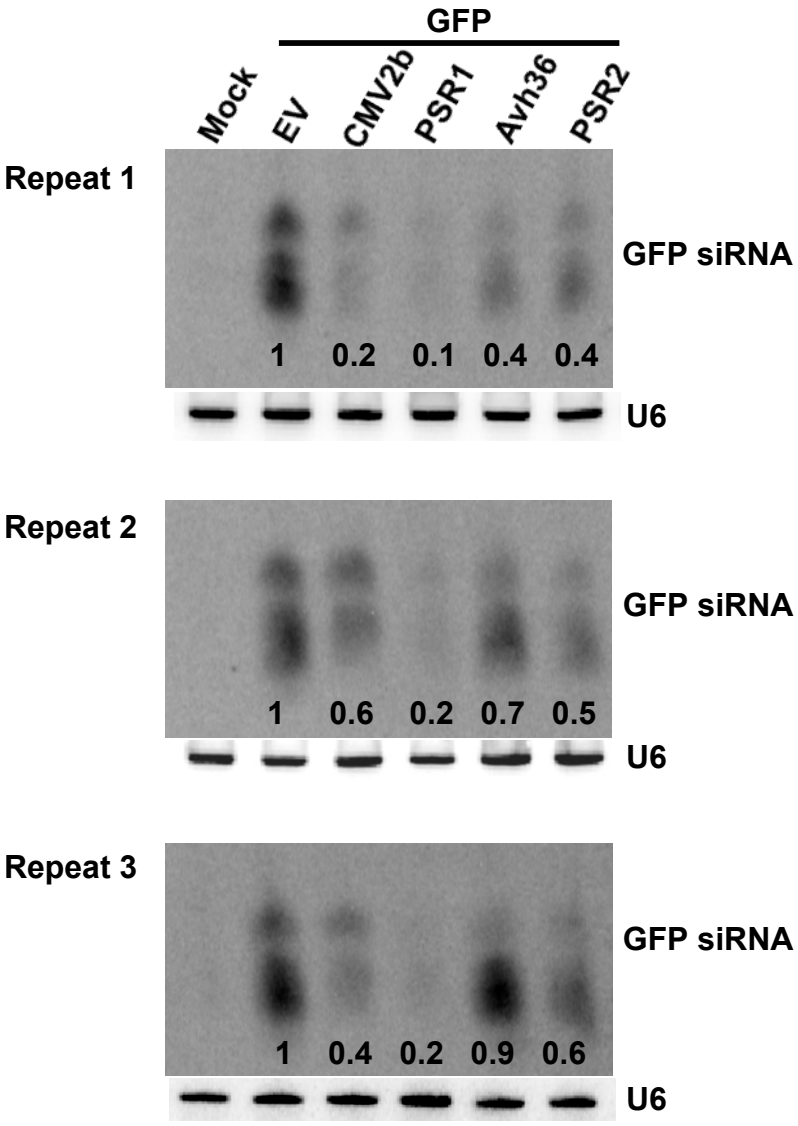

Supplementary Figure 2. *GFP* siRNA accumulation in the leaves of *N. benthamiana* 16c plants infiltrated with *Agrobacterium tumefaciens* carrying *35S-GFP* and individual viral or *Phytophthora* effectors. Leaves at a similar developmental stage and without *Agro*-infiltration were used as the mock control. The numbers represent relative siRNA abundance. Data from one representative experiment are shown in Fig. 1b and results from three additional independent biological replicates are shown here. Avh36 is a *P. sojae* effector that may also possess RNA silencing suppression activity but has not been focused in this paper because it did not show consistent suppression of RNA silencing as the other two PSRs did.

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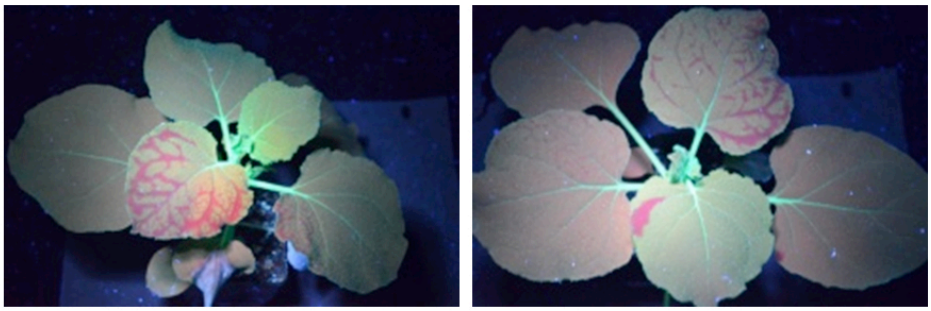
	Constructs	No. plants infiltrated	No. plants systemically silenced	No. plants systemically partial silenced
1	GFP+EV	30	30	0
2	GFP+CMV2b	30	0	8
3	GFP+PSR1	40	0	13
4	GFP+PSR1M	30	30	0
5	GFP+PSR2	36	36	0



GFP+CMV2b GFP+PSR1

Supplementary Figure 3. Systemic silencing of GFP suppressed by PSR1 in *N. benthamiana* 16c plants. Leaves of *N. benthamiana* 16c plants were infiltrated with *Agrobacterium tumefaciens* carrying *35S-GFP* and one of the following constructs: empty vector (EV), *35S-CMV2b* (positive control), *35S-PSR1*, *35S-PSR1M* and *35S-PSR2*. The table specified the numbers of completely and partially silenced plants on the systemic tissues. For leaves expressing PSR1 or CMV2b, both complete and partial suppression of *GFP* silencing (lower panel) were observed. Systemic silencing for these partially silenced plants was restricted to the regions on and around the veins of the leaves**.**

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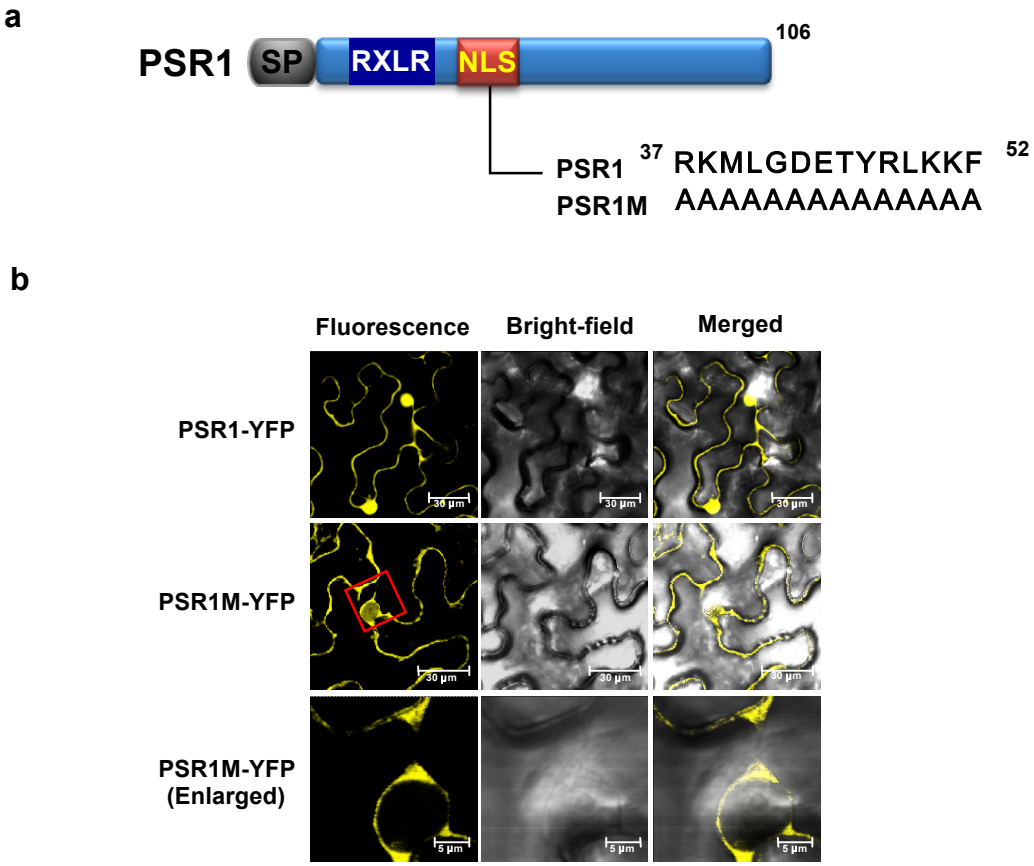

Supplementary Figure 4. PSR1 contains a potential nuclear localization signal (NLS) and is located in the nucleus in plant cells. (**a)** Schematic map showing motif architectures of PSR1. SP, signal peptide region; RXLR, RxLR/dEER host targeting motif; and NLS, bipartite nuclear localization signal. The mutant PSR1M was generated by replacing all 16 amino acid residues in the NLS motif to alanines. (**b)** Subcellular localizations of the PSR1-YFP and PSR1M-YFP fusion proteins were determined in *N. benthamiana* leaves after *Agrobacterium*-mediated transient expression. Yellow fluorescence was detected by confocal microscopy from infiltrated tissues 48 hours post *Agro*-infiltration. Nuclear region in the red box was enlarged to better visualize the lack of PSR1M proteins in the nucleoplasm.

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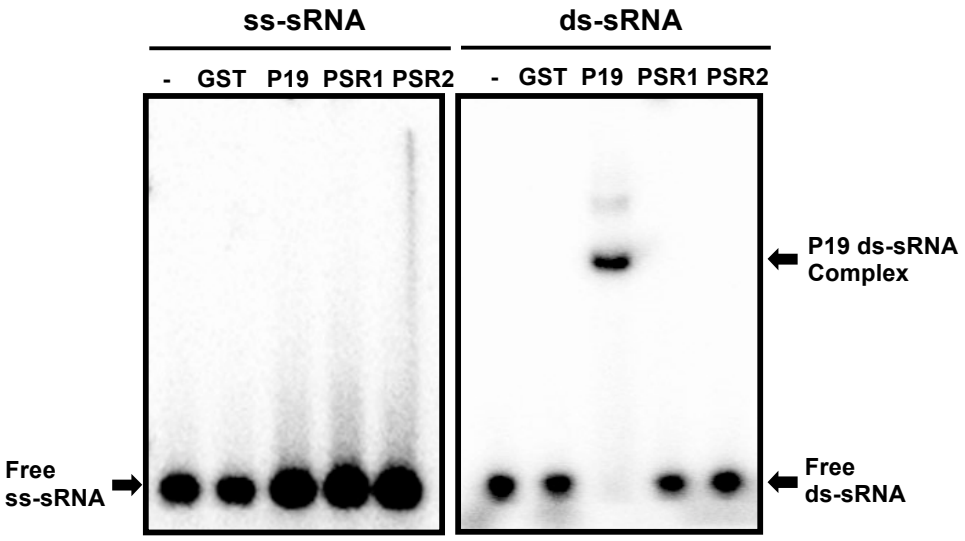

Supplementary Figure 5. PSR1 and PSR2 do not possess small RNA binding activity in vitro**.** Electrophoretic mobility shift assays were performed using purified GST-tagged recombinant proteins and ^{32}P-labelled synthetic small RNA oligos that were 21 nt in length. The viral RNA silencing suppressor P19 that is known to bind to ds-sRNA was used as a positive control.

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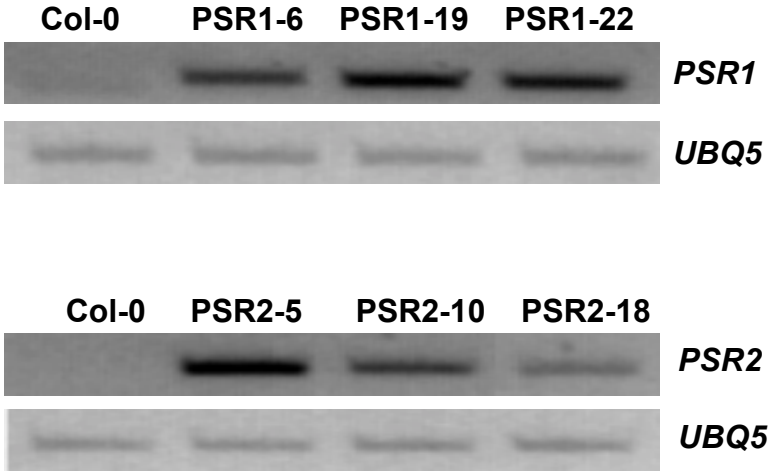

Supplementary Figure 6. Semi-quantitative RT-PCR showing the expression of *PSR1* and *PSR2* in transgenic *Arabidopsis* plants. Three independent transgenic lines (as indicated by the different numbers) harboring *35S-PSR1-YFP* or *35S-PSR2-Flag* were assayed for transgene expression. Col-0, wild-type. Inflorescence tissues were used to confirm the expression of *PSR1-YFP* and *PSR2-Flag* in the transgenic lines by RT-PCR with *UBQ5* as an internal control. The three lines with varying expression levels for each construct were further investigated for small RNA levels (Fig. S7, S9).

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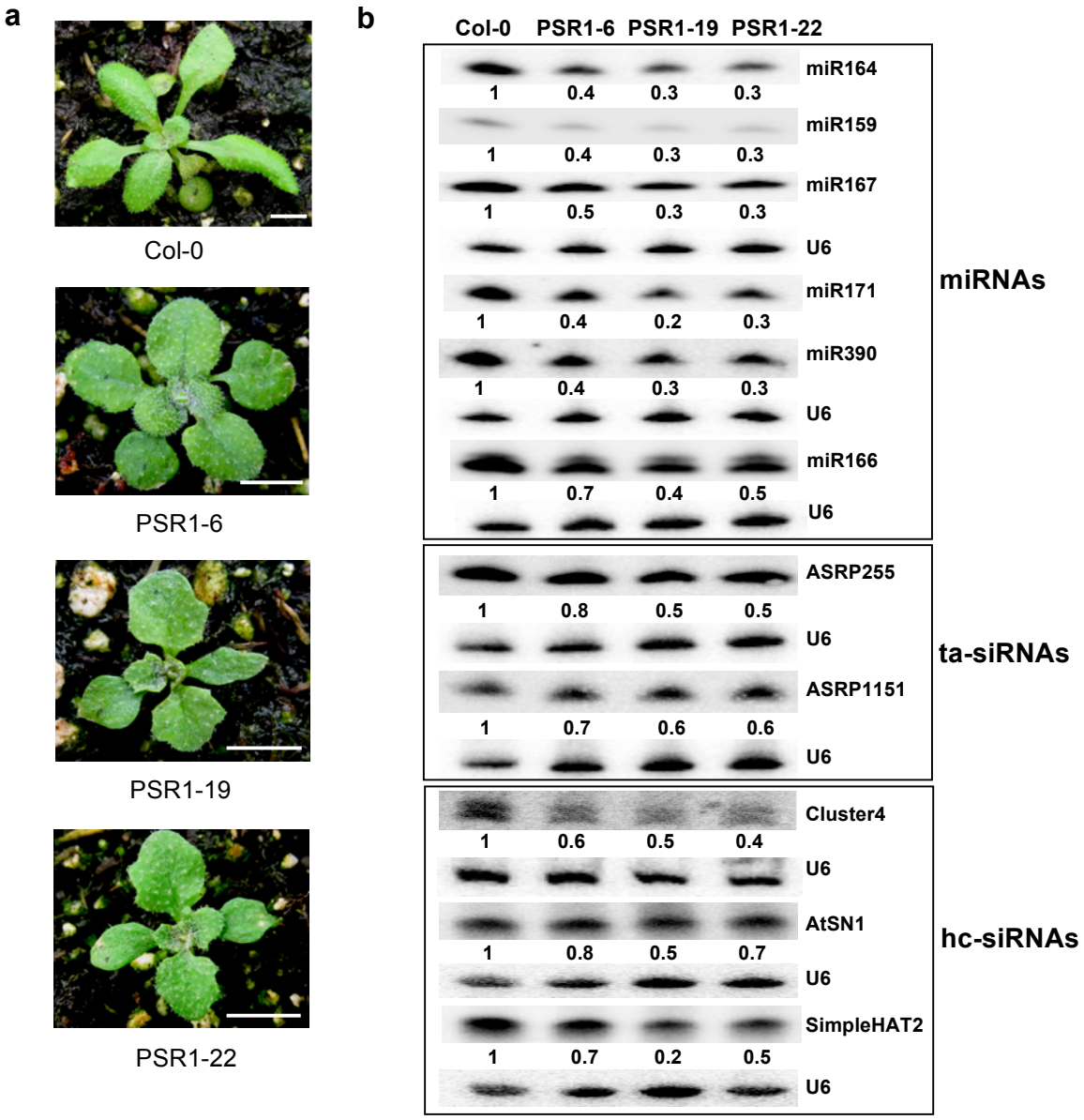

Supplementary Figure 7. Reduced accumulation of small RNAs in *Arabidopsis* transgenic lines expressing PSR1. **a,** *PSR1*-expressing plants are much smaller with other morphological phenotypes. Pictures were taken on 20-day-old *Arabidopsis* plants grown in soil. Col-0, wild-type; PSR1-6, PSR1-19, PSR1-22, three independent *35S-PSR1-YFP Arabidopsis* transgenic lines. **b,** Levels of representative miRNA, trans-acting siRNA (ta-siRNA) and heterochromatic siRNA (hc-siRNA) species in wild-type (Col-0) and the transgenic lines were determined by northern blots using total RNAs extracted from inflorescences. U6 served as an internal control for each blot. The numbers below the gel images represent relative small RNA abundance.

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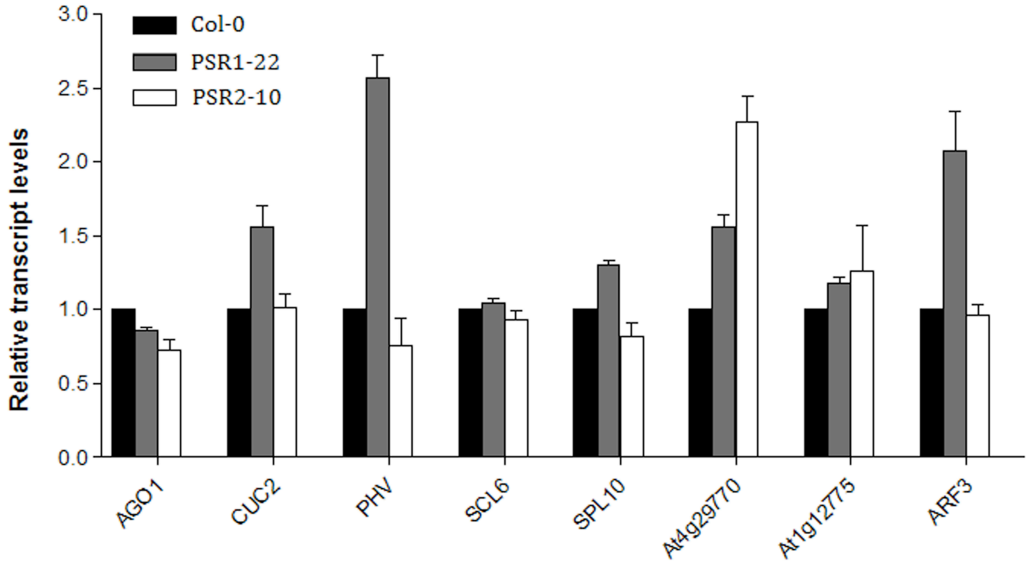

Supplementary Figure 8. Transcript levels of miRNA or ta-siRNA target genes were determined by quantitative RT-PCR in wild-type (Col-0) and representative transgenic *Arabidopsis* lines expressing PSR1 or PSR2. *AGO1*, *CUC2*, *PHV*, *SCL6*, and *SPL10* are targets of miR168, miR164, miR165/166, miR171, and miR156/157, respectively. At4g29770, At1g12775, and *ARF3* are targets of ta-siRNAs from *TAS1*, *TAS2*, and *TAS3*, respectively. Total RNAs extracted from inflorescences were used for this analysis.

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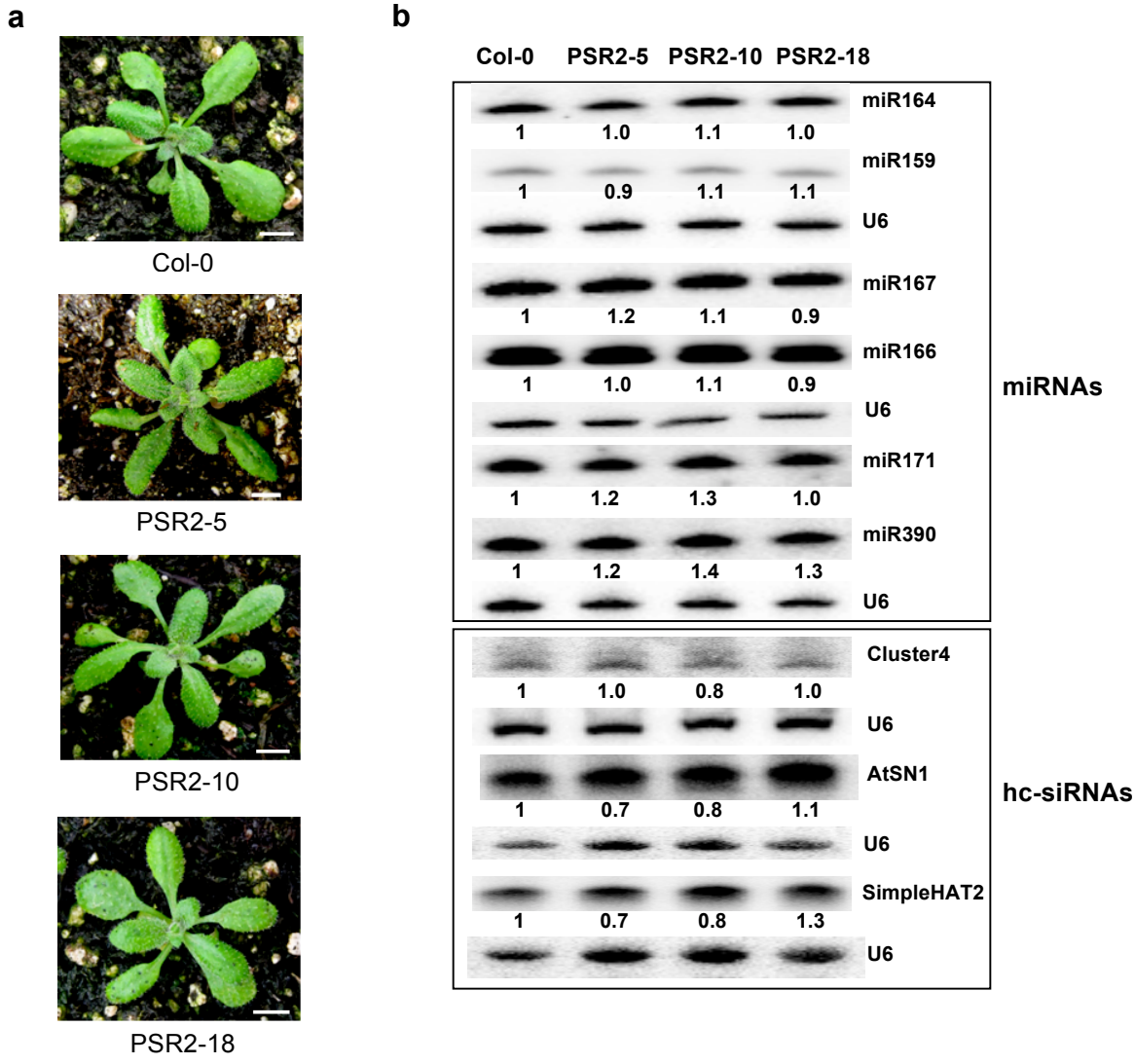

Supplementary Figure 9. PSR2 does not affect the accumulation of miRNAs or heterochromatic siRNAs (hc-siRNAs) in *Arabidopsis*. (**a)** Expression of PSR2 does not significantly change the morphological phenotype in *Arabidopsis*. Pictures were taken on 20-day-old *Arabidopsis* plants grown in soil. Col-0, wild-type; PSR2-5, PSR2-10, PSR2-18, three independent *35S-PSR2-Flag Arabidopsis* transgenic lines. (**b)** Levels of representative miRNAs and heterochromatic siRNAs (hc-siRNAs) in wild-type and *35S-PSR2-Flag* transgenic *Arabidopsis* lines were determined by northern blotting. The numbers below the images represent relative abundance of the small RNAs compared to wild-type (Col-0). U6 served as an internal loading control.

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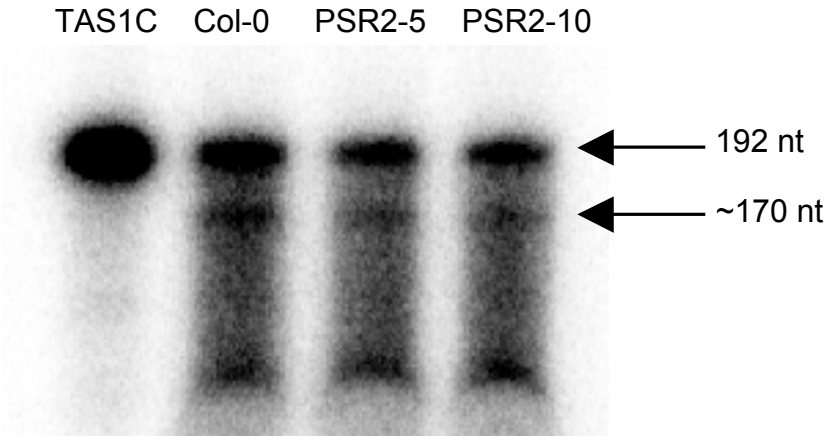

Supplementary Figure 10. AGO1-miR173 slicer activity assay in wild-type (Col-0) and two independent *PSR2*-expressing *Arabidopsis* lines. A portion of the *TAS1c* locus containing the miR173 target site was amplified by PCR with one of the primers containing a T7 promoter sequence. The PCR fragment was used as a template for in vitro transcription by T7 polymerase to generate a 192 nt *TAS1c* transcript, which was incubated with AGO1 immunoprecipitates from Col-0 or two *PSR2*-expressing lines. AGO1/miR173-mediated cleavage of the 192 nt transcript results in a 170 nt fragment and a 22 nt fragment, the latter beyond the range of detection. This assay showed that PSR2 did not affect the slicer activity of AGO1/miR173 in *Arabidopsis*.

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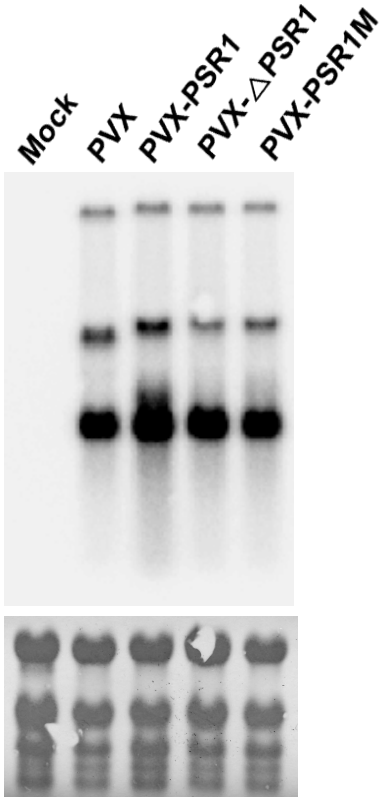

Supplementary Figure 11. PSR1 promotes the infection of *N. benthamiana* by potato virus X (PVX). *N. benthamiana* plants were infiltrated with *Agrobacterium tumefaciens* carrying *35S-PVX*, *35S-PVX-PSR1, PVX-PSR1M* or *35S-PVX-ΔPSR1*. The accumulation of PVX genomic and subgenomic RNAs was analyzed at four days post inoculation. This experiment was repeated twice with similar results. Data from one representative experiment are shown in Fig. 3b; additional data from the other replicate are shown here.

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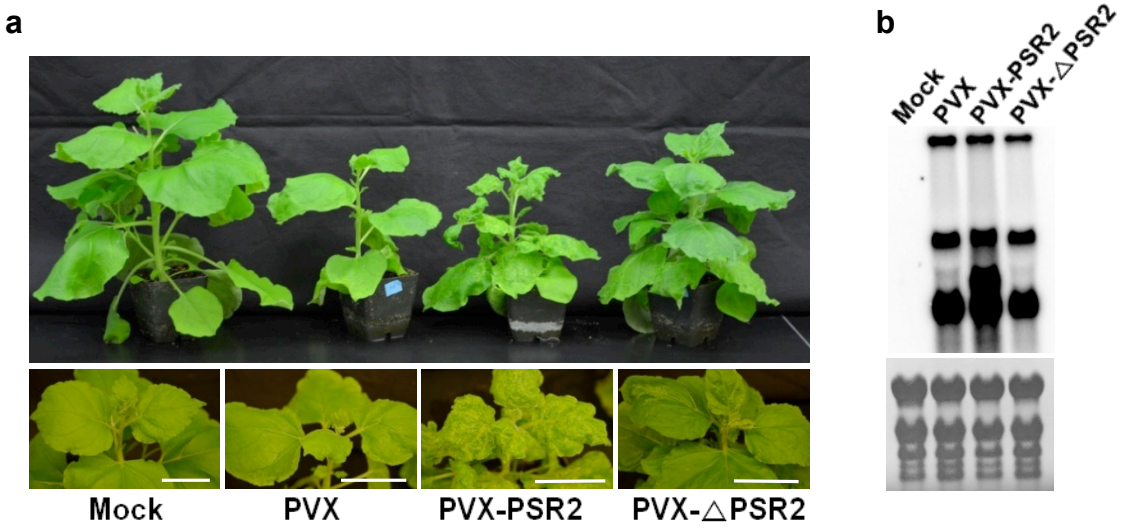

Supplementary Figure 12. PSR2 promotes PVX infection of *N. benthamiana*. **(a)** Disease symptoms of *N. benthamiana* plants infiltrated with *Agrobacterium tumefaciens* strains carrying *35S-PVX*, *35S-PVX-PSR2* or *35S-PVX-ΔPSR2*. The plants infected with PVX-PSR2 exhibited more severe mosaic symptoms compared to those expressing wild-type *35S-PVX* or *35S-PVX-ΔPSR2*. The photograph was taken at 21 days post-infiltration (dpi). **(b)** Northern blot analysis showing the accumulation of viral genomic (gRNA) and subgenomic mRNAs at 21 dpi. This experiment was repeated twice with similar results.

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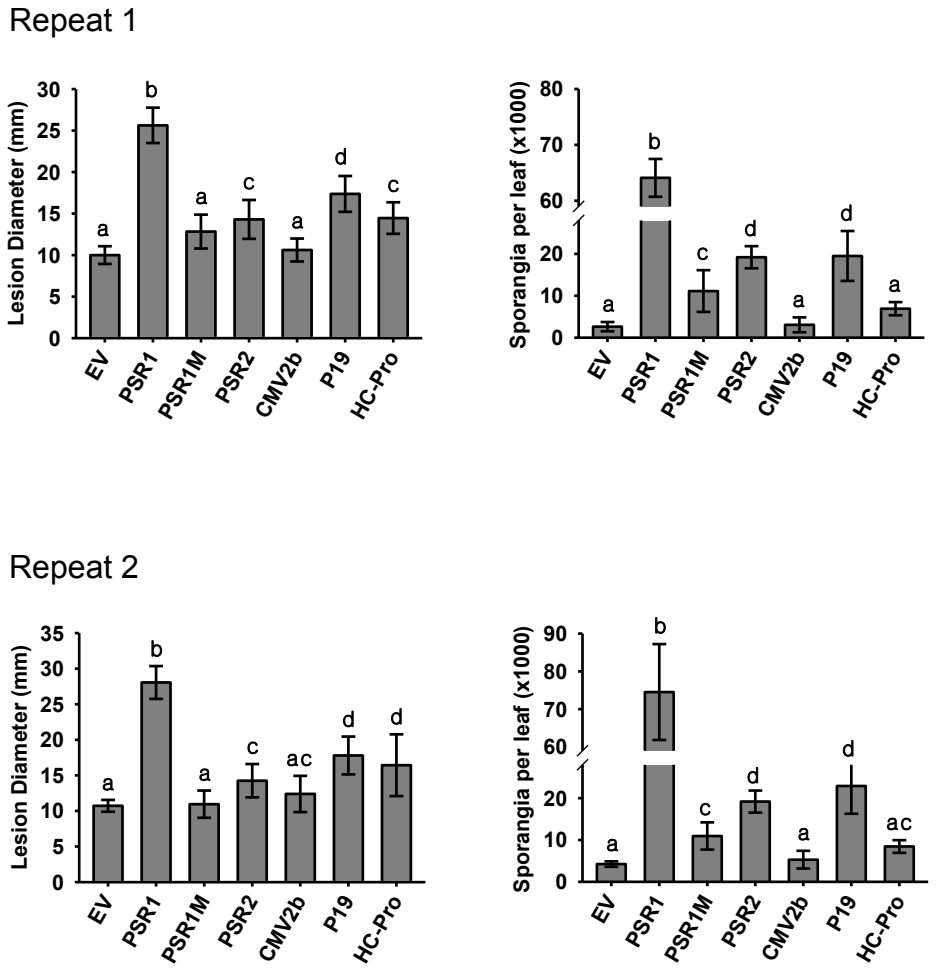

Supplementary Figure 13. Over-expression of *Phytophthora* and viral RNA silencing suppressors in *N. benthamiana* increases plant susceptibility to the infection of *Phytophthora infestans* isolate 1306. Data from two independent biological replicates are presented showing the lesion sizes and numbers of sporangia from *N. benthamiana* leaves expressing PSRs and VSRs. In each experiment, leaves from 6-10 plants were used for each treatment. Error bars represent standard errors and different letters indicate values with significant differences.

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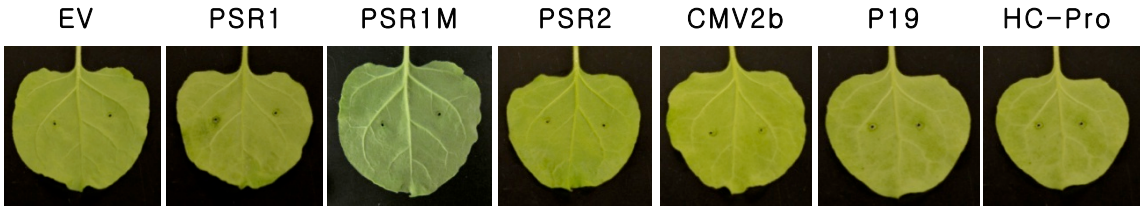

Supplementary Figure 14. Expression of the PSRs and VSRs in *N. benthamiana* leaves did not elicit visible lesions or cell death symptoms without the subsequent infection of *P. infestans*. The effector proteins were expressed in *N. benthamiana* by *Agro*-infiltration. 24 hours after the plants were *Agro*-infiltrated, the leaves were detached and incubated in a growth chamber at 18°C. The photograph was taken seven days after *Agro*-infiltration.

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Supplementary Table 1. Bacterial strains and plasmids used in this study

Strains or Plasmids	Description	Source/ reference
Escherichia coli DH5α	F- Φ80d*lac*ZΔM15 Δ(*lac*ZYA-*arg*F) U169 *rec*A1 *end*A1, *hsd*R17(rk-, mk+) *pho*A *sup*E44 λ- *thi*-1 *gyr*A96 *rel*A1	Invitrogen
Escherichia coli BL21(DE3)	F– ompT gal dcm lon hsdS_B(r_B- m_B -) λ(DE3 [lacI lacUV5-T7 gene 1 ind1 sam7 nin5]	Invitrogen
Agrobacterium tumefaciens GV3101 (pMP90)	Rif^R, $Gent^R$	26
Agrobacterium tumefaciens C58C1 (pCH32)	Rif^R, Tet^R	26
Phytophthora infestans isolate 1306	A1 mating type	
Phytophthora sojae strain P6497	Race 2 strain with full genome sequenced	3, 27, Michael Coffey
pCAMBIA1300::*CMV2b*	pCAMBIA1300 carrying *CMV2b*, Kan^R	30, Shou-Wei Ding
pCAMBIA1300::*P19*	pCAMBIA1300 carrying *P19*, Kan^R	Shou-Wei Ding
pCAMBIA1300::*HC-Pro*	pCAMBIA1300 carrying *HC-Pro*, Kan^R	Shou-Wei Ding
pEG100	pEarleyGate100, a Gateway binary vector with cauliflower mosaic virus *35S* promoter, Kan^R	28
pEG100::*PSR1*	pEG100 carrying *PSR1*, Kan^R	This study
pEG100::*PSR1-YFP*	pEG100 carrying *PSR1-YFP*, Kan^R	This study
pEG100::*PSR1M*	pEG100 carrying *PSR1M* (lacking the putative NLS motif), Kan^R	This study
pEG100::*PSR2*	pEG100 carrying *PSR2*, Kan^R	This study
pEG100::*PSR2-Flag*	pEG100 carrying *PSR2-Flag*, Kan^R	This study
pEG101	pEarleyGate101, a Gateway binary vector with cauliflower mosaic virus *35S* promoter and *yfp*, Kan^R	28
pEG101::*PSR1*	pEG101 carrying *PSR1* tagged with YFP at the C-terminus, Kan^R	This study
pEG101::*PSR1M*	pEG101 carrying *PSR1M* tagged with YFP at the C-terminus, Kan^R	This study
pGR106	a binary vectors carrying the Potato virus X genome, Kan^R	37, Sophien Kamoun
pGR106::*PSR1*	pGR106 carrying *PSR1*, Kan^R	This study
pGR106::*PSR1M*	pGR106 carrying *PSR1M* (lacking the putative NLS motif), Kan^R	This study
pGR106::*ΔPSR1*	pGR106 carrying *ΔPSR1* (stop codon at the 3 aa position of the ORF), Kan^R	This study
pGR106::*PSR2*	pGR106 carrying *PSR2*, Kan^R	This study
pGR106::*ΔPSR2*	pGR106 carrying *ΔPSR2* (stop codon at the 8 aa position of the ORF), Kan^R	This study
pGEX4T-2	*E. coli* expression vector with C-terminal GST tag, Amp^R	Amersham
pGEX4T-2::*PSR1*	pGEX4T-2 carrying *PSR1* that expresses GST-PSR1, Amp^R	This study
pGEX4T-2::*PSR2*	pGEX4T-2 carrying *PSR2* that expresses GST-PSR2, Amp^R	This study
pGEX4T-2::*P19*	pGEX4T-2 carrying *P19* that expresses GST-P19, Amp^R	This study
pTH209	*Phytophthora* transformation vector used as a helper vector for dsRNA-induced silencing of *PSR2*, $G418^R$, Kan^R	40

Supplementary Table S2. *Phytophthora sojae* PsAvh genes screened for RNA silencing suppression activity in this study

Index	Effector ID	VMD ID	Primers used in this study for gene cloning	
			Forward sequence	Reverse sequence
1*	Avr1b	AAM2093(		
2*	PsAvh_5	158993		
3*	PsAvh_6	158994		
4	PsAvh_8	158999	CTACGTCGACATGTTGTCAGCGGGCCCCGGCAAGG	TCTACGAATTCGACTATGGGTACTGTCTAGTGTTCC
5	PsAvh_16	159004	TCTACGTCGACATGGCCCTCCCTTCGGCCACGGCAT	TCTACGAATTCGATCACATGCCCATCTTCTTTGCTT
6	PsAvh_18	159006	CTACGAATTCGCATGACTAAACCGTCGACGGAGGC	CTACGATATCTTCATTGTTCTAGCCACGCCT
7	PsAvh_23	159011	TCTACGTCGACATGCTCGCCACCGCCGAGGCCCCCA	TCTACGAATTCGATCATGCATTGTCGGAAAGTTTGA
8	PsAvh_29	159017	TCTACGAATTCGCATGGCCGTCGCGGGTGCCAGCGA	TCTACGATATCTTCAATGGAAATTATTGATGAGCCA
9	PsAvh_35	159022	CTACGAATTCGCATGGCCGCGGGTGGTACTGCCGT	CTACGATATCTCTAATGGTTGGTGAACCAGTA
10	PsAvh_36	159023	CTACGAATTCGCATGCTCTCGACCACCACGGACTC	CTACGATATCTTCAAGTGAGAGTCCTTCCTTCG
11	PsAvh_38	159025	TCTACGAATTCGCATGGTTCAGGTCTCTCCAGCTGC	TCTACCTCGAGTGCTATGTCCGTGTCGGATGTGCCT
12	PsAvh_39	159026	CTACGTCGACATGGCTCAACCCACGCCAGTGAA	CTACGAATTCGATTAGTCGATCTTCACCTTTCC
13	PsAvh_42	159029	CTACGTCGACATGTTGAGTATCACCAATTCCGA	CTACGAATTCGATCACTTTTTTGGGATCGACG
14	PsAvh_52	159037	TCTACGTCGACATGCTCACGTTGACCAAGGATTCCA	TCTACGAATTCGATCAGTTGGCCGCCTTATAAACCT
15	PsAvh_63	159042	CTACGTCGACATGCAATCCTCCGCCACCGCCAC	CTACGAATTCGATTACGTAGTCCTCTGGTGCA
16	PsAvh_66	159045	CTACGTCGACATGGTTCCTGCTTCCGCAGACAT	CTACGAATTCGATTAGGGGTATCTCAGGGGCC
17	PsAvh_67	159046	CTACGAATTCGCATGCTCCCCGCAGACCAGGCGGC	CTACGATATCTTTACAAAATCTCGCCAGTCTG
18*	PsAvh_73	159050		
19*	PsAvh_92(Avr3a)	159064		
20	PsAvh_94	159065	TCTACGTCGACATGGCCAACACCAAGGTGGAGTCGT	TCTACGAATTCGACTAAGCGGTGTCCTCCTCCTCCT
21	PsAvh_105	159073	CTACGTCGACATGGCCTCCGATCTGACCACGACG	CTACGAATTCGATTAAATCTTAAACTTGCCGAC
22	PsAvh_109	159076	TCTACGAATTCGCATGCTTCAGGTCCTCCCCAATTC	TCTACGATATCTTTAATCAGCGGTTTGTCGCCTGTA
23	PsAvh_115	159081	TCTACGTCGACATGGTCCCAGACTCGAAGGTCTCAA	TCTACGAATTCGACTAGGCGGCATTCTTGCGAGCAT
24	PsAvh_122	159085	CTACGTCGACATGTTGGTCCTTGGGGAAGGTGT	CTACGAATTCGATTAGACTGCCTCCATGACGC
25	PsAvh_127	159090	CTACGTCGACATGGCGGGGCTCGCTGATGCATC	CTACGAATTCGATTACTTTTTGCCAGAGTTGG
26	PsAvh_137	159094	TCTACGTCGACATGGCCGTGGCCGATCCCAAGAGCT	TCTACGAATTCGATCACGGGTAAATGTATCCCTCAA
27	PsAvh_138	159095	CTACGAATTCGCATGCTTCAACCGGGACAGATCGC	CTACGATATCTTTACTGCTTACTACACCAATA
28	PsAvh_139	159096	CTACGTCGACATGGCGTCTGCTGCCACAGAATC	CTACGAATTCGATTAGGGATTGAAAGAAAATA
29	PsAvh_140	159097	TCTACGGATCCGGATGGAATTCCCTTCGGTGGCTTC	TCTACGATATCTCTACTGTTTGCTGCTGGTCAGCTC
30	PsAvh_145	159101	CTACGAATTCGCATGACACCAGCATCTCTACTAAC	CTACGATATCTCTACTTCTTGAGACGTTTGAAGGC
31	PsAvh_146	159102	CTACGAATTCGCATGACACATGCTCCTCCTAACGT	CTACGATATCTTTACCCCCACCTGACTTTGAACTT
32	PsAvh_147	159103	CTACGTCGACATGTCGGTGACAGATAACTCAATCG	CTACGAATTCGATTAGGTCGCCCTGTTCGTGCGCA
33	PsAvh_148	159104	CTACGTCGACATGGAATCTGTAGGCCCCATCAC	CTACGAATTCGATCACCGCCTAGAAGGATATTTCA
34	PsAvh_151	159107	CTACGAATTCGCATGGCACCCGAGTCTGCCACCGC	CTACGATATCTTCACTGAATCTTCGTACCTTTGAG
35	PsAvh_156	159112	CTACGTCGACATGGCCTCGGCGACTTCGACGCT	CTACGAATTCGACTACAGCAGATACAGCTTGTATG
36	PsAvh_162	159118	CTACGTCGACATGAGCACCGACTCCAAGATCGT	CTACGAATTCGATTATTTTGCCCTTACGTTCATGG
37	PsAvh_163	159119	CTACGTCGACATGGCTACCGTATCAGCACTCCAGC	CTACGAATTCGACTAAGAGCGCCGAGGGGTCACAT
38	PsAvh_170	159126	CTACGTCGACATGAGGACTGTGGTGACGCCTAGTT	CTACGAATTCGACTAGGCACTGTATGCAGCTCGGA
39*	PsAvh_171(Avr4/	159127		
40	PsAvh_172	159128	TCTACGAATTCGCATGGAGGTCGACTCGAAGACGGC	TCTACGATATCTCTATTCCCCCCGTAATTTGTAAAA
41	PsAvh_180	159136	TCTACGTCGACATGGAGACCCAGCTTTCGCAGACGA	TCTACGAATTCGACTAAGCGATGTTCGTCTGCTTCT
42*	PsAvh_181	159137		
43	PsAvh_182	159138	CTACGTCGACATGCTACCGGCCTCCGACCAACT	CTACGAATTCGACTAGTGGGGGAATTGTTGCGAGT
44	PsAvh_183	159139	CTACGTCGACATGGAGTCTGCGTCCACAGATACTA	CTACGAATTCGATTACGGGTAGCTTCTATGGATCG
45	PsAvh_189	159145	CTACGTCGACATGACCGCGACCGTCACCGACTCGA	CTACGAATTCGATCAGTAGTCGCAACCCGAACTTA
46	PsAvh_190	159146	CTACGAATTCGCATGACTACCGTCTCGGACAAGTC	CTACGATATCTCTACGGGGCAAAGATGTGGATGGG
47	PsAvh_194	159150	CTACGTCGACATGCTTTCAGCGGCCACGGAGATCGA	CTACGAATTCGATTATCCTCGTGGGGCACCACCTG
48	PsAvh_196	159152	TCTACGAATTCGCATGTCGGTTAGCAACCTCCAAAC	TCTACCTCGAGTGTTAGCTGAGCTTGGACGTCCACC
49	PsAvh_205	159161	CTACGTCGACATGGACGTCGTCTCGGCCTCCATCG	CTACGAATTCGATTAAACGACGAGCTTGCTAGCCC
50	PsAvh_231	159187	TCTACGAATTCGCATGGCCGTGCATACCCAAGAAGG	TCTACCTCGAGTGCTAGCGAATCGATGTGCTTCTGG
51*	PsAvh_238	159194		
52	PsAvh_240	159196	TCTACGTCGACATGGACGCCGGCCGAGTTCTCGAGA	TCTACGAATTCGACTAGTTTGCGGGTTGGTTCCGGA
53	PsAvh_244	159200	CTACGTCGACATGGCCAGGAACCGTGCACCCGGGA	CTACGAATTCGATCACGCGACTTTCGCCACAGCTT
54	PsAvh_254	159210	CTACGAATTCGCATGGAGAAGGCCGTCGACGTGGA	CTACGATATCTTCAGTTGGCTCGTGGGCTGCCCAT
55	PsAvh_256	159212	TCTACGTCGACATGGAGTCCGCAAACGGCGTTTCCC	TCTACGAATTCGATTAAGTGGTCTTTTTAGCAGCGG
56	PsAvh_260	159216	TCTACGTCGACATGGCCACGTGCATCAGTGCCTCGA	TCTACGAATTCGATTAGTCCTTGAGCATGGCGGTGA
57	PsAvh_263	159219	TCTACGTCGACATGGATGACACGCACCGAAGCCT	TCTACGAATTCGACTAATGGATATAACTGTTCTTGA
58*	PsAvh_275(Avr1a	159231		
59*	PsAvh_331(Avr1k	159287		

* effector constructs provided by Dr.Brett Tyler

Supplementary Table 3. Primers used in this study.

	Primer name	Primer sequence (5' to 3')
PVX assay	pGR-PSR1ClaI-F	CTACATCGATATGACTAAACCGTCGACGGAGGC
	pGR-ΔPSR1ClaI-F	CTACATCGATATGACTAAACCGTAGACGGAGGC
	pGR-PSR1NotI-R	CTACGCGGCCGCTTTGTTCTAGCCACGCCTTGT
	pGR-PSR2AscI-F	CTACGGCGCGCCATGACACATGCTCCTCCTAACGTTAAG
	pGR-ΔPSR2AscI-F	CTACGGCGCGCCATGACACATGCTCCTCCTAACGTTTAG
	pGR-PSR2NotI-R	CTACGCGGCCGCACCCCCACCTGACTTTGAACTT
Northern blotting	PVXCP-F	GTCAACTACCTCAACTACCAC
	PVXCP-R	TATGTAGACGTAGTTATGGTGG
	GFP-F	GAAGGTGATGCAACATACGG
	GFP-R	TCCATGCCATGTGTAATCCC
RT-PCR	PSR1-RT-F	ACTAAACCGTCGACGGAGGCGACTG
	PSR1-RT-R	TCATTGTTCTAGCCACGCCTTGTAC
	PSR2-RT-F	ACGAGGTTCTGTCGGGTATG
	PSR2-RT-R	GTCAAGCGATAGCAACGTGA
	AtUBQ5-F	GGTGCTAAGAAGAGGAAGAAT
	AtUBQ5-R	CTCCTTCTTTCTGGTAAACGT
	GmActin-F	GTTCTCTCCTTGTATGCAAGTG
	GmActin-R	CCAGACTCATCATATTCACCTTTAG
Q-RT-PCR	PSR2-QPCR-F	TGTTCGCGGCAAAGAAGGAC
	PSR2-QPCR-R	CCTGACTTTGAACTTGGCGG
	PsojaeActin-F	ACTGCACCTTCCAGACCATC
	PsojaeActin-R	CCACCACCTTGATCTTCATG
sRNA target real-time RT-PCR	AGO1-F	TGGACCACCGCAGAGACAAT
	AGO1-R	CATCATACGCTGGAAGACGACT
	CUC2-F	TTTTCCTCGTTTCGTTTCTA
	CUC2-R	TCCAAATACAGTCAAGTCCA
	PHV-F	CAAGGCTACAGGAACTGC
	PHV-R	TGAGGATTTCAGCGACCT
	SCL6-F	ACTCAAGACAACCTCAAGCA
	SCL6-R	GATAGATGCTTCACGAAACG
	SPL10-F	TGAGACAAAGCCTACACAGATGGA
	SPL10-R	GATGATGCAACCCGACTTTTTTATG
	At4G27990-F	CCGTCAGGTAATGAAACAC
	At4G27990-R	TGGGATACAGAAGTCAACAA
	At1G12775-F	GCTTTTTCTACTATGGGGAAG
	At1G12775-R	ATGAGAGTGGGTTTATGTCC
	ARF3-F	GGTGGCCTGGTTCAAAATGGAG
	ARF3-R	CGGAAGAGGGTGATGATGATAC
pre-miRNA northern blotting	pre-miR164bF	GATGGAGAAGCAGGGCACGT
	pre-miR164bR	GTGAAGATGGGCACATGAAG
	pre-miR166aF	AGATATATATTCAGAAACCCTAG
	pre-miR166aR	GGTTCATTCACTGGATCTGAAAC
***PSR2* silencing**	PSR2-F	GTAATACGACTCACTATAGGGACGAAGAGCGGGGAATCAACT
	PSR2-R	GTAATACGACTCACTATAGGGGTCCCTGTCTTGTCAAGTCGT

	Primer name	Primer sequence (5' to 3')
miRNA Northern blotting	miR159	TAGAGCTCCCTTCAATCCAAA
	miR167	TAGATCATGTTGGCAGTTTCA
	miR319	GGGAGCTCCCTTCAGTCCAA
	miR163	ATCGAAGTTCCAAGTCCTCTTCAA
	miR164	TGCACGTGCCCTGCTTCTCCA
	miR171	CGTGATATTGGCACGGCTCAA
	miR172	ATGCAGCATCATCAAGATTCT
	ASRP255	TACGCTATGTTGGACTTAGAA
	ASRP1151	AAGTATCATCATTCGCTTGGA
	ASR5D8	AAAGGCCTTACAAGGTCAAGA
	miR173	GTGATTTCTCTCTGTAAGCGA
	miR393	GATCAATGCGATCCCTTTGGA
	miR166	GGGGAATGAACGCTGTTCGCT
	miR390	GGCGCTATCCCTCCTGAGCTT
	miR168	TTCCCGACCTGCACCAAGCGA
	siR1003	ATGCCAAGTTTGGCCTCACCGTC
	AtSN1	ACCAACGTGTTGTTGGCCCAGTGGTAAATCTCTCAGAT
	SimpleHAT2	TGGGTTACCCATTTTGACACCCCTA
	Cluster4	AAGATCAAACATCAGCAGCGTCAGAGGCTT
	U6	AGGGGCCATGCTAATCTTCTC
pri-miRNA Q-RT-PCR	pri-miR159F	GGAGCTCTACTTCCATCGTCA
	pri-miR159R	CCACGTTCTCATCAAAACTTT
	pri-miR163F	GCATAGGTCTTGATTGGTGGA
	pri-miR163R	CGTTGTCGTTGAAGAGGTTG
	pri-miR164F	CCATTGACGATTGCATCCTCG
	pri-miR164R	TTGATGGAGAAGCAGGGCAC
	pri-miR166aF	CACCACTCACTTATCTTCTTC
	pri-miR166aR	CAGTCGAAATTATAGAATCTAGGGT
	pri-miR167F	GAAGCTGCCAGCATGATCTA
	pri-miR167R	GGGTTTATAGAAGGGTGCGA
	pri-miR171F	CCGCGCCAATATCTCAGTA
	pri-miR171R	TGTCTCCATTTCAACACACACA
	pri-miR172F	TAGGGTTAGCATGTTGATGAC
	pri-miR172R	CCTCAAGTTATCATATCGGAG
	pri-miR173F	CTTCTTCTCACAAATAAACCCA
	pri-miR173R	AAGATCTCTAACATTAAATCAT
	pri-miR319F	AGAGGTTAGCATGTTGATGAC
	pri-miR319R	CCTCAAGTTATCATATCGGAG
AGO1 slicer assay	TAS1CF	TAATACGACTCACTATAGGGTTAGTTTGAGATTGCGTTTGTC
	TAS1CR	CTAAGATCCACCGATAAATGGTC
EMSA	si21-1	CGUACGCGGAAUACUUCGAUU
	si21-2	UCGAAGUAUUCCGCGUACGUU
	P19-BamHI-F	CTACGGATCCATGGAACGAGCTATACAAGG
	P19-XhoI-R	CTACCTCGAGTTTACTCGCTTTCTTTCTTGA
	PSR1-BamHI-F	CTACGGATCCATGACTAAACCGTCGACGGAGGC
	PSR1-EcoRI-R	CTACGAATTCGTCATTGTTCTAGCCACGCCT
	PSR2-BamHI-F	CTACGGATCCATGACACATGCTCCTCCTAACGTTAAG
	PSR2-EcoRI-R	CTACGAATTCGTTACCCCCACCTGACTTTGA