Small RNA derived from the virulence modulating region of the *Potato spindle tuber viroid* silences *callose synthase* genes of tomato plants

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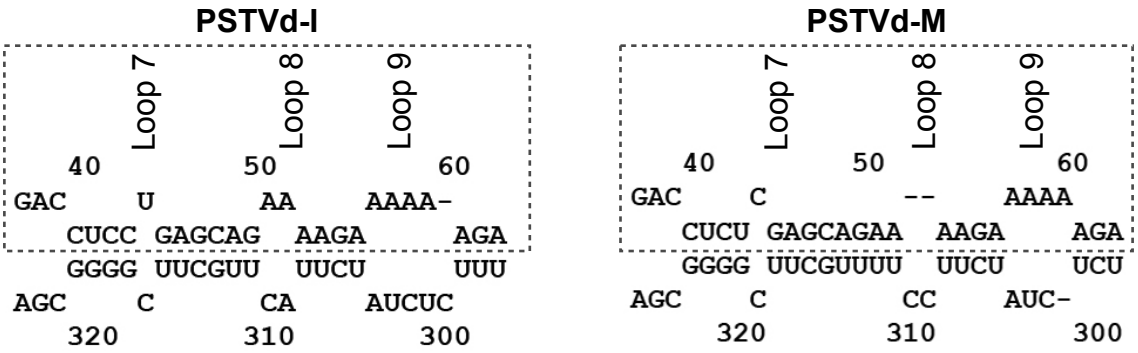
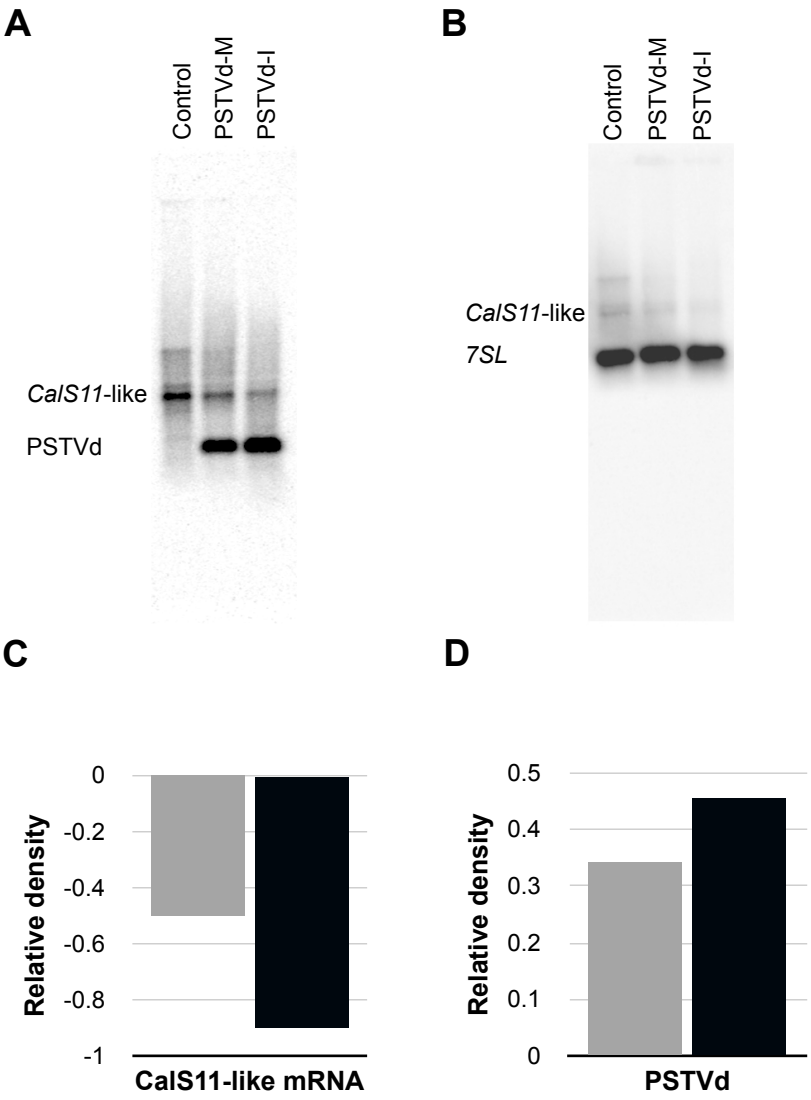


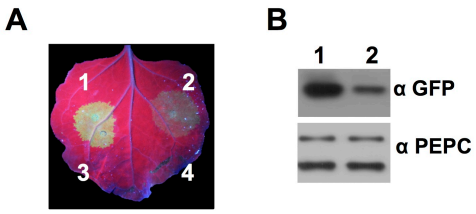
Supplemental Figure 1. Thermodynamically predicted secondary structure of PSTVd variants.

Thermodynamically predicted secondary structure of PSTVd-I and PSTVd-M by mfold (Zuker, 2003). The structures at the VMRs are shown for comparison. The boxed region indicates the VMR sequence.



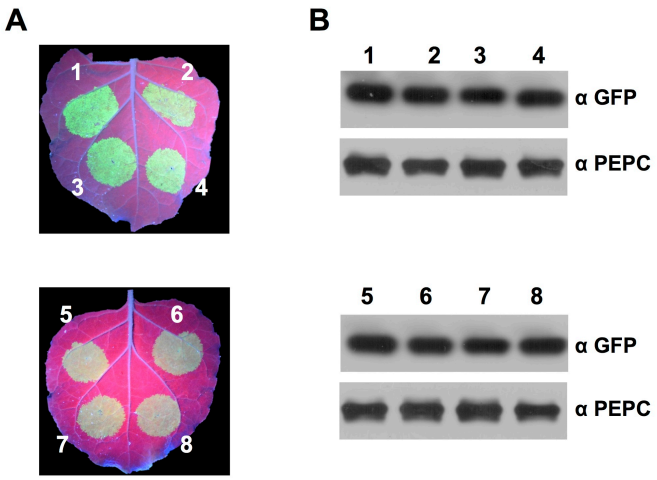
Supplemental Figure 2. RNA gel blot analysis of *CalS11-like* mRNA in PSTVd infected tomato plants.

Total RNA from plants infected with PSTVd-M or PSTVd-I was extracted at 14 dpi and subjected to RNA gel blot using probes against the (**A**) *CalS11-like* mRNA and PSTVd, or (**B**) the 7SL RNA as an internal control. RNA Gel blot signals for *CalS11 -like* mRNA and PSTVd were quantified and expressed as relative densities in (**C**) and (**D**), respectively.



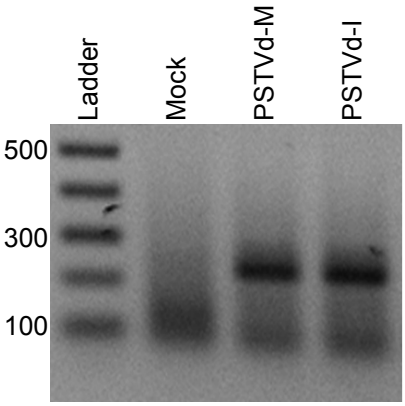
Supplemental Figure 3. Demonstration of feasibility of amiRNA technique.

(A) *N. benthamiana* leaves were agro-infiltrated with (1), empty pBIN61 vector plus GFP:UPF1; (2), amiR:UPF1 plus GFP:UPF1. Three dpi, leaves were photographed under UV illumination. (**B**) *N. benthamiana* leaves were agro-infiltrated with the same combination as in (A). Three dpi, total protein extracts were subjected to immune-blotting with anti-GFP (top panel) and anti-PEPC (lower panel) antibodies.



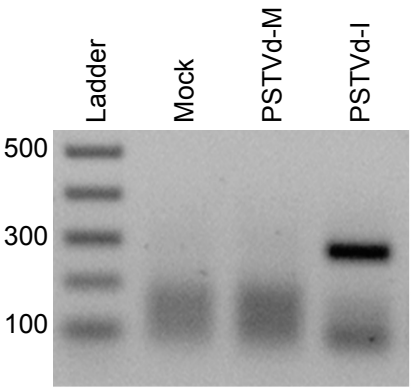
Supplemental Figure 4. Evaluation of effect of vd-sRNA:amiRNAs on GFP.

(A) *N. benthamiana* leaves were agro-infiltrated with pBIN61-GFP together with (1), empty pBIN61 vector (EV); (2), amiR:M39; (3), EV; (4), amiR:I39; (5), EV; (6), amiR:M40; (7), EV; (8), amiR:I40. At three dpi, leaves were photographed under UV illumination. (**B**) *N. benthamiana* leaves were agro-infiltrated with the same combinations as in (A). Three dpi, total protein extracts were subjected to immune-blotting with anti-GFP (top panel) and anti-PEPC (lower panel) antibodies.



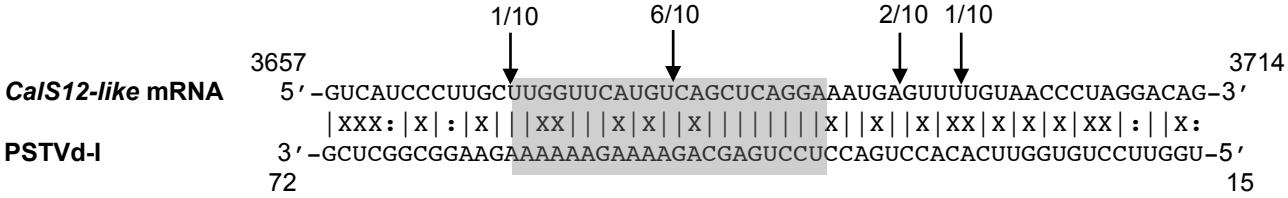
Supplemental Figure 5. 5' RLM-RACE products obtained with primers targeting the *CalS11-like* mRNA.

Total RNA was purified from mock-infected tomato plants, or plants infected with PSTVd-M or PSTVd-I, as indicated. Nested PCR products obtained from 5' RLM-RACE using primers targeting the *CalS11*-like mRNA were separated by 2.0% agarose gel electrophoresis.



Supplemental Figure 6. 5' RLM-RACE products obtained with primers targeting the *CaIS12-like* mRNA.

Total RNA was purified from mock-infected tomato plants, or plants infected with PSTVd-M or PSTVd-I, as indicated. Nested PCR products obtained from 5' RLM-RACE using primers targeting the *CaIS12*-like mRNA were separated by 2.0% agarose gel electrophoresis.

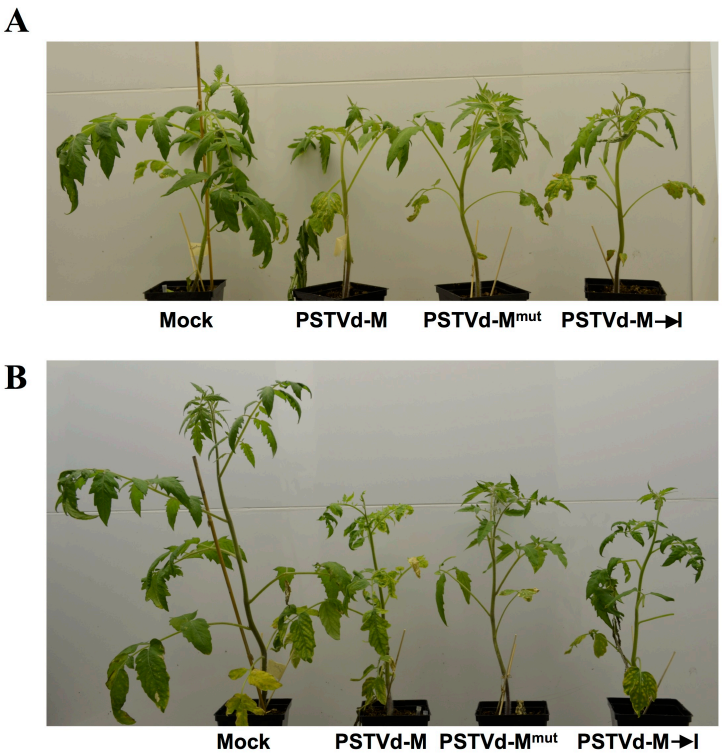


Supplemental Figure 7. Alignment of the *CalS12-like* mRNA with the portion of PSTVd-I located in the vicinity of the predicted mRNA/vd-sRNA duplexes.

Alignment of the *CalS12-like* mRNA with the portion of PSTVd-I located in the vicinity of the predicted mRNA/vd-sRNA duplexes. The shaded regions denote the predicted mRNA/vd-sRNA duplexes. The arrows indicate the 5' termini of *CalS12-like* mRNA fragments isolated from the PSTVd infected plants, as identified by 5’ RLM-RACE products, with the frequency of clones shown (ex. 1/10, indicates that 1 cleavage was found out of analyzed 10 clones). Sequences are shown in the complementary polarity.

Supplemental Figure 8. Effect of PSTVd-I mutants on tomato plants at 21 dpi.

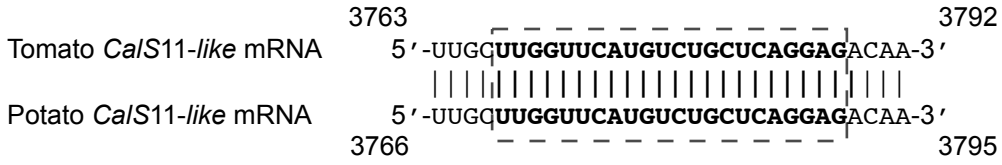
Tomato plants exhibiting symptoms after being mock inoculated or inoculated with the PSTVd-I mutants, PSTVd-I^{mut} and PSTVd-I→M, as indicated at 21 dpi.



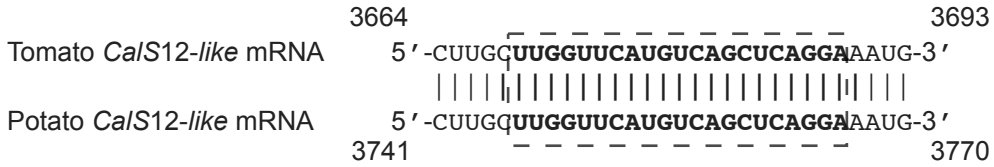
Supplemental Figure 9. Effect of PSTVd-M mutants on tomato plants at 14 and 21 dpi.

PSTVd-M mutants obtained by altering nucleotides from positions 47-50 (PSTVd-M^{mut}) and by interchanging the VMRs (PSTVd-I→M) were inoculated onto tomato plants in order to analyze the effect of vd-sRNA on the phenotype. **(A)** and **(B)** show symptoms induced by PSTVd-M, PSTVd-M^{mut} and PSTVd-M→I at 14 and 21 dpi, respectively, compared to mock-inoculated plants.

A



B



Supplemental Figure 10. Alignment of the PSTVd-sRNA binding sites of the tomato *CalS* genes against the potato *CalS* genes.

The sequences encompassing the PSTVd-sRNA binding sites of the tomato *CalS* genes were aligned against the potato *CalS* genes in order to determine the possibility of RISC-mediated degradation of this target by PSTVd-sRNA. Identical sequences were found at the vd-sRNA binding sites of (**A)** *CalS11-like* and (**B)** *CalS12-like*. Bold letters inside the box represent the target sequence.

A

```
                3768                        3788
GFP:C11-vd39     5′-UGGUUCAUGUCUGCUCAGGAG-3′
                    |xx|||x|x||||||||||||
amiR:I39         3′-AAAAAGAAAAGACGAGUCCUC-5′
                59                          39
```

B

```
                3766                         3787
GFP:C11-vd40     5′-CUUGGUUCAUGUCUGCUCAGGA-3′
                    |||xx|||x|x||||||||||||
amiR:I40         3′-GAAAAAAGAAAAGACGAGUCCU-5′
                61                           40
```

C

```
                3768                        3788
GFP:C11-vd39     5′-UGGUUCAUGUCUGCUCAGGAG-3′
                    |xx|||x|x|||||||x:|||
amiR:M39         3′-AAAAAGAAAAGACGAGCUCUC-5′
                59                          39
```

D

```
                3766                         3787
GFP:C11-vd40     5′-CUUGGUUCAUGUCUGCUCAGGA-3′
                    |||xx|||x|x|||||||x:||
amiR:M40         3′-GAAAAAAGAAAAGACGAGCUCU-5′
                61                           40
```

E

```
                3670                        3690
GFP:C12-vd39     5′-UGGUUCAUGUCAGCUCAGGAA-3′
                    |xx|||x|x||x||||||||x
amiR:I39         3′-AAAAAGAAAAGACGAGUCCUC-5′
                59                          39
```

F

```
                3668                         3689
GFP:C12-vd40     5′-CUUGGUUCAUGUCAGCUCAGGA-3′
                    |||xx|||x|x||x||||||||
amiR:I40         3′-GAAAAAAGAAAAGACGAGUCCU-5′
                61                           40
```

G

```
                 3670                        3690
GFP:C12-vd39      5′-UGGUUCAUGUCAGCTCAGGAA-3′
                     |xx|||x|x||x||||x:||x
amiR:M39          3′-AAAAAGAAAAGACGAGCUCUC-5′
                 59                          39
```

H

```
                3668                         3689
GFP:C12-vd40     5′-CUUGGUUCAUGUCAGCUCAGGA-3′
                    |||xx|||x|x||x||||:|||
amiR:M40         3′-GAAAAAAGAAAAGACGAGCUCU-5′
                61                           40
```

Supplemental Figure 11. Duplexes predicted to be formed by complexes of amiRNAs and GFP-expressing reporter constructs.

(A) amiR:I39 plus GFP:C11-vd39; (B) amiR:I40 plus GFP:C11-vd40; (C) amiR:M39 plus GFP:C11-vd39; (D) amiR:M40 plus GFP:C11-vd40; (E) amiR:I39 plus GFP:C12-vd39; (F) amiR:I40 plus GFP:C12-vd40; (G) amiR:M39 plus GFP:C12-vd39 and, (H) amiR:M40 plus GFP:C12-vd40.

Supplemental Table 1. Predicted duplex formation between tomato *CalS11-like* mRNA targets and all possible vd-sRNA derived from the VMR of PSTVd variants.

Viroid	Position on viroid (nt)	*CalS11* gene/vd-sRNA alignment	vd-sRNA binding site	ΔG	% pair
PSTVd-I	39-59 (21)	5′-UGGUUCAUGUCUGCUCAGGAG-3′ 3′-AAAAAGAAAAGACGAGUCCUC-5′ * *** * **********	3664-3684	-27.9	80.9
	39-60 (22)	5′-UUGGUUCAUGUCUGCUCAGGAG-3′ 3′-AAAAAAGAAAAGACGAGUCCUC-5′ ** *** * **********	3663-3684	-28.3	81.8
	39-61 (23)	5′-CUUGGUUCAUGUCUGCUCAGGAG-3′ 3′-GAAAAAAGAAAAGACGAGUCCUC-5′ *** *** * **********	3662-3684	-29.8	82.6
	39-62 (24)	5′-GCUUGGUUCAUGUCUGCUCAGGAG-3′ 3′-AGAAAAAAGAAAAGACGAGUCCUC-5′ *** *** * **********	3661-3684	-31.3	79.2
	40-60 (21)	5′-UUGGUUCAUGUCUGCUCAGGA-3′ 3′-AAAAAAGAAAAGACGAGUCCU-5′ ** *** * **********	3663-3683	-26.4	80.9
	40-61 (22)	5′-CUUGGUUCAUGUCUGCUCAGGA-3′ 3′-GAAAAAAGAAAAGACGAGUCCU-5′ *** *** * **********	3662-3683	-27.8	81.8
	40-62 (23)	5′-GCUUGGUUCAUGUCUGCUCAGGA-3′ 3′-AGAAAAAAGAAAAGACGAGUCCU-5′ *** *** * **********	3661-3683	-29.4	78.3
	40-63 (24)	5′-UGCUUGGUUCAUGUCUGCUCAGGA-3′ 3′-AAGAAAAAAGAAAAGACGAGUCCU-5′ * *** *** * **********	3660-3683	-29.4	79.2
PSTVd-M	39-59 (21)	5′-UGGUUCAUGUCUGCUCAGGAG-3′ 3′-AAAAAGAAAAGACGAGCUCUC-5′ * *** * ******* ***	3664-3684	-22.1	71.4
	39-60 (22)	5′-UUGGUUCAUGUCUGCUCAGGAG-3′ 3′-AAAAAAGAAAAGACGAGCUCUC-5′ ** *** * ******* ***	3663-3684	-22.5	72.7
	39-61 (23)	5′-CUUGGUUCAUGUCUGCUCAGGAG-3′ 3′-GAAAAAAGAAAAGACGAGCUCUC-5′ *** *** * ******* ***	3662-3684	-23.9	73.9
	39-62 (24)	5′-GCUUGGUUCAUGUCUGCUCAGGAG-3′ 3′-AGAAAAAAGAAAAGACGAGCUCUC-5′ *** *** * ******* ***	3661-3684	-25.5	70.8
	40-60 (21)	5′-UUGGUUCAUGUCUGCUCAGGA-3′ 3′-AAAAAAGAAAAGACGAGCUCU-5′ ** *** * ******* **	3663-3683	-20.5	71.4
	40-61 (22)	5′-CUUGGUUCAUGUCUGCUCAGGA-3′ 3′-GAAAAAAGAAAAGACGAGCUCU-5′ *** *** * ******* **	3662-3683	-22.0	72.7
	40-62 (23)	5′-GCUUGGUUCAUGUCUGCUCAGGA-3′ 3′-AGAAAAAAGAAAAGACGAGCUCU-5′ *** *** * ******* **	3661-3683	-23.5	69.6
	40-63 (24)	5′-UGCUUGGUUCAUGUCUGCUCAGGA-3′ 3′-AAGAAAAAAGAAAAGACGAGCUCU-5′ * *** *** * ******* **	3660-3683	-23.5	70.8

Supplemental Table 2. Details of the primers used in the RT-qPCR reactions for the evaluation of both the PSTVd titer and the *callose synthase* gene expression.

Target	Forward primer (5′-3′)	Reverse primer (5′-3′)
Potato spindle tuber viroid (PSTVd)[a]	PSTVd-231F (GCCCCCTTTGCGCTGT)	PSTVd-296R (AAGCGGTTCTCGGGAGCTT)
CalS11-like mRNA	CalS-11F (GAAGGACGAGAGAGAGATATGG)	CalS-11R (CTGAAGCAGAATCAAGGAACG)
CalS12-like mRNA	CalS-12F (TGAGGAGGCACTGAAAATGAGG AAC)	CalS-12R (CGGATTTTCAGGGGGTTGGCT)
Ubiquitin-conjugating enzyme (*UBC*)[b,c]	UBC-F (GCAATCTTCTTCGATCCGGT)	UBC-R (GCTACAGAACACCAAGCAGA)
Transducing/WD40 repeat family protein[b,c]	WD40rfp-F (ATAAGCTCCCTGGACACAC)	WD40rfp-R (CCTCACCTTCTTCTCAAATCTC)
ARF-like GTPase family protein (*ASAR1*)[b,c]	ASAR1-F (GGAGGTGTTTATGTGCAGTATT)	ASAR1-R (CCAGACGGGAAAAAATAGTTGT)

[a]Boonham et al., 2005,

[b]Dekkers et al., 2012,

[c]house-keeping genes

Supplemental Table 3. Details of the primers used for constructing amiRNAs on the osa-MIR528 backbone.

Osa-MIR528 amiRNA construct	Name of the primer	Primer Sequence (5′-3′)
ami-UPF1	U15F	tctctagactgtagcagcagcagTTTGAATTAAACTGATGGGCCcaggagattc agtttgaa
	U15R	gaggatccgcctagcagcaggaaCTTGAATTAAACTGATGGGCTagagagg caaaagtgaa
amiR:I39	PSTVd-I39-59F	tctctagactgtagcagcagcagCTCCTCAGCTGAAAAGAAAAAcaggagattc agtttgaa
	PSTVd-I39-59R	gaggatccgcctagcagcaggaaCTCCTGAGCAGAAAAGAAAAAagagagg caaaagtgaa
amiR:I40	PSTVd-I40-61F	tctctagactgtagcagcagcagTCCTGTGCACAAAAGAAAAAAGcaggagat tcagtttgaa
	PSTVd-I40-61R	gaggatccgcctagcagcaggaaTCCTGAGCAGAAAAGAAAAAAGagagag gcaaaagtgaa
amiR:M39	PSTVd-M39-59F	tctctagactgtagcagcagcagCTCTCCAGCTGAAAAGAAAAAcaggagattc agtttgaa
	PSTVd-M39-59R	gaggatccgcctagcagcaggaaCTCTCGAGCAGAAAAGAAAAAagagagg caaaagtgaa
amiR:M40	PSTVd-M40-61F	tctctagactgtagcagcagcagTCTCGTGCACAAAAGAAAAAAGcaggagat tcagtttgaa
	PSTVd-M40-61R	gaggatccgcctagcagcaggaaTCTCGAGCAGAAAAGAAAAAAGagagag gcaaaagtgaa

*Bam*HI (ggatcc) and *Xba*I (tctaga) sites are used in PCR primers for cloning of amplified products into pCambia-1300.

Capital letters in the primers indicates amiRNA sequence of vd-sRNA.

[a]Modified from Yan et al., 2012

Supplemental Table 4. Oligonucleotides of the predicted target sequence of the *CalS11-like* mRNA used as inserts in to the binary vector pBIN61-GFP.

Target	Construct	Oligonucleotide 1[a] (5′-3′)	Oligonucleotide 2[b] (5′-3′)	amiRNA construct
UPF1	**GPF:UPF1**	gatccCTTGAATTAAACTGATGGGCTc	ccgggAGCCCATCAGTTTAATTCAAGg	ami-UPF15[c]
CalS11	**GFP:C11-vd39**	gatccCTCCTGAGCAGACATGAACCAc	ccgggTGGTTCATGTCTGCTCAGGAGg	amiR:M39, amiR:I39
	GFP:C11-vd40	gatccTCCTGAGCAGACATGAACCAAGc	ccgggCTTGGTTCATGTCTGCTCAGGAg	amiR:M40, amiR:I40
CalS12	**GFP:C12-vd39**	gatccTTCCTGAGCTGACATGAACCAc	ccgggTGGTTCATGTCAGCTCAGGAAg	amiR:M39, amiR:I39
	GFP:C12-vd40	gatccTCCTGAGCTGACATGAACCAAGc	ccgggCTTGGTTCATGTCAGCTCAGGAg	amiR:M40, amiR:I40

[a] Lower case nucleotides at the 5' end of the oligonucleotide is similar to the *Bam*HI restricted site and a single nucleotide at the 3' end is similar to the product obtained after *Xma*I restriction digestion.

[b] Five nucleotides at 5' end of the is oligonucleotides are similar to the *Xma*I restriction enzyme digestion product whereas single nucleotide at 3' end is similar to the product obtained after *Bam*HI restriction digestion.

[c]Modified from Yan et al., 2012

Upper case nucleotide sequences in the oligonucleotide represent target nucleotides.

Supplemental Table 5. Details of the primers used for the 5' RLM-RACE experiment used to prove RISC-mediated cleavage site of the *CaIS11-like* mRNA.

Primer name	Target gene	Primer sequence (5′-3′)	Primer binding site	Expected amplicon size (bp)
RNA adapter[a]		GUUCAGAGUUCUACAGUCCGACG AUC	5'- end of the cleaved mRNA	
RACE For primer[a]		AATGATACGGCGACCACCGACAGG TTCAGAGTTCTAC AGTCCGA		
RACE nFor primer[a]		CCGACAGGTTCAGAGTTCTAC		
CaIS11-RACE R	*CaIS11*-like mRNA	CATCCCTTCCCTTACCCACT	4001-4020[b]	252
CaIS11-RACE nR		GTGGGTAACATTGCCACCTC	3967-3986[b]	218

[a]Navarro et al., 2012

[b]GenBank Acc. No. XM_004232828

Supplemental Table 6. Predicted duplexes formed between the tomato *CalS12-like* mRNA targets and all possible vd-sRNAs derived from the VMR of PSTVd variants.

Viroid	Position on viroid (nt)	*CalS12* gene/vd-sRNA alignment	vd-sRNA binding site	ΔG	% pair
PSTVd-I	39-59 (21)	5′-UGGUUCAUGUCAGCUCAGGAA-3′ 3′-AAAAAGAAAAGACGAGCUCUC-5′ * *** * ** **** ***	3670-3690	-22.0	71.4
	39-60 (22)	5′-UUGGUUCAUGUCAGCUCAGGAA-3′ 3′-AAAAAAGAAAAGACGAGCUCUC-5′ ** *** * ** **** ***	3669-3690	-22.4	72.7
	39-61 (23)	5′-CUUGGUUCAUGUCAGCUCAGGAA-3′ 3′-GAAAAAAGAAAAGACGAGCUCUC-5′ *** *** * ** **** ***	3668-3690	-23.9	73.9
	39-62 (24)	5′-GCUUGGUUCAUGUCAGCUCAGGAA-3′ 3′-AGAAAAAAGAAAAGACGAGCUCUC-5′ *** *** * ** **** ***	3667-3690	-25.4	70.8
	40-60 (21)	5′-UUGGUUCAUGUCAGCUCAGGA-3′ 3′-AAAAAAGAAAAGACGAGCUCU-5′ ** *** * ** **** ***	3669-3689	-21.5	76.2
	40-61 (22)	5′-CUUGGUUCAUGUCAGCUCAGGA-3′ 3′-GAAAAAAGAAAAGACGAGCUCU-5′ *** *** * ** **** ***	3668-3689	-22.9	77.3
	40-62 (23)	5′-GCUUGGUUCAUGUCAGCUCAGGA-3′ 3′-AGAAAAAAGAAAAGACGAGCUCU-5′ *** *** * ** **** ***	3667-3689	-24.4	73.9
	40-63 (24)	5′-UGCUUGGUUCAUGUCAGCUCAGGA-3′ 3′-AAGAAAAAAGAAAAGACGAGCUCU-5′ * *** *** * ** **** ***	3666-3689	-24.4	75
PSTVd-M	39-59 (21)	5′-UGGUUCAUGUCAGCUCAGGAA-3′ 3′-AAAAAGAAAAGACGAGCUCUC-5′ * *** * ** **** **	3670-3690	-16.2	66.7
	39-60 (22)	5′-UUGGUUCAUGUCAGCUCAGGAA-3′ 3′-AAAAAAGAAAAGACGAGCUCUC-5′ ** *** * ** **** **	3669-3690	-16.6	68.2
	39-61 (23)	5′-CUUGGUUCAUGUCAGCUCAGGAA-3′ 3′-GAAAAAAGAAAAGACGAGCUCUC-5′ *** *** * ** **** **	3668-3690	-18.0	69.6
	39-62 (24)	5′-GCUUGGUUCAUGUCAGCUCAGGAA-3′ 3′-AGAAAAAAGAAAAGACGAGCUCUC-5′ *** *** * ** **** **	3667-3690	-19.6	66.7
	40-60 (21)	5′-UUGGUUCAUGUCAGCUCAGGA-3′ 3′-AAAAAAGAAAAGACGAGCUCU-5′ ** *** * ** **** **	3669-3689	-15.6	66.7
	40-61 (22)	5′-CUUGGUUCAUGUCAGCUCAGGA-3′ 3′-GAAAAAAGAAAAGACGAGCUCU-5′ *** *** * ** **** **	3668-3689	-17.1	68.2
	40-62 (23)	5′-GCUUGGUUCAUGUCAGCUCAGGA-3′ 3′-AGAAAAAAGAAAAGACGAGCUCU-5′ *** *** * ** **** **	3667-3689	-18.6	65.2
	40-63 (24)	5′-UGCUUGGUUCAUGUCAGCUCAGGA-3′ 3′-AAGAAAAAAGAAAAGACGAGCUCU-5′ * *** *** * ** **** **	3666-3689	-18.6	66.7

Supplemental Table 7. Details of the primers used for the 5' RLM-RACE experiment used to prove the RISC-mediated cleavage site of the *CalS12-like* mRNA.

Primer name	Primer sequence (5′-3′)	Primer binding site[a]	Expected amplicon size (bp)
CalS12-RACE R	CTTGTTCTCCATTGCCACTGG	3962-3982	317
CalS12-RACE nR	CCCAACATCCCTTCCTTTGC	3908-3927	262

[a]GenBank Acc. No. XM_004243304

Supplemental Table 8. Details of the oligonucleotides used to generate, by PCR, both PSTVd mutants and chimeric constructs.

Primer	Sequence (5′-3′)	Remark
PSTVd-81F	CAGGGATCCCCGGGGAAACCTGGAG CG	Forward primer
PSTVd-I^{mut}	CCCGGGGATCCCTGAAGCGCTCCTCC GAGCCGCCTTCTTTTTTCTTTagacCTC AGGAGGTC	Nucleotide sequence of PSTVd-I is mutated at position 47-50. Used in combination of PSTVd-81F to obtain monomer PSTVd-I^{Mut}.
PSTVd-M^{mut}	CCCGGGGATCCCTGAAGCGCTCCTCC GAGCCGCCATTCTTTTTTCTTTagacCT CGAGAGGTC	Nucleotide sequence of PSTVd-M is mutated at position 47-50. Used in combination of PSTVd-81F to obtain monomer PSTVd-M^{Mut}.
PSTVd-I→M	CCCGGGGATCCCTGAAGCGCTCCTCC GAGCCGCCTTCTTTTTTCTTTTCTGCT CgaGAGGTC	Nucleotide sequence of PSTVd-I is mutated at position 42and 43. Used in combination of PSTVd-81F to obtain monomeric PSTVd-I→M.
PSTVd-M→I	CCCGGGGATCCCTGAAGCGCTCCTCC GAGCCGCCATTCTTTTTTCTTTTCTGC TCagGAGGTC	Nucleotide sequence of PSTVd-M is mutated at position 42 and 43. Used in combination of PSTVd-81F to obtain monomeric PSTVd-M→I.

Bold letter indicate *Bam*HI endonuclease cleavage site.

Small letters indicates the mutated nucleotides.

Underlined letters indicates sRNA sequence.

Supplemental Table 9. Details of the oligonucleotides used to amplify, by PCR, the *CalS11-like* and *CalS12-like* mRNA of tomato.

Primer name	**Primer sequence (5′-3′)**	**Primer binding site**	**Target gene**
CalS11-F	CAGAGGTGATGCAGTTCAAAC	3599-3619[b]	*CalS11*-like
CalS11-RACE R[a]	AATGATACGGCGACCACCGACAGG TTCAGAGTTCTAC AGTCCGA	4001-4020[b]	
CalS11-RACE nR[a]	CCGACAGGTTCAGAGTTCTAC	3967-3986[b]	
CalS12-F	CGTGGTGATGCAGTTCAGAC	3502-3521[c]	*CalS12*-like
CalS12-RACE R[a]	CTTGTTCTCCATTGCCACTGG	3962-3982[c]	
CalS12-RACE nR[a]	CCCAACATCCCTTCCTTTGC	3908-3927[c]	

[a]Primers used for 5'-RLM RACE

[b]GenBank Acc. No. XM_004232828

[c]GenBank Acc. No. XM_004243304

Supplemental Data References

Boonham, N., Pérez, L.G., Mendez, M.S., Peralta, E.L., Blockley, A., Walsh, K., Barker, I., and Mumford, R.A. (2005). Development of a real-time RT-PCR assay for the detection of Potato spindle tuber viroid. J. Virol. Methods **116:** 139-146.

Dekkers, B.J., Willems, L., Bassel, G.W., van Bolderen-Veldkamp, R.P., Ligterink, W., Hilhorst, H.W., and Bentsink, L. (2012). Identification of Reference Genes for RT–qPCR Expression analysis in Arabidopsis and tomato seeds. Plant Cell Physiol. **53:** 28-37.

Navarro, B., Gisel, A., Rodio, M.E., Delgado, S., Flores, R., and Di Serio, F. (2012). Small RNAs containing the pathogenic determinant of a chloroplast-replicating viroid guide the degradation of a host mRNA as predicted by RNA silencing. Plant J.**70:** 991-1003.

Yan, F., Lu, Y., Wu, G., Peng, J., Zheng, H., Lin, L., and Chen, J. (2012). A simplified method for constructing artificial microRNAs based on the osa-MIR528 precursor. J. Biotechnol. **160:** 146-150.

Zuker, M. (2003). Mfold web server for nucleic acid folding and hybridization prediction. Nucleic Acids Res. **31**: 3406–3415.