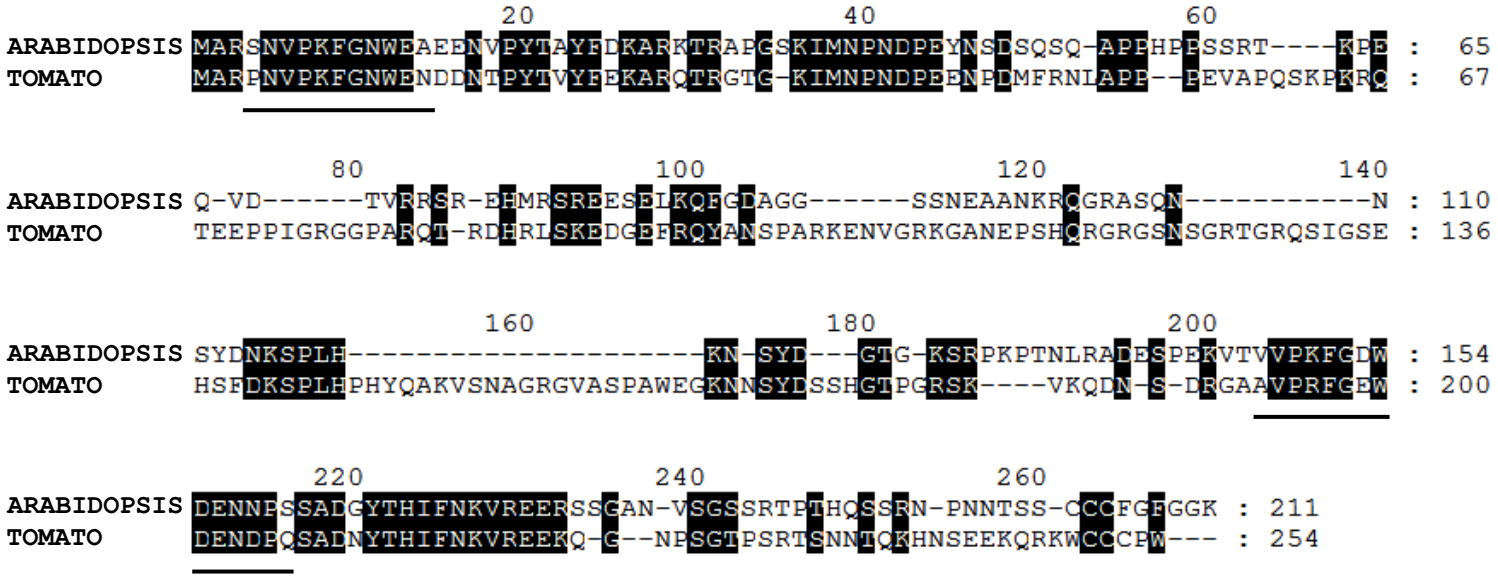
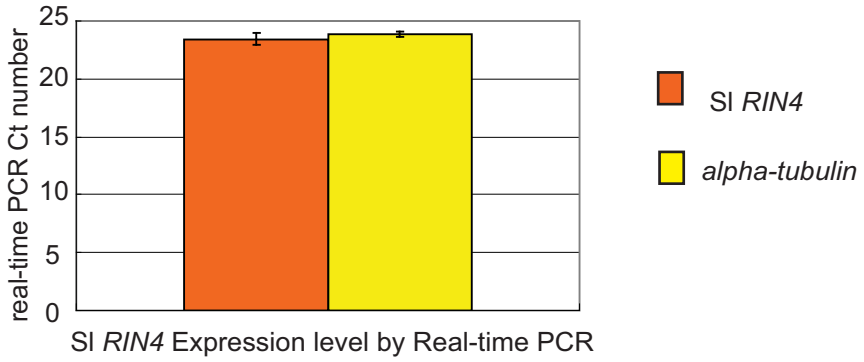
Supplemental Data Luo et al., (2009) Proteolysis of a Negative Regulator of Innate Immunity is Dependent on Resistance Genes in Tomato and *Nicotiana benthamiana* and Induced by Multiple Bacterial Effectors

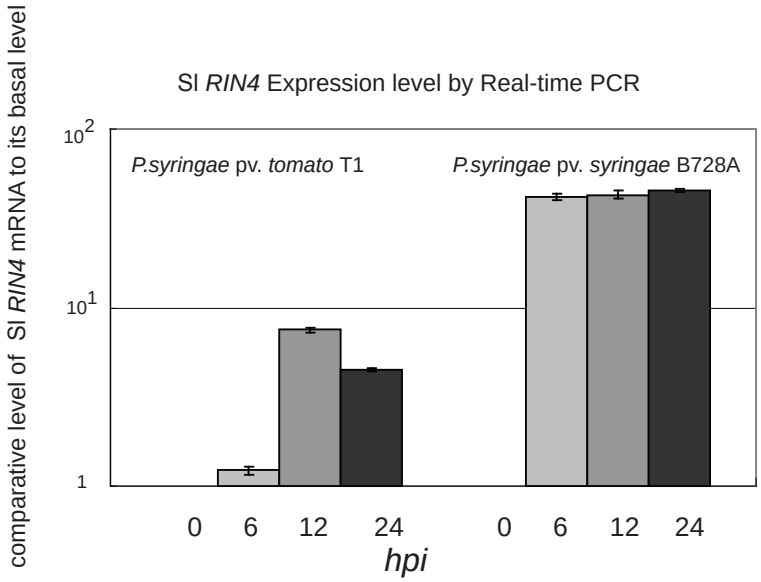


Supplemental Figure 1. **The amino acid sequence of At RIN4 and Sl RIN4.** Conserved residues are highlighted in black, the conserved cleavage sites are underlined.

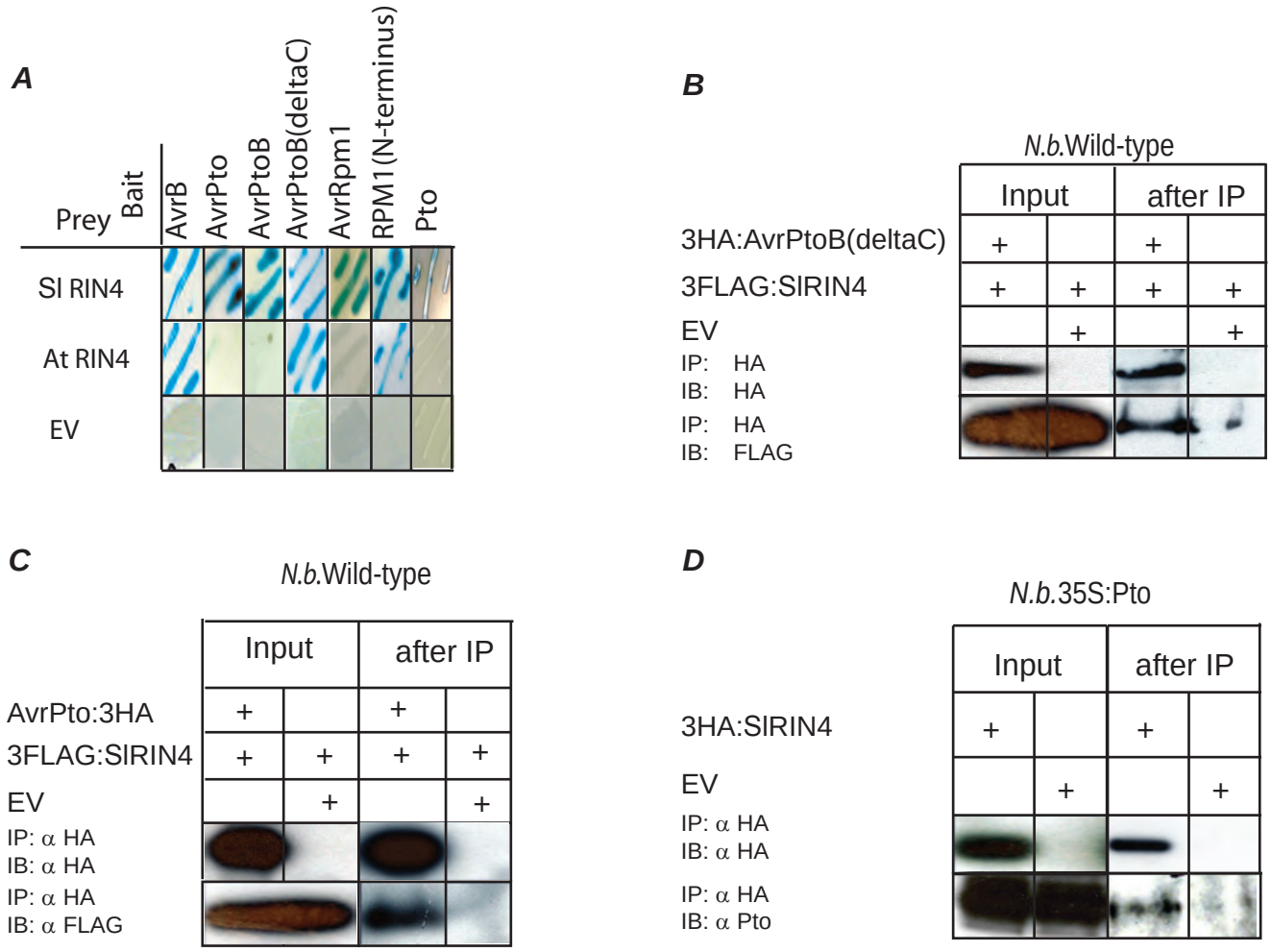


Supplemental Figure 2. Real- time PCR analysis showing the similar expression level of Sl *RIN4* to the house-keeping gene (*alpha-tubulin*).

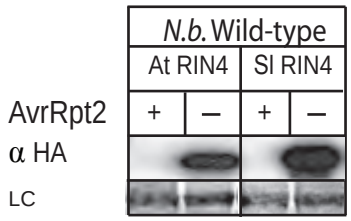
Ct (threshold cycle) is the cycle number at which enough amplified product accumulates to yield a detectable fluorescent signal. Two sets of independent samples were prepared. Each set had three replicates. Error bars show standard deviations.



Supplemental Figure 3. Enhanced transcription of Sl *RIN4* after challenge with virulent or avirulent strains. The first-strand cDNA was generated from total RNA isolated from leaves of tomato cv. Rio Grande 76R 0, 6, 12, and 24 hr after inoculation with the virulent or avirulent tomato pathogens *P. syringae* pv. *tomato* T1 or *P. syringae* pv. *syringae* B728A, respectively. Real-time PCR was performed using SyBr Green to detect the fold-difference in accumulation of Sl *RIN4* mRNA compared to the basal level (regarded as 1). Sl *RIN4* mRNA levels were normalized using the gene encoding alpha-tubulin as the reference gene. Two independent sets of samples were harvested and three replicates of each sample were analyzed in each round of QT-PCR.

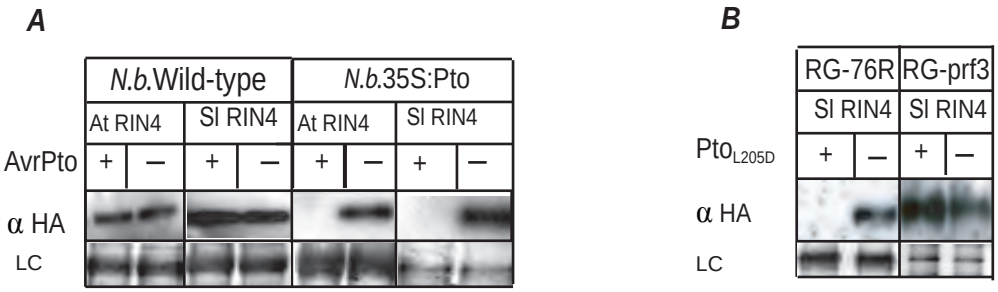


Supplemental Figure 4. Protein-protein interactions in yeast and *in planta*. (A) Interactions between At RIN4, Sl RIN4, effectors, the N terminus of RPM1, and Pto in the yeast two-hybrid assay, the C terminus of AvrPtoB (amino acids 308-543) was unstable in yeast and, therefore, could not be tested using the yeast two-hybrid assay and other resistance signaling components such as At NPR1 and multiple bacteria effectors such as HopPsyE showed no interactions with either At RIN4 or Sl RIN4 (data not shown); **(B)** Immunoblot experiment in which *Agrobacterium* carrying constructs to express 3HA:AvrPtoB(deltaC) and 3FLAG:SlRIN4 or an empty vector were co-inoculated into *N. benthamiana* and which shows co-immunoprecipitation of Sl RIN4 with the N terminus of AvrPtoB; **(C)** Immunoblot experiment in which *Agrobacterium* carrying constructs of AvrPto:3HA and 3FLAG:LeRIN4 or an empty vector were co-inoculated into *N. benthamiana* and which shows association of AvrPto and Sl RIN4 in *N. benthamiana*, all suspensions of *Agrobacterium* were inoculated at OD = 1.0 and leaf tissues were harvested 22 hr after inoculation; **(D)** Immunoblot experiment in which *Agrobacterium* carrying constructs to express 3HA:SlRIN4 or an empty vector were inoculated into transgenic *N. benthamiana* overexpressing Pto and which shows association of Pto and Sl RIN4 in *N. benthamiana*. AvrPtoB(deltaC) = N terminus only (amino acids 1-308) of AvrPtoB and RPM1 N term = N terminus (amino acids 1-176) of RPM1 (A), EV = empty vector negative control (A)-(D), protein complexes in (B)-(D) were immunoprecipitated (IP) using anti-HA antibody matrix (α-HA) and immunoblotted (IB) using α-HA, anti-FLAG (α-FLAG) or anti-Pto (α-Pto) (made in the Michelmore Lab) antibodies.



Supplemental Figure 5. Degradation of RIN4 by AvrRpt2 in *N. benthamiana*.

RIN4 homologs from *Arabidopsis* (At RIN4) or tomato (Sl RIN4) were cœxpressed in *N. benthamiana* with AvrRpt2 (+) or without AvrRpt2 (-). Hemaglutin (HA)-tagged RIN4 was detected by immunoblotting using anti-HA antibody (α-HA). The mobility differences between At RIN4 and Sl RIN4 are consistent with a difference in size of 43 amino acids. LC: Loading control; Ponceau staining of the membrane to show equal loading.



Supplemental Figure 6. Degradation of RIN4 was triggered by two bacterial effectors, AvrPto and AvrPtoB, and an autoactive Pto mutant and was dependent on Pto and Prf.

(A) Immunoblot experiment in which HA-tagged At RIN4 or Sl RIN4 were each co-expressed, with (+) or without (-) AvrPto, in *N. benthamiana* not expressing (*N.b.* Wild-type) or expressing (*N.b.*:35S:*Pto*) the *Pto* transgene from the cauli-flower mosaic virus (CaMV) 35S promoter and which shows AvrPto-triggered degradation of RIN4 is dependent on Pto in *N. benthamiana*; **(B)** Immunoblot experiment in which Pto_{L205D} (+) or an empty vector (-) and HA-tagged Le RIN4 were transiently co-expressed in wild-type tomato RG-76R or the isogenic mutant (RG-prf3) and which shows that constitutively active Pto mutant-triggered degrad-ation of RIN4 is dependent on Prf in tomato. α-HA = anti-HA antibody (A) and (B), LC = Ponceau staining of the membrane (A) or silver staining of the gel (B) to show equal loading.

Supplemental Table 1. Oligonucleotide primers used for molecular constructions.

Primer	sequences
Gateway-SlRIN4F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGCTCGTCCAAATGTCC
Gateway-SlRIN4R	GGGGACCACTTTGTACAAGAAAGCTGGGTCTTACCAGGGACAACAACACCACTTCCTTTG
Gateway-AtRIN4F	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGGCTAGCATGACTGGTGGACAGCAAAT GGGT
Gateway-AtRIN4R	GGGGACCACTTTGTACAAGAAAGCTGGGTCTCATTTTCCTCCAAAGCCAAAGCAGCAACA
CyscleaveF	GGGGACAATGTTGTACAAAAAAGCAGGCTCCATGAATGTCCCAAAGTTTGGC
Cyscleave+GFPF	ATGAATGTCCCAAAGTTTGGCAATTGGGAAATGAGTAAAGGAGAACTTTTC
GFPR	GGGGACCACTTTGTACAAGAAAGCTGGGTTCTATCTCTTTTCGTTGGGATCTTTCGA
MutCyscleaveF	ATGAATCTCCCA**GCTGCTGCT**AATTGGGAAATGAGTAAAGGAGAACTTTTC
GatewayMutCysF	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGAATGTCCCAGCT**GCTGCT**
pBDfor	GGACTAGTAATGAAGCTACTGTCTTCTATCGAACAA
pBDrev	GCTTCTAGATCACGGCGATACAGTCAACTGTCTTTGACC
pADfor	GGACTAGTAATGGATAAAGCGGAATTAATTCCCGAGCCT
pADrev	GCTTCTAGATCAAGCGTAATCTGGAACATCGTATGGGTA
3FLAG+SlRIN4F	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGGACTATAAAGACGACGACGACAAAGA CTATAAAGACGACGACGACAAAGACTATAAAGACGACGACGACAAAATGGCTCGTCCAAAT GTCC

Supplemental Table 2. Primers used for Quantitative PCR analysis of Sl *RIN4* expression in transgenic plants.

QtRIN4F	AGGTGTTGCATCTCCTGCTT
QtRIN4R	CCACTCTCCGAATCTTGGAA
Alpha-tubulinF	CACCACAGGTCTCCAACTTCT
Alpha-tubulinR	TGAGATTGGTGTAGGTAGGG

Supplemental Table 3. The list of the constructs in this paper.

Gene	vector	application in this paper
Sl RIN4	pGWB15[a]	Agrobacterium-mediated transient assay
avrPto	pCB302-3[b]	Agrobacterium-mediated transient assay
avrPtoB	pCB302-3	Agrobacterium-mediated transient assay
avrPto:3HA	pGWB14[c]	Agrobacterium-mediated transient assay
3FLAG:LeRIN4	pCB302-3	Agrobacterium-mediated transient assay
3HA:AvrPtoB(deltaC)	pGWB15	Agrobacterium-mediated transient assay
DEX-AvrPto	pTA7002[d]	Agrobacterium-mediated transient assay
HA-RCS-GFP	pGWB15	Agrobacterium-mediated transient assay
Sl *MEKK2* $_{T125D/S221D}$	pCB302-3	Agrobacterium-mediated transient assay
RPP8	pCB301[e]	Agrobacterium-mediated transient assay
HopAM1 $_{DC3000}$	pBAV139[f]	Agrobacterium-mediated transient assay
RPS2	pCB301	Agrobacterium-mediated transient assay
avrB	pCB302-3	Agrobacterium-mediated transient assay
avrPto $_{I96T}$	pCB302-3	Agrobacterium-mediated transient assay
avrPto $_{I96T}$	pGWB14	Agrobacterium-mediated transient assay
avrRpm1:3HA	pGWB14	Agrobacterium-mediated transient assay
Pto $_{D164N/L205D}$	pCB302-3	Agrobacterium-mediated transient assay
avrRpt2	pCB301	Agrobacterium-mediated transient assay
Pto $_{L205D}$	pCB302-3	Agrobacterium-mediated transient assay
HA-RCS-GFP*	pGWB15	Agrobacterium-mediated transient assay
avrPto	pDSK519[g]	a broad-host vector for expressing in *Pto*, *Pto* $_{T1}$ and *Pfl*

[a]pGWB15: a binary vector with 3 HA tags at its N terminus and a 35S promoter.
[b]pCB302-3: a binary vector without any tag and a 35S promoter.
[c]pGWB14: a binary vector with 3 HA tags at its C terminus and a 35S promoter.
[d]pTA7002: a binary vector with DEX-inducible promoter.
[e]pCB301: a binary vector without any tag and a 35S promoter.
[f]pBAV139: a binary vector with one HA tag at its C terminus and a 35S promoter.
[g]pDSK519: a broad-host vector to express genes in *Pseudomonas*.