PNAS

# Supporting Information for

# Mycoviral gene integration converts a plant pathogenic fungus into a biocontrol agent

Hong Liu[a,b], Hui Wang[a,b], Xiao Lan Liao[a], Bida Gao[a], Xun Lu[a], Donghui Sun[a], Weijie Gong[a], Jie Zhong[a], Hongjian Zhu[a], Xianting Pan[a], Li Guo[b1], Xing Wang Deng[b,1] and Qian Zhou[a,1]

[1]co-correspondence authors:

Li Guo: li.guo@pku-iaas.edu.cn

Xing Wang Deng: deng@pku.edu.cn;

Qian Zhou: zhouqian2617@hunau.edu.cn

**This PDF file includes:**

## SI Materials and Methods

### Treatment of the virus-free strains with SlAV1

Infection of a virus-free *Stemphylium lycopersici* strain with SlAV1 as described by Urayama *et al.* (1) with minor modifications. Firstly, virus-infected strain SlHN-03 was cultured in 50 mL potato dextrose broth (PDB) medium with shaking (180 rpm) at 28℃ for five days. Mycelia were collected by filtering through four layers of sterilized gauze, ground in a sterilized mortar, then transferred to fresh PDB broth for four weeks. Fungal culture supernatants were centrifuged at 10,000 g to remove cell debris, and the resultant supernatant was ultra-centrifuged at 100,000 g for 2 hours. The sediment was resuspended in 10 mL of 0.01 M sodium-phosphate buffer (pH 7.0) and filtered through a 0.22 μm nylon filter. Similarly, virus-free *S. lycopersici* strain SlHN-10 and *Alternaria alternata* strain AaHN-06 were cultured for five days in 50 mL PDB. Mycelia were then collected by filtering through four layers of sterilized gauze, ground with a sterilized mortar, and then transferred to fresh PDB broth with 10 mL suspension of SlHN-03 prepared as described above for co-cultivation. After five days of co-cultivation, mycelia were transferred to fresh PDA plates for subculture. Double-strand RNA (dsRNA) extraction and RT-PCR were used to verify infection of SlAV1. Another 10 mL culture suspension of SlHN-03 was added to 50 mL PDB as control for experiments.

### Construction of the RNA-interference vector pSilent-1+AsPKS1

Vector pSilent-1 with inverted repeats of *Aspks1* coding gene segment (419 bp) was used to build the silencing vector for *Aspks1*, pSilent-1+AsPKS1 (Fig S14*B*). The forward fragment and plasmid pSilent-1 were digested with restriction enzymes, *Xho*I and *Hind*III, respectively. After purification, they were ligated with T4 ligase to generate pSilent-1-AsPKS1F which was then transformed into *Escherichia coli* DH5α cells. Then the plasmid and reverse fragment were then digested with *Kpn*I and *Stu*I, ligated with T4 ligase and transformed into *E. coli* DH5α cells again (Fig S14*A*). The final plasmid was validated by a double digest with *Xho*I and *Hind*III, and *Kpn*I and *Stu*I, respectively, and designed as pSilent-1+AsPKS1. The protoplast preparation and transformation of *A. solani* ZYAS-11 strain was conducted according to materials and methods as described in main text.

### Metabolic analysis of Altersolanol A using HPLC-MS

Altersolanol A extraction was conducted following procedures described by Zheng *et al.* (2). Metabolic analysis of Altersolanol A standards and fungal samples were conducted using a HPLC-DAD apparatus Agilent 1229 Infinity LC system (Agilent®, Waldbronn, Germany) model G6530A equipped with an auto sampler and a column oven set at 35℃. A Diode Array Detector model G4212A set at 215 and 220 nm was used as the detector. An Sharpsil-AR C18 analytical column (250 × 4.6 mm i.d., 5 μm) was used. The mobile phases contain

A: 25% methanol (HPLC grade) and B: 75% water with a flow rate of 1.0 mL/min. Altersolanol A peak was identified by comparing with chromatograms of Altersolanol A standards and quantified based on an Altersolanol A standard curve. The standard curve was created by analyzing 0.1 ng/μL, 0.2 ng/μL, 0.4 ng/μL, 0.5 ng/μL, 0.8 ng/μL, 5 ng/μL, 10 ng/μL, 20 ng/μL, 40 ng/μL, 80 ng/μL and 160 ng/μL Altersolanol A standard solutions (AdipoGen, Inc). Ergosterol was analyzed by Agilent 1229 HPLC with UV detection with a C18 column as described above. The mobile phase was 100 % methanol with a flow rate of 1 mL/min. Ergosterol was detected at 282 nm by a diode array detection and quantified based on the ergosterol standard curve which was created by analyzing 5 ng/μL, 10 ng/μL, 20 ng/μL, 40 ng/μL, 80 ng/μL, 120 ng/μL and 200 ng/μL ergosterol standard solutions (Sigma). Ten microliters of each sample were injected and analyzed. Mass spectra were collected in the electrospray ionization-positive and electrospray ionization-negative modes using a TOF/Q-TOF Mass Spectrometer.

**Fungal hyphae staining**

Leaves were inoculated with the virulent strain SlHN-10 and ORF3-transgenic SlHN-10 strain, respectively, stained with Wheat Germ Agglutinin, Alexa Fluor™ 488 Conjugate (Invitrogen, W11261) and observed under a confocal microscope (Zeiss, LSM710), as described previously (3).

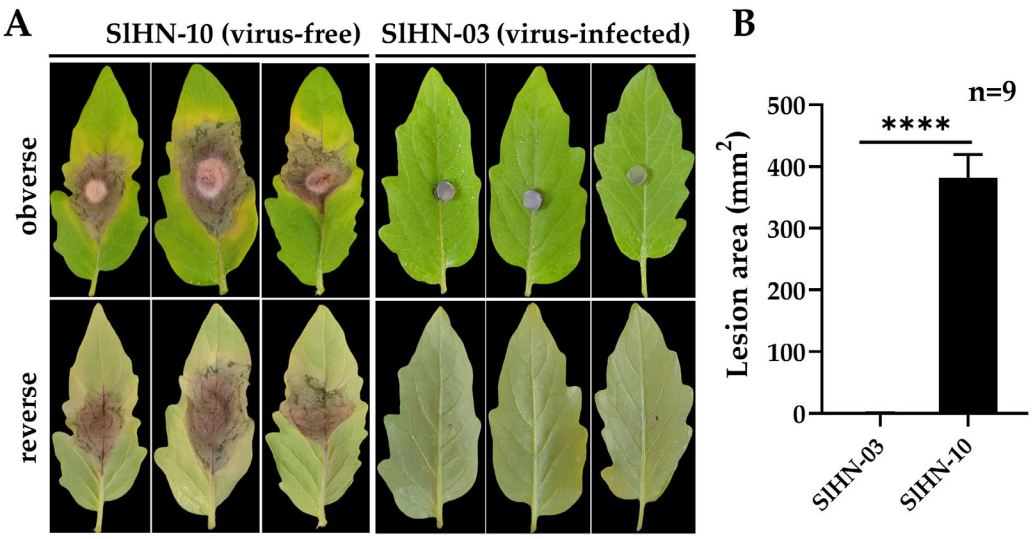


**Figure S1**. **The pathogenicity of SlHN-10 (virus-free) and SlHN-03 (virus-infected). (*A*)** and **(*B*)** Virulence assay on detached tomato leaves. Data are represented as the mean ± SD from nine replicates (5d after inoculation), ****: statistically significant (P < 0.0001).

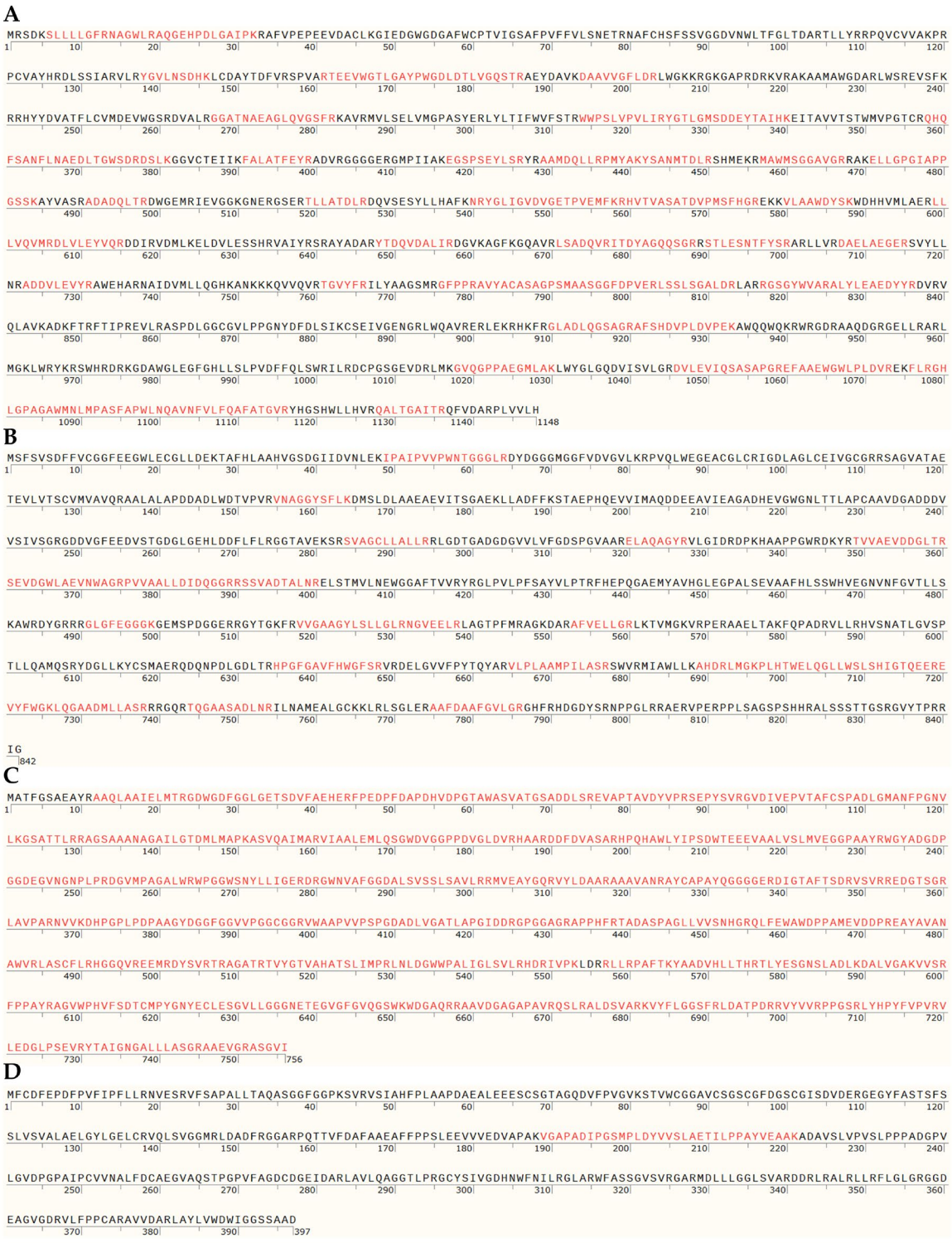


**Figure S2. Peptides of the major protein band in SDS-PAGE mapped on SIAV1 protein.** The coverage of ORF1 (***A***), ORF2 (***B***), ORF3 (***C***) and ORF4 (***D***) are 45.2%, 27.9%, 98.1% and 8.3%, respectively. The mapped amino acids are highlighted in red.

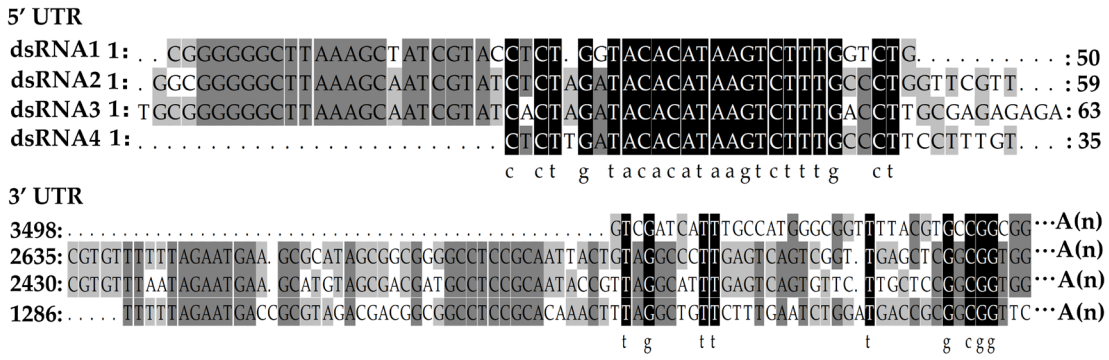


**Figure S3. Multiple sequence alignment of the 5'- and 3'-terminal regions of the four SlAV1 dsRNA segments**. Black, 100% nucleotide identity; grey, 75% nucleotide identity; light grey, 50% nucleotide identity.

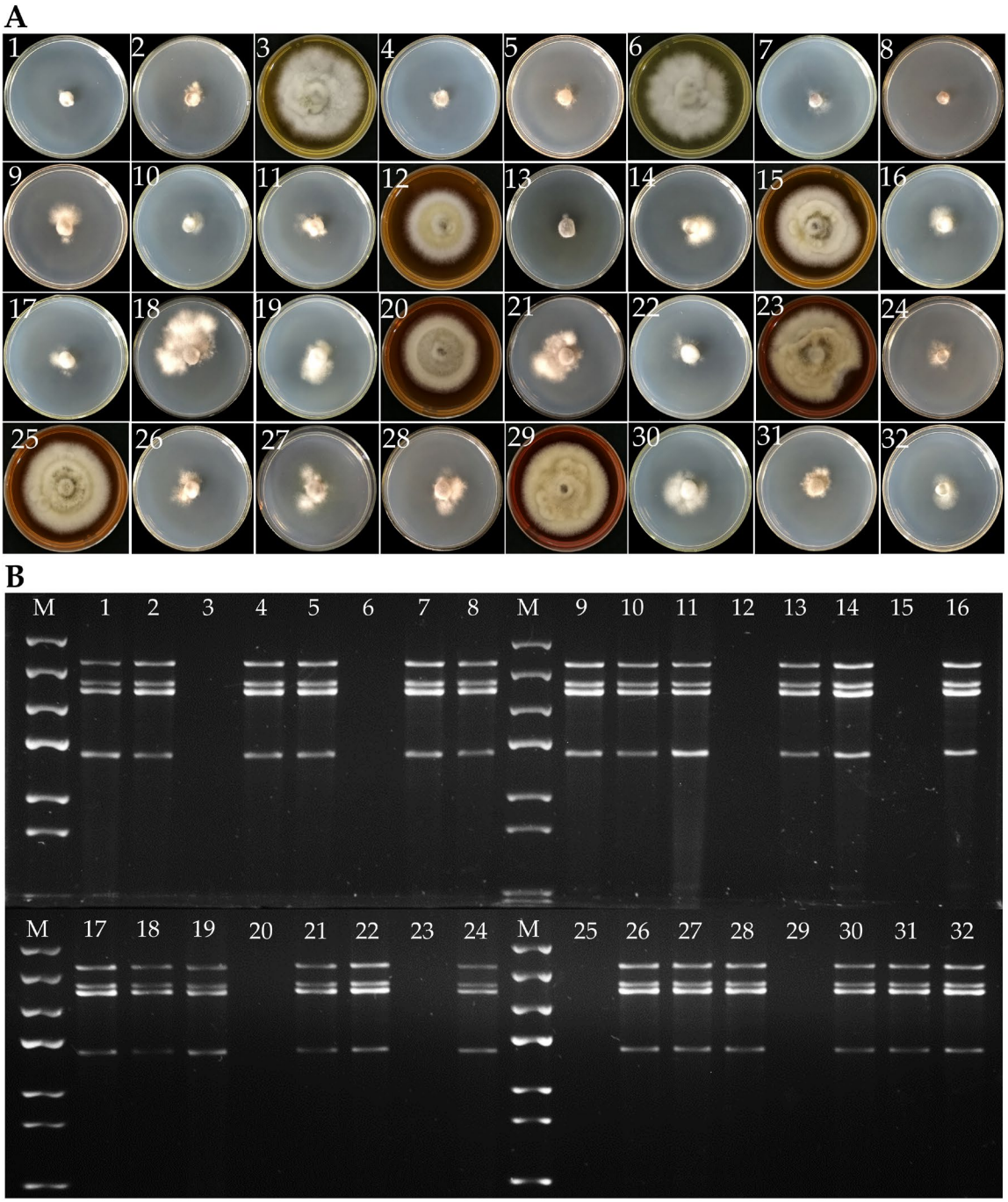


**Figure S4. Colony morphology and dsRNA detection in SlHN-03 derivative isolates. (*A*)** Colony morphology on potato dextrose agar of the SlHN-03 derivative isolates (#1-#32) obtained from hyphal tip isolation and protoplast regeneration experiment. **(*B*)** dsRNA detection of these isolates (**M**: 5000 bp marker).

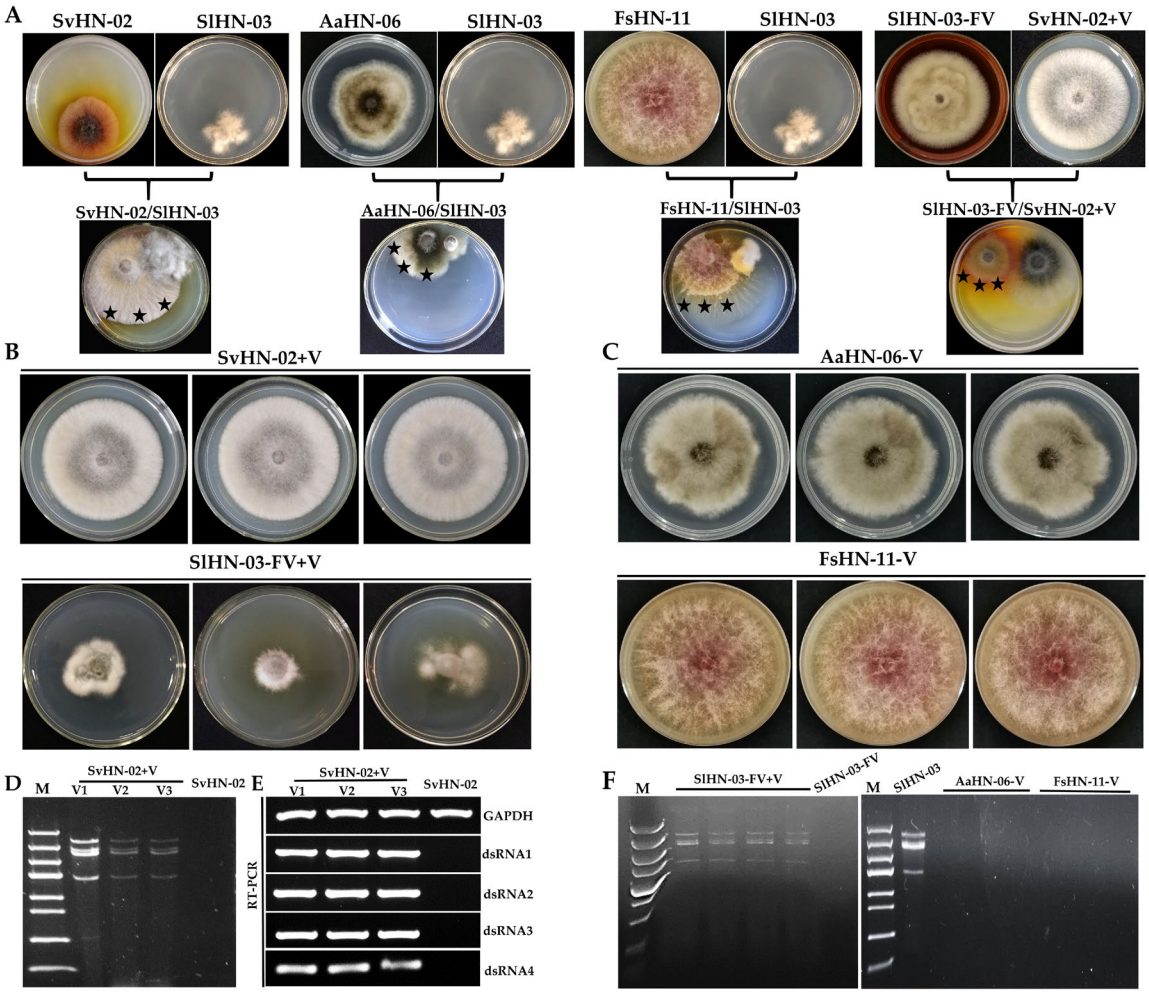


**Figure S5. Horizontal transmission of SlAV1.** Virus transmission from strain SlHN-03 (the donor) to other recipients including wild type dsRNA-free strains SvHN-02, AaHN-06, FsHN-11 is examined. Virus transmission from strain SvHN-02+V (the donor) to virus-cured strain SlHN-03-FV (recipient) is also checked. **(*A*)** Strains SvHN-02, AaHN-06 and FsHN-11 were dually cultured with strain SlHN-03, respectively. SvHN-02+V (virus-infected strain) was dually cultured with SlHN-03-FV (a virus-cured strain); the stars represent the locations where a mycelial agar plug was removed to generate a derivative isolate. **(*B* and *C*)** Colony morphology of sub-isolates derived from the colony margins of the recipient strains in A. **(*D*, *E* and *F*)** Agarose gel electrophoresis of dsRNAs extracted from mycelia of derivative isolates mentioned above (**M**: 5000 bp marker). The *GAPDH* (glyceraldehyde 3-phosphate dehydrogenase) gene was used as an internal control.

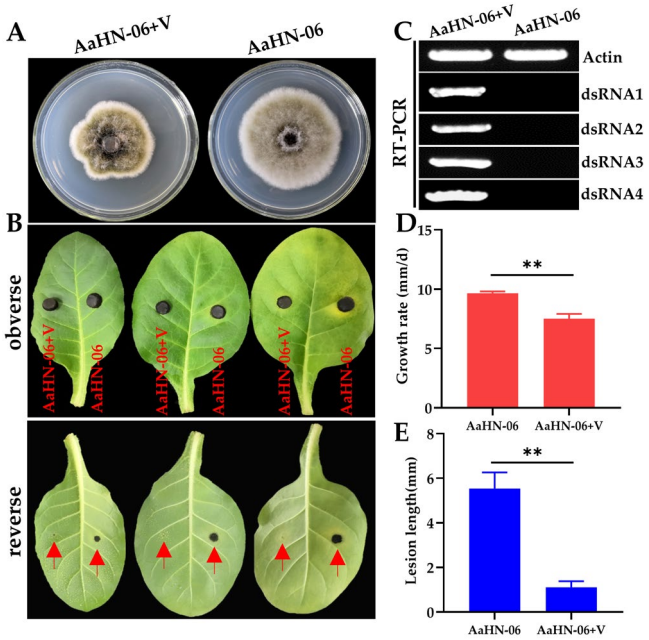


**Figure S6. Comparison of the biological characteristics between virus-infected and virus-free *Alternaria alternata* strains. (*A*)** Colony morphology of *A. alternata* strain AaHN-06 and its virus-infected strain AaHN-06+V. **(*B*)** The pathogenicity on detached tobacco leaves at the fifth day post inoculation. **(*C*)** RT-PCR detection for virus-infection. **(*D*)** Growth rate on potato dextrose agar medium. **(*E*)** Measurement of the lesions induced on detached tobacco leaves. Red arrows denote the leaf lesions.

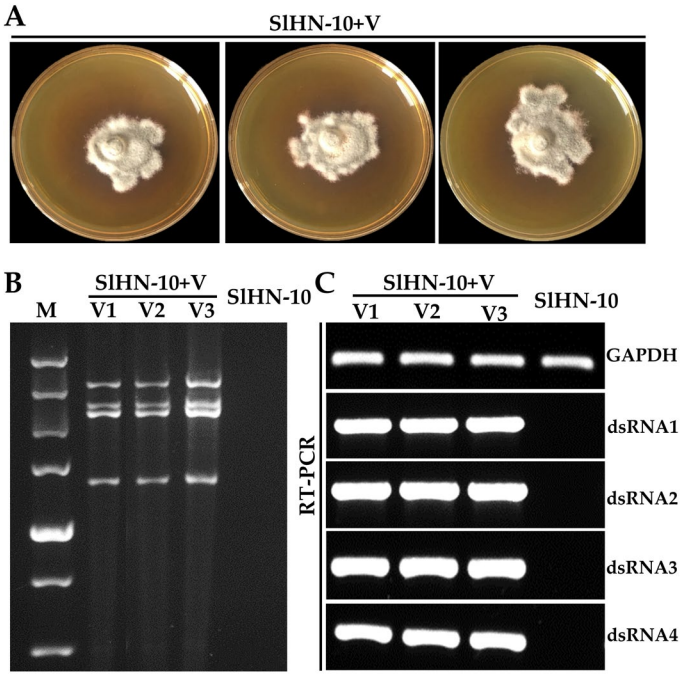


**Figure S7. Colony morphology and virus detection of virus-infected *Stemphylium lycopersici* SlHN-10 strains. (*A*)** Colony morphology of *S. lycopersici* SlHN-10 strains. **(*B* and *C*)** dsRNA extraction and RT-PCR detection of these virus-infected strains (**M**: 5000 bp marker).

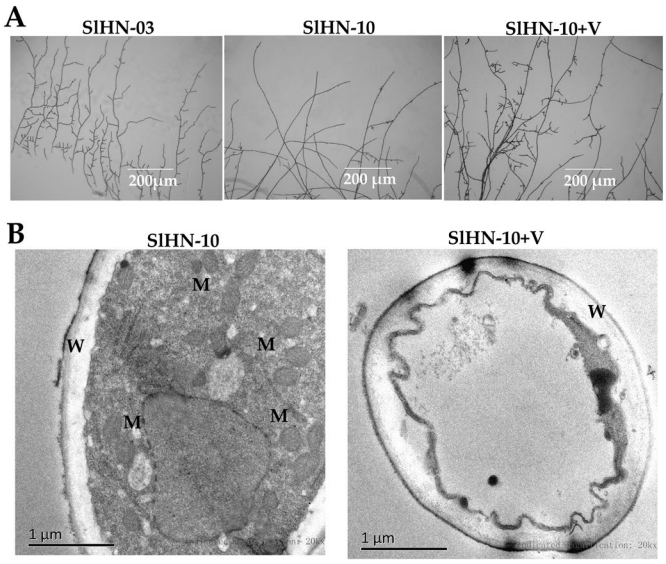


**Figure S8. Phenotypes of hypovirulent *Stemphylium lycopersici* strains. (*A*)** Morphology of hyphal tips. Virus-infected strains SlHN-03 and SlHN-10+V exhibited excessive and shorter hyphal branching compare to virus-free strain SlHN-10. **(*B*)** Ultrastructure of SlHN-10 and SlHN-10+V cells examined by transmission electron microscopy. SlHN-10 shows a well-distributed cytoplasm with many mitochondria and normal cell membranes. By contrast, SlHN-10+V exhibited a granulated cytoplasm and shrinkled cell membranes with few normal mitochondria. W, cell wall; M, mitochondria.

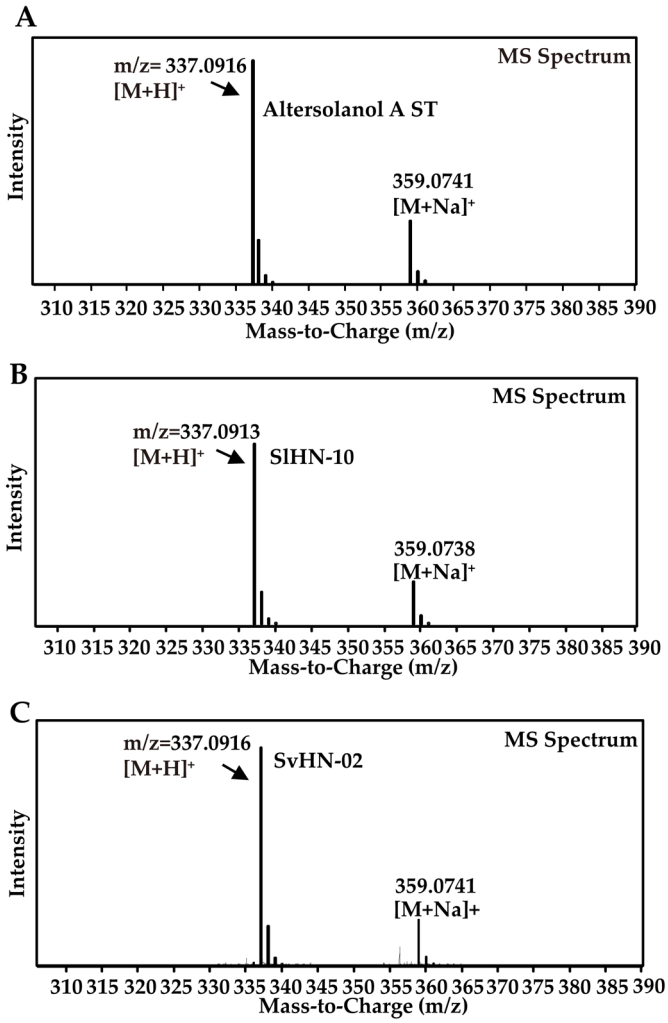


**Figure S9. Mass spectrometry analysis of Altersolanol A in different fungal strains. (*A*)** Mass spectra from the Altersolanol A standard peak (19.87 min). **(*B*)** and **(*C*)** Mass spectra from the major peak (19.87 min) of SlHN-10 and SvHN-02, respectively.

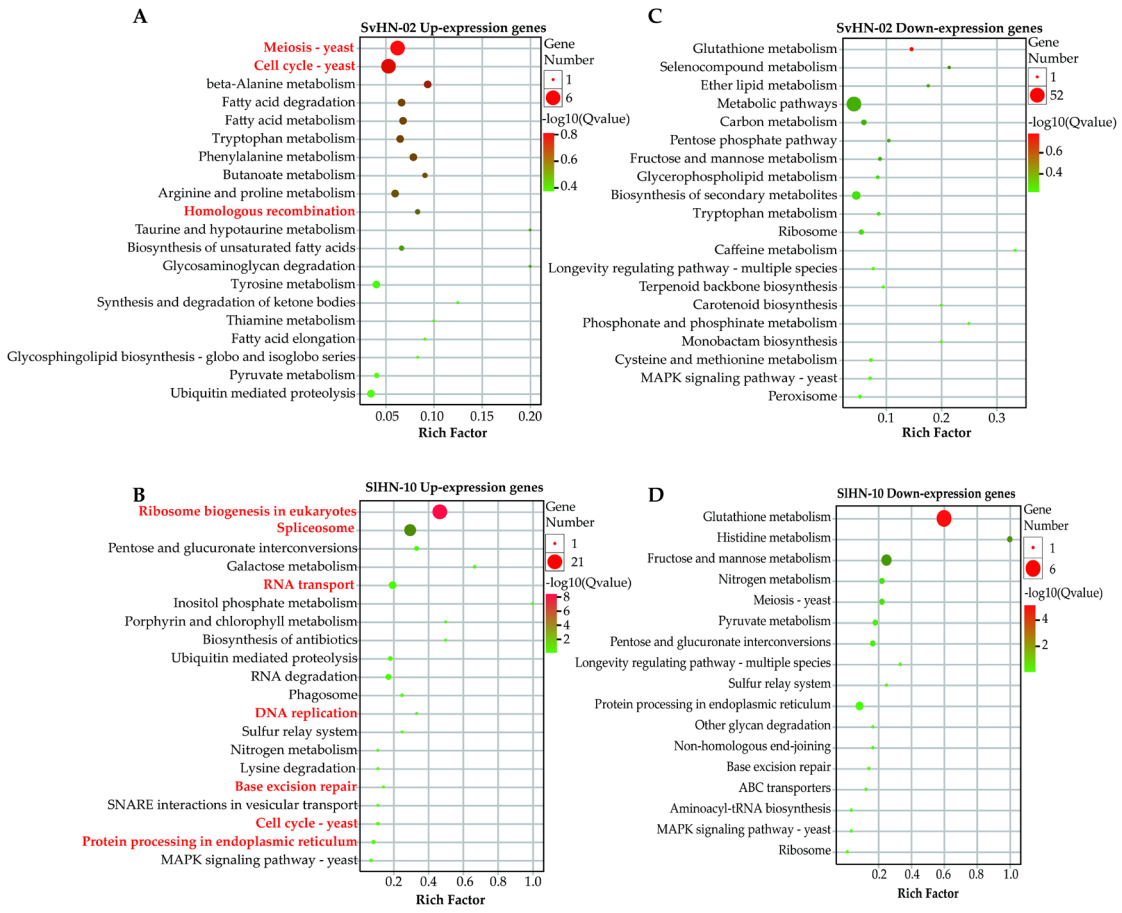


**Figure S10. Functional enrichment analysis of differentially expressed genes (DEGs) of SlHN-10 and SvHN-02 compared with their virus-infected strains SlHN-10+V and SvHN-02+V.** KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment of DEGs of SvHN-02 compared with the virus-infected strain SvHN-02+V (***A*** and ***C***), and of SlHN-10 compared with the virus-infected strain SlHN-10+V (***B*** and ***D***). Categories related to virus replication were highlighted in red.

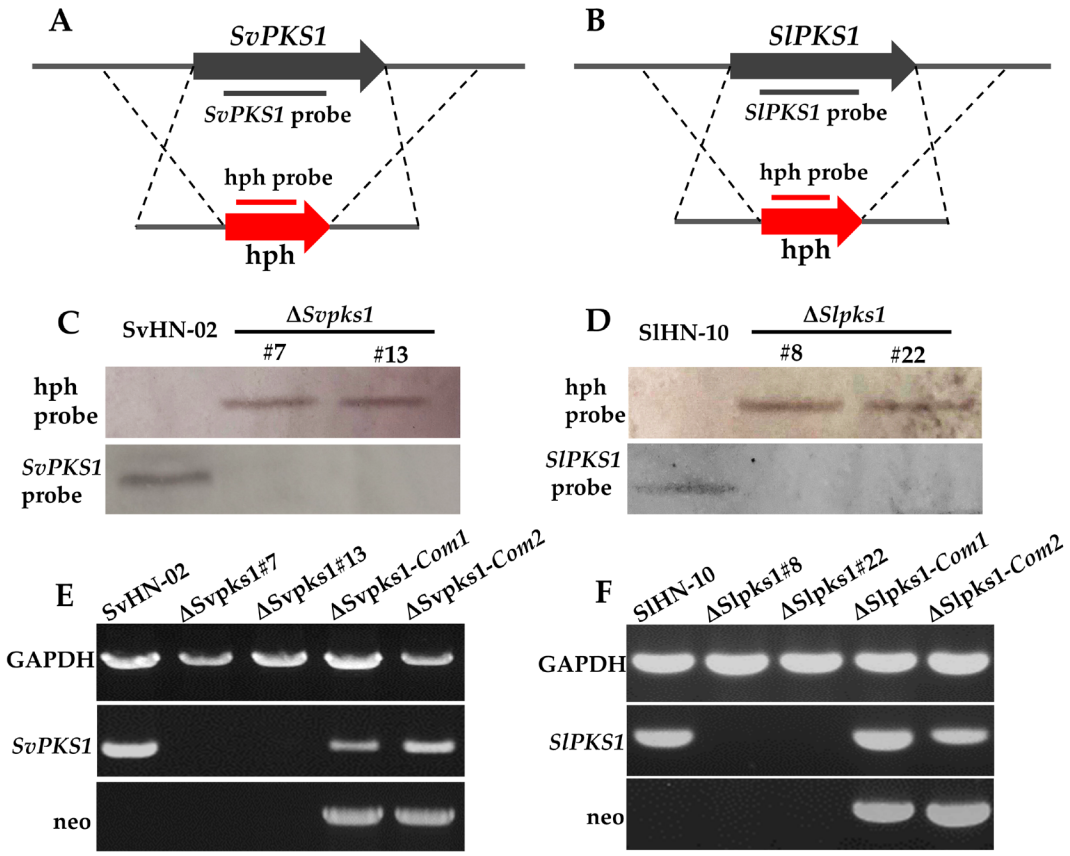


**Figure S11. Targeted gene replacement and complementation. (*A* and *B*).** A 5.63 kb fragment of the *SvPKS1* coding region and a 5.64 kb fragment of the *SlPKS1* coding region were replaced by a 1.4 kb fragment containing the hygromycin B resistance cassette to create the Δ*SvPKS1* and Δ*SlPKS1* alleles respectively. **(*C* and *D*).** Southern hybridization analysis was used to validate the deletion of *SvPKS1* and *SlPKS1* genes and the addition of a single copy integration of the hygromycin resistance gene (*HPH)*. **(*E* and *F*).** RT-PCR was carried out to confirm the deletion and complementation of *SvPKS1* and *SlPKS1* genes.

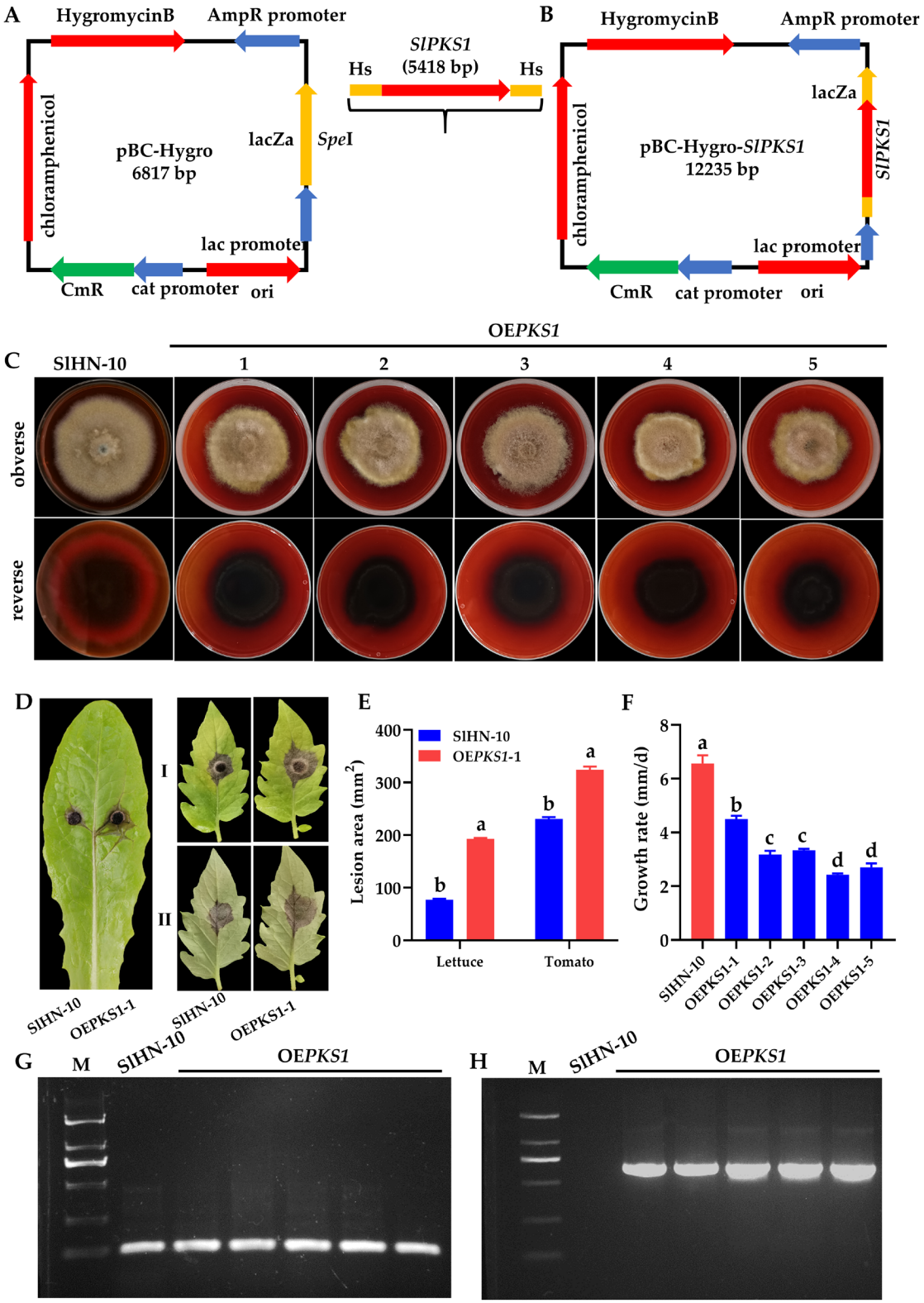


**Figure S12. Construction and biological characterization of *SlPKS1*-overexpressing (OE) strains.** (***A***) Schematic diagram of empty overexpression vector PBC-Hygro. (***B***) The *SlPKS1*

were inserted into PBC-Hygro to produce plasmid PBC-Hygro-*SlPKS1*. Hs: Homologous sequences on both sides of *SpeI* restriction site. (***C***) Colony morphology of SlHN-10 and OE-*PKS1* strains. (***D***) The pathogenicity on detached lettuce and tomato leaves at the third day post inoculation. (***E***) Measurement of lesions induced on detached lettuce and tomato leaves. (***F***) Growth rate measurement of fungal strains grown on potato dextrose agar medium. (***G*** and ***H***) RT-PCR amplification of actin gene and hygromycin resistance gene segment in wild type and overexpression strains, respectively (**M**: 5000 bp marker).

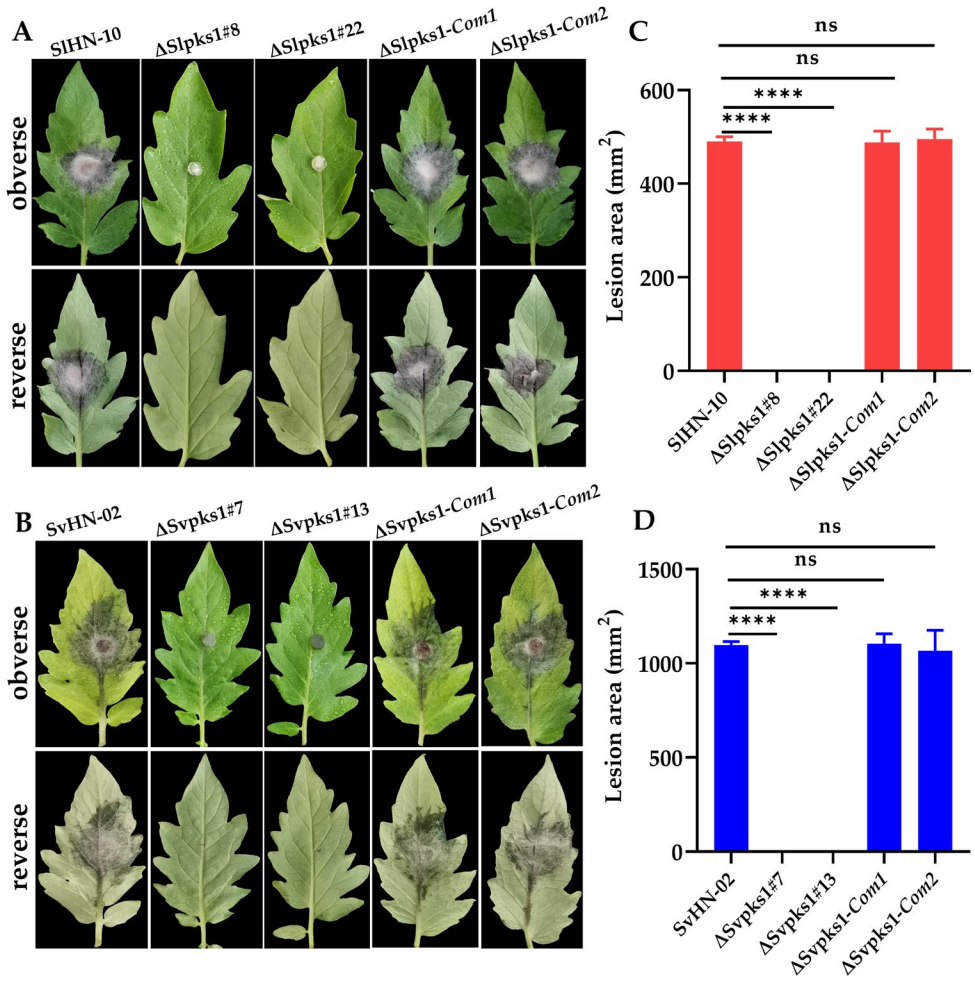


**Figure S13. The pathogenicity of wild type, *PKS1* mutants and complemented strains were inoculated on detached tomato. (*A*)** and **(*C*)** Virulence assay on detached tomato leaves by inoculation with *S. lycopersici* strains. **(*B*)** and **(*D*)** Virulence assay on detached tomato leaves by inoculation with *S. vesicarium* strains. ****: statistically significant (P < 0.0001). ns: statistically not significant.

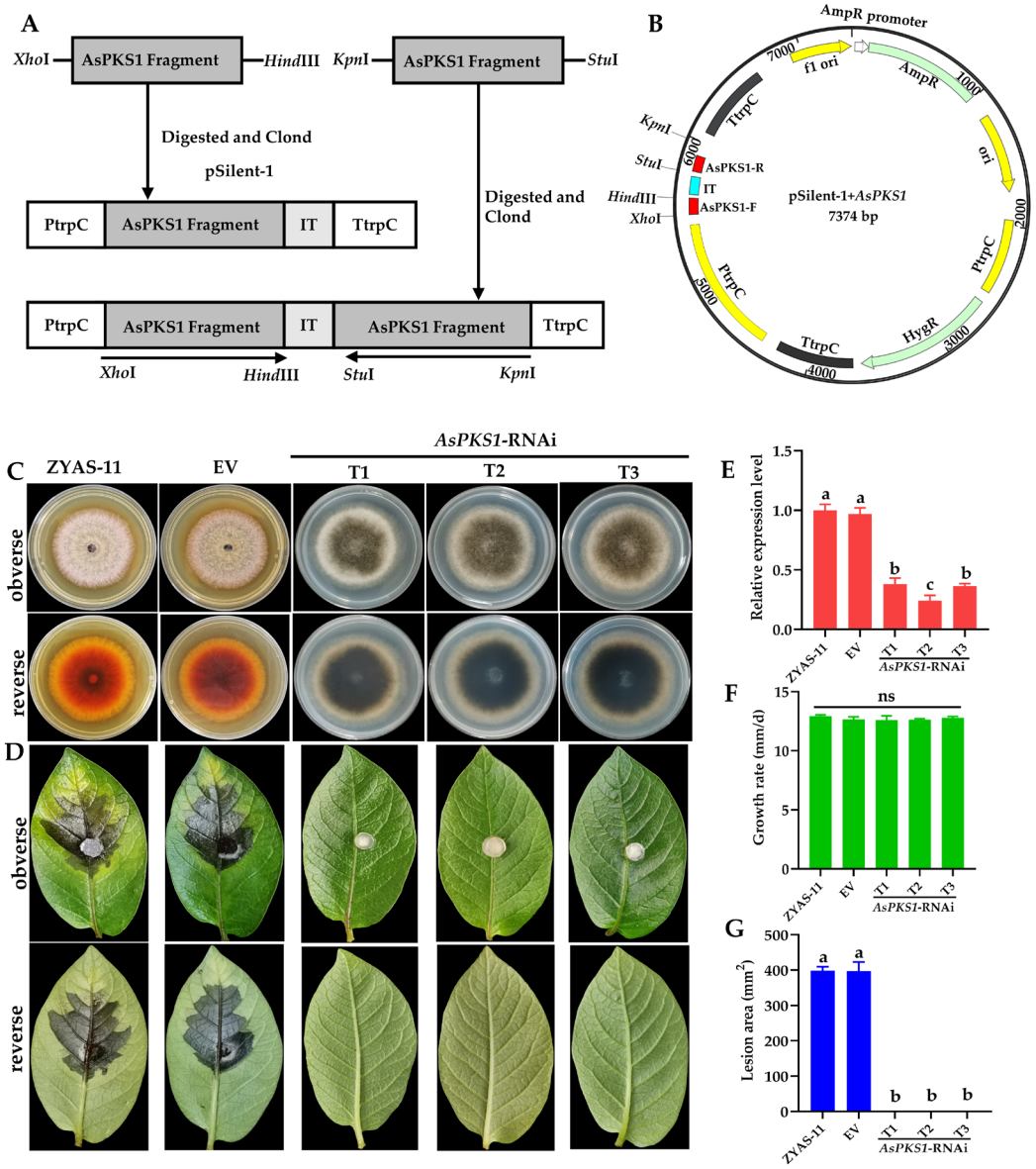


**Figure S14. Schematic representations of the silencing construct pSilent-1+AsPKS1 encoding As-pks1 hairpin and *AsPKS1* silencing in *Alternaria solani* strain ZYAS-11. (*A* and *B*)** Schematic diagram of silencing vector construction. Arrows indicate the direction of the *AsPKS1* coding region**.** IT, intron 2 of the cutinase (CUT) gene from *Magnaporthe oryzae*. *TtrpC*, terminator; *PtrpC*, promoter; *AmpR*, Ampicillin resistance gene; *HygR*, hygromycin B phosphotransferase resistance gene. **(*C*)** Colony phenotypes of *A. solani* WT (ZYAS-11) and *AsPKS1* silencing transformants growing on potato dextrose agar for five days. ***(D)*** Symptoms on detached tomato leaves infected by *A. solani* WT (ZYAS-11) and *AsPKS1* silencing transformants. **(*E*)** RT-qPCR analysis of total RNAs from WT and transformants. **(*F*)** Growth rate measurement of WT and silencing transformants. **(*G*)**. Measurement of lesions on detached tomato leaves. EV: WT transformed with empty vector (pSilent-1); T1-T3: WT transformed with the silencing vector pSilent-1+*AsPKS1*.

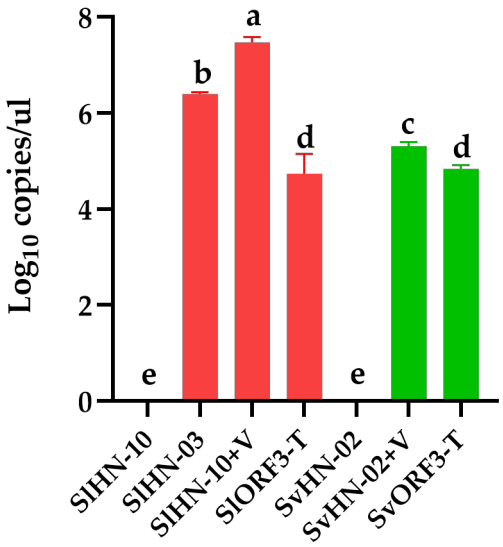


**Figure S15. ORF3 expression level in virus-infected and ORF3-transgenic strains.** The virus-infected strains have significant more ORF3 expression than ORF3 transgenic strains do. Statistical significance is indicated by different letters labelled on top of bars (one-way ANOVA: $P<0.05$).

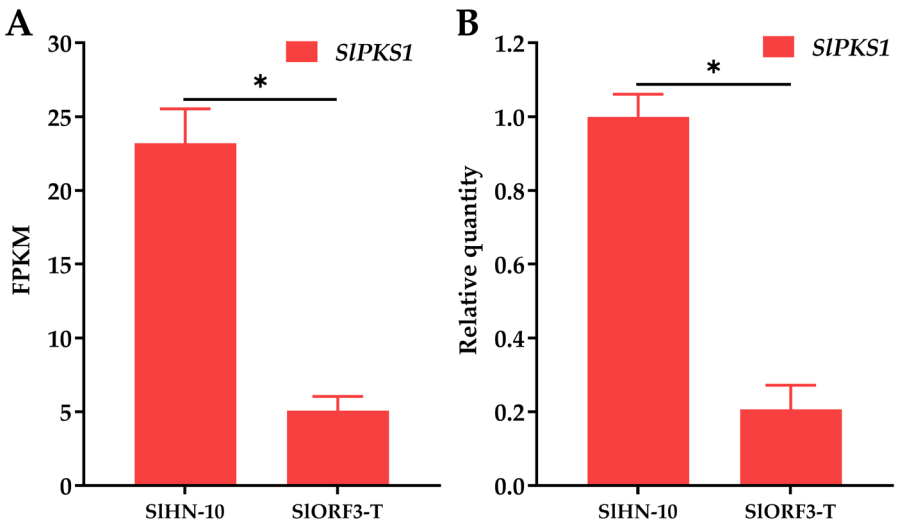


**Figure S16. *SlPKS1* expression is suppressed by transgenic expression of ORF3-SlAV1 in *S. lycopersici* strain SlHN-10 as shown in RNA-seq data (*A*) and RT-qPCR result (*B*).** FPKM, fragments per kilobase per million mapped reads. The asterisks indicate statistically significant difference based on Student's t-test ($P < 0.05$).


**Figure S17. *PKS1* deletion mutants do not induce disease resistance of tomato plants.** Spraying hyphal suspensions of Δ*SlPKS1* (***A***) or Δ*SvPKS1* mutants (***B***) does not protect plants against their virulent strains, while spraying hyphal suspensions of SlHN10-ORF3-T ***(C)*** or SvHN-02-ORF3-T (***D***) enhances plant resistance against their virulent strains. (***E, F, G, H***) represent the leaf closeup photos of (***A, B, C, D***), respectively. Photos were taken five days post inoculation.

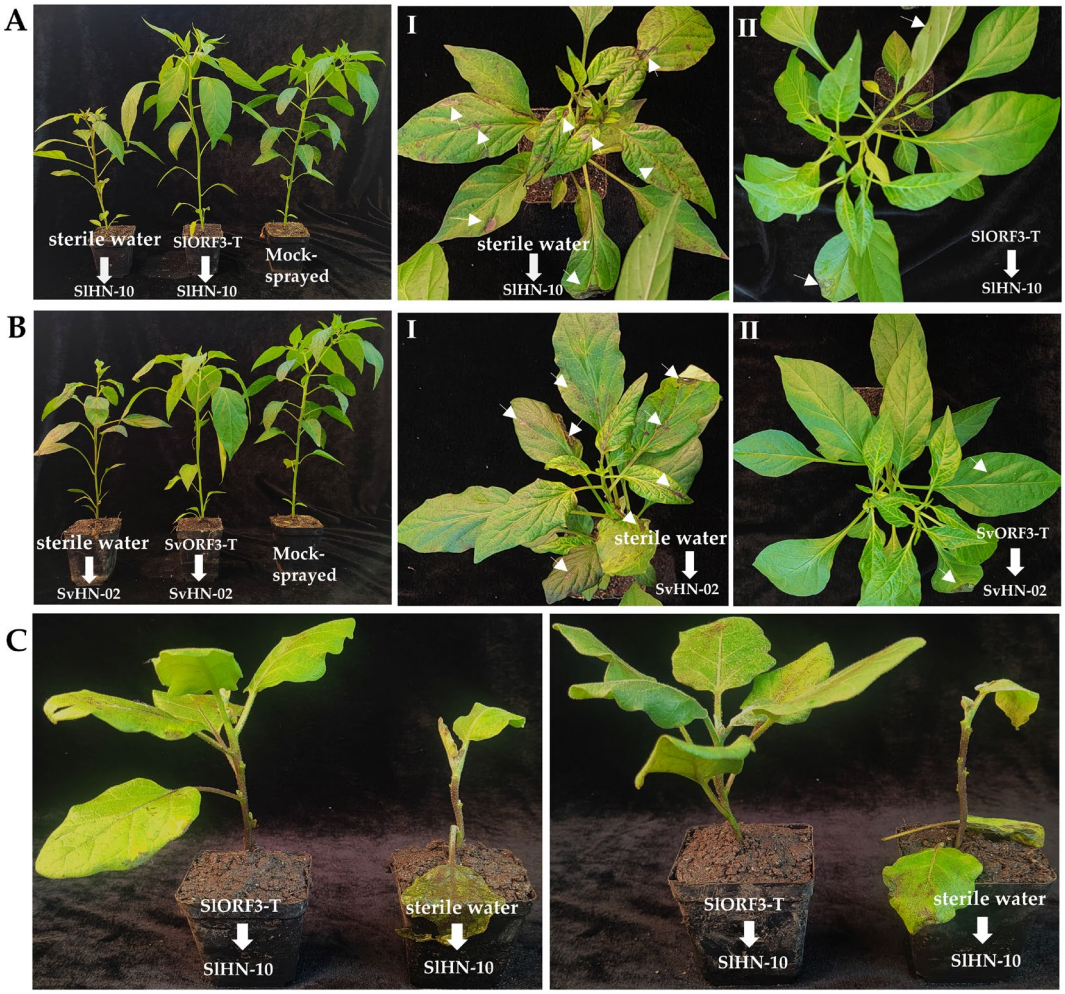


**Figure S18. Transgenic expressing ORF3 of *S. lycopersici* and *S.vesicarium* strains enhances disease resistance of pepper and eggplant plants.** Spraying hyphal suspensions of **(*A*)** SlHN-10- ORF3-T and **(*B*)** SvHN-02 -ORF3-T promoted the resistance of pepper plants to *S. lycopersici* and *S. vesicarium*, respectively, but spraying sterile water did not. **(*C*)**. Similarly, spraying hyphal fragment suspensions of the virulent strain SlHN-10 destroyed the whole eggplant plant, while spraying plants with ORF3-T promoted the resistance to virulent strain SlHN-10. (I) and (II) indicated the leaves observed from above. Typical leaf spots and lesions were induced on pepper leaves by virulent strains after spraying sterile water. However, no or very few leaf spot was observed in plants infected with virulent strains after spraying ORF3-transgenic hyphal suspensions. White arrows represent the leaf spots and lesions.

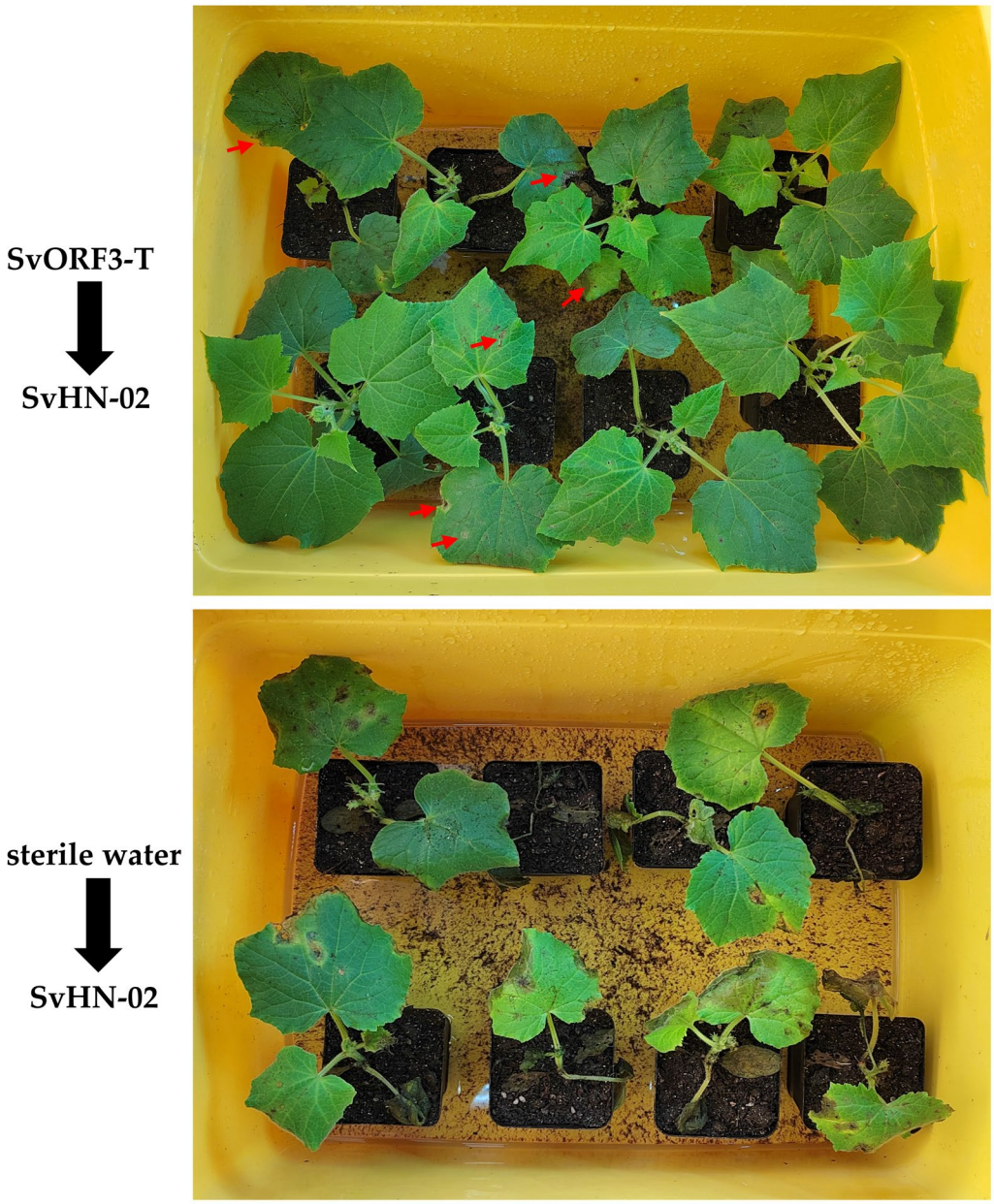


**Figure S19. Transgenic expressing ORF3 of *S. vesicarium* strain enhanced the resistance of cucumber plants.** The virulent strain SvHN-02 almost destroyed the whole cucumber plant pre-sprayed with sterile water (bottom), while pre-spraying plants with ORF3-T strain showed enhanced resistance to virulent strain SvHN-02 (top). Red arrows denote the leaf lesions.

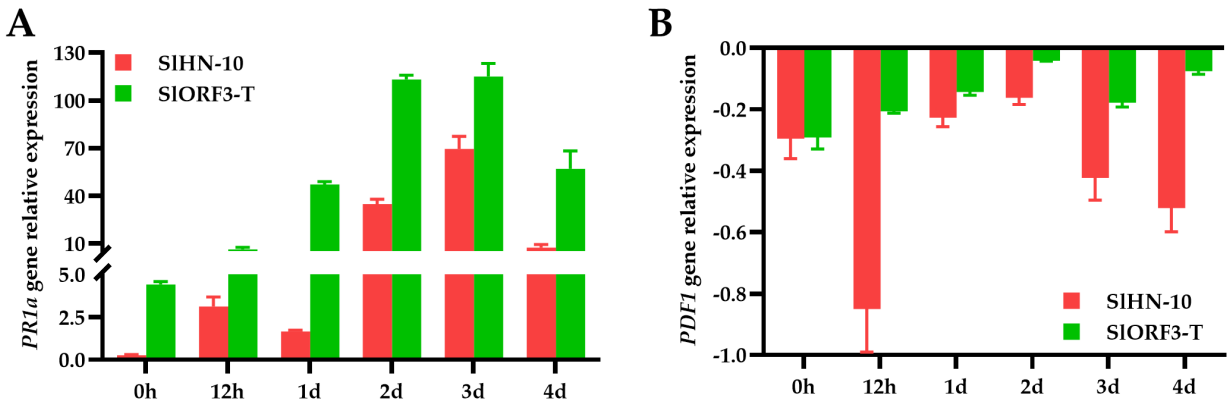


**Figure S20. RT-qPCR of *PR1a* and *PDF1* in tomato sprayed with SlHN-10 and SlORF3-T (sterile water as control). (*A*)** The salicylic acid inducible *PR1a* gene was strongly induced in plants inoculated with SlORF3-T compared with being inoculated with SlHN-10. **(*B*)** Jasmonic acid inducible *PDF1* gene of plants inoculated with ORF3-T or SlHN-10 was downregulated.

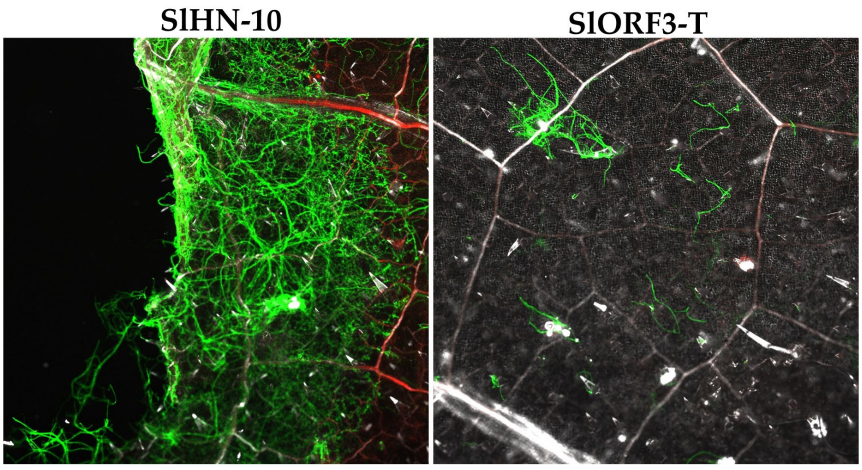


**Figure S21. Confocal microscopic observation of the fungal hyphae colonization of plant leaves (five days post inoculation) by pathogenic SlHN-10 strain (left) and ORF3-transgenic strain (right).**

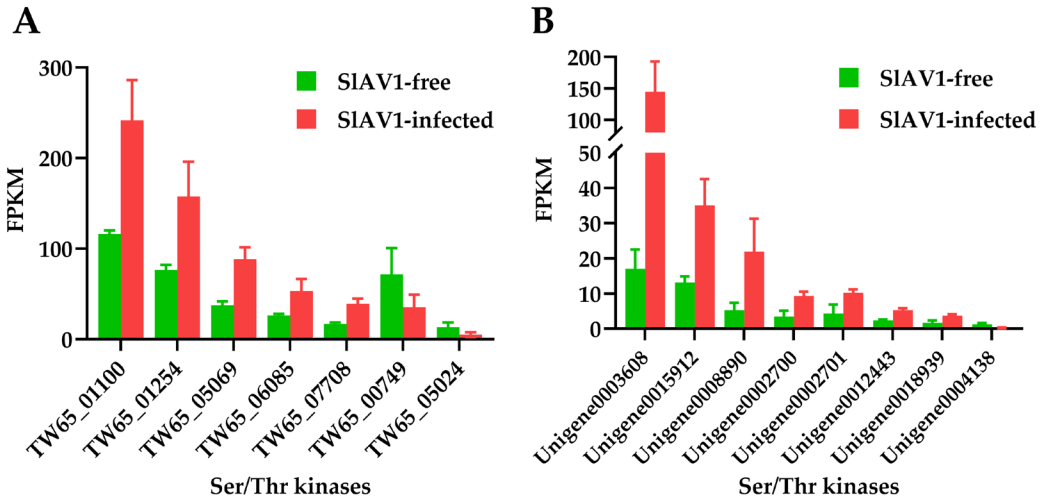


**Figure S22**. The Ser/Thr kinase expression level in response to virus-infection of SlHN-10 (***A***) and SvHN-02 (***B***).

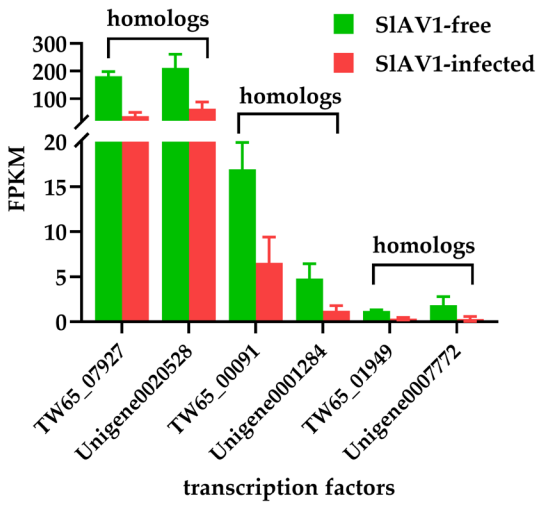


**Figure S23**. The transcription factor expression level in response to virus-infection.

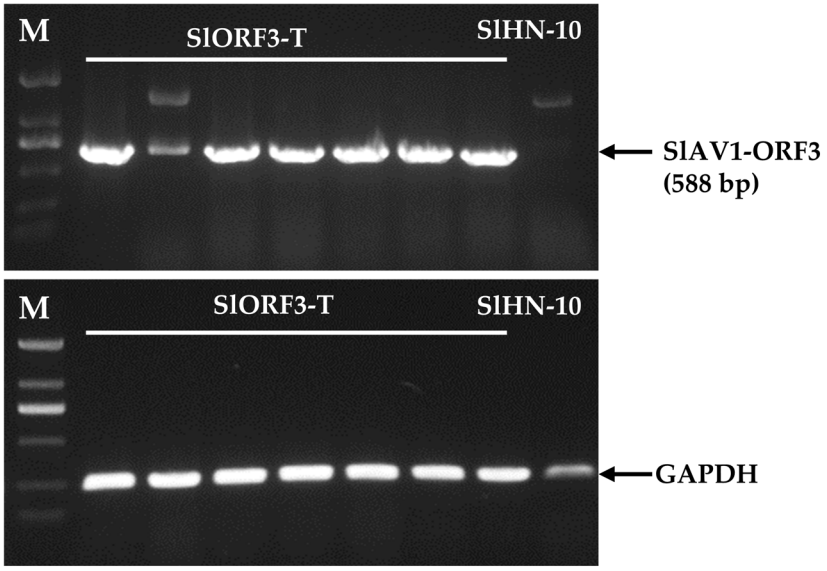


**Figure S24. A stable expression of SlAV1 ORF3 transgene in *S. lycopersici.*** After 50 generations of subculture, the SlAV1 ORF3 transcript remains detectable by RT-PCR. M:2000 bp marker.

**Table S1. Summary of results of four ORFs BLASTp.**

| No. | Species | Total score | Query Cover(%) | E value | Identity (%) | GenBank accession no. |
|---|---|---|---|---|---|---|
| ORF1 | Alternaria alternata virus 1 | 2050 | 100 | 0 | 85.38 | YP001976142.1 |
| | Cordyceps chanhua alternavirus 1 | 648 | 96 | 0 | 37.27 | YP001976142.1 |
| | Aspergillus heteromorphus alternavirus 1 | 645 | 96 | 0 | 36.40 | AZT88575.1 |
| | Aspergillus foetidus dsRNA mycovirus | 643 | 96 | 0 | 36.28 | YP007353985.1 |
| | Aspergillus mycovirus 341 | 642 | 96 | 0 | 36.19 | ABX79997.1 |
| | Fusarium solani alternavirus 1 | 619 | 97 | 0 | 37.10 | UQZ09636.1 |
| | Fusarium poae alternavirus 2 | 590 | 98 | 0 | 35.48 | UWK02066.1 |
| | Fusarium incarnatum alternavirus 1 | 597 | 95 | 0 | 35.95 | AYJ09265.1 |
| | Fusarium poae alternavirus 1 | 596 | 98 | 0 | 35.30 | YP009272952.1 |
| | Fusarium graminearum alternavirus 1 | 592 | 98 | 0 | 35.21 | YP009449439.1 |
| | Fusarium oxysporum alternavirus 1 | 416 | 57 | 7.00E-180 | 38.97 | QYY49562.1 |
| | Dactylonectria torresensis alternavirus 1 | 545 | 87 | 7.00E-171 | 36.52 | URY16699.1 |
| ORF2 | Alternaria alternata virus 1 | 1336 | 100 | 0 | 80.92 | YP001976150.1 |
| | Aspergillus heteromorphus alternavirus 1 | 118 | 59 | 4.00E-23 | 27.68 | AZT88576.1 |
| | Aspergillus foetidus dsRNA mycovirus | 117 | 59 | 8.00E-23 | 28.74 | YP_007353982.1 |
| | Cordyceps chanhua alternavirus 1 | 104 | 57 | 1.00E-18 | 27.29 | UPH33985.1 |
| | Fusarium solani alternavirus 1 | 99 | 57 | 6.00E-17 | 27.13 | UQZ09637.1 |
| | Dactylonectria torresensis alternavirus 1 | 93.6 | 50 | 3.00E-15 | 26.59 | URY16700.1 |
| | Fusarium incarnatum alternavirus 1 | 95.9 | 56 | 5.00E-16 | 26.49 | AYJ09266.1 |
| | Fusarium poae alternavirus 1 | 90.9 | 56 | 1.00E-14 | 26.98 | YP_009272949.1 |
| | Fusarium oxysporum alternavirus 1 | 86.7 | 25 | 2.00E-13 | 31.31 | QYY49563.1 |
| | Fusarium graminearum alternavirus 1 | 86.3 | 27 | 4.00E-13 | 31.38 | YP_009449446.1 |
| | Fusarium poae alternavirus 2 | 84 | 28 | 3.00E-12 | 30.86 | YP_009272949.1 |
| ORF3 | Alternaria alternata virus 1 | 1164 | 100 | 0 | 81.23 | YP_001976151.1 |
| | Aspergillus foetidus dsRNA mycovirus | 183 | 94 | 1.00E-44 | 28.45 | YP_007353983.1 |
| | Cordyceps chanhua alternavirus 1 | 109 | 25 | 3.00E-20 | 38.14 | UPH33986.1 |
| | Aspergillus heteromorphus alternavirus 1 | 179 | 94 | 4.00E-43 | 28.73 | AZT88577.1 |
| | Fusarium oxysporum alternavirus 1 | 118 | 36 | 8.00E-24 | 32.36 | QYY49564.1 |
| | Fusarium solani alternavirus 1 | 94 | 28 | 2.00E-15 | 34.55 | UQZ09638.1 |
| | Dactylonectria torresensis alternavirus 1 | 80.5 | 25 | 3.00E-11 | 34.18 | URY16701.1 |
| | Fusarium poae alternavirus 1 | 74.3 | 34 | 2.00E-09 | 30.04 | YP_009272950.1 |
| | Fusarium poae alternavirus 2 | 57.8 | 27 | 3.00E-04 | 30.66 | YP_009272950.1 |
| | Fusarium graminearum alternavirus 1 | 72 | 34 | 9.00E-09 | 29.28 | AUI80777.1 |
| | Fusarium incarnatum alternavirus 1 | 66.6 | 27 | 4.00E-07 | 29.19 | AYJ09267.1 |
| ORF4 | Alternaria alternata virus 1 | 153 | 93 | 2.00E-38 | 35.53 | YP_001976152.1 |

**Table S2. Summary of the peptide mass spectrometry analysis of the major protein band in SDS-PAGE.**

| Sequence | Modifications | # PSMs | Master Protein Accessions | # Missed Cleavages | Theo. MH+ [Da] | XCorr (by Search Engine): Sequest HT |
|---|---|---|---|---|---|---|
| AGSAAANAGAILGTDMLMAPKASVQAIMAR | 1xOxidation [M] | 8 | P3 | 1 | 2874.46343 | 10.11 |
| DHPGPLPDPAAGYDGGFGGVVPGGCGGR | 1xCarbamidomethyl [C25] | 37 | P3 | 0 | 2636.19466 | 10.09 |
| FPEDPFDAPDHVDPGTAWASVATGSADDLSR | | 5 | P3 | 0 | 3243.45014 | 9.91 |
| HPQHAWLYIPSDWTEEEVAALVSLMVEGGPAAYR | 1xOxidation [M25] | 21 | P3 | 0 | 3838.85337 | 9.59 |
| NVVKDHPGPLPDPAAGYDGGFGGVVPGGCGGR | 1xCarbamidomethyl [C29] | 8 | P3 | 1 | 3076.46938 | 9.42 |
| AGSAAANAGAILGTDMLMAPKASVQAIMAR | 2xOxidation [M18; M] | 10 | P3 | 1 | 2890.45834 | 9.39 |
| AGSAAANAGAILGTDMLMAPKASVQAIMAR | 3xOxidation [M16; M18; M28] | 3 | P3 | 1 | 2906.45326 | 9.15 |
| YAADVHLLTHRTLYESGNSLADLKDALVGAK | | 6 | P3 | 2 | 3341.74883 | 9.1 |
| LLRPAFTKYAADVHLLTHRTLYESGNSLADLK | | 37 | P3 | 2 | 3613.94893 | 9.06 |
| GDWGDFGGLGETSDVFAEHER | | 91 | P3 | 0 | 2280.97923 | 8.84 |
| AGSAAANAGAILGTDMLMAPK | | 10 | P3 | 0 | 1930.97249 | 8.83 |
| AGSAAANAGAILGTDMLMAPK | 1xOxidation [M] | 49 | P3 | 0 | 1946.96741 | 8.78 |
| LLRPAFTKYAADVHLLTHR | | 5 | P3 | 1 | 2222.25567 | 8.69 |
| AGSAAANAGAILGTDMLMAPKASVQAIMAR | | 2 | P3 | 1 | 2858.46851 | 8.69 |
| AGATRTVYGTVAHATSLIMPR | | 13 | P3 | 1 | 2173.15463 | 8.67 |
| YAADVHLLTHRTLYESGNSLADLK | | 12 | P3 | 1 | 2687.37875 | 8.57 |
| AGSAAANAGAILGTDMLMAPK | 2xOxidation [M16; M18] | 194 | P3 | 0 | 1962.96232 | 8.55 |
| TLYESGNSLADLKDALVGAKVVSR | | 2 | P3 | 2 | 2506.35114 | 8.49 |
| AGATRTVYGTVAHATSLIMPR | 1xOxidation [M19] | 10 | P3 | 1 | 2189.14955 | 8.33 |
| AGVWPHVFSDTCMPYGNYECLESGVLLGGGNETEGVGFGVQGSWK | 2xCarbamidomethyl [C12; C20]; 1xOxidation [M13] | 1 | P3 | 0 | 4864.17525 | 8.12 |
| DRGWNVAFGGDALSVSSLSAVLR | | 18 | P3 | 1 | 2377.22588 | 8.08 |
| VWAAPVVPSPGDADLVGATLAPGIDDRGPGGAGR | | 7 | P3 | 1 | 3211.64945 | 8.05 |
| GVDIVEPVTAFCSPADLGMANFPGNVLK | 1xCarbamidomethyl [C12]; 1xOxidation [M19] | 29 | P3 | 0 | 2934.43759 | 7.99 |
| VIAALEMLQSGWDVGGPPDVGLDVR | 1xOxidation [M7] | 217 | P3 | 0 | 2610.32321 | 7.95 |
| RAGSAAANAGAILGTDMLMAPK | 1xOxidation [M] | 48 | P3 | 1 | 2103.06852 | 7.93 |
| VWAAPVVPSPGDADLVGATLAPGIDDR | | 21 | P3 | 0 | 2659.37261 | 7.81 |
| RLLRPAFTKYAADVHLLTHR | | 4 | P3 | 2 | 2378.35678 | 7.77 |
| HPQHAWLYIPSDWTEEEVAALVSLMVEGGPAAYR | | 4 | P3 | 0 | 3822.85846 | 7.68 |
| VIAALEMLQSGWDVGGPPDVGLDVR | | 175 | P3 | 0 | 2594.3283 | 7.55 |
| DRGWNVAFGGDALSVSSLSAVLRR | | 4 | P3 | 2 | 2533.32699 | 7.36 |
| TLYESGNSLADLKDALVGAK | | 12 | P3 | 1 | 2065.08118 | 7.09 |
| RAGSAAANAGAILGTDMLMAPK | 2xOxidation [M17; M19] | 30 | P3 | 1 | 2119.06343 | 7.06 |
| AAAVANRAYCAPAYQGGGGER | 1xCarbamidomethyl [C10] | 2 | P3 | 1 | 2109.9883 | 7.03 |
| RAGSAAANAGAILGTDMLMAPK | | 11 | P3 | 1 | 2087.0736 | 6.96 |
| VLEDGLPSEVRYTAIGNGALLLASGR | | 4 | P3 | 1 | 2671.44135 | 6.83 |
| WGYADGDPGGDEGVNGNPLPR | | 14 | P3 | 0 | 2142.94754 | 6.72 |
| GWNVAFGGDALSVSSLSAVLR | | 67 | P3 | 0 | 2106.09783 | 6.44 |
| TRAGATRTVYGTVAHATSLIMPR | 1xOxidation [M21] | 11 | P3 | 2 | 2446.29834 | 6.39 |
| TADASPAGLLVVSNHGR | | 36 | P3 | 0 | 1664.87146 | 6.31 |
| VYVVRPPGSRLYHPYFVPVRVLEDGLPSEVR | | 7 | P3 | 2 | 3595.95362 | 6.23 |
| TVYGTVAHATSLIMPR | 1xOxidation [M14] | 62 | P3 | 0 | 1732.90507 | 6.2 |
| ALDSVARKVYFLGGSFR | | 10 | P3 | 2 | 1886.02829 | 5.91 |
| HVTVASATDVPMSFHGR | 1xOxidation [M12] | 2 | P1 | 0 | 1827.88064 | 5.78 |
| TVYGTVAHATSLIMPR | | 42 | P3 | 0 | 1716.91015 | 5.77 |
| HVTVASATDVPMSFHGR | | 1 | P1 | 0 | 1811.88573 | 5.73 |
| LNLDGWWPALIGLSVLR | | 17 | P3 | 0 | 1923.08508 | 5.7 |
| VLEDGLPSEVRYTAIGNGALLLASGRAAEVGR | | 8 | P3 | 2 | 3254.74916 | 5.64 |
| LNLDGWWPALIGLSVLRHDR | | 6 | P3 | 1 | 2331.27204 | 5.64 |
| TRAGATRTVYGTVAHATSLIMPR | | 12 | P3 | 2 | 2430.30342 | 5.59 |
| LNLDGWWPALIGLSVLRHDRIVPK | | 8 | P3 | 2 | 2768.57225 | 5.52 |
| VYVVRPPGSRLYHPYFVPVR | | 6 | P3 | 1 | 2401.32917 | 5.51 |
| DGVMPAGALWRWPGGWSNYLLIGER | 1xOxidation [M4] | 3 | P3 | 1 | 2817.39296 | 5.51 |
| YGTLGMSDDEYTAIHK | 1xOxidation [M6] | 2 | P1 | 0 | 1816.80581 | 5.5 |

| Sequence | Modifications | # PSMs | Master Protein Accessions | # Missed Cleavages | Theo. MH+ [Da] | XCorr (by Search Engine): Sequest HT |
|---|---|---|---|---|---|---|
| HAARDDFDVASAR | | 5 | P3 | 1 | 1430.67711 | 5.48 |
| QLFEWAWDPPAMEVDDPR | 1xOxidation [M12] | 29 | P3 | 0 | 2217.99098 | 5.45 |
| QLFEWAWDPPAMEVDDPR | | 7 | P3 | 0 | 2201.99607 | 5.44 |
| YTAIGNGALLLASGR | | 76 | P3 | 0 | 1476.8169 | 5.41 |
| VIAALEMLQSGWDVGGPPDVGLDVRHAAR | 1xOxidation [M7] | 2 | P3 | 1 | 3045.55746 | 5.36 |
| GVDIVEPVTAFCSPADLGMANFPGNVLK | 1xCarbamidomethyl [C12] | 6 | P3 | 0 | 2918.44268 | 5.26 |
| HPGFGAVFHWGFSR | | 2 | P2 | 0 | 1601.77604 | 5.25 |
| YTAIGNGALLLASGRAAEVGRASGVI | | 4 | P3 | 2 | 2487.3678 | 5.1 |
| KVYFLGGSFRLDATPDR | | 6 | P3 | 2 | 1942.01812 | 5.01 |
| GWNVAFGGDALSVSSLSAVLRRMVEAYGQR | 1xOxidation [M23] | 3 | P3 | 2 | 3212.62694 | 4.98 |
| VYLDAARAAAVANRAYCAPAYQGGGGER | 1xCarbamidomethyl [C17] | 2 | P3 | 2 | 2898.40638 | 4.95 |
| GGATNAEAGLQVGSFR | | 4 | P1 | 0 | 1534.76084 | 4.93 |
| QSLRALDSVARK | | 2 | P3 | 2 | 1343.77537 | 4.82 |
| YTAIGNGALLLASGRAAEVGR | | 6 | P3 | 1 | 2060.12471 | 4.81 |
| AAQLAAIELMTR | 1xOxidation [M10] | 26 | P3 | 0 | 1303.70385 | 4.79 |
| YAADVHLLTHR | | 61 | P3 | 0 | 1295.68549 | 4.78 |
| AFSHDVPLDVPEK | | 4 | P1 | 0 | 1453.73217 | 4.71 |
| TADASPAGLLVVSNHGRQLFEWAWDPPAMEVDDPR | 1xOxidation [M29] | 4 | P3 | 1 | 3863.8446 | 4.7 |
| AAQLAAIELMTR | | 11 | P3 | 0 | 1287.70893 | 4.68 |
| GSATTLRRAGSAAANAGAILGTDMLMAPK | 1xOxidation [M] | 4 | P3 | 2 | 2789.43966 | 4.64 |
| LYHPYFVPVRVLEDGLPSEVR | | 7 | P3 | 1 | 2485.32381 | 4.64 |
| WPGGWSNYLLIGER | | 16 | P3 | 0 | 1647.8278 | 4.63 |
| TEEVWGTLGAYPWGDLDTLVGQSTR | | 1 | P1 | 0 | 2751.32605 | 4.57 |
| GFPPRAVYACASAGPSMAASGGFDPVER | 1xCarbamidomethyl [C10] | 9 | P1 | 1 | 2825.31339 | 4.4 |
| GSATTLRRAGSAAANAGAILGTDMLMAPK | | 2 | P3 | 2 | 2773.44474 | 4.29 |
| IPAIPVVPWNTGGGLR | | 4 | P2 | 0 | 1646.93769 | 4.24 |
| EVAPTAVDYVPR | | 35 | P3 | 0 | 1316.68449 | 4.23 |
| DVLEVIQSASAPGR | | 2 | P1 | 0 | 1441.76453 | 4.21 |
| VVSRFPPAYR | | 10 | P3 | 1 | 1191.6633 | 4.13 |
| DGVMPAGALWRWPGGWSNYLLIGER | | 2 | P3 | 1 | 2801.39805 | 4.01 |
| GWNVAFGGDALSVSSLSAVLRR | | 1 | P3 | 1 | 2262.19894 | 4 |
| QSLRALDSVAR | | 4 | P3 | 1 | 1215.68041 | 4 |
| ELLGPGIAPPGSSK | | 2 | P1 | 0 | 1322.73144 | 3.99 |
| TLYESGNSLADLK | | 51 | P3 | 0 | 1410.7111 | 3.98 |
| RMVEAYGQRVYLDAAR | 1xOxidation [M2] | 2 | P3 | 2 | 1913.96504 | 3.93 |
| RAAVDGAGAPAVR | | 8 | P3 | 1 | 1210.66509 | 3.86 |
| RMVEAYGQRVYLDAAR | | 2 | P3 | 2 | 1897.97013 | 3.78 |
| AAQLAAIELMTRGDWGDFGGLGETSDVFAEHER | 1xOxidation [M10] | 2 | P3 | 1 | 3565.66524 | 3.74 |
| AHDRLMGKPLHTWELQGLLWSLSHIGTQEEREVYFWGK | | 3 | P2 | 2 | 4548.30337 | 3.72 |
| AAMDQLLRPMYAK | | 2 | P1 | 0 | 1507.77597 | 3.71 |
| VYFLGGSFRLDATPDRR | | 2 | P3 | 2 | 1970.02427 | 3.71 |
| AAEVGRASGVI | | 2 | P3 | 1 | 1029.56873 | 3.7 |
| KVYFLGGSFR | | 29 | P3 | 1 | 1173.6415 | 3.69 |
| AAVDGAGAPAVRQSLRALDSVAR | | 5 | P3 | 2 | 2251.22655 | 3.68 |
| EAYAVANAWVR | | 265 | P3 | 0 | 1249.6324 | 3.66 |
| REDGTSGRLAVPAR | | 4 | P3 | 2 | 1484.79281 | 3.65 |
| GSATTLRRAGSAAANAGAILGTDMLMAPK | 2xOxidation [M24; M26] | 2 | P3 | 2 | 2805.43457 | 3.62 |
| VVGAAGYLSLLGLR | | 2 | P2 | 0 | 1388.82601 | 3.62 |
| FLRGHLGPAGAWMNLMPASFAPWLNQAVNFVLFQAFATGVR | 1xOxidation [M] | 10 | P1 | 1 | 4519.3107 | 3.61 |
| AAVDGAGAPAVR | | 29 | P3 | 0 | 1054.56398 | 3.6 |
| VLEDGLPSEVR | | 75 | P3 | 0 | 1213.64229 | 3.59 |
| RVYVVRPPGSR | | 2 | P3 | 1 | 1285.74876 | 3.55 |
| ASVQAIMARVIAALEMLQSGWDVGGPPDVGLDVR | 1xOxidation [M7] | 1 | P3 | 1 | 3537.81923 | 3.55 |
| LYHPYFVPVR | | 22 | P3 | 0 | 1290.69935 | 3.51 |
| MVEAYGQRVYLDAARAAAVANR | 1xOxidation [M1] | 2 | P3 | 2 | 2411.22484 | 3.46 |

| Sequence | Modifications | # PSMs | Master Protein Accessions | # Missed Cleavages | Theo. MH+ [Da] | XCorr (by Search Engine): Sequest HT |
|---|---|---|---|---|---|---|
| GFPPRAVYACASAGPSMAASGGFDPVERLSSLSGALDR | 1xCarbamidomethyl [C10] | 5 | P1 | 2 | 3824.8483 | 3.46 |
| RMVEAYGQR | 1xOxidation [M2] | 14 | P3 | 1 | 1125.54695 | 3.46 |
| AQGEHPDLGAIPK | | 2 | P1 | 0 | 1332.69064 | 3.43 |
| QHQFSANFLNAEDLTGWSDRDSLK | | 8 | P1 | 1 | 2779.30705 | 3.43 |
| MVEAYGQRVYLDAAR | 1xOxidation [M1] | 2 | P3 | 1 | 1757.86393 | 3.42 |
| AAVDGAGAPAVRQSLR | | 3 | P3 | 1 | 1538.83976 | 3.41 |
| ASVQAIMAR | | 50 | P3 | 0 | 946.51386 | 3.39 |
| EDGTSGRLAVPARNVVK | | 1 | P3 | 2 | 1768.96642 | 3.37 |
| DGVMPAGALWR | 1xOxidation [M4] | 52 | P3 | 0 | 1188.583 | 3.36 |
| SSVADTALNR | | 2 | P2 | 0 | 1033.52726 | 3.36 |
| STLESNTFYSR | | 2 | P1 | 0 | 1304.61172 | 3.34 |
| REDGTSGR | | 55 | P3 | 1 | 877.41223 | 3.33 |
| ASVQAIMAR | 1xOxidation [M7] | 59 | P3 | 0 | 962.50878 | 3.29 |
| LSSLSGALDR | | 2 | P1 | 0 | 1018.55275 | 3.23 |
| VSVRREDGTSGR | | 2 | P3 | 2 | 1318.6822 | 3.22 |
| AAFDAAFGVLGR | | 2 | P2 | 0 | 1194.62658 | 3.21 |
| YTDQVDALIR | | 2 | P1 | 0 | 1193.61608 | 3.18 |
| VYFLGGSFRLDATPDR | | 3 | P3 | 1 | 1813.92316 | 3.18 |
| EEMRDYSVR | | 8 | P3 | 1 | 1184.53645 | 3.16 |
| RMVEAYGQR | | 8 | P3 | 1 | 1109.55204 | 3.13 |
| AYCAPAYQGGGGER | 1xCarbamidomethyl [C3] | 8 | P3 | 0 | 1456.62739 | 3.12 |
| GLADLQGSAGR | | 2 | P1 | 0 | 1044.54325 | 3.12 |
| GVQGPPAEGMLAK | | 2 | P1 | 0 | 1254.65108 | 3.1 |
| DDFDVASAR | | 208 | P3 | 0 | 995.44286 | 3.08 |
| DAAVVGFLDR | | 2 | P1 | 0 | 1062.55783 | 3.08 |
| DIGTAFTSDRVSVR | | 4 | P3 | 1 | 1523.78125 | 3.07 |
| FLRGHLGPAGAWMNLMPASFAPWLNQAVNFVLFQAFATGVR | | 3 | P1 | 1 | 4503.31579 | 3.07 |
| DIGTAFTSDRVSVRR | | 2 | P3 | 2 | 1679.88236 | 3.07 |
| ADDVLEVYR | | 2 | P1 | 0 | 1079.53677 | 3.06 |
| GPGGAGRAPPHFRTADASPAGLLVVSNHGR | | 2 | P3 | 2 | 2922.51938 | 3.06 |
| ITDYAGQQSGR | | 2 | P1 | 0 | 1195.57019 | 3.04 |
| AYCAPAYQGGGGERDIGTAFTSDR | 1xCarbamidomethyl [C3] | 2 | P3 | 1 | 2520.12083 | 3.04 |
| SVAGCLLALLR | 1xCarbamidomethyl [C5] | 2 | P2 | 0 | 1172.68199 | 3.03 |
| DGVMPAGALWR | | 17 | P3 | 0 | 1172.58809 | 3.02 |
| VLPLAAMPILASR | | 2 | P2 | 0 | 1351.813 | 2.97 |
| LLRPAFTK | | 17 | P3 | 0 | 945.58801 | 2.96 |
| ALYLEAEDYYR | | 2 | P1 | 0 | 1405.66342 | 2.96 |
| RLLRPAFTK | | 2 | P3 | 1 | 1101.68912 | 2.94 |
| FALATFEYR | | 2 | P1 | 0 | 1117.56767 | 2.94 |
| LDATPDRR | | 13 | P3 | 1 | 943.49557 | 2.92 |
| LASCFLR | 1xCarbamidomethyl [C4] | 30 | P3 | 0 | 866.45528 | 2.91 |
| ADADQLTR | | 2 | P1 | 0 | 889.43739 | 2.9 |
| VLPLAAMPILASR | 1xOxidation [M7] | 1 | P2 | 0 | 1367.80792 | 2.89 |
| DIGTAFTSDR | | 29 | P3 | 0 | 1082.51128 | 2.87 |
| HGGQVREEMR | | 2 | P3 | 1 | 1198.57456 | 2.85 |
| EEMRDYSVRTR | | 2 | P3 | 2 | 1441.68524 | 2.85 |
| QALTGAITR | | 2 | P1 | 0 | 930.53671 | 2.83 |
| LDATPDRRVYVVRPPGSR | | 1 | P3 | 2 | 2054.12538 | 2.81 |
| LLLVQVMR | | 1 | P1 | 0 | 971.60703 | 2.77 |
| AAMDQLLRPMYAK | 1xOxidation [M] | 3 | P1 | 0 | 1523.77088 | 2.75 |
| VYFLGGSFR | | 18 | P3 | 0 | 1045.54654 | 2.73 |
| YSANMTDLR | | 2 | P1 | 0 | 1070.49352 | 2.69 |
| EVAPTAVDYVPRSEPYSVR | | 1 | P3 | 1 | 2135.07676 | 2.69 |
| LQGAADMLLASR | 1xOxidation [M7] | 2 | P2 | 0 | 1261.6569 | 2.68 |
| VGAPADIPGSMPLDYVVSLAETILPPAYVEAAK | | 1 | P4 | 0 | 3354.75416 | 2.66 |

| Sequence | Modifications | # PSMs | Master Protein Accessions | # Missed Cleavages | Theo. MH+ [Da] | XCorr (by Search Engine): Sequest HT |
|---|---|---|---|---|---|---|
| NGVEELR | | 2 | P2 | 0 | 816.42101 | 2.66 |
| GPGGAGRAPPHFR | | 1 | P3 | 1 | 1276.66576 | 2.66 |
| LLLVQVMR | 1xOxidation [M7] | 2 | P1 | 0 | 987.60195 | 2.64 |
| NRYGLIGVDVGETPVEMFKR | 1xOxidation [M17] | 2 | P1 | 2 | 2296.17543 | 2.64 |
| LMGKPLHTWELQGLLWSLSHIGTQEER | 1xOxidation [M2] | 1 | P2 | 0 | 3175.63571 | 2.62 |
| VYVVRPPGSR | | 11 | P3 | 0 | 1129.64765 | 2.61 |
| TVVAEVDDGLTRSEVDGWLAEVNWAGRPVVAALLDIDQGGRR | | 1 | P2 | 2 | 4505.31716 | 2.61 |
| EEMRDYSVR | 1xOxidation [M3] | 5 | P3 | 1 | 1200.53136 | 2.6 |
| GVQGPPAEGMLAK | 1xOxidation [M10] | 2 | P1 | 0 | 1270.646 | 2.6 |
| DAELAEGER | | 3 | P1 | 0 | 989.45343 | 2.6 |
| ALDSVARK | | 2 | P3 | 1 | 859.49959 | 2.59 |
| SLLLLGFR | | 2 | P1 | 0 | 918.57711 | 2.55 |
| MVEAYGQR | 1xOxidation [M1] | 23 | P3 | 0 | 969.44584 | 2.54 |
| VNAGGYSFLK | | 2 | P2 | 0 | 1055.55202 | 2.52 |
| LDATPDR | | 13 | P3 | 0 | 787.39446 | 2.51 |
| GLGFEGGGK | | 2 | P2 | 0 | 821.41519 | 2.5 |
| ELAQAGYR | | 2 | P2 | 0 | 907.46321 | 2.48 |
| YGVLNSDHK | | 2 | P1 | 0 | 1032.51088 | 2.45 |
| EEMRDYSVRTR | 1xOxidation [M3] | 2 | P3 | 2 | 1457.68015 | 2.44 |
| LAVPARNVVK | | 3 | P3 | 1 | 1066.67314 | 2.44 |
| GSATTLR | | 9 | P3 | 0 | 705.38898 | 2.44 |
| MVEAYGQR | | 4 | P3 | 0 | 953.45093 | 2.42 |
| WDGAQRRAAVDGAGAPAVR | | 1 | P3 | 2 | 1923.98962 | 2.41 |
| TQGAASADLNR | | 2 | P2 | 0 | 1103.54398 | 2.4 |
| WDGAQR | | 4 | P3 | 0 | 732.34236 | 2.37 |
| VLAAWDYSK | | 2 | P1 | 0 | 1052.54112 | 2.36 |
| LASCFLRHGGQVR | 1xCarbamidomethyl [C4] | 1 | P3 | 1 | 1500.78523 | 2.35 |
| AFVELLGR | | 2 | P2 | 0 | 904.52508 | 2.34 |
| DVLEVIQSASAPGREFAAEWGWLPLDVR | | 1 | P1 | 1 | 3111.58981 | 2.32 |
| ALDSVAR | | 21 | P3 | 0 | 731.40463 | 2.31 |
| TLLATDLR | | 2 | P1 | 0 | 902.53056 | 2.28 |
| GSGYWVAR | | 2 | P1 | 0 | 895.44208 | 2.26 |
| NAGWLR | | 1 | P1 | 0 | 716.38383 | 2.26 |
| LSADQVR | | 2 | P1 | 0 | 788.42609 | 2.22 |
| MAWMSGGAVGR | 1xOxidation [M1] | 1 | P1 | 0 | 1138.51321 | 2.22 |
| VYLDAAR | | 7 | P3 | 0 | 807.43593 | 2.21 |
| EGSPSEYLSR | | 1 | P1 | 0 | 1124.52184 | 2.21 |
| EDGTSGR | | 3 | P3 | 0 | 721.31112 | 2.18 |
| DLVLEYVQR | | 2 | P1 | 0 | 1134.61535 | 2.18 |
| HDRIVPK | | 1 | P3 | 1 | 864.50501 | 2.08 |
| TGVYFR | | 1 | P1 | 0 | 742.38825 | 2.07 |
| FPPAYR | | 1 | P3 | 0 | 750.39334 | 2.02 |
| RGSGYWVAR | | 1 | P1 | 1 | 1051.54319 | 2.01 |
| WWPSLVPVLIR | | 1 | P1 | 0 | 1365.80415 | 1.95 |
| APPHFR | | 1 | P3 | 0 | 724.38892 | 1.93 |
| GSATTLRR | | 1 | P3 | 1 | 861.49009 | 1.93 |

**Table S3. Summary of Altersolanol A production in various *S. lycopersici*, *S.vesicarium* and *A. solani* strains, each normalized by inherent ergosterol production proportional to fungal biomass.**

| Strain | Altersolanol A (μg/mL) | Ergosterol (μg/mL) | Altersolanol A/ Ergosterol |
|---|---|---|---|
| SlHN-03 | ND | 22.747±3.120 | ND |
| SlHN-03-FV | 42.525±4.485 | 72.193±8.202 | 0.589±0.145 [a] |
| SlHN-10 | 197.670±8.496 | 36.634±1.879 | 5.396±0.047[b] |
| SlHN-10+V | 5.500±0.053 | 26.402±2.161 | 0.208±0.017[e] |
| Δ*Slpks1* | ND | 40.403±3.215 | ND |
| Δ*Slpks1-Com* | 186.346±5.793 | 42.326±1.111 | 4.403±0.037[d] |
| SlHN-10-OE-PKS1 | 343.49±30.255 | 31.217±2.762 | 11.003±0.174[a] |
| SlHN-10-EV | 173.622±8.496 | 35.177±2.509 | 4.936±0.177[c] |
| SlHN-10-ORF1-T | 189.61±9.262 | 38.698±0.796 | 4.900±0.143[c] |
| SlHN-10-ORF2-T | 176.457±23.656 | 39.874±1.857 | 4.425±0.388[d] |
| SlHN-10-ORF3-T | 0.578±0.106 | 15.497±2.978 | 0.037±0.001[e] |
| SlHN-10-ORF4-T | 161.157±5.832 | 34.893±2.199 | 4.619±0.125[d] |
| SvHN-02 | 55.002±3.041 | 53.174±1.215 | 1.034±0.036[a] |
| SvHN-02+V | 0.495±2.010 | 50.077±4.105 | 0.077±0.025[c] |
| Δ*Svpks1* | ND | 55.094±2.701 | ND |
| Δ*Svpks1-Com* | 54.383±2.614 | 59.694±1.990 | 0.911±0.028[b] |
| SvHN-02-ORF3-T | ND | 53.24±2.219 | ND |
| ZYAS-11 | 65.485±3.663 | 7.116±0.266 | 9.203±0.342[a] |
| ZYAS-11-EV | 65.560±2.778 | 7.421±0.212 | 8.834±0.260[a] |
| AsPKS1-RNAi-T | ND | 9.386±0.292 | ND |
| PSB blank | ND | ND | ND |

**Table S4. Primers used for PCR analysis in this study.**

| Primer name | Primer sequence (5'-3') | Purpose |
|---|---|---|
| ORF1-check-F | GCGTGGTTCAGGCTATTGG | RT-PCR detection for virus-infection and expression of four ORFs |
| ORF1-check-R | TCTACCGAGCACGGAAATGA | |
| ORF2-check-F | TAGGTCTGTTGCTGGATGTTTG | |
| ORF2-check-R | CCGTGTCTGCGACTGATGAA | |
| ORF3-check-F | TCCGTCCTGCCTTTACCA | |
| ORF3-check-R | CAGAAGCCCTACCAACCTCA | |
| ORF4-check-F | GGAGGAGTCTTGTTCTGGCAC | |
| ORF4-check-R | TTGACGACGCAGGGTATGG | |
| Svgapdh-F | TCAACGGCAAGACCATCC | GAPDH for *Stemphylium vesicarium* |
| Svgapdh-R | CCGCCCTTCAAGTGAGC | |
| Slgapdh-F | GTCAACGGCAAGACCATCC | GAPDH for *S. lycopersici* |
| Slgapdh-R | CCGCCCTTCAAGTGAGC | |
| Asactin-F | AGCCTTCCGTCTTGGGTCT | Actin for *Alternaria solani* |
| Asactin-R | AGGGCGGTGATTTCCTTCTG | |
| AsPKS1-RNAi-F1 | CAAGTTCCTGGGACAGTATGG | RT-qPCR for silencing transformants |
| AsPKS1-RNAi-R1 | GCAAAGCGTAATGAAGTGGG | |
| AsPKS1-RNAi-F2 | ACCGTTGCTTTCGCCTTTAC | |
| AsPKS1-RNAi-R2 | GATGCCTAGATCAAATGACTGTAC | |
| TW65_03508-F | GCTGCTTCCGTGTCCCTCA | RT-qPCR for PKSs |
| TW65_03508-R | GGTTACCGTCCGCATCTGG | |
| Unigene0000727-F | GTCTACATCTCCAGCCCACAAAG | |
| Unigene0000727-R | TCTCCCAGGGTCGGAACTTACT | |
| hph-F | ATGAAAAAGCCTGAACTCACC | Amplification, detection and labeled probe |
| hph-R | TACACATGGGGATCAGCAATC | |
| SlPKS1-check-F | TCCGCAACTCCGTTATCC | |
| SlPKS1-check-R | GCGTCAGCCGAATGGT | |
| SvPKS1check-F | CACGAGGATGCCCAGTTAG | |
| SvPKS1check-R | GACGGTGGTTGGCTTCAGTA | |
| Neo-F | CTCTTCAGCAATATCACGGGTA | Detection of the neomycin segment |
| Neo-R | GGCTATGACTGGGCACAACA | |
| Ef1α-F | GATTGACAGGCGTTCAGGTAAG | RT-qPCR for SA and JA marker gene |
| Ef1α-R | CCCAATGGAGGGTATTCAGC | |
| PR1a-F | GAGGGCAGCCGTGCAA | |
| PR1a-R | CACATTTTTCCACCAACACATTG | |
| PDF1-F | GGACCATGTGTGAGTGAGAAG | |
| PDF1-R | GCCTAGTGCAAAAGCAACGG | |

**Table S5. Primers used for vector construction in this study.**

| | | |
|---|---|---|
| SlPKS1-UF | CGGggtaccGCATCACACATCCATCACAT | Construction of the targeted gene deletion vector |
| SlPKS1-UR | CCGgaattcCGTTGTGTTGAATAGATGACTG | |
| SlPKS1-DF | CGCggatccTTTCCGTCCACTAAGGTCAT | |
| SlPKS1-DR | CTAGtctagaCACACTATGGGAGGTAAGCAG | |
| SvPKS1-UF | CCCaagcttTGACGGTTGAATTGGTGGT | |
| SvPKS1-UR | CCGgaattcGCTCATGTCGGAGTTGGTT | |
| SvPKS1-DF | CGCggatccTTTCTCCTCTCATTCTTCAATCA | |
| SvPKS1-DR | CTAGtctagaCCAAACACAGGCATCACTTTA | |
| KSTNP-SlPKS1-HB-F | TGGGTACCCGGGggatccATGAGCAGTAGCAAAGCCGAT | Construction of the complementation vector |
| KSTNP-SlPKS1-HB-R | GATGATTTCAGTAACGTTAAGTggatccTCTGCTCATCGATTCTCTTCCA | |
| KSTNP-SvPKS1-HB-F | TGGGTACCCGGGggatccATCGTTGTTAGCCAGCATGT | |
| KSTNP-SvPKS1-HB-F | GATGATTTCAGTAACGTTAAGTggatccTCGGTTCTCTTCCAGCCACT | |
| ORF1-T-F | TGGCGGCCGCTCTAGAactagtATGCGAAGTGACAAATCC | Construction of the overexpression vector |
| ORF1-T-R | AGCCCGGGGGATCCactagtATGTAGCACCACTAAGGG | |
| ORF2-T-F | TGGCGGCCGCTCTAGAactagtATGTCTTTTTCCGTTTCTGA | |
| ORF2-T-R | AGCCCGGGGGATCCactagtTCCGATCCTGCGCG | |
| ORF3-T-F | TGGCGGCCGCTCTAGAactagtATGGCGACGTTTGGAAGTG | |
| ORF3-T-R | AGCCCGGGGGATCCactagtGATGACACCAGAAGCCCTAC | |
| ORF4-T-F | TGGCGGCCGCTCTAGAactagtATGTTTTGCGATTTTGAGCC | |
| ORF4-T-R | AGCCCGGGGGATCCactagtATCAGCAGCAGAAGAGCCTCC | |
| OESlPKS1-F | TGGCGGCCGCTCTAGAactagtATGCCTTCTTCAAGTTCC | |
| OESlPKS1-R | AGCCCGGGGGATCCactagtTCTGCTCATCGATTCTCTTCCA | |
| AsPKS1-RNAi-ZF | CCctcgagCCAGCGACGACATCAATCA | Construction of the RNAi vector |
| AsPKS1-RNAi-ZR | CCCaagcttATAGACACGAACCGTATCACAA | |
| AsPKS1-RNAi-FF | GGggtaccCCAGCGACGACATCAATCA | |
| AsPKS1-RNAi-FR | GAaggcctATAGACACGAACCGTATCACAA | |

## References


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