

FIG S1 Bioinformatics analysis of VdGAL4. (A) The signal peptide sites of VdGAL4 protein. (B)

Transmembrane domain analysis of VdGAL4. (C) The conserved domain of VdGAL4.



**FIG S2** Subcellular localizations of VdGAL4 in *N. benthamiana* on *A. tumefaciens*-mediated transient expression. Fluorescence was detected by confocal microscopy after 48 h of injection. Scale bar = 100 μm.



**FIG S3** Identification of deletion and complementary mutants. (A) The principle of obtaining the mutant of *VdGAL4* by homologous recombination method. (B) The deletion mutant strains of *VdGAL4* were determined by PCR. (C) The complementary mutant strains of *VdGAL4* were determined by PCR. (D) Southern blotting was used to identify the deletion mutant strains.



**FIG S4** (A) The conidia of WT,  $\Delta V dGAL4$  and  $C - \Delta V dGAL4$  strains germinated at 25°C. Bar = 200 µm. (B) Assays of the relative expression of conidia-related genes in all tested strains by RT-qPCR. The error bar represents standard error of means. \*p < 0.05, \*\*p < 0.01.

## Table S1:Primers used in this study.

Primer name	Sequence (5'-3')	Enzyme loci	Purpose
pGR107-VdGAL1-F	GGTCAGCACCAGCTAGC <u>ATCGAT</u> ATGGGTG TCATTCGCAAC	Cla I	
pGR107-VdGAL1-R	TCGCCCTTGCTCACCAT <u>CCCGGG</u> CTCGGTCT	Smal I	-
	CCTGATCAGCCAG		
pGR107-VdGAL2-F	GGTCAGCACCAGCTAGC <u>ATCGAT</u> ATGCCTTC	Cla I	-
	CAACACAGCG		
pGR107-VdGAL2-R	TCGCCCTTGCTCACCAT <u>CCCGGG</u> CGCATAAG	Smal I	-
	TCGCGAAGCCTAC		
pGR107-VdGAL3-F	GGTCAGCACCAGCTAGCATCGATATGGCAC	Cla I	-
	CACTTGCCACA		
pGR107-VdGAL3-R	TCGCCCTTGCTCACCAT <u>CCCGGG</u> CAGCTCCT	Smal I	- 
	CCTTGGTCATGGA		Transient gene
pGR107-VdGAL4-F	GGTCAGCACCAGCTAGCATCGATATGGGTTC	Cla I	expression
	AAAGTCCACT		_
pGR107-VdGAL4-R	TCGCCCTTGCTCACCAT <u>CCCGGG</u> GCAAGAA	Smal I	-
	AGCAGCAACACCTT		
pGR107-VdGAL5-F	GGTCAGCACCAGCTAGC <u>ATCGAT</u> ATGGCCC	Cla I	-
	GACACTCCACC		
pGR107-VdGAL5-R	TCGCCCTTGCTCACCAT <u>CCCGGG</u> CAGCTCAT	Smal I	
	CCTTTTCCTCGCG		
pGR107-VdGAL4(Δ SP)-F	GGTCAGCACCAGCTAGC <u>ATCGAT</u> ATGACTG	Cla I	
	ACCGGTCCTTTC		_
pGR107-VdGAL4(∆ SP)-R	TCGCCCTTGCTCACCAT <u>CCCGGG</u> GCAAGAA	Smal I	
	AGCAGCAACACCTT		
pSUC2-VdGAL4(SP)-F	CCG <u>GAATTC</u> ATGGGTTCAAAGTCC	Ecor I	Yeast signal
pSUC2-VdGAL4(SP)-R	CCG <u>CTCGAG</u> CCCGAAAACGGGAGC	Xho I	<ul> <li>sequence trap</li> <li>system</li> </ul>
PBI121(RFP)-VdGAL4-F	AGAACACGGGGGGAC <u>TCTAGA</u> ATGAAATTTA	Xba I	
	CCGGCCTTGCTTCG		Subcellular
PBI121(RFP)-VdGAL4-R	GTTTTGTTAATTAA <u>GGATCC</u> GAAGGTCAGC	BamH I	localization
	GTCTGGCC		
TRV-BAK1-F	CG <u>GGATCC</u> GTGAGGGTGGTGAGCGGGATA		
	AT		
TRV-BAK1-R	CG <u>GAATTC</u> GCTCATAACTGGGCAAAGGGCT		-
	т		VIGS in <i>N.</i>
TRV-SOBIR1-F	CG <u>GGATCC</u> AATCTTTATCCACCAGATCATGC		benthamiana
TRV-SOBIR1-R	CG <u>GAATTC</u> CAGAAAGTTTTCCAATGGCAG		_

qPCR- <i>NbActin</i> -F	TGGTCGTACCACCGGTATTGTGTT		
qPCR- <i>NbActin</i> -R	TCACTTGCCCATCAGGAAGCTCAT		
qPCR- <i>NbBAK1</i> -F	GAGGTGGGAGGAATGGCAAA		
qPCR- <i>NbBAK1</i> -R	TTGGCCCCGACAATTCATCT		
qPCR- <i>NbSOBIR1</i> -F	CCAGCAAGTCACAGAAGGGA		
qPCR- <i>NbSOBIR1</i> -R	CCAACACCACACCAAAGCTG		
B303-VdGAL4-UP-F	GGACCGGACGGGGC <u>GGTACC</u> CCAGGATTC	Kpn I	
	GAICAAIAAIAI		
B303-VdGAL4-UP-R	CTTCAATATCAGTTAACGTCTTTCTCCTTGTT		
	CAAAGTCCA		
B303-VdGAL4-Hyg-F	TGGACTTTGAACAAGGAGAAAGACGTTAAC		Generation of
	TGATATTGAAG		VdGAL4 deletion
B303-VdGAL4-Hyg-R	CTGACTGAATTATTTCCTTTACTATTCCTTTG		mutant in V.
	CCCTCGGACG		dahliae
B303-VdGAL4-Down-F	CGTCCGAGGGCAAAGGAATAGTAAAGGAA		
	ATAATTCAGTCAG		
B303-VdGAL4-Down-R	TAGTCCCGGGTCTTAATTAACGTGAAAGACC	Pac I	
	AGCCAATGGC		
pSULPH- VdGAL4(+UP)-F	ACGGCCAGTGCCAAGCTTCCAGGATTCGAT	BamH I	Generation of
pSULPH- <i>VdGAL4</i> (+UP)-F	ACGGCCAGTGCC <u>AAGCTT</u> CCAGGATTCGAT CAATAA	BamH I	Generation of VdGAL4
pSULPH- <i>VdGAL4</i> (+UP)-F pSULPH- <i>VdGAL4</i> (+UP)-R	ACGGCCAGTGCC <u>AAGCTT</u> CCAGGATTCGAT CAATAA ATTCACTAGTCAGGATCCCTGCAAGAAAGC	BamH I Hind III	Generation of VdGAL4 complementation
pSULPH- <i>VdGAL4</i> (+UP)-F pSULPH- <i>VdGAL4</i> (+UP)-R	ACGGCCAGTGCC <u>AAGCTT</u> CCAGGATTCGAT CAATAA ATTCACTAGTCA <u>GGATCC</u> CTGCAAGAAAGC AGCAACACCTTG	BamH I Hind III	Generation of VdGAL4 complementation mutant in V. dahliae
pSULPH- VdGAL4(+UP)-F pSULPH-VdGAL4(+UP)-R Test-Hyg-F	ACGGCCAGTGCC <u>AAGCTT</u> CCAGGATTCGAT CAATAA ATTCACTAGTCA <u>GGATCC</u> CTGCAAGAAAGC AGCAACACCTTG TCGTTATGTTTATCGGCACT	BamH I Hind III	Generation of VdGAL4 complementation mutant in V. dahliae
pSULPH- <i>VdGAL4</i> (+UP)-F pSULPH- <i>VdGAL4</i> (+UP)-R Test-Hyg-F Test-Hyg-F	ACGGCCAGTGCC <u>AAGCTT</u> CCAGGATTCGAT CAATAA ATTCACTAGTCA <u>GGATCC</u> CTGCAAGAAAGC AGCAACACCTTG TCGTTATGTTTATCGGCACT TCGGTCGGCATCTACTCT	BamH I Hind III	Generation of VdGAL4 complementation mutant in V. dahliae
pSULPH- VdGAL4(+UP)-F pSULPH-VdGAL4(+UP)-R Test-Hyg-F Test-Hyg-F Test-VdGAL4-F	ACGGCCAGTGCC <u>AAGCTT</u> CCAGGATTCGAT CAATAA ATTCACTAGTCA <u>GGATCC</u> CTGCAAGAAAGC AGCAACACCTTG TCGTTATGTTTATCGGCACT TCGGTCGGCATCTACTCT GGACATCCACGCCAAGTT	BamH I Hind III	Generation of VdGAL4 complementation mutant in V. dahliae Validation of VdGAL4 deletion
pSULPH- VdGAL4(+UP)-F pSULPH-VdGAL4(+UP)-R Test-Hyg-F Test-Hyg-F Test-VdGAL4-F Test-VdGAL4-R	ACGGCCAGTGCC <u>AAGCTT</u> CCAGGATTCGAT CAATAA ATTCACTAGTCA <u>GGATCC</u> CTGCAAGAAAGC AGCAACACCTTG TCGTTATGTTTATCGGCACT TCGGTCGGCATCTACTCT GGACATCCACGCCAAGTT TCAGAGCAAGCGCAACAT	BamH I	Generation of VdGAL4 complementation mutant in V. dahliae Validation of VdGAL4 deletion and complementation
pSULPH- VdGAL4(+UP)-F pSULPH-VdGAL4(+UP)-R Test-Hyg-F Test-Hyg-F Test-VdGAL4-F Test-VdGAL4-R Test-VdGAL4-R	ACGGCCAGTGCC <u>AAGCTT</u> CCAGGATTCGAT CAATAA ATTCACTAGTCA <u>GGATCC</u> CTGCAAGAAAGC AGCAACACCTTG TCGTTATGTTTATCGGCACT TCGGTCGGCATCTACTCT GGACATCCACGCCAAGTT TCAGAGCAAGCGCAACAT TCGCCCTTCCTCCCTTTA	BamH I Hind III	Generation of VdGAL4 complementation mutant in V. dahliae Validation of VdGAL4 deletion and complementation mutant in V. dahlia
pSULPH- VdGAL4(+UP)-FpSULPH-VdGAL4(+UP)-RTest-Hyg-FTest-Hyg-FTest-VdGAL4-FTest-VdGAL4-RTest-Hyg- southern -FTest-Hyg- southern -R	ACGGCCAGTGCC <u>AAGCTT</u> CCAGGATTCGAT CAATAA ATTCACTAGTCA <u>GGATCC</u> CTGCAAGAAAGC AGCAACACCTTG TCGTTATGTTTATCGGCACT TCGGTCGGCATCTACTCT GGACATCCACGCCAAGTT TCAGAGCAAGCGCAACAT TCGCCCTTCCTCCCTTTA CGCTGTTATGCGGCCATT	BamH I Hind III	Generation of VdGAL4 complementation mutant in V. dahliae Validation of VdGAL4 deletion and complementation mutant in V. dahlia
pSULPH- VdGAL4(+UP)-F pSULPH-VdGAL4(+UP)-R Test-Hyg-F Test-Hyg-F Test-VdGAL4-F Test-VdGAL4-R Test-Hyg- southern -F Test-Hyg- southern -R $\beta$ -tubulin-F	ACGGCCAGTGCC <u>AAGCTT</u> CCAGGATTCGAT CAATAA ATTCACTAGTCA <u>GGATCC</u> CTGCAAGAAAGC AGCAACACCTTG TCGTTATGTTTATCGGCACT TCGGTCGGCATCTACTCT GGACATCCACGCCAAGTT TCAGAGCAAGCGCAACAT TCGCCCTTCCTCCCTTTA CGCTGTTATGCGGCCATT TCACCAGCCGTGGCAAGGTTG	BamH I Hind III	Generation of VdGAL4 complementation mutant in V. dahliae Validation of VdGAL4 deletion and complementation mutant in V. dahlia
pSULPH- VdGAL4(+UP)-F pSULPH-VdGAL4(+UP)-R Test-Hyg-F Test-Hyg-F Test-VdGAL4-F Test-VdGAL4-R Test-Hyg- southern -F Test-Hyg- southern -R $\beta$ -tubulin-F $\beta$ -tubulin-R	ACGGCCAGTGCCAAGCTTCCAGGATTCGATCAATAAATTCACTAGTCAGGATCCCTGCAAGAAAGCAGCAACACCTTGTCGTTATGTTTATCGGCACTTCGGTCGGCATCTACTCTGGACATCCACGCCAAGTTTCAGCAGCAAGCGCAACATTCGCCCTTCCTCCCTTTACGCTGTTATGCGGCCATTTCACCAGCCGTGGCAAGGTTGAGCAAAGGGCGGTCTGGACGTTG	BamH I Hind III	Generation of VdGAL4 complementation mutant in V. dahliae Validation of VdGAL4 deletion and complementation mutant in V. dahlia Quantification of
pSULPH- VdGAL4(+UP)-F pSULPH-VdGAL4(+UP)-R Test-Hyg-F Test-Hyg-F Test-VdGAL4-F Test-VdGAL4-R Test-VdGAL4-R Test-Hyg- southern -F 6-tubulin-F 6-tubulin-F GhUBQ7-F	ACGGCCAGTGCCAAGCTTCCAGGATTCGATCAATAAATTCACTAGTCAGGATCCCTGCAAGAAAGCAGCAACACCTTGTCGTTATGTTTATCGGCACTTCGGTCGGCATCTACTCTGGACATCCACGCCAAGTTTCAGAGCAAGCGCAACATCGCTGTTATGCGGCCATTCGCCGTTATGCGGCCATTTCACCAGCCGTGGCAAGGTTGAGCAAAGGGCGGTCTGGACGTTGGAAGGCATTCCACCTGACCAAC	BamH I Hind III	Generation of VdGAL4 complementation mutant in V. dahliae Validation of VdGAL4 deletion and complementation mutant in V. dahlia Quantification of fungal biomass
pSULPH- VdGAL4(+UP)-F pSULPH-VdGAL4(+UP)-R Test-Hyg-F Test-Hyg-F Test-VdGAL4-F Test-VdGAL4-R Test-Hyg- southern -F fest-Hyg- southern -R b-tubulin-F b-tubulin-F GhUBQ7-F GhUBQ7-R	ACGGCCAGTGCCAAGCTTCCAGGATTCGATCAATAAATTCACTAGTCAGGATCCCTGCAAGAAAGCAGCAACACCTTGTCGTTATGTTTATCGGCACTTCGGTCGGCATCTACTCTGGACATCCACGCCAAGTTTCAGCAGCAAGCGCAACATTCGCCTTCCTCCCTTTACGCTGTTATGCGGCCATTTCACCAGCCGTGGCAAGGTTGAGCAAAGGGCGGTCTGGACGTTGGAAGGCATTCCACCTGACCAACCTTGACCTTCTTCTTCTTCTTGTGCTTG	BamH I Hind III	Generation       of         VdGAL4       complementation         mutant in V. dahliae       mutant in V. dahliae         Validation       of         VdGAL4       deletion         and       complementation         mutant in V. dahlia       mutant in V. dahlia         Quantification       of         fungal biomass       of

VdNLP1-R	GCCTGGTTTGCGTTGTTC	related conidial
VdPLP-F	GCTGACCAGTATCTGTCGGAGG	production
VdPLP-R	ATGACGACTGGCTTCTCGGCCT	
Vdpf-F	ACCATTTTCAACAGTCGGGTACGCG	
<i>Vdpf</i> -R	ACCATTTTCAACAGTCGGGTACGCG	
VdNoxB-F	TGCGTGGCAAGCATAAGACATAC	
<i>VdNoxB</i> -R	GACAGCACGAGTGAAATCACCAAC	
VdPls1-F	ATGGTCAACAAGATCCTCGCGA	
VdPls1-R	TCCGGCTGCTCAAACATGTTGT	
VdSep5-F	AGCTCGACCTGGACGAGGA	
VdSep5-R	GAGGCTTCGTTATCAATCTCGTCTC	
VdCrz1-F	ATGGATCAGCAAGCTCAACATCG	qPCR of genes
VdCrz1-R	GATCCAGACCGAGACCGAGAC	penetration
VdCSIN1-F	CTTTGATTGTGGTATGGGTTCT	
VdCSIN1-R	GTGGTGGGTTTGCCTTGT	
Som1-F	GTCGTGACAACCGAAAGCAG	
Som1-R	TCCCTCGTGGAGCGCAAA	
Vta3-F	GATGTCTGCCCTGCGTAA	
Vta3-R	GATCTGAGCCTGGTCAAAGT	
Vayg1-F	GTTGCGACGAGTTCTTGT	
Vayg1-R	ACCATCACCTTGCCCATA	
VT4HR-F	TGGTGGCATCAAGACAGACA	qPCR of genes
VT4HR-R	CGAAGCGAGGAAGCAAACAA	related melanin formation
VaflM-F	GACTGTCAATGCCATCGCC	
<i>VaflM-</i> R	CGGTGACCTTGATAACTT	
VdSCD-F	ATGCCCGCTTCCGAGTTC	

VdSCD-R	TTCCACACGCCGTCAATCTT	
VDH1-F	GTCTATTCATCTGGTTCCTCCCTA	-
VDH1-R	CAAACCTCTTACAATGTTGACGC	-
VdLAC-F	CGTTTCCTCACTTTAGCCACAGC	_
VdLAC-R	CACCCAGTCCACCGTCCATTTGT	-