

Supplementary Material

Differential suppression of *Nicotiana benthamiana* innate immune responses by transiently expressed *Pseudomonas syringae* type III effectors

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		Section 1				
	(1)	10	20	30	46	
HopO1-1 <i>Pta</i> 11528	(1)	MGNICGTSGSNHVYSPFI	SPQHASGSSTFVPSASGTMLSLSHQIL			
HopO1-1 <i>Pto</i> DC3000	(1)	MGNICGTSGSNHVYSPFI	SPQHASGSSTFVPSASGTMLSLSHQIL			
<i>Consensus</i>	(1)	MGNICGTSGSNHVYSPFI	SPQHASGSSTFVPSASGTMLSLSHQIL			
		Section 2				
	(47)	60	70	80	92	
HopO1-1 <i>Pta</i> 11528	(47)	SQNYASNIKGYRTNPRKGFSPRLSDTLMKQALSSVITQEKRLKS				
HopO1-1 <i>Pto</i> DC3000	(47)	SQNYASNIKGYRTNPRKGFSPRLSDTLMKQALSSVITQEKRLKS				
<i>Consensus</i>	(47)	SQNYASNIKGYRTNPRKGFSPRLSDTLMKQALSSVITQEKRLKS				
		Section 3				
	(93)	100	110	120	138	
HopO1-1 <i>Pta</i> 11528	(93)	QPKSIAQDIQFPNSMIKNALDEKDSHPPGDCFSDDFLAIHLYTSC				
HopO1-1 <i>Pto</i> DC3000	(93)	QPKSIAQDIQFPNSMIKNALDEKDSHPPGDCFSDDFLAIHLYTSC				
<i>Consensus</i>	(93)	QPKSIAQDIQFPNSMIKNALDEKDSHPPGDCFSDDFLAIHLYTSC				
		Section 4				
	(139)	150	160	170	184	
HopO1-1 <i>Pta</i> 11528	(139)	LYRPINHHRLRYAPKNDVAPVVEAMNSGLAKLAQYVDYQVSGQLHRG				
HopO1-1 <i>Pto</i> DC3000	(139)	LYRPINHHRLRYAPKNDVAPVVEAMNSGLAKLAQYVDYQVSGQLHRG				
<i>Consensus</i>	(139)	LYRPINHHRLRYAPKNDVAPVVEAMNSGLAKLAQYVDYQVSGQLHRG				
		Section 5				
	(185)	190	200	210	220	230
HopO1-1 <i>Pta</i> 11528	(185)	IKQKMDGDEVMSRFPKPGNTYRDDAFMSTSTRMDVTEFTSDVTLHL				
HopO1-1 <i>Pto</i> DC3000	(185)	IKQKMDGDEVMSRFPKPGNTYRDDAFMSTSTRMDVTEFTSDVTLHL				
<i>Consensus</i>	(185)	IKQKMDGDEVMSRFPKPGNTYRDDAFMSTSTRMDVTEFTSDVTLHL				
		Section 6				
	(231)	240	250	260	276	
HopO1-1 <i>Pta</i> 11528	(231)	QSSSAVNIGPFSKPNFYEDALIPPLTPFKVTSLHKQDDRWVHLNE				
HopO1-1 <i>Pto</i> DC3000	(231)	QSSSAVNIGPFSKPNFYEDALIPPLTPFKVTSLHKQDDRWVHLNE				
<i>Consensus</i>	(231)	QSSSAVNIGPFSKPNFYEDALIPPLTPFKVTSLHKQDDRWVHLNE				
		Section 7				
	(277)	284				
HopO1-1 <i>Pta</i> 11528	(277)	IAESSDE-				
HopO1-1 <i>Pto</i> DC3000	(277)	IAESSDE-				
<i>Consensus</i>	(277)	IAESSDE				

Figure S1. Sequence alignment of HopO1-1_{*Pta*11528}.

Amino acid alignment of HopO1-1_{*Pta*11528} and HopO1-1_{*Pto*DC3000}. Identical residues are labelled in yellow.

		Section 1			
		(1) 1	10	20	30
HopT1-1 Pta 11528	(1)	MRTVSNHSIPSTNLVVDACA	ETL	AQKSPQV	SEIQRNSKIEKAVIE
HopT1-1 Pto DC3000	(1)	MRTVSNHSIPSTNLVVDAGT	ETS	AQKSPQVC	SEIQRNSKIEKAVIE
Consensus	(1)	MRTVSNHSIPSTNLVVDAG	ET	AQKSPQV	SEIQRNSKIEKAVIE
		Section 2			
		(47) 47	60	70	80
HopT1-1 Pta 11528	(47)	HIADHPAAKMTISALVD	TLTDV	FVRAHG	EVKGWAEIVQAVSRPHDS
HopT1-1 Pto DC3000	(47)	HIADHPAAKMTISALVD	TLTDV	FVRAHG	EVKGWAEIVQAVSRPHDS
Consensus	(47)	HIADHPAAKMTISALVD	TLTDV	FVRAHG	EVKGWAEIVQAVSRPHDS
		Section 3			
		(93) 93	100	110	120
HopT1-1 Pta 11528	(93)	NRHGS	CVLSP	PRFDV	MGSVGVWNAAAI
HopT1-1 Pto DC3000	(93)	NRHGS	CVLSP	PRFDV	MGSVGVWNAAAI
Consensus	(93)	NRHGS	CVLSP	PRFDV	MGSVGVWNAAAI
		Section 4			
		(139) 139	150	160	170
HopT1-1 Pta 11528	(139)	SNNFRHL	LKR	VVSD	PALQ
HopT1-1 Pto DC3000	(139)	SNNFRHL	LKR	VVND	PALQ
Consensus	(139)	SNNFRHL	LKR	VVD	PALQ
		Section 5			
		(185) 185	190	200	210
HopT1-1 Pta 11528	(185)	ASERREQ	IGKARYET	ASNLS	QTLISARE
HopT1-1 Pto DC3000	(185)	ASERREQ	IGKARYET	ASNLS	QTLISARE
Consensus	(185)	ASERREQ	IGKARYET	ASNLS	QTLISARE
		Section 6			
		(231) 231	240	250	260
HopT1-1 Pta 11528	(231)	CFDKCLPEE	SDLQVLR	CHGCS	SVWSVK
HopT1-1 Pto DC3000	(231)	CFDKCLPEE	SDLQVLR	CHGCS	SVWSVK
Consensus	(231)	CFDKCLPEE	SDLQVLR	CHGCS	SVWSVK
		Section 7			
		(277) 277	290	300	310
HopT1-1 Pta 11528	(277)	SGTAS	RMVAVAR	FLAPACLK	SLGIES
HopT1-1 Pto DC3000	(277)	SGTAS	RMVAVAR	FLAPACLK	SLGIES
Consensus	(277)	SGTAS	RMVAVAR	FLAPACLK	SLGIES
		Section 8			
		(323) 323	330	340	350
HopT1-1 Pta 11528	(323)	HHSML	EVNL	GVASHG	MPEQWDD
HopT1-1 Pto DC3000	(323)	HHSML	EVNL	GVASHG	MPEQWDD
Consensus	(323)	HHSML	EVNL	GVASHG	MPEQWDD
		Section 9			
		(369) 369	379		
HopT1-1 Pta 11528	(369)	QVVRQAA	QKS	-	
HopT1-1 Pto DC3000	(369)	QVVRQAA	QKS	-	
Consensus	(369)	QVVRQAA	QKS	-	

Figure S2. Sequence alignment of HopT1-1_{Pta11528}.

Amino acid alignment of HopT1-1_{Pta11528} and HopT1-1_{PtoDC3000}. Identical residues are labelled in yellow and conservative substitutions in green.

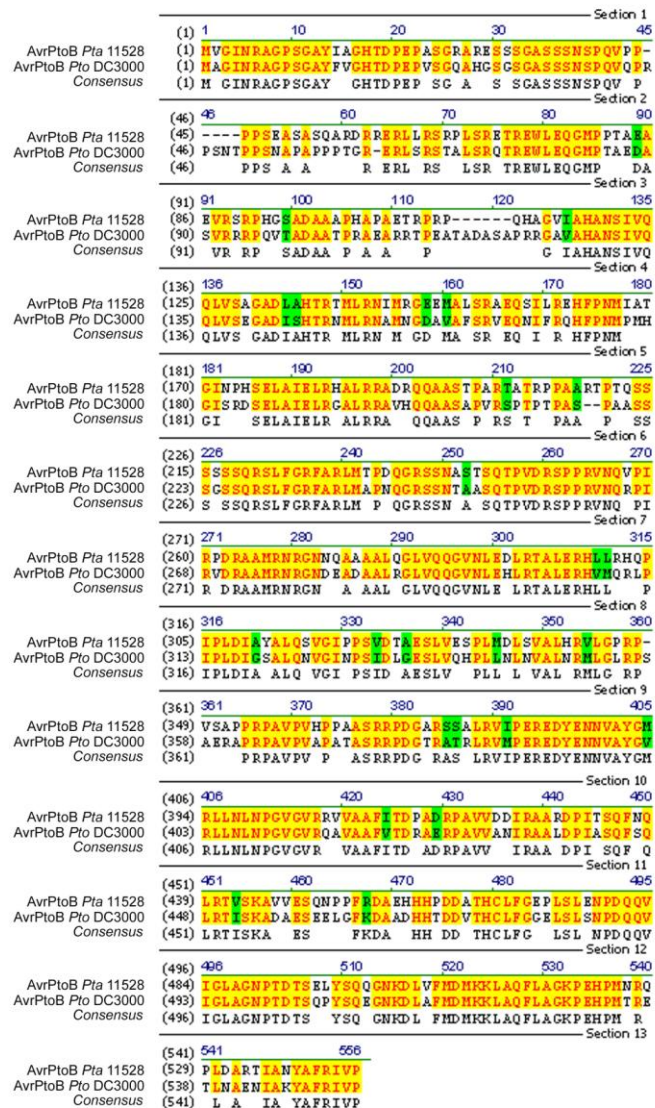


Figure S3. Sequence alignment of AvrPtoB_{Pta11528}.

Amino acid alignment of AvrPtoB_{Pta11528} and AvrPtoB_{PtoDC3000}. Identical residues are labelled in yellow and conservative substitutions in green.

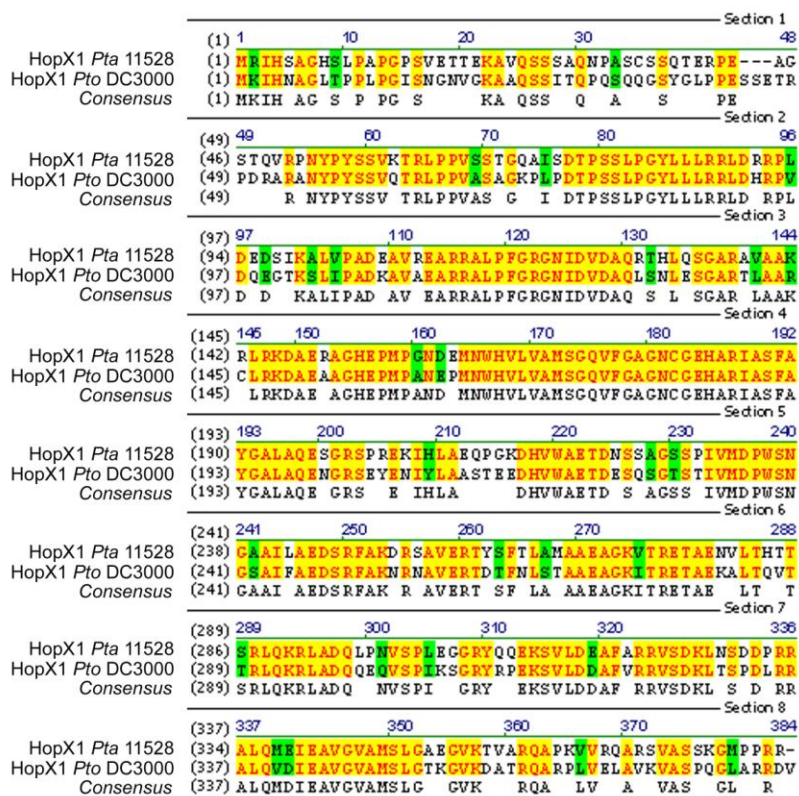


Figure S4. Sequence alignment of HopX1_{*Pta*11528}.

Amino acid alignment of HopX1_{*Pta*11528} and HopX1_{*Pto*DC3000}. Identical residues are labelled in yellow and conservative substitutions in green.

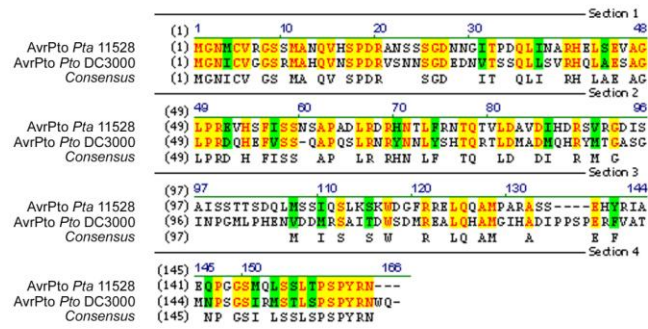


Figure S5. Sequence alignment of AvrPto_{Pta11528}.

Amino acid alignment of AvrPto_{Pta11528} and AvrPto_{PtoDC3000}. Identical residues are labelled in yellow and conservative substitutions in green.

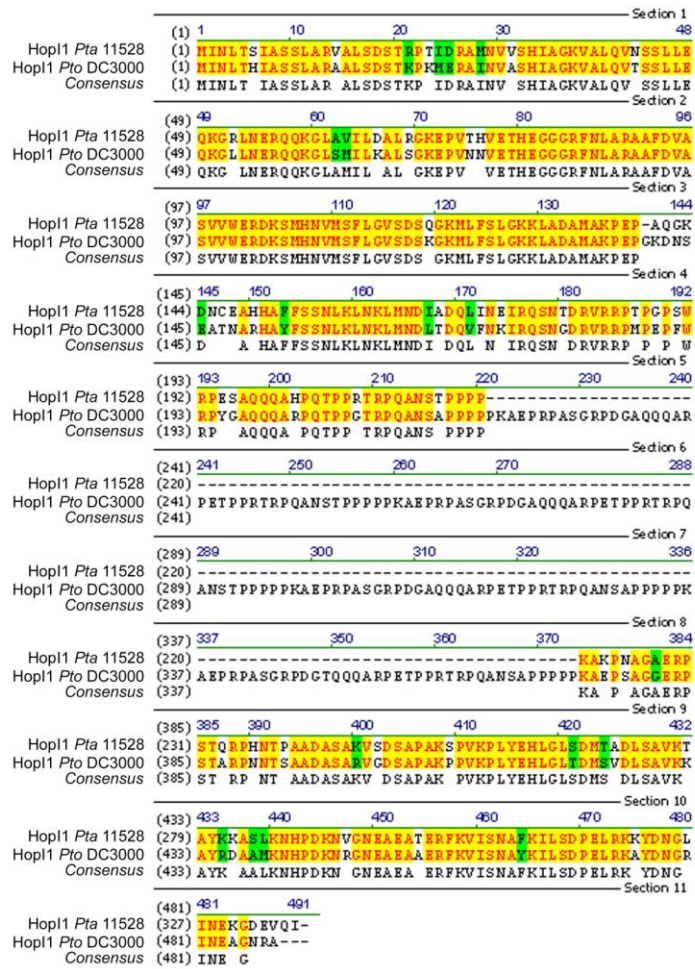


Figure S6. Sequence alignment of HopI1_{Pta11528}.

Amino acid alignment of HopI1_{Pta11528} and HopI1_{PtoDC3000}. Identical residues are labelled in yellow and conservative substitutions in green.

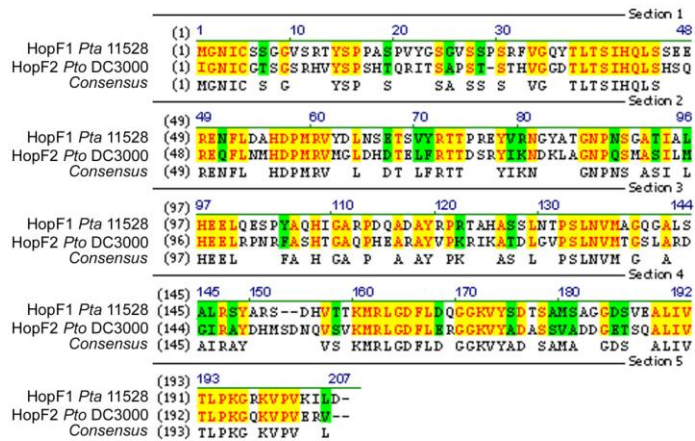


Figure S7. Sequence alignment of HopF1_{*Pta*11528}.

Amino acid alignment of HopF1_{*Pta*11528} and HopF2_{*Pto*DC3000}. Identical residues are labelled in yellow and conservative substitutions in green.

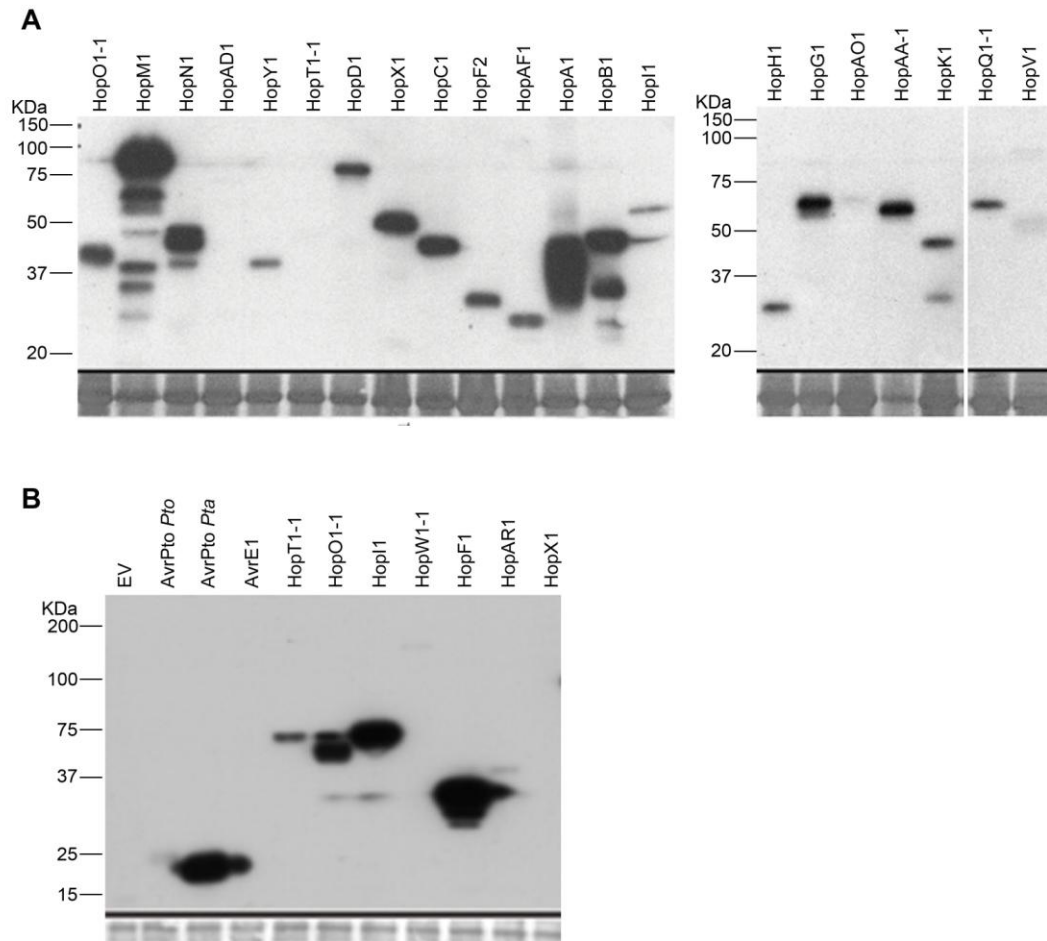


Figure S8. Accumulation of *Pto* DC3000 and *Pta* 11528 effector proteins in *N. benthamiana*.

(A) Western blot showing accumulation of *Pto* DC3000 effector proteins in leaf tissue after *A. tumefaciens* mediated transient expression in *N. benthamiana* for two days. A Coomassie brilliant blue loading control is shown in the lower panel.

(B) Western blot showing accumulation of all *Pta* 11528 effector proteins in leaf tissue after *A. tumefaciens* mediated transient expression in *N. benthamiana* for two days. A Coomassie brilliant blue loading control is shown in the lower panel.

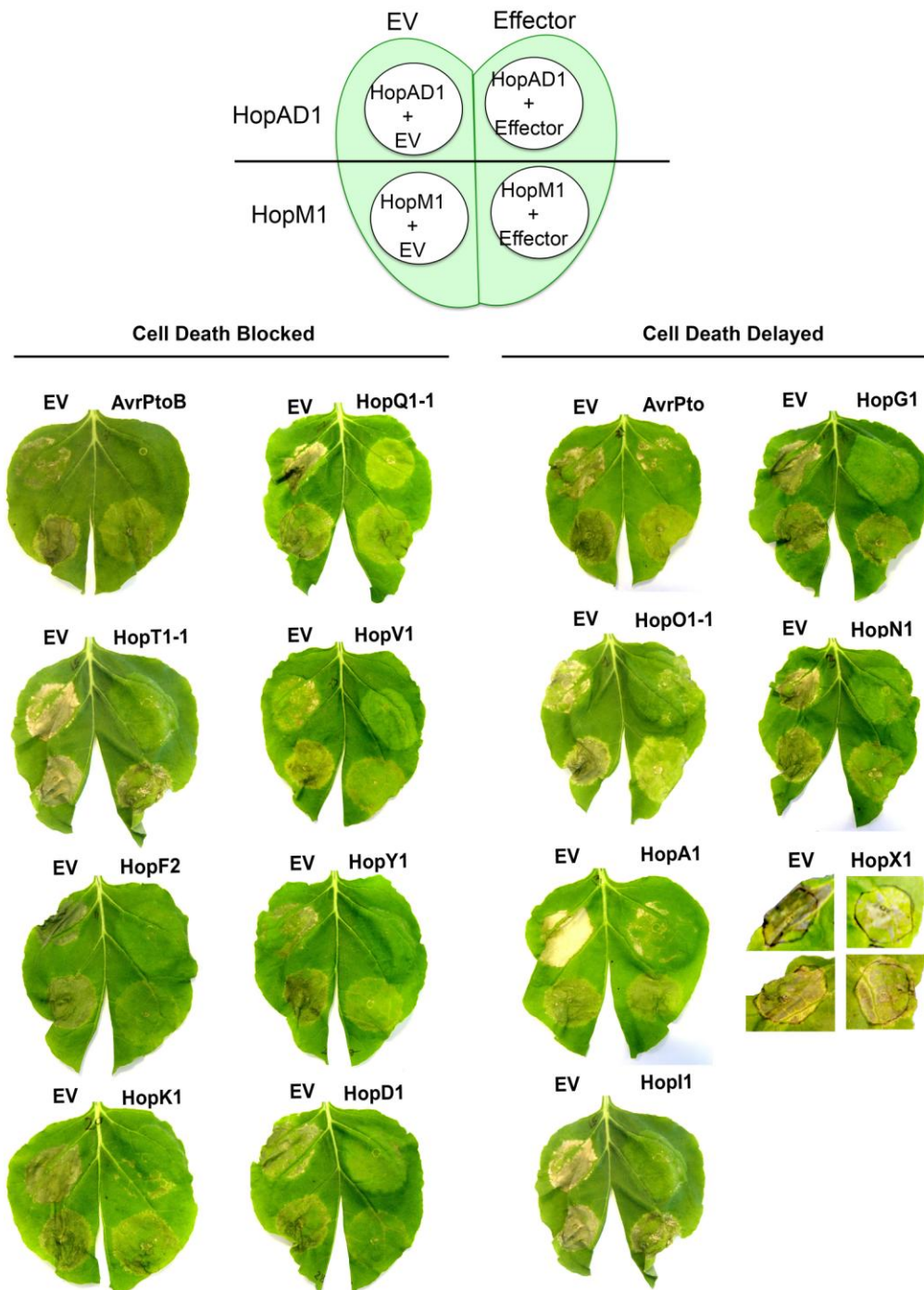


Figure S9. Most *Pto* DC3000 effectors can interfere with *hopMI*_{*Pto*DC3000}-induced cell death.

Suppression of *hopMI*_{*Pto*DC3000} and *hopAD1*_{*Pto*DC3000} induced cell death by *Pto* DC3000 effectors. The effector *hopMI*_{*Pto*DC3000} or *hopAD1*_{*Pto*DC3000} was co-expressed in *N. benthamiana* leaves with each effector in the *Pto* DC3000 repertoire, or an EV control, as depicted in the upper schematic diagram. Each of the two *A. tumefaciens* strains containing the respective effector gene or an EV control were prepared at OD₆₀₀=0.5 then mixed in equal volumes. Cell death induced by the effectors was scored 6 days after infiltration. Similar results were obtained in two independent experiments.

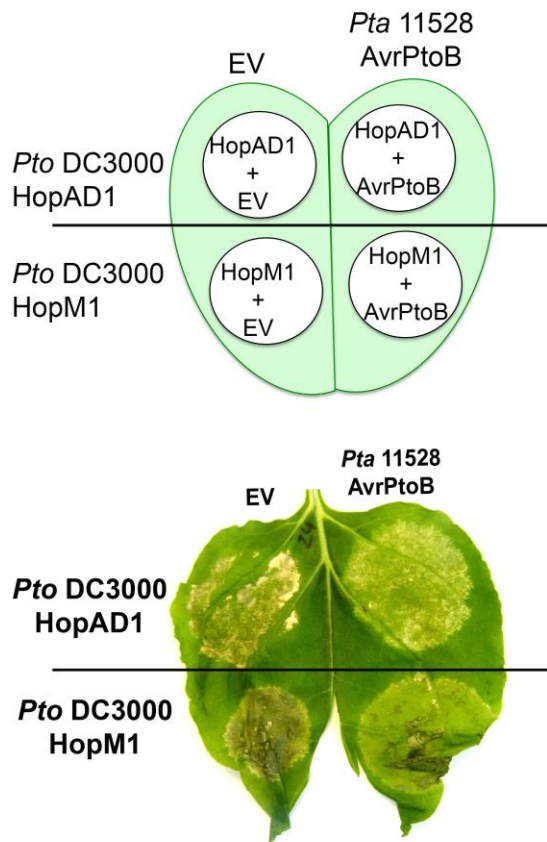


Figure S10. *Pta 11528 AvrPtoB* can interfere with *hopAD1*_{*PtoDC3000*} or *hopMI*_{*PtoDC3000*}-induced cell death.

Suppression of *hopMI*_{*PtoDC3000*} and *hopAD1*_{*PtoDC3000*} induced cell death by the *Pta 11528* effector *AvrPtoB*. The effector *hopMI*_{*PtoDC3000*} or *hopAD1*_{*PtoDC3000*} was co-expressed in *N. benthamiana* leaves with *avrPtoB*_{*Pta11528*} or an *EV* control, as depicted in the upper schematic diagram. Each of the two *A. tumefaciens* strains containing the respective effector gene or an *EV* control were prepared at $OD_{600}=0.5$ then mixed in equal volumes. Cell death induced by the effectors was scored 6 days after infiltration. Similar results were obtained in two independent experiments.

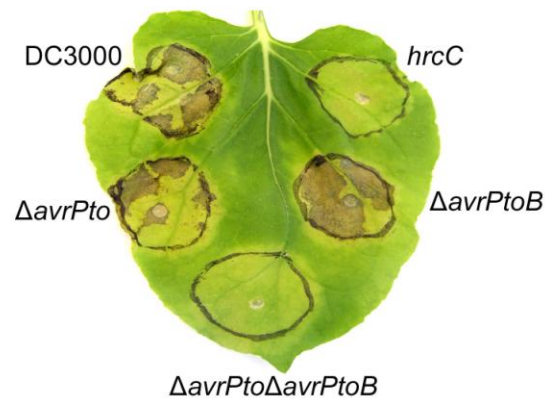


Figure S11. *Pto* DC3000 effectors cannot collectively suppress the induction of the hypersensitive response (HR) triggered upon recognition of *AvrPto*_{*Pto*DC3000} and/or *AvrPtoB*_{*Pto*DC3000} in transgenic *N. benthamiana* plants expressing the tomato *Pto* and *Prf* genes (*N. benthamiana* R411A).

Transgenic *N. benthamiana* plants were infiltrated with a high dose (5×10^7 cfu/ml) of *Pto* DC3000 (DC3000), *Pto* DC3000 *hrcC* (*hrcC*), *Pto* DC3000 Δ *avrPto* (Δ *avrPto*), *Pto* DC3000 Δ *avrPtoB* (Δ *avrPtoB*) and *Pto* DC3000 Δ *avrPto* Δ *avrPtoB* (Δ *avrPto* Δ *avrPtoB*). The HR was visible 24-48 h after infiltration, and photographed at 3 days post infiltration. Similar results were obtained in two independent experiments.