CRISPR-Cas12a genome editing at the whole-plant level using two compatible RNA virus vectors

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Abstract

The use of viral vectors that can replicate and move systemically through the host plant to deliver bacterial clustered, regularly interspaced, short palindromic repeats (CRISPR) components enables genome editing at the whole-plant level and avoids the requirement for labor-intensive stable transformation. However, this approach usually relies on previously transformed plants that stably express a CRISPR-associated (Cas) nuclease. Here we describe successful DNA-free genome editing of *Nicotiana benthamiana* using two compatible RNA virus vectors, derived from tobacco etch virus (TEV; genus *Potyvirus*) and potato virus X (PVX; genus *Potexvirus*), which replicate in the same cells. The TEV and PVX vectors respectively express a Cas12a nuclease and the corresponding guide RNA. This novel two-virus vector system improves the toolbox for transformation-free virus-induced genome editing in plants and will advance efforts to breed more nutritious, resistant, and productive crops.

Introduction

Systems derived from bacterial clustered, regularly interspaced, short palindromic repeats (CRISPR) and CRISPR-associated (Cas) proteins (Cong *et al.*, 2013) have revolutionized biotechnology. In plants, CRISPR-Cas holds great promise for unprecedented genome engineering of both model species and crops (Nekrasov *et al.*, 2013; Zhang *et al.*, 2016; Li *et al.*, 2017; Zhu *et al.*, 2020; Huang and Puchta, 2021). Most common CRISPR/Cas arrangements include a Cas endonuclease, such as *Streptococcus pyogenes* SpCas9, and a single-guide RNA

(sgRNA), which specifically directs the nuclease to a sequence of interest in the genome. As in other taxonomic groups (Platt et al., 2014; Senís et al., 2014; Lau and Suh, 2017; Xu et al., 2019), virus-derived vectors have been reported as a powerful alternative to express the CRISPR/Cas components at the whole-plant level avoiding the labor-intensive and time-consuming tissue culture approaches required for stable transformation. These strategies are commonly termed as virus-induced genome editing (VIGE) and have focused on the delivery of one or more sgRNAs using RNA or DNA virus vectors in transgenic plants that stably express the Cas nuclease (Ali et al., 2015; Yin et al., 2015; Cody et al., 2017; Ali et al., 2018; Hu et al., 2019; Jiang et al., 2019; Ellison et al., 2020; Lei et al., 2021).

Expression of a Cas nuclease using a plant virus-derived vector able to move systemically through a plant was long considered unachievable due to cargo constraints. However, the innovative work by Ma et al. (2020) demonstrated efficient genome editing by delivering both the sgRNA and SpCas9 at the whole-plant level using a vector derived from *sonchus yellow net virus* (SYNV; family *Rhabdoviridae*). Despite this unprecedented achievement, additional virus-based systems for the co-expression of Cas nucleases and sgRNAs at the whole-plant level are still required to improve the current toolbox for crop engineering. Notably, each viral vector has its own unique properties, particularly a specific host range.

To expand the virus-based tools for tissue culture-free genome editing in plants, we co-expressed the Cas nuclease and the guide RNA using two compatible viral vectors that replicate in the same cells and coordinately move systemically through the whole-plant. We chose a potyvirus vector to express the Cas nuclease. Potyviruses (genus *Potyvirus*) are the largest group of plus-strand RNA viruses, with more than 200 currently known species that collectively can infect a large range of host plants (Wylie et al., 2017). We also focused on a Cas12a (formerly Cpf1) nuclease, which is a component of a class 2 type V CRISPR system, isolated from *Lachnospiraceae bacterium* ND2006 (LbCas12a) (Zetsche et al., 2015). Genome editing using LbCas12a has been demonstrated in plants (Endo et al., 2016; Tang et al., 2017; Wang et al., 2017; Xu et al., 2017), and the complementary DNA (cDNA) corresponding to this nuclease is smaller than that of SpCas9. We also co-expressed the guide RNA using a recently described potato virus X (PVX, genus *Potexvirus*, family *Virgaviridae*) vector, which efficiently induces heritable gene editing in *Nicotiana benthamiana* plants that stably express SpCas9 (Uranga et al., 2021). Our results demonstrated efficient DNA-free *N. benthamiana* genome editing using the two compatible RNA virus vectors.

Methods

Viral vectors

Guide RNAs to target *N. benthamiana* Flowering locus *T* (*NbFT*; SolGenomics Niben101Scf01519g10008.1) and *Xylosyl transferase 1* (*NbXT1*; Niben101Scf04205g03008.1) were selected using the CRISPR-P online tool as described by Bernabé-Orts et al. (2019) (Table S1). Nucleotide (nt) sequences of recombinant viral clones are shown in Figs. S1 and S2. These clones were built using the primers shown in Tables S2 and S3.

Plant inoculation

N. benthamiana wild-type and transformed plants expressing tobacco etch virus (TEV, genus *Potyvirus*) nuclear inclusion *b* (NIb) protein (Martí et al., 2020), were grown at 25°C under a 12 h/12 h day/night photoperiod. Plants that were 4- to 6-weeks-old were agroinoculated as previously described (Bedoya et al., 2010; Uranga et al., 2021). Tissue samples (approximately 100 mg) from the first symptomatic upper non-inoculated leaf were collected at different days post inoculation (dpi), as indicated, for virus progeny and plant genome-editing analyses.

Reverse transcription (RT)-polymerase chain reaction (PCR) analysis of viral progeny

RNA was purified from leaf samples using silica gel columns (Uranga et al., 2021). cDNA was synthesized using RevertAid reverse transcriptase (Thermo Scientific) and primer D179 (Table S4). PCR with *Thermus thermophilus* DNA polymerase (Biotools) was used to amplify the TEV coat protein (CP) cistron (primers D178 and D211; Table S4) or a fragment of the LbCas12a open reading frame (ORF) (primers D3604 and D3605; Table S4). PCR products were separated by electrophoresis in 1% agarose gels followed by staining with ethidium bromide.

Analysis of *N. benthamiana* genome editing

DNA from leaf samples was purified using silica gel columns (Uranga et al., 2021). *N. benthamiana* genome fragments were amplified by PCR using high-fidelity Phusion DNA polymerase (Thermo Scientific) (Table S5). PCR products were separated by agarose gel electrophoresis, purified from the gel, and subjected to Sanger sequencing (Table S5). The presence of sequence modifications was analyzed using the inference of CRISPR edits (ICE) software (<http://www.synthego.com/products/bioinformatics/crispr-analysis>).

Results

A dual virus-based vector system to co-express Cas nucleases and guide RNAs in plants

Initially, we built a TEV recombinant clone in which the cDNA of a human codon-optimized LbCas12a replaced that of the viral NIb protein (TEVΔN::LbCas12a). Our previous work showed that this vector could express large exogenous sequences, such as a whole bacterial metabolic pathway to biosynthesize lycopene or the saffron carotenoid cleavage dioxygenase (Majer *et al.*, 2017; Martí *et al.*, 2020). However, since the virus lacks the viral RNA-dependent RNA polymerase NIb, it replicates only in plants that express this protein (Majer *et al.*, 2017). In our recombinant clone, the sequence coding for LbCas12a replaced most of the NIb cistron and was flanked by the native nuclear inclusion *a* protease (NIaPro) cleavage sites that mediate the release of the nuclease from the viral polyprotein (Fig. 1A and Fig. S1).

Next, we built a recombinant version of PVX in which the LbCas12a CRISPR RNA (crRNA) was expressed under the control of the viral CP promoter, and the 29 initial codons of PVX CP were deleted to improve the stability of the recombinant clone (Dickmeis *et al.*, 2014). The viral CP was expressed from a heterologous promoter derived from that of the CP of bamboo mosaic virus (BaMV; genus *Potexvirus*). Based on previous work by Bernabé-Orts *et al.* (2019) assessing the efficiency of the Cas12a-mediated gene editing of several *N. benthamiana* loci, *NbFT* was selected as the target gene (PVX::crFT) (Fig. 1B and Fig. S2). The 65-nt crRNA was cloned downstream of the PVX CP promoter and consisted of a 23-nt protospacer sequence specific to the target gene flanked on both 5' and 3' ends by a conserved 21-nt scaffold, also known as a direct repeat.

N. benthamiana plants constitutively expressing TEV NIb under the control of cauliflower mosaic virus (CaMV) 35S promoter and terminator (Martí *et al.*, 2020) were co-inoculated with a 1:1 mix of two cultures of *Agrobacterium tumefaciens* transformed with plasmids harboring TEVΔNIb::LbCas12a and PVX::crFT constructs. Controls included inoculation of TEVΔNIb::LbCas12a alone and inoculation of TEVΔNIb::crtB, which allows visual tracking of virus systemic movement due to the yellow pigmentation of infected tissue induced by *Pantoea ananatis* phytoene synthase (crtB) (Majer *et al.*, 2017). At 7 dpi, typical symptoms of TEV infection emerged in the upper non-inoculated leaves of all plants. Notably, at 14 dpi irregular chlorotic spots appeared in the leaves of plants co-inoculated with TEVΔNIb::LbCas12a and PVX::crFT, but not in those inoculated with TEVΔNIb::LbCas12a alone (Fig. S3). PVX infection is characterized by the appearance of vein banding, ring spots

and leaf atrophy (Loebenstein and Gaba, 2012). Therefore, the observed phenotypic alteration could have been due to LbCas12a-mediated editing of *NbFT* or an effect of the coinfection. Samples from the first systemically infected upper leaf were collected at 7 and 14 dpi, and TEVΔNIb progeny were studied by RT-PCR analysis (Fig. 1C). An 804-bp specific region of the TEV genome corresponding to the CP cistron was amplified in all virus-inoculated plants, confirming the presence of the virus (Fig. 1C, top panel). However, for viral vectors carrying big cargos, genomes larger than wild type are likely to recombine to smaller sizes, thus triggering the loss of heterologous genes (Gilbertson et al., 2003). An additional RT-PCR analysis was performed, and a 600-bp cDNA corresponding to a fragment of the LbCas12a ORF was exclusively amplified from plants inoculated with TEVΔNIb::LbCas12a alone or co-inoculated with TEVΔNIb::LbCas12a and PVX::crFT, regardless of sampling time (Fig. 1C, bottom panel). These results suggested that the LbCas12a nuclease was expressed from the onset of viral infection and throughout the rest of the experiment.

Next, DNA was purified from leaf samples collected at 7 and 14 dpi, and a 550-bp fragment of the *NbFT* gene covering the LbCas12a target site was amplified by PCR. Sanger sequencing of the PCR products and ICE analysis revealed robust gene editing at 14 dpi in plants co-inoculated with TEVΔNIb::LbCas12a and PVX::crFT, reaching an indel percentage of up to 75% (Fig. 1D and Fig. S4). No significant differences were observed at 21 and 28 dpi. The indel distribution was consistent with the deletion-enriched mutagenesis profile characteristic of Cas12a activity, being mainly 5- to 10-bp deletions (Bernabé-Orts et al., 2019). These results indicate that the simultaneous delivery of CRISPR-Cas12a components through two compatible viral vectors (i.e., TEVΔNIb and PVX) allows highly efficient, DNA-free targeted mutagenesis in *N. benthamiana*.

Multiplex genome editing using the dual virus-based vector system in plants

A key advantage of CRISPR-Cas genome editing is the capacity to target several loci at once by the simultaneous expression of several guide RNAs (i.e., multiplexing). Based on our observations in Cas9-expressing plants (Uranga et al., 2021), we wondered whether the PVX vector could allow the delivery of multiple, functional crRNAs for Cas12a-mediated editing. To investigate this, we selected *Xylosyl transferase 1* (*NbXT1*) as the second target gene. As Cas12a can self-process crRNAs due to its RNase III activity, *NbXT1* and *NbFT* crRNAs were arranged in tandem under the control of the same CP promoter, thus creating the PVX::crXT1:crFT construct (Fig. 1E). *N. benthamiana* plants constitutively expressing TEV

Nlb were co-inoculated with a 1:1 mix of two *A. tumefaciens* cultures carrying TEVΔNlb::LbCas12a and PVX::crXT1:crFT. *A. tumefaciens* transformed with TEV::crtB was used as a control in this assay. The first systemically infected leaf was sampled at 14, 21, and 28 dpi, following extraction of genomic DNA and PCR amplification of the target sites. ICE analysis revealed efficient gene editing on both *NbXT1* and *NbFT*, with average indel percentages ranging from 76% to 88%, which was maintained regardless of the sampling time (Fig. 1F). In addition, the absence of statistically relevant differences in gene editing among *NbXT1* and *NbFT* suggested that LbCas12a can efficiently self-process tandemly arrayed crRNAs. Time-course comparison with the single-crRNA construct, in the case of *NbFT*, also revealed that multiplexing does not affect the editing efficiency, since the mutation rates achieved with both strategies were similar (Fig. 1D and F).

Dual vector CRISPR-Cas genome editing in wild-type plants

The fact that TEVΔNlb infectivity depends on the supplementation of viral Nlb from a transgene may be perceived as a limitation of this genome-editing system, as the approach is still bound to a previously transformed plant. An alternative strategy for supplying Nlb activity consists of the co-inoculation of TEVΔNlb with a recombinant PVX expressing Nlb (Bedoya et al., 2010). We wondered whether a single PVX vector could: (i) provide Nlb activity for the systemic movement of TEVΔNlb; and (ii) deliver the crRNA for LbCas12a-mediated gene editing. Thus, the coding sequence of the TEV Nlb cistron plus an additional amino-terminal Met was inserted within the expression cassette in PVX. *NbFT*-specific crRNA was added downstream of Nlb without any linker sequence, so that both Nlb and crFT expression were under the control of the PVX CP promoter (PVX::Nlb:crFT) (Fig. 2A). Wild-type *N. benthamiana* plants were co-inoculated with a 1:1 mix of two cultures of *A. tumefaciens* carrying TEVΔNlb::LbCas12a and PVX::Nlb:crFT. *A. tumefaciens* transformed with TEV::crtB was again used as a control. At 7 dpi, the apical leaves of the co-inoculated *N. benthamiana* plants became symptomatic (i.e., leaf curling). At 14 dpi, necrotic spotting and interveinal mottling were observed in systemic leaves, which became more noticeable over time (Fig. S5). Samples from the first systemically infected upper leaf were collected at 14 dpi and the 550-bp fragment of the *NbFT* gene covering the LbCas12a target site was amplified by PCR. ICE analysis of the PCR products exhibited 20% indels in plants co-inoculated with TEVΔNlb::LbCas12a and PVX::Nlb:crFT. These results indicate that a single PVX vector can

supply the viral Nib activity that allows TEVΔNib to systemically spread in wild-type *N. benthamiana*, as well as to perform crRNA delivery for LbCas12a-mediated genome editing.

Discussion

In this study we describe the engineering of a dual RNA-virus system for the delivery of CRISPR-Cas12a components for genome editing in plants. The system consists of two compatible plus-strand RNA viruses, specifically the potyvirus TEV and the potexvirus PVX, to express the Cas nuclease and the guide RNA, respectively. Notably, the TEV vector (TEVΔNib) contains the deletion of the RNA-dependent RNA polymerase (Nib) in order to accommodate the large ORF of the Cas nuclease. Our previous efforts to express a functional Cas nuclease using a full-length potyvirus vector in plants have been unsuccessful. However, recent works have reported successful Cas9 expression and DNA-free genome editing using single SYMV (Ma et al., 2020) and PVX (Ariga et al., 2020) vectors in *N. benthamiana*. These contrasting results emphasize the diverse properties of vectors derived from viruses belonging to different genera and families, and the necessity for a large spectrum of molecular tools, operating in a wide range of host species, to tackle challenging VIGE goals in crop plants. Notably, SYNIV is a minus-strand RNA virus the inoculation of which entails some complexity (Peng et al., 2021) and, as with each plant virus, it exhibits a particular host range (Jackson and Christie, 1977).

In this work, we aimed to express a Cas nuclease using a potyvirus vector for DNA-free plant-genome editing. The genus *Potyvirus* is the largest among the plant RNA viruses, and comprises more than 200 species that infect a wide range of host plants from many different botanical families (Wylie et al., 2017). Therefore, it offers a wealth of genetic resources for VIGE. Although we were unable to successfully express the Cas nuclease using a full-length potyvirus vector, we demonstrated that TEVΔNib allows the transient expression of LbCas12a, and that PVX can perform both single or multiple crRNA delivery as well as providing the Nib activity. Our two-virus delivery system resulted in efficient DNA-free targeted editing both in Nib-expressing and wild-type *N. benthamiana* plants, reaching indel percentages of up to 80% and 20%, respectively (Figs. 1 and 2). This dual vector system not only incorporates the enormous genetic resources of potyviruses for VIGE, but also demonstrates that compatible RNA virus vectors can be used to simultaneously deliver several CRISPR-Cas components. This could be useful for more sophisticated arrangements in DNA-free plant-genome editing and gene-expression regulation studies. Recently, we developed a PVX vector to efficiently

express multiplex SpCas9 sgRNAs at the whole-plant level. Current results with LbCas12a confirm that PVX can be easily engineered for the simultaneous delivery of guide RNAs regardless of the nature of the Cas nuclease, which highlights its usefulness in a variety of multiplexing approaches.

In contrast to previous reports of DNA-free VIGE that used SpCas9 (Ariga *et al.*, 2020; Ma *et al.*, 2020), we selected Cas12a. This was due to the following unique features of the nuclease (Zaidi *et al.*, 2017): (i) the cleavage of target DNA is directed by a single crRNA shorter than that of SpCas9 sgRNA; (ii) the protospacer adjacent motif (PAM) is T-rich (5'-TTTN-3'); (iii) DNA cleavage results in cohesive ends with 4- or 5-nt overhangs, which might facilitate homology-directed repair (HDR); (iv) it exhibits RNase III activity useful to facilitate multiplex gene editing; and (v) it is smaller than SpCas9 (3.8 kb vs 4.2 kb), which is important in terms of viral delivery. Moreover, the unique characteristics of the CRISPR-Cas12a system, unlike that of Cas9, such as the recognition of a T-rich PAM and the induction of staggered ends that facilitate homologous recombination, limit the range of target sequences and thus reduce off-target activity (Zaidi *et al.*, 2017). Cas12a orthologs from *Francisella novicida* U112 (FnCas12a), *Acidaminococcus* sp. BV3L6 (AsCas12a) and LbCas12a were first experimentally validated in mammalian cells (Kim *et al.*, 2016, 2017a; Zetsche *et al.*, 2017). In plants, targeted mutagenesis was achieved in rice and tobacco using any of the orthologs (Endo *et al.*, 2016; Tang *et al.*, 2017; Wang *et al.*, 2017; Xu *et al.*, 2017). A DNA-free approach based on the delivery of AsCas12a or LbCas12a loaded with crRNA was also validated in wild tobacco and soy-bean protoplasts (Kim *et al.*, 2017b). Here we focused on LbCas12a, since previous works reported that this nuclease possesses higher efficiency than FnCas12a or AsCas12a (Tang *et al.*, 2017; Bernabé-Orts *et al.*, 2019), and is also effective for plant-genome editing when virally delivered.

In conclusion, our dual RNA-virus-based system broadens the current toolbox for DNA-free VIGE and will contribute to applications in plant functional genomics and crop improvement.

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Supplementary Material

Figure S1. Full sequence of wild-type tobacco etch virus (TEV, Genbank accession number DQ986288), the defective viral vector TEVΔNIb and their derived recombinant viruses, TEV::crtB, TEVΔNIb::crtB and TEVΔNIb::LbCas12a.

Figure S2. Full sequence of wild-type potato virus X (PVX; GenBank accession number MT799816) and its derived recombinant viruses PVX::crFT and PVX::NIb:crFT.

Figure S3. 35S::NIb *N. benthamiana* plants constitutively expressing TEV NIb and representative leaves from these plants at 14 dpi inoculated with TEVΔNIb::LbCas12a or co-inoculated with TEVΔNIb::LbCas12a and PVX::crFT.

Figure S4. Example of a sequence electropherogram and mutagenesis profile from 35S::NIb *N. benthamiana* plants co-inoculated with TEVΔNIb::LbCas12a and PVX::crFT.

Figure S5. Representative leaves from wild-type *N. benthamiana* plants at 14 dpi inoculated with TEVΔNIb::LbCas12a alone, PVX::NIb:crFT alone, or coinoculated with TEVΔNIb::LbCas12a and PVX::NIb:crFT.

Table S1. *N. benthamiana* genes targeted by the CRISPR-Cas12a system. PAMs are highlighted with a grey background.

Table S2. Primers used for the construction of recombinant viruses.

Table S3. Primer combinations used for the construction of recombinant viruses.

Table S4. Primers used for TEVΔNIb diagnosis by RT-PCR.

Table S5. Primers used for Cas12a-crRNA gene editing analysis.

References

- Ali, Z., Eid, A., Ali, S., and Mahfouz, M.M. (2018) Pea early-browning virus-mediated genome editing via the CRISPR/Cas9 system in *Nicotiana benthamiana* and *Arabidopsis*. *Virus Res.*, **244**, 333–337.
- Ariga, H., Toki, S., and Ishibashi, K. (2020) Potato virus X vector-mediated DNA-free genome editing in plants. *Plant Cell Physiol.*, **61**, 1946–1953.
- Bedoya, L., Martínez, F., Rubio, L., and Daròs, J.A. (2010) Simultaneous equimolar expression of multiple proteins in plants from a disarmed potyvirus vector. *J.*

Biotechnol., **150**, 268–275.

- Bernabé-Orts, J.M., Casas-Rodrigo, I., Minguet, E.G., Landolfi, V., Garcia-Carpintero, V., Gianoglio, S., et al. (2019) Assessment of Cas12a-mediated gene editing efficiency in plants. *Plant Biotechnol. J.*, **17**, 1971–1984.
- Cody, W.B., Scholthof, H.B., and Mirkov, T.E. (2017) Multiplexed gene editing and protein overexpression using a tobacco mosaic virus viral vector. *Plant Physiol.*, **175**, 23–35.
- Cong, L., Ran, F.A., Cox, D., Lin, S., Barretto, R., Habib, N., et al. (2013) Multiplex genome engineering using CRISPR/Cas systems. *Science* (80-.), **339**, 819–823.
- Dickmeis, C., Fischer, R., and Commandeur, U. (2014) Potato virus X-based expression vectors are stabilized for long-term production of proteins and larger inserts. *Biotechnol. J.*, **9**, 1369–1379.
- Ellison, E.E., Nagalakshmi, U., Gamo, M.E., Huang, P. jui, Dinesh-Kumar, S., and Voytas, D.F. (2020) Multiplexed heritable gene editing using RNA viruses and mobile single guide RNAs. *Nat. Plants*, **6**, 620–624.
- Endo, A., Masafumi, M., Kaya, H., and Toki, S. (2016) Efficient targeted mutagenesis of rice and tobacco genomes using Cpf1 from Francisella novicida. *Sci. Rep.*, **6**, 38169.
- Gilbertson, R.L., Sudarshana, M., Jiang, H., Rojas, M.R., and Lucas, W.J. (2003) Limitations on Geminivirus Genome Size Imposed by Plasmodesmata and Virus-Encoded Movement Protein: Insights into DNA Trafficking. *Plant Cell*, **15**, 2578–2591.
- Hu, J., Li, S., Li, Z., Li, H., Song, W., Zhao, H., et al. (2019) A barley stripe mosaic virus-based guide RNA delivery system for targeted mutagenesis in wheat and maize. *Mol. Plant Pathol.*, **20**, 1463–1474.
- Huang, T.K. and Puchta, H. (2021) Novel CRISPR/Cas applications in plants: from prime editing to chromosome engineering. *Transgenic Res.*
- Jackson, A.O. and Christie, S.R. (1977) Purification and some physicochemical properties of sonchus yellow net virus. *Virology*, **77**, 344–355.
- Jiang, N., Zhang, C., Liu, J.Y., Guo, Z.H., Zhang, Z.Y., Han, C.G., and Wang, Y. (2019) Development of Beet necrotic yellow vein virus-based vectors for multiple-gene expression and guide RNA delivery in plant genome editing. *Plant Biotechnol. J.*, **17**, 1302–1315.
- Kim, D., Kim, J., Hur, J.K., Been, K.W., Yoon, S.-H., and Kim, J.-S. (2016) Genome-wide analysis reveals specificities of Cpf1 endonucleases in human cells. *Nat. Biotechnol.*, **34**, 863–8.
- Kim, H., Kim, S.T., Ryu, J., Kang, B.C., Kim, J.S., and Kim, S.G. (2017) CRISPR/Cpf1-

mediated DNA-free plant genome editing. *Nat. Commun.*, **8**, 14406.

Kim, H.K., Song, M., Lee, J., Menon, A.V., Jung, S., Kang, Y.M., et al. (2017) In vivo high-throughput profiling of CRISPR-Cpf1 activity. *Nat. Methods*, **14**, 153–159.

Lau, C.H. and Suh, Y. (2017) In vivo genome editing in animals using AAV-CRISPR system: Applications to translational research of human disease. *F1000Research*, **6**, 2153.

Lei, J., Dai, P., Li, Y., Zhang, W., Zhou, G., Liu, C., and Liu, X. (2021) Heritable gene editing using FT mobile guide RNAs and DNA viruses. *Plant Methods*, **17**.

Li, J., Zhang, H., Si, X., Tian, Y., Chen, K., Liu, J., et al. (2017) Generation of thermosensitive male-sterile maize by targeted knockout of the ZmTMS5 gene. *J. Genet. Genomics*, **44**, 465–468.

Loebenstein, G. and Gaba, V. (2012) Viruses of Potato. In: *Advances in Virus Research*, pp. 209–246. Academic Press Inc.

Ma, X., Zhang, X., Liu, H., and Li, Z. (2020) Highly efficient DNA-free plant genome editing using virally delivered CRISPR–Cas9. *Nat. Plants*, **6**, 773–779.

Majer, E., Llorente, B., Rodríguez-Concepción, M., and Daròs, J.A. (2017) Rewiring carotenoid biosynthesis in plants using a viral vector. *Sci. Rep.*, **7**, 41645.

Martí, M., Diretto, G., Aragonés, V., Frusciante, S., Ahrazem, O., Gómez-Gómez, L., and Daròs, J.A. (2020) Efficient production of saffron crocins and picrocrocin in *Nicotiana benthamiana* using a virus-driven system. *Metab. Eng.*, **61**, 238–250.

Nekrasov, V., Staskawicz, B., Weigel, D., Jones, J.D.G., and Kamoun, S. (2013) Targeted mutagenesis in the model plant *Nicotiana benthamiana* using Cas9 RNA-guided endonuclease. *Nat. Biotechnol.*, **31**, 691–693.

Peng, X., Ma, X., Lu, S., and Li, Z. (2021) A Versatile Plant Rhabdovirus-Based Vector for Gene Silencing, miRNA Expression and Depletion, and Antibody Production. *Front. Plant Sci.*, **11**, 627880.

Platt, R.J., Chen, S., Zhou, Y., Yim, M.J., Swiech, L., Kempton, H.R., et al. (2014) CRISPR-Cas9 knockin mice for genome editing and cancer modeling. *Cell*, **159**, 440–455.

Senís, E., Fatouros, C., Große, S., Wiedtke, E., Niopek, D., Mueller, A.-K., et al. (2014) CRISPR/Cas9-mediated genome engineering: an adeno-associated viral (AAV) vector toolbox. *Biotechnol. J.*, **9**, 1402–12.

Tang, X., Lowder, L.G., Zhang, T., Malzahn, A.A., Zheng, X., Voytas, D.F., et al. (2017) A CRISPR-Cpf1 system for efficient genome editing and transcriptional repression in plants. *Nat. Plants*, **3**, 17103.

Uranga, M., Aragonés, V., Selma, S., Vázquez-Vilar, M., Orzáez, D., and Daròs, J. (2021)

Efficient Cas9 multiplex editing using unspaced sgRNA arrays engineering in a Potato virus X vector. *Plant J.*

Wang, M., Mao, Y., Lu, Y., Tao, X., and Zhu, J.-K. (2017) Multiplex Gene Editing in Rice Using the CRISPR-Cpf1 System. *Mol. Plant*, **10**, 1011–1013.

Wylie, S.J., Adams, M., Chalam, C., Kreuze, J., López-Moya, J.J., Ohshima, K., et al. (2017) ICTV virus taxonomy profile: Potyviridae. *J. Gen. Virol.*, **98**, 352–354.

Xu, C.L., Ruan, M.Z.C., Mahajan, V.B., and Tsang, S.H. (2019) Viral delivery systems for CRISPR. *Viruses*, **11**, 28.

Xu, R., Qin, R., Li, H., Li, D., Li, L., Wei, P., and Yang, J. (2017) Generation of targeted mutant rice using a CRISPR-Cpf1 system. *Plant Biotechnol. J.*, **15**, 713–717.

Yin, K., Han, T., Liu, G., Chen, T., Wang, Y., Yu, A.Y.L., and Liu, Y. (2015) A geminivirus-based guide RNA delivery system for CRISPR/Cas9 mediated plant genome editing. *Sci. Rep.*, **5**, 14926.

Zahir Ali, Aala Abul-faraj, Lixin Li, Neha Ghosh, Marek Piatek, Ali Mahjoub, Mustapha Aouida, Agnieszka Piatek, Nicholas J Baltes, Daniel F Voytas, Savithramma Dinesh-Kumar, M.M.M. (2015) Efficient Virus-Mediated Genome Editing in Plants Using the CRISPR/Cas9 System. *Mol. Plant*, **8**, 1288–91.

Zaidi, S.S.-A., Magdy M. Mahfouz, A., and Mansoor, S. (2017) CRISPR-Cpf1: A New Tool for Plant Genome Editing. *Trends Plant Sci.*, **22**, 549–550.

Zaidi, S.S. e. A., Mahfouz, M.M., and Mansoor, S. (2017) CRISPR-Cpf1: A New Tool for Plant Genome Editing. *Trends Plant Sci.*, **22**, 550–553.

Zetsche, B., Gootenberg, J.S., Abudayyeh, O.O., Slaymaker, I.M., Makarova, K.S., Essletzbichler, P., et al. (2015) Cpf1 Is a Single RNA-Guided Endonuclease of a Class 2 CRISPR-Cas System. *Cell*, **163**, 759–771.

Zetsche, B., Heidenreich, M., Mohanraju, P., Fedorova, I., Kneppers, J., Degennaro, E.M., et al. (2017) Multiplex gene editing by CRISPR-Cpf1 using a single crRNA array. *Nat. Biotechnol.*, **35**, 31–34.

Zhang, Y., Liang, Z., Zong, Y., Wang, Y., Liu, J., Chen, K., et al. (2016) Efficient and transgene-free genome editing in wheat through transient expression of CRISPR/Cas9 DNA or RNA. *Nat. Commun.*, **7**, 12617.

Zhu, H., Li, C., and Gao, C. (2020) Applications of CRISPR–Cas in agriculture and plant biotechnology. *Nat. Rev. Mol. Cell Biol.*, **21**, 661–677.

Figures

FIG. 1. DNA-free gene editing in *N. benthamiana* based on virally delivered LbCas12a and crRNA. **(A)** Schematic representation of recombinant virus TEVΔNIb::LbCas12a. Cas12a ORF from *Lachnospiraceae bacterium* ND2006 (LbCas12a), containing a carboxy-terminal nucleoplasmic nuclear localization signal (NLS, grey box), replaces the TEV NIb cistron (light blue box) and is flanked by proteolytic processing sites of viral NIaPro. TEV cistrons P1, HC-Pro, P3, P3N-PIPO, 6K1, CI, 6K2, VPg, NIaPro, NIb, and CP are represented by boxes. 5' and 3' untranslated regions (UTRs) are represented by lines. **(B)** Schematic representation of

417 recombinant clone PVX::crFT. RNA-dependent RNA polymerase (RdRp), triple gene block
418 (TGB), and coat protein (CP) are represented by open boxes. Heterologous *Bamboo mosaic*
419 *virus* CP promoter (CP_{BaMV}) is represented by a black arrow. 5' and 3' UTRs are represented
420 by black lines. crRNA consists of a gene-specific 23-nt protospacer (pink diamond) and
421 conserved 21-nt direct repeats (grey boxes). CP_{BaMV}, protospacer and scaffold are not shown at
422 scale. (C) RT-PCR analysis of TEVΔNIb progeny at 7 and 14 dpi in 35S::NIb *N. benthamiana*
423 plants inoculated with TEVΔNIb::LbCas12a alone or co-inoculated with TEVΔNIb::LbCas12a
424 and PVX::crFT. Amplification products were separated by electrophoresis in an agarose gel
425 stained with ethidium bromide. Lane 1, 1-kb ladder DNA marker with the length of some
426 components (in bp) indicated on the left; lane 2, non-inoculated plant; lanes 3 to 7, plants
427 inoculated with TEVΔNIb::crtB (lane 3) or TEVΔNIb::LbCas12a (lanes 4 and 6), and co-
428 inoculated with TEVΔNIb::LbCas12a and PVX::crFT (lanes 5 and 7). The amplification
429 products corresponding to cDNA regions of TEV CP (804 bp) (top) or LbCas12a (600 bp)
430 (bottom) are indicated by arrows. (D) ICE analysis of the first systemically infected upper leaf
431 of *N. benthamiana* plants co-inoculated with TEVΔNIb::LbCas12a and PVX::crFT at the
432 indicated dpi (n=6). TEVΔNIb::crtB was used as a negative control. (E) Schematic
433 representation of recombinant clone PVX::crXT1:crFT. Protospacers for NbXT1 and NbFT are
434 represented by orange and pink diamonds, respectively. Other details are indicated above. (F)
435 ICE analysis of the first systemically infected upper leaf of *N. benthamiana* plants (n=6) co-
436 inoculated with TEVΔNIb::LbCas12a and PVX::crXT1:crFT at the indicated dpi. Columns and
437 error bars represent average indels (%) and standard deviation, respectively.

FIG. 2. Engineering of a single PVX vector for complementation of defective TEVΔNlB and expression of LbCas12a crRNA. **(A)** Schematic representation of recombinant virus PVX::NlB:crFT. TEV NlB cistron is represented by a light blue box. Other details are as described in the legend to Fig. 1. **(B)** RT-PCR analysis of TEVΔNlB progeny at 14 dpi in wild-type *N. benthamiana* plants co-inoculated with TEVΔNlB::LbCas12a and PVX::crFT or with TEVΔNlB::LbCas12a and PVX::NlB:crFT. Amplification products were separated by electrophoresis in an agarose gel that was stained with ethidium bromide. Lane 1, DNA marker ladder with the length of some components (in bp) indicated on the left; lane 2, non-inoculated plant; lane 3 to 5, plants inoculated with TEVΔNlB::crtB (lane 3) and co-inoculated with TEVΔNlB::LbCas12a and PVX::crFT (lane 4) or with TEVΔNlB::LbCas12a and PVX::NlB:crFT (lane 5). The amplification products corresponding to cDNA regions of TEV CP (804 bp) (top) or LbCas12a (600 bp) (bottom) are indicated by arrows. **(c)** ICE analysis of the first systemically infected upper leaf of *N. benthamiana* plants co-inoculated with TEVΔNlB::LbCas12a and PVX::NlB:crFT at 14 dpi (n=6). TEVΔNlB::crtB was used as a

453 negative control. Columns and error bars represent average indels (%) and standard deviation,
454 respectively.

455

SUPPLEMENTAL DATA

CRISPR-Cas12a genome editing at the whole-plant level using two compatible RNA virus vectors

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Figure S1. Full sequence of the wild-type tobacco etch virus (TEV), the defective viral vector TEVΔNIb and their derived recombinant viruses, TEVΔNIb::crtB and TEVΔNIb::LbCas12a. TEV-wt sequence corresponds to Genbank accession number DQ986288 including two silent mutations (G273A and A1119G, in red). Limits between TEV cistrons are marked on blue background. cDNAs corresponding to *P. ananatis* **phytoene synthase** (crtB) and **Cas12a from *Lachnospiraceae bacterium ND2006*** (LbCas12a) are on yellow and dark green backgrounds, respectively. Nucleoplasmin nuclear localization signal (NLS) and human influenza hemagglutinin (3xHA) tag are underlined and dotted, respectively. In the inserted cDNAs, sequences corresponding to **native** and **artificial** TEV NIaPro cleavage sites are in black or blue, respectively.

>TEV

AAAATAACAAATCTCAACACAACATATACAAAACAAACGAATCTCAAGCAATCAAGCATTCTACTTCTATTGCAG
CAATTTAAATCATTTCTTTTAAAGCAAAAGCAATTTTCTGAAAATTTTACCATTACGAACGATAGC**CA**TGGCA
CTCATCTTTGGCACAGTCAACGCTAACATCCTGAAGGAAGTGTTCGGTGGAGCTCGTATGGCTTGCCTTACCAGC
GCACATATGGCTGGAGCGAATGGAAGCATTTTGAAGAAGGCAGAAGA**A**ACCTCTCGTGCAATCATGCACAAACCA
GTGATCTTCGGAGAAGACTACATTACCGAGGCAGACTTGCCTTACACACCACTCCATTAGAGGTCGATGCTGAA
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CATTATAGCGACAAATCAATCTCTGAGGCATTCTTACATACCATACTTAAGAAATCTTGGAGTTGAG**C**CGAGAT
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507 CGTAAAGAAATTGGGAATCACTGCATTTCAATGCGGAATGGTAATTACGTGTACCCATGTTGTTGTGTTACTCTT
508 GAAGATGGTAAGGCTCAATATTCGGATCTAAAGCATCCAACGAAGAGACATCTGGTCATTGGCAACTCTGGCGAT
509 TCAAAGTACCTAGACCTTCCAGTTCTCAATGAAGAGAAAATGTATATAGCTAATGAAGGTTATTGCTACATGAAC
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646 ATAGTCTCCCCTTCAATTTTAATTGCCATGTACAACCTCTGGAACTTTTGAGCAGGCGTTACAAATGTGGTTGCCA
647 AATACAATGAGGTTAGCTAACCTCGCTGCCATCTTGTGAGCCTTGGCGCAAAAGTTAACTTTGGCAGACTTGTTC
648 GTCCAGCAGCGTAATTTGATTAATGAGTATGCGCAGGTAATTTTGGACAATCTGATTGACGGTGTGAGGGTTAAC
649 CATTGCTATCCCTAGCAATGGAATTTGTTACTATTAAGCTGGCCACCCAAGAGATGGACATGGCGTTGAGGGAA
650 GGTGGCTATGCTGTGACCTCTGAAAAGGTGCATGAAATGTTGGAAAAAACTATGTAAAGGCTTTGAAGGATGCA
651 TGGGACGAATTAACCTTGGTTGGAAAAATTTCTCCGCAATCAGGCATTCAAGAAAGCTCTTGAATTTGGGCGAAAG
652 CCTTTAATCATGAAAAACCCGTAGATTGCGGCGGACATATAGACTTGTCTGTGAAATCGCTTTTCAAGTTCCAC
653 TTGGAATCCTGGAAGGAACCATCTCAAGAGCGTAATGTTGGTGCAGAAAGGTAAGAGTAGCGAAGAATGCC
654 ATGACAAAAGGGGTTTTTCTCAAAATCTACAGCATGCTTCTGAGCTCTACAAGTTTATACAGTCTCGAGTGTC
655 CTTTCCTTGTGTTGATCATTCTTATTTCAAATTGACTGCATGATAAGGGCACACCGGAGAGGCGAAGGTTGCTGCA
656 CAGTTGCAGAAAGAGAGCGAGTGGGACAATATCATCAATAGAATTTTCCAGTATTCTAAGCTTGAAAAATCCTATT
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658 GGAAAGGAAGACCTCGTTGAACAAGGCAAAACAACCGGAGATAGCATACTTTGAAAAGATTATAGCTTTTCATCACA
659 CTTGTATTAATGGCTTTTTCAGCTGAGCGGAGTGATGGAGTGTTCAGATACTCAATAAGTTCAAAGGAATACTG
660 AGCTCAACGGAGAGGGAGATCATCTACACGCAAGTTTGGATGATTACGTTACAACCTTTGATGACAATATGACA
661 ATCAACCTCGAGTTGAATATGGATGAATCCACAAGACGAGCCTTCTGAGTCACTTTTAAGCAATGGTGGAAAC
662 AACCAAATCAGCCGAGGCAACGTGAAGCCACATTATAGAAGTGAAGGGGCACTTCATGGAGTTTACCAGAGATACT
663 GCGGCATCGGTTGCCAGCGAGATATCACACTACCCGCAAGAGATTTTCTTGTGAGAGGTGCTGTTGGATCTGGA
664 AAATCCACAGGACTTCCATACCATTTATCAAAGAGAGGGGAGAGTGTTAATGCTTGAGCCTACCAGACCACTCACA
665 GATAACGTGCACAAGCAACTGAGAAGTGAACCATTTAACTGCTTCCCAACTTTGAGGATGAGAGGGGAAGTCAACT
666 TTTGGGTGATCACCAGATTACAGTCATGACTAGTGGATTGCTTTACACCATTTTGCACGAAACATAGCTGAGGTA
667 AAAACATACGATTTTGTGATAATTGATGAATGTGATGTTGATGCTTCTGCTATAGCGTTTAGGAATCTACTG
668 TTTGAACATGAATTTGAAGGAAAAGTCTCAAAGTGTGAGCCACACCAAGGTTAGAGAGTTGAATTCACAACCT
669 CAGTTTCCCGTGAAGTCAAGATAGAAGAGGCTCTTAGCTTTGAGGAATTTGTAAGTTTACAAGGGACAGGTGCC
670 AACGCCGATGTGATTAGTTGTGGCGACAACATAGTATGTTGCTAGCTACAATGATGTTGATAGTCTTGGC
671 AAGCTCCTTGTGCAAAAGGGATACAAAGTGTGCAAGATGTTGAGGAAGAACAATGAAGAGTGGAGGAATGAAATA
672 ATCACTGAAGGTACTTTCAGTGAAAAAGCATTTTCATAGTTCGCAACTAATATTATTGAGAATGGTGTAACCATTGAC
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674 AAAACTGTGGTGAGTTATGGGGAGCGCATCCAAAGACTCGGTAGAGTTGGGCGACACAAGGAAGGAGTAGCACTT
675 CGAATTGGCCAAACAATAAAACACTGGTTGAAATTCAGAAATGGTTGCCACTGAAGCTGCCTTTCTATGCTTC
676 ATGTACAATTTGCCAGTGACAACACAGAGTGTTCACACCACTGCTGGAAAATGCCACATTATTACAAGCTAGA
677 ACTATGGCACAGTTTTCAGCTATCATATTTTACACAATTAATTTTGTGCGATTGATGGTAGTATGCATCCAGTC
678 ATACATGACAAGCTGAAGCGCTTTAAGCTACACACTTGTGAGACATTCCTCAATAAGTTGGCGATCCCAAATAAA
679 GGCTTATCCTCTTGGCTTACGAGTGGAGAGTATAAGCGACTTGGTTACATAGCAGAGGATGCTGGCATAAGAATC
680 CCATTGCTGTGCAAGAAATTCAGACTCCTTGCATGAGGAAATTTGGCACATTGTAGTCGCCCATAAAGGTGAC
681 TCGGGTATTGGGAGGCTCACTAGCGTACAGGCAGCAAAGGTTGTTTATACTCTGCAAACGGATGTGCACTCAATT
682 GCGAGGACTCTAGCATGCATCAATAGACTCATAGCACATGAACAAATGAAGCAGAGTCATTTTGAAGCCGCAACT
683 GGGAGAGCATTTTCTTCCAAATTAATCAATAACAGCATATTTGACACGCTGAAAGCAAATATGCTACAAAG
684 CATACGAAAGAAAATATTGCAAGTGTTCAGCAGGCAAAGATCAATTGCTAGAGTTTTCGAACCTAGCAAAGGAT
685 CAAGATGTCACGGGTATCATCCAAGACTTCAATCACCTGGAAACTATCTATCTCCAATCAGATAGCGAAGTGGCT
686 AAGCATCTGAAGCTTAAAAGTCACTGGAATAAAAGCCAAATCACTAGGGACATCATAATAGCTTTGTCTGTGTTA
687 ATTGGTGGTGGATGGATGCTTGAACGTAAGTCAAGGACAAGTTCAATGAACCAGTCTATTTCCAAGGAAGAAG


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688 AATCAGAAGCACAAAGCTTAAGATGAGAGAGGCGCGTGGGGCTAGAGGGCAATATGAGGTTGCAGCGGAGCCAGAG
689 GCGCTAGAACATTACTTTGGAAGCGCATATAATAACAAAGGAAAGCGCAAGGGCACCACGAGAGGAATGGGTGCA
690 AAGTCTCGGAAATTCATAAACATGTATGGGTTTGATCCAACCTGATTTTTTCATACATTAGGTTTGTGGATCCATTG
691 ACAGGTCACACTATTGATGAGTCCACAAACGCACCTATTGATTTAGTGCAGCATGAGTTTGGAAAGGTTAGAACA
692 CGCATGTTAATTGACGATGAGATAGAGCCTCAAAGTCTTAGCACCCACACCACAATCCATGCTTATTTGGTGAAT
693 AGTGGCACGAAGAAAGTTCTTAAGGTTGATTTAACACCACACTCGTCGCTACGTGCGAGTGAGAAATCAACAGCA
694 ATAATGGGATTTCTGAAAGGGAGAATGAATTGCGTCAAACCGGCATGGCAGTGCCAGTGCGCTTATGATCAATTG
695 CCACCAAAGAGTGAGGACTTGACGTTTGAAGGAGAAAGCTTGTAAAGGGACCACGTGATTACAACCCGATATCG
696 AGCACCATTTGTCACTTGACGAATGAATCTGATGGGCACACAACATCGTTGTATGGTATTGGATTGGTCCCTTC
697 ATCATTACAAACAAGCACTTTGTTTAGAAGAAATAATGGAACACTGTTGGTCCAATCACTACATGCTGTATTCAAG
698 GTCAAGAACACCACGACTTTGCAACAACACCTCATTGATGGGAGGGACATGATAATTATTTCGCATGCCTAAGGAT
699 TTCCACCATTCTCTCAAAGCTGAAATTTAGAGAGCCACAAAGGGAAGAGCGCATATGTCTTGTGACAACCAAC
700 TTCCAAACTAAGAGCATGTCTAGCATGGTGTGACAGACTAGTTGCACATTCCCTTCATCTGATGGCATATTCTGG
701 AAGCATTGGATTCAAACCAAGGATGGGCAGTGTGGCAGTCCATTAGTATCAACTAGAGATGGGTTTATTGTTGGT
702 ATACACTCAGCATCGAATTTACCAACACAAACAATTATTTTACAAGCGTGCCGAAAAACTTCATGGAATTGTTG
703 ACAAATCAGGAGGCGCAGCAGTGGGTTAGTGGTTGGCGATTAAATGCTGACTCAGTATTGTGGGGGGGCCATAAA
704 GTTTTCATGAGCAAACCTGAAGAGCCTTTTCAGCCAGTTAAGGAAGCGACTCAACTCATGAGTGAATTGGTGTAC
705 TCGCAAGTGGCACTGTGGGTGCTGGTGTGACGCTGGTAAGAAGAAAGATCAAAGGATGATAAAGTCGCTGAG
706 CAGGCTTCAAAGGATAGGGATGTTAATGCTGGAACCTTCAGGAACATTCTCAGTTCCACGAATAAATGCTATGGCC
707 ACAAACCTTCAATATCCAAGGATGAGGGGAGAGGTGGTTGTAAACTTGAATCACCTTTTAGGATACAAGCCACAG
708 CAAATTGATTTGTCAAATGCTCGAGCCACACATGAGCAGTTTGCCGCGTGGCATCAGGCAGTGATGACAGCCTAT
709 GGAGTGAATGAAGAGCAAATGAAAATATTGCTAAATGGATTTATGGTGTGGTGCATAGAAAAATGGGACTTCCCCA
710 AATTTGAACGGAACCTTGGGTTATGATGGATGGTGAAGGAGCAAGTTTCATACCCGCTGAAACCAATGGTTGAAAA
711 GCGCAGCCAACACTGAGGCAAATTATGACACACTTCAGTGACCTGGCTGAAGCGTATATTGAGATGAGGAATAGG
712 GAGCGACCATAACATGCCTAGGTATGGTCTACAGAGAAACATTACAGACATGAGTTTGTACGCTATGCGTTTCGAC
713 TTCTATGAGCTAACTTCAAAAACACCTGTTAGAGCAGGGAGGCGCATATGCAAATGAAAGCTGCTGCAGTACGA
714 AACATGGAACCTAGGTTATTTGGTCTTGTAGGCAACGTGGGTACTGCAGAGGAAGACATGAACGGCACACAGCG
715 CAGATGTGAACCGTAACATGCACACACTATTAGGGGTCCGCCAGTGATAGTTTCTGCGTGTCTTTGCTTTCCGC
716 TTTTAAGCTTATTGTAATATATATGAATAGCTATTACAGTGGGACTTGGTCTTGTGTTGAATGGTATCTTATAT
717 GTTTTAATATGTCTTATTAGTCTCATTACTTAGGCGAACGACAAAGTGAGGTACCTCGGTCTAATTCTCCTATG
718 TAGTGCGAGAAAAA
719
720 >TEVΔIb::crtB (insert between positions 6981-6982 of TEVΔIb)
721 GGGGAGAAGATGAATAATCCGTCGTTACTCAATCATGCGGTGCAACGATGGCAGTTGGCTCGAAAAGTTTGC
722 ACAGCCTCAAAGTTATTTGATGCAAAAACCCGGCGCAGCGTACTGATGCTCTACGCCTGGTGCCGCCATTGTGAC
723 GATGTTATTGACGATCAGACGCTGGGCTTTTCAGGCCCCGCGCAGCCTGCCTTACAAACGCCCCGAACAACGTC
724 CAACCTTGAGATGAAAACGCGCCAGGCCTATGCAGGATCGCAGATGCACGAACCGGCGTTTTCAGGAA
725 GTGGCTATGGCTCATGATATCGCCCCGGCTTACGCGTTTATCATCTGGAAGGCTTCGCCATGGATGTACGCGAA
726 GCGCAATACAGCCAACCTGGATGATACGCTGCGCTATTGCTATCACGTTGCAGGCGTTGTGCGCTTGATGATGGCG
727 CAAATCATGGGCGTGCGGGATAACGCCACGCTGGACCGCGCTGTGACCTTGGGCTGGCATTTTCAAGTTGACCAAT
728 ATTGCTCGCGATATTGTGGACGATGCGCATGCGGGCCGCTGTTATCTGCCGGAAGCTGGCTGGAGCATGAAGGT
729 CTGAACAAAGAGAATTATGCGGCACCTGAAAACCGCTCAGGCGCTGAGCCGTATCGCCCGTCGTTTGGTGCAGGAA
730 GCAGAACCTTACTATTCTGCTGCCAGCGCGCTGGCAGGGTTGCCCTGCGTTCCGCTGGGCAATCGCTACG
731 GCGAAGCAGGTTTACCAGGAAATAGGTGACAGCTTGAACAGGCGGTCAGCAAGCCTGGGATCAGCGGCAGTCA
732 ACGACCAGCGCCGAAAAATTAACGCTGCTGTGCGCGCTCTGGTGCAGGCCCTTACTTCCCGGATGCGGGCTCAT
733 CCTCCCCGCCCTGCGCATCTCTGGCAGCGCCCGCTCACGACTGAGAATCTTTATTTTCAAG
734
735 >TEVΔIb::LbCas12a (insert between positions 6981-6982 of TEVΔIb)
736 GATGAGCAAGCTGGAGAAGTTTACAACTGCTACTCCCTGTCTAAGACCCTGAGGTTCAAGGCCATC
737 CCTGTGGGCAAGACCCAGGAGAATCGACAATAAGCGGCTGCTGGTGGAGGACGAGAAGAGAGCCGAGGATTAT
738 AAGGGCGTGAAGAAGCTGCTGGATCGCTACTATCTGTCTTTTATCAACGACGTGCTGCACAGCATCAAGCTGAAG
739 AATCTGAACAATTACATCAGCCTGTTCCGGAAGAAAACGAGAACCGAGAAGGAGAATAAGGAGCTGGAGAACCTG
740 GAGATCAATCTGCGGAAGGAGATCGCCAAGGCCTTCAAGGGCAACGAGGGCTACAAGTCCCTGTTTAAAGAGGAT
741 ATCATCGAGACAATCTGCCAGAGTTCTTGGACGATAAGGACGAGATCGCCCTGGTGAACAGCTTCAATGGCTTT
742 ACCACAGCCTTACCAGGCTTCTTTGATAACAGAGAGAATATGTTTTCCGAGGAGGCCAAGAGCACATCCATCGCC
743 TTCAGGTGTATCAACGAGAATCTGACCCGCTACATCTCTAATATGGACATCTTCGAGAAGGTGGACGCCATCTTT
744 GATAAGCACGAGGTGCAGGAGATCAAGGAGAAGATCCTGAACAGCGACTATGATGTGGAGGATTTCTTTGAGGGC
745 GAGTTCTTTAACTTTGTGCTGACACAGGAGGGCATCGACGTGTATAACGCCATCATCGGCGGCTTCGTGACCGAG
746 AGCGGCGAGAAGATCAAGGGCCTGAACGAGTACATCAACCTGTATAATCAGAAAACCAAGCAGAAGCTGCCTAAG
747 TTTAAGCCACTGTATAAGCAGGTGCTGAGCGATCGGGAGTCTCTGAGCTTCTACGGCGAGGGCTATACATCCGAT
748 GAGGAGGTGCTGGAGGTGTTTAGAAACACCCTGAACAAGAACAGCGAGATCTTCACTCCATCAAGAAGCTGGAG

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749 AAGCTGTTCAAGAATTTTGCAGGACTCTAGCGCCGGCATCTTTGTGAAGAACGGCCCCGCCATCAGCACAAATC
750 TCCAAGGATATCTTCGGCGAGTGGAACGTGATCCGGGACAAGTGAATGCCGAGTATGACGATATCCACCTGAAG
751 AAGAAGGCCGTGGTGACCGAGAAGTACGAGGACGATCGGAGAAAGTCCCTTCAAGAAGATCGGCTCCTTTTCTCTG
752 GAGCAGCTGCAGGAGTACGCCGACGCCGATCTGTCTGTGGTGGAGAAGCTGAAGGAGATCATCATCCAGAAGGTG
753 GATGAGATCTACAAGGTGTATGGCTCCTCTGAGAAGCTGTTTCGACGCCGATTTTGTGCTGGAGAAGAGCCTGAAG
754 AAGAACGACGCCGTGGTGGCCATCATGAAGGACCTGCTGGATTCTGTGAAGAGCTTCGAGAATTACATCAAGGCC
755 TTCTTTGGCGAGGGCAAGGAGACAAACAGGGACGAGTCCTTCTATGGCGATTTTGTGCTGGCCTACGACATCCTG
756 CTGAAGGTGGACCACATCTACGATGCCATCCGCAATTATGTGACCCAGAAGCCCTACTCTAAGGATAAGTTCAAG
757 CTGTATTTTCAAGACCCTCAGTTTCATGGGCGGCTGGGACAAGGATAAGGAGACAGACTATCGGGCCACCATCCTG
758 AGATACGGCTCCAAGTACTATCTGGCCATCATGGATAAAGAAGTACGCCAAGTGCTGCAGAAGATCGACAAGGAC
759 GATGTGAACGGCAATTACGAGAAGATCAACTATAAGCTGCTGCCCGGCCCTAATAAGATGCTGCCAAAGGTGTTT
760 TTTTCTAAGAAGTGGATGGCCTACTATAACCCAGCGAGGACATCCAGAAGATCTACAAGAATGGCACATTCAAG
761 AAGGGCGATATGTTTAACCTGAATGACTGTCAAGCTGATCGACTTCTTTAAGGATAGCATCTCCCGGTATCCA
762 AAGTGGTCCAATGCCTACGATTTCAACTTTTCTGAGACAGAGAAGTATAAGGACATCGCCGGCTTTTACAGAGAG
763 GTGGAGGAGCAGGGCTATAAGGTGAGCTTCGAGTCTGCCAGCAAGAAGGAGGTGGATAAGCTGGTGGAGGAGGGC
764 AAGCTGTATATGTTCCAGATCTATAACAAGGACTTTTCCGATAAGTCTCACGGCACACCCAATCTGCACACCATG
765 TACTTCAAGCTGCTGTTTGCAGAGAACAATCACGGACAGATCAGGCTGAGCGGAGGAGCAGAGCTGTTTCATGAGG
766 CGCGCCTCCCTGAAGAAGGAGGAGCTGGTGGTGCACCCAGCCAACCTCCCTATCGCCAACAAGAATCCAGATAAT
767 CCCAAGAAAACCACAACCCTGTCTACGACGTGTATAAGGATAAGAGGTTTCTGAGGACCAGTACGAGCTGCAC
768 ATCCCAATCGCCATCAATAAGTGCCCCAAGAACATCTTCAAGATCAATACAGAGGTGCGCGTGCTGCTGAAGCAC
769 GACGATAACCCCTATGTGATCGGCATCGATAGGGGCGAGCGCAATCTGCTGTATATCGTGGTGGTGGACGGCAAG
770 GGCAACATCGTGGAGCAGTATTCCTTGAACGAGATCATCAACAACCTTCAACGGCATCAGGATCAAGACAGATTAC
771 CACTCTCTGCTGGACAAGAAGGAGAAGGAGAGGTTTCGAGGCCCCGAGAACTGGACCTCCATCGAGAATATCAAG
772 GAGCTGAAGGCCGGCTATATCTCTCAGGTGGTGCACAAGATCTGCGAGCTGGTGGAGAAGTACGATGCCGTGATC
773 GCCCTGGAGGACCTGAACCTCTGGCTTTAAGAATAGCCGCGTGAAGGTGGAGAAGCAGGTGTATCAGAAGTTCGAG
774 AAGATGCTGATCGATAAGTGAACCTGATGGTGGACAAGTCTAATCCTTGTGCAACAGCGCGCCCTGAAG
775 GGCTATCAGATCAACCAATAAGTTTCAGAGCTTTAAGTCCATGTCTACCCAGAAGCGCTTCATCTTTTACATCCCT
776 GCCTGGCTGACATCCAAGATCGATCCATCTACCGCTTTGTGAACCTGCTGAAAACCAAGTATACACGACATCGCC
777 GATTCCAAGAAGTTCATCAGCTCCTTTGACAGGATCATGTACGTGCCCCGAGGAGGATCTGTTTCGAGTTTGCCCTG
778 GACTATAAGAAGTCTCTCGCACAGACGCCGATTACATCAAGAAGTGAAGCTGTACTCTACGGCAACCGGATC
779 AGAATCTTCCGGAATCCTAAGAAGAACAACGTGTTTCGACTGGGAGGAGGTGTGCTGACCAGCGCCTATAAGGAG
780 CTGTTCAACAAGTACGGCATCAATTATCAGCAGGGCGATATCAGAGCCCTGCTGTGCGAGCAGTCCGACAAGGCC
781 TTCTACTCTAGCTTTATGGCCCTGATGAGCCTGATGCTGCAGATGCGGAACAGCATCACAGGCCGACCGACGTG
782 GATTTTCTGATCAGCCCTGTGAAGAAGTCCGACGGCATCTTCTACGATAGCCGGAAGTATGAGGCCAGGAGAAT
783 GCCATCCTGCCAAGAAGCGGACGCCAATGGCGCCTATAACATCGCCAGAAAGGTGCTGTGGGCCATCGGCCAG
784 TTCAAGAAGGCCGAGGACGAGAAGCTGGATAAGGTGAAGATCGCCATCTCTAACAAGGAGTGCTGGAGTACGCC
785 CAGACCAGCGTGAAGCACAAAAGGCCGGCCAGCAAAAAGGCCGGCCAGGCAAAAAGAAAAGGGATCCTTAC
786 CCATACGATGTTCCAGATTACGCTTATCCCTACGACGTGCCTGATTATGCATACCCATATGATGTCCCCGACTAT
787 GCCACGACTGAGAATCTTTATTTTCAG

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Figure S2. Full sequence of wild-type potato virus X (PVX; GenBank accession number MT799816) and its derived recombinant viruses PVX::crFT and PVX::Nib:crFT. In recombinant PVX clones, heterologous sequences are transcribed from coat protein (CP) promoter and a deleted version of PVX CP, lacking the 29 initial codons, is transcribed from an heterologous promoter derived from *Bamboo mosaic virus* (BaMV) CP (Dickmeis et al., 2014). **PVX CP promoter** with a ATG-AGG mutation to abolish start codon is underlined. **BaMV CP promoter** is in blue. crRNA sequences corresponding to **NbFT** and **NbXT1** are on pink and red background, respectively, and **protospacer** region is underlined (dotted). cDNA corresponding to **TEV nuclear inclusion b (Nib) cistron** is on dark blue background.

>PVX (MT799816)

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800 GAAACTAAACCATAACACCACCAACACAACCAACCCACCACGCCCAATTGTTACACACCCGCTTGAAAAAGAAA
801 GTTTAACAATGGCCAAGGTGCGCGAGGTTTACCAATCTTTTACAGACTCCACCACAAAACCTCTCATCCAAGAT
802 GAGGCTTATAGAAACATTCGCCCATCATGGAAAAACACAACTAGCTAACCCCTACGCTCAAACGGTTGAAGCG
803 GCTAATGATCTAGAGGGGTTTCGGCATAGCCACCAATCCCTATAGCATTGAATTGCATACACATGCAGCCGCTAAG
804 ACCATAGAGAATAAACTTCTAGAGGTGCTTGGTTCCATCCTACCACAAGAACCTGTTACATTTATGTTTCTTAAA
805 CCCAGAAAGCTAACTACATGAGAAGAAACCCGCGGATCAAGGACATTTTCCAAAATGTTGCCATTGAACCAAGA
806 GACGTAGCCAGGTACCCCAAGGAAACAATAATTGACAACTCACAGAGATCACAACGGAAACAGCATACATTAGT
807 GACACTCTGCACTTCTTGATCCGAGCTACATAGTGGAGACATTCCAAAACCTGCCCAAAATTGCAACATTTGTAT

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808 GCGACCTTAGTTCTCCCCGTTGAGGCAGCCTTTAAAATGGAAAGCACTCACCCGAACATATACAGCCTCAAATAC
809 TTCGGAGATGGTTTTCCAGTATATACCAGGCAACCATGGTGGCGGGGCATACCATCATGAATTCGCTCATCTACAA
810 TGGCTCAAAGTGGGAAAGATCAAGTGGAGGGACCCCAAGGATAGCTTTCTCGGACATCTCAATTACACGACTGAG
811 CAGGTTGAGATGCACACAGTGACAGTACAGTTGCAGGAATCGTTTCGCGGCAAACCACTTGTACTGCATCAGGAGA
812 GGAGACTTGCTCACACCGGAGGTGCGCACTTTTCGGCCAACCTGACAGGTACGTGATTCCACCACAGATCTTCCTC
813 CCAAAGTTTCAAACTGCAAGAAGCCGATTCTCAAGAAAATATGATGCAGCTCTTCTGTATGTTAGGACAGTC
814 AAGGTCGCAAAAAATTGTGACATTTTTGCCAAAGTCAGACAATTAATTAATCATCTGACTTGGACAAATACTCT
815 GCTGTGGAACCTGGTTTACTTAGTAAGCTACATGGAGTTCCTTGCCGATTTACAAGCTACCACCTGCTTCTCAGAC
816 ACACCTTCTGGTGGCTTGCTAACAAGACCCCTTGCACCGGTGAGGGCTTGGATACAAGAGAAAAAGATGCAGCTG
817 TTTGGTCTTGAGGACTACGCGAAGTTAGTCAAAGCAGTTGATTTCCACCCGGTGGATTTTCTTTCAAAGTGGAA
818 ACTTGGGACTTCAGATTCCACCCCTTGCAAGCGTGGAAGCCTTCCGACCAAGGGAAGTGTGCGGATGTAGAGGAA
819 ATGGAAGTTTGTCTCAGATGGGGACTTGCTTGATTGCTTCACAAGAATGCCAGCTTATGCGGTAAACGCGAGAG
820 GAAGATTTAGCTGCAATCAGGAAAACGCCCAGATGGATGTGCGGTCAAGAAGTTAAAGAACCTGCAGGAGACAGA
821 AATCAATACTCAAACCTGCAGAACTTTCTCAACAAGCTCCACAGGAAACACAGTAGGGAGGTGAAAACACCAG
822 GCCGCAAAGAAAGCTAAACGCCTAGCTGAAATCCAGGAGTCAATGAGAGCTGAAGGTGATGCCGAACCAATGAA
823 ATAAGCGGGGGCATGGGGGCAATACCCAGCAACGCCGAACCTTCTGGCACGAATGATGCCAGACAAGAACTCACA
824 CTCCCAACCACTAAACCTGTCCCTGCAAGGTGGGAAGATGCTTCATTACAGATTCTAGTGTGGAAGAGGAGCAG
825 GTTAAACCCCTTGGAAAAGAAACCGTTGAAACAGCGACGCAACAAGTCATCGAAGGACTTCTTGGAAACACTGG
826 ATTCCTCAATTAATGCTGTTGGATTCAAGGCGCTGGAAATTCAGAGGGATAGGAGTGGAAACAATGATCATGCC
827 ATCAGAGAAATGGTCTCCGGGCTGGAAAAGAGGACTTCCCTGAAGGAACCTCCAAAAGAGTTGGCAGGAGAAATG
828 TTCGCTATGAACAGAAGCCCTGCCACCATCCCTTTGGACCTGCTTAGAGCCAGAGACTACGGCAGTGATGTAAG
829 AACAGAGAATTGGTGCCATCACAAGACACAGGCAACGAGTTGGGGCGAATACTTGACAGGAAAGATAGAAAGC
830 TTGACTGAGAGGAAAGTTGCGACTTGTGTCAATTCATGGAGCTGGAGGTCTTGGAAAAAGTCATGCCATCCAGAAG
831 GCATTGAGAGAAATTGGCAAGGGCTCGGACATCACTGTAGTCTTCCGACCAATGAAGTGCAGGCTAGATTGGAGT
832 AAGAAAGTGCCTAACACTGAGCCCTATATGTTCAAGACCTATGAAAAGGCGTTAATTGGGGGAACAGGCAGCATA
833 GTCATCTTTGACGATTACTCAAACCTTCTCCCGGTTACATAGAAAGCCTTAGTCTGTTTCTACTCTAAATCAAG
834 CTAATCTATTCAACAGGAGATAGCAGACAAGCGCTTACCATGAAACTGCTGAGGACGCGCTCCATCAGGCACTTG
835 GGACAGCAACAGAGTACTTCTCAAAATACTGCCGATACTATCTCAATGCCACACACCCGAACAAAGAGATCTT
836 GCGAACATGCTTGGTGTCTACAGTGAGAGAACGGGAGTCACCGAAATCAGCATGAGCGCCGAGTTCTTAGAAGGA
837 ATCCCAACTTTGGTACCCTCGGATGAGAAGAGAAAGCTGTACATGGGCACCGGGAGGAATGACACGTTTACATAC
838 GCTGGATGCCAGGGGCTAACTAAGCCGAAGGTACAAATAGTGTGGACCACAACACCCCAAGTGTGTAGCGGAAT
839 GTGATGTACACGGCACTTTCTAGAGCCACCGATAGGATTCACTTCGTGAACACAAGTGCAAAATCTCTCGGCCTT
840 TGGGAAAAGTTGGACAGCACCCCTTACCTCAAGACTTTCTATCAGTGGTGAGAGAAACAAGCACTCAGGGAGTAC
841 GAGCCGGCAGAGGCAGAGCCAATTCAAGAGCCTGAGCCCCAGACACACATGTGTGTCGAGAATGAGGAGTCCGTG
842 CTAGAAGAGTACAAAGAGGAACCTTTGGAAAAGTTTGACAGAGAGATCCACTCTGAATCCCATGGTCAATCAAC
843 TGTGTCCAACTGAAGACACAACCATTCAAGTTGTTTTTCGCATCAACAAGCAAAAGATGAGACCCCTCTCTGGGCG
844 ACTATAGATGCGCGGCTCAAGACCAGCAATCAAGAGACAACTTCCGAGAATTCCTGAGCAAGAAGGACATTGGG
845 GACGTTCTGTTTTTAACTACCAAAAAGCTATGGGTTTACCCAAAGAGCGTATTCCTTTTTTCCCAAGAGGTCTGG
846 GAAGCTTGTGCCCACGAAGTACAAAGCAAGTACCTCAGCAAGTCAAAGTGCAACTTGATCAATGGGACTGTGAGA
847 CAGAGCCCAGACTTCGATGAAAATAAGATTATGGTATTCCTCAAGTCGAGTGGGTACAAAGGTGGAAAAACTA
848 GGTCTACCCAAGATTAAGCCAGGTCAAACCATAGCAGCCTTTTACCAGCAGACTGTGATGCTTTTTTGGAACTATG
849 GCTAGGTACATGCGATGGTTTCAAGCAGGCTTTCCAGCCAAAAGAAGTCTTCATAAACTGTGAGACCACGCCAGAT
850 GACATGTCTGCATGGGCTTGAACAACCTGGAATTTTCAAGCAGACCTAGCTTGGCTAATGACTACACAGCTTTCGAC
851 CAGTCTCAGGATGGAGCCATTGTTGCAATTTGAGGTCTTCAAGCCAAACACCAGTGCATACCAAGGAAATCATT
852 CAGGCATACATAGATATTAAGACTAATGCAGATTTTCTAGGACGTTATCAATTATGCGCCTGACTGGTGAA
853 GGTCCCCTTTTGTATGCAAAACACTGAGTGCAACATAGCTTACACCCATACAAAGTTTGACATCCCAGCCGGAAC
854 GCTCAAGTTTATGCAGGAGACGACTCCGCACTGGACTGTGTTCCAGAAGTGAAGCATAGTTTCCACAGGCTTGAG
855 GACAAATTACTCCTAAAGTCAAAGCCTGTAATCACGCAGCAAAAAGAAGGGCAGTTGGCCTGAGTTTTGTGGTTGG
856 CTGATCACACCAAAAGGGGTGATGAAAGACCAATTAAGCTCCATGTAGCTTAAAATTTGGCTGAAGCTAAGGGT
857 GAACTCAAGAAATGTCAAGATTCTATGAAATTGATCTGAGTTATGCCTATGACCACAAGGACTCTCTGCATGAC
858 TTGTTTCGATGAGAAACAGTGTGAGGCACACACACTCACTTGCAAGCACTAATCAAGTCAGGGAGAGGCAGTGT
859 TCACTTTCCCGCCTCAGAACTTTCTTTAACCGTTAAGTTACCTTAGAGATTTGAATAAGATGGATATTCTCATC
860 AGTAGTTTGAAGTTTGGTTATTCTAGGACTTCCAAATCTTTAGATTACAGGACCTTTGGTAGTACATGCAGTA
861 GCCGGAGCCGGTAAGTCCACAGCCCTAAGGAAGTTGATCCTCAGACACCCAAACATTACCGTGCATACACTCGGT
862 GTCCCTGACAAGGTGAGTATCAGAACTAGAGGCATACAGAAGCCAGGACCTATTCTGAGGGCAACTTCGCAATC
863 CTCGATGAGTATACTTTGGACAACACCACAAGGAACCTACATACCAGGCACTTTTTGCTGACCCCTATCAGGCACCG
864 GAGTTTAGCCTAGAGCCCCACTTCTACTTGGAACATCATTTTCGAGTTCGAGGAAAGTGGCAGATTTGATAGCT
865 GGCTGTGGCTTCGATTTTCGAGACCAACTCACCGGAAGAAGGGCACTTAGAGATCACTGGCATATTCAAAGGGCCC
866 CTACTCGGAAAGGTGATAGCCATTGATGAGGAGTCTGAGACAACACTGTCCAGGCATGGTGTGAGTTTGTAAAG
867 CCCTGCCAAGTGACGGGACTTGAGTTCAAAGTAGTCACTATTGTGTCTGCCGCACCAATAGAGGAAATTGCCAG
868 TCCACAGCTTTCTACAACGCTATCACCAGGTCAAAGGGATTGACATATGTCCGCGCAGGGCCATAGGCTGACCGC

869 TCCGGTCAATTCTGAAAAAGTGACATAGTATTAGGTCTATCATTTTGCTTTAGTTTCAATTACCTTTCTGCTTTC
870 TAGAAATAGCTTACCCACGTCGGTGACAACATTACAGCTTGCCACACGGAGGAGCTTACAGAGACGGCACC
871 AGCAATCTTGTACAACCTCCCAATCTAGGGTCACGAGTGAGTCTACACAACGGAAAGAACGCAGCATTTGCTGC
872 CGTTTTGCTACTGACTTTGCTGATCTATGGAAGTAAATACATATCTCAACGCAATCATACTTGTGCTTGTGGTAA
873 CAATCATAGCAGTCATTAGCACTTCCTTAGTGAGGACTGAACCTTGTGTCATCAAGATTACTGGGGAATCAATCA
874 CAGTGTGGCTTGCAAACCTAGATGCAGAAACCATAGGGCCATTGCCGATCTCAAGCCACTCTCCGTTGAACGGT
875 TAAGTTTCCATTGATACTCGAAAGATGTCAGCACCAGCTAGCACAACACAGCCCATAGGGTCAACTACCTCAACT
876 ACCACAAAACTGCAGGCGCAACTCCTGCCACAGCTCAGGCCTGTTCACTATCCCGGATGGGGATTCTTTAGT
877 ACAGCCCGTGCCATAGTACCCAGCAATGCTGTGCAACAAATAGGACCTCAGCAAGATTGAGGCTATTTGGAAG
878 GACATGAAGGTGCCACAGACACTATGGCAGGCTGCTGGGACTTAGTCAGACACTGTGCTGATGTAGGATCA
879 TCCGCTCAAACAGAAATGATAGATACAGGTCCCTATTCCAACGGCATCAGCAGAGCTAGACTGGCAGCAGCAATT
880 AAAGAGGTGTGCACACTTAGGCAATTTTGCATGAAGTATGCCCCAGTGGTATGGAACCTGGATGTTAACTAACAAC
881 AGTCCACCTGCTAACTGGCAAGCACAAGGTTTCAAGCCTGAGCACAATTCGCTGCATTGCACTTCTTCAATGGA
882 GTCACCAACCCAGCTGCCATCATGCCAAAGAGGGGCTCATCCGGCCACCGTCTGAAGCTGAAATGAATGCTGCC
883 CAACTGCTGCCTTTGTGAAGATTACAAAGGCCAGGGCACAATCCAACGACTTTGCCAGCCTAGATGCAGCTGTC
884 ACTCGAGGTGCTATCACTGGAACAACAACCGCTGAGGCTGTTGTCACTCTACCACCACCATAACTACGTCTACAT
885 AACCAGCGCTACCCAGTTTCATAGTATTTTCTGGTTTGATTGTATGAATAATATAAATAAAAAAAAAAAAAAA
886 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
887
888 >PVX::crFT (insert between A5585 and T5737 of PVX-wt)
889 AGGGCCATTGCCGATCTCAAGCCACTCTCCGTTGAACGGTTAAGTTTCCATTGATACTCGAAAGAGGTCAGCACC
890 AGCTAGCATAAATTTCTACTAAGTGTAGATCAAGATCTATTGGCCTAAGAGTTAATTTCTACTAAGTGTAGATTA
891 GGGTTTGTAAAGTTTCCCTTTTTACTCGAAAGATG
892
893 >PVX::crXT1:crTFL1:crFT (insert between A5585 and T5737 of PVX-wt)
894 AGGGCCATTGCCGATCTCAAGCCACTCTCCGTTGAACGGTTAAGTTTCCATTGATACTCGAAAGAGGTCAGCACC
895 AGCTAGCATAAATTTCTACTAAGTGTAGATTATGTAGGTGTTTGGAAATTTCTTAATTTCTACTAAGTGTAGATCA
896 AGATCTATTGGCCTAAGAGTTTAATTTCTACTAAGTGTAGATTAGGGTTTGTAAAGTTTCCCTTTTTACTCGAA
897 GATG
898
899 >PVX::Nib:crFT (insert between A5585 and T5737 of PVX-wt)
900 AGGGCCATTGCCGATCTCAAGCCACTCTCCGTTGAACGGTTAAGTTTCCATTGATACTCGAAAGAGGTCAGCACC
901 AGCTAGCATATGGGGGAGAAGAGGAAATGGGTGCTGGAAGCACTGTGAGGAACTTGAGGCCAGTGGCTGAGTGTG
902 CCAGTCAGTTAGTCACAAAGCATGTGGTTAAAGGAAAGTGTCCTCTTTGAGCTCTACTTGCAGTTGAATCCAG
903 AAAAGGAAGCATATTTTAAACCGATGATGGGAGCATATAAGCCAAGTCGACTTAATAGAGAGGCGTTCCTCAAG
904 ACATTCTAAATATGCTAGTGAAATTGAGATTGGGAATGTGGATTGTGACTTGCTGGAGCTTGCAATAAGCATGC
905 TCATCACAAGCTCAAGGCGTTAGGATTCCCAACTGTGAACTACATCACTGACCCAGAGGAAATTTTGTAGTGCAT
906 TGAATATGAAAGCAGCTATGGGAGCACTATACAAAGGCAAGAAGAAAGCTCTCAGCGAGCTCACACTAGATG
907 AGCAGGAGGCAATGCTCAAAGCAAGTTGCCTGCGACTGTATACGGGAAAGCTGGGAATTTGGAATGGCTCATTGA
908 AAGCAGAGTTGCGTCCAATTGAGAAGGTTGAAAACAACAAACGCGAACTTTACAGCAGCACCACATAGACACTC
909 TTCTTGCTGGTAAAGTTTGCCTGGATGATTTCAACAATCAATTTTATGATCTCAACATAAAGGCACCATGGACAG
910 TTGGTATGACTAAGTTTATCAGGGGTGGAATGCAATTGATGGAGGCTTTACCAAGTGGGTGGTGTATTGTGACG
911 CTGATGGTTGCAATTCGACAGTTCTTGACTCAATTCCTCATTAATGCTGTATTGAAAGTGCAGCTTGCCCTTCA
912 TGGAGGAATGGGATATTGGTGAGCAATGCTGCGAAATTTGTACACTGAGATAGTGATACACCAATCCTCACAC
913 CGGATGGTACTATCATTAAGAAGCATAAAGGCAACAATAGCGGGCAACCTTCAACAGTGGTGGACAACACACTCA
914 TGGTCATTATTGCAATGTTATACACATGTGAGAAGTGTGGAATCAACAAGGAAGAGATTGTGTATTACGTCAATG
915 GCGATGACCTATTGATTGCCATTACCCAGATAAAGCTGAGAGGTTGAGTGGATTCAAAGAATCTTTCGGAGAGT
916 TGGGCCCTGAAATATGAATTTGACTGCACCACCAGGGACAAGACACAGTTGTGGTTTCATGTACACAGGGCTTTGG
917 AGAGGGATGGCATGTATATACCAAAGCTAGAAGAAGAAAGGATTGTTTCTATTTTGAATGGGACAGATCCAAAG
918 AGCCGTCACATAGGCTTGAAGCCATCTGTGCATCAATGATCGAAGCATGGGGTTATGACAAGCTGGTTGAAGAAA
919 TCCGCAATTTCTATGCATGGGTTTTGGAACAAGCGCGTATTACAGCTTGCAGAAGAAGAAAGCGCCATATC
920 TGGCTGAGACTGCGCTTAAGTTTTGTACACATCTCAGCAGGAACAACTCTGAGATAGAAGATATTTAAAG
921 TGTTGTATGATTACGATATTCCAACGACTGAGAATCTTTATTTTCAGTAATAATTTCTACTAAGTGTAGATCAAG
922 ATCTATTGGCCTAAGAGTTTAATTTCTACTAAGTGTAGATTAGGGTTTGTAAAGTTTCCCTTTTTACTCGAAAG
923 TG
924

925 **Figure S3.** 35S::Nib *N. benthamiana* plants and representative leaves from these plants at 14
926 dpi inoculated with TEVΔNib::LbCas12a (left) or co-inoculated with TEVΔNib::LbCas12a
927 and PVX::crFT (right). Chlorotic spots in leaves from co-inoculated plants are indicated with
928 red arrows.

TEVΔNlb::LbCas12a

TEVΔNlb::LbCas12a + PVX::crFT

Figure S4. ICE analysis results of target site mutations in 35S::Nlb *N. benthamiana* plants co-inoculated with TEVΔNlb::LbCas12a and PVX::crFT. Examples of sequencing chromatograms (top) and mutagenesis profiles (bottom) are shown.

MU237:

MU371:

Figure S5. Representative leaves from wild-type *N. benthamiana* plants at 14 dpi inoculated with TEVΔNlb::LbCas12a alone (left), PVX::Nlb:crFT alone (middle), or coinoculated with TEVΔNlb::LbCas12a and PVX::Nlb:crFT (right).

Table S1. *N. benthamiana* genes targeted by the CRISPR-Cas12a system. Note that PAM sequence recognised by LbCas12a nuclease is highlighted in grey.

Target gene	SolGenomics Accession No	Target site
<i>NbFT</i>	Niben101Scf01519g10008.1	TTTA CAAGATCTATTGGCCTAAGAGTT
<i>NbXT1</i>	Niben101Scf04205g03008.1	TTTA TATGTAGGTGTATTGGAATTCT

Table S2. Primers used for the construction of recombinant viruses.

Construct element	Primer*	Primer sequence (5'→3')	Purpose
LbCas12a	D2521 (F)	attggtgtactcgcaaggggagaagATG AGCAAGCTGGAGAAG	Amplification of <i>LbCas12a</i> for TEVΔNlb::LbCas12a
	D2508 (R)	tctgaaaataaagattctcagtcgtGGCA TAGTCGGGGACATC	Amplification of <i>LbCas12a</i> for TEVΔNlb::LbCas12a
crFT	D3591 (F)	gaggtcagcaccagctagcaTAATTT CTACTAAGTGTAGATCAAG ATCTATTGGCCTAAG	Amplification of crFT for PVX::crFT
	D3592 (R)	gggaaacttaacaaaccctaATCTACA CTTAGTAGAAATTAACTC TTAGGCCAATAGAT	Amplification of crFT for PVX::crFT

	D3712 (F)	tttcagtaaTAATTTCTACTAAG TG TAGATCAAGATCTATTG GCCTAAG	Amplification of crFT for PVX::Nlb:crFT
	D3713 (R)	gggaaacttaacaaaccctaATCTACA CTTAGTAGAAATTAACTC TTAGGCCAATAGAT	Amplification of crFT for PVX::Nlb:crFT
pBPVX:: crFT	D4036 (F)	TAATTTCTACTAAGTGTAG ATCAAGATCTATTGG	Amplification of pBPVX::crFT for PVX::crXT1:crTFL1:crFT
	D3536 (R)	TGCTAGCTGGTGCTGACC	Amplification of pBPVX::crFT for PVX::crXT1:crTFL1:crFT
crXT1	D4037 (F)	gaggtcagcaccagctagcaTAATTT CTACTAAGTGTAGATTATG TAGGTGTATTGG	Amplification of crXT1 for PVX::crXT1:crFT
	D4038 (R)	ATCTACACTTAGTAGAAAT TAAGAATTCCAAATACACC	Amplification of crTFL1 3.1/14.1 for PVX::crXT1:crFT
Nlb	D3710 (F)	gaggtcagcaccagctagcaTGGGGG AGAAGAGGAAATG	Amplification of TEV Nlb cistron for PVX::Nlb:crFT
	D3711 (R)	gtagaaattaTTACTGAAAATAA AGATTCTCAGTCG	Amplification of TEV Nlb cistron for PVX::Nlb:crFT

*F, forward primer; R, reverse primer

Table S3. Primer combinations used for the construction of recombinant viruses.

Construct	Primer names
TEVΔNlb::LbCas12a	D2521 + D2508 (template: pcDNA3.1::hLbCpf1)
PVX::crFT	D3591 + D3592 (no template)
PVX::crXT1:crFT	D4037 + D4038 D4036 + D3536 (template: pBPVX::crFT)
PVX::Nlb:crFT	D3710 + D3711 (template: pGTEVa) D3712 + D3713

Table S4. Primers used for TEVΔNlb diagnosis by RT-PCR.

Primer*	Primer sequence (5'→3')	Purpose
D179 (R)	CTCGCACTACATAGGAGAATTAGAC	Reverse transcription of TEVΔNlb mRNA into cDNA
D178 (F)	AGTGGCACTGTGGGTGCTGGTGTTG	Analysis of TEVΔNlb infection
D211 (R)	GGCGCGGCGTCGACCTGGCGGACCCC TAATAG	Analysis of TEVΔNlb infection
D3604 (F)	ATAAGGAGACAGACTATCGGGC	Expression of <i>LbCas12a</i> nuclease in TEVΔN progeny
D3605 (R)	TGATCTGTCCGTGATTGTTCTC	Expression of <i>LbCas12a</i> nuclease in TEVΔN progeny

*F, forward primer; R, reverse primer, S, sequencing primer

Table S5. Primers used for Cas12a-crRNA gene editing analysis.

Gene	Primer name	Primer sequence (5'→3')	Purpose
<i>NbFT</i>	D3667 (F)	CTAGAAAACCTATGGCTATAAGGG	Amplification of a 550-nt DNA fragment flanking <i>NbFT</i> target region
	D3668 (R)	GTTCTCGAGAGGTATAATATAGGC	Amplification of a 550-nt DNA fragment flanking <i>NbFT</i> target region
	D3669	CACAAGCACGCATAGAAC	Sequencing of the 550-nt PCR product for the analysis of <i>NbFT</i> editing
<i>NbXT1</i>	JO16 JUN05 (F)	AACCACTTTTCCTCGTCGGAAA	Amplification of a 1286-nt DNA fragment flanking <i>NbXT1</i> target region
	JO16 JUN06 (R)	TAAC TAT TCA ACT AAAGCTTCAAACAG	Amplification of a 1286-nt DNA fragment flanking <i>NbXT1</i> target region
	JO16 NOV05	TGTTTAATGAAGATTGTCTGG	Sequencing of the 1286-nt PCR product for the analysis of <i>NbXT1</i> editing

*F, forward primer; R, reverse primer