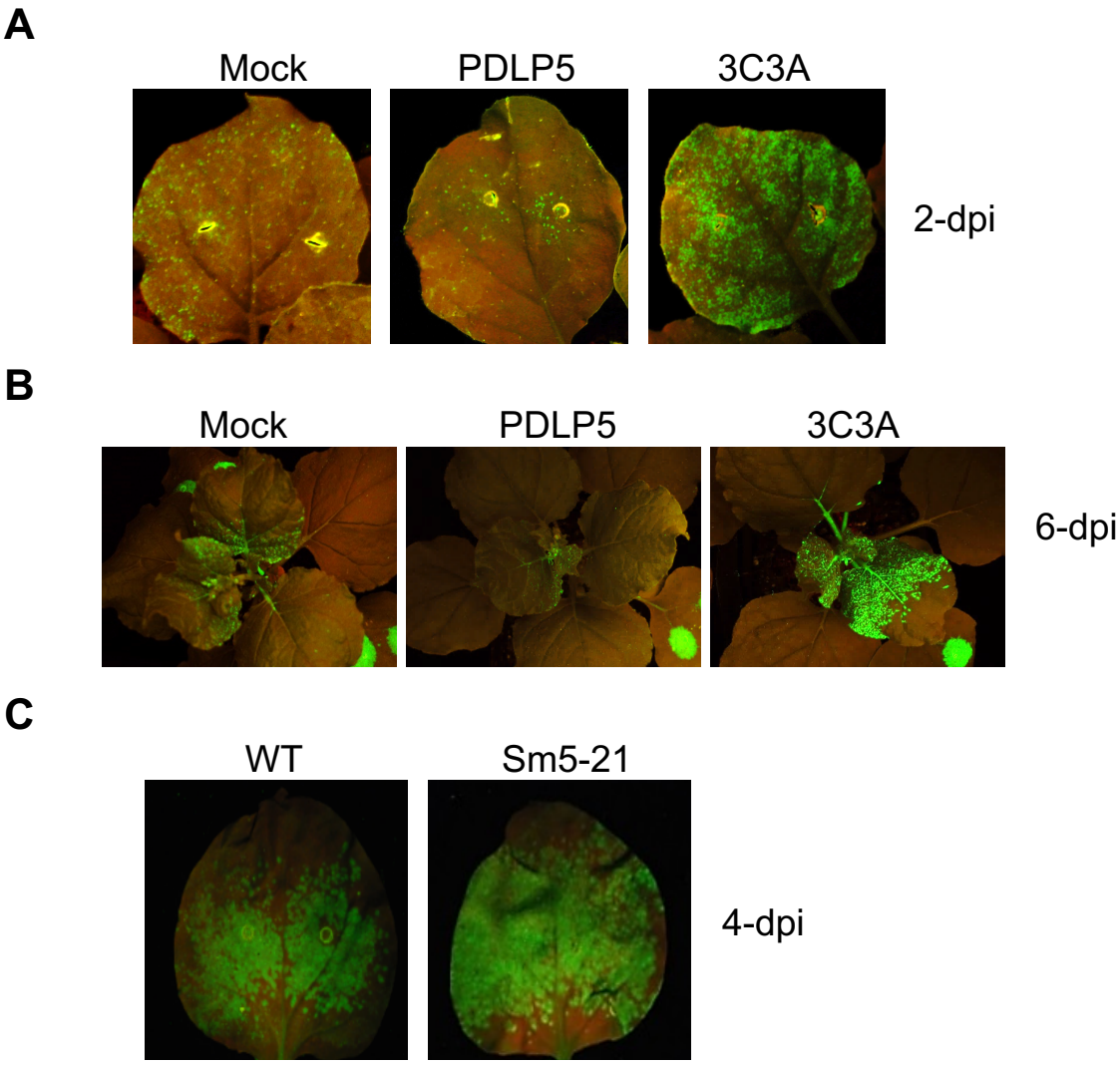
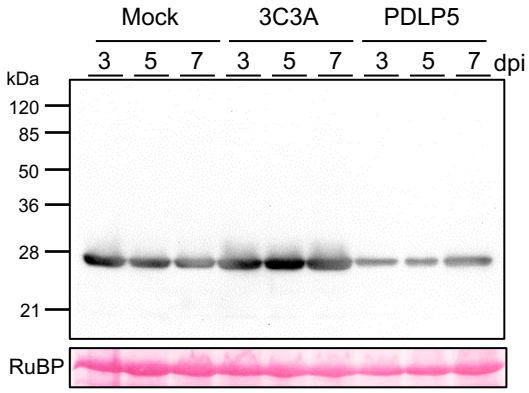
Supplementary Figure 1



Representative photographs showing the effect of 3C3A on PVX-GFP movement. A) Spread of PVX-GFP in local leaves. WT plants (3.5-week-old) were pre-treated with mock (empty vector), PDLP5 or 3C3A mixed 1:1 with p19 by infiltrating mature leaves fully with agrobacterial suspension carrying each plasmid. Three days later, the same leaves were infiltrated fully with a suspension of agrobacteria carrying PVX-GFP vector (pGR-PVX-GFP). B) Effect of 3C3A on the systemic movement of PVX-GFP. Initial treatment as for (A), but secondary infiltration of PVX-GFP comprised two spots of 0.1 mL per leaf (one of which is visible in the lower right region of each image). Final ODs of agrobacterial suspension used in A and B are 0.35 for mock (empty vector), PDLP5 or 3C3A; 0.5 for p19; and 0.01 for PVX-GFP. C) Leaves of WT and SM5-21 infected with PVX-GFP by agroinfiltration (final OD_{600nm}=0.5). Photos were taken under a BlackRay UV lamp using a D3500 Nikon camera at different days post infiltration (dpi) of the viral vector.

Supplementary Figure 2

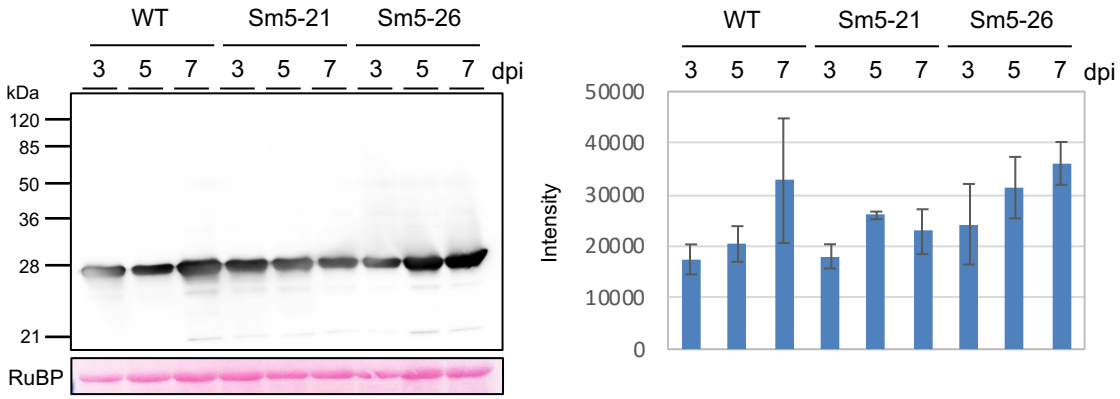


Representative image of Western blot analysis. The effect of introducing 3C3A by transient expression in WT *N. benthamiana* plants for viral vector driven protein expression using TMV-GFP. Leaves were first infiltrated with Agrobacteria carrying 3C3A at final OD_{600nm}=0.35 for pre-treatment and 3-days later the same leaves were infiltrated with *Agrobacteria* carrying TMV-GFP at final OD_{600nm}=0.2. Infiltrated leaves were collected at 3, 5, and 7 dpi. Protein extracts were prepared in a buffer containing 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5 mM EDTA, and protease inhibitors. Western blot analysis was performed using anti-GFP as primary and HRP-conjugated anti-rabbit as secondary antibodies, respectively. RuBP, Rubisco. The experiment was performed on multiple plants per treatment and repeated three times.

Supplementary Figure 3

Representative images of *N. benthamiana* WT (WT *N.b.*) and transgenic Sm5-21 and Sm5-26 individual plants and trays of plants.

Supplementary Figure 4



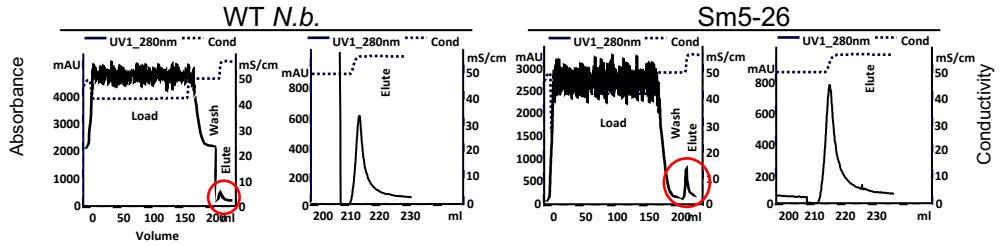
Representative image of Western blot analysis and quantification. The effect of 3C3A on non-viral vector driven protein expression using a binary vector carrying EGFP (pART-GFP). GFP expression levels were compared using WT and Sm5-21 and Sm5-26 transgenic plants. Leaves were infiltrated with *Agrobacteria* carrying pART-GFP at a final OD_{600nm}=0.7 and collected at 3, 5, and 7 dpi. Protein extracts were prepared in a buffer containing 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5 mM EDTA, and protease inhibitors. Western blot analysis was performed using anti-GFP as primary and HRP-conjugated anti-rabbit as secondary antibodies, respectively. RuBP, Rubisco. The experiment was performed on multiple plants per treatment and repeated 3 times, and the quantification result of the experiment is presented in a bar graph. The signal intensity of protein bands detected on Western blots was measured using Image J software (Fiji). Error bars, standard error (±SE).

Supplementary Figure 5

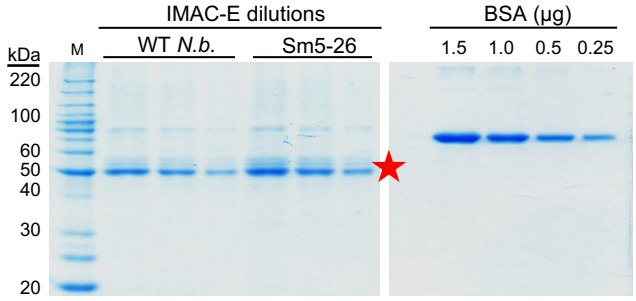
A

Plant host	Protein accumulation at 7 dpi (mg/kg leaf)	
	YFE-1	PA83
WT	183.5 (100%)	223.7 (100%)
Sm5-21	260 (140%)	261.3 (117%)
Sm5-26	301.5 (163%)	325.7 (146%)

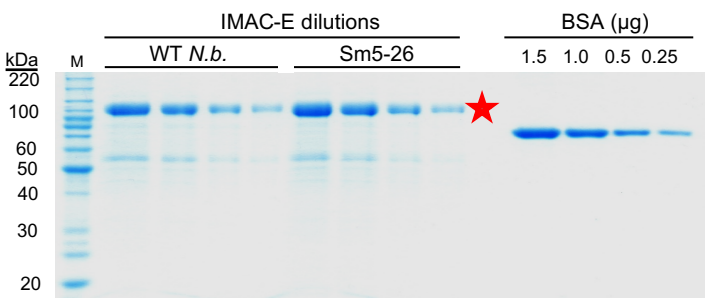
B



C



D



A) A summary of target protein accumulation for YFE-1 and PA83 from WT, Sm5-21 and Sm5-26 plants from the data presented in main Figure 4 C-F. B) Protein absorbance profiles for IMAC of soluble extracts from WT and Sm5-26 plants expressing PA83. C and D) Recovery of expressed recombinant protein targets from aerial biomass of transgenic 3C3A plants. SDS-PAGE analysis of IMAC purified YFE-1 (C) and PA83 (D), respectively. Soluble proteins were extracted from WT and Sm5-26 plants at 6 dpi and purified using IMAC. Peak elution fractions (IMAC-E) were pooled and serially diluted to resolve by SDS-PAGE to quantify the protein yields, with bovine serum albumin (BSA) protein standards. Red stars indicate the expressed recombinant proteins.

Supplementary Table 1. Information related to vectors used in this study.

Plasmids or genes	References and/or sources
pGWB	Provided by T. Nakagawa (Nakagawa et al., J Biosci Bioeng. 2007 Jul;104(1):34-41).
cYFP	CitrineYFP (https://www.fpbase.org/protein/citrine/) provided by R. Tsien (Griesbeck et al., 2001, JBC 276:29188-29194).
TMV-GFP	pSPDK661 (TMV-GFP) provided by S.P. Dinesh-Kumar (Liu et al., 2002, Plant J 30:415-429).
pBI121	https://www.snapgene.com/resources/plasmid-files/?set=plant_vectors&plasmid=pBI121 (Chen et al., 2003, Mol Breeding 11:287-293).
pART	Provided by J. Bowman (Gleave AP, 1992, Plant Mol Biol 20 1203–1207)
p19	pBIN35S:p19 provided by J. Caplan (Voinnet et al., 2003, Plant J. 33: 949–956).
P1/HC-Pro	Provided by V. Dolja (Chapman et al., Genes & Dev 18: 1179-1186).
pGreen-based expression vector carrying TMV genome sequences	TMV genome sequences of pBID4 (Musiychuk et al., 2007, Influenza Other Respir. Viruses, 1:19-25) introduced into the pGreen binary vector (Hellens et al., 2000, Plant Mol Biol 42: 819–832).
pBI-D	*Cauliflower mosaic virus* (CaMV) 35S promoter and *A. tumefaciens* nopaline synthase (Nos) terminator of pBI121 replaced with CaMV 35S promoter with dual enhancer sequences plus the 5' non-translated leader sequence of Tobacco etch virus and with 35S terminator, respectively.
pGR-PVX-GFP	PVX genome sequences and GFP reporter introduced into the pGreen binary vector (Hellens, et al., 2000,Plant Mol Biol 42: 819–832).

Supplementary Table 2. An evaluation summary of transgenic Sm5 *N. benthamiana* lines.

Expression construct	Plant ID	Confirmed by RT-PCR	Confirmed by PCR	Segregation‡ (Kan plates)	TMV-GFP foci growth
pBI-S-PDLP5m5	Sm5-5	+	n.d.	42R : 8S	n.d.
	Sm5-8	+	+	45R : 5S	n.d.
	Sm5-9	+	+	46R : 2S	n.d.
	Sm5-10	+	+	42R : 8S	+++
	Sm5-13	+	n.d.	49R : 1S	n.d.
	Sm5-16	+	n.d.	50R	+++
	Sm5-17	+	n.d.	n.d.	n.d.
	Sm5-20	+	+	n.d.	n.d.
	Sm5-21	+	+	32R : 18S	**++++**
	Sm5-26	+	n.d.	40R : 10S	**++++**
	Sm5-34	n.d.	+	50R	++
	Sm5-63	n.d.	+	38R : 10S	++
	Sm5-64	n.d.	+	28R : 10S	+++
	Sm5-65	n.d.	+	28R : 12S	++
	Sm5-66	n.d.	+	46R : 1S	+++

n.d., not determined. +, relative extent to which foci growths are observed (**++++**, high; +++, moderate; ++, mild). ‡T1 seeds were analyzed.