Supporting Information

Cao et al. 10.1073/pnas.1407131111

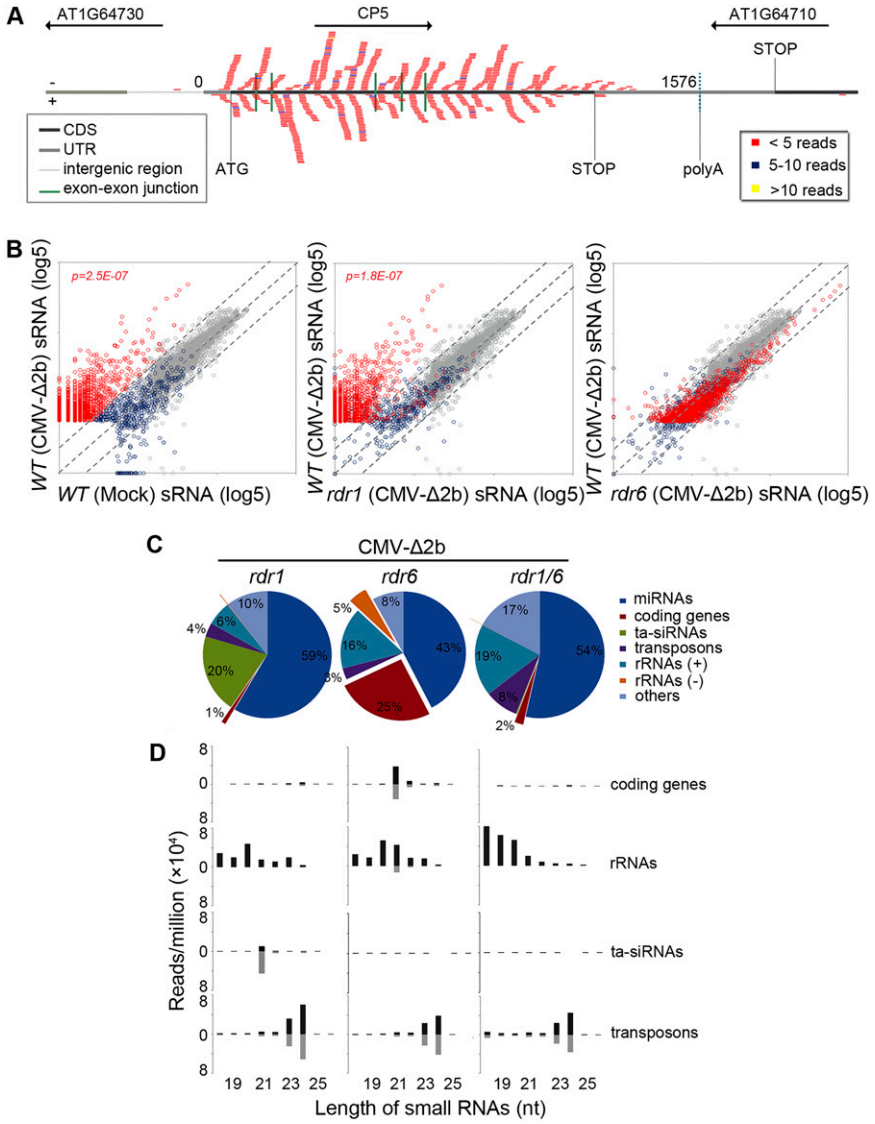

Fig. S1. Properties and biogenesis of vasiRNAs. (*A*) Distribution patterns of sense (bottom) and antisense (top) vasiRNAs specific to the RDR1 target gene encoding membrane related protein CP5 (CP5). Various regions of the target gene and the neighboring gene(s) are indicated by colored lines. Only the mature mRNA is shown with exon junctions represented by green vertical lines. CDS, the protein-coding region; UTR, untranslated region. (*B*) Scatter plots depicting locus-by-locus comparisons of small RNA (sRNA) abundance between pairs of small RNA libraries, with the 1,172 RDR1 target genes labeled as red circles and transposons and the remaining genes as gray and blue circles, respectively. The *x* and *y* values of each circle corresponded to the number of small RNA reads from this locus in the respective libraries. The loci within the outside pair of the dotted lines exhibited less than threefold difference in the abundance of the locus-specific small RNAs between the two libraries. (*C*) Relative abundance of total *Arabidopsis* small RNAs divided in specific sequence groups from *rdr1*, *rdr6*, or *rdr1 rdr6* plants after CMV-Δ2b infection. (*D*) Length distribution (in nucleotides) and abundance (reads per million of total reads) of the total *Arabidopsis* small RNAs derived from protein-coding genes, rRNAs, tasiRNAs, and transposons from *rdr1*, *rdr6*, or *rdr1 rdr6* plants after CMV-Δ2b infection.

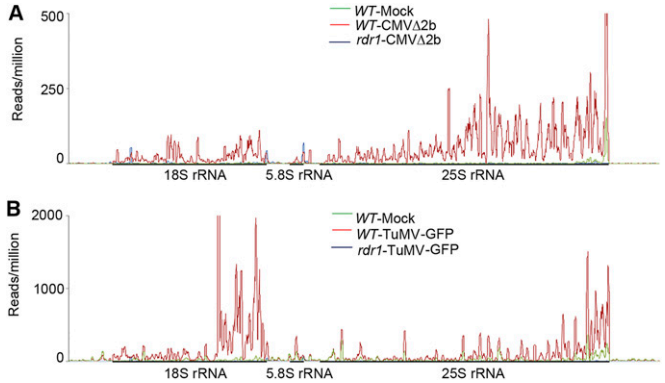

Fig. S2. Distribution patterns of antisense vasiRNAs derived from the 45S ribosomal DNA (rDNA) locus that encodes the 5.8S, 18S, and 25S rRNAs in mock-inoculated wild type (WT) plants and in WT and rdr1 plants after infection (*A*) with CMV-Δ2b of the Fny strain or (*B*) with the recombinant isolate of TuMV that expresses green fluorescent protein (TuMV-GFP).

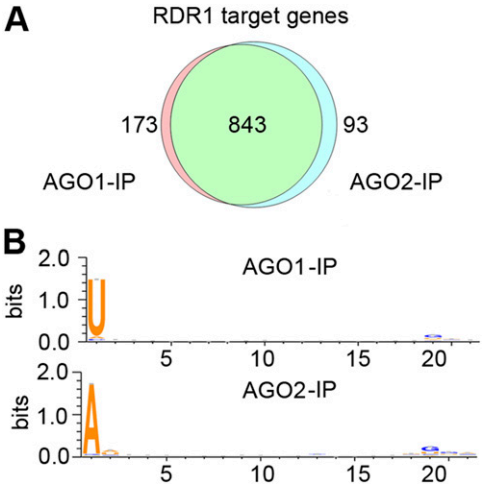

Fig. S3. Enrichment and properties of vasiRNAs in AGO1 and AGO2 complexes. (*A*) Venn diagram depicting the proportion of the 1172 RDR1 target loci that are enriched twofold or more in both of the AGO1 and AGO2 complexes coimmunoprecipitated (IP) from WT plants after CMV-Δ2b infection. (*B*) The 5′ terminal nucleotide bias of vasiRNAs loaded into AGO1 and AGO2 examined by Weblogo software.

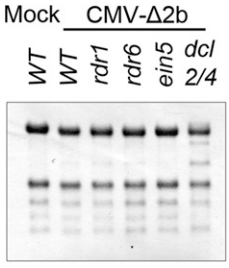

Fig. S4. Infection with CMV-Δ2b did not induce RDR1-dependent changes in the accumulation of rRNAs in *Arabidopsis* plants. WT, *rdr1*, *rdr6*, *ein5*, and *dcl2*/*dcl4* mutant seedlings were inoculated with CMV-Δ2b virions (5 μg/mL) as described for Fig. 3 *B*–*D*. Two weeks after infection, the upper systemically infected leaves were harvested from the inoculated plants and the fresh tissues weighted before the extraction of total RNAs. High molecular weight RNAs from 10 mg fresh tissues of each sample were loaded in each lane and fractionated by denaturing agarose gel electrophoresis. The experiments were repeated multiple times, and a reprehensive gel stained by methylene blue was shown. The two dominant RNA bands corresponded to 25S and 18S rRNA. Lane 1 contained total RNAs from WT plants inoculated with buffer (mock).

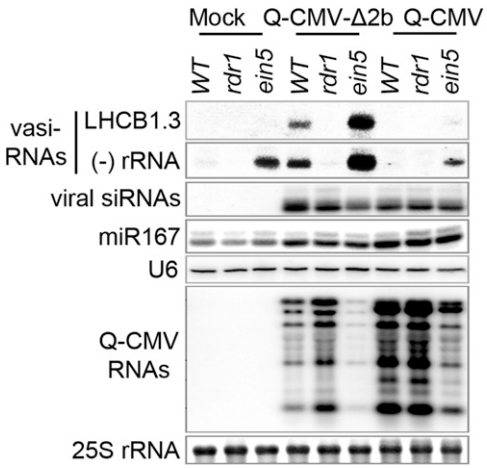

Fig. S5. Production of vasiRNAs induced by Q strain of CMV was RDR1 dependent and was enhanced by the presence of the null allele (*ein5-6*) of *Arabidopsis* exoribonuclease gene *XRN4/EIN5*. The accumulation of vasiRNAs derived from LHCB1.3 and the antisense 25S rRNA in WT, *rdr1*, and *ein5* plants after infection with the Q strain of CMV and its CMV-Δ2b was detected by Northern blotting analysis. Viral siRNAs, miR167, and U6 RNA as well as the Q-CMV RNAs were also probed.

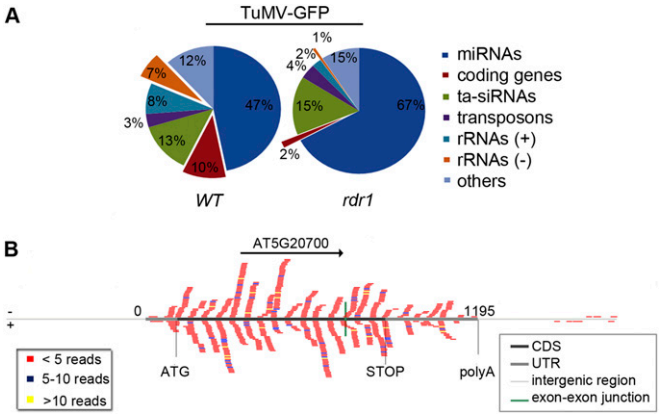

Fig. S6. Properties of vasiRNAs induced by TuMV-GFP. (*A*) Relative abundance of the total 21-nt RNAs from different sequence groups in WT and *rdr1* plants after infection by the TuMV-GFP isolate. (*B*) Distribution patterns of sense (bottom) and antisense (top) vasiRNAs specific to one of RDR1 target genes, AT5G20700, in the TuMV-GFP-infected WT plants. Various regions of the target gene and the neighboring gene(s) are indicated by colored lines. Only the mature mRNA is shown, with exon junctions represented by green vertical lines. CDS, the protein-coding region; UTR, untranslated region.

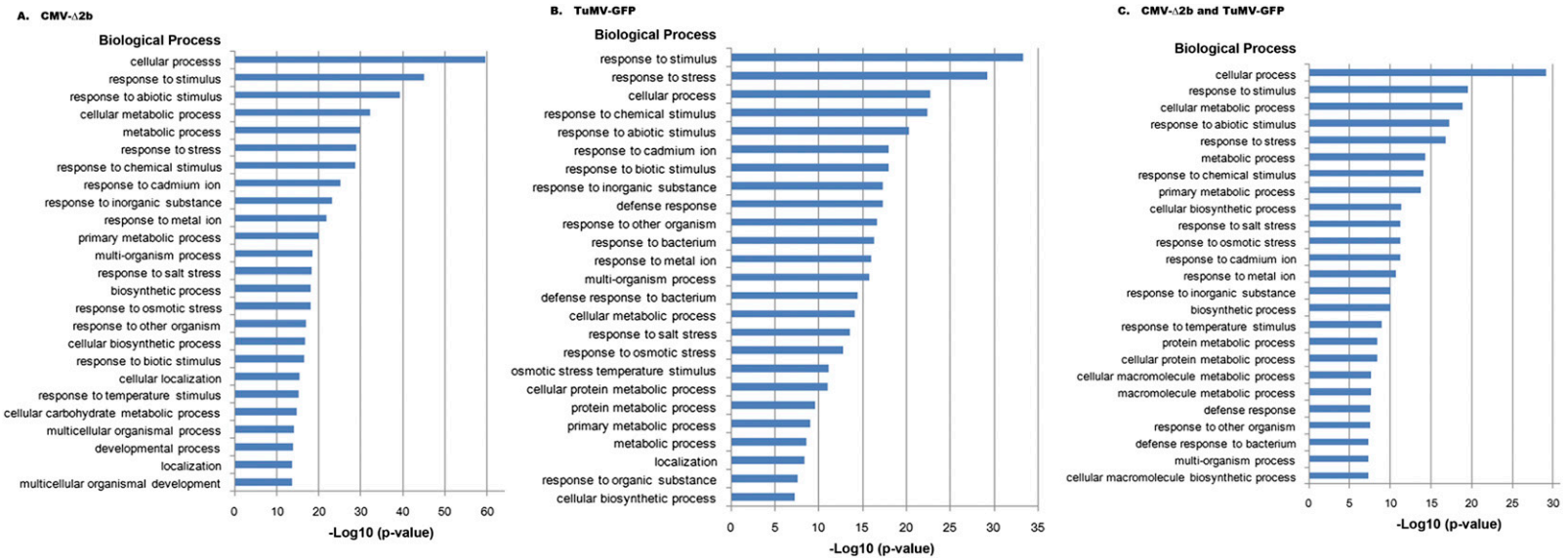

Fig. S7. Gene ontology (GO) annotation of *Arabidopsis* genes in biological processes targeted by RDR1-dependent vasiRNAs in response to infection by CMV-Δ2b (*A*), TuMV-GFP (*B*), or both (*C*). Top 25 enriched GO terms ranked by the corrected *P* values were listed.

Table S1. Probes and primers used in this paper

Primer or probe	Sequence (5′-3′)
Probes used for Northern blot detection of host small RNAs	
DNA oligo probe	
LHCB1.3-130-180-R	AGTCTTCCTCATTGTCACACGGCCGCTTCCAAGGACTTCAGATGCCGCGG
LHCB1.3-200-250-F	ATCAGGCAGCCCATGGTACGGATCTGACCGTGTCAAGTACTTGGGTCCAT
LHCB1.3-250-300-F	TCTCTGGCGAATCACCGAGCTACCTTACCGGAGAGTTCCCCGGAGACTAC
LHCB1.3-330-380-F	CCCGAGACATTCGCAAGGAACCGTGAACTAGAAGTTATCCACAGCAGGTG
LHCB1.3-460-510-R	TCCCAAGTAATCGAGCCCTCCATCGCTGAAGATCTGTGAACCGGCCTTGA
LHCB1.3-390-440-R	TTGACTCCGTTTCTAGCCAAAAGCTCAGGGAAGACGCAGCCTAGGGCTCC
LHCB1.3-590-640-R	GGTAAAGCAAGTCCTCGGCCTCTCCCAATGGCCCATTTCCTGCGACTCTG
LHCB1.3-540-590-R	TAGCCTTCAACGGCTCCCATCAAAATAACTTGTGTGGCCCAAATGGCCAA
LHCB1.3-640-690-F	CCGGTGGCAGCTTCGACCCATTGGGTTTGGCTACCGACCCAGAGGCATTC
LHCB1.3-690-740-R	GAGAACATAGCCAATCTTCCGTTCTTGAGCTCCTTCACCTTCAACTCAGC
LHCB1.3-750-800-F	TTCTTCGTTCAAGCCATCGTCACTGGTAAGGGACCGATAGAGAACCTTGC
LHCB1.3-800-850-F	TGACCATTTGGCCGATCCAGTTAACAACAACGCATGGGCCTTCGCCACCA
CP5-100-150-R	AGAATCGAGATAAGCCCACTTGGGCCTCCAAACCCAACCAACCAAAACAC
CP5-50-100-R	CGGCAGCCACAGCAATCCAGAGTGGCGCAACAAAAACTATAAGGTCAGAG
CP5-150-200-F	AAACTCTTGTCCAATTCACCTAAATTCCTCAATCTTCAGCTTCCCACTTC
CP5-370-430-R	CAAACACAGTTCTGCTACGGTACTGGGGAGGCCCATTCTCAGGATCTCGCCTCCAAGCTT
CP5-500-550-F	GCTTTTGTATTCTTCTACTCTCGAGCGTTGCAAGGACACGGGCACTATGG
CP5-340-370-F	TGGACCGATCAACCCCAACCTTCTCATATC
CP5-280-340-R	TCATTTGAATCCAACAAGGACCGCCATCTTTGACCTCAACCAACTTCCATAGATGCCTAA
CP5-720-780-R	TACCTCACATGATGTCATCTCACCATCGCCTCTTTTTGATTCAACTGCACGGATACACCA
CP5-940-990-R	GCTTTAGCCCTCTGATATGCACGTAGGCCTGGCTCAATCTTCTTGACTGC
CP5-650-700-F	GAAAGTTCCCCTTCTTTTGTAGCGACAGGGAGTATATTATAGGCCGTCGT
CP5-480-530-F	TGGGCCTCCCCAGTACCGTAGCAGAACTGTGTTTGAGGATGCCACTCCCG
CP5-430-480-F	CGATCAACCCCAACCTTCTCATATCAAGCTTGGAGGCGAGATCCTGAGAA
CP5-280-330-F	GAAGAAAGGCTGGGGTTAACGTGGTGAGAAGAAGTTTTGAGGATGGAAGT
CP5-320-370-R	CCTAAAATCATCGTCCGTTACAAACCCAGTTTTCTCTTTGGAAGAAAGGC
CP5-50-200-F	CTCCCAAGACCCAACCATCGGAGGGAGGGGGCGAGATGATGGGATTGAGT
HSP70-1-1030-1083-F	GTGGTTCTACCCGTATCCCTAAGGTTCAGCAATTGCTCCAGGACTTCTTCAAC
HSP70-1-1130-1180-R	CCTTCTCGTTTCCTTCACCGCTGAGAATAGCTCCCTGGACAGCAGCACCG
HSP70-1-1490-1530-F	GGACAAGACCACCGGACAGAAGAACAAGATCACCATCACC
HSP70-1-150-180-R	ATTCTTAGCTGCGTCACCGATCAACCTCTC
HSP70-1-1560-1610-R	TCCTCGTCTTCGGACTTGTACTTCTCAGCCTCTTGAACCATCTTCTCAAT
HSP70-1-1730-1680-R	TCGATCTTCTTCTTGTCTGCAGCCGGGAGCTTCTCACCAATCTTCTCGTC
HSP70-1-1760-1810-R	CCTTCATCTTGTCTTCGAACTCATCAGCCTCAGCCAACTGGTTACCCTCG
HSP70-1-1920-1950-R	GACCTCCTCGATCTTAGGTCCAGCACCGCC
HSP70-1-220-260-R	TGAACAGAGCTGTCAGAGAAACGACGACCGATCAACCTCT
HSP70-1-840-870-F	TCTTCCACTGCTCAGACCACCATCGAGATT
HSP70-1-890-930-F	CGACTTCTACTCCACCATCACCCGTGCTAG TTTGAGGAG
HSP70-1-980-1030-R	CAACAAGGACAACATCATGAACAGTGCTCTTGTCCATCTTAGCATCACGA
HSP70-1-1960-2010-R	ATCGTCCATACCAGAGGCACCTGGACCACCGGCTTCACCACCAGCTCCTT
HSP70-1-1750-1800-F	TCGAGAACTACGCTTACAACATGAGGAACACCATCCAAGACGAGAAGATT
HSP70-1-1240-1290-F	GTGCTGCTGTCCAGGGAGCTATTCTCAGCGGTGAAGGAAACGAGAAGGTT
HSP70-1-1590-1640-R	TTGGTGATGGTGATCTTGTTCTTCTGTCCGGTGGTCTTGTCCTCAGCAGA
HSP70-1-1910-1960-F	GATGAAGGAATTGGAGAGCATCTGCAACCCAATCATTGCCAAGATGTACC
HSP70-1-1470-1520-R	GGAATTCCGGAGAGCTCAAATTTACCAAGAAGGTTGTTGTCCTTGGTTCT
HSP70-1-1070-1120-R	GAACAGTGCTCTTGTCCATCTTAGCATCACGAAGACACTTCTCAACTGGC
AT5G20700-80-130-F	AATCTGATCATCAAAACTCTGCTCTCTCAAGATCATGCTTACTAAAAGAA
AT5G20700-190-240-F	CTCCTTTCTTCGACGTCTTGATGACGAGCCCTAAAAGCCCACTGGATTTC
AT5G20700-280-330-R	GGCTACAATCCCTAGACCAACAGAGCCACCAAGATTGTCATCGTAGAATC
AT5G20700-330-380-R	TCCGATCTACAAACGCTCGTGATTCGACGAGTGTTTGAGTTCTCGAGCGC
AT5G20700-430-490-R	CCAACGTGTAATCCTCCTCGTCCATTATGAACATCTCCTCGTCTTCTCCGTCCGTGCTTC
AT5G20700-600-640-R	CTGGCGAATTCTCTGGAGATTCCGTAACGACATCGACCAC
AT5G20700-790-830-F	GTAGATCAAAATTCTCAACCTCTCCTTACACCGCCGGCCA
AT5G20700-840-880-R	ATCAGTAGAAGAACTCCGGCTAAGTCACTAGAACTCCGGT
AT5G20700-840-880-F	ACCGGAGTTCTAGTGACTTAGCCGGAGTTCTTCTACTGAT
AT5G20700-730-780-R	CCTTTCATCATTTGCTATATGACTCGATCGACACTCTGTGCTGCAAAATG
AT5G20700-670-720-R	TCTGTAAATAAATATGTCTTGACCATGAAGTTTCTTCCTGCACAAGTAAC
AT5G20700-530-580-R	CATCGTTGATCTTACTTGAGAAACACTCAAACCCATCTTTGTCATAAACC
AT5G20700-480-530-F	TACACGTTGGTGACATGCCACCATGGACCAAGTGGATCTTGTAATACAAG
AT5G20700-430-480-F	GAAGCACGGACGGAGAAGACGAGGAGATGTTCATAATGGACGAGGAGGAT
AT5G20700-330-380-F	GCGCTCGAGAACTCAAACACTCGTCGAATCACGAGCGTTTGTAGATCGGA

Table S1. Cont.

Primer or probe	Sequence (5′-3′)
AT5G20700-280-330-F	GATTCTACGATGACAATCTTGGTGGCTCTGTTGGTCTAGGGATTGTAGCC
25S rRNA-4170-F	GGCCTTGCTGCCACGATCCACTGAGATTCAGCCCTTTGTC
25S rRNA-4130-F	ACGACTTAAATACGCGACGGGGTATTGTAAGTGGCAGAGT
25S rRNA-4070-F	GGTCGTTCGGACCGCCTTGAATTATAATTACCACCGAGCG
25S rRNA-4030-F	CCAAAGGCACGTGTCGTTGGCTAAGTCCGTTCGGCGGAAC
25S rRNA-3990-F	CCCGCCGCCCGATTGCCGACCCTCAGTAGGAGCTTAGGCT
25S rRNA-3950-F	CGCCTCTAAGTCAGAATCCGGGCTAGAAGCGACGCATGC
25S rRNA-3910-F	CCAGTGGCGCGAAGCTACCGTGCGCTGGATTATGACTGAA
25S rRNA-3860-F	CGAGAGGAACCGTTGATTCGCACAATTGGTCATCGCGCTT
25S rRNA-3820-F	CCTACTGATGCCCGCGTCGCGATAGTAATTCAACCTAGTA
25S rRNA-3690-F	TTGCTTTTTGATCCTTCGATGTCGGCTCTTCCTATCATTG
25S rRNA-3650-F	GGGATAACTGGCTTGTGGCAGCCAAGCGTTCATAGCGACG
25S rRNA-3550-F	TGATTCTGATTTTCAGTACGAATACGAACCGTGAAAGCGT
25S rRNA-3240-F	ACCCTGTTGAGCTTGACTCTAGTCCGACTTTGTGAAATGA
25S rRNA-3120-F	CCTCGTCATCTAATTAGTGACGCGCATGAATGGATTAACG
25S rRNA-3020-F	CCTCGTCATCTAATTAGTGACGCGCATGAATGGATTAACG
25S rRNA-2940-F	CAGAACTGGTACGGACAAGGGGAATCCGACTGTTTAATTA
25S rRNA-2800-F	GGGTCCCAGTTCCGAACCCGTCGGCTGTCAGCGGACTGCT
25S rRNA-2700-F	GGTGAACAGCCTCTGGTCGATGGAACAATGTAGGCAAGGG
25S rRNA-2650-F	CCGAGTGCCGCTCACGCCCGGTCGTACTCATAACCGCATC
miR167	TAGATCATGCTGGCAGCTTCA
ASRP255	TACGCTATGTTGGACTTAGAA
U6	AGGGGCCATGCTAATCTTCTC
Primers used for PCR amplification of cDNA probes to detect host mRNA by Northern blotting	
Primer	
LHCB1.3-F	AACGGAGTCAAGTTCGGA
LHCB1.3-R	TCCCTTACCAGTGACGATG
CP5-F	GATGATGAGTTCCGTTCCA
CP5-R	GGTGTTGATGTGAGCCATT
AT5G20700-F	TTCATAATGGACGAGGAGG
AT5G20700-R	CGGTGTAAGGAGAGGTTGAG
HSP70-1-F	GTGTCATCGCTGGTTTGA
HSP70-1-R	TGATGGTGGAGTAGAAGTCG
RBCS-1A-F	TACTATGGTTGCCTCTCCG
RBCS-1A-R	GCACTCTTCCACTTCCTTCA
RCA-F	GAGGCAAAGGTCAAGGTAAA
RCA-R	ACGGATGAGAGGAGCGTAT
TUB2-F	CGAGTTGCGGTAGATTCGT
TUB2-R	AGAGTAGCGTTGTAAGGCTCA
Primers used for obtaining cDNA fragments as probes to detect viral RNAs	
Primer	
Fny-CMV-RNA2-F	TCTGTGGCGGGAGCTGAGTTGGC
Fny-CMV-RNA2-R	TGGTCTCCTTTTGGAGGCCCCAC
Q-CMV-RNA3-F	CGTCCGAAGACGTTAAACTAC
Q-CMV-RNA3-R	TGGTCTCCTTATGGAGAACCT
TuMV-CP-F	CGAACTGACGGAGGACAAA
TuMV-CP-R	TTCCATCCAAGCCGAACA
Primers used for obtaining the cDNA fragment as probes to detect viral siRNAs	
Primer	
TuMV-CI-F	ACTCTCAATGATATAGAGGATG
TuMV-CI-R	TTGATGGTGAACTGCCTCAAG
Probes used for Northern blot detection of viral siRNAs	
DNA oligo probe	
Q-CMV-RNA3-1-40-F	GTAATCTTACCACTTTCTTTCACGTCGTGTCGCGTCAGTC
Q-CMV-RNA3-241-280-F	CGGATAACGCCATCTCTGTCAGACCTCTCGTTCCCCAAGT
Q-CMV-RNA3-741-780-F	GCCGTCGCTCGCCTGTTGAAGTCGCAGTTAAACAACGTTA
Q-CMV-RNA3-1041-1080-F	GACGCGATGCCGCTTGAAGATTTCCCATCTGGGGTTAGTA
Q-CMV-RNA3-1341-1380-F	GACTCAATAAAACCCTCGCCATTGGTCGTCCCACTCTTAA
Q-CMV-RNA3-1641-1680-F	CTATGTTTGGCGATGGTAACTCACCGGTTTTGGTTTATCA
Q-CMV-RNA3-2131-2170-F	TACACTGATATTACCAAGAGTGCGGGTATCGCCTGTGGTT
Fny-CMV-RNA3-241-280-F	TAGGCCGGCATCATTGGATGCGCGCTGATAATGCTATTTC
Fny-CMV-RNA3-741-780-F	TTAGCTGAGCAAACAAAACCGTCAGCTGTCGCTCGCCTGT

Table S1. Cont.

Primer or probe	Sequence (5′-3′)
Fny-CMV-RNA3-1041-1080-F	AGTAGACATCTGTGACGCGATGCCGTGTTGAGAAGGGAAC
Fny-CMV-RNA3-1341-1380-F	GATGCTAACTTTAGAGTCTTGTCGCAGCAGCTTTCGCGAC
Fny-CMV-RNA3-1600-1640-F	GAAATTTGATTCTACCGTGTGGGTGACAGTCCGTAAAGTT
Fny-CMV-RNA3-1681-1710-F	TGTTCGCGGACGGAGCCTCACCGGTACTGG
Fny-CMV-RNA3-1731-1770-F	GGAGTCCAAGCCAACAACAAACTGTTGTATGATCTTTCGG

Other Supporting Information Files

Dataset S1 (XLS)