

**Table S1.** *Verticillium dahliae* strains used in this study

strain <sup>1</sup>	background (VCG)	genotype <sup>6</sup>	constructed using plasmid(s)
<b>Analyses of CAT-mediated fusion (Fig. 1) and post-fusion cellular behavior (Fig. 2)</b>			
PH H1-mCherry (a)	PH (2A)	<i>nitM</i> , <i>VdH1-mCherry</i> , <i>neo</i> <sup>R</sup>	pVV19
PH H1-sGFP (b)	PH (2A)	<i>nit1</i> , <i>NcH1-sgfp</i> , <i>hph</i>	pMF357
Ls.17 H1-mCherry <sup>2</sup> (c)	Ls.17 (2B)	<i>nit1</i> , <i>VdH1-mCherry-ble</i> <sup>R</sup>	fusion PCR
Ls.17 H1-mCherry neo	Ls.17 (2B)	<i>nit1</i> , <i>VdH1-mCherry-ble</i> <sup>R</sup> , <i>neo</i> <sup>R</sup>	fusion PCR; pBS-gen
Ls.17 H1-sGFP (d)	Ls.17 (2B)	<i>nitM</i> , <i>NcH1-sgfp</i> , <i>hph</i>	pMF357
Cf.38 H1-sGFP (e)	Cf.38 (2B <sup>3</sup> )	<i>nitM</i> , <i>NcH1-sgfp</i> , <i>hph</i>	pMF357
115 H1-sGFP (f)	115 (2B)	<i>nitM</i> , <i>NcH1-sgfp</i> , <i>hph</i>	pMF357
BB H1-sGFP (g)	BB (4A)	<i>nit1</i> , <i>NcH1-sgfp</i> , <i>hph</i>	pMF357
Ca.146 H1-sGFP (h)	Ca.146 (6)	<i>nitM</i> , <i>NcH1-sgfp</i> , <i>hph</i>	pMF357
Ca.148 H1-mCherry (i)	Ca.148 (6)	<i>nit1</i> , <i>VdH1-mCherry</i> , <i>neo</i> <sup>R</sup>	pVV19
Ls.17 sGFP <sup>2</sup>	Ls.17 (2B)	<i>sgfp</i> , <i>hph</i>	pIGPAPA
Cf.38 sGFP	Cf.38 (2B <sup>5</sup> )	<i>sgfp</i> , <i>hph</i>	pIGPAPA
PH sGFP	PH (2A)	<i>sgfp</i> , <i>hph</i>	pIGPAPA
<b>Complementation assays (Fig. 3)</b>			
T9.M <sup>3</sup> (A)	T9 (1A)	<i>nitM</i>	–
V607I.M <sup>3</sup> (B)	V607I (1B)	<i>nitM</i>	–
123V.M <sup>2</sup> (C)	123V (2A)	<i>nitM</i>	–
115.M <sup>3</sup> (D)	115 (2B)	<i>nitM</i>	–
Cf.38.M <sup>3</sup> (E)	Cf.38 (2B <sup>5</sup> )	<i>nitM</i>	–
PCW.M <sup>3</sup> (F)	PCW (3)	<i>nitM</i>	–
Dvd-E6.M <sup>3</sup> (G)	Dvd-E6 (4A)	<i>nitM</i>	–
S39.M <sup>3</sup> (H)	S39 (4B)	<i>nitM</i>	–
Ca.146.M <sup>3</sup> (I)	Ca.146 (6)	<i>nitM</i>	–
V13.M <sup>3</sup> (J)	V13 (HSI <sup>4</sup> )	<i>nitM</i>	–
Ms.102.M <sup>3</sup> (K)	<i>V. nonalfalae</i> Ms.102	<i>nitM</i>	–
V44.1 <sup>3</sup> (L)	V44 (1A)	<i>nit1</i>	–
V661I.1 <sup>3</sup> (M)	V661I (1B)	<i>nit1</i>	–
PH.1 <sup>3</sup> (N)	PH (2A)	<i>nit1</i>	–
Ls.17.1 <sup>3</sup> (O)	Ls.17 (2B)	<i>nit1</i>	–
70-21.1 <sup>3</sup> (P)	70-21 (3)	<i>nit1</i>	–

BB.1 <sup>3</sup> (Q)	BB (4A)	<i>nit1</i>	–
V684I.1 <sup>3</sup> (R)	V684I (4B)	<i>nit1</i>	–
Ca.148.1 <sup>3</sup> (S)	Ca.148 (6)	<i>nit1</i>	–
Cf.162.1 <sup>3</sup> (T)	Cf.162 (HSI <sup>4,5</sup> )	<i>nit1</i>	–
T2.1 <sup>3</sup> (U)	<i>V. nonalfalfae</i> T2	<i>nit1</i>	–

#### Mixed infection experiments

Ls.17 <i>neo</i> <sup>R</sup>	Ls.17 (2B)	<i>neo</i> <sup>R</sup>	pBS-genR
Ls.17 <i>hph</i>	Ls.17 (2B)	<i>hph</i>	pUCATPH
Cf.38 <i>hph</i>	Cf.38 (2B <sup>5</sup> )	<i>hph</i>	pUCATPH
PH <i>hph</i>	PH (2A)	<i>hph</i>	pUCATPH
BB <i>hph</i>	BB (4A)	<i>hph</i>	pUCATPH
Ca.146 <i>hph</i>	Ca.146 (6)	<i>hph</i>	pUCATPH

#### Analyses of autophagic genes

Ls.17 mCherry- <i>atg8</i>	Ls.17 (2B)	<i>mCherry-atg8</i>	pVV27; pVV25
Ls.17 H1-mCherry sGFP- <i>atg8</i>	Ls.17 H1-mCherry <i>neo</i>	<i>nit1</i> , <i>VdH1-mCherry-ble</i> <sup>R</sup> , <i>neo</i> <sup>R</sup> , <i>sGFP-atg8</i>	pVV27; pVV26
Ls.17- $\Delta$ <i>atg1</i>	Ls.17 H1-mCherry <i>neo</i>	<i>nit1</i> , <i>VdH1-mCherry-ble</i> <sup>R</sup> , <i>neo</i> <sup>R</sup> , $\Delta$ <i>atg1::hph</i>	pOSCAR- <i>atg1</i>
Ls.17- $\Delta$ <i>atg8</i>	Ls.17 H1-mCherry	<i>nit1</i> , <i>VdH1-mCherry-ble</i> <sup>R</sup> , <i>atg8::neo</i> <sup>R</sup>	pVV27; 5' <sup>H</sup> <sub><i>atg8</i></sub> - <i>neo</i> <sup>R</sup> -3' <sup>H</sup> <sub><i>atg8</i></sub> (H: 100 bp-long homology arms)
PH- $\Delta$ <i>atg1</i>	PH (2A)	$\Delta$ <i>atg1::hph</i>	pOSCAR- <i>atg1</i>
Ls.17- $\Delta$ <i>atg1-c</i>	Ls.17- $\Delta$ <i>atg1</i>	$\Delta$ <i>atg1::hph</i> , <i>neo</i> <sup>R</sup>	pBS-genR, wild-type <i>atg1</i>
Ls.17- $\Delta$ <i>atg8-c</i>	Ls.17- $\Delta$ <i>atg8</i>	<i>atg8::neo</i> <sup>R</sup> , <i>hph</i>	pUCATPH, wild-type <i>atg8</i>

<sup>1</sup> Single-letter codes (in brackets) correspond to the strains shown in Fig. 1 and 3.

<sup>2</sup> Reference: [37].

<sup>3</sup> Reference: [36].

<sup>4</sup> HSI: Heterokaryon Self-Incompatible.

<sup>5</sup> Isolates Cf.38 and Cf.162 were originally classified into VCG 6, but their classification was later re-assessed [38].

<sup>6</sup> *Vd*: *Verticillium dahliae*; *Nc*: *Neurospora crassa*.