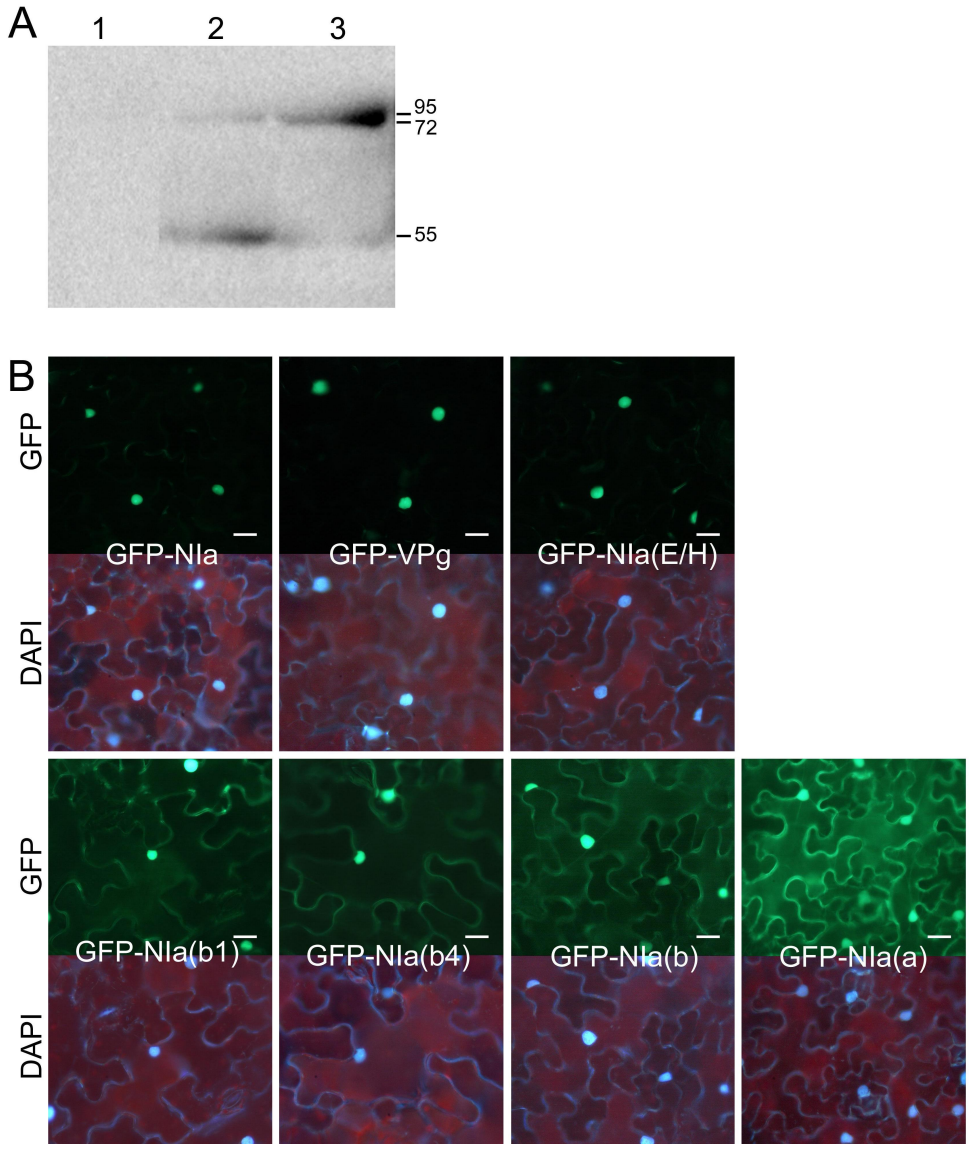
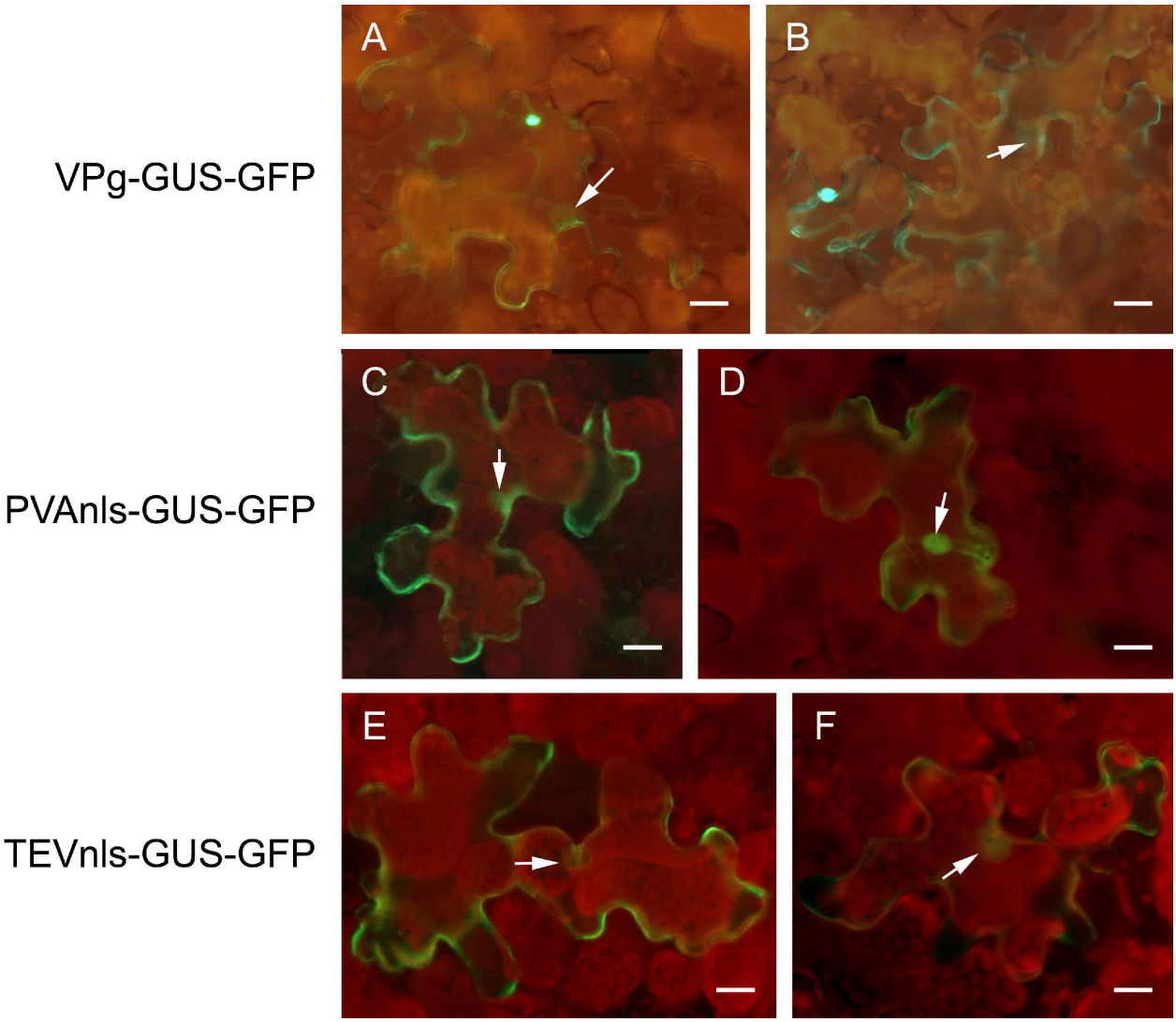
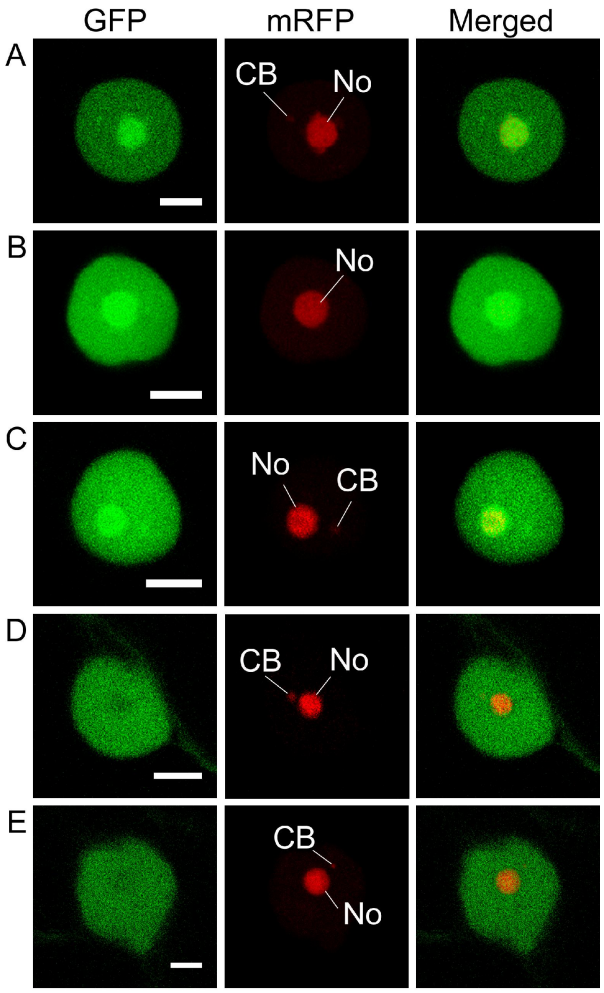
Supplemental Data. Rajamäki and Valkonen (2009). Control of Nuclear and Nucleolar Localization of Nuclear Inclusion Protein a of Picorna-Like *Potato virus A* in *Nicotiana* Species.



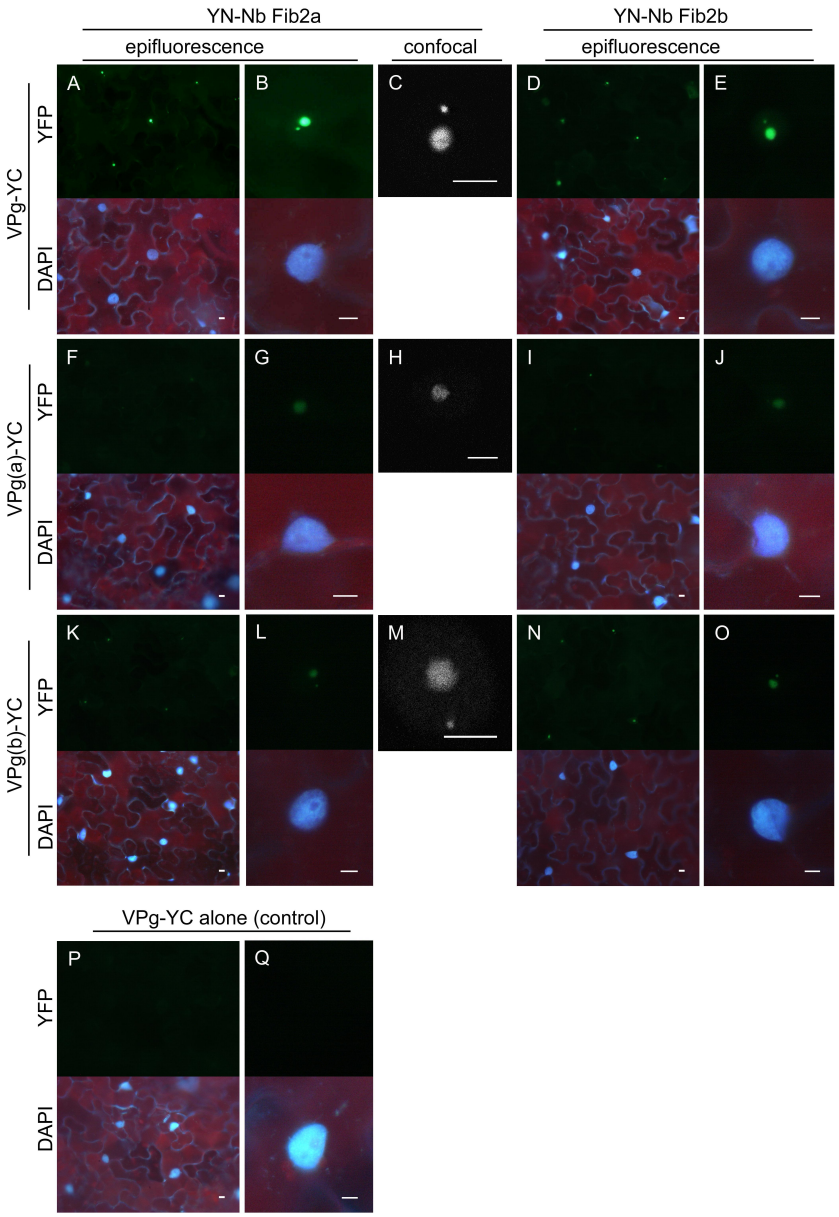
Supplemental Figure 1. Protein Gel Blot Analysis and Subcellular Localization of GFP-NIa Fusion Proteins Expressed in Leaves of *Nicotiana benthamiana*. **A**, Protein gel blot analysis of expression of GFP-NIa and GFP-NIa(E/H) in leaves of *N. benthamiana* agroinfiltrated for expression of GFP-NIa and GFP-NIa(E/H) constructs, respectively, as detected with antibodies against VPg. GFP-NIa(E/H) contains an amino acid substitution E189H in the internal proteolytic cleavage site to prevent separation of the VPg and NIa-Pro domains. 1, Uninfiltrated plant; 2, GFP-NIa; 3, GFP-NIa(E/H). The sizes of markers (kDa) are indicated to the right. Results show that the majority of NIa in GFP-NIa has been internally processed to GFP-VPg (ca. 50-kDa), whereas little processing is evident in GFP-NIa(E/H) containing a mutation in the internal proteolytic cleavage site. **B**, Subcellular localization of GFP-NIa and GFP-VPg fusion proteins. The constructs were delivered by agroinfiltration and visualized by epifluorescence microscopy 2 to 3 days after infiltration. Mutations in NLS I [GFP-NIa(a)] and NLS II [GFP-NIa(b1), GFP-NIa(b4) and GFP-NIa(b)] are shown in Figure 1. DAPI staining was used to detect nuclei. Scale bars = 25 µm.



Supplemental Figure 2. Subcellular Localization of VPg-GUS-GFP (A and B), PVAnls-GUS-GFP (C and D) and TEVnls-GUS-GFP (E and F) Fusion Proteins as Observed by Epifluorescence Microscopy 3 Days After Inoculation. Arrows indicate the positions of nuclei. Expression of the VPg-GUS-GFP was weak, and the protein tended to aggregate as bright spots. Scale bars = 25 µm.

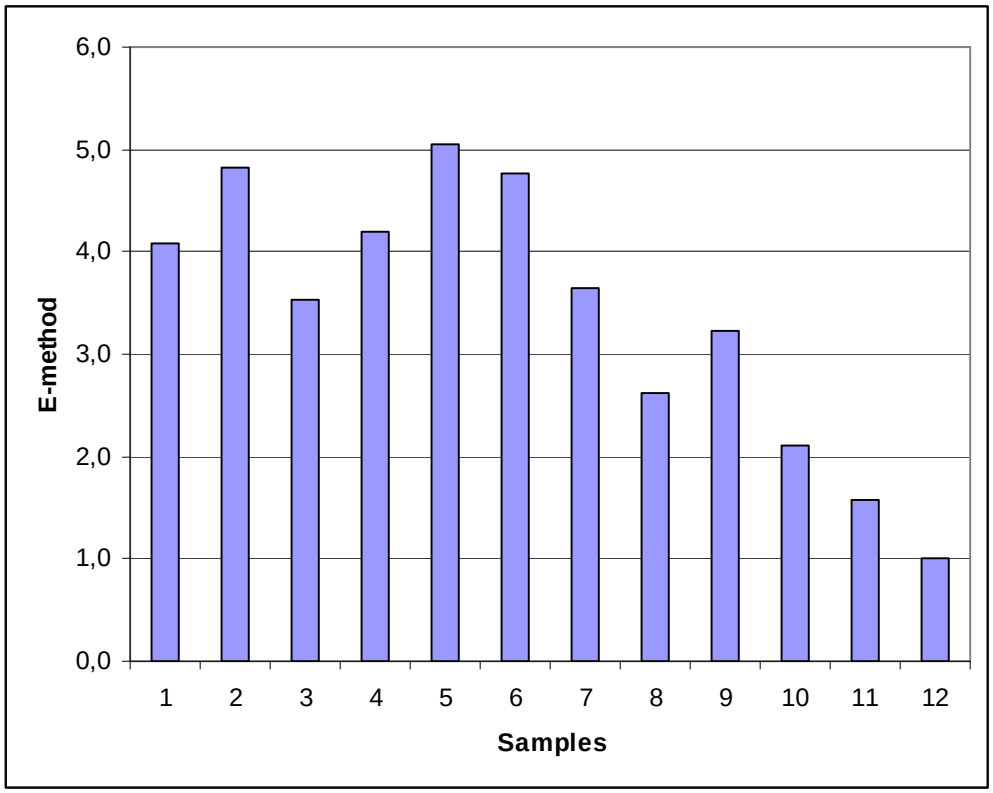


Supplemental Figure 3. Subnuclear Localization of GFP-NIa and GFP-VPg Fusion Proteins Expressed in Leaves of *Nicotiana benthamiana*. The constructs were delivered to epidermal cells by agroinfiltration and the fluorescence detected by confocal microscopy 2 to 3 days after infiltration. Fibrillarin (Fib2 of *Arabidopsis thaliana*) fused to red fluorescent protein (mRFP) was co-expressed with GFP-NIa (or GFP-VPg) to visualize the nucleolus (No) and Cajal bodies (CB). Results show that GFP-NIa (**A**), GFP-VPg (**B**), and GFP-NIa(E/H) (**C**) localize to the nucleolus, in contrast to GFP-NIa(a) (**D**) and GFP-NIa(b) (**E**). Some of the GFP-NIa (**A**) and GFP-NIa(E/H) (**C**) localize to CBs. NIa(E/H) denotes a construct with the substitution E189H in the internal proteolytic cleavage site of NIa, which prevents cleavage between the VPg and NIa-Pro domains. Mutations in NLS I [GFP-NIa(a)] and NLS II [GFP-NIa(b)] are indicated in Figure 1. Scale bars = 5 µm.

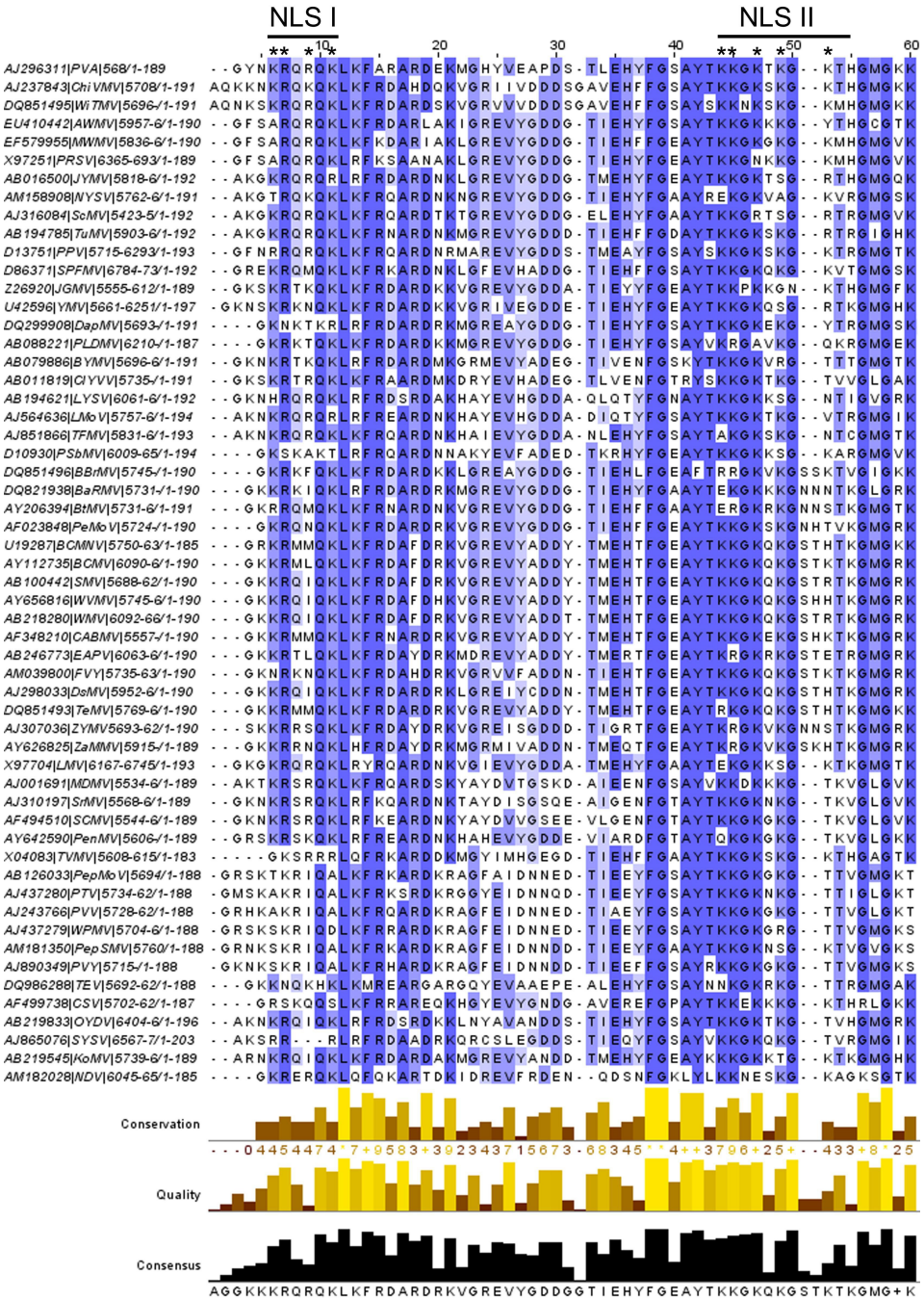


Supplemental Figure 4. Visualization by Green Fluorescence of the Interaction between *Potato virus A* VPg and Fibrillarin in Nucleoli and Cajal Bodies Using a Bimolecular Fluorescence Complementation (BiFC) Assay. Agroinfiltration of **A-C**, VPg-YC and YN-Nb Fib2a; **D-E**, VPg-YC and YN-Nb Fib2b; **F-H**, VPg(a)-YC and YN-Nb Fib2a; **I-J**, VPg(a)-YC and YN-Nb Fib2b; **K-M**, VPg(b)-YC and YN-Nb Fib2a; **N-O**, VPg(b)-YC and YN-Nb Fib2b; or **P-Q**, VPg-YC alone. A, B, D-G, I-L and N-Q show interactions detected under epifluorescence microscope. DAPI staining (blue) was used to detect nuclei. C, H and M show interactions detected by confocal microscopy. Bars = 5 µm. The YFP halves were fused to VPg and Nb Fib2a or Nb Fib2b, and BiFC was detected by reconstruction of YFP. Fluorescence is observed specifically in the nucleolus and Cajal bodies. The cultures of *Agrobacterium tumefaciens* strains carrying the indicated YN- and YC-fusion protein constructs were combined in a ratio 1:1 and infiltrated into epidermal cells of *Nicotiana benthamiana*. Fluorescence was observed by epifluorescence microscopy 2 to 3 days after infiltration. The same exposure times were used to acquire all epifluorescence images. No fluorescence was observed when only VPg-YC was expressed in leaves. Mutations in NLS I [VPg(a)] and NLS II [VPg(b)] are indicated in Figure 1.

Supplemental Figure 5. Virus-Induced Gene Silencing of Fibrillarin (Fib; A and C) and Phytoene Desaturase (PDS) (D) in *Nicotiana benthamiana* Using *Tobacco rattle virus* (TRV) as a Vector. **A**, A plant inoculated with TRV alone (control) to the left, and a plant inoculated with TRV-Fib2 to the right. **B** and **C**, Close-ups of the plants in (A). Note the severe stunting and leaf curling in the plant infected with TRV-Fib2 (C) as compared to the plant infected with TRV (B). **D**, A plant infected with TRV-PDS. The arrow indicates the position of leaves that were inoculated with *Potato virus A* (PVA) in non-silenced (TRV-infected) and Fib-silenced (TRV-Fib2-infected) plants. **E**, A non-silenced and **F**, Fib-silenced plant inoculated with green fluorescent protein-tagged PVA photographed under UV-light at 6 days after inoculation. IL, inoculated leaf.



Supplemental Figure 6. Expression of Fibrillarin mRNA in the Leaves of TRV-Fib2-Infected Plants as Compared to the Leaves of TRV-Infected Plants. Samples 1-6 are from TRV-infected plants (mean E-method value = 4.4 ± 0.6). Samples 7-12 are from the leaves of TRV-Fib2-infected plants (mean E-method value = 2.7 ± 1.2).



Supplemental Figure 7. Multiple Sequence Alignment of the 60 N-Proximal Amino Acid Residues of VPg from 56 Potyviruses Shows That the NLS Regions Are Conserved in Genus *Potyvirus*. VPg sequences were derived from the full-length sequences of potyviruses (www.dpvweb.net/), aligned using ClustalW and edited with Jalview 2.4 (Clamp et al., 2004). Intensity of color indicates the percentage of residues that agree with the consensus sequence. Quality refers to the likelihood of observing mutations in a particular position. Asteriks above the columns denote basic amino acid residues mutated in this study.

Supplemental Reference:

Clamp, M., Cuff, J., Searle, S.M., and Barton, G.J. (2004). The Jalview java alignment editor. Bioinformatics **20**: 426-427.

Supplemental Table 1. Primers Used for Cloning or Site-Directed Mutagenesis.

Primer		Sequence[b]	Cloning/mutagenesis
BamHI-NIaF	F[a]	5'-ATAGGATCCATGGGCTATAATAAGCGACAGAGG-3'	pRT-GFP-NIa, pRT-GFP-VPg
XbaI-NIaR	R	5'-ATATCTAGATTATTGGGTGTATACTGCCTCTCC-3'	pRT-GFP-NIa
XbaI-VPgR	R	5'-ATATCTAGATTACTCGAATTCAACCGACTCTTTC-3'	pRT-GFP-VPg
BamHI-VPgAF	F	5'-ATAGGATCCATGGGCTATAATGCGGCACAGGC-3'	pRT-GFP-NIa(a)
FGUS	F	5'-AATGGTACCATGTTACGTCCTGTAGAAACC-3'	pRT-GUS-GFP
RGUS	R	5'-ATCACCATGGTTTGTTTGCCTCCCTGCTGC-3'	pRT-GUS-GFP
FnlsGUS	F	5'-AATGGTACCATGAAGAAAGGAAAAACAAAGGGCAAA ACCCATATGTTACGTCCTGTAGAAACC-3'	pRT-PVAnls-GUS-GFP
FtevnlsGUS	F	5'-AATGGTACCATGAACAAAGGAAAGCGCAAGGGCACC ACGAGAATGTTACGTCCTGTAGAAACC-3'	pRT-TEVnls-GUS-GFP
FVPg	F	5'-AATCTCGAGATGGGCTATAATAAGCGACAGAGG-3'	pRT-VPg-GUS-GFP, pRT-VPgN-GUS-GFP
RVPg	R	5'-TCATGGTACCCTCGAATTCAACCGACTCTTTC-3'	pRT-VPg-GUS-GFP
RVPgN	R	5'-TCATGGTACCCTTTTTCCCCATTCCATGGG-3'	pRT-VPgN-GUS-GFP
VPg-NotIF	F	5'-GGTGGCGGCCGCGGGCTATAATAAGCGACA-3'	pA-VPg, pA-VPg-b1 to b4
VPgA-NotIF	F	5'-ATAGCGGCCGCATGGGCTATAATGCGGCACAGGCG-3´	pA-VPg(a)
VPg-FseI	R	5'-AAACGGCCGGCCTCACTCGAATTCAACCGACTC-3'	pA-VPg, pA-VPg(a) pA-VPg-b1 to b4
4143NLSF	F	5'-GCGCGTACACAAAGGCCGGAGCAACAAAGGGCAAA ACCCATGG-3'	b1-mutagenesis
4143NLSR	R	5'-CCATGGGTTTTGCCCTTTGTTGCTCCGGCCTTTGTGTA CGCGC-3'	b1-mutagenesis
4144NLSF	F	5'-GGAAGCGCGTACACAAAGGCCGGAAAAGCAAAGGGC AAAACCC-3'	b2-mutagenesis
4144NLSR	R	5'-GGGTTTTGCCCTTTGCTTTTCCGGCCTTTGTGTACGC GCTTCC-3'	b2-mutagenesis
4344NLSF	F	5'-CGCGTACACAAAGAAAGGAGCCGCAAAGGGCAAA ACCCATGG-3'	b4-mutagenesis
4344NLSR	R	5'-CCATGGGTTTTGCCCTTTGCGGCTCCTTTCTTTGTGTA CGCG-3'	b4-mutagenesis
414344NLSF	F	5'-GCGCGTACACAAAGGCCGGAGCAGCAAAGGGCAAA ACCCATGG-3'	b3-mutagenesis
414344NLSR	R	5'-CCATGGGTTTTGCCCTTTGCTGCTCCGGCCTTTGTGTA CGCGC-3'	b3-mutagenesis
B1VPgm4-9-F	F	5´-GAAGTGGTGGCATTTCAG GGCTATAATGCGGCACAG GCGCAAGCACTGAAGTTTGCCAGAGCC-3'	a-mutagenesis
B1VPgm4-9-R	R	5'-GGCTCTGGCAAACTTCAGTGCTTGCGCCTGTGCCGCA TTATAGCC CTGAAATGCCACCACTTC-3'	a-mutagenesis
nls5KA-F	F	5'-GGAAGCGCGTACACAGCGGCCGGAGCAACAGCGGGC GCAACCCATGGAATGG-3'	b-mutagenesis
nls5KA-R	R	5'-CCATTCCATGGGTTGCGCCCGCTGTTGCTCCGGCCG CTGTGTACGCGCTTCC-3'	b-mutagenesis
VPgm54-55F	F	5'-GCGCAACCCATGGAATGGGGGCAGCGGGTACCATGT TACGTCCTG-3'	c-mutagenesis in pRT-VPgN-GUS-GFP
VPgm54-55R	R	5'-CAGGACGTAACATGGTACCCGCTGCCCCCATTCCAT GGGTTGCGC-3'	c-mutagenesis in pRT-VPgN-GUS-GFP
NIaE/H-F	F	5-GAGTCGGTTGAATTCCACTCAACATCTATGTTTAGA GGGG-3'	E/H-mutagenesis
NIaE/H-R	R	5'-CCCCTCTAAACATAGATGTTGAGTGGAATTCAA CCGACTC-3'	E/H-mutagenesis

[a] F, forward primer; R, reverse primer. [b] Restriction sites are underlined. Mutated nucleotides are shown in red.