

Figure S1. The colony morphology and growth rate of *VdaSm* and *VdaSm*-GFP on PDA plates. (a) PCR assays differentiated the *Verticillium* species. The primer pair Df/Dr amplified *V. dahliae* and *V. longisporum* strains, whereas A1f/A1r was specific to *V. longisporum*. V592 was used as a control. M indicated the marker; (b) The colony morphology of *VdaSm* and *VdaSm*-GFP on PDA plates; (c) The diameter of colonies at 5, 7, 9, 11 and 13 days. For each strain, 9 plates were repeated. No significant differences were noticed ($P > 0.05$, t-test). Error bar showed the standard deviations.

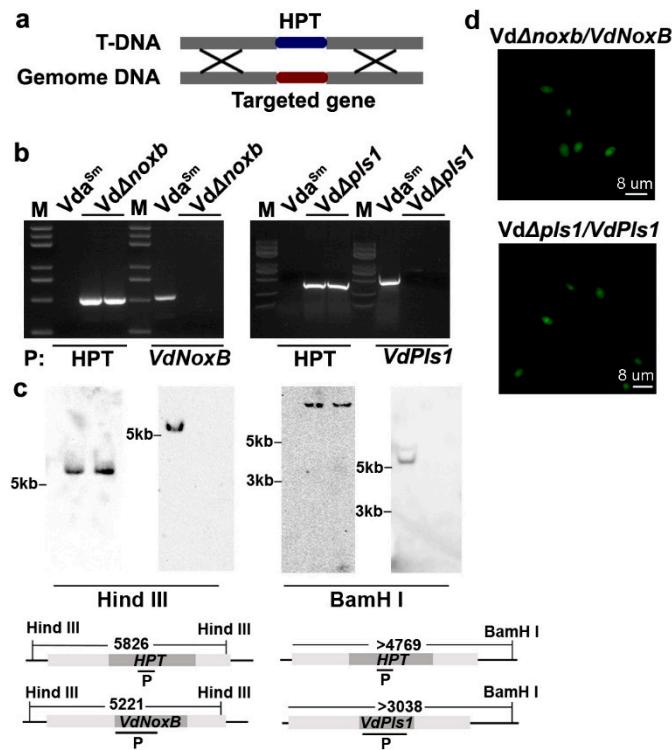


Figure S2. Construction of knockout mutants *VdΔnoxb* and *VdΔpls1*, and complemented strains *VdΔnoxb/VdNoxB* and *VdΔpls1/VdPls1*. (a) Schematic description of the homologous recombination event; (b) PCR analysis of targeted gene deletion mutants with special primers as indicated; (c) Southern blot identification of mutants with the HPT, *VdNoxB* and *VdPls1* probes as indicated. Genomic DNA was digested by Hind III and BamH I, respectively. The schematic diagrams showed the cleavage sites of

restriction enzymes and the expected fragment sizes; (d) Confocal micrograph of green fluorescence by Vd Δ nox b /VdNoxB and Vd Δ pls 1 /VdPls 1 .

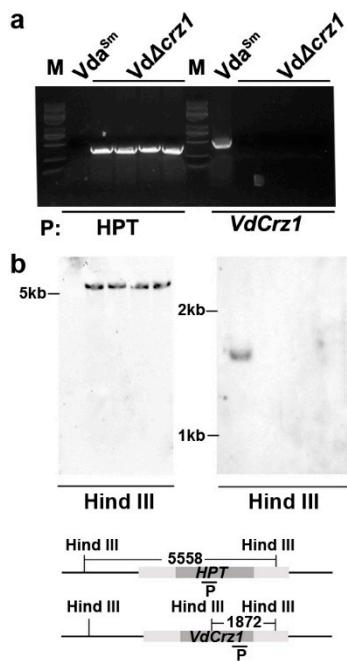


Figure S3. Identification of the *VdCrz1* knockout mutant. (a) PCR identification of the *VdCrz1* knockout mutant with the primers HPT and *VdCrz1* as indicated; (b) Southern blot analysis of *VdCrz1* gene deletion with *HPT* and *VdCrz1* probes as indicated. Genomic DNA was digested by Hind III. The schematic diagrams showed the cleavage sites of restriction enzymes and the expected fragment sizes.

Table S1. Primers were used in this study.

Name	Primer Sequence(5'-3')	Purpose
ITS1	TCCGTAGGTGAACCTGCGG	Sequencing
ITS4	TCCTCCGCTTATTGATATGC	Sequencing
A1f	AAGTGGAGCCCCGTATCTTGAAT	Sequencing
A1r	CAACTGGCAACAGGGCTTGAAT	Sequencing
Df	CCGGTCCATCAGTCTCTCTG	Sequencing
Dr	CTGTTGCCGCTTCACTCG	Sequencing
KONoxB-U-F	CTTGCTGAGGTCTTAATTAAACAACAAGAGGCCGA ATCAA	Cloning
KONoxB-U-R	AGTGCTGAGGCATTAATTAAACGTGCTGGTAGTGC TATAATCGT	Cloning
KONoxB-D-F	CCCGCTGAGGACTTAATTAAAGGGGGCAAGGCG TGGAGCA	Cloning
KONoxB-D-R	CTCGCTGAGGGTTAATTAAATGGAACACGCTCA AACACGTCG	Cloning
KOPIs1-U-F	CTTGCTGAGGTCTTAATTAAAGCGCACTAGGTGC TTGCAAGT	Cloning
KOPIs1-U-R	AGTGCTGAGGCATTAATTAAAGTTGCTGGCGGTCT CGAGGACT	Cloning
KOPIs1-D-F	CCCGCTGAGGACTTAATTAAACGACCGTAAAGCC GATCTC	Cloning
KOPIs1-D-R	CTCGCTGAGGGTTAATTAAAGAGGCAAGGAAATG ATGATCAGT	Cloning
KOCrz1-U-F	CTTGCTGAGGTCTTAATTAACTCCAGCGCTCATCT ACCCT	Cloning
KOCrz1-U-R	AGTGCTGAGGCATTAATTAAAGGTTGGGACGTGT CGTGAGTA	Cloning
KOCrz1-D-F	CCCGCTGAGGACTTAATTAAAGTGTGCCCTTGTCT TCTTC	Cloning
KOCrz1-D-R	CTCGCTGAGGGTTAATTAAAGAGGACCATGTT CATAACG	Cloning
pGKO-check-F	GTCGACGGTATCGATAAGCTTGCTC	Checking
pGKO-check-R	CGGACATGCAGCTCACGCCTA	Checking
HPT-F	ATAAGAGTCACACTTCGAGCGCC	Checking
HPT-R	GCTCATGAGCGCTTGTTCGG	Checking
Pnoxb-NoxB-F	TAAAACGACGCCAGTGCCAAGCTTCTCCAGGT AACAGGGAAGA	Cloning
Pnoxb-NoxB-R	GCCCATCGAGTAGTAGTCCATCGTGCTGGTAGTG CTATAAT	Cloning

NoxB-GFP-F	AGCACGATGGACTACTACTCGATGGGCTC	Cloning
NoxB-GFP-R	AATTAAGCCGCCTCCGCCGAAGTTCTCCT TGCCCCATT	Cloning
Ppls1-Ppls1-F	TAAAACGACGGCCAGTGCCAAGCTTGGGCACGG CACAAAGGGTCA	Cloning
Ppls1-Ppls1-R	GATCTTGTGACCATGTTGCTGGCGGTCTCGAGG ACTGT	Cloning
Pls1-GFP-F	GCCAGCAACATGGTCAACAAGATCCTCGCGACC TTCG	Cloning
Pls1-GFP-R	AATTAAGCCGCCTCCGCCGAGGCTGCGGTATCC GCT	Cloning
pTef-Kar2-RFP-F	AACCTCTAGAGGATCCATGTCGAGGTCTC GCAAC	Cloning
pTef-Kar2-RFP-R	CACCAGCACCGAATTGAGCTCGTCGTGG GAGGC	Cloning
HPT-probe-F	CTTCTGCGGGCGATTGTGTAC	Southern Blotting
HPT-probe-R	CATGTGTATCACTGGCAAATGTTG	Southern Blotting
NoxB-probe-F	GACTACTACTCGATGGGCTC	Southern Blotting
NoxB-probe-R	AATCTTCCTCAGGCGCACTCGT	Southern Blotting
Pls1-probe-F	GATGTGCTTTCTGATCAC	Southern Blotting
Pls1-probe-R	CGGTCCCTCCAGTAGCACAGCAAG	Southern Blotting
Crz1-probe-F	ACGTTCCAGTGCAACCTGTGT	Southern Blotting
Crz1-probe-R	ATCAAACGAAGACCGTCCCGAAA	Southern Blotting
NAT-probe-F	TGGAGCTAGTGGAGGTCAAC	Southern Blotting
NAT-probe-R	GAGGCCCTTCGTCTCAAG	Southern Blotting
β-tublin-qF	AGCTCACCCAGCAGATGTT	RT-qPCR
β-tublin-qR	TCGACCTCCTCATGGCAAC	RT-qPCR
VdCrz1-qF	TTTACTCGCGCGTACAACCT	RT-qPCR
VdCrz1-qR	AACTCTTCTCGCCCGAGTG	RT-qPCR
VdLcc-qF	TACCTCTTCCACTGCCACATC	RT-qPCR
VdLcc-qR	GTTTCCGCCAGCATTACCA	RT-qPCR
VdRhom-qF	CCCATGATTGGCCCTCTT	RT-qPCR
VdRhom-qR	CACCAAATGGGCTGTTGAGC	RT-qPCR