Supplementary Material

The C-terminal half of *Phytophthora infestans* RXLR effector AVR3a is sufficient to trigger R3a-mediated hypersensitivity and suppress INF1-induced cell death in *Nicotiana benthamiana*

Jorunn I. B. Bos[1], Thirumala-Devi Kanneganti[1], Carolyn Young[1], Cahid Cakir[1], Edgar Huitema[1], Joe Win[1], Miles R. Armstrong[2], Paul R. J. Birch[2], and Sophien Kamoun[1]

[1]Department of Plant Pathology, The Ohio State University, Ohio Agricultural Research and Development Center, Wooster, Ohio 44691, USA

[2]Plant Pathology Department, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK

Figure S1. R3a and AVR3a agroinfiltration controls. Symptoms of *Nicotiana benthamiana* agroinfiltration sites expressing the R3a and AVR3a combinations. Symptoms induced by the R3a and AVR3a^{KI} combination (a, b) and R3a and AVR3a^{EM} combination (e, f), as well as R3a (I, j), AVR3a^{KI} (c, d), and AVR3a^{EM} (g, h) alone. Panels b, d, f, h, and j were examined under ultraviolet (UV) light (480/40 nm excitation filter; 510 barrier nm) to visualize the accumulation of autofluorescent compounds. Photographs were taken 6 days post infiltration (dpi).

Figure S2. Virus induced gene silencing (VIGS) Controls. (a) RT-PCR analysis of SGT1 expression in leaf tissue of control- and SGT1-silenced plants using gene specific primers. Amplification of the constitutively expressed tubulin gene was used to ensure equal amounts of RNA template were used. (b) Accumulation of HSP90 protein was determined by Western blot analysis of leaf extracts from control- and HSP90-silenced plants using polyclonal HSP90 antibodies. Bottom panel shows a Coomassie stained gel as a control for equal loading. (c) Phenotypes of control-, SGT1- and HSP90-silenced *Nicotiana benthamiana* plants 3 weeks after inoculation with the *Tobacco rattle virus* constructs. Note the stunted phenotype of the SGT1- and HSP90- silenced plants.

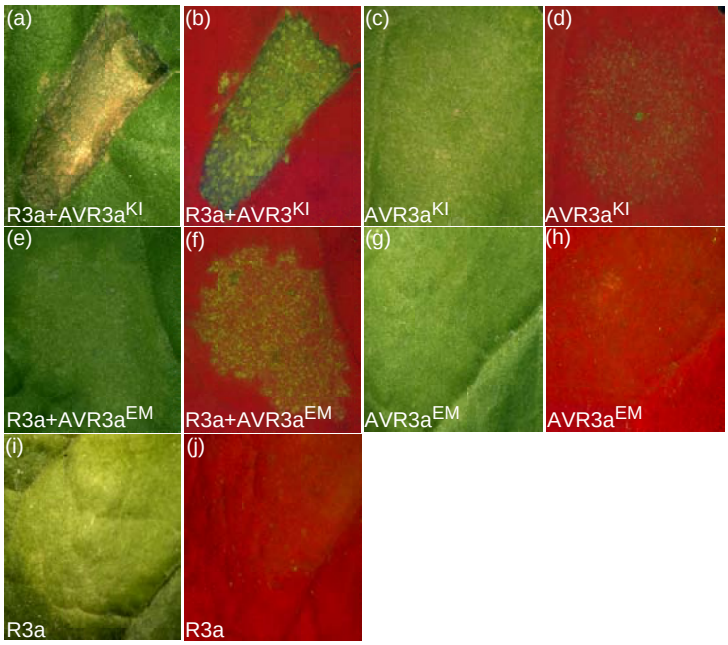
Figure S3. Suppression of INF1-induced cell death in *Nicotiana benthamiana* plants systemically infected with recombinant *Potato virus X* (PVX) expressing AVR3a. (a) Expression of recombinant FLAG-AVR3a^{EM} and FLAG-AVR3a^{KI} protein in *N. benthamiana* plants systemically infected with recombinant PVX. The proteins were

extracted three weeks after agroinfection with *Agrobacterium tumefaciens* strains carrying pGR106-FLAG-AVR3a^{EM} or pGR106-FLAG-AVR3a^{KI}. (b) Symptoms induced by INF1 on *N. benthamiana* leaves systemically infected with PVX constructs _GFP (control), AVR3a^{EM} or AVR3a^{KI}. The INF1 treatments consisted of agroinfiltration with *A. tumefaciens* strains carrying p35S-INF1, or recombinant wild-type INF1 protein or the inactive mutant INF1S1, produced from *E. coli* carrying plasmids pFB53-INF1 and pFB52-INF1S1, respectively. (c) Percentages of infiltration sites showing INF1 cell death based on three independent experiments similar to the one depicted in panel (b). Symptoms were scored 5 and 7 days after infiltration. Error bars indicate the standard errors.

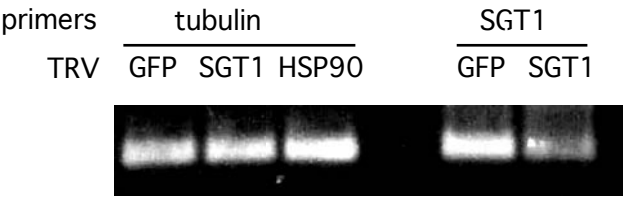
Table S1. Primers used for cloning.

Table S2. Bacterial strains and plasmids used in this study.

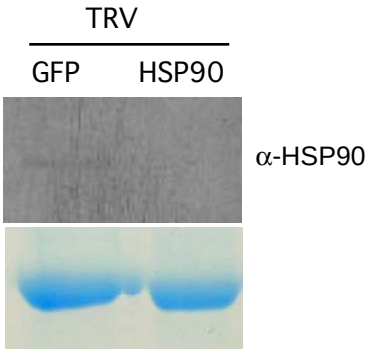
Ratcliff, F., Montserrat Martin-Hernandez, A. and Baulcombe, D.C. (2001) Tobacco rattle virus as a vector for analysis of gene func27 tion by silencing. *Plant J.* **25**, 237–245. (cited in Table S2)


(a)
R3a+AVR3a^{KI}
(b)
R3a+AVR3^{KI}
(c)
AVR3a^{KI}
(d)
AVR3a^{KI}
(e)
R3a+AVR3a^{EM}
(f)
R3a+AVR3a^{EM}
(g)
AVR3a^{EM}
(h)
AVR3a^{EM}
(i)
R3a
(j)
R3a

(a)

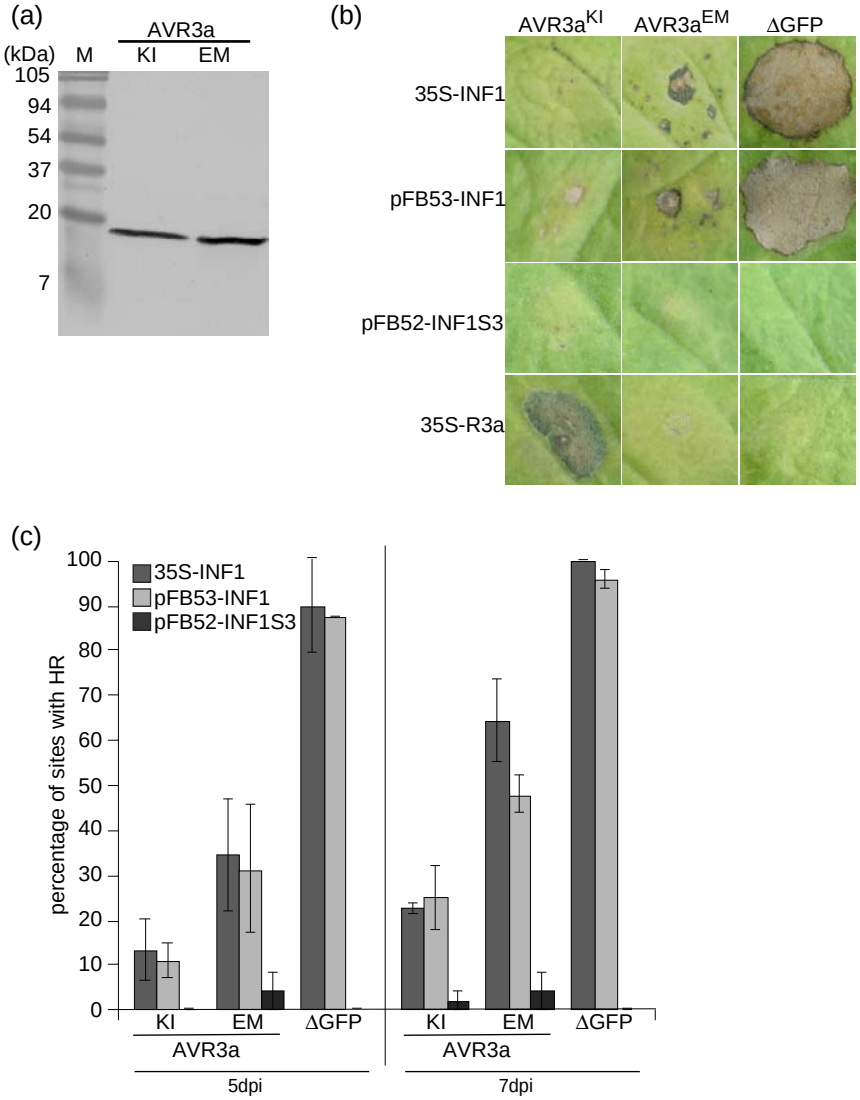

primers
tubulin
SGT1
TRV
GFP
SGT1
HSP90
GFP
SGT1

(b)


TRV
GFP
HSP90
α-HSP90

(c)


TRV-GFP
TRV-SGT1
TRV-HSP90


(a)
AVR3a
(kDa)
M
KI
EM
105
94
54
37
20
7
(b)
AVR3a^KI
AVR3a^EM
ΔGFP
35S-INF1
pFB53-INF1
pFB52-INF1S3
35S-R3a
(c)
35S-INF1
pFB53-INF1
pFB52-INF1S3
percentage of sites with HR
100
90
80
70
60
50
40
30
20
10
0
KI
EM
ΔGFP
KI
EM
ΔGFP
AVR3a
AVR3a
5dpi
7dpi

Supplemental Table 1. Primers used for cloning and their corresponding plasmids.

Name	*Sequence (5'-3')*	*Plasmid*
AVR3a_Δ23-F	GAAAATCGATATGGACCAAACCAAGGTCTTGGTG	pGR106-AVR3a^{KI} Δ23-147
		pGR106-AVR3a^{EM}Δ23-147
		pGR106-AVR3a^{EI} Δ23-147
		pGR106-AVR3a^{KM}Δ23-147
AVR3a_Δ60-F	GCGATCGATGGCCCCAAATTTCAATTTGGCGAATCTA	pGR106-AVR3a^{KI} Δ60-147
AVR3a_Δ73-F	GCGATCGATGTTTAATGTGGCTGCGTTGACGAAGAGA	pGR106-AVR3a^{KI} Δ73-147
AVR3a_Δ87-F	GCGATCGATGCTAGCGAAACAGCTTATGGGTAATGAT	pGR106-AVR3a^{KI} Δ87-147
AVR3a_147-R	GCGGCGGCCGCCTAATATCCAGTGAGCCCCAGGTGCATCAGGTA	pGR106-AVR3a^{KI} Δ23-147
		pGR106-AVR3a^{EM}Δ23-147
		pGR106-AVR3a^{KI}Δ60-147
		pGR106-AVR3a^{KI}Δ73-147
		pGR106-AVR3a^{KI}Δ87-147
		pGR106-AVR3a^{EI} Δ23-147
		pGR106-AVR3a^{KM}Δ23-147
		pGR106-SP-AVR3a^{KI}
		pGR106- SP-AVR3a^{KI} AxAA
		pGR106-FLAG-AVR3a^{KI}
		pGR106-FLAG-AVR3a^{EM}
AVR3a_Δ131-R	GCGGCGGCCGCCTAGTACTTTGCGCCTTGCGTCTTGCGG	pGR106-AVR3a^{KI}Δ60-131
		pGR106-AVR3a^{KI}Δ73-131
AVR3a_EI-R	AGCTTTTTGGCATCTGCTCTCTCCGTCAACGCAGC	pGR106-AVR3a^{EI} Δ23-147
AVR3a_KM-R	AGCTTTTTGGCATCTGCTCTCTTCGTCAACGCAGC	pGR106-AVR3a^{KM}Δ23-147
AVR3a-C-F1	AGAGAGCAGATGCCAAAAAGCTAGCGAAACAGCTT	pGR106-AVR3a^{EI} Δ23-147
		pGR106-AVR3a^{KM}Δ23-147
AVR3a_FLAG-F	GCGGAATTCCATCGACCAAACCAAGGTCCTGG	FLAG-ATS-AVR3a^{KI}
		FLAG-ATS-AVR3a^{EM}
AVR3a_FLAG-R	GCGGGTACCCTAATATCCAGTGAGCCCCAGGT	FLAG-ATS-AVR3a^{KI}
		FLAG-ATS-AVR3a^{EM}

AVR3a+SP-F	GCGATCGATGCGTCTGGCAATTATGCTGTCGC	pGR106-SP-AVR3a^{KI} pGR106-SP-AVR3a^{KI}AxAA
AVR3a-N-R	TTCTCTTCGTTCGCAGCAAGTGCTCTGCCGGCTGAATCGTGTAT	pGR106-SP-AVR3a^{KI} AxAA
AVR3a-C-F2	GAGCACTTGCTGCGAACGAAGAGAATGAAGAAACGTCT	pGR106-SP-AVR3a^{KI} AxAA
PVX-FLAG-F	GGAATCGATGGACTACAAGGACGACGATGACAAAG	pGR106-FLAG-AVR3a^{KI} pGR106-FLAG-AVR3a^{EM}
GFP-F	GGAGCGGCCGCTCTTCTTCAAGGACGACGGG	pGR106-ΔGFP
GFP-R	GGAATCGATGAAAGGGCAGATTGTGTGGAC	pGR106-ΔGFP
TRV_ GFP-F	GCGCCCGGGTTCTTCAAGGACGACGGGAA	PTV-GFP
TRV_GFP-R	GCGGGATCCGAAAGGGCAGATTGTGTGGAC	PTV-GFP

Table 2. Bacterial strains and plasmids used in this study.

Strain	*Plasmid*	*Description*	*Reference*
AGL0	pBINplus-I2GA-SH23-1 (R3-1)	pBINplus vector with R3a paralog insert	Huang *et al.*, 2005
AGL0	pBINplus-I2GA-SH23-2 (R3a)	pBINplus vector with R3a insert	Huang *et al.*, 2005
AGL0	pBINplus-I2GA-SH23-3 (R3-3)	pBINplus vector with R3a paralog insert	Huang *et al.*, 2005
AGL0	pBINplus-I2GA-SH194-2 (R3-4)	pBINplus vector with R3a paralog insert	Huang *et al.*, 2005
GV3101	PTV00	TRV RNA 2 vector	Ratcliff *et al.*, 2001
GV3101	PTV-GFP	TRV RNA 2 vector PTV00 with GFP insert	This work
GV3101	PTV-SGT1	TRV RNA 2 vector PTV00 with NbSGT1 insert	Peart *et al.*, 2002
GV3101	PTV-HSP90	TRV RNA 2 vector PTV00 with LeHSP90 insert	Kanneganti *et al.*, 2006
GV3101	pBINTRA6	pBINTRA6, binary TRV RNA 1 construct	Ratcliff *et al.*, 2001
GV3101	p35S-INF1	pAvr9 with INF1 insert	Kamoun *et al.*, 2003
GV3101	p35S-CRN2	pCB302-3 with CRN2 insert	E. Huitema and S. Kamour unpublished
GV3101	p35S-PiNPP1.1	pCB302-3 with PiNPP1.1 insert	Kanneganti *et al.*, 2006
GV3101	pGR106-ΔGFP	pGR106 with mutated and reversed GFP fragment	This work
LBA4404	pGR106-AVR3a^{KI} Δ23-147	pGR106 with AVR3a^{KI} insert minus signal peptide sequence.	Armstrong *et al.*, 2005
LBA4404	pGR106-AVR3a^{EM}Δ23-147	pGR106 with AVR3a^{EM} insert minus signal peptide sequence.	Armstrong *et al.*, 2005
GV3101	pGR106-AVR3a^{KI} Δ23-147	pGR106 with AVR3a^{KI} insert minus signal peptide sequence.	This work
GV3101	pGR106-AVR3a^{EM}Δ23-147	pGR106 with AVR3a^{EM} insert minus signal peptide sequence.	This work
GV3101	pGR106-SP-AVR3a^{KI}	pGR106 with full length AVR3a^{KI} insert	This work
GV3101	pGR106-SP-AVR3a^{KI}AxAA	pGR106 with full length AVR3a^{KI} insert with	This work

		a mutated RxLR motif sequence	
GV3101	pGR106-AVR3a^{KI}Δ60-147	pGR106 with AVR3a^{KI} insert lacking signal peptide and RxLR motif sequence	This work
GV3101	pGR106-AVR3a^{KI}Δ73-147	pGR106 with AVR3a^{KI} insert encoding amino acid 73 to 147	This work
GV3101	pGR106-AVR3a^{KI}Δ87-147	pGR106 with AVR3a^{KI} insert encoding amino acid 87 to 147	This work
GV3101	pGR106-AVR3a^{KI}Δ60-131	pGR106 with AVR3a^{KI} insert encoding amino acid 60 to 131	This work
GV3101	pGR106-AVR3a^{KI}Δ73-131	pGR106 with AVR3a^{KI} insert encoding amino acid 73 to 131	This work
GV3101	pGR106-AVR3a^{KM}Δ23-147	pGR106 with the AVR3a^{KM} insert minus signal peptide sequence	This work
GV3101	pGR106-AVR3a^{EI} Δ23-147	pGR106 with the AVR3a^{EI} insert minus signal peptide sequence	This work
DH5α	pFLAG-ATS	pFLAG-ATS vector	Sigma (St. Louis, MO)
DH5α	pFLAG-ATS-AVR3a^{KI}	pFLAG-ATS vector with the AVR3a^{KI} insert minus the signal peptide sequence	This work
DH5α	pFLAG-ATS-AVR3a^{EM}	pFLAG-ATS vector with the AVR3a^{EM} insert minus the signal peptide sequence	This work
GV3101	pGR106-FLAG-AVR3a^{KI}	pGR106 with AVR3a^{KI} insert containing an N-terminal FLAG-tag	This work
GV3101	pGR106-FLAG-AVR3a^{EM}	pGR106 with AVR3a^{EM} insert containing an N-terminal FLAG-tag	This work
GV3101	pJL3-p19	pJL3 with p19 ORF	J. Lindbo, unpublished