Supplementary Methods

Plasmids. A DNA fragment including PPV P1-P3-6K1 without HCPro was engineered to include *Eco*RV and *Sbf*I restriction sites, as well as PPV P1 and PPV NIa cleavage sites between the P1 and P3 coding sequences, by using the gene splicing via overlap-extension method (Horton *et al.*, 1989). Primers and templates used for PCRs are listed in Tables S1 and S2. The P1-P3-6K1 PCR fragment was cloned into a pGEMT vector by using *Nco*I and *Sal*I restriction sites, to generate the pGEMTp1p3 intermediate clone (Fig. 1a). pGEMTp1p3 was further used as parental plasmid to generate intermediate clones encoding proteins with reported silencing-suppression activity [tomato bushy stunt virus (TBSV) P19, a 360 aa N-terminal region of the P1 protein ($P1^N$) of sweet potato mild mottle virus (SpMMV), cauliflower mosaic virus (CaMV) P6, and influenza A virus NS1] (Table 1). Sequences of interest were amplified by PCR using appropriate primers (Tables S1 and S2), digested with *Pst*I or *Sbf*I and inserted into pGEMTp1p3 digested with *Eco*RV and *Sbf*I (Fig. 1). pICPPV-NK-GFPn (P. Sáenz, M. R. Fernández-Fernández & J. A. García, unpublished results), a PPV full-length cDNA clone derived from pICPPV-NK-GFP (Fernández-Fernández *et al.*, 2001) in which the eGFP had been replaced by the wild-type GFP from *Aequorea victoria*, was used as backbone to generate different PPV chimeras. Hence, the *Bpu*1102I–*Sal*I fragment from PPV that encodes the P1-HCPro-P3 region of the viral polyprotein was replaced with the corresponding fragments from pGEMTp1-P19-p3 and pGEMTp1-NS1-p3 to produce pICPPV-P19 and pICPPV-NS1, respectively (Fig. 1b). pICPPV-$P1^N$ and pICPPV-P6 (Fig. 1b) were constructed by replacing the *Rsr*II–*Dra*III fragment from PPV with the corresponding fragment from pGEMTp1-$P1^N$-p3 and pGEMTp1-P6-p3, respectively. pICPPV-ΔHC and pICPPV-P1b have been described previously (P1ΔHC and P1P1b, respectively; Carbonell *et al.*, 2012).

pBIN-P1HC, a pBin19 derivative carrying the cDNA corresponding to the 5′ region of the PPV genome (nt 1–3627, including the P1, HCPro, P3 and 6K1 coding sequence; M. O. Delgadillo, J. A. García & C. Simón-Mateo, unpublished results) under the control of the CaMV 35S promoter, was used as parental plasmid to produce the generic pBIN-P1RSS constructs, which were employed in the agroinfiltration assays. These clones were constructed by replacing the *Xma*I–*Bsr*GI fragment from pBIN-P1HC that encodes the 5′ region of the viral genome (nt 1–3586) preceded by the CaMV 35S promoter, with the corresponding fragments from pICPPV-P19, pICPPV-$P1^N$ and pICPPV-NS1 to generate pBIN-P1P19, pBIN-$P1P1^N$ and pBIN-P1NS1, respectively. pBIN-P1P6 was constructed by a triple ligation of the *Xma*I–*Bsr*GI fragment from pBIN-P1HC with *Xma*I–*Dra*III and *Dra*III–*Bsr*GI fragments from pICPPV-P6. pBIN-P1P1b has been described previously (Carbonell *et al.*, 2012).

To construct binary plasmids expressing CaMV P6, either with their precise ends or with the extra amino acids derived from P1 cleavage (SD at the N terminus) and NIa cleavage (QVVVHQ at the C

terminus) that remain linked to the RSSs expressed from the pICPPV-RSS chimerical clones, as well as a binary plasmid expressing the protease domain of PPV NIa (NIaPro), the GATEWAY technology (Invitrogen) was applied. Hence, pDONR-207 (Invitrogen) was used as donor vector, whereas pMDC32 (Curtis & Grossniklaus, 2003) was used as destination vector. Primers and templates used for PCR amplifications to generate the different entry vectors are listed in Tables S3 and S4. Expression vectors pMDC32-P6, pMDC32-SD-P6-QVVVHQ and pMDC32-NIaPro were constructed by LR clonase reactions between the corresponding pDONR entry vectors and the destination vector pMDC32 (table S3). Plasmid expressing CVYV P1b (pMDC32-P1b) will be described elsewhere (Valli *et al.*, submitted).

The accuracy of all the constructions was verified by restriction digestion analysis and DNA sequencing of all regions derived from PCR amplification.

Agrobacterium tumefaciens C58C1 strain carrying p35S:GFP (Haseloff *et al.*, 1997) plus pCH32 (Hamilton *et al.*, 1996) was kindly provided by David Baulcombe (University of Cambridge, UK).

Table S1. Primers and templates used for PCRs

Sequences of primers are shown in Table S2.

Generated plasmid	Forward*	Reverse*	Template (origin)
1-pGEMTp1p3			
PCR1	#976	#2	pICPPV-NK-GFP
PCR2	#3	#210	pICPPV-NK-GFP
PCR3	#976	#210	PCR1+PCR2
2-pGEMTp1-NS1-p3			
PCR4	NS1_For	NS1_Rev	pBIN-NS1 Delgadillo *et al.* (2004)
3-pGEMTp1-P19-p3			
PCR5	P19_For	P19_Rev	pBIN61-P19 Voinnet *et al.* (2003)
5-pGEMTp1-P6-p3			
PCR7	P6_For-II	P6_Rev	pUC-BJI Cecchini *et al.* (1997)
6-pGEMTp1-P1^{N}-p3			
PCR8	SPMMV_For	SPMMV_Rev	pGEMT-P1 Giner *et al.* (2010)

Table S2. Sequences of PCR primers used in the construction of chimerical viruses

Sequences corresponding to PPV are in upper case, sequences corresponding to other viruses are in upper italic case, restriction sites used for cloning are indicated in bold and sequences as a tail are in lower case. The sequence corresponding to the NIa-Pro cleavage site is underlined.

Primer	Sequence (5′–3′)
#976	gtcaccATGGCAACCATTG
#2	tcagcttgatgaaccacaa**cctgcagg**tacgc**gatATC**AGAGTAGTGGATTATCTCATTGC
#3	gcgta**cctgcagg**ttgtggttcatcaagctgacgaaGGTCTTGAAGTGGATAAGTGTGACG
#210	GGGACAGTTGGTGCAAC
NS1_For	*GATTCCAACACTGTGTCAAG*
NS1_Rev	tata**ctgcag***ATCAGCCATCTTATCTCTTC*
P19_For	*ATGGAACGAGCTATACAAGG*
P19_Rev	tata**ctgcag***CTCGCTTTCTTTTTCGAAG*
P6_For-II	*GAGAACATAGAAAAACTCCTCATGCAAG*
P6_Rev	tata**ctgcag***ATCCACTTGCTTTGAAGACG*
SPMMV_For	*GGGAAATCCAAACTCACTTAC*
SPMMV_Rev	tata**ctgcag***CACCGTGATGGGACACAC*

Table S3. List of primers and templates used in PCRs to generate plasmids based on pMDC32

Sequences of primers are shown in Table S4.

Entry plasmid	Forward	Reverse	Template
pDONR-NIaPro	NIaPro-For	NIaPro-Rev	pICPPV-NK-GFP
pDONR-P6	#1641	#1642	pICPPV-P6
pDONR-SD-P6-NIa_cut	#1643	#1644	pICPPV-P6

Table S4. Sequences of PCR primers used to construct plasmids based on pMDC32

Recombination sequences are indicated in lower case.

Primer	Sequence (5′–3′)
NIaPro-For	ggggacaagtttgtacaaaaaagcaggctccATGGCAAGTAAATCACTGTTCAGAGGC
NIaPro-Rev	ggggaccactttgtacaagaaagctgggttaCTGAGTGTAAACAAATTCCCC
#1637	ggggacaagtttgtacaaaaaagcaggctccATGCAATTTCTCGCTCACGATAACTTTC
#1638	ggggaccactttgtacaagaaagctgggttaTACAAACATTTCGGTGTAGACCG
#1639	ggggacaagtttgtacaaaaaagcaggctccATGTCTGATCAATTTCTCGCTCACGATAAC
#1640	ggggaccactttgtacaagaaagctgggttaTTGATGAACCACAACCTGCAG
#1641	ggggacaagtttgtacaaaaaagcaggctccATGGAGAACATAGAAAAACTCCTCATGC
#1642	ggggaccactttgtacaagaaagctgggttaATCCACTTGCTTTGAAGACGTGG
#1643	ggggacaagtttgtacaaaaaagcaggctccATGTCTGATGAGAACATAGAAAAACTCC
#1644	ggggaccactttgtacaagaaagctgggttaTTGATGAACCACAACCTGCAGATCC

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(a)

Virus	Inoculated leaves	
	GFP appearance	Fluorescence detection
PPV	16/16	5-7 dpi
PPV-ΔHC	0/8	-
PPV-P1b	8/8	7 dpi
PPV-P19	16/16	5-7 dpi

(b)

Virus	Upper non -inoculated leaves		
	GFP appearance	Fluorescence detection	Fluorescence level
PPV	16/16	7-8 dpi	+++
PPV-ΔHC	0/8	-	-
PPV-P1b	8/8	8 dpi	++
PPV-P19	16/16	7-10 dpi	++

Fig. S1. Infectivity of chimerical viruses based on plum pox virus. The tables show the number of plants showing GFP fluorescence and the time at which it appears on inoculated (a) and upper non-inoculated (b) leaves.

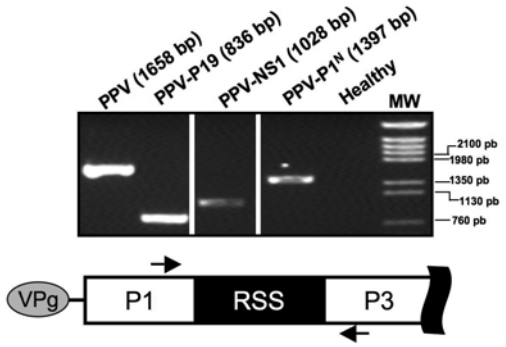
(a)

Virus	Inoculated leaves		
	GFP appearance	Fluorescence detection	PPV CP detection
PPV	8/8	5-9 dpi	Yes
PPV-P19	8/8	5-7 dpi	Yes
PPV-P1	0/8	-	Yes
PPV-NS1	6/8	7-10 dpi	Yes
PPV-P6	0/8	-	No

(b)

Virus	Upper non-inoculated leaves		
	GFP appearance	Fluorescence detection	PPV CP detection
PPV	8/8	7-13 dpi (+++)	Yes
PPV-P19	8/8	10-14 dpi (+++)	Yes
PPV-P1	2/8	18 dpi (+/-)	Yes
PPV-NS1	2/8	15-18 dpi (+/-)	Yes
PPV-P6	0/8	15-18 dpi (-)	No

(c)



(d)

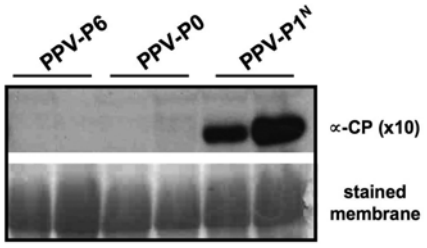


Fig. S2. Infectivity of chimerical viruses based on plum pox virus. (a, b) The tables show the number of plants showing GFP fluorescence and the time at which it appears in inoculated (a) and upper non-inoculated (b) leaves. Detection of viral CP by Western blot is also indicated. (c) Amplification by IC-RT-PCR of cDNA fragments from the different chimerical viruses. Primers targeting the 3′ end of PPV P1 and 5′ end of PPV P3 were used in the assay. DNA of the bacteriophage ø29 digested with *Hin*dIII was used as molecular mass marker [MW, sizes (nt): 4545, 2850, 2480, 2200, 1950, 1346, 1150, 763]. The expected size of the different chimerical fragments is indicated above each lane. (d) Western blot analysis of protein extracts prepared from upper non-inoculated leaves (two plants per construct) collected at 21 days post-inoculation. Membrane stained with Ponceau red showing the Rubisco is included as loading control.

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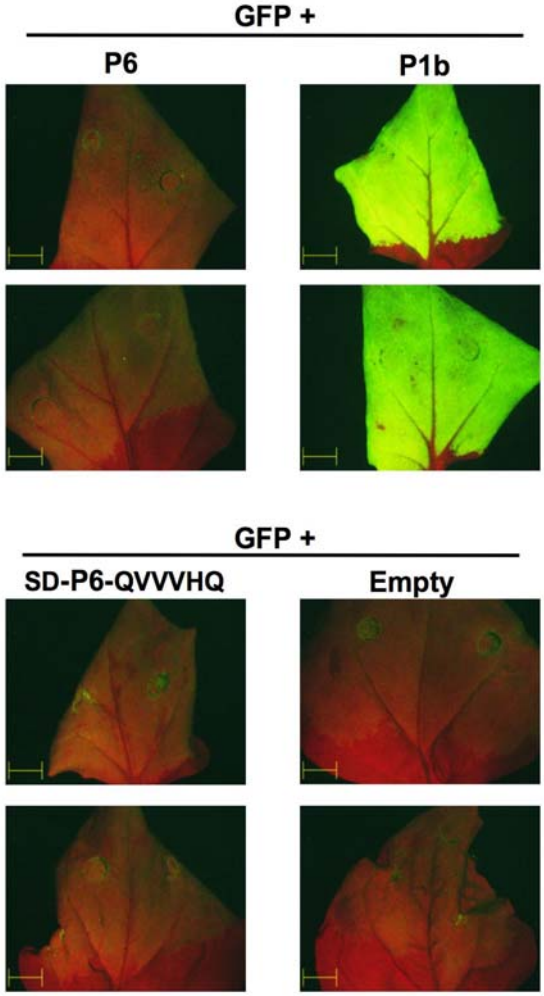

Fig. S3. Anti-silencing activity of CaMV P6 proteins. GFP fluorescence pictures of agroinfiltrated leaves expressing the indicated proteins, taken under an epifluorescence microscope at 6 days post-agroinfiltration. Yellow bars, 5 mm.

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