

Supplementary Materials:

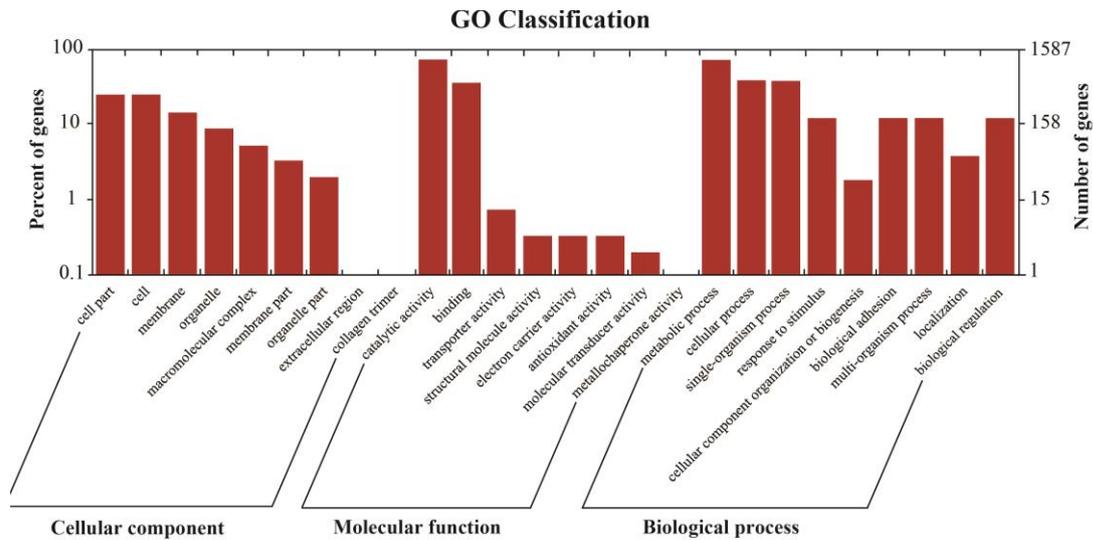


Figure S1. GO classification of predicted protein-encoding genes of *V. dahliae* strain VdLs.17.

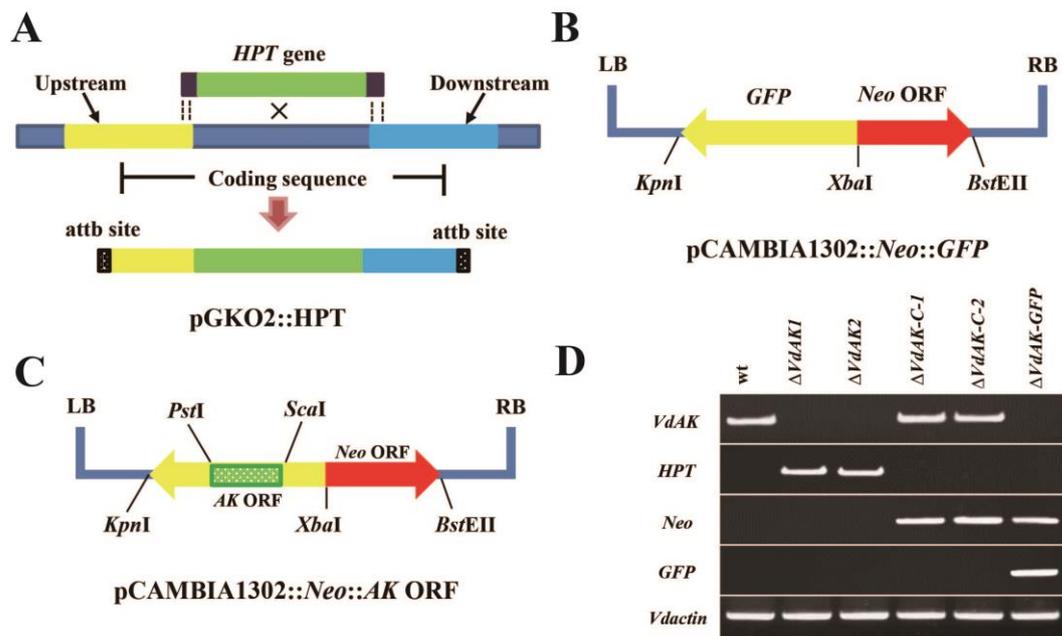


Figure S2. Generation of disruption ($\Delta VdAK$), complementation ($\Delta VdAK-C$) and $\Delta VdAK-GFP$ strains via homologous recombination. (A) Construction of *VdAK*-disrupted mutant $\Delta VdAK$. About 1.5 kb upstream and downstream of targeted gene were fused with the hygromycin resistance cassette. The whole fragment, with attb sites, was cloned using nested PCR. (B) Construction of $\Delta VdAK-GFP$. The neomycin resistance cassette, containing *Xba*I and *Bst*EII restriction sites, was cloned into the pCAMBIA1302 vector. The GFP expression cassette was then cloned into the intermediate plasmid by *Xba*I/*Kpn*I double-digestion (designated pCAMBIA1302::Neo::GFP). (C) Construction of $\Delta VdAK-C$. The GFP ORF of plasmid pCAMBIA1302::Neo::GFP was replaced with *VdAK* ORF via *Sca*I/*Pst*I double digestion

(designated pCAMBIA1302::Neo::VdAK). (D) Expression of the targeted genes was confirmed by RT-PCR.

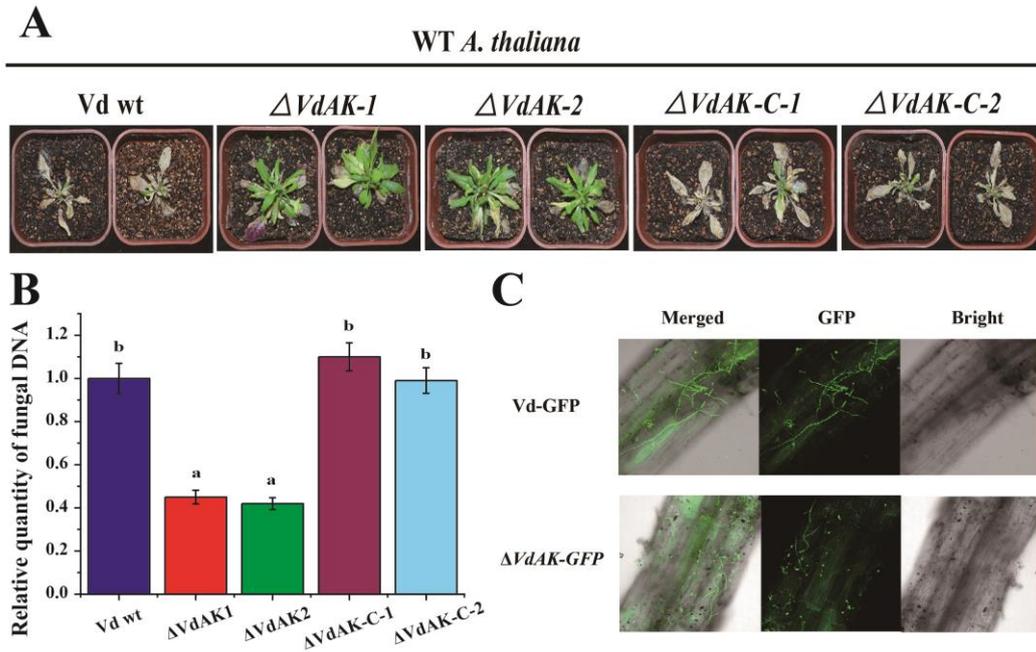


Figure S3. Virulence analysis of disruption ($\Delta VdAK$) and complementation ($\Delta VdAK-C$) mutants and wild-type *Vd* in *A. thaliana*. (A) Symptoms on seedlings at 12 days after roots were dipped in 10^6 spores/mL of $\Delta VdAK$, $\Delta VdAK-C$ or wild-type *Vd*. (B) Relative amounts of fungal DNA determined by RT-qPCR. Means (\pm SE) from three independent experiments that significantly differed among treatments in Duncan's test ($P < 0.05$) are indicated with different letters. (C) Micrographs of fluorescing fungal hyphae in *A. thaliana* root tips at 5 days post inoculation with *Vd-GFP* or $\Delta VdAK-GFP$.

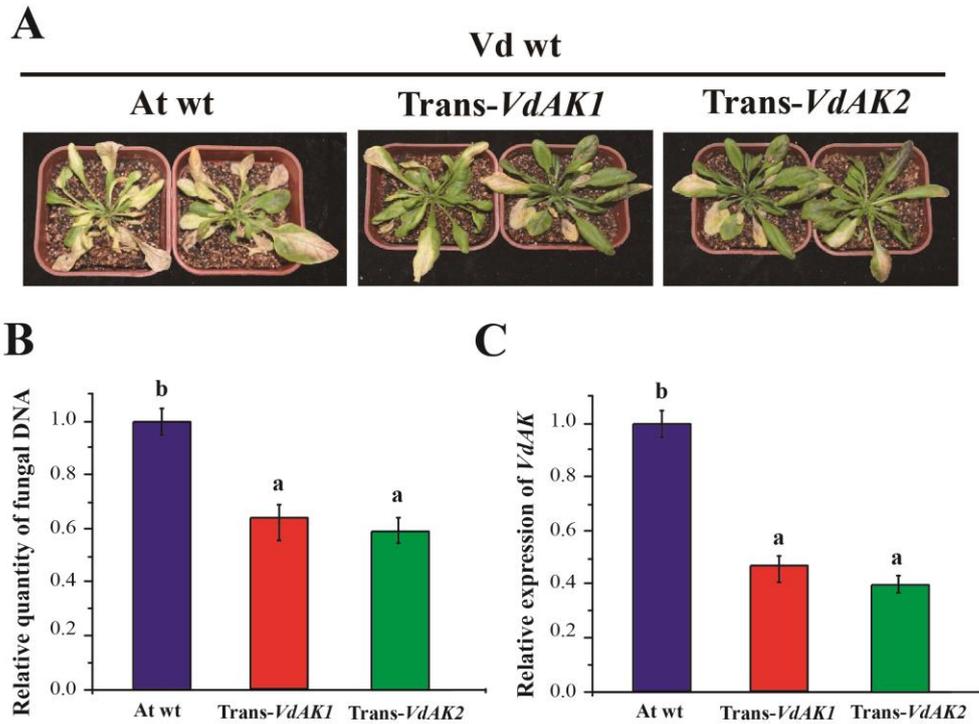


Figure S4. Expression of dsRNAs of *VdAK* in *A. thaliana* led to increased resistance against *Vd*. (A) Symptoms on transgenic and wild-type plants at 12 days after roots were dipped in 10^6 spores/mL of the respective strains. (B) Relative amounts of fungal DNA determined by RT-qPCR. (C) Transcript levels of *VdAK* in stems of transgenic and wild-type plants. Means (\pm SE) from three independent experiments were analyzed for significant differences among treatments using Duncan's test ($P < 0.05$).

Table S1. Information on RNAi constructs targeting 92 *V. dahliae* genes selected for HIGS and disease index from 10-12 days post inoculation of construct-treated plants

| RNAi constr | Primer sequences (5' to 3') | Gene IDs | Annotations | Disease index | | |
|-------------|--|------------------|---|---------------|--------|--------|
| | | | | 10 dpi | 11 dpi | 12 dpi |
| TRV:00 | | | | 100 | 100 | 100 |
| 1 | ATAGAATTCGGTTATCCT CGTCGGTGC ATAGGATCCAGACACGG CCCGAG | VDAG_01 040.1 | Adenylate kinase | 15 | 20 | 25 |
| 2 | ATAGAATTCATTCAG ACTGCCAG ATAGGATCCCTCCTCCA AAGATCC | VDAG_05 976.1 | Alpha-amylase A type-1/2 | 15 | 55 | 65 |
| 3 | ATAGAATTCATGGCGCA GAACCCC GCGTATGGAAATCCTCT CGTCCC | VDAG_07 833.1 | Ankyrin repeat-containing protein | 60 | 80 | 90 |

| | | | | | | |
|----|---|------------------|---|----|----|----|
| 4 | ATAGAATTCTTGTCCTCT CCAACG GGTGTAGATGAAGACAT CGTACAAG AGTGAATTCGTGCTTCAA | VDAG_07 739.1 | Aquaporin-3 | 18 | 20 | 24 |
| 5 | GCGTAC AGAGGATCCCCCTTGAA GAGAGAC AGAGAATTCATGGATTA | VDAG_07 535.1 | ADP, ATP carrier protein | 20 | 30 | 35 |
| 6 | CCAGGGTTC TGAGGATCCCACCTTGTA GCAGATC ATAGAATTCATGTCGGA | VDAG_00 201.1 | Cellular nucleic acid-binding protein | 20 | 55 | 70 |
| 7 | GAACCTCGC ATAGGATCCGACAGTCA AGATGCC ATAGAATTCGGTCATCG | VDAG_02 332.1 | Aconitate hydratase | 20 | 45 | 70 |
| 8 | AGCCTGG ATATGGATCCGGAATCG TGTTCTCC AGAGAATTCTCGTCATC | VDAG_01 206.1 | Pyruvate kinase | 20 | 40 | 55 |
| 9 | CGCAGTG ATAGGATCCCTTGTGGCT CCTCTG ATAGAATTCACCGGAGG | VDAG_09 094.1 | Peroxisomal membrane protein PEX30 | 30 | 40 | 45 |
| 10 | ACTCGGG ATAGGATCCCCTTGATC GTCCAGC AGAGAATTCTGCTAACA | VDAG_03 613.1 | NADPH dehydrogenase | 25 | 50 | 65 |
| 11 | AGGCCGGC AGAGGATCCAGTGGATG ACCGAGG ATAGAATTCCAATCGAC | VDAG_04 294.1 | 60S Acidic ribosomal protein P0 | 40 | 55 | 75 |
| 12 | GGCATCC ATAGGATCCTGCAATCG ATGCTCC AGAGAATTCCAACCTCT | VDAG_01 123.1 | Hard-surface induced protein | 35 | 50 | 75 |
| 13 | ACGACGCC ATAGGATCCCACGGAGC TTATCGG ATAGAATTCATCAAGGC | VDAG_01 774.1 | Malate synthase | 60 | 70 | 80 |
| 14 | GCTCAAGC AGAGGATCCAGTGAGCA TGAGACG | VDAG_03 232.1 | Oligosaccharyl transferase STT3 subunit | 10 | 25 | 35 |

| | | | | | | |
|----|---|------------------|---|----|----|-----|
| 15 | ATAGAATTCGTGCCGAC ATCGACC ATAGGATCCGCCCACTG AATAGAGC | VDAG_08 270.1 | Transport protein BOS1 | 75 | 90 | 100 |
| 16 | ATAGAATTCGACACGGA GTACCTCCG AGAGGATCCTGCTGGTG ATATGCC | VDAG_05 521.1 | Vacuolar protein sorting-associated protein | 50 | 75 | 90 |
| 17 | ATAGAATTCATCAAGCG ACCGAGAGG ATAGGATCCATGCTCCCT GTCAGC | VDAG_01 656.1 | Viral A-type inclusion protein repeat protein | 25 | 60 | 75 |
| 18 | ATAGAATTCGAGCCAGC CCAACAG AGAGGATCCTGGACAAG GTGTACTCG | VDAG_06 474.1 | cAMP-dependent protein kinase | 45 | 65 | 75 |
| 19 | ATAGAATTCACCTTCCA GGACTCC ATAGGATCCTCTCTTCTG GGTACCG | VDAG_06 474.1 | cAMP-dependent protein kinase | 80 | 85 | 95 |
| 20 | AGAGAATTCGCAGGCTA CATCAGCG ATAGGATCCGTGCTCAG CTCGTACG | VDAG_02 311.1 | Solute carrier family 25 member 45 | 50 | 70 | 85 |
| 21 | AGAGAATTCCTGGAAAG GCACCTGC ATAGGATCCAGGCATGA GAACGCC | VDAG_00 714.1 | Argininosuccinate lyase | 20 | 25 | 30 |
| 22 | TGTGAATTCGGCCTCGA GTATAAGG ATAGGATCCGCCTTAC GTACTCG | VDAG_00 317.1 | Cryptochrome DASH | 65 | 75 | 85 |
| 23 | AGAGAATTCGTACGTCC GCTCCTGC ATAGGATCCGAGATCTG GGTCACGG | VDAG_05 375.1 | Covalently-linked cell wall protein | 70 | 80 | 95 |
| 24 | ATAGAATCCAACGTGA CTCCTGGC AGTGGATCCTTAACACT CGTCCGTC | VDAG_10 495.1 | Endoglucanase B | 65 | 75 | 85 |
| 25 | ATAGAATTCAACGCTGT CTACTTCACG ATAGGATCCTGAGGTAG TAGTTGACG | VDAG_04 416.1 | Chitinase A | 60 | 70 | 90 |

| | | | | | | |
|----|--|------------------|---|----|----|----|
| 26 | AGAGAATTCGGAGCATA CTGACGTCC ATAGGATCCTGTGCACA GACTGATGC | VDAG_09 689.1 | Guanine nucleotide-binding protein subunit beta | 55 | 65 | 80 |
| 27 | AGAGAATTCGCCTACCA GGAAGACC ATAGGATCCGGACTTGA TGTAGGCC | VDAG_07 192.1 | 1,3-Beta-glucanosylt ransferase gel4 | 55 | 65 | 80 |
| 28 | ATAGAATTCCACGCTCA ACACGACC TTAGGATCCCTCCATGGT GTACTCG | VDAG_02 243.1 | 1,3-Beta-glucanosylt ransferase gel1 | 55 | 75 | 85 |
| 29 | ATAGAATTCCTTGGCCTT AGCTGGC TTAGGATCCTCTAGGGTT GAGCCG | VDAG_02 071.1 | Glucosyltransferase H | 60 | 70 | 85 |
| 30 | TGAGAATTCTACAGCCA GCTCTCGC ATAGGATCCCGCTGGAT ATCGAGC | VDAG_06 369.1 | GTP-binding protein GTR2 | 40 | 50 | 65 |
| 31 | AGAGAATTCCTGAGGAC TCAGCAGGC ATAGGATCCCGGATGTG CTTCAGG | VDAG_10 151.1 | NADH-ubiquinone oxidoreductase 21 kDa subunit | 60 | 70 | 80 |
| 32 | TGAGAATTCACAGTCGA GCTCGACG ATAGGATCCGGCTGAGA GAACAGGC | VDAG_04 456.1 | Nuclear pore glycoprotein p62 | 50 | 60 | 70 |
| 33 | TTAGAATTCGACCACA GCATCACG ATAGGATCCGGATCATC TCAGTGCG | VDAG_03 673.1 | Nuclear distribution protein nudeE | 20 | 30 | 35 |
| 34 | TGAGAATTCCTTCTCCA ACGTCCG AGAGGATCCTAATGTTG GACATGCG | VDAG_09 326.1 | GTP-binding protein GTR1 | 40 | 45 | 55 |
| 35 | AGAGAATTCOAACCGTC TGGCTACC TTAGGATCCCAATGATC AGAGGGC | VDAG_09 877.1 | Aspartyl/glutamyl-t RNA (Asn/Gln) amidotransferase subunit B | 50 | 65 | 75 |
| 36 | TTAGAATTCGAGCAGGA TTACGTGGC ATAGGATCCATACGAGC CTGCACG | VDAG_02 981.1 | Methyltransferase domain-containing protein | 45 | 55 | 65 |

| | | | | | | |
|----|---|------------------|--|-----------|-----------|-----------|
| 37 | ATAGAATCCAAGCGGA GACTGATGG TTAGGATCCTGAATGTGC AGGTGG TGTGAATTCTCTCCTACT | VDAG_05 486.1 | Atrial gland-specific antigen | 31.2 5 | 56.2 5 | 62.5 |
| 38 | CCGTCCG ATAGGATCCGGATATGT CGAGGTCTG AGAGAATCCTCTTATCT | VDAG_00 647.1 | Inner centromere protein | 6.25 | 12.5 | 18.7 5 |
| 39 | GTCGGCGG ATAGGATCCGTTGACGT GAGTCCG ATAGAATTCATCGGGCC | VDAG_00 787.1 | Hypothetical protein | 43.7 5 | 50 | 56.2 5 |
| 40 | AAGGTGG TTAGGATCCTCACTCAGC ATGCTCG AGAGAATTCTCGCCGAG | VDAG_06 790.1 | PSP1 domain-containing protein | 37.5 | 43.7 5 | 56.2 5 |
| 41 | ATGCTCG ATAGGATCCGGCGTCCA CATTATGG ATAGAATTCGACGAGAT | VDAG_08 982.1 | Mitogen-activated protein kinase HOG1 | 68.7 5 | 81.2 5 | 87.5 |
| 42 | TGACGAGG ATAGGATCCTCTCAAGC TCATCAGC AGAGAATTCAGCTCTGG | VDAG_03 613.1 | NADPH dehydrogenase | 25 | 31.2 5 | 37.5 |
| 43 | GATCAAGG ATAGGATCCTTGGTCTCG TCCTCTG ATAGAATTCGCAAGATC | VDAG_04 020.1 | NADPH dehydrogenase D | 40 | 56 | 68 |
| 44 | GCTCTGGC ATAGGATCCGAGGTAGC ACGAGC ATAGAATTCGCCTTGCTT | VDAG_08 067.1 | Pectate lyase B | 50 | 56.2 5 | 62.5 |
| 45 | CGTTCG TAAGGATCCCTGCGAAT GTTGTACC ATAGAATTCGGACGAAG | VDAG_05 402.1 | Pectate lyase | 56.2 5 | 62.5 | 68.7 5 |
| 46 | CAGACACG AGAGGATCCTCTCGTGA TAGGGAGG ATAGAATTCGCTTCGAG | VDAG_08 663.1 | cAMP-dependent protein kinase regulatory subunit | 50 | 55 | 69 |
| 47 | CGTCATGG ATAGGATCCGATCCCGT CATCTCG | VDAG_08 656.1 | Cell pattern formation-associate d protein stua | 56.2 5 | 68.7 5 | 75 |

| | | | | | | |
|----|---|-------------------|--|----|----|----|
| 48 | AGAGAATTCCAATGGCA GCAGAGC ATAGGATCCGGTTCGAGG TATAGGGC TTAGAATTCGTCAACCA | VDAG_06 649.1 | Dual specificity protein kinase YAK1 | 20 | 30 | 35 |
| 49 | GCTCGAGG ATAGGATCCCTTGATCAT GGCAGG ATAGAATTCCAGAAGCC | VDAG_07 142.1 | Polysaccharide deacetylase family protein | 65 | 75 | 80 |
| 50 | TGTCGTGC ATAGGATCCAATCTCGTT CGTGACG ATAGAATTCGTCACCA | VDAG_08 977.1 | ATP-dependent protease La | 25 | 40 | 45 |
| 51 | TCAACAAGGC ATAGGATCCACCTTGCTC GTGTTGG ATAGAATTCGTCAAGA | VDAG_08 977.1 | ATP-dependent protease La | 55 | 65 | 70 |
| 52 | TACCACGGC ATAGGATCCGTAGCTCT GCGTCAGG ATAGAATTCACGTACGC | VDAG_00 279.1 | Import receptor | 35 | 45 | 50 |
| 53 | TGCGTATCG TTAGGATCCTGATGGTGT ACTCGGC ATAGAATTCGTCGACCA | VDAG_05 060.1 | Multidrug resistance protein | 65 | 75 | 85 |
| 54 | GGAAGTCG ATAGGATCCTACGGTAA GCCGTCG ATAGAATTCCAACGGTG | VDAG_05 084.1 | ATP-dependent bile acid permease | 25 | 30 | 35 |
| 55 | ACCTCAAGG ATAGGATCCCTCCTCAG CCTTCTCC ATAGAATTCGGCAACCG | VDAG_04 294.1, | 60S Acidic ribosomal protein P0 | 55 | 70 | 75 |
| 56 | AGATAGCC ATAGGATCCCTGGAGTA CGGTAACC AGAGAATTCATGCTCTC | VDAG_00 335.1 | Secreted protein K | 60 | 65 | 70 |
| 57 | CAGCCTC ATAGGATCCTCTCTCTGC AGCACG AGAGAATTCGACGACAT | VDAG_07 788.1 | Microsomal signal peptidase 18 kDa subunit | 60 | 65 | 70 |
| 58 | GATCTCCCAG ATAGGATCCGGTGAAGG TGACCTCC | VDAG_07 546.1 | Stress protein | 55 | 65 | 75 |

| | | | | | | |
|----|---|------------------|--|----|----|----|
| 59 | ATAGAATTCACCTGGCC ATGAGGTC ATAGGATCCTGCGACAG CATTAC AGAGAATTCGCAAGACG | VDAG_03 187.1 | General stress protein | 55 | 65 | 85 |
| 60 | AACGTTCC ATAGGATCCACTCGTGG AAGTGCG ATAGAATTCCTTGGGG | VDAG_01 389.1 | Glycogen synthase | 65 | 80 | 85 |
| 61 | CTACACCC ATAGGATCCTGCTGCTGC TAGTGCC AGAGAATTCCTCGTTCAT | VDAG_01 389.1 | Glycogen synthase | 55 | 70 | 90 |
| 62 | CTACGTCG ATAGGATCCCTCTGGTA CGTACTGCC AGAGAATTCCTGGTATCT | VDAG_01 254.1 | Tryptophan synthase | 50 | 55 | 65 |
| 63 | CAAGGCG ATAGGATCCAGTCGTCG TGGTAGG AGAGAATTCCTGGGACT | VDAG_07 000.1 | Killer toxin subunits alpha/beta | 45 | 60 | 65 |
| 64 | ACAAGAGCG ATAGGATCCGAGAGGCT CACAAGC AGAGAATTCTGTCAAC | VDAG_00 901.1 | Killer toxin subunits alpha/beta | 55 | 65 | 70 |
| 65 | AGCAAGCC AGAGGATCCTGTCAAGA ATGGCAC GAGGAATTCAGAATACG | VDAG_00 825.1 | Vacuolar protease A | 62 | 72 | 80 |
| 66 | TGGAACGGG ATAGGATCCGATGCTGC TCGTTGAC ATAGAATTCCTCCAATCC | VDAG_08 621.1 | Carbon catabolite-derepress ing protein kinase | 50 | 60 | 75 |
| 67 | GTTGTCC ATAGGATCCAGGAGCAG CTGGTC AGAGAATTCAGTCTGGC | VDAG_04 529.1 | Thioredoxin | 16 | 23 | 28 |
| 68 | TCATACCG ATAGGATCCAGGTTGGC AACGATG AGAGAATTCGTCGACG | VDAG_09 532.1 | Serine 3-dehydrogenase | 60 | 75 | 85 |
| 69 | GCAAGC AGAGGATCCTTGTGCCA GCTGAGG | VDAG_05 692.1 | Uricase | 70 | 85 | 95 |

| | | | | | | |
|----|---|------------------|---|----|----|----|
| 70 | AGAGAATTCGCAAGCGT CCAGAAG ATAGGATCCAGACCCAG GACCCTC TATGAATTCGGTCGCAC | VDAG_09 026.1 | NADH-ubiquinone oxidoreductase 29.9 kDa subunit | 25 | 30 | 35 |
| 71 | AGTACAACG ATAGGATCCCGACTCAA TGTCGAGG AGAGAATTCGCGAGGAG | VDAG_01 642.1 | Pyruvate dehydrogenase E1 component subunit beta | 70 | 90 | 95 |
| 72 | ACTCTGCG ATAGGATCCGGTTGGAG CCGTACC ATAGAATTCGGTTC AAG | VDAG_03 252.1 | Glycine-rich RNA-binding protein | 60 | 70 | 75 |
| 73 | AATATGCCG TATGGATCCACCGTGAG ACTTGAC CTAGAATTC AAGGGCAT | VDAG_07 546.1 | Stress protein | 10 | 15 | 20 |
| 74 | CCTCGTTC ATAGGATCCCCAGAAGT CTTCAGCC ATAGAATTC CCTCCGGC | VDAG_06 669.1 | Hypothetical protein | 70 | 85 | 90 |
| 75 | ATGAAGG TTAGGATCCTGAGCACC TCCATCG AGTGAATTC TGCGATTGC | VDAG_08 238.1 | Glycerate kinase | 80 | 85 | 90 |
| 76 | GACATCC ATAGGATCCCTATCCGA CCCATGGC ATAGAATTCGTCATCTGA | VDAG_04 980.1 | Thymidylate kinase | 60 | 70 | 85 |
| 77 | GCAGCAGC ATAGGATCCGCATTGTA GCTCATGG ATAGAATTC CGGCACCA | VDAG_01 726.1 | Choline kinase | 70 | 90 | 95 |
| 78 | CGGTCAT ATAGGATCCCGAGGACA CGAGCAC ATAGAATTCGTGTTGGTT | VDAG_02 259 | Glutamate decarboxylase | 65 | 75 | 95 |
| 79 | GAGTCGA ATAGGATCCAACAGCCC CGTGCCGA ATAGAATTC TCCGCTCCT | VDAG_06 992 | Ferric reductase transmembrane component 4 | 60 | 70 | 85 |
| 80 | GCGTCCC ATAGGATCCGATCTGGG TCACGGC | VDAG_04 380 | Cytochrome P450 51 | 65 | 75 | 85 |

| | | | | | | |
|----|--|-------------------|---|----|----|-----|
| 81 | ATAGAATTCATTCCAAC GTGACTC ATAGGATCCCTTAACAC TCGTCCG | VDAG_10 495 | Endoglucanase B | 30 | 35 | 40 |
| 82 | ATAGAATTCTCGAGCCA GCCCAAC ATAGGATCCCTGGACAA GGTGTA | VDAG_06 474 | cAMP-dependent protein kinase | 60 | 70 | 75 |
| 83 | ATAGAATTCGGTCAACC AGCTCGA ATAGGATCCCCTTGATC ATGGCAGG | VDAG_07 142 | Polysaccharide deacetylase family protein | 40 | 45 | 60 |
| 84 | ATAGAATTCGCTCAAGC CCTACGGCC ATAGGATCCAGGATCTG GCCGAAGCC | VDAG_01 845.1 | Branched-chain-ami no-acid aminotransferase | 65 | 75 | 95 |
| 85 | ATAGAATTCGTGGTGCG CGTCTACC ATAGGATCCGATGGCCA GCTCGAGG | VDBG_07 510.1 | Xylulose-5-phospha te phosphoketolase | 60 | 70 | 75 |
| 86 | ATAGAATTCCTGACTTC CGGCAAC ATAGGATCCGCGACAGC GTAACGA | VDAG_05 940.1, | 60S Ribosomal protein L2 | 55 | 60 | 65 |
| 87 | ATAGAATTCGTGTTGGTT GAGTCGA ATAGGATCCAACAGATC CGTGCCG | VDAG_06 992.1 | Ferric reductase transmembrane component 4 | 60 | 80 | 100 |
| 88 | ATAGAATTCOAAGTGGT ACGGCACCA ATAGGATCCCAATGGTC GAGGACAC | VDAG_01 692.1 | Endoglucanase | 55 | 65 | 75 |
| 89 | ATAGAATTCACGGACT TCCTGAC ATAGGATCCAGAGACGG AGTGGGT | VDBG_00 534.1 | 1,3-Beta-glucanosylt ransferase gel1 | 70 | 80 | 90 |
| 90 | ATAGAATTCGAGACCGA GAAGTCG ATAGGATCCACAGAGCG GGCGGGG | VDBG_07 986.1 | PH domain-containing protein | 65 | 70 | 80 |
| 91 | ATAGAATTCGCACATTGT CGCTCTCG ATAGGATCCCAGGTAGT GGCTCAAGG | VDAG_09 595.1 | Chitin synthase export chaperone | 55 | 64 | 78 |

| | | | | | | |
|----|-------------------|---------|--------------|----|----|----|
| | ATAGAATTCGGCCTCGA | | | | | |
| 92 | GTATAAGGA | VDAG_00 | Cryptochrome | 50 | 70 | 85 |
| | ATAGGATCCGCCTTAC | 317.1 | DASH | | | |
| | GTACTCGC | | | | | |

Table S2. Primers used to produce the *VdAK* RNAi construct

| Primer | Sequences (5' to 3') |
|------------|--|
| trans-VdAK | <u>AAAAAAGCAGGCT</u> CGTTATCCTCGTCGGTGC AAGAAAGCTGGGTAGACACGGCCCGAA |
| BP site | GGGACAAGTTTGTACAAAAAAGCAGGCT GGGACCACTTTGTACAAGAAAGCTGGGT |
| Det-VdAK | ACTCGCCTAACTGTGCCTCTA GGAACCCATCCAGAATGAAGG |

Table S3. Primers used to construct mutant strains of *V. dahliae*

| Primer | Sequences (5' to 3') |
|--|---|
| Hygromycin resistant expression cassette | TTGAAGGAGCATTTTTGGGC TTATCTTTGCGAACCCAGGG 5F: CAGAGTACCCTGACGGCATCGGC 5R: GCCCAAAAATGCTCCTTCAAATCAAGCGCATGATAAGG TCG 3F: CCCTGGGTTTCGCAAAGATAACTTCGACGTGATCATGCAG CGC |
| Δ VdAK | 3R: GGAAGTCTCCTGGGGTGGTGGTGG NEST-F: <u>GGGGACAAGTTTGTACAAAAAAGCAGGCTCGTCATTCC</u> ATTCCACGGACCC NEST-R: <u>GGGGACCACTTTGTACAAGAAAGCTGGGTCAAGGTACA</u> ATGTCACATACGG |
| Neomycin resistant expression cassette | aattctagaGTTTGC GGGCTGTCTTGACG ataggtcaccTACCTGTGCATTCTGGGTAA |
| gGFP expression cassette | ggctctagaCTTTCGACACTGAAATACGTCG ataggtaccGCATCAGAGCAGATTGTA CTGAGAG |
| Δ VdAK-com | aaaagtactATGAGACTTCGAAAGGCAG aaactgcagTCAAGCCGCAAAGCGTTT |
| Det-HPH | TTCGACAGCGTCTCCGACCTGA |

| | |
|-----------|---|
| Det-ΔVdAK | AGATGTTGGCGACCTCGTATTGGG TATAACAAGAATGGTTGGCTGCGTCCC GTGGATGGAAGGTGGTATTGTAGACACG |
| Det-neo | GTTGTCACTGAAGCGGGAAGGG GCGATACCGTAAAGCACGAGGAA CGACGTAAACGGCCACAAGTT |
| Det-GFP | TCTTTGCTCAGGGCGGACTGG |

Note: Restriction sites are in bold italic font.

Table S4. Primers used for RT-qPCR

| Primer | Sequences (5' to 3') |
|--------------------|--|
| qRT-VdAK | GTGTCTACAATACCACCTTCCA CTCATCACTGCTCATAACCTC |
| qRT-VA | GGGTATTCAGACCCTATTGGACG CGAACTTCTTGTACTCAGCCTCC |
| qRT-VdATP6 | CTAGACCAATTTGAAATAAGA AAAGATTCTTGGCTAATAGAT |
| qRT-VdAC | TCTCCATCGTCTTCACCGACATCA TCTGCACGGCGAAACACCACA |
| qRT-VdATP-PRT | CGACGCCAACGTGCGGTCTACAA GCCCCGAGAAGCTCGTGCCAAT |
| qRT-Vdactin | GGCTTCCTCAAGGTCGGCTATG GCTGCATGTCATCCCACCTTCTC |
| qRT-VdITS | CCGCCGGTCCATCAGTCTCTCTGTTTATAC CGCCTGCGGGACTCCGATGCGAGCTGTAAC |
| RT-Nbactin | GGACCTTTATGGAAACATTGTGCTCAGT CCAAGATAGAACCTCCAATCCAGACAC |
| RT-AtEF-1 α | TGAGCACGCTCTTCTTGCTTTC GGTGGTGGCATCCATCTTGTTA |

Table S5. Information on sRNAs from *V. dahliae* isolated from *Vd*-infected seedlings

| Sample | Total sRNAs | Mapped sRNAs | Total mapped sRNAs |
|---------------|---------------------|---------------------|---------------------------|
| Col_1 | 10786798 (100.00 %) | 6919974 (64.15 %) | 6650 (0.10 %) |
| RNAi_1 | 4068549 (100.00 %) | 3039943 (74.72 %) | 5015 (0.16 %) |
| RNAi_2 | 8235986 (100.00 %) | 5599526 (67.99 %) | 5090 (0.09 %) |
| RNAi_3 | 9820717 (100.00 %) | 6890613 (70.1 %) | 9929 (0.14 %) |

Table S6. Sixteen *Vd* candidate pathogenicity/virulence factor genes identified by HIGS

| Gene ID | Annotation | Disease index | | |
|--------------|---|---------------|--------|--------|
| | | 10 dpi | 11 dpi | 12 dpi |
| VDAG_01040.1 | Adenylate kinase | 15 | 20 | 25 |
| VDAG_07535.1 | ADP, ATP carrier protein | 20 | 30 | 35 |
| VDAG_01206.1 | Pyruvate kinase | 20 | 40 | 55 |
| VDAG_09094.1 | Peroxisomal membrane protein PEX30 | 30 | 40 | 45 |
| VDAG_03232.1 | Oligosaccharyl transferase STT3 subunit | 10 | 25 | 35 |
| VDAG_00714.1 | Argininosuccinate lyase | 20 | 25 | 30 |
| VDAG_03673.1 | Nuclear distribution protein nudeE | 20 | 30 | 35 |
| VDAG_00647.1 | Inner centromere protein | 6.25 | 12.5 | 18.75 |
| VDAG_03613.1 | NADPH dehydrogenase | 25 | 31.25 | 37.5 |
| VDAG_06649.1 | Dual specificity protein kinase YAK1 | 20 | 30 | 35 |
| VDAG_08977.1 | ATP-dependent protease La | 25 | 40 | 45 |
| VDAG_05084.1 | ATP-dependent bile acid permease | 25 | 30 | 35 |
| VDAG_04529.1 | Thioredoxin | 16 | 23 | 28 |
| VDAG_09026.1 | NADH ubiquinone oxidoreductase | 25 | 30 | 35 |
| VDAG_07546.1 | Stress protein | 10 | 15 | 20 |
| VDAG_10495.1 | Endoglucanase B | 30 | 35 | 40 |
| Control | | 100 | 100 | 100 |