Supporting Information

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SI Materials and Methods

Viroid RNA Transcripts. Exact full-length strands of (+) polarity from *Potato spindle tuber viroid* (PSTVd; GenBank accession no. U23058) between positions G2 and C1, G16 and U15, G36 and U35, G96 and G95, G98 and G97, G198 and G197, and G265 and C264 (Fig. 2*A*) were produced by in vitro transcription from plasmids containing the corresponding cDNA flanked by a bacteriophage RNA polymerase promoter and an Eco31I restriction site. The promoter (SP6 or T3) was positioned next to the viroid cDNA to produce the exact transcript initiation in each case. The recognition site of the type-IIS restriction enzyme Eco31I was properly placed at the end of the viroid cDNAs to produce the exact full-length PSTVd transcripts by run-off transcription on a DNA template linearized by Eco31I (Fermentas). Transcripts were purified by denaturing PAGE in 5% (wt/vol) gels, including 8 M urea in TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA), eluted from the gel, and quantified spectrophotometrically. The 5′-triphosphorylated group of the resulting transcripts was subsequently converted to 5′-phosphomonoester (see below).

Another exact full-length PSTVd transcript between positions G96 and G95 was produced by in vitro transcription of a conveniently linearized plasmid containing the PSTVd cDNA flanked by a modified version of the hammerhead ribozyme of *Tobacco ringspot virus* satellite RNA (sTRSV) (1) and a modified version of the ribozyme of *Hepatitis delta virus* minus RNA strand (2). Transcripts, with 5′-hydroxyl and 2′,3′-cyclic phosphodiester termini, were also purified and quantified as indicated above.

A third version of an exact full-length PSTVd RNA between positions G96 and G95 with 5′-hydroxyl and 3′-hydroxyl termini was obtained by in vitro transcription using a linearized plasmid in which the cDNA was flanked by the modified sTRSV hammerhead ribozyme and a properly positioned Eco31I restriction site. Following the same strategy, exact full-length transcripts with 5′-hydroxyl and 3′-hydroxyl termini were also obtained of *Hop stunt viroid* (HSVd) (GenBank accession no. D13764) from position G83 to G82, *Coconut cadang-cadang viroid* (CCCVd) (GenBank accession no. J02050) from G63 to G62, *Apple scar skin viroid* (ASSVd) (GenBank accession no. AF421195) from A91 to G90, and *Coleus blumei viroid 1* (CbVd-1) (GenBank accession no. X69293, with an additional G inserted in 146–147) from C74 to G73. All these transcripts were also purified by elution from a gel after denaturing PAGE separation and phosphorylated at their 5′ end with ^{32}P (see below).

In Vitro Transcription of Viroid RNA. RNAs were transcribed in vitro in 20-μL reactions in transcription buffer [40 mM Tris·HCl (pH 8.0), 6 mM $MgCl_2$, 20 mM DTT, 2 mM spermidine], including 0.5 mM NTPs, 20 U RNase inhibitor (RiboLock; Fermentas), 0.1 U yeast inorganic pyrophosphatase (Fermentas), 50 U of T3 or SP6 RNA polymerase (Epicentre), and 1 μg of the corresponding linearized plasmid DNA. Reactions were incubated for 2 h at 37 °C. To produce radioactive RNA probes, the 0.5 mM NTPs were replaced by 2 mM each of ATP, CTP, and GTP, and 40 μCi of [α-^{32}P]UTP (800 Ci/mmol). After transcription, the DNA template was digested with 4 U DNase I (Fermentas) for 10 min at 37 °C, and the probe was purified by chromatography using a Sephadex G-50 column (Mini Quick Spin Column; Roche).

Terminal Group Modification of in Vitro Transcribed Viroid RNAs. When indicated, the 5′-phosphorylated group of some in vitro transcribed viroid RNAs was converted into 5′-phosphomonoester by treatment with RNA 5′ polyphosphatase (Epicentre). RNAs (25 μg) were incubated for 1 h at 37 °C in a 100-μL reaction mixture containing 50 mM Hepes/KOH (pH 7.5), 100 mM NaCl, 1 mM EDTA, 0.1% 2-mercaptoethanol, 0.01% Triton X-100, 80 U RNase inhibitor, and 20 U of the enzyme. RNAs were recovered by phenol/chloroform extraction and ethanol precipitation, and quantified by spectrophotometry.

Proteinase K Digestion and Thermal Inactivation of the Tomato RNA Ligase Activity. An aliquot (4 μL) of the tomato protein chromatographic fraction showing the highest viroid circularizing activity was incubated with 20 μg proteinase K in a 10-μL reaction in 10 mM Tris·HCl (pH 8.0), 5 mM EDTA, 1 mM 2-mercaptoethanol, 0.05% SDS for 30 min at 42 °C followed by 30 min at 55 °C. Thermal treatments of equivalent aliquots (4 μL) in a final volume of 10 μL were performed for 15 min at 55, 70, or 95 °C. Aliquots (10 U) of a purified preparation of bacteriophage T4 RNA ligase 1 (Fermentas) were treated in parallel as controls. PSTVd RNA circularizing activity after proteinase K or thermal treatments was assayed as indicated above.

Protein Identification by Mass Spectrometry Analysis. A piece of a preparative polyacrylamide gel stained with Coomassie Blue was excised and subjected to digestion with sequencing grade trypsin (Promega) as described (3). A gel plug with 75 ng of BSA was treated in parallel as a control of the digestion process. The gel piece was first incubated for 30 min at 60 °C in 10 mM DTT, 50 mM NH_4HCO_3, then for 30 min (in the dark) in 55 mM iodoacetamide and 50 mM NH_4HCO_3, and finally washed for 2 min in acetonitrile:H_2O (1:1) and for 5 min in acetonitrile. Proteins in the gel plug were incubated on ice with 100 ng trypsin for 30 min. The supernatant was removed and 20 μL of 50 mM NH_4HCO_3 added. After an overnight digestion at 37 °C, the reaction was stopped with 2 μL of 10% TFA. Peptides were extracted twice with acetonitrile, and the solvent of the combined extracts evaporated under vacuum. The digestion mixture was dried in a vacuum centrifuge, resuspended in 20 μL of 0.1% TFA, and then 1 μL was spotted onto the MALDI target plate. After the droplets were air-dried at room temperature, 0.5 μL of matrix [5 mg/mL CHCA (Sigma) in 0.1% trifluoroacetic acid in acetonitrile:H_2O (1:1)] was added and allowed to air-dry at room temperature. The resulting mixture was analyzed in a 4700 Proteomics Analyzer (AB Sciex) in positive reflectron mode (2000 shots every position).

Peptide separation by liquid chromatography-MS/MS was performed using an UltiMate NanoLC system (LC Packings) and a QSTAR XL Q-TOF hybrid mass spectrometer (AB Sciex). Samples (5 μL) were delivered to the system using a FAMOS autosampler (LC Packings) at 30 μL/min, and, the peptides were trapped onto a PepMap C18 precolumn (5 mm, 300 μm i.d.; LC Packings). Peptides were then eluted onto the PepMap C18 analytical column (15 cm, 75 μm i.d.; LC Packings) at 300 nL/min and separated using a 120-min gradient of 5–50% acetonitrile. The QSTAR XL was operated in information-dependent acquisition mode, in which a 1-s TOF MS scan from 400 to 2,000 *m/z* was performed, followed by 3-s product ion scans from 65 to 2,000 *m/z* on the three most intense doubly or triply charged ions. The combined MS/MS information was sent to Mascot via the Mascot Daemon software (Matrix Science), and a database search was performed using the NCBI database restricted to green plants (Viridiplantae). Carbamidomethylation of Cys was used as a fixed modification, and oxidation of Met and deami-

dation of Asn and Gln as variable modifications. Mascot sequence query searches were done with tryptic specificity allowing two maximum missed cleavages, a peptide mass tolerance of ±100 ppm, and a fragment mass tolerance of ±0.6 Da.

Tomato RNA Extraction and Purification. Tomato (*Solanum lycopersicum* L. cv. Rutgers) young leaves (5 g) were ground with a mortar in the presence of liquid N_2 and homogenized with 5 vol of extraction buffer [0.1 M Tris·HCl (pH 9.0), 0.1 M NaCl, 10 mM EDTA, 0.1 M 2-mercaptoethanol, 5 M urea]. The extract was clarified by centrifugation for 5 min at 12,000 × *g* and extracted with buffered (pH 8.0) phenol:chloroform (1:1). Total RNAs in the aqueous phase were precipitated with isopropanol and recovered by centrifugation. An aliquot of the tomato RNAs (one-fourth of the total) was purified by chromatography using a silica gel spin column (Centrifuge Plant RNA Mini Kit; Nëdken) and eluted in 60 μL H_2O.

RT-PCR Amplification and Cloning of Tomato DNA Ligase 1 cDNA. Purified tomato RNA (4 μL) was reverse transcribed using 5 pmol of primer I (5′-CGCAAGTGCTCTTTGATTAGTCTTC-3′, complementary to positions 2,470–2,494 of GenBank accession no. BT014510) in a 10-μL reaction containing 50 mM Tris·HCl (pH 8.3), 50 mM KCl, 4 mM $MgCl_2$, 10 mM DTT, 0.5 mM each dNTP, 10 U RNase inhibitor, and 50 U of M-MuLV reverse transcriptase (Fermentas) for 45 min at 42 °C, 10 min at 50 °C, and 5 min at 60 °C. An aliquot of the RT reaction (1 μL) was subjected to nested PCR amplification in two consecutive reactions of 20 μL with 0.4 U of Phusion High-Fidelity DNA polymerase (Finnzymes) in the presence of HF Buffer (Finnzymes), 3% dimethyl sulfoxide, and 0.2 mM each dNTP. The cycling profile consisted of an initial denaturation of 30 s at 98 °C, 30 cycles of 10 s at 98 °C, 30 s at 55 °C, and 1.5 min at 72 °C, and a final incubation of 10 min at 72 °C. The first PCR contained 0.5 μM of primers I and II (5′-CTTCTTTCCAAATTG-AATGTTGTG-3′, homologous to positions 54–77 of BT014510), and the second, 1 μL of first reaction products and 0.5 μM of primers III (5′-GGCGCGGCCCATGGGA*ATGTTGTGTTTG-AGTTC*-3′, with the sequence homologous to positions 70–86 of BT014510 in italics and the site NcoI underlined) and IV (5′-GGCGCGGCCTCGAG*GTCTTCATCGTTGTC*-3′, with the sequence complementary to positions 2,461–2,475 of BT014510 in italics and the XhoI site underlined). The PCR products were separated by electrophoresis in a 1% agarose gel in Tris-acetate-EDTA buffer [40 mM Tris, 20 mM sodium acetate, 1 mM EDTA (pH 7.2)] that was stained with ethidium bromide. The amplified cDNA product was eluted from the gel, digested with NcoI and XhoI (Fermentas), and ligated into plasmid pET-23d(+) (Novagen) linearized with NcoI and XhoI to produce plasmid pESlDNL1.

Expression and Purification of Recombinant Tomato DNA Ligase 1. A recombinant version of tomato DNA ligase 1, including amino-terminal Met-Gly and carboxyl-terminal Leu-Glu-His_6 extensions was expressed in *Escherichia coli* Rosetta 2(DE3)pLysS (Novagen) transformed with pESlDNL1. A 250-mL culture of 0.6 U at OD_{600} was induced with 400 μM isopropyl β-D-1-thiogalactopyranoside for 3 h at 28 °C. Cells were harvested by centrifugation, washed, resuspended in 2.5 mL H_2O containing a mixture of proteinase inhibitors, and frozen. Once thawed, cell suspension was brought to 50 mM Tris·HCl (pH 7.5), 1% Nonidet P-40, 2.5 mM $MgCl_2$, and 10 mM 2-mercaptoethanol. After adding 125 U of Benzonase (Novagen), the mix was incubated for 45 min at 4 °C. Solid NaCl was added to the solution to a final concentration of 0.5 M, and the mix was shaken 15 more min at 4 °C. The extract was clarified by centrifugation at 100,000 × *g* for 30 min and the supernatant brought to 20 mM imidazole. The recombinant protein was purified by chromatography using a 1-mL Ni-Sepharose column (HisTrap HP; GE Healthcare) with an ÄKTA Prime Plus liquid chromatography system operated at 4 °C with a flow rate of 1 mL/min. The column was equilibrated with 10 mL of buffer [50 mM Tris·HCl (pH 7.5), 0.5 M NaCl, 1% Nonidet P-40, 10 mM 2-mercaptoethanol, 20 mM imidazole], and the extract loaded. The column was washed with 20 mL of buffer and eluted with a 1:1 mix of equilibration buffer and 1 M imidazole (pH 8.0).

Plasmid Constructs for RNA Silencing Assays. Plasmid pCdPSTVd+ contains a dimeric PSTVd cDNA (M16826 from position G87 to G86) replacing the GFP coding sequence in pCAMBIA-1302 (GenBank accession no. AF234298) under the control of *Cauliflower mosaic virus* (CaMV) 35S promoter and *Agrobacterium tumefaciens nos* terminator. A fragment of the tomato DNA ligase 1 sequence between positions 1,213 and 1,609 (GenBank accession no. BT014510) was inserted in sense orientation between the XhoI and KpnI sites, and in reverse orientation between the XbaI and ClaI sites of pHANNIBAL (GenBank accession no. AJ311872) to produce a hairpin expression cassette, including a CaMV 35S promoter and an *A. tumefaciens ocs* terminator. This cassette, flanked by two NotI restriction sites, was inserted into the unique NotI site of a modified version (XhoI and SalI sites eliminated) (4) of binary plasmid pCLEAN-G181 (GenBank accession no. EU186083) to generate pGhpSlDNL1. The empty pHANNIBAL NotI cassette was also inserted into the modified version of pCLEAN-G181 to produce the control plasmid pGhpEMPTY. pGhpSlDNL1 and pGhpEMPTY were electroporated into *A. tumefaciens* C58C1 containing the auxiliary plasmid pCLEAN-S48 (5). In the construct pTRV2-GFP-DNL1, two cDNAs corresponding to positions 219–480 of GFP variant mgfp5 (GenBank accession no. U87973) (6) and to positions 1,173–1,388 of tomato DNA ligase 1 (GenBank accession no. BT014510) were inserted in tandem in sense orientation between the EcoRI and XhoI sites of the virus-induced gene silencing (VIGS) vector pTRV2 (GenBank accession no. AF406991) (7). In the control plasmid pTRV2-GFP, only the mgfp5 cDNA fragment was cloned between the EcoRI and XhoI sites of pTRV2. pTRV1 (GenBank accession no. AF406990) (7), pTRV2-GFP-DNL1, and pTRV2-GFP were independently electroporated into *A. tumefaciens* C58C1 and cultures mixed for different VIGS experiments (see below).

Transient Expression of PSTVd and DNA Ligase 1 Constructs in *Nicotiana benthamiana*. Young, expanded leaves of *Nicotiana benthamiana* Domin plants (5 wk old) were infiltrated with a mix of *A. tumefaciens* C58C1 cultures. The individual cultures were grown to 0.5 OD at 600 nm, and cells were recovered by centrifugation, resuspended at 0.5 OD at 600 nm in 10 mM Mes-NaOH (pH 5.6), 10 mM $MgCl_2$, and 150 μM acetosyringone, and induced by incubation for 3 h at 28 °C. Infiltration solution consisted of a 999:1 mix of the cultures in which *A. tumefaciens* was transformed with a plasmid to express a DNA ligase 1 hairpin construct (pGhpSlDNL1, see above) and a culture of *A. tumefaciens* transformed with a plasmid to express a dimeric (+) PSTVd transcript (pCdPSTVd+). In the control infiltration, the *A. tumefaciens* culture transformed with pGhpSlDNL1 was replaced by another transformed with the empty plasmid (pGhpEMPTY). Infiltrated areas were harvested at several time points, RNA extracted and purified, and PSTVd RNA separated by denaturing PAGE and analyzed by Northern blot hybridization as described above. PSTVd monomeric circular and linear forms were quantified by phosphorimetry.

Virus-Induced Gene Silencing of *N. benthamiana* DNA Ligase 1. Transgenic *N. benthamiana* plants expressing the GFP variant mgfp5 (line 16c) (6) were agroinoculated at the four-leaf stage with a 1:1 mix of *A. tumefaciens* C58C1 cultures transformed with plasmids pTRV1 and pTRV2-GFP-DNL1 (see above). Control infiltration was done with a 1:1 mix of *A. tumefaciens* transformed with pTRV1 and pTRV2-GFP. Cultures were grown and induced as indicated

above. At 3–4 wk after agroinoculation, plants were screened for GFP silencing and infiltrated with an *A. tumefaciens* culture transformed with pCdPSTVd+. This culture was also grown and induced at 0.5 OD at 600 nm, as indicated above, and then diluted at 10^{-4} OD_{600} with induction buffer before infiltration. In other experiments, the 1:1 mix of *A. tumefaciens* transformed with pTRV1 and pTRV2-GFP-DNL1 (0.5 OD_{600}; or pTRV2-GFP in the control infiltration) was subsequently mixed in the proportion 999:1 with the *A. tumefaciens* culture transformed with pCdPSTVd+ (0.5 OD_{600}), and plants were infiltrated.

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3. Shevchenko A, et al. (1996) Linking genome and proteome by mass spectrometry: Large-scale identification of yeast proteins from two dimensional gels. *Proc Natl Acad Sci USA* 93:14440–14445.
4. Bedoya LC, Daròs JA (2010) Stability of *Tobacco etch virus* infectious clones in plasmid vectors. *Virus Res* 149:234–240.
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7. Liu Y, Schiff M, Marathe R, Dinesh-Kumar SP (2002) Tobacco *Rar1, EDS1* and *NPR1/NIM1* like genes are required for *N*-mediated resistance to tobacco mosaic virus. *Plant J* 30:415–429.

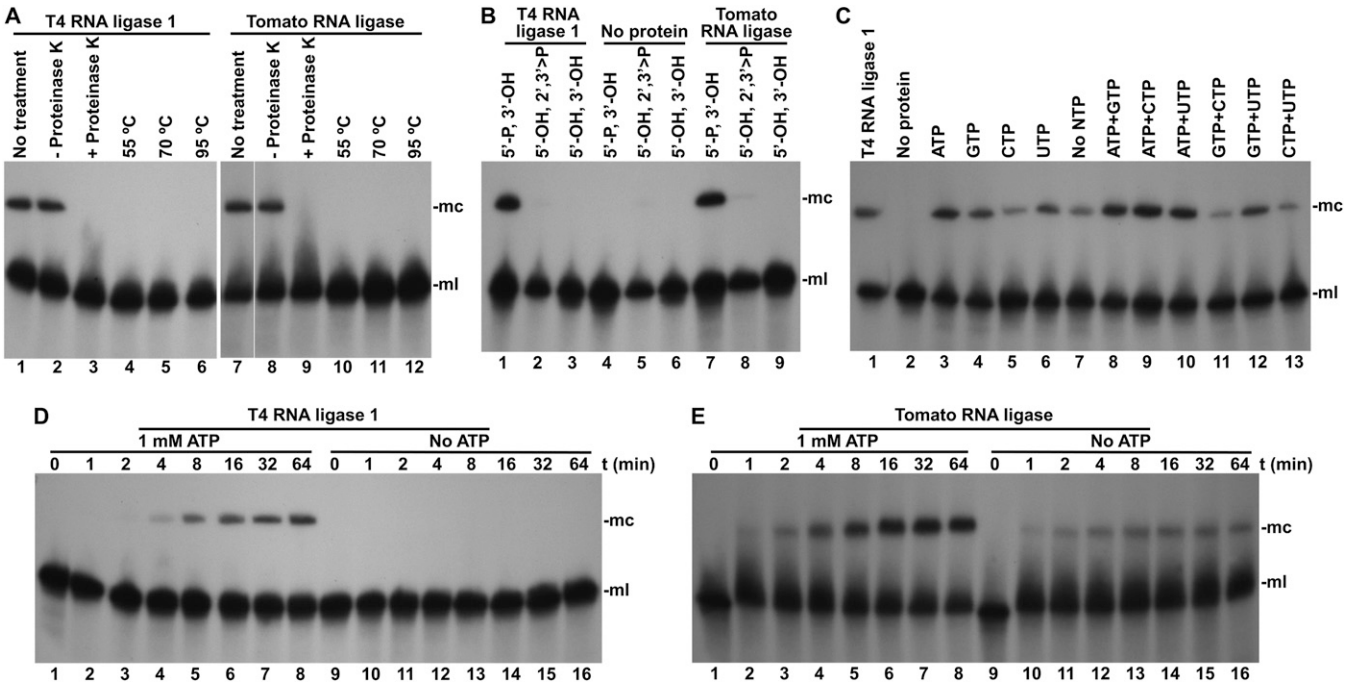

Fig. S1. Characterization of the PSTVd circularizing activity from tomato. Monomeric linear PSTVd RNAs were subjected to the circularization assay, and the reaction products were separated by denaturing PAGE and visualized by Northern blot hybridization. (*A*) Proteinase K and thermal inactivation treatments of T4 RNA ligase 1 (lanes 1–6) and the tomato protein fraction showing maximum PSTVd circularization activity (lanes 7–12). Lanes 1 and 7, control reactions in standard conditions. Lanes 3 and 9, reactions in which protein preparations were treated with proteinase K. Lanes 2 and 8, controls without proteinase K added. Lanes 4–6 and 10–12, reactions with the protein fraction heated at 55, 70, and 95 °C for 15 min. (*B*) Circularization of different monomeric linear PSTVd forms (opened at position G95–G96) by T4 RNA ligase 1 (lanes 1–3) and the tomato protein fraction (lanes 7–9). Lanes 4–6, controls with no protein added. Lanes 1, 4, and 7, monomeric linear PSTVd RNA with 5′-phosphomonoester and 3′-hydroxyl termini; lanes 2, 5, and 8, with 5′-hydroxyl, 2′,3′-cyclic phosphodiester termini; lanes 3, 6, and 9, with 5′-hydroxyl, 3′-hydroxyl termini. (*C*) Effect of different nucleoside triphosphate combinations on PSTVd circularization. Lane 1, control with T4 RNA ligase 1 and ATP. Lane 2, control with ATP and no protein added. Lanes 3–13, product generated by the tomato chromatographic fraction in the presence of 1 mM ATP, GTP, CTP, or UTP (lanes 3–6, respectively), no NTP (lane 7), and combinations of two different NTPs as indicated (lanes 8–13). (*D* and *E*) Time course of PSTVd circularization with T4 RNA ligase 1 (*D*) and the tomato protein fraction (*E*) in the presence of 1 mM ATP (lanes 1–8) or with no ATP added (lanes 9–16). Reaction aliquots were taken at 0, 1, 2, 4, 8, 16, 32, and 64 min (lanes 1–8 and 9–16, respectively). Positions of monomeric circular (mc) and monomeric linear (ml) PSTVd RNAs are indicated to the right of the images.

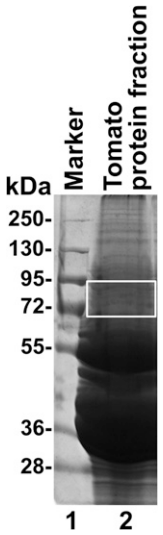

Fig. S2. Electrophoretic analysis of the tomato proteins contained in the fraction showing the highest PSTVd circularizing activity. Proteins were separated by SDS/PAGE in a preparative gel, and the gel fragment containing proteins with apparent sizes from ~70–90 kDa (white window) was cut. The proteins contained in the gel slice were subjected to protein identification by mass spectrometry analysis. Lane 1, protein markers with the sizes in kilodaltons indicated on the left. Lane 2, tomato protein fraction with the highest viroid circularizing activity.

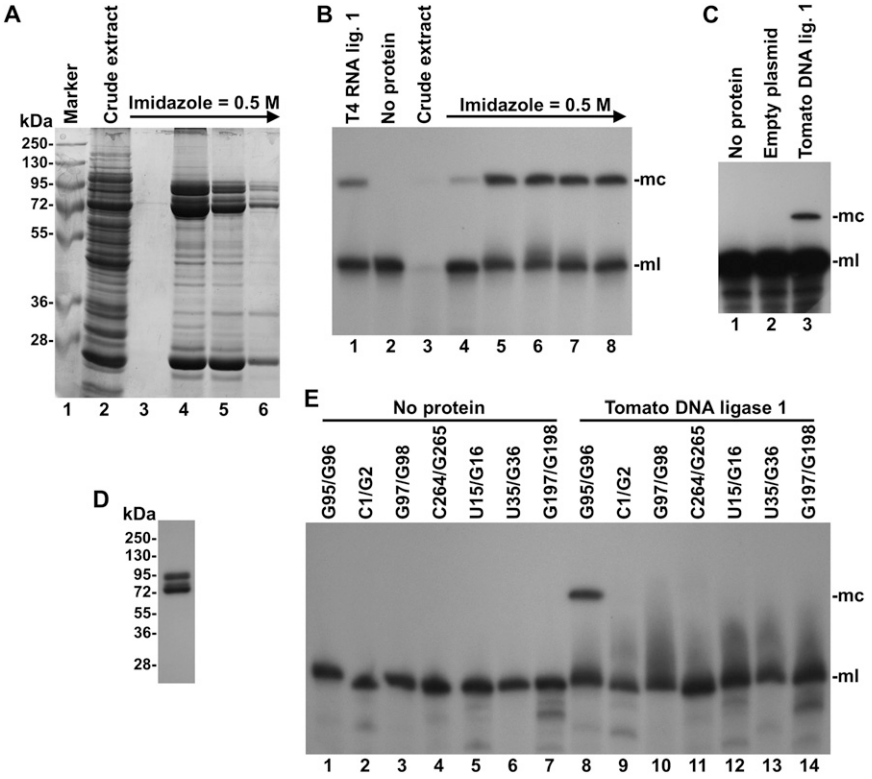

Fig. S3. Purification and analysis of the recombinant tomato DNA ligase 1. A recombinant version of tomato DNA ligase 1 was expressed in *E. coli*, purified, and assayed for its PSTVd circularizing activity and protein adenylation. (*A*) Proteins in the *E. coli* crude extract (lane 2) and in chromatographic fractions eluted with 0.5 M imidazole (lanes 3–6) were separated by SDS/PAGE and the gel was stained with Coomassie Blue. Lane 1, protein markers with the sizes in kilodaltons indicated on the left. (*B*, *C*, and *E*) Different monomeric linear PSTVd RNAs were incubated with the recombinant tomato DNA ligase 1. Reactions products were separated by denaturing PAGE, and PSTVd was visualized by Northern blot hybridization. (*B*) Analysis of the *E. coli* crude extract (lane 3) and different chromatographic fractions eluting from the Ni-Sepharose column with 0.5 M imidazole (lanes 4–8), using the standard substrate opened between positions G95 and G96. Lane 1, control with T4 RNA ligase 1. Lane 2, control with no protein added. (*C*) Comparison of the PSTVd circularizing activity of the recombinant tomato DNA ligase 1 purified from *E. coli* (lane 3) and of an equivalent protein fraction obtained from an *E. coli* culture transformed with the empty plasmid (lane 2). Lane 1, control with no protein added. (*D*) Adenylation assay of the recombinant tomato DNA ligase 1. Proteins were separated by SDS/PAGE and the gel autoradiographed. Positions of protein markers with their sizes in kilodaltons are indicated on the left of the image. (*E*) Circularization assays using monomeric linear PSTVd forms opened at seven different positions (Fig. 2*A*). Lanes 1–7, controls with no protein added. Lanes 8–14, reactions including the recombinant tomato DNA ligase 1. Positions of PSTVd monomeric circular (mc) and monomeric linear (ml) forms are indicated to the right of the images.

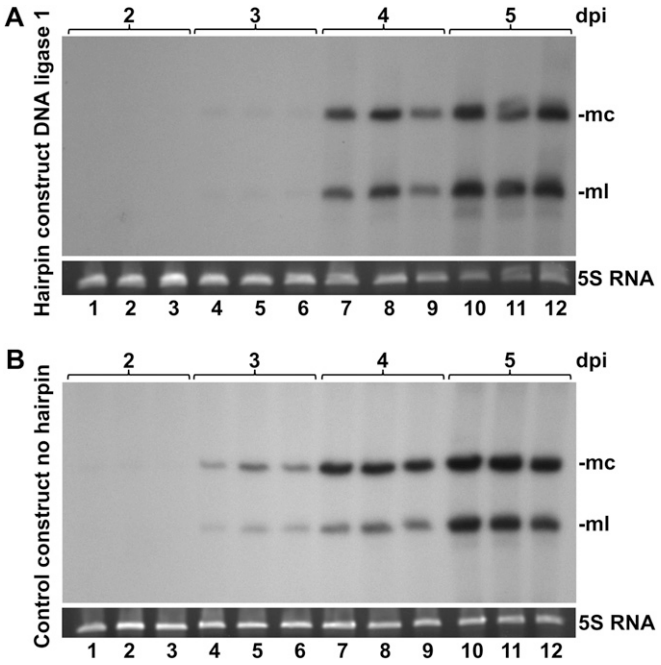

Fig. S4. Interference of the expression of a DNA ligase 1 hairpin construct on the in vivo circularization of PSTVd RNA. (*A*) A dimeric PSTVd construct and a tomato DNA ligase 1 hairpin construct, designed to induce the silencing of the endogenous gene, were coexpressed in *N. benthamiana*. (*B*) Control treatment in which the dimeric PSTVd construct was coinfiltrated with the empty hairpin vector. RNAs were purified from the agroinfiltrated areas from triplicate plants and separated by denaturing PAGE. Monomeric circular (mc) and linear (ml) PSTVd forms were detected by Northern blot hybridization. Tissue samples were harvested at 2 (lanes 1–3), 3 (lanes 4–6), 4 (lanes 7–9), and 5 (lanes 10–12) days postinfiltration (dpi). A fragment of the polyacrylamide gel stained with ethidium bromide containing a band corresponding to *N. benthamiana* 5S rRNA is included below each panel as a loading control. Positions of mc and ml PSTVd forms are indicated to the right of the images.

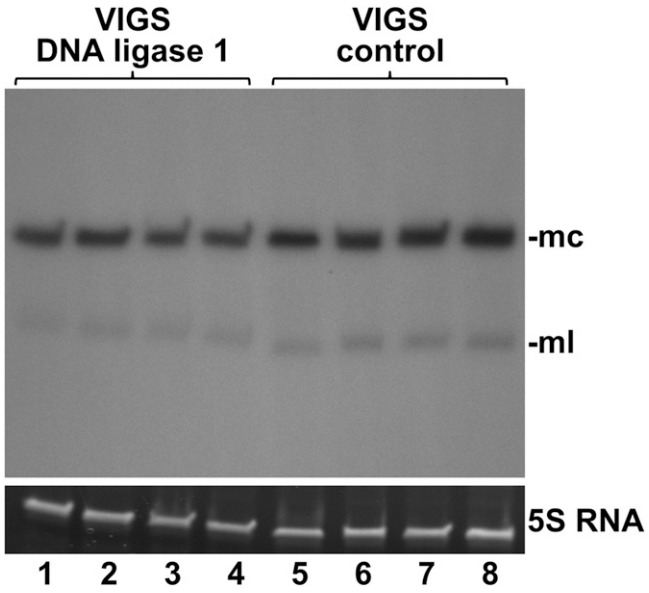

Fig. S5. PSTVd accumulation in plants subjected to VIGS of the host DNA ligase 1. *N. benthamiana* 16c plants constitutively expressing GFP were coagroinfiltrated with a dimeric PSTVd construct and a *Tobacco rattle virus* construct to silence the endogenous DNA ligase 1 as well as GFP (as a silencing reporter; lanes 1–4). A control coinfiltration with a VIGS vector to silence only GFP is also included (lanes 5–8). Tissue samples were harvested from quadruplicate plants 1 mo postinfiltration. (*Upper*) RNA was purified and separated by denaturing PAGE, and PSTVd forms were visualized by Northern blot hybridization. Positions of monomeric circular (mc) and linear (ml) PSTVd forms are indicated on the right side of the image. (*Lower*) Fragment of the polyacrylamide gel stained with ethidium bromide containing a band corresponding to *N. benthamiana* 5S rRNA as a loading control.

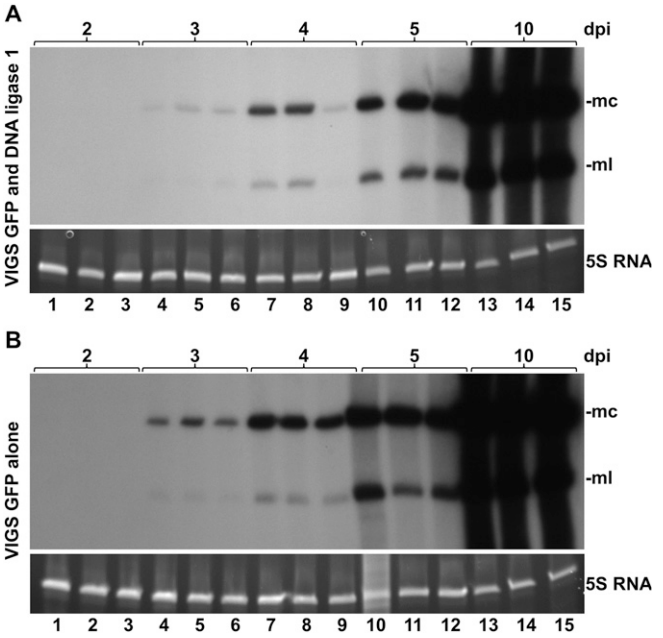

Fig. S6. PSTVd accumulation and circularization in plants previously subjected to VIGS of host DNA ligase 1. *N. benthamiana* 16c plants constitutively expressing GFP were infiltrated with a *Tobacco rattle virus* construct for VIGS of (*A*) GFP (as a silencing reporter) and the endogenous DNA ligase 1, or (*B*) GFP alone. At 3–4 wk later, once GFP silencing was confirmed, plants were infiltrated with a dimeric PSTVd construct. Tissue samples were harvested from the infiltrated areas of triplicate plants at 2 (lanes 1–3), 3 (lanes 4–6), 4 (lanes 7–9), 5 (lanes 10–12), and 10 (lanes 13–15) days after PSTVd infiltration (dpi). RNA was purified and separated by denaturing PAGE, and PSTVd forms were visualized by Northern blot hybridization. Positions of monomeric circular (mc) and linear (ml) PSTVd forms are indicated on the right. Fragments of the polyacrylamide gels stained with ethidium bromide containing a band corresponding to *N. benthamiana* 5S rRNA are included below each panel as a loading control.

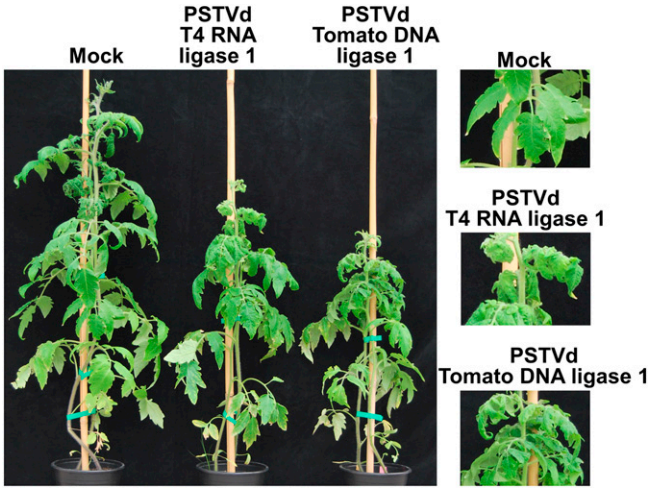

Fig. S7. Inoculation of tomato plants with circular PSTVd RNAs produced in vitro by the recombinant DNA ligase 1. The reaction products of a PSTVd circularization reaction catalyzed by either the T4 RNA ligase 1 or the recombinant tomato DNA ligase 1 were separated by denaturing PAGE. The circular forms were eluted from the gel and used to mechanically inoculate tomato seedlings. Representative PSTVd- and mock-inoculated plants were photographed 1 mo postinoculation.

Table S1. Proteins identified by mass spectrometry analysis in the piece of polyacrylamide gel likely containing the tomato PSTVd circularizing activity

gi|1707998 RecName: full = serine hydroxymethyltransferase, mitochondrial; short = SHMT
gi|1346155 RecName: full = serine hydroxymethyltransferase 1, mitochondrial; short = SHMT
gi|1781348 Homologous to plastidic aldolases (*Solanum tuberosum*)
gi|31126793 Putative glycine hydroxymethyltransferase [*Oryza sativa* (Japonica group)]
gi|15809972 AT4g37930/F20D10_50 (*Arabidopsis thaliana*)
gi|61969078 Putative ferredoxin-NADP reductase (*Solanum peruvianum*)
gi|3721540 Sulfite reductase (*Nicotiana tabacum*)
gi|462187 RecName: full = serine hydroxymethyltransferase, mitochondrial; short = SHMT; short = serine methylase; AltName:
gi|3913651 RecName: full = ferredoxin–NADP reductase, leaf-type isozyme, chloroplastic; short = FNR; flags: precursor
gi|3687301 Subtilisin-like protease (*Solanum lycopersicum*)
gi|120661 RecName: full = Glyceraldehyde-3-phosphate dehydrogenase A, chloroplastic; AltName:
gi|4827253 Plastidic aldolase (*Nicotiana paniculata*)
gi|169039 Aldolase (*Pisum sativum*)
gi|18420348 Fructose-bisphosphate aldolase, putative (*Arabidopsis thaliana*)
gi|18072795 Glyceraldehyde-3-phosphate dehydrogenase (*Capsicum annuum*)
gi|145203152 Heat shock protein 70 (*Cyclamen persicum*)
gi|108864705 Heat shock cognate 70-kDa protein, putative, expressed (*Oryza sativa* (Japonica cultivar group)]
gi|26453355 mRNA binding protein precursor (*Solanum lycopersicum*)
gi|30025966 Heat shock protein 70 (*Nicotiana tabacum*)
gi|23477636 Grp94 (*Xerophyta viscosa*)
gi|6899972 Chloroplast ferredoxin-NADP+ oxidoreductase precursor (*Capsicum annuum*)
gi|4827251 Plastidic aldolase NPALDP1 (*Nicotiana paniculata*)
gi|225427768 Predicted: hypothetical protein (*Vitis vinifera*)
gi|18072799 Glyceraldehyde-3-phosphate dehydrogenase (*Capsicum annuum*)
gi|17017273 Serine hydroxymethyltransferase (*Zea mays*)
gi|8919178 Alpha-glucosidase (*Solanum tuberosum*)
gi|77540212 Glyceraldehyde-3-phosphate dehydrogenase B subunit (glycine max)
gi|462013 RecName: full = endoplasmin homolog; AltName: full = glucose-regulated protein 94 homolog
gi|336390 Glyceraldehyde 3-phosphate dehydrogenase B subunit (*Arabidopsis thaliana*)
gi|56122688 Chloroplast latex aldolase-like protein (*Manihot esculenta*)
gi|12658639 Ferredoxin:sulfite reductase precursor (glycine max)
gi|147865450 Hypothetical protein (*Vitis vinifera*)
gi|108705994 Glyceraldehyde-3-phosphate dehydrogenase B, chloroplast precursor, putative, expressed [*Oryza sativa* (Japonica cultivar)]
gi|4930123 Chain A, wild-type pea Fnr
gi|3687303 Subtilisin-like protease (*Solanum lycopersicum*)
gi|544242 RecName: full = endoplasmin homolog; AltName: full = glucose-regulated protein 94 homolog; short = GRP-94 homolog
gi|111162651 Chloroplast aldolase (*Nicotiana attenuata*)
gi|20733 Unnamed protein product (*Pisum sativum*)
gi|18252211 Aspartate–tRNA ligase-like protein (*Arabidopsis thaliana*)
gi|115446545 Os02g0538000 [*Oryza sativa* (Japonica cultivar group)]
gi|62320472 Putative protein (*Arabidopsis thaliana*)
gi|475600 BiP isoform B (glycine max)
gi|3608173 Acid invertase (*Solanum lycopersicum*)
gi|77745483 Unknown (*Solanum tuberosum*)
gi|51989592 PEN2-like protein (*Solanum tuberosum*)
gi|115478034 Os09g0127800 [*Oryza sativa* (Japonica cultivar group)]
gi|147838425 Hypothetical protein (*Vitis vinifera*)
gi|15226538 Coatomer protein complex, subunit alpha, putative (*Arabidopsis thaliana*)
gi|67043517 Subtilisin-like serine protease (*Solanum tuberosum*)
gi|147855642 Hypothetical protein (*Vitis vinifera*)
gi|1841704 Histidyl tRNA synthetase [*Oryza sativa* (Japonica cultivar group)]
gi|79677444 Glyceraldehyde-3-phosphate dehydrogenase (*Chara vulgaris*)
gi|37222955 Putative fructose-bisphosphate aldolase protein (*Solanum tuberosum*)
gi|147770508 Hypothetical protein (*Vitis vinifera*)
gi|40716077 Fructose-bisphosphate aldolase (*Pandanus amaryllifolius*)
gi|13111324 110 kDa 4SNc-Tudor domain protein (*Pisum sativum*)
gi|218157 Cytoplasmic aldolase [*Oryza sativa* (Japonica group)]
gi|2765081 g5bf (*Arabidopsis thaliana*)
gi|115488340 Os12g0420200 [*Oryza sativa* (Japonica cultivar group)]
gi|79677457 Glyceraldehyde-3-phosphate dehydrogenase (*Klebsormidium flaccidum*)
gi|32492578 RNA binding protein Rp120 [*Oryza sativa* (Japonica group)]
gi|2493321 RecName: full = L-ascorbate oxidase; short = ascorbase; short = ASO; flags: precursor
gi|3047084 Similar to aminoacyl-tRNA synthetases (*Arabidopsis thaliana*)

Table S1. Cont.

gi|115434198 Os01g0118000 [*Oryza sativa* (Japonica cultivar group)]
gi|15236768 Fructose-bisphosphate aldolase, putative (*Arabidopsis thaliana*)
gi|71534902 Histidyl-tRNA synthetase (*Medicago sativa*)
gi|22328958 Aspartyl-tRNA synthetase, putative/aspartate–tRNA ligase, putative (*Arabidopsis thaliana*)
gi|6728986 Putative histidyl tRNA synthetase (*Arabidopsis thaliana*)
gi|2341032 EST gb|ATTS0956 comes from this gene (*Arabidopsis thaliana*)
gi|110289257 Dehydration-responsive protein, putative, expressed [*Oryza sativa* (Japonica cultivar group)]
gi|225450527 Predicted: hypothetical protein (*Vitis vinifera*)
gi|1359495 DNA ligase (*Arabidopsis thaliana*)
gi|115438604 Os01g0617500 [*Oryza sativa* (Japonica cultivar group)]
gi|3913525 RecName: full = DNA polymerase delta catalytic subunit
gi|2191162 Similar to threonyl-tRNA synthetase; coded for by *A. thaliana* cDNA R65376 (*Arabidopsis thaliana*)
gi|108707116 Subtilisin proteinase, putative, expressed [*Oryza sativa* (Japonica cultivar group)]
gi|8843758 ABC transporter-like (*Arabidopsis thaliana*)
gi|15221411 PTAC2 (plastid transcriptionally active 2) (*Arabidopsis thaliana*)
gi|3426038 Unknown protein (*Arabidopsis thaliana*)
gi|147786974 Hypothetical protein (*Vitis vinifera*)
gi|3451071 Beta adaptin-like protein (*Arabidopsis thaliana*)
gi|84688912 AGO4-2 (*Nicotiana benthamiana*)
gi|9945085 F5A9.21 (*Arabidopsis thaliana*)
gi|9294698 Unnamed protein product (*Arabidopsis thaliana*)
gi|3695406 Similar to isoleucyl-tRNA synthetases (*Arabidopsis thaliana*)
gi|119150 RecName: full = elongation factor 1-α; short = EF-1-α
gi|115529201 Isoflavone conjugate-specific beta-glucosidase (glycine max)
gi|3193299 T14P8.19 (*Arabidopsis thaliana*)
gi|159486781 Gln-Glu nondiscriminatory tRNA synthetase (*Chlamydomonas reinhardtii*)
gi|5918016 Myosin-like protein (*Arabidopsis thaliana*)
gi|5531937 Acetoacetyl CoA thiolase (*Zea mays*)
gi|28201554 Hypothetical protein [*Oryza sativa* (Japonica group)]
gi|37932212 Pectin acetylesterase (*Lactuca sativa*)
gi|34393513 Hypothetical protein [Oryza sativa (Japonica group)]
gi|28209456 Putative polyprotein [*Oryza sativa* (Japonica group)]
gi|29367425 Heat shock-related protein [*Oryza sativa* (Japonica group)]
gi|32488074 OSJNBa0084A10.2 [*Oryza sativa* (Japonica cultivar group)]
gi|145345433 Predicted protein (*Ostreococcus lucimarinus* CCE9901)
gi|10129647 Putative protein (*Arabidopsis thaliana*)
gi|115450355 Os03g0119300 [*Oryza sativa* (Japonica cultivar group)]
gi|147815446 Hypothetical protein (*Vitis vinifera*)
gi|145348499 Predicted protein (*Ostreococcus lucimarinus* CCE9901)
gi|115436430 Os01g0348600 [*Oryza sativa* (Japonica cultivar group)]
gi|13435254 Putative ankyrin [*Oryza sativa* (Japonica group)]
gi|108773101 Hypothetical protein ScobCp071 (*Scenedesmus obliquus*)
gi|147773688 Hypothetical protein (*Vitis vinifera*)
gi|110739422 Hypothetical protein (*Arabidopsis thaliana*)
gi|116308918 OSIGBa0131F24.2 [*Oryza sativa* (Indica cultivar group)]
gi|12322998 Unknown protein (*Arabidopsis thaliana*)

The hit corresponding to *A. thaliana* DNA ligase 1 is in boldface.