Supplementary information

SI Experimental Procedures

Fungal protoplast isolation and virus transfection

R. solani protoplasts were prepared as described previously (1), and protoplasts of *V. mali*, *C. parasitica*, and *F. graminearum* strains were prepared following the method described previously (2). Transfection of protoplasts with virions was performed as described previously (3).

RNA extraction, RT-PCR detection, and RNA blot analysis

Extraction of ssRNA and dsRNA from fungal mycelia followed the procedure described previously (4). dsRNA was separated on 8% PAGE. Total RNAs were extracted from leaves of potato and *N. benthamiana* plants using Trizol (Invitrogen). For RT-PCR detection, first strand cDNAs were synthesized using ReverTra Ace reverse transcriptase (Toyobo) and amplified by using 2× mixture DNA polymerase (Kangwei). For Northern blot analysis, Digoxigenin (DIG)-labelled DNA probes specific for CMV RNA1 (nt 2589 to 3100), RNA2 (nt 2060 to 2560), and RNA3 (nt 1631 to 2220) were used. The probes were prepared using the PCR DIG Probe Synthesis Kit (Roche). Gel electrophoresis and blotting were carried out as described previously (5). Hybridization conditions and detection of mRNAs were as described in the DIG Application Manual supplied by Roche. All of the primers used in this study are listed in Table S2.

Western blot analysis

Preparation of protein samples, SDS-PAGE, electroblotting, and immunodetection for Western blot analysis were carried out as described previously (4). CMV CP was detected using primary anti-CP (1:3,000) polyclonal serum and secondary polyclonal HRP-conjugated mouse anti-rabbit IgG (1:10,000) (Abcam). Protein bands were detected with a western ECL substrate kit (Bio-Rad). Chemiluminescent signals were visualized with a Hamamatsu Photonics real-time image processor model Argus-50 (Hamamatsu Photonics).

References

1. Liu T-H, Lin M-J, & Ko W-H (2010) Factors affecting protoplast formation by Rhizoctonia solani. *New biotechnology* 27(1):64-69.
2. Churchill A, Ciuffetti L, Hansen D, Van Etten H, & Van Alfen N (1990) Transformation of the fungal pathogen Cryphonectria parasitica with a variety of heterologous plasmids. *Current Genetics* 17(1):25-31.
3. Hillman BI, Supyani S, Kondo H, & Suzuki N (2004) A reovirus of the fungus Cryphonectria parasitica that is infectious as particles and related to the Coltivirus genus of animal pathogens. *Journal of virology* 78(2):892-898.
4. Sun L & Suzuki N (2008) Intragenic rearrangements of a mycoreovirus induced by the multifunctional protein p29 encoded by the prototypic hypovirus CHV1-EP713. *RNA* 14(12):2557-2571.
5. Andika IB, Kondo H, & Tamada T (2005) Evidence that RNA silencing-mediated resistance to Beet necrotic yellow vein virus is less effective in roots than in leaves. *Molecular Plant-Microbe Interactions* 18(3):194-204.

Supplementary tables

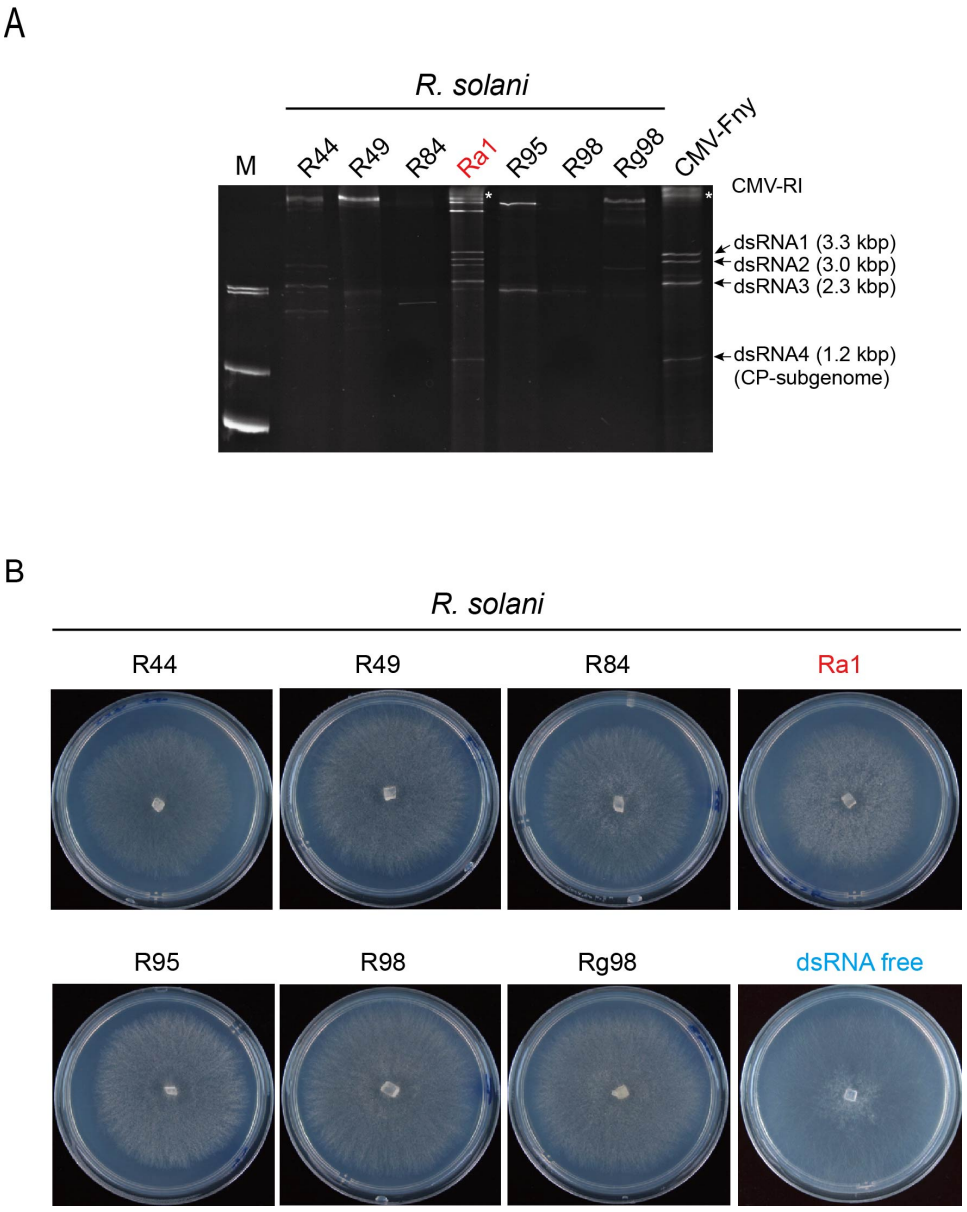
Table S1. **A list of viruses identified in *R. solani* Ra1 strain.**

Contig name	Contig length, nt	Most-similar virus				
		Name	Protein_ID	Identity	E-value	Genus
DN711_c0_gl	3309	Cucumber mosaic virus (RNA1)	BAK61797.1	99.2 %	0	*Cucumovirus*
DN711_c0_g3	3053	Cucumber mosaic virus (RNA2)	BAK61793.1	99.3 %	0	*Cucumovirus*
DN711_c0_g2	2274	Cucumber mosaic virus (RNA3)	P18028.1	100 %	1E-157	*Cucumovirus*
DN1679	22405	Sclerotinia sclerotiorum endornavirus 2	YP_009022070.1	24.4 %	6E-59	*Betaendornavirus*
DN3740	9445	Phaseolus vulgaris endornavirus 2	BAM68540.1	24.7 %	2E-60	*Alphaendornavirus*
DN328	2812	Tuber excavatum mitovirus	AEP83726.1	33.8 %	0	*Mitovirus*

Table S2. A list of primers used in this study.

Primer Name	Oligonucleotide sequence (5′- 3′)	Usage
ITS1	TCCGTAGGTGAACCTGCGG	For the fungal ITS1 region
ITS2	GCTGCGTTCTTCATCGATGC	
ITS3	GCATCGATGAAGAACGCAGC	For the fungal ITS2 region
ITS4	TCCTCCGCTTATTGATATGC	
Rs-tubF	CACTCACTCTCTTGGTGGTGG	For *R. s.* ß-tublin
Rs-tubR	CGTTGTCGATGCAGAATGTCTC	(Acc. no. GU372727)
Nb-18SF	GCAAGACCGAAACTCAAAGG	For *N. b.* 18S rRNA
Nb-18SR	TGTTCATATGTCAAGGGCTGG	(Acc. no. TC23401)
Pt-18SF	GGGCATTCGTATTTCATAGTCAGAG	For potato 18S rRNA
Pt-18SR	CGGTTCTTGATTAATGAAAACATCCT	(Acc. no. X67238)
CMV-Rs RNA3F	TCCCTTGCCGAAATTCGATTCTAC	For CMV RNA3
CMV-Rs RNA3R	CGTACCCTGAAACTAGCACGTTG	(contig DN711_c0_g2)
CMV-Rs RNA2F	AGATCCATTGCGCGAGGTTCAGC	For CMV RNA2
CMV-Rs RNA2R	ACATGGCGGTATGACCCTGTCAG	(contig DN711_c0_g3)
CMV-Rs RNA1F	AGTGATGCGGACACCACATTCCG	For CMV RNA1
CMV-Rs RNA1R	TTGCAGATTACCGCCAACTCAGG	(contig DN711_c0_gl)
PV1679-620F	ACAGAGCCCTAAGCATCATGGCC	For endorna-like virus
PV1679-1140R	AGGTGGCGTAAGGTCCACGTGG	(contig DN1679)

Supplementary figures



Supp. Figure S1. (A) dsRNA profiles of *R. solani* strains isolated from potato plants grown in the fields. dsRNAs were extracted from total RNAs of fungi, run on 8% PAGE and stained with ethidium bromide (EtBr). dsRNA extracted from leaves of CMV-Fny-infected *N. benthamiana* plants was included in the gel electrophoresis (CMV-Fny). Asterisks mark genomic DNA bands. (B) Colony of *R. solani* strains carrying dsRNAs on PDA medium. Fungi were grown for 3 days and photographed.

ORF 1a

aa position	247	363	550	560	564	661	695	858	886	895
CMV 1a (Rs)	S	M	A	E	K	V	F	T	V	P
CMV 1a (fny)	P	T	T	D	R	I	S	A	A	S

ORF 2a

aa position	4	64	92	168	170	270	512	581	617	631	777	631	784	785	808	829	846	857
CMV 1a (Rs)	S	Q	T	V	M	N	Y	F	Q	Y	K	Y	R	D	A	V	I	A
CMV 1a (fny)	P	R	D	A	I	D	H	L	L	F	N	F	C	N	T	I	M	V

ORF 2b

aa position	21	63	69	81	108
CMV 1a (Rs)	V	P	Y	P	V
CMV 1a (fny)	A	S	C	S	A

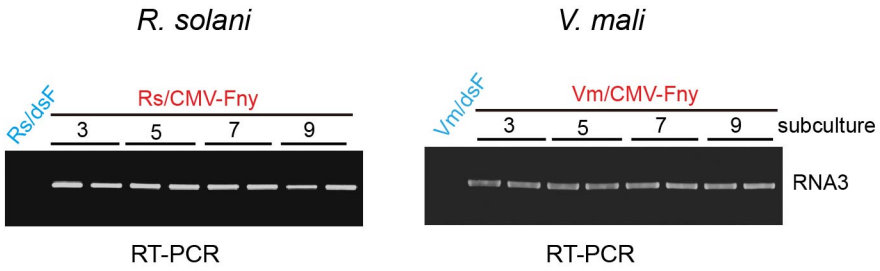
ORF 3a

aa position	251
CMV 1a (Rs)	E
CMV 1a (fny)	D

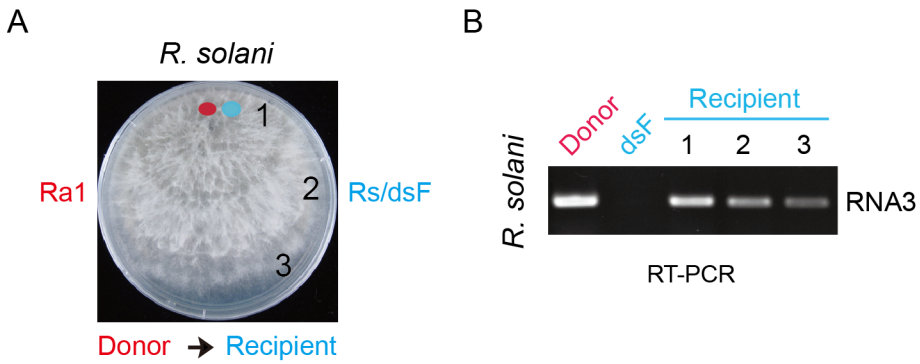
ORF CP

aa position	25	28	65	97	205
CMV 1a (Rs)	S	S	K	M	V
CMV 1a (fny)	P	A	R	T	I

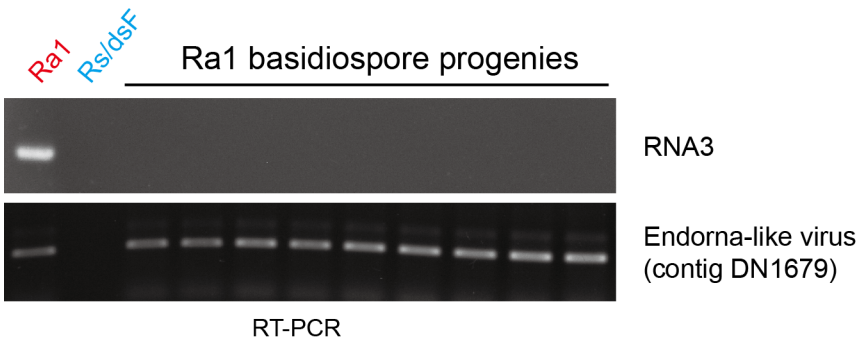
Supp. Figure S2. Amino acid sequence comparison of proteins encoded by CMV-Rs and CMV-Fny.



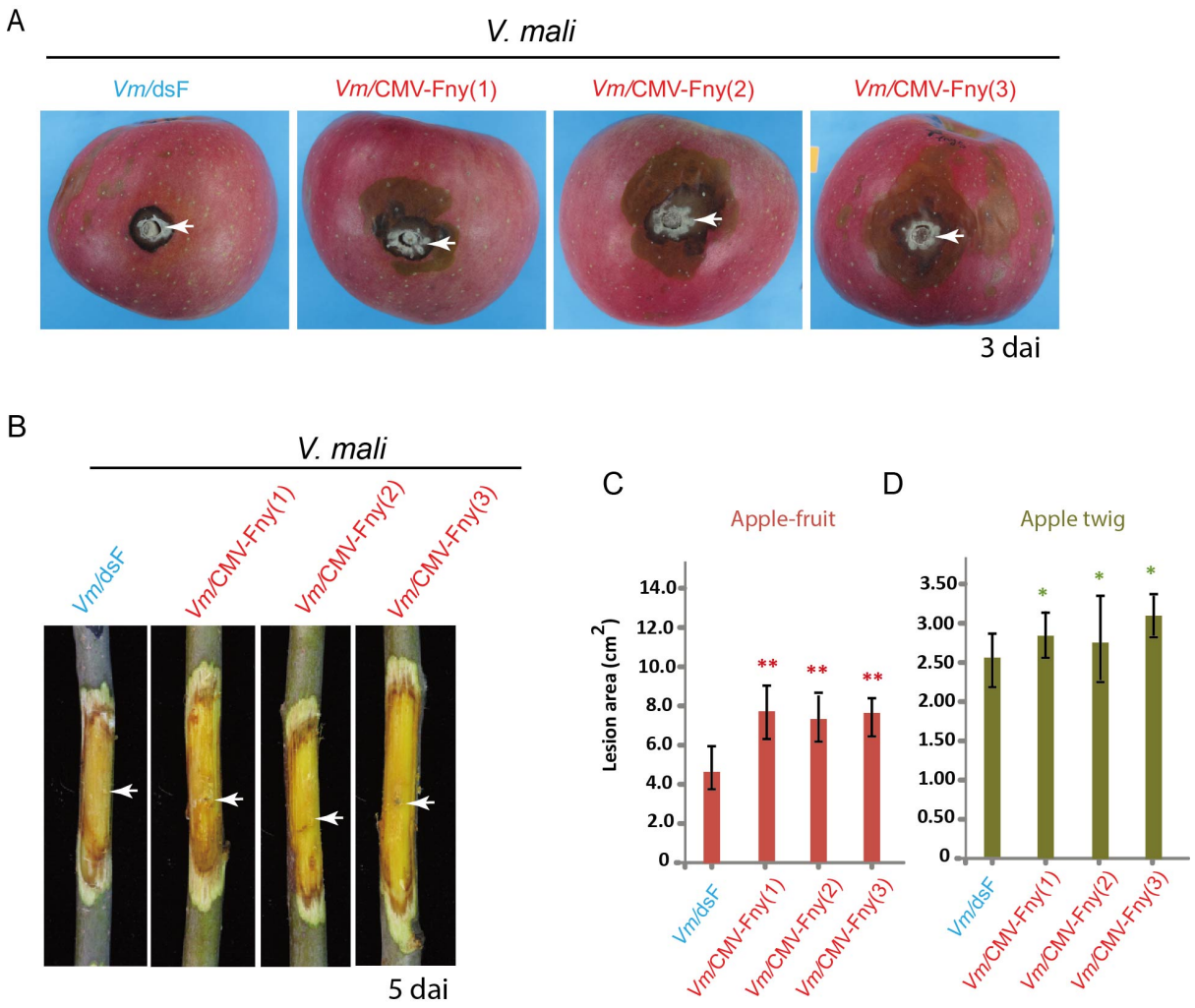
Supp. Figure S3. RT-PCR detection of CMV RNA3 accumulation in *R. solani* and *V. mali* strains after successive subcultures.



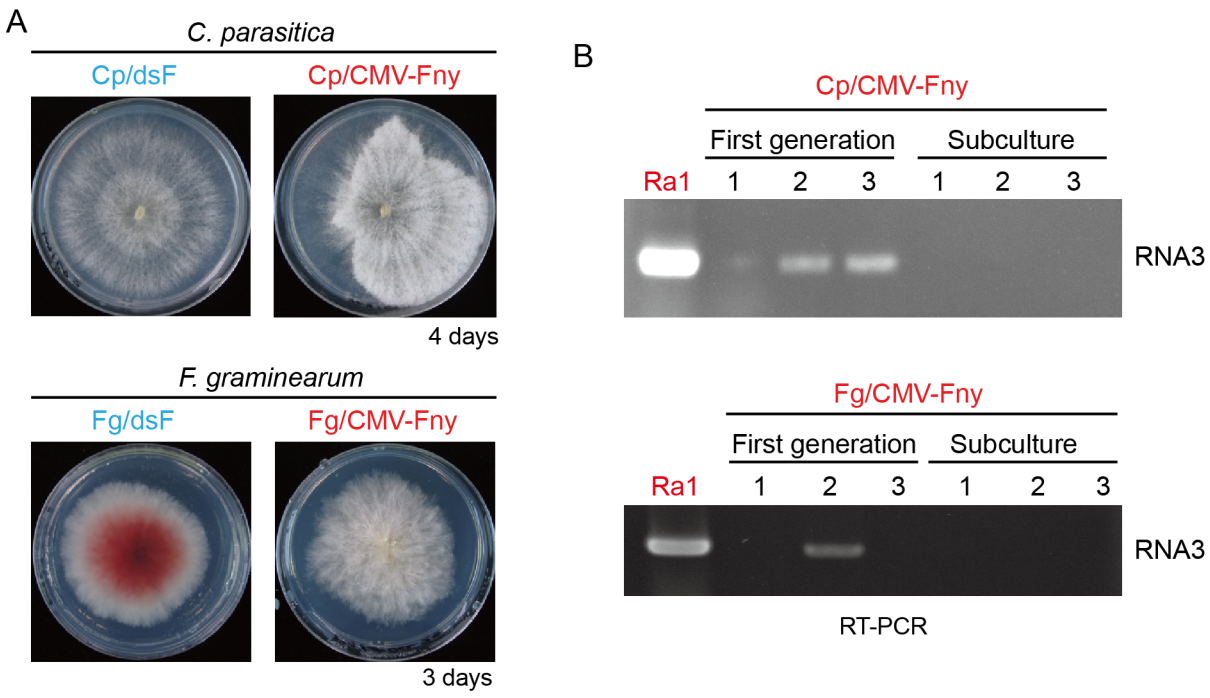
Supp. Figure S4. Horizontal transmission of CMV. (A) Co-culturing of virus-infected and virus-free fungal strains on PDA medium for investigating virus horizontal transfer through hyphal fusion. The transmission of the virus from donor (Ra1 strain) to recipient fungi is indicated by arrows. Number (1, 2 and 3) indicates the positions where the mycelia of fungal recipients were taken for subsequent culture. Plate was photographed at 6 days after co-culture. (B) RT-PCR detection of CMV RNA3 accumulation in fungal recipients described in A.



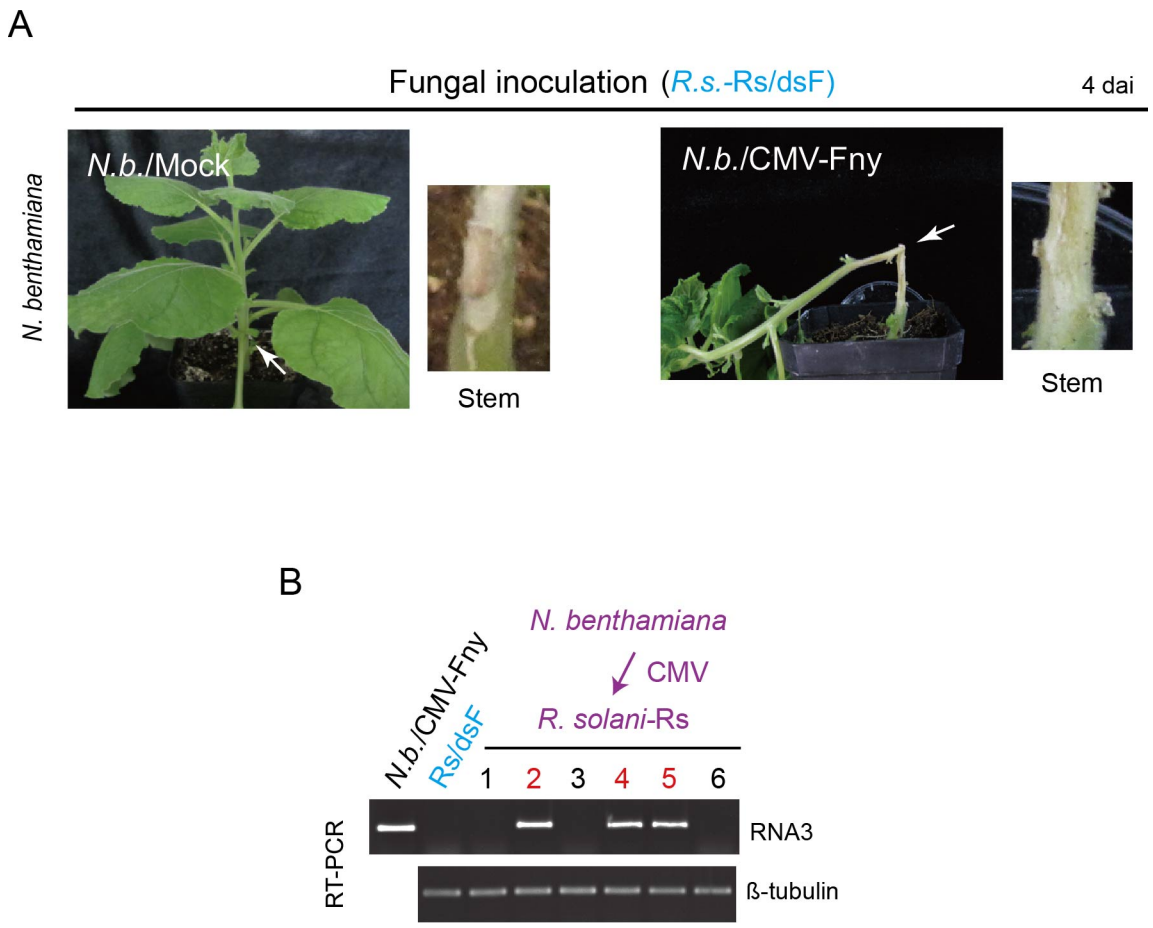
Supp. Figure S5. RT-PCR detection of CMV RNA3 and endorna-like virus accumulations in basidiospore progenies of Ra1 strain.



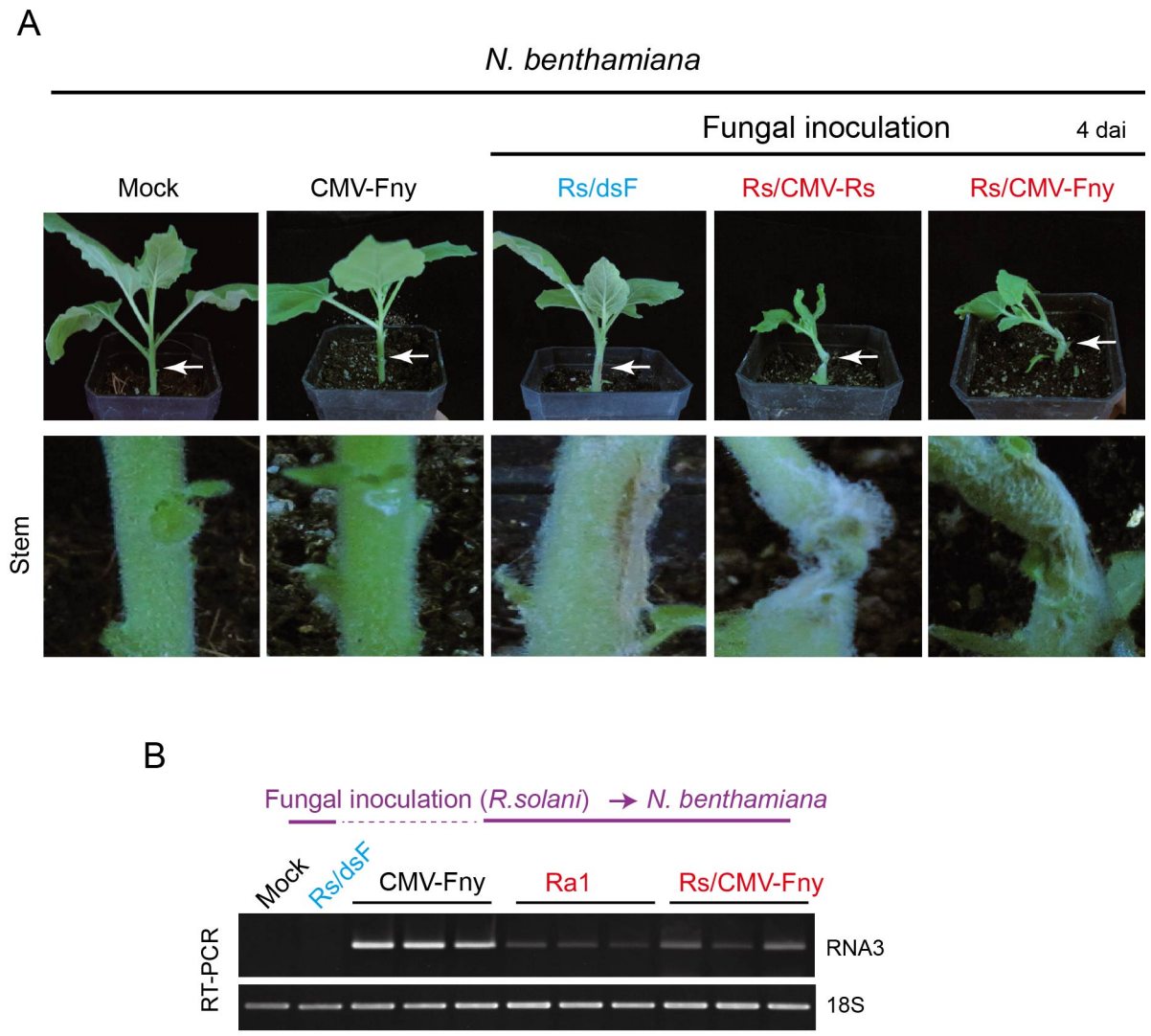
Supp. Figure S6. Virulence assay of *V. mali* strain carrying CMV-Fny. (A and B) Representative lesions produced by virus-free (dsF) and virus-infected *V. mali* strains on apple fruits (A) and apple twigs (B). (C and D) Mean of lesion area on apple fruits (C) and apple twigs (D). ** and * indicate significantly different (Student's t-test) at $p<0.05$ and $p<0.1$, respectively.



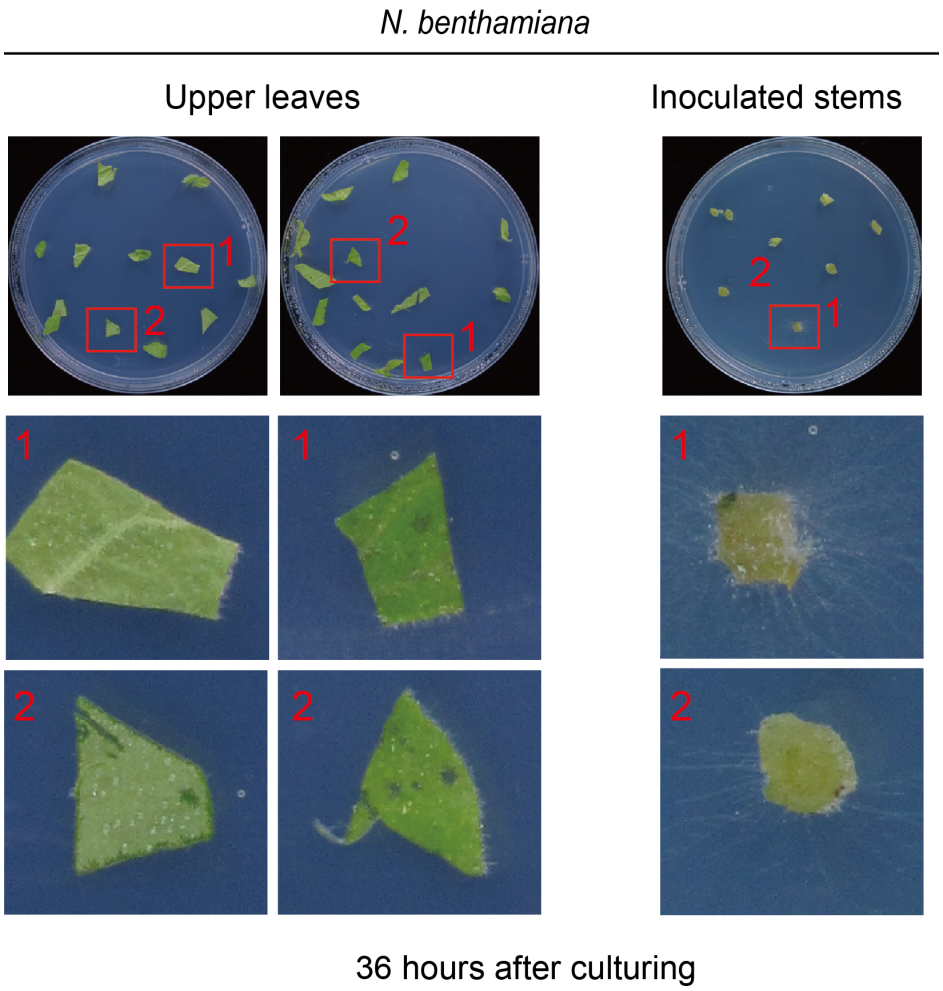
Supp. Figure S7. The introduction of CMV into fungal protoplasts. (A) Colony of *C. parasitica* and *F. graminearum* strains transfected with CMV-Fny particles on PDA medium. Fungi were grown for 4 or 3 days and photographed. (B) RT-PCR detection of CMV RNA3 accumulation in *C. parasitica* and *F. graminearum* strains after transfection and subsequent subcultures.



Supp. Figure S8. Acquisition of CMV by *R. solani.* (A) Stem rot disease in virus-free and CMV-infected *N. benthamiana* plants inoculated with virus-free *R. solani* (dsF). The insets are close-up views of the inoculated stems. Plants were photographed at 4 days after fungal inoculation. (B) RT-PCR detection of CMV RNA3 accumulation in *R. solani* strains isolated from inoculated stem of *N. benthamiana* plants.



Supp. Figure S9. Transmission of CMV from *R. solani* to the plant. (A) Development of stem rot disease in *N. benthamiana* plants infected with virus-free (dsF) and virus-infected *R. solani* strains. Plants were photographed at 4 dai. (B) The detection of CMV RNA3 accumulation in uppermost leaves of *N. benthamiana* plants by RT-PCR.



Supp. Figure S10. Examination of the systemic spread of *R. solani*. *N. benthamiana* plants were inoculated with *R. solani* at the stem. Five days after inoculation, uppermost leaves were placed on growth medium and the growth of *R. solani* colony was monitored. Plates were photographed at 36 hours after culturing.