TABLE S1 List of primers used in this study

Primer	**Sequence 5′ to 3′**	**Description**	**PCR**[a]
primer-1	GCGTCGATGCAGATTCTTACTTA	flanking region upstream of s*ol4* FP[b]	838
primer-2	TTGACCTCCACTAGCTCCAGCCAAGCCCATCGTGTTGTACTCATGTGGTT	flanking region upstream of *sol4* RP[c] with overhang sequence of 5'end of *hph*	
primer-3	GCAAAGGAATAGAGTAGATGCCGACCGTTAGGCGATGGAGTAGAGAGAAG	flanking region downstream of *sol4* RP with overhang sequence of 3'end of *hph*	1,094
primer-4	CATCCAAGCGAGCGAGAATGGAG	flanking region downstream of *sol4* FP	
HYG-F	GGCTTGGCTGGAGCTAGTGGAG	FP to amplify *hph* from pDWJ5	1,372
HYG-R	CGGTCGGCATCTACTCTATTCCTT	RP to amplify *hph* from pDWJ5	
primer-7	AGAGTAAGCGGAGCGGATCAAAG	flanking region upstream of *sol4* FP nested primer	
primer-8	CCAGAAGTGGACCTCTGATGCGA	flanking region downstream of *sol4* RP nested primer	
YG-F	CGATGTAGGAGGGCGTGGATATGTCC	FP coding region of *hph* for overlapping extension, pair with primer-8	
HY-R	GTATTGACCGATTCCTTGCGGTCCGAA	RP coding region of *hph* for overlapping extension, pair with primer-7	
primer-11	GTGTGTCCACCTGCCTATGTATC	FP upstream of *sol4*, verification primer	1,950 (in wild type)
primer-12	CACGCTTCTTCCCGTGCATCTG	RP downstream of *sol4*, verification primer	1,581 (in *sol4*)
Sol4BamH5	GGATCCATGATGCCGTCCACCCTCATC	FP at ATG with *Bam*HI cut site for 4OE construction	2,029
Sol4Hind3	AAGCTTTCGCAAGGTCTGGGAAATTC	RP at +276 bp from stop codon with *Hin*dIII cut site for 4OE construction	

[a] PCR = PCR product length in basepair; [b] FP = forward primer; [c] RP = reverse primer

TABLE S1 Continued

Primer	Sequence 5′ to 3′	Description	PCR[a]
Sol4OE5	GTTGTGTGTCCACCTGCCTATG	FP at +92 bp from ATG for confirmation of 4OE strains	2,187 (with Sol4OE5 and Sol4OE3 primer pair)
Sol4OE3	CGGGAAGCTGCGAGAAGATAAG	4OE RP at +354 bp from stop codon for confirmation of 4OE strains	
DW38	AGATGGTCAACGCTGCTTAC	*pelA* promoter primer for confirmation of 4OE strains, 141 bp away from *Pvu*II site	≈ 2,200 (with DW38 and Sol4OE3 primer pair)
Sol1RT-F	TTGGTATTGGTTCGCTCGAGGT	RT-PCR for *sol1*	530 (615, for gDNA or pre-mRNA)
Sol1RT-R	TCAACAGCGGTTGACATCCTCT		
Sol2RT-F	CACATCTCCATGGCTTTGGCTC	RT-PCR for *sol2*	390 (470, for gDNA or pre-mRNA)
Sol2RT-R	GTTCGCTGCTTAGCACCCAGAA		
Sol3RT-F	GTTCGCCTTGATGGCAAGACTG	RT-PCR for *sol3*	572 (622, for gDNA or pre-mRNA)
Sol3RT-R	CGCGCATCCAGAGGATGTTCAA		
Sol4RT-F	TGGATCAACCAGATCGTCCATC	RT-PCR for *sol4*	522 (588, for gDNA or pre-mRNA)
Sol4RT-R	CTTACGGTGCAGTACGCATCTA		
Sol5RT-F	AGAACCCAGCGTGCATCTATAC	RT-PCR for *sol5*	534 (631, for gDNA or pre-mRNA)
Sol5RT-R	GATCATGGAACCTCCCCATATC		
Sol6RT-F	GCAAAGTGCTAACACCCGCTCT	RT-PCR for *sol6*	540 (606, for gDNA or pre-mRNA)
Sol6RT-R	CGTTTAGCTGTTCTAGGCTTGG		

[a] PCR = PCR product length in basepair.

TABLE S1 Continued

Primer	**Sequence 5′ to 3′**	**Description**	**PCR**[a]
Actin1RT-F	CAATGGTTCGGGTATGTGCAAG	RT-PCR for *Actin1*	482 (633, for gDNA or pre-mRNA)
Actin1RT-R	GAAGAGCGAAACCCTCGTAGAT		
Sol1realt-F	GTTGGCATGGGCTGTAGATG	Real-time RT-PCR for *sol1*	129
Sol1realt-R	ATGGTGGAATCCCTTGCGAG		
Sol2realt-F	CACTACATGCTCGATGAATGC	Real-time RT-PCR for *sol2*	123
Sol2realt-R	GAACATTGGCACACCGAAG		
Sol3realt-F	CGTTCAGGTTAATCACCTGG	Real-time RT-PCR for *sol3*	126
Sol3realt-R	CAGAAGTGGACCTCTGATG		
Sol4realt-F	CAACCTTCGCCTTGCAAAAG	Real-time RT-PCR for *sol4*	140
Sol4realt-R	GAAGTACTCTCTCGGTGAAC		
Sol5realt-F	CGCGAACAATTTTGGCATTG	Real-time RT-PCR for *sol5*	162
Sol5realt-R	ATGGCAGACTTCTTGTCCAG		
Sol6realt-F	CAAGCAATACGGACCGGTG	Real-time RT-PCR for *sol6*	135
Sol6realt-R	GGAAACGAGATGCATCGATAG		
ORF2realt-F	ATTGGACCCGCACCAAATAC	Real-time RT-PCR for ORF2	140
ORF2realt-R	GTCTTCATGGGATCTCCAAGG		

[a] PCR = PCR product length in basepair.

TABLE S1 Continued

Primer	**Sequence 5′ to 3′**	**Description**	**PCR**[a]
ORF3realt-F	CCGCTCTAGCGATAAGAAGG	Real-time RT-PCR for ORF3	136
ORF3realt-R	CGTAGCAACCTGATGCAAC		
ORF10realt-F	CACTGCCATCCTTGAGACAG	Real-time RT-PCR for ORF10	127
ORF10realt-R	GAGACTTCGCTGTTCTTGCC		
Peudo6 realt-F	GCACTTTGACAGGCATACAAAG	Real-time RT-PCR for pseudogene 6	144
Peudo6 realt-R	GAGTGTGGAGGCATGCATAG		
PKS1realt-F	CAACATGTCTCCACGTGAAG	Real-time RT-PCR for PKS1	130
PKS1realt-R	AATGCGGTTCAGCTTTGTGG		
PKS2realt-F	GAACCTTGCTGGTGCATCG	Real-time RT-PCR for PKS2	123
PKS2realt-R	GCTTTGGACAGCGACTTGAG		
Actin1realt-F	GTATCATGATCGGTATGGGACAG	Real-time RT-PCR for *Actin1*	134
Actin1realt-R	CCAGATCTTCTCCATGTCGTCC		

[a] PCR = PCR product length in basepair.

TABLE S2 The locations of putative binding motifs for a Zn(II)2Cys6 zinc cluster transcription factor in the promoter regions of biosynthetic genes for solanapyrone production

Gene[a]	Binding site I[b]	Binding site II[c]
AR *sol*1	285[d],[GC**A**(N_{10})GGC]	None
AR *sol*2	320, [**A**CC(N_{10})GGC]	321, [CCG(N_8)CGG]
AR *sol*3	252, [GCC(N_{10})GGC]	253, [CCG(N_8)CGG]
AR *sol*5	355, [GCC(N_{10})GGC]	356, [CCG(N_8)CGG]
AR *sol*6	224, [GCC(N_{10})GGC]	225, [CCG(N_8)CGG]
AS *sol*1	438, [G**A**C(N_{10})GGC]	439, [**A**CG(N_8)CGG]
AS *sol*2	320, [**A**CC(N_{10})GGC]	321, [CCG(N_8)CGG]
AS *sol*3	259, [GCC(N_{10})GGC]	260, [CCG(N_8)CGG]
AS *sol*5	357, [GCC(N_{10})GGC]	358, [CCG(N_8)CGG]
AS *sol*6	310, [GCC(N_{10})GGC]	311, [CCG(N_8)CGG]

[a] AR and AS prefixes indicate *Ascochyta rabiei* and *Alternaria solani*, respectively.

[b] Perfect sites contain the 5′-GCC(N_{10})GGC-3′ consensus motif. Imperfect sites contain a single base pair mismatch in one of the two triplet half sites (GCC or GGC), highlighted in bold.

[c] Perfect sites contain the 5′-CCG(N_8)CGG-3′ consensus motif. Imperfect sites contain a single base pair mismatch in one of the two triplet half sites (CCG or CGG), highlighted in bold.

[d] The sites represent those found within 500 bp upstream of the ATG start codon. The numbers indicate relative position of the binding site from ATG start codon.

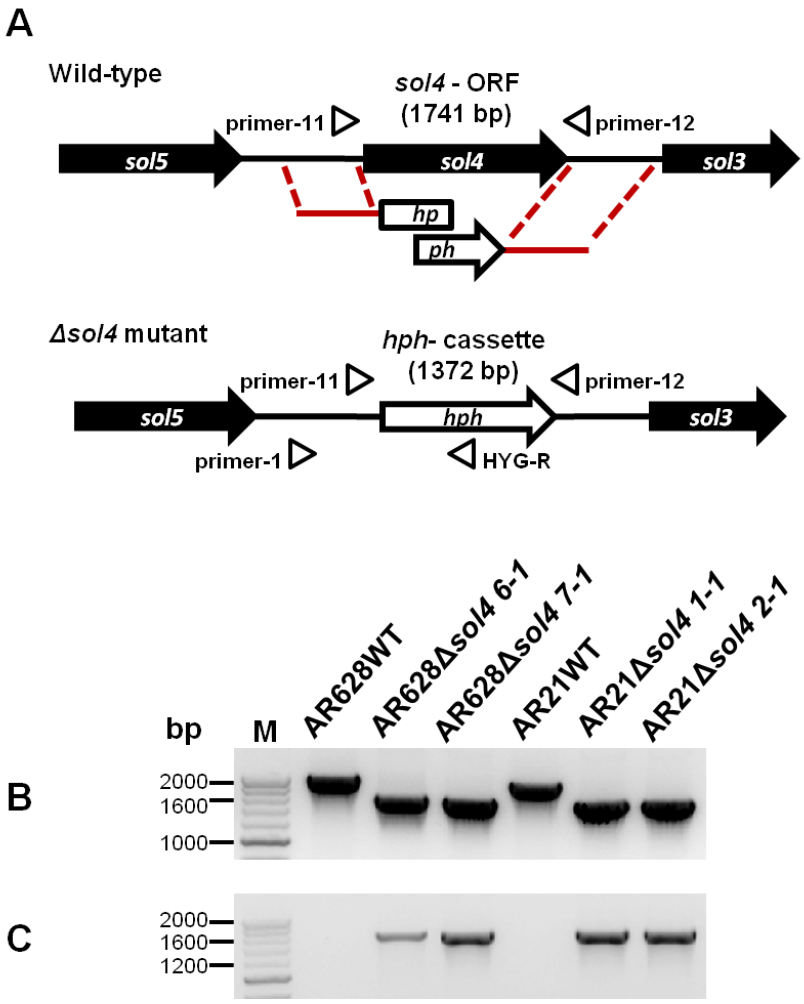

FIG S1 Gene replacement via split-marker strategy and PCR analysis of *sol4*-deletion. (A) Schematic diagram of targeted gene replacement strategy. (B) PCR analysis of *A. rabiei* wild-type isolates and their Δ*sol4* mutants. Replacement of the *sol4* gene by the *hph* cassette was verified in two independent mutants from each isolate using a primer pair, primer-11 and primer-12 (wild-types, 1,950 bp; Δ*sol4* mutants, 1,581 bp). (C) PCR verification for homologous integration of the replacement fragment to the correct genomic site with a primer pair, primer-1 and HY-R (1,604 bp).

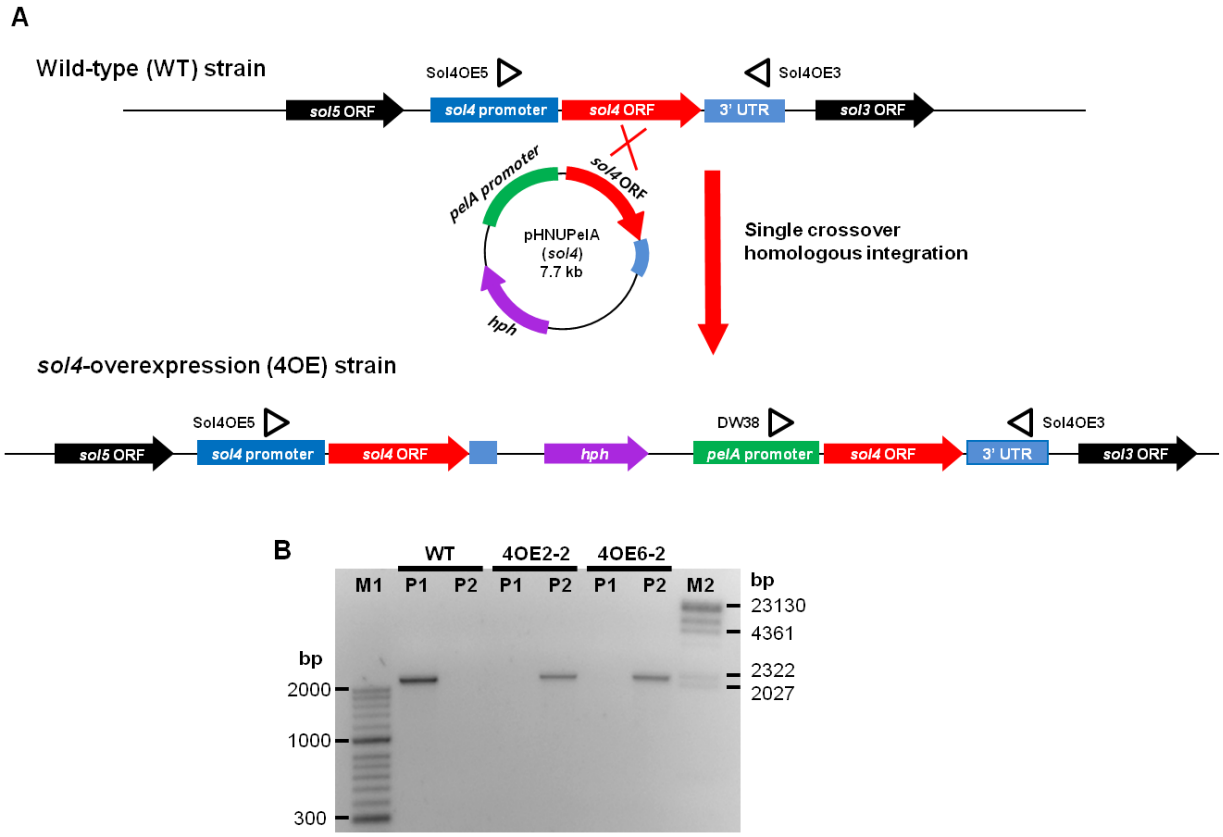

FIG S2 Verification of integration of pHNU3PelA plasmid carrying *sol4* open reading frame in *sol4*-overexpression (4OE) strains. (A) Strategy to overexpress the *sol4* gene and positioning of the primers used for a diagnostic PCR. (B) PCR analysis of AR628 wild-type (WT) strain and two independent 4OE strains confirmed a single crossover event at the *sol4* genomic locus in 4OE strains, using two primer pairs, Sol4OE5 and Sol4OE3 (P1) and DW38 and Sol4OE3 (P2). M1 = 100 bp DNA ladder, M2 = λ DNA digested with *HindIII*.

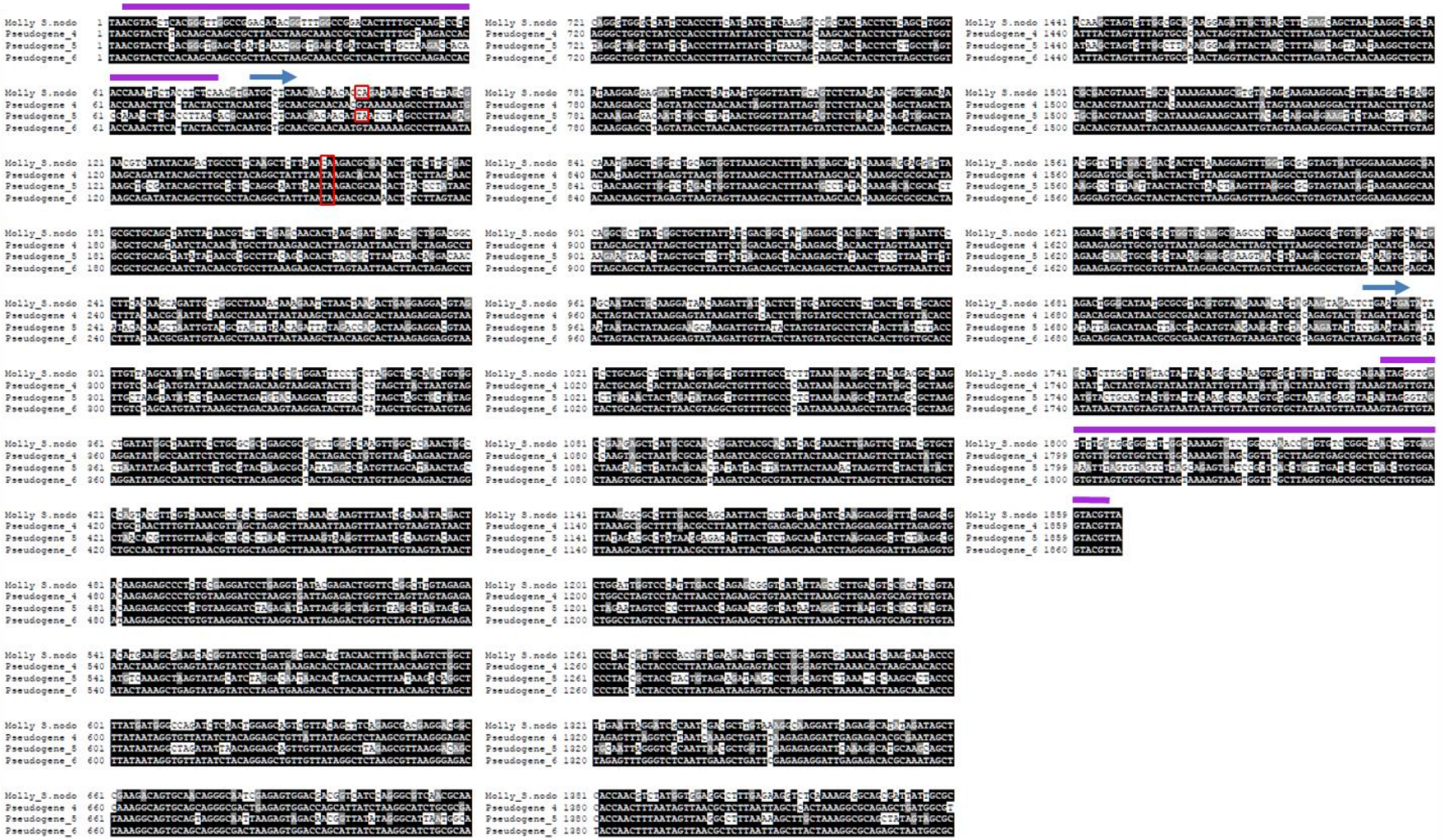

FIG S3 Multiple DNA sequence alignment of *Molly* transposon from *Stagonospora nodorum* and degenerated transposons (pseudogene 4–6) located proximal to the solanapyrone gene cluster in *A. rabiei*. Identical nucleotides are shaded in black. The regions marked with purple line indicate terminal inverted repeat. The coding regions of putative transposase are indicated by arrows. The first RIP mutation (CpA to TpA) which introduced premature stop codon in the transposase domain of pseudogene 4, 5, or 6 are indicated by red boxes in comparison to the functional *Molly* transposon. Note that the four elements are of nearly identical length and include 5′ and 3′ TA insertion sites.

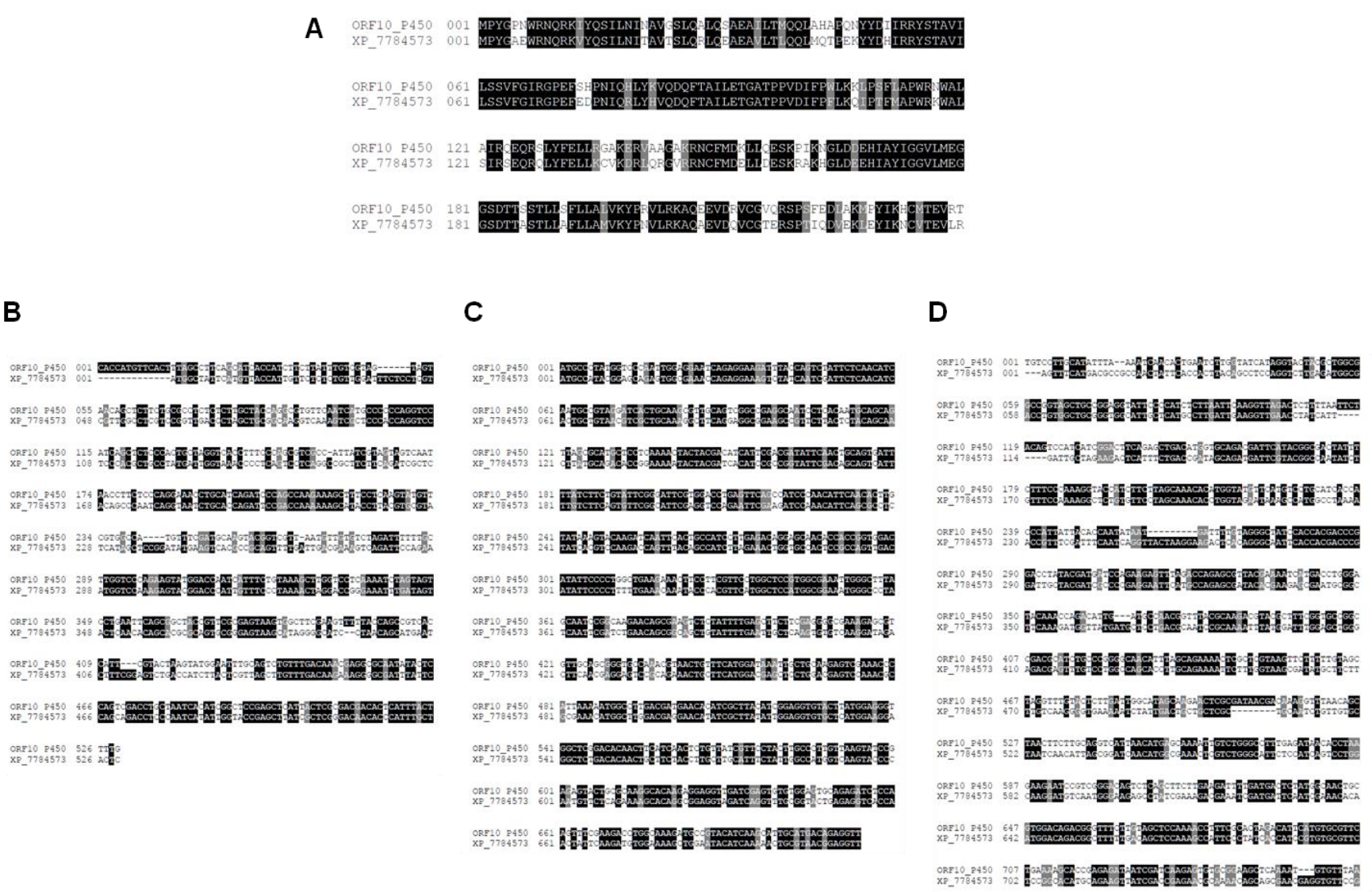
A
B
C
D

FIG S4 Pairwise sequence alignment of *ORF10* and a *P450* homologous gene found in *Coniosporium apollinis*. (A) Pairwise comparison of deduced amino acid sequence of *ORF10* and the homologous *P450* gene from *Coniosporium apollinis* (GenBank accession: XP_007784573). Only a region conserved with *ORF10* (240 out of 535 residues) was shown for the *P450*. Alignment shaded to indicate similarity with black corresponding to blocks of identical residues, and with grey corresponding to conservative substitutions. Pairwise DNA sequence comparison between upstream of *ORF10* and the corresponding region of the hypothetical protein (B), between the coding region of *ORF10* and the corresponding region of the hypothetical protein (C) and between downstream of *ORF10* and the corresponding region of the hypothetical protein (D). Note that the coding region of *ORF10* has no indels and is more conserved with the hypothetical protein compared with the upstream or downstream regions.

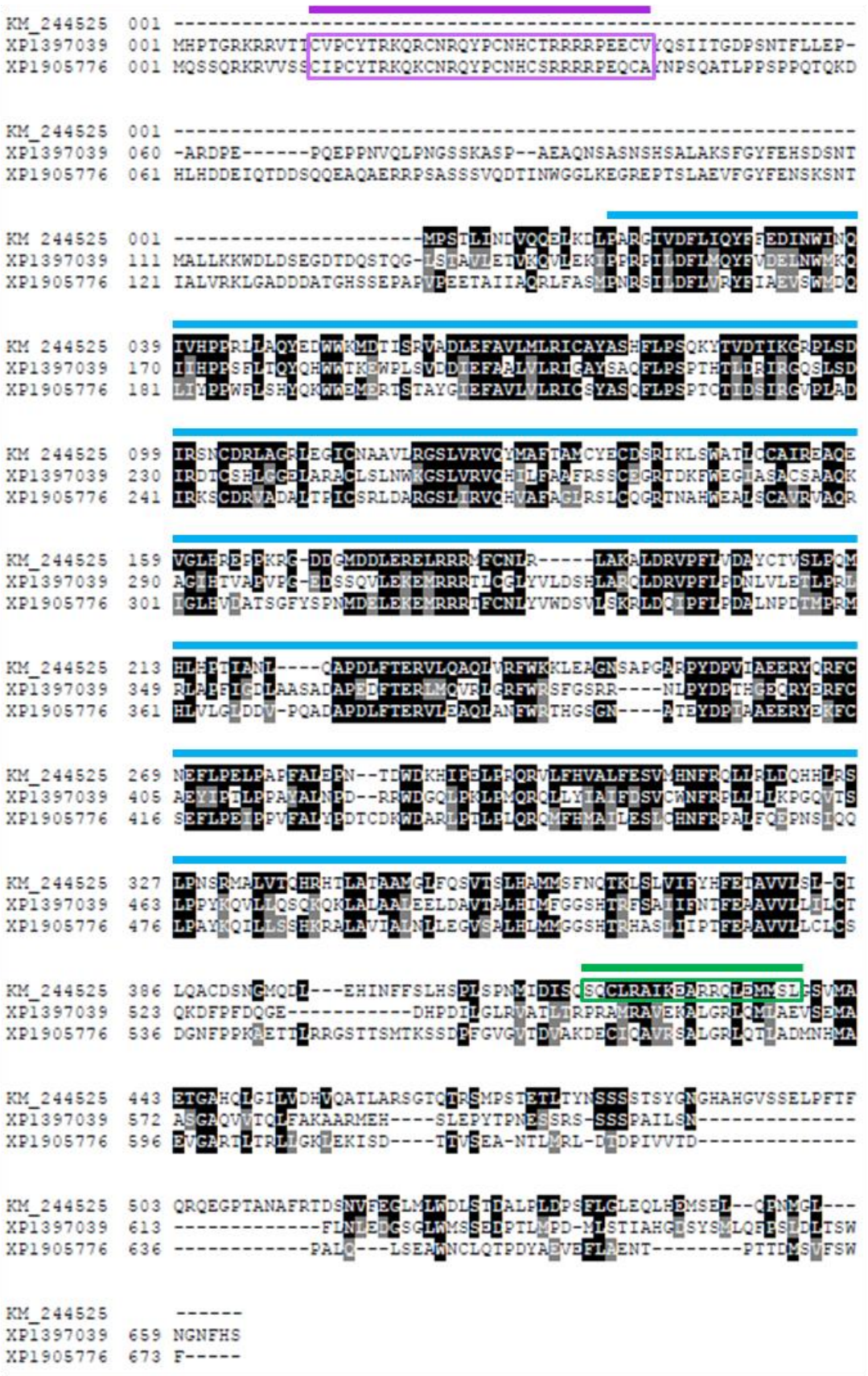
KM_244525
XP1397039
XP1905776

FIG S5 Multiple amino acid sequence alignment of *A. rabiei* Sol4 (KM244525) and two hypothetical proteins from *Aspergillus niger* (XP_001397039) and *Podospora anserina* (XP_001905776). Alignment was shaded to indicate similarity with black corresponding to blocks of identical residues, and with grey corresponding to conservative substitutions. Zn(II)2Cys6 (C_6) zinc cluster DNA-binding domain is boxed in purple and middle homology region (MHR) is marked with blue line. The coiled coil region in Sol4 amino acid sequence detected by COILS program (1) is boxed in green (a region with prediction values greater than 0.8 in scanning windows of 14). No coiled coil region was predicted for the two hypothetical proteins. Note that Sol4 lacks the C_6 zinc cluster DNA-binding domain.

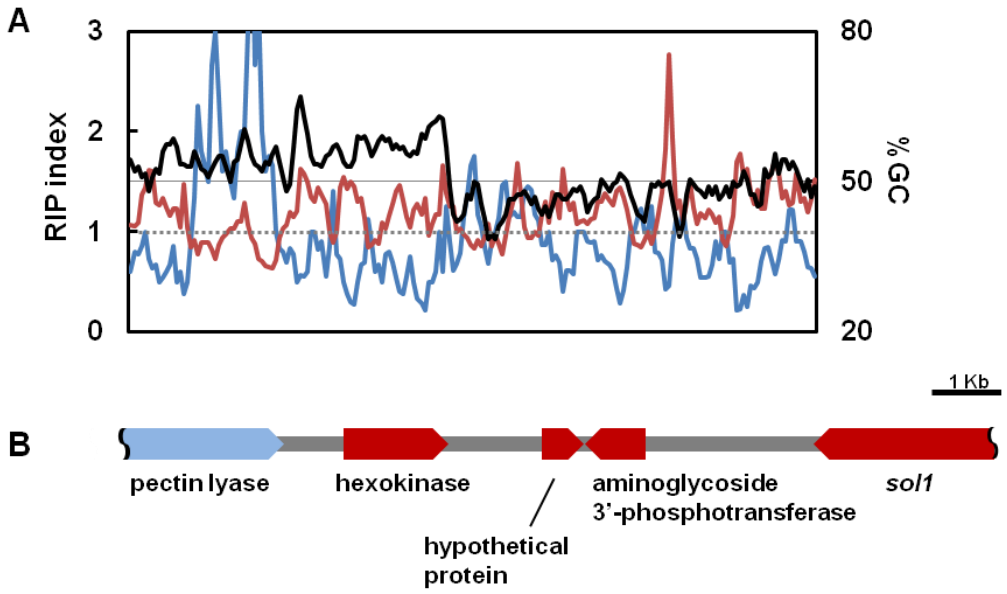

FIG S6 Gene content of the flanking region of *sol1* gene in *Alternaria solani*. (A) GC content (%, black line), RIP index I (TpA/ApT, blue line) and RIP index II (CpA+TpG/ApC+ GpT, red line) were calculated in a 200-bp window, which was slid in 50-bp increments across ≈ 10 kb of the flanking sequence. Horizontal solid line are provided to show the 50% GC mark, while dashed line shows a RIP index value of 1. (B) Schematic diagram of arrangement and orientation of three open reading frames (red boxes) and one pseudogene (blue box). Genes found were pectin lyase (similar to XM_001796354, 67% identity, *E*-value = 4e-155), hexokinase (similar to XP_008029266, 94% identity, *E*-value = 0), hypothetical protein (similar to XP_007692661, 88% identity, *E*-value = 1e-119), and aminoglycoside 3′-phosphotransferase (similar to XP_007692660, 85% identity, *E*-value = 0). Note that the partial coding region of pectin lyase gene showed strong RIP response.

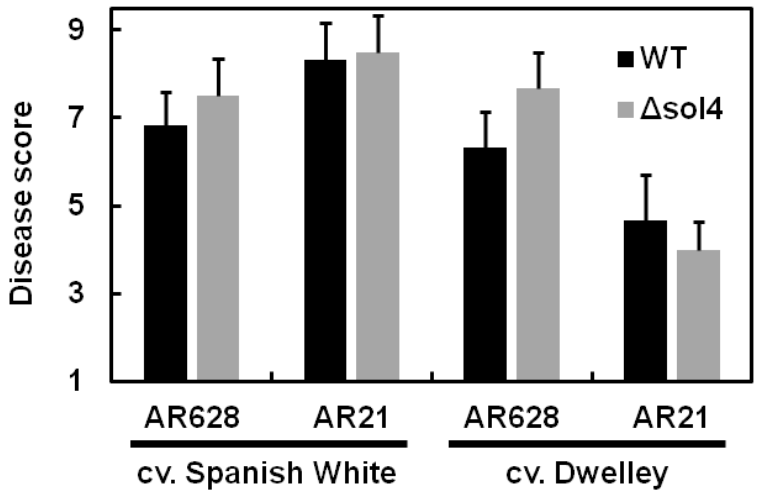

FIG S7 Comparison of virulence between Δ*sol4* mutants and their wild-type progenitors. Mean disease severity of two chickpea cultivars Spanish White and Dwelley caused by wild-type strains AR628 (pathotype II), AR21 (pathotype I), and their corresponding Δ*sol4* mutants. Disease score was assessed 2 weeks after inoculation, based on the 1–9 rating scale (1 = no symptom, 9 = dead plants) as described previously (2). Student's *t*-test showed that the disease severity caused by the Δ*sol4* mutants was not different from that caused by their respective wild-type progenitors.

REFERENCES

1. **Lupas A, Van Dyke M, Stock J.** 1991. Predicting coiled coils from protein sequences. Science **252:**1162-1164.

2. **Chen W, Coyne CJ, Peever TL, Muehlbauer FJ.** 2004. Characterization of chickpea differentials for pathogenicity assay of ascochyta blight and identification of chickpea accessions resistant to *Didymella rabiei*. Plant Pathol. **53:**759-769.