Supporting Information

Qiao et al. 10.1073/pnas.1421475112

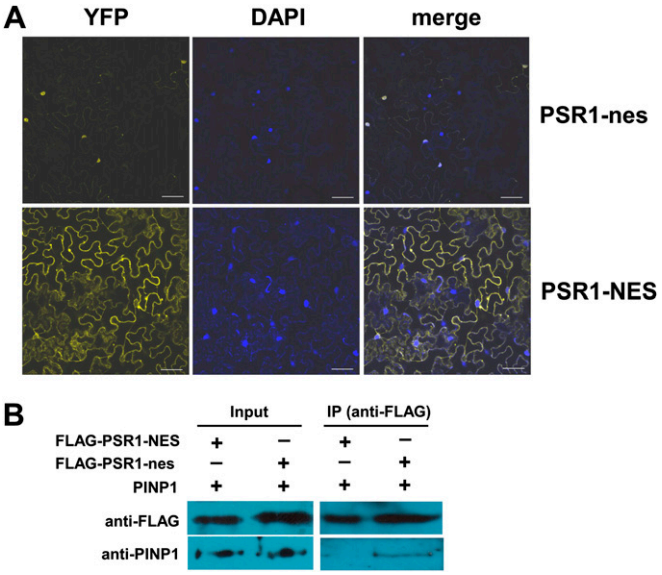

Fig. S1. Fusion to a NES impaired the interaction of PSR1 with PINP1 in plant cells. (*A*) Fusion to NES changed the localization of PSR1. Confocal microscopy of *N. benthamiana* epidermal cells showing the subcellular localization of PSR1–NES–YFP and PSR1–nes–YFP. DAPI staining was used to visualize the nuclei. Please note that the overall fluorescence was decreased to better visualize the predominant nuclear localization of PSR1–nes–YFP; the nuclear localization was completely lost by PSR1–NES–YFP. (Scale bars: 50 μm.) (*B*) Coimmunoprecipitation of PSR1–NES or PSR1–nes with PINP1. The 3x–FLAG–PSR1–NES/nes and PINP1 were coexpressed in *N. benthamiana*. The protein complex was pulled down by using anti-FLAG resins, and the enrichment of PINP1 was detected by an anti-PINP1 antiserum. This experiment was repeated twice with similar results.

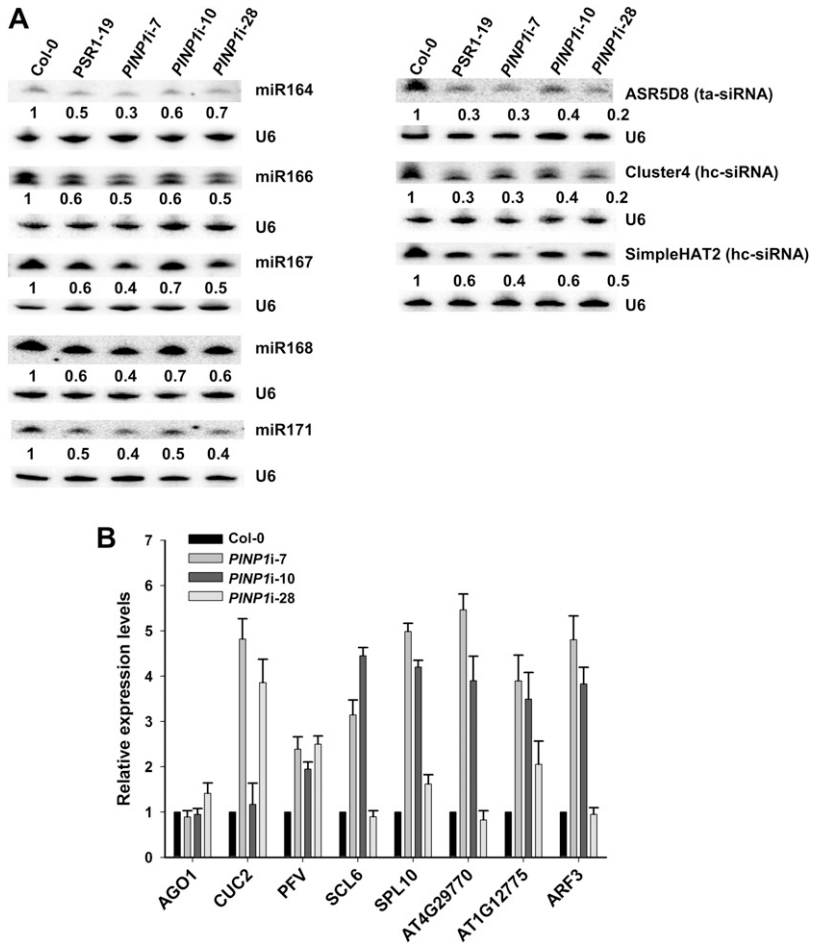

Fig. S2. PINP1 affects small RNA accumulation in *Arabidopsis*. (*A*) Abundances of endogenous small RNAs in wild-type (Col-0), *PSR1*-expressing (PSR1-19), and *PINP1*-silenced plants were determined by Northern blotting. U6 served as an internal control. The numbers below the gel images represent relative small RNA abundance. (*B*) Transcript levels of small RNA target genes were determined by qRT-PCR. *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 generated from *TAS1, TAS2*, and *TAS3,* respectively. *AtUBQ10* was used as the internal standard. This experiment was repeated twice with similar results.

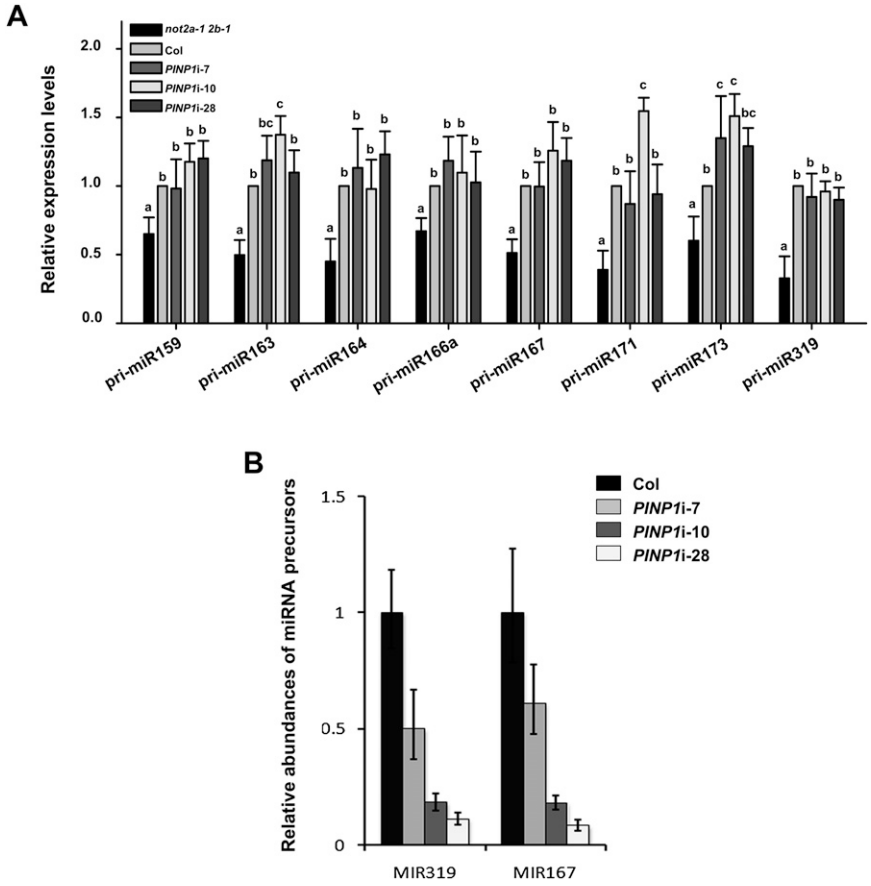

Fig. S3. PINP1 affects the pre-miRNA, but not pri-miRNA, levels. (*A*) Silencing of *PINP1* does not interfere with the transcription of *MIR* genes. Transcript abundances of pri-miRNAs in wild-type (Col-0), *PINP1*-silenced plants, and the *not2a-1 2b-1* mutant were determined by qRT-PCR. *AtUBQ10* was used as the internal standard. Values are means ± SDs (as error bars) from three independent replicates. Letters represent differences with statistical significance ($P < 0.05$) as determined by Duncan's multiple range test. (*B*) Levels of miR319 and miR167 precursors were determined by qRT-PCR in wild-type (Col-0) and *PINP1*-silenced plants. Primers were designed to anneal to the stem portion of the hairpins to amplify the reverse-transcribed products of both pri-miRNAs and pre-miRNAs. Because the abundances of pri-miR167 and pri-miR319 remain unchanged in *PINP1*-silenced plants (Fig. 2*D*), the decreased levels of miRNA precursors observed here were presumably due to lower levels of pre-miRNAs. This experiment was repeated twice with similar results.

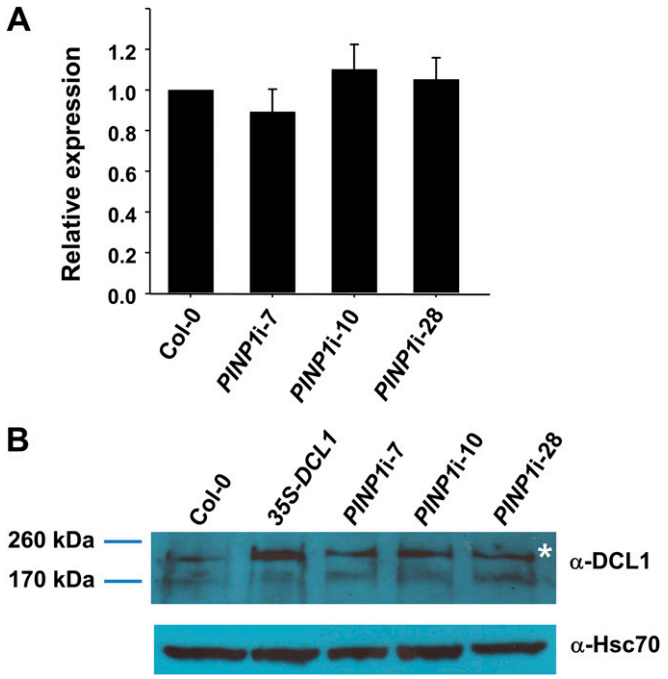

Fig. S4. Transcript and protein levels of DCL1 in *PINP1*-silenced plants. (*A*) *dcl1* transcript levels were determined by qRT-PCR in wild-type (Col-0) and *PINP1*-silenced lines. (*B*) DCL1 protein abundances were determined by Western blotting using an anti-DCL1 antibody (Agrisera) in wild-type (Col-0), *p35S–DCL1*, and *PINP1*-silenced plants. * labels the protein band corresponding to DCL1 with a predicted molecular mass of 210 kDa. Hsc70 was detected by using an anti-Hsc70 antibody as a loading control. Protein sample extracted from *Arabidopsis* plants overexpressing DCL1 was used as a control. This experiment was repeated twice with similar results.

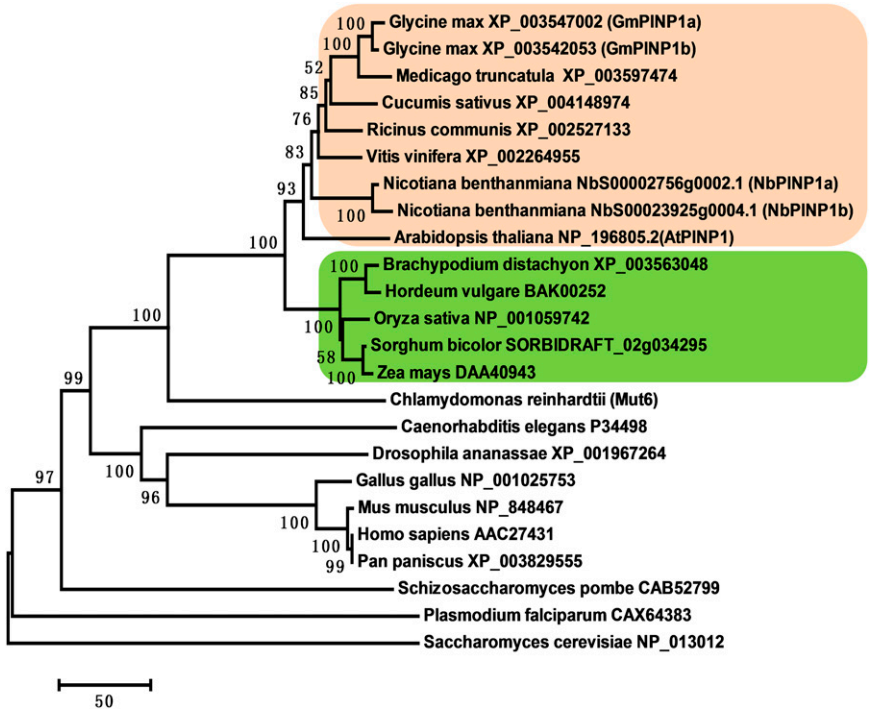

Fig. S5. Phylogenetic analysis of PINP1 homologs in fungi, plants, and animals. Neighbor-joining tree of 24 PINP1 homologs, all containing the DEAH-box RNA helicase domain, was constructed by using full-length amino acid sequences. The clade containing PINP1 homologs in dicots is highlighted in yellow, and the clade with PINP1 homologs in monocots is highlighted in green. The soybean homologs GmPINP1a and GmPINP1b can also interact with PSR1 (Fig. 1*A*).

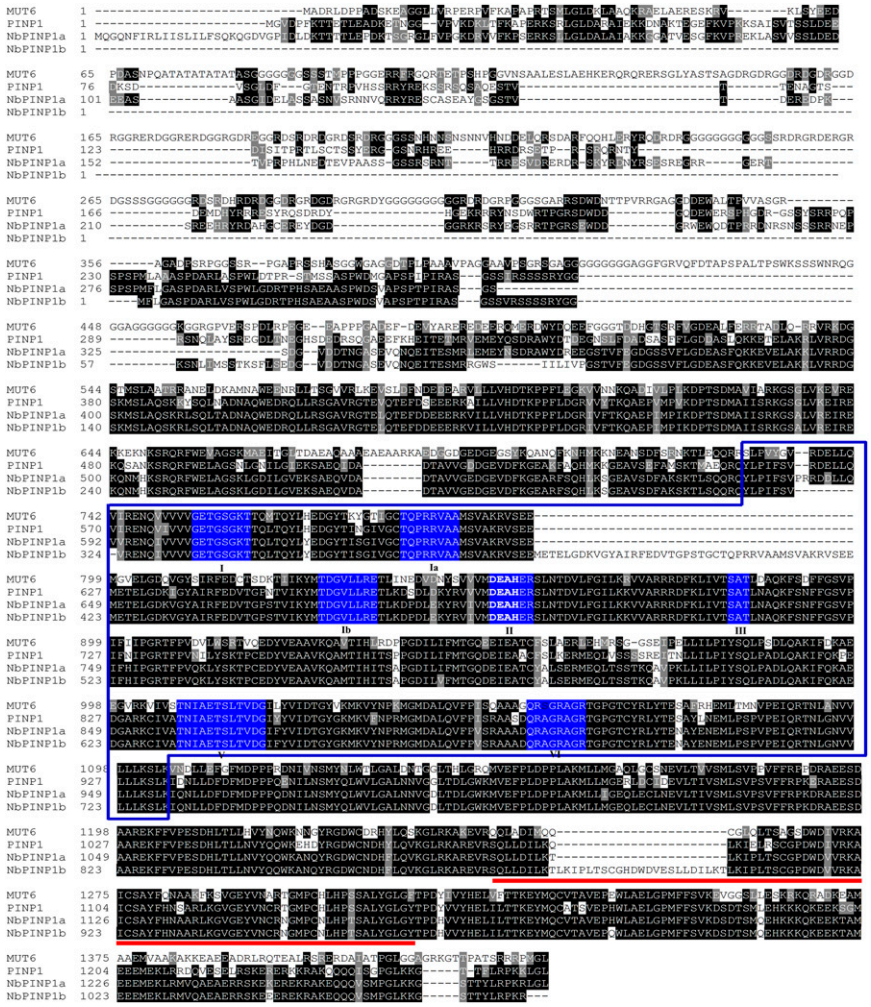

Fig. S6. Amino acid sequence alignment of MUT6 and PINP1 homologs in *Arabidopsis* (PINP1) and in *N. benthamiana* (NbPINP1a and NbPINP1b). The blue box denotes the DEAH RNA helicase domain, and the conserved motifs are highlighted in blue. The red line labels the region of a conserved DUF1605 domain with unknown functions. Identical residues are shaded in black, and similar residues are shaded in gray.

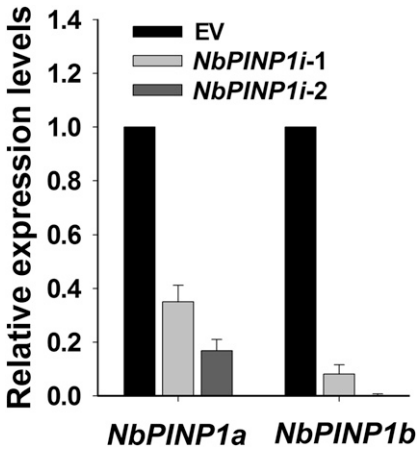

Fig. S7. qRT-PCR analysis of the transcript abundances of *NbPINP1a* and *NbPINP1b* in *N. benthamiana* inoculated with *Agrobacterium* harboring either the empty VIGS vector TRV2–LIC (EV) or TRV2–LIC carrying the silencing constructs *NbPINP1i*-1 or *NbPINP1i*-2. 18S rRNA was used as the internal standard. Error bars are SEs of three biological replicates. This experiment was repeated twice with similar results.

Table S1. Characterization of T-DNA mutants of *PINP1* in *Arabidopsis* eco. Col-0

T-DNA lines	Homozygous plants	Heterozygous plants	Wild-type	χ^2 (2:1)	*P* value
CS24359	0	35	18	0.01	0.9203
SALK_062354	0	38	18	0.02	0.8875
SALK_019541	0	34	17	0.00	1.0000

Table S2. Strains and plasmids used in this study

Strains or plasmids	Description	Source
E. 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
E. coli BL21(DE3)	F– *ompT gal dcm lon hsdSB* (rB- mB -) λ(DE3 [lacI lacUV5-T7 gene 1 *ind1 sam7 nin5*]	Invitrogen
Agrobacterium tumefaciens GV3101(pMP90)	Deletion of T-DNA of pTiC58, Rif^R, Gent^R	Holsters (1)
A. tumefaciens C58C1 (pCH32)	Rif^R, Tet^R	Mudgett et al. (2)
P. infestans isolate 1306	Tomato and potato pathogen, can infect *N. benthamiana*	Cvitanich and Judelson (3)
Phytophthora capsici strain LT263	Isolated from pumpkin, can infect *Arabidopsis*	Donahoo and Lamour (4)
pEG100	pEarleyGate100, a Gateway binary vector with cauliflower mosaic virus 35S promoter, Kan^R	Earley et al. (5)
pEG100::*PSR1A*	pEG100 carrying *PSR1A*, Kan^R	This study
pEG100::*3×FLAG–PSR1*	pEG100 carrying *PSR1* tagged with 3×FLAG at N terminus, Kan^R	This study
pEG100::*3×FLAG–PSR1–NES*	pEG100 carrying *PSR1–NES* (LALKLAGLDI) tagged with 3×FLAG at N terminus, Kan^R	This study
pEG100::*3×FLAG–PSR1–nes*	pEG100 carrying *PSR1–nes* (LALKAAGADA) tagged with 3×FLAG at N terminus, Kan^R	This study
pEG100::*3×FLAG–PSR1A*	pEG100 carrying *PSR1A* tagged with 3×FLAG at N terminus, Kan^R	This study
pEG100::*3×FLAG–PSR1M*	pEG100 carrying *PSR1M* tagged with 3×FLAG at N terminus, Kan^R	This study
pEG100::*amiRPINP1*	pEG100 carrying amiRNA for *PINP1* silencing, Kan^R	This study
pENTR1A	An entry vector for gateway system, Kan^R	Invitrogen
pENTR1A::*PSR1A*	pENTR/D-TOPO carrying the *PSR1* gene, Kan^R	This study
pENTR1A::*3XFLAG–PSR1*	pENTR/D-TOPO carrying the *PSR1* gene with 3xFLAG tag at the N terminus, Kan^R	This study
pENTR1A::*3XFLAG–PSR1A*	pENTR/D-TOPO carrying the *PSR1A* gene with 3xFLAG tag at the N terminus, Kan^R	This study
pENTR1A::*3XFLAG–PSR1M*	pENTR/D-TOPO carrying the *PSR1M* gene with 3xFLAG tag at the N terminus, Kan^R	Qiao et al. (6)
pENTR1A::*PINP1*	pENTR/D-TOPO carrying the *PINP1* gene, Kan^R	This study
pEG101	pEarleyGate101, a Gateway binary vector carrying cauliflower mosaic virus (CaMV) *35S* promoter and *YFP*, Kan^R	Earley et al. (5)
pEG101::*PSR1*	pEG101 carrying PSR1 in-frame fused to YFP, Kan^R	Qiao et al. (6)
pEG101::*PINP1*	pEG101 carrying PINP1 in-frame fused to YFP, Kan^R	This study
pEG104	pEarleyGate104, a Gateway binary vector carrying *P35S-YFP*, Kan^R	Earley et al. (5)
pEG301	pEarleyGate301, a Gateway binary vector without a promoter, Kan^R	Earley et al. (5)
pEG301-pUBQ10::*PSR1*	pEG301 carrying *PSR1* with the UBQ10 promoter, Kan^R	This study
pEG301-pUBQ10::*amiRPINP1*	pEG301 carrying an amiRNA for *PINP1* silencing with the UBQ10 promoter, Kan^R	This study
pGEX4T-2	*E. coli* expression vector with a C-terminal GST tag, Amp^R	Amersham
pGEX4T-2::*PSR1*	pGEX4T-2 carrying *PSR1* which expresses GST-PSR1, Amp^R	This study
pET-mal	*malE* gene from pMAL-c2 is cloned into NdeI-XhoI site of pET28a, Kan^R	Sweeney et al. (7)
pET-mal::*PINP1*	pET-mal carrying *PINP1*, Kan^R	This study
pGBKT7	A yeast bait vector expressing proteins fused to the GAL4 DNA binding domain, Kan^R	Clontech
pGBKT7::*PSR1*	pGBKT7 carrying *PSR1*, Kan^R	This study
pGADT7	A yeast prey vector expressing proteins fused to the GAL4 activation domain, Amp^R	Clontech
pGADT7::*PINP1*	pGADT7 carrying *PINP1*, Amp^R	This study
pGADT7::*GmPINP1a*	pGADT7 carrying *GmPINP1a*, Amp^R	This study
pGADT7::*GmPINP1b*	pGADT7 carrying *GmPINP1b*, Amp^R	This study
pSPYNE	A binary vector carrying CaMV 35S promoter and the N-terminal (1-155 aa) domain of YFP for BiFC analysis, Kan^R	Walter et al. (8)
pSPYCE	A binary vector carrying CaMV 35S promoter and the C-terminal (156-239 aa) domain of YFP for BiFC analysis, Kan^R	Walter et al. (8)
pSPYNE::*PSR1*	pSPYNE carrying *PSR1* in frame fused with nYFP, Kan^R	This study
pSPYNE::*PSR1A*	pSPYNE carrying *PSR1A* in frame fused with nYFP, Kan^R	This study
pSPYNE::*PSR1M*	pSPYNE carrying *PSR1M* in frame fused with nYFP, Kan^R	This study
pSPYCE::*PINP1*	pSPYCE carrying *PINP1* in frame fused with cYFP, Kan^R	This study
TRV1	A VIGS vector with 2x*35S* promoter and the cDNA sequence of TRV strain Ppk20 RNA1, Kan^R	Dong et al. (9)
TRV2-LIC	A VIGS vector with 2x*35S* promoter and the cDNA sequence of TRV strain Ppk20 RNA2, Kan^R	Dong et al. (9)
TRV2::*NbPINP1i-1*	TRV2 carrying fragment 1 between *NbPINP1a/b* genes for gene silencing, Kan^R	This study
TRV2::*NbPINP1i-2*	TRV2 carrying fragment 2 between *NbPINP1a/b* genes for gene silencing, Kan^R	This study

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2. Mudgett MB, et al. (2000) Molecular signals required for type III secretion and translocation of the *Xanthomonas campestris* AvrBs2 protein to pepper plants. *Proc Natl Acad Sci USA* 97(24):13324–13329.
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4. Donahoo RS, Lamour KH (2008) Interspecific hybridization and apomixis between *Phytophthora capsici* and *Phytophthora tropicalis*. *Mycologia* 100(6):911–920.
5. Earley KW, et al. (2006) Gateway-compatible vectors for plant functional genomics and proteomics. *Plant J* 45(4):616–629.
6. Qiao Y, et al. (2013) Oomycete pathogens encode RNA silencing suppressors. *Nat Genet* 45(3):330–333.
7. Sweeney MC, et al. (2005) Decoding protein-protein interactions through combinatorial chemistry: Sequence specificity of SHP-1, SHP-2, and SHIP SH2 domains. *Biochemistry* 44(45):14932–14947.
8. Walter M, et al. (2004) Visualization of protein interactions in living plant cells using bimolecular fluorescence complementation. *Plant J* 40(3):428–438.
9. Dong Y, Burch-Smith TM, Liu Y, Mamillapalli P, Dinesh-Kumar SP (2007) A ligation-independent cloning tobacco rattle virus vector for high-throughput virus-induced gene silencing identifies roles for NbMADS4-1 and -2 in floral development. *Plant Physiol* 145(4):1161–1170.

Table S3. Primers used in this study

Experiment	Primer name	Primer sequence (5′ to 3′)
Y2H	AD-PINP1 EcoRI-F	CTACGAATTCATGGGGGTTGATCCATTCAAAACTA
	AD-PINP1 XhoI-R	CTACCTCGAGTCACAGTCCAAGCTTCTTAGGCCTG
	BD-PSR1-EcoRI-F	CTACGAATTCATGACTAAACCGTCGACGGAGGC
	BD-PSR1-BamHI-R	CTACGGATCCGTCATTGTTCTAGCCACGCCT
	AD-GmPINP1a-EcoRI-F	CTACGAATTCATGGAGAAGGATGGAGCTGGAGCTG
	AD-GmPINP1a-ClaI-R	CTACATCGATTTACAAACCAAACTTCTTTGGTCTC
	AD-GmPINP1b-EcoRI-F	CTACGAATTCATGGAGAAGGATGGAACTGGTGCTG
	AD-GmPINP1b-ClaI-R	CTACATCGATTTACAAACCAAACTTCTTTGGCCTC
Pull-down assay	GST-PSR1-BamHI-F	CTACGGATCCATGACTAAACCGTCGACGGAGGC
	GST-PSR1-EcoRI-R	CTACGAATTCGTCATTGTTCTAGCCACGCCT
	MBP-PINP1-EcoRI-F	CTACGAATTCATGGGGGTTGATCCATTCAAAACTA
	MBP-PINP1-XhoI-R	CTACCTCGAGTCACAGTCCAAGCTTCTTAGGCCTG
BiFC assay	pSPYNE-PSR1-XbaI-F	CTACTCTAGAATGACTAAACCGTCGACGGA
	pSPYNE-PSR1-KpnI-R	CTACGGTACCTTGTTCTAGCCACGCCTTGT
	pSPYCE-PINP1-SalI-F	CTACGTCGACATGGGGGTTGATCCATTCAAAACTA
	pSPYCE-PINP1-KpnI-R	CTACGGTACCCAGTCCAAGCTTCTTAGGCCTGAGG
Co-IP assay	pENTR-PINP1-SalI-F	CTACGTCGACATGGGGGTTGATCCATTCAAAACTA
	pENTR-PINP1-XhoI-R	CTACCTCGAGTGCAGTCCAAGCTTCTTAGGCCTG
	TSK108-PSR1-EcoRI-F	CTACGAATTCATGACTAAACCGTCGACGGA
	TSK108-PSR1-XbaI-R	CTACTCTAGATCATTGTTCTAGCCACGCCT
VIGS assay	NbPINP1-VIGS1-F	CGACGACAAGACCCTGCTGCATCTTCTGGGAGTTC
	NbPINP1-VIGS1-R	GAGGAGAAGAGCCCTTGGAACGATTATCACGACGA
	NbPINP1-VIGS2-F	CGACGACAAGACCCTCGTGTGGCAGCTATGAGTGT
	NbPINP1-VIGS2-R	GAGGAGAAGAGCCCTTGGAGAATTTTTGGGCATT
qRT-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
	NbPINP1a/b-F	AGGGACTTGTTACCGGCTTT
	NbPINP1a/b-R	TAGCCGCATCACTTTCCTCT
	NbPINP1a-F	CCTGGTATGACCGAGAAGAA
	NbPINP1a-R	CAAACTCCGTCTGCAACTCA
	NbPINP1b-F	TTTTCTGTCCGAGGATGGAG
	NbPINP1b-R	CGATCTTCCCATTGAGCATT
	Nb18S-rRNA-F	TGACGGAGAATTAGGGTTCG
	Nb18S-rRNA-R	CCTCCAATGGATCCTCGTTA
	PINP1-RT-F	ACATGACCGATGGAGTACTACTGA
	PINP1-RT-R	TCTACTGGAAGATGATACGAGCTG
	AtUBQ10-F	AAATCTCGTCTCTGTTATGCTTAAGAAG
	AtUBQ10-R	AAAGAGATAACAGGAACGGAAACATAGT
	DCL1-F	AATGGGCATCAGCCGTTTACGAGA
	DCL1-R	AAATCTCTTTGCATGAGCCGGTCC
	premiR319-F	TGAGTCCATTCACAGGTCGT
	premiR319-R	TGGCGACTCGGTATTTGGAT
	premiR167-F	TGTTGTGTTTCATGACGATGGT
	premiR167-R	CAACGGGTGAAACTGCGAA
	pri-miR159-F	GGAGCTCTACTTCCATCGTCA
	pri-miR159-R	CCACGTTCTCATCAAAACTTT
	pri-miR163-F	GCATAGGTCTTGATTGGTGGA
	pri-miR163-R	CGTTGTCGTTGAAGAGGTTG
	pri-miR164-F	CCATTGACGATTGCATCCTCG

Table S3. Cont.

Experiment	Primer name	Primer sequence (5′ to 3′)
	pri-miR164-R	TTGATGGAGAAGCAGGGCAC
	pri-miR166a-F	CACCACTCACTTATCTTCTTC
	pri-miR166a-R	CAGTCGAAATTATAGAATCTAGGGT
	pri-miR167-F	GAAGCTGCCAGCATGATCTA
	pri-miR167-R	GGGTTTATAGAAGGGTGCGA
	pri-miR171-F	CCGCGCCAATATCTCAGTA
	pri-miR171-R	TGTCTCCATTTCAACACACACA
	pri-miR173-F	CTTCTTCTCACAAATAAACCCA
	pri-miR173-R	AAGATCTCTAACATTAAATCAT
	pri-miR319-F	AGAGGTTAGCATGTTGATGAC
	pri-miR319-R	CCTCAAGTTATCATATCGGAG
miRNA Northern blotting	miR159	TAGAGCTCCCTTCAATCCAAA
	miR167	TAGATCATGTTGGCAGTTTCA
	miR164	TGCACGTGCCCTGCTTCTCCA
	miR171	CGTGATATTGGCACGGCTCAA
	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
Silencing of *PINP1*	amiRPINP1	TCAGTATTCCAAAAAGGACGT
NES	NES	CTGGCTTTGAAGTTAGCTGGTTTGGATATC
	nes	CTTGCTCTTAAGGCGGCTGGAGCTGATGCT

For PSR1, PSR1A, and PSR1M, the same primer sets were used in each experiment.