## Supplementary Material Supplementary file 1

	1	10	20	30	40
KAH6840552.1	LNTLLTT	LGLAVPAVAQ	RDFCAAMSGE	PWAESATGFR	FQ
RYO54054.1	LNTLLLAT	LGLALPAVAQ	RDFCAAMSGE	PWAESA <b>TGF</b> R	FQ
RII22952.1	LNTLLLTT	LGLALPAVAQ	RDFCAAMSGE	PWAESATGFR	FQ
EFQ86255.1	LNILVLTA	LGLAVPAVAQ	TNFCTALSHT	PYATSATGFR	FS
KNG46207.1	TTLILTTA	LSLSLPAAAQ	VDFCAGIQGS	PWSESATGFR	FR
XP_014551076.1	FNTLVLAT	IGLALPAAAQ	KNFCASKDGT	PWARASTGFG	FK
KAH/000011.1		IGLAIPAVAQ	KNYCASKDGI	PWARSSIGFR	г і гт
XP_014062330.1	MKI GVI VP	IGLAIPAVAQ	ANNCNOAKUT	PTPRSTTCFN	도도
Vullae		TIMEVSSVS	ANNONVARVI		E I
		50	60	7 <u>0</u>	80
KAH6840552.1	STSAGTWN	WFSRDRGTDL	KIYQDCNVVQ	NNSQRALRTV	CI
RYO54054.1	STSAGTWN	WFSRDRGTDL	KIYQDCNVVQ	NNSQRALRTV	CI
RII22952.1	STSTSTWN	WFSRDRGTDL	KIYQDCNVVQ	NNSQRALRTV	CI
EFQ86255.1	DTGSGTWH	WWSRDKGTDL	KIYQDCKVVQ	NGSRRPLRTF	CL
KNG46207.1	STGSSTWN	WSSRDRGTDL	QIFQDCMVRQ	NNSQRALRQF	CI
XP_014551076.1	STSSSTWQ	WYSVDYNTDL	NIYODCNVVQ	RGSQRALRQF	CI
KAH/000011.1	STSSSTWQ	WSSVDINIDL	VVYQDCNVVQ	HGSQRALRQF	OF
VdR30	STOSSIWU	WRSPDRCSAV	TUNOKCELRO	CACKTTTATU	Qr
Vultoe		MISINDINGSAV		GAGRETTATV	CI
		90	100	110	
KAH6840552.1	ATSNNYRC	WEAPGQNDPN	GSCFIPDCSN	IRNVWGW	
RYO54054.1	ATSNNYRC	WEAPGQNDPS	GSCFIPDCSN	IRNVWGW	
RII22952.1	ATSNNYRC	WEAPGQNEPN	GSCFIPDCSN	IRNVWGW	
EFQ86255.1	DISDNYQC	WEAPGQNDPQ	GECTLLKCNQ	IVNAWGW	
KNG46207.1	ATSNDYNC	WEAPGQNEPS	GSCRLPDCSN	IANVWGW	
XP_014551076.1	ATSNNYQC	WEAPGLNDPT	GSCFLPDCSN	INNVWAW	
KAH/559611.1	AISNNYQC	WEAPGLNDPS	GSCYLPDCSN		
XP_014082330.1	DVCCOVVC	WIAPGLND WIAPGLND	F D G S C I L P D C	NMWCW	
vurtse	DIDGVIKC	WIAFAVNDRC	STEGIVCINAL	MINIM GW	

**Fig S1** Multiple sequence alignment of VdR3e and homologous proteins. Multiple sequence alignments of VdR3e and its homologous proteins on the same evolutionary clade (KAH6840552.1, RYO54054.1, RII22952.1, EFQ86255.1, KNG46207.1, XP\_014551076.1, KAH7559611.1 and XP\_014082330.1) were performed based on the online tool ESPript. Conserved residues were written in white in red box. Red characters represent similarity in a group. A blue frame indicates similarity across groups.



Fig S2 Analysis of the virulence function of VdR3e from Verticillium dahliae on N. benthamiana and cotton. (A) Phenotypes of the N. benthamiana plants inoculated with ΔVdR3e-HoMCLT, EC<sup>VdR3e</sup>-HoMCLT and OE<sup>VdR3e</sup>-HoMCLT strains. The 3-week-old N. benthamiana plants were inoculated by a root-dipping method with  $\Delta V dR3e$ -HoMCLT, EC<sup>VdR3e</sup>-HoMCLT and OE<sup>VdR3e</sup>-HoMCLT strains, the wild type (HoMCLT) and sterile water (Mock) as control. Individual transformants were characterized for each mutant. Symptoms were recorded 21 days after inoculation. (B) Detection of the fungal biomass of the  $\Delta VdR3e$ -HoMCLT, EC<sup>VdR3e</sup>-HoMCLT and OE<sup>VdR3e</sup>-HoMCLT strains following inoculations of N. benthamiana plants. Three weeks after inoculation, the roots and stems of the N. benthamiana were harvested. The genomic DNA of the harvested tissue was extracted and the fungal colonization of V. dahliae in the N. *benthamiana* roots were detected by quantitative PCR. Values represent the means  $\pm SE$ of three independent samples. Statistical significance was calculated by an unpaired Student's t tests. (C) Detection of pathogenicity of HoMCLT on cotton. Cultivar Junmian No. 1 was selected for testing in this experiment. Cotton was inoculated with HoMCLT by the root-dip inoculation just after the second true leaf appeared. The phenotypes were photographed 3 weeks later. (D) Phenotypes of the  $\Delta$ VdR3e-HoMCLT, EC<sup>VdR3e</sup>-HoMCLT and OE<sup>VdR3e</sup>-HoMCLT strains inoculated on cotton. After 3 weeks, cotton plants inoculated with the mutant strains were photographed.



Fig S3 The radial growth of the  $\Delta$ VdR3e-HoMCLT, EC<sup>VdR3e</sup>-HoMCLT and OE<sup>VdR3e</sup>-HoMCLT strains under different carbon sources and stress conditions. The growth diameter of the mutant strains on plate medium was measured. There are two separates independent transformants for each mutant. Values represent the means  $\pm SE$  of three independent samples. There was no significant difference between data at p > 0.05based on unpaired Student's *t* tests.