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Supplemental Information

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Includes:
Supplemental table (Table S1)
Supplemental figures (S1-S9)

Table S1: Primers used in this study for RipE1 cloning and qRT-PCR.

Gene	Forward primers	Reverse primers
Primers for site-directed amino acid mutation		
RipE1-C172A	AGGGGCGGGGAACGCCGGCGAACACGCC	GGCGTGTTCGCCGGCGTTCCCCGCCCCT
RipE1 ΔAD	GATACTGACGCACATCGACGCCACCCA	TGGGTGGCGTCGATGTGCGTCAGTATC
Primers for qRT-PCR in *N. benthamiana*		
NbEF1a	CCCAAGAGGCCCTCAGACA	CACACGACCAACAGGGACAGT
NbPR-1	GGTCAACACGGCGAAAACC	GCCTTAGCAGCCGTCATGA
NbICS1	GTGTCGGCTCTGCTGTCTTCT	CTGCGTATAGCACGCCAATC
NbPAL05	AAGGGAGCTGAAATCGCCAT	TCCGCACTTTGGACATGGTT
NbPAL08	TATCACCCCATGCTTGCCTC	AGTGGCCTTGGAATTGGGTC
NbPAL10	GTCACTCCATGTTTGCCCCT	GACCTGTGAGTAAACCGGCA
NbLOX2	TCTTGGGTGGCTCCTCTGACT	TGTTGGAGGTCTGCCTGTTCT
NbAOS	CTGGGGTCAAACTCCACACT	TTGTGATGCAACTGGTGGTT
Primers for qRT-PCR in *A. thaliana*		
AtACTIN2	TGCTGGACGTGACCTTACTG	TTCTCGATGGAAGAGCTGGT
AtPR1	TGATCCTCGTGGGAATTATGT	TGCATGATCACATCATTACTTCAT
AtICS1	GCGTCGTTCGGTTACAGG	ACAGCGAGGCTGAATCTCAT
AtPAL1	TATCCCGAACAGGATCAAGG	TCTCCGGTCAAAAGCTCTGT
AtPDF1.2	ACTATGTCTTCCCAGCACAC	AACAACAACGGGAAAATAAA
AtVSP2	GTTTGGATCTTTGACCTAGACGA	CTCTAACCACGACCAGTACGC
Primers for cloning RipE1 to sXVE:GFPc:Bar estradiol inducible vector		
RipE1	TCAGGATCCATGCCGCCCGTCCTGCCGT	ACTCTCGAGGCTTTCCGTGGCGGGCGGCT

Figure S1

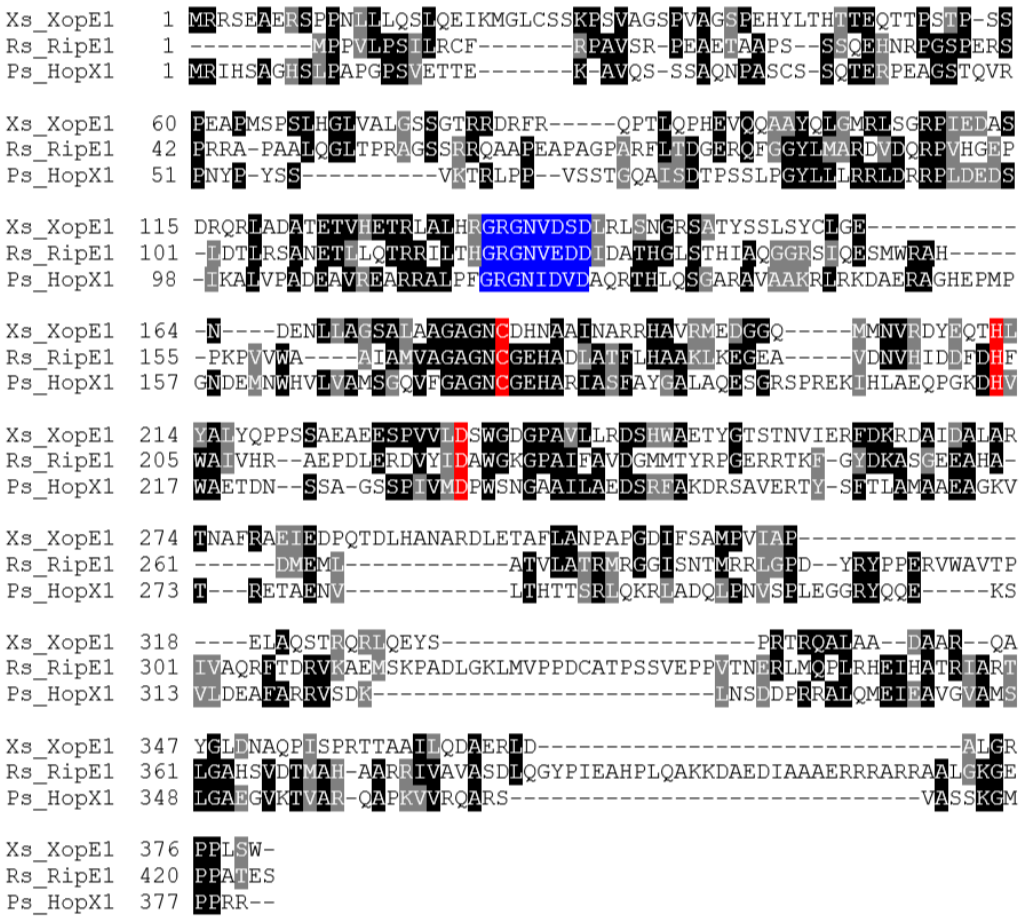

Figure S1. Phylogenetic analysis of RipE1

Alignment of the amino acid sequence of RipE1 from *R. solanacearum* GMI1000 (Rs_RipE1), XopE1 from *Xanthomonas campestris* pv. *vesicatoria* (Xs_XopE1), and HopX1 from *Pseudomonas syringae* pv tabaci 11528 (Ps_HopX1). Residues forming the predicted catalytic triad are indicated in red, and the conserved domain A is indicated in blue. The black shaded amino acids are identical among the three effectors.

Figure S2

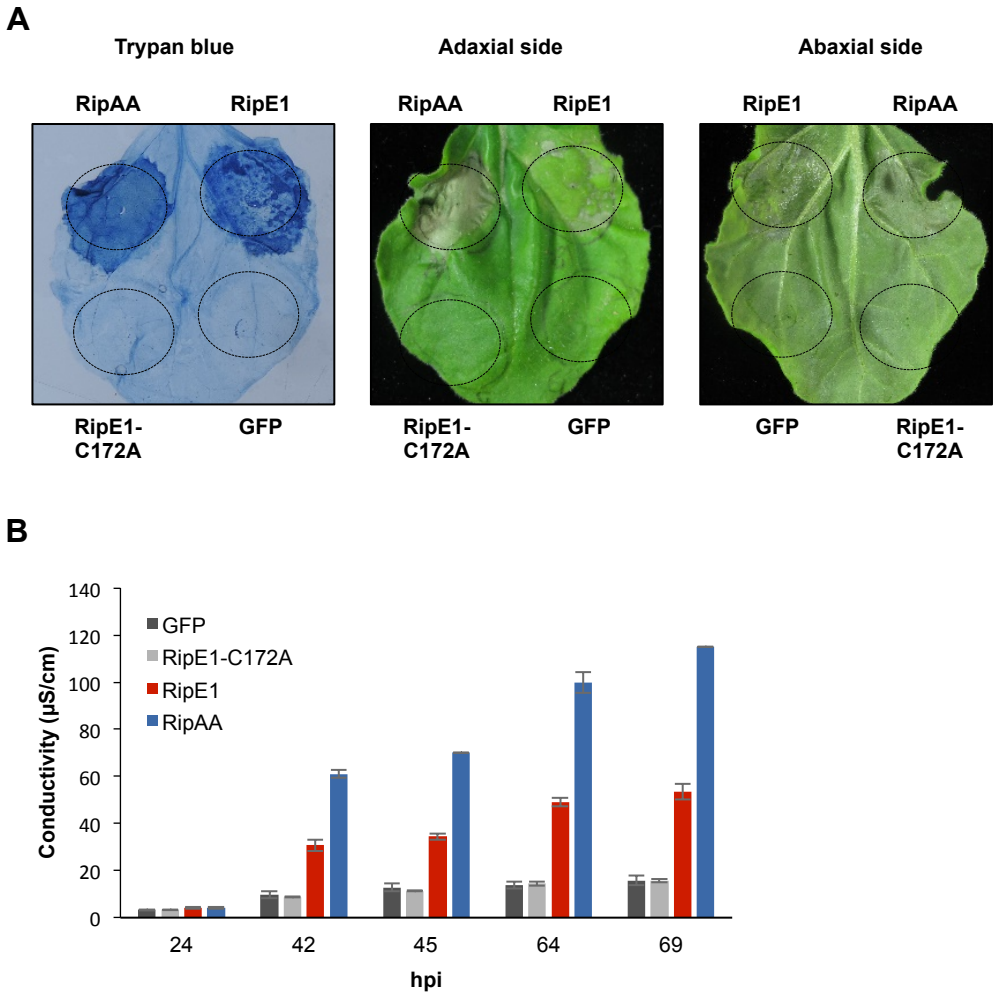

Figure S2. RipE1 induces cell death in *N. benthamiana*.
RipE1-GFP, RipE1-C172A-GFP, RipAA-GFP (as positive control), and GFP (as negative control) were expressed in the same leaf of *N. benthamiana* using Agrobacterium with an OD_{600} of 0.5. (a) Trypan blue staining was performed 4 days post-inoculation, and additional photos of an independent unstained leaf is shown for reference. (b) Ion leakage measured in leaf discs taken from *N. benthamiana* tissues expressing the indicated constructs, at the indicated time points. Values indicate mean ± SE (n=3 biological replicates). These experiments were repeated 3 times with similar results.

Figure S3

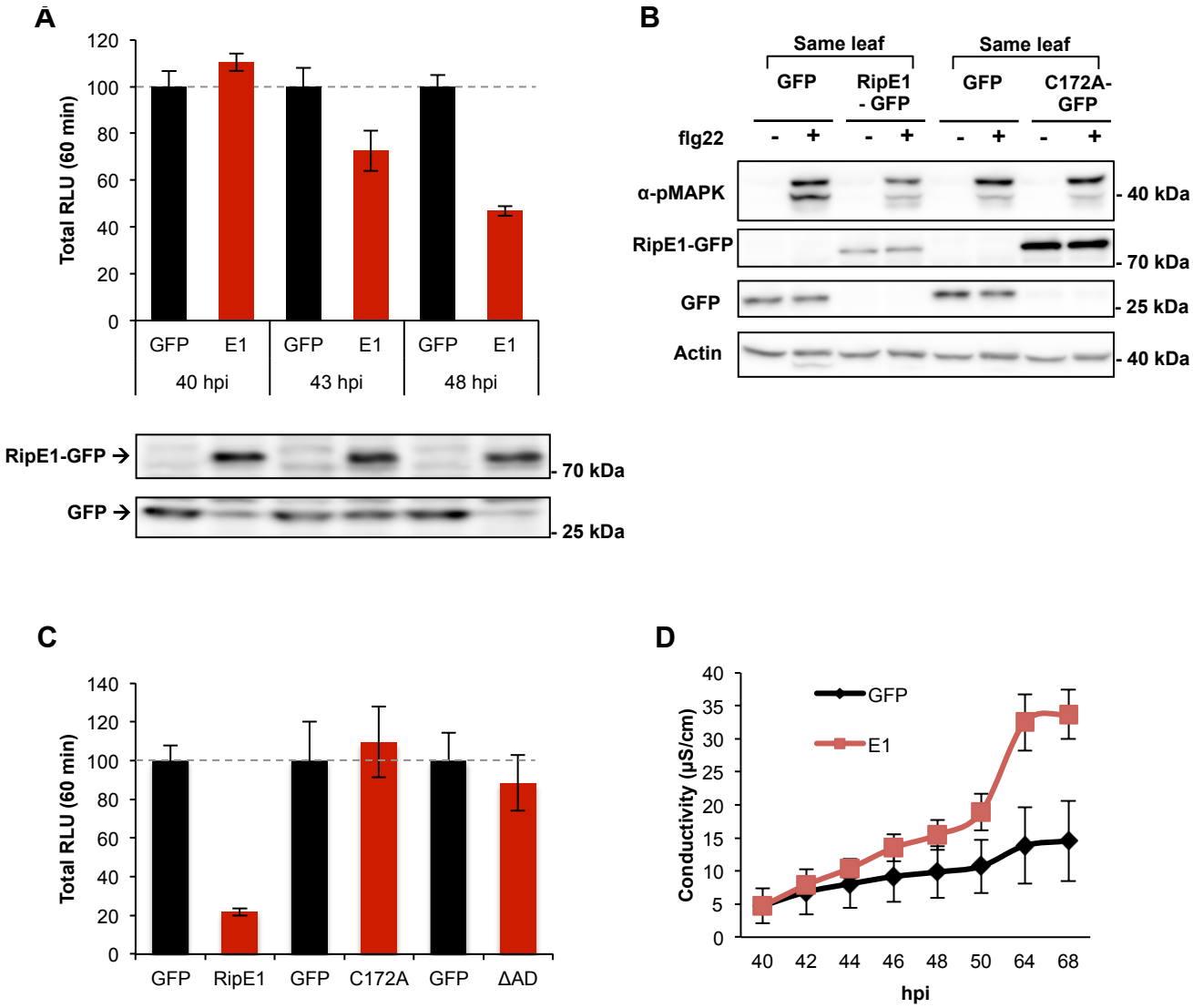

Figure S3. RipE1 expression inhibits PTI responses in *N. benthamiana*, which correlates with the induction of cell death.
Agrobacterium was used to induce the transient expression of RipE1-GFP in half of the leaf and GFP in the other half. (a) Oxidative burst triggered by 50 nM flg22 in *N. benthamiana* tissues at the indicated time points, measured in a luminol-based assay as relative luminescence units (RLU). Values are average ± SE (n=24), and are represented as % of the GFP control in each time point. Western blot with anti-GFP is shown for reference of protein accumulation at each time point. (b) MAPK activation was induced 40 hours after Agrobacterium infiltration with 100 nM flg22 and analysed 15 minutes after flg22 treatment using anti-phosphorylated MAPK antibody (anti-pMAPK). Immunoblots were also analysed using anti-GFP antibody to verify protein accumulation. Anti-actin was used to verify equal loading. Molecular weight (kDa) marker bands are indicated for reference. (c) Oxidative burst was induced as in (a) and measured 2 days post-Agrobacterium infiltration. Mutant variants are described in the Figure 1. (d) Ion leakage was measured as in the Figure 1. Measurement over time after RipE1 expression reflects that the induction of cell death correlates in time with the suppression of PTI responses. The experiments were repeated three times with similar results.

Figure S4

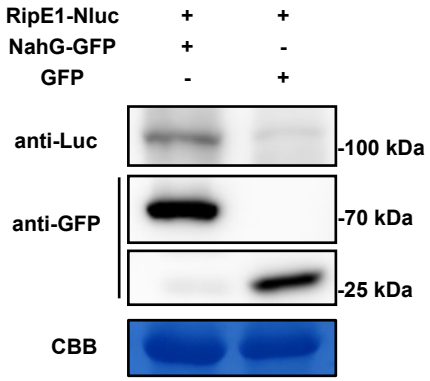

Figure S4. Protein accumulation upon co-expression of RipE1 and NahG.
Western blot showing protein accumulation in the experiments shown in the figure 2. Molecular weight (kDa) marker bands are indicated for reference.

Figure S5

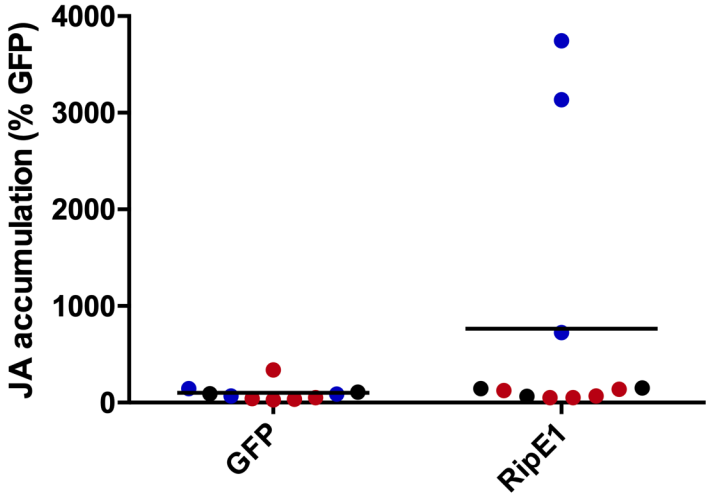

Figure S5. RipE1 expression leads to an increase in JA contents.
Measurement of JA accumulation in *N. benthamiana* tissues expressing GFP or RipE1, using Agrobacterium with an OD_{600} of 0.5. Samples were taken 42 hours after Agrobacterium infiltration. Three independent biological repeats were performed, and the different colors indicate values from different replicates. Values are represented as % of the GFP control in each replicate.

Figure S6

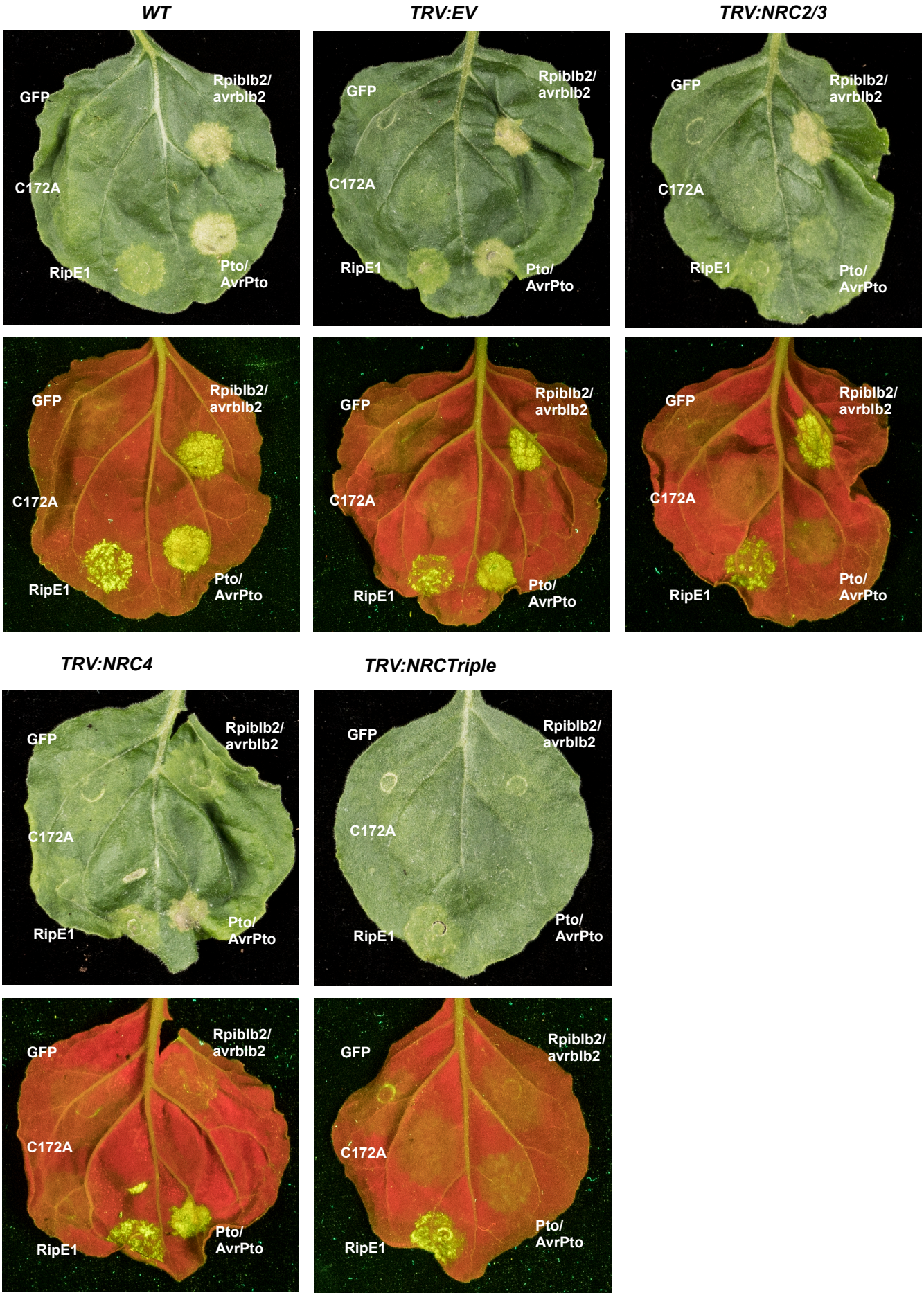
WT
TRV:EV
TRV:NRC2/3
TRV:NRC4
TRV:NRCTriple
GFP
Rpiblb2/
avrblb2
C172A
RipE1
Pto/
AvrPto

Figure S6. RipE1-triggered cell death does not require NRC proteins.
RipE1-GFP, RipE1-C172A-GFP or GFP (as control) were transiently expressed using agrobacterium into wild type (WT) *N. benthamiana*, leaves silenced with EV (as control) and those silenced with different NRC homologs (NRC2/3, NRC4 and NRC2/3/4-Triple), using VIGS. For RipE1-GFP, RipE1-C172A-GFP and GFP an OD_{600} of 0.5 was used. Rpiblb2 (OD_{600} 0.2)/AVRblb2 (OD_{600} 0.1) and Pto (OD_{600} 0.6)/AVRPto (OD_{600} 0.1), which are NRC4 and NRC2/3 dependent, respectively, were included as controls. Photos were taken 5 days post inoculation under natural or UV light. UV images were taken from the abaxial side and flipped horizontally for representation.

Figure S7

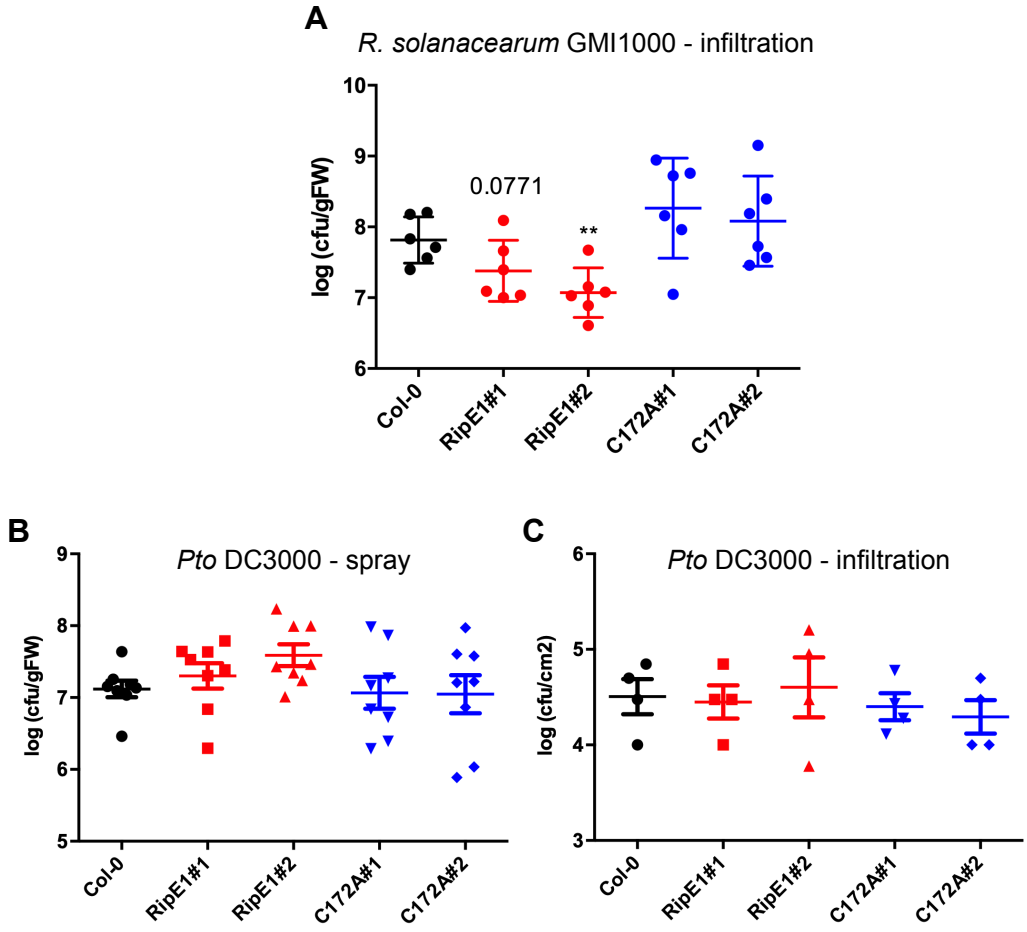

Figure S7. Leaves of RipE1-expressing Arabidopsis plants show enhanced resistance against inoculation with *R. solanacearum*, but not *P. syringae pv tomato* (*Pto*) DC3000.

(a) Growth of *R. solanacearum* GMI1000 (inoculated at 10^7 cfu/ml) infiltrated with a needleless syringe into wild-type Col-0 and RipE1-expressing Arabidopsis plants. Four week-old plants were sprayed with 100 µM estradiol and then covered for 2 days prior inoculation. Bacterial colony-forming units (cfu) were determined 2 days post-inoculation (dpi). Values indicate mean ± SE (n=6 biological replicates). Asterisks indicate significant differences compared to Col-0 WT plants according to a Student's t test (** $p < 0.01$). A specific p value is indicated for the RipE1#1 plant to indicate a reproducible (but not statistically significant) difference in bacterial growth compared to Col-0 WT plants. (b) Growth of surface (spray)-inoculated *Pto* DC3000 (inoculated at 10^8 cfu/ml) in wild-type Col-0 and RipE1-expressing Arabidopsis seedlings. Three week-old seedlings were sprayed with 100 µM estradiol and then covered for 2 days prior inoculation. Bacterial colony-forming units (cfu) were determined 3 days post-inoculation (dpi). Values indicate mean ± SE (n=8 biological replicates). (c) Growth of *Pto* DC3000 (inoculated at 10^5 cfu/ml) infiltrated with a needleless syringe into wild-type Col-0 and RipE1-expressing Arabidopsis plants. Four week-old plants were sprayed with 100 µM estradiol and then covered for 2 days prior inoculation. Bacterial colony-forming units (cfu) were determined 3 days post-inoculation (dpi). Values indicate mean ± SE (n=4 biological replicates). Experiments were repeated more than three times with similar results.

Figure S8

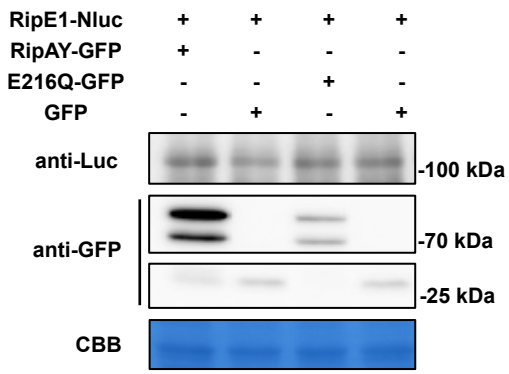

Figure S8. Protein accumulation upon co-expression of RipE1 and RipAY.
Western blot showing protein accumulation in the experiments shown in the figure 6. Molecular weight (kDa) marker bands are indicated for reference.

Figure S9

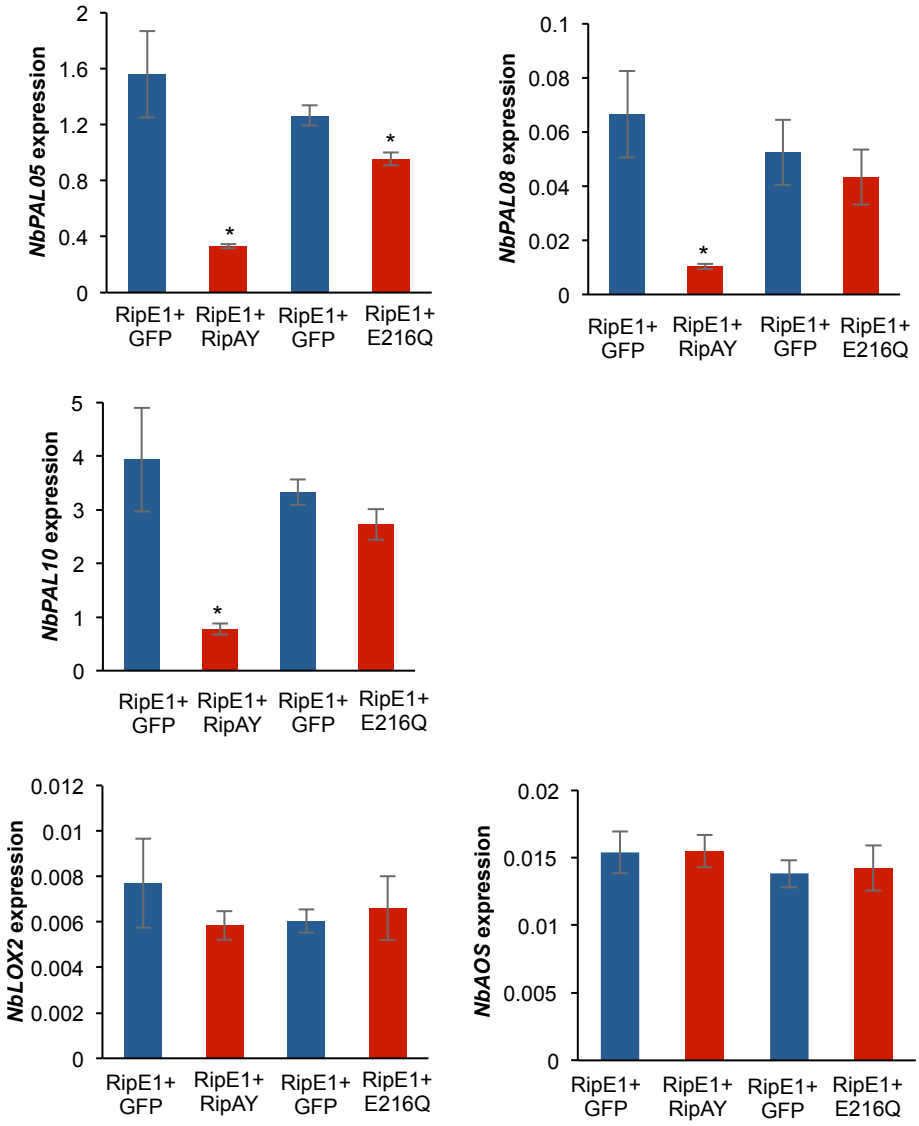

Figure S9. RipAY suppresses the overexpression of SA-related genes triggered by RipE1.
Quantitative RT-PCR to determine the expression of *NbPAL05, NbPAL08, NbPAL10, NbLOX2,* and *NbAOS* in *N. benthamiana* tissues 48 hours after Agrobacterium infiltration. Expression values are relative to the expression of the housekeeping gene *NbEF1a*. Values indicate mean ± SE (n=3 biological replicates). Asterisks indicate significant differences compared to the mock control according to a Student's t test (* $p < 0.05$).