

SUPPORTING INFORMATION

Efficient production of antifungal proteins in plants using a new transient expression vector derived from tobacco mosaic virus

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Running title: A new viral system for efficient AFP production

Keywords: antifungal proteins, viral vector, tobacco mosaic virus, plant biofactory, *Nicotiana benthamiana*, Gibson assembly.

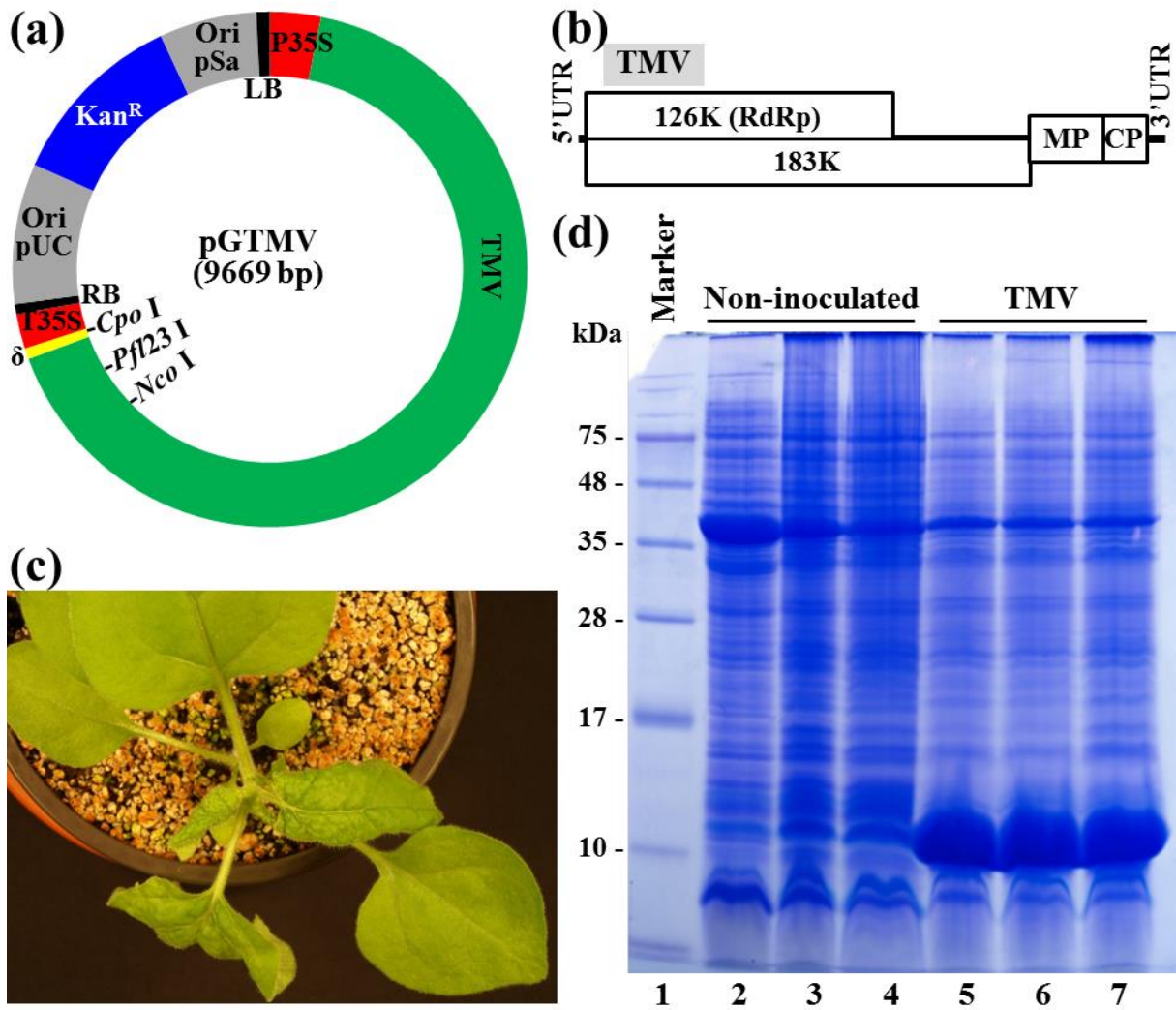


Figure S1. *Tobacco mosaic virus* (TMV) infectious clone. (a) Schematic representation of plasmid pGTMV that contains the whole TMV cDNA. See more details in the legend to Figure 1. (b) Schematic representation of wild-type TMV genome. Boxes represent the 126K and 183K replication proteins, movement protein (MP) and coat protein (CP). Lines represent the 5' and 3' untranslated regions (UTRs). (c) *Nicotiana benthamiana* plant infected with wild-type TMV. Picture was taken 5 days post-inoculation (dpi). (d) Coomassie blue-stained polyacrylamide gel showing the protein content of *N. benthamiana* leaves from plants mock-inoculated (lanes 2 to 4) or agroinoculated with TMV (lanes 5 to 7). Lane 1, marker proteins with sizes in kDa indicated on the left.

Figure S2. Full sequences of plasmid pGTMV, which contains a full-length TMV infectious clone (GenBank accession number MK087763), and intermediate plasmids pMTMVi-N and pMTMVi-M, which can be used to construct recombinant TMV clones unable and able to move systemically throughout the plant, respectively.

>pGTMV (9669 bp)

GCGGCCGCGATTCCATTGCCAGCTATCTGTCACTTTATTGTGAAGATAGTGAAAAAGGAAGGTGGCTCCTACAA
 ATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCC
 ACCCAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGCTTCAAAGCAAGTGGATTGATGTGATATCTC
 CACTGACGTAAGGGATGACGCACAATCCCACCTATCCTTCGCAAGACCCCTCCTCTATATAAGGAAGTTCATTTCA
 TTTGGAGAGGTATTTTTTACAACAATTACCAACAACAACAACAACAACAACAACAATTACAATTAATAAATTA
 CAATGGCATAACACAGACAGCTACCACATCAGCTTTGCTGGACACTGTCCGAGGAAAACAACCTCTGGTCAATG
 ATCTAGCAAAGCGTCGTCTTTACGACACAGCGGTTGAAGAGTTTAAACGCTCGTGACCGCAGGCCAAAGGTGAACT
 TTTCAAAGTAATAAGCGAGGAGCAGACGCTTATTGCTACCCGGCGTATCCAGAATTCCAAATTACATTTTATA
 ACACGCAAAATGCCGTGCATTGCTTGCAGGTGGATTGCGATCTTTAGAACTGGAATATCTGATGATGCAAAATC
 CCTACGGATCATTGACTTATGACATAGGCGGGAATTTTGCATCGCATCTGTTCAAGGGACGAGCATATGTACACT
 GCTGCATGCCAACCTGGACGTTTCGAGACATCATGCGGCACGAAGGCCAGAAAGACAGTATTGAACTATACCTTT
 CTAGGCTAGAGAGAGGGGGGAAAACAGTCCCCAACTTCCAAAAGGAAGCATTGACAGATACGCAGAAATTCCTG
 AAGACGCTGTCTGTACAATACTTTCCAGACATGCGAACATCAGCCGATGCAGCAATCAGGCAGAGTGTATGCCA
 TTGCGCTACACAGCATATATGACATAACAGCCGATGAGTTCCGGGGCGGCACCTCTTGAGGAAAAATGTCATACGT
 GCTATGCCGCTTTCCACTTCTCCGAGAACCTGCTTCTTGAAGATTCATGCGTCAATTTGGACGAAAATCAACGCGT
 GTTTTTCGCGCGATGGAGACAAGTTGACCTTTTCTTTTGCATCAGAGAGTACTCTTAATTAATGTCATAGTTATT
 CTAATATTCTTAAGTATGTGTGCAAACTTACTTCCCGCCTCTAATAGAGAGGTTTACATGAAGGAGTTTTTAG
 TCACCAGAGTTAATACCTGGTTTTGTAAAGTTTTCTAGAATAGATACTTTTTCTTTTGTACAAAGGTGTGGCCATA
 AAAGTGTAGATAGTGAGCAGTTTTATACTGCAATGGAAGACGCATGGCATTACAAAAGACTCTTGCAATGTGCA
 ACAGCAGAGAGAATCCTCCTTGGAGATTCATCAGTCAGTCAATTAATGTTTTCCAAAATGAGGGATATGGTCAATG
 TACCATTATTCGACATTTCTTTGGAGACTAGTAAGAGGACGCGCAAGGAAGTCTTAGTGTCCAAGGATTCGTGT
 TTACAGTGCTTAACCACATTGCAACATAACAGGCGAAAGCTCTTACATACGCAAAATGTTTTGTCCTTCGTGCAAT
 CGATTGATCGAGGGTAATCATTAAACGGTGTGACAGCGAGGTCCGAATGGGATGTGGACAAAATCTTTGTTACAAT
 CCTTGTCCATGACGTTTTTACCTGCATACTAAGCTTGCCGTTCTAAAGGATGACTTACTGATTAGCAAGTTTAGTC
 TCGGTTCGAAAACGGTGTGCCAGCATGTGTGGGATGAGATTTGCTGGCGTTTGGGAACGCATTTCCCTCCGTGA
 AAGAGAGGCTCTTGAACAGGAACTTATCAGAGTGGCAGGCGACGCATTAGAGATCAGGGTGCCTGATCTATATG
 TGACCTTCCACGACAGATTAGTGACTGAGTACAAGGCCTCTGTGGACATGCCTGCGCTTGACATTAGGAAGAAGA
 TGAAGAAACGGAAGTGTGATGACAATGCACTTTTCAAGATTATCGGTGTTAAGGGAGTCTGACAAAATTCGATGTTG
 ATGTTTTTTCCAGATGTGCCAATCTTTGGAAGTTGACCCAATGACGGCAGCGAAGGTTATAGTCGCGGTTCATGA
 GCAATGAGAGCGGTCTGACTCTCACATTTGAACGACCTACTGAGGCGAATGTTGCGCTAGCTTTACAGGATCAAG
 AGAAGGCTTCAAGAGGTGCATTGGTAGTTACCTCAAGAGAAAGTTGAAGAACCCTCCATGAAGGGTTCGATGGCCA
 GAGGAGAGTTACAATTAGCTGGTCTTGTGAGATCATCCGGAGTGCCTTATTCTAAGAACCAGGAGATAGAGT
 CTTTAGAGCAGTTTTCATATGGCGACGGCAGATTGCTTAATTCGTAAGCAGATGAGCTCGATTGTGTACACGGGTC
 CGATTAAAGTTCAGCAAATGAAAACTTTATCGATAGCCTGGTAGCATCACTATCTGCTGCGGTGTGCAATCTCG
 TCAAGATCCTCAAAGATACAGCTGCTATTGACCTTGAACCCGTCAAAAGTTTGGAGTCTTGGATGTTGCATCTA
 GGAAGTGGTTAATCAAACCAACGGCCAAGAGTCATGCATGGGGTGTGTTGAAACCCACGCGAGGAAGTATCATG
 TGGCGCTTTTGGAAATGATGAGCAGGGTGTGGTGCATGCGATGATTGGAGAAGAGTAGCTGTTAGCTCTGAGT
 CTGTTGTTTTATTCCGACATGGCGAAACTCAGAACCTGCGCAGACTGCTTCGAAACGGAGAACCAGCATGTCAGTA
 CGCGAAAAGTTGTTCTTGTGGACGGAGTTCCGGGCTGTGGAAAAACCAAAGAAATCTTTCCAGGGTTAATTTTG
 ATGAAGATCTAATTTTTAGTACCTGGGAAGCAAGCCGCGGAAATGATCAGAAGACGTGCGAATTCCTCAGGGATTA
 TTGTGGCCACGAAGGACAACGTTAAAACCGTTGATTCTTTTCATGATGAATTTTTGGGAAAAACACACGCTGTGAGT
 TCAAGAGGTTATTTCATTGATGAAGGGTTGATGTTGCATACTGGTTGTGTTAATTTTTCTTGTGGCGATGTCATTGT
 GCGAAATTGCATATGTTTACGGAGACACACAGCAGATTCCATACATCAATAGAGTTTCCAGGATTCCTGATACCCCG
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 AACTTAGCTCGTACTTGTAGATATGTATAAGGTCGATGCAGGAACACAATAGCAATTACAGATTGACTCGGTGT
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ACGCACCCGAGTTGTCTGGCATCATTGATATTGAAAATACTGCATCTTTGGTTGTAGATAAGTTTTTTGATAGTT
ATTTGCTTAAAGAAAAAGAAAACCAAATAAAAAATGTTTCTTTGTTTCAGTAGAGAGTCTCTCAATAGATGGTTAG
AAAAGCAGGAACAGGTAACAATAGGCCAGCTCGCAGATTTTGGATTTTGTGGATTTGCCAGCAGTTGATCAGTACA
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TTGTGTACCATTCAAAAAAGATCAATGCAATATTCGGCCCCGTTGTTTAGTGAGCTTACTAGGCAATTAAGTGGACA
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ACAGTCATGTGCCGATGGATGTCTTGGAGCTGGATATATCAAAAATACGACAAAATCTCAGAATGAATCCACTGTG
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ATAAATTCGTCAGCCAGTTTAGTCTGACCATCTCATCTGTAACAACATTTGGCAACGCTACCTTTGCCATGTTTC
AGAAACAACCTCTGGCGCATCGGGCTTCCCATACAATCGGTAGATTGTGCGACCTGATTGCCCGACATTATCGCGA

GCCCATTATACCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGGCCCTTGAGCAAGACGTTTCCCGTTG
ATATGGCTCATAACACCCCTTGTATTACTGTTTATGTAAGCAGACAGTTTTATTGTTTCATGATGATATATTTTTA
TCTTGTGCAATGTAACATCAGAGATTTTTGAGACACAACGTGGCTTTGTTGAATAAATCGAACTTTTTGCTGAGTTG
AAGGATCAGATCACGCATCTTCCCACAAACGCAGACCGTTCCCGTGGCAAAGCAAAAAGTTCAAAAATCACCAACTGG
TCCACCTACAACAAAGCTCTCATCAACCGTGGCTCCCTCACTTTCTGGCTGGATGATGGGGCGATT**CAGGC**GATC
CCCATCCAACAGCCCGCGTTCGAGCGGGCTTTTTTATCCCCGGAAGCCTGTGGATAGAGGGTAGTTATCCACGTG
AAACCGCTAATGCCCGCAAAGCCTTGATTCACGGGGCTTTCCGGCCCGCTCCAAAACTATCCACGTGAAATCG
CTAATCAGGGTACGTGAAATCGCTAATCGGAGTACGTGAAATCGCTAATAAGGTCACGTGAAATCGCTAATCAAA
AAGGCAGTGAGAACGCTAATAGCCCTTTCAGATCAACAGCTTGCAAACACCCCTCGCTCCGGCAAGTAGTTACA
GCAAGTAGTATGTTCAATTAGCTTTTCAATTATGAATATATATATCAATTATTGGTCGCCCTTGGCTTGTGGACA
ATGCGCTACGCGCACCGGCTCCGCCCGTGGACAACCGCAAGCGGTTGCCACCGTCGAGCGCCTTTGCCACAAC
CCGGCGGCCGGCCGCAACAGATCGTTTTATAAATTTTTTTTTTTTGA AAAAGAAAAAGCCCGAAAGGCGGCAACCT
CTCGGGCTTCTGGATTTCCGATCCCGGAATTAGATCCGTTTAAACTACGTAAGATCGATCT**FGGCAGGATATA**
TGTGCTATAACGTTTCTGCGGCGGTCGAGATGGATCT**FGGCAGGATATAATTGTGGTATAAC**GTTCTT

TMV-MK087763 is in bold. **Cauliflower mosaic virus (CaMV) 35S promoter** is in red with the transcription **+1** nucleotide on yellow background. **Hepatitis delta virus (HDV)-derived ribozyme** is in red. **CaMV 35S terminator** is in fuchsia with the processing and polyadenylation site underlined. *E. coli* **pUC replication origin** is in blue on gray background. **Kanamycin** selection marker is in blue on dark gray background (complementary strand). *A. tumefaciens* **pSa replication origin** is in blue on gray background. *A. tumefaciens* T-DNA **RB** with **overdrive** (underlined) is in blue on yellow background and **double LB** is in blue on red background.

>pMTMVi-N (2376 bp)

AAGATTACAAACGTGAGAGACGGAGGGCCCATGGAACTTACAGAAGAAGTCGTTGATGAGTTCATGGAAGATGTC
CCTATGTCGATCAGGCTTGC**AAAGTTTCGATCTCGA**ACCGGAAAAAAGAGTGATGTCCGCAAAGGGAAAAATAGT
AGTAGTGATCGGTCAGTGCCGAACAAGAACTATAGAAATGTTAAGGATTTTGGAGGAATGAGTTTTAAAAAGAA
AATTTAATCGATGATGATT**CGGAGGCTACTGT**CGCCGAATCGGATTCGTTT**TAAATA**GATCTTACAGTATCACTA
CTCCATCTCAGTTCGTGT**CTTTGTCA**ACCGGT**GAAAGCGGGCAGT**GAGCGCAACGCAATTAATGTGAGTTAGCTCA
CTCATTAGGCAACCCAGGCTTTACACTTTATGCTCCCGCTCGTATGTTGTGTGGAATTTGAGCGGATAACAAT
TTCACACAGGAAACAGCTATGACCATGATTACGCCAAGCGCGCAATTAACCTCACTAAAGGGAACAAAAGCTGG
GTACCCAATTCGCCCTATAGTGAGTCGTATTACGCGCGCTCACTGGCCGTGTTTTACAACGTCGTGACTGGGAA
AACCTTGGCGTTACCCA**ACTTAATCGCCTTGC**AGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCC
CGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGGACCGC**CTCGAG**GTCTCGCAACT**TGAG**
GTAGTCAAGATGCATAATAAATAACGGATTGTGTCCGTAATCACACGTGGT**GCCTACG**GATAACGCATAGT**GTTTT**
TCCCTCCACTTG**GAGACC**GCCTTGGCTGGCGT**TTTTCC**ATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGA
CGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCTGGAAAGCTCCCTCGTG
CGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTCTCCCTTCGGGAAGCGTGGCGCTTTCT
CATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTGTTGCTCCAAAGCTGGGCTGTGTGCACGAACCCCC
GTTACAGCCGACCGCTGCGCCTTATCCGGTAACATATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCA
CTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTGAAGTGGTGG
CCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGA
GTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTTGCAAGCAGCAGATTACG
CGCAGAAAAAAGGATCTCAAGAAGATCCTTT**TTACCAAT**GCCTTAATCAGTGAGGCACCTATCTCAGCGATCTGT
CTATTT**CGTT**CATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGC
CCCAGTGTGCAATGATACCGCGAGAGCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGA
AGGGCCGAGCGCAGAAGTGGTCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGA
GTAAGTAGTTCGCCAGTTAATAGTTTGC**GCAACGTTGTT**GCCATTGCTACAGGCATCGTGGTGTACGCTCGTCCG
TTTGGTATGGCTT**CATT**CAGCTCCGGTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAA
GCGGTTAGCTCCTTCGGTCTCCGATCGTTGT**CAGAAGTAAGTTG**CCCGCAGTGTATCACTCATGGTTATGGCA
GCACTGCATAATTCTTACTGT**CATGCCATCCGTAAGATGCT**TTTTCTGTGACTGGTGAGTACTCAACCAAGTCA
TTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGGCCGGCGTCAATACGGGATAATACCGCGCCACATAGC
AGA**ACTTTAA**AGTGCTC**ATCATTGG**AAAACGTTCTTCCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGA
TCCAGTTCGATGTAACCCACTCGTGCACCCA**ACTGATCTT**CAGCATCTTTACTTTACCAGCGTTCTGGGTGA
GCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTC
CTTTTTCAATATTATTGAAGCATTATCAGGGTATTGTCTCAT**GGTCTC**G

TMV-MK087763 genome fragment is in bold. The ATG to AGA mutation of the TMV CP initiation codon is indicated. Unique restriction sites *NcoI* and *Pfl23II* are on green background. A polylinker is inserted between TMV positions 5756 and 6177. The polylinker consists of two unique restriction sites *AgeI* and *XhoI* (yellow background) separated by a blue-white *LacZ* marker (in blue and italics). Two *BsaI* recognition sites to release the TMV fragment, once manipulated, are indicated on blue background. Hybridization sites for PI and PII oligonucleotides to alternatively produce the manipulated TMV fragment by PCR are doubly underlined. Plasmid also contains pUC replication origin (in gray) and ampicillin resistance marker (on gray background).

>pMTMVi-M (3373 bp)

AAGATTACAAACGTGAGAGACGGAGGGCCCATGGAACTTACAGAAGAAGTCGTTGATGAGTTCATGGAAGATGTC
CCTATGTGCGATCAGGCTTGCAAAGTTTCGATCTCGAACCAGGAAAAAGAGTGATGTCCGCAAAGGGAAAAATAGT
AGTAGTGATCGGTCAGTGCCGAACAAGAAGTATAGAAATGTTAAGGATTTTGGAGGAATGAGTTTAAAAAGAAT
AATTTAATCGATGATGATTCGGAGGCTACTGTGCGCGAATCGGATTCGTTT**TAAATAGATCTTACAGTATCACTA**
CTCCATCTCAGTTTCGTGTTCTTGTCAACCGGTGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCA
CTCATTAGGCACCCAGGCTTTACACTTTATGCTCCCGCTCGTATGTTGTGGAATTTGAGCGGATAACAAT
TTACACAGGAAACAGCTATGACCATGATTACGCCAAGCGCGCAATTAACCTCACTAAAGGGAACAAAAGCTGG
GTACCCAATTCGCCCTATAGTGAGTCGTATTACGCGCGCTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAA
AACCTGGCGTTACCCAACCTAATCGCCTTGCAGCACATCCCCCTTTCCGCAGCTGGCGTAATAGCGAAGAGGCC
CGCACCGATCGCCCTTCCAACAGTTGCGCAGCCTGAATGGCGAATGGGACCGCTCGAGGTCTCTGCAACTTGAG
GTAGTCAAGATGCATAATAAATAACGGATTGTGTCCGTAATCACACGTGGTGCGTACGATAACGCATAGTGTTTT
TCCCTCCACTTAAATCGAAGGTTGTGTCTTGGATCGCGGGTCAAATGTATATGGTTCATATACATCCGCAGG
CACGTAATAAAGCGAGGGTTTCGGAACCAAATCCTCAAAAAGAGGTCGAAAAATAATAATAATTTAGGTAAGGG
GCGTTTCAGGCGGAAGGCCTAAACCAAAAAGTTTTGATGAAGTTGAAAAAGAGTTTGATAAATTTGATGAAGATGA
AGCCGAGACGTCGGTCGCGGATTCTGATTCTATTAAAT**ATG**TCTTACTCAATCACTTCTCCATCGCAATTTGTG
TTTTTGTATCTGTATGGGCTGACCCTATAGAATTGTTAAACGTTTGTACAAATTCGTTAGGTAACCAGTTTCAA
ACACAGCAAGCAAGAAGTACTGTTCAACAGCAGTTCAGCGAGGTGTGGAAACCTTTCCCTCAGAGCACCAGTCCAG
TTTCCCTGGCGATGTTTATAAGGTGTACAGGTACAATGCAGTTTTAGATCCTCTAATTAAGTGGTGGGGTCT
TTCGATACTAGGAATAGAATAATCGAAGTAGAAAACCAGCAGAATCCGACAACAGCTGAAACGTTAGATGCTACC
CGCAGGGTAGACGACGCTACGGTTGCAATTCGGTCTGCTATAATAATTTAGTAAATGAAGTAAAGAGTACT
GGACTGTACAATCAAATACTTTTTGAAAGTATGTCTGGGTTGGTCTGGACCTCTGCACCTGCATCT**TAAATGCAT**
AGGTGCTGAAATATAAAGTTTTGTGTTCTAAAACACACGTGGTACGTACGATAACGTATAGTGTTTTTCCCTCCA
CTTAAATCGAAAGGGTAGTGTCTTGGAGCGCGGAGTAAACATATATGGTTCATATATGTCCTAGCAGCTAA
AAAGCGAGGGATTGCAATTCCTCCCGGAACCCCGGGTTGGGGCCCAGGGTCGGCATGGCATCTCCACCTCCTCG
GGTCCACCTGGGCTACTTCGGTAGGCTAAGGGGGAGACCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCT
TGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTT
TCCCCCTGGAAGCTCCCTCGTGGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCC
TTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCCAAGCT
GGGCTGTGTGCACGAACCCCGTTCCAGCCGACCCGCTGCGCCTTATCCGGTAACATATCGTCTTGAGTCCAACCC
GGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGTTAACAAGGATTAGCAGAGCGAGGATGTAGGCGGTGC
TACAGAGTTCTGAAGTGGTGGCCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAA
GCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGTGGTAGCGGTGGTTTTTT
TGTTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTT**TACCAATGCTTAATCAGTGA**
GGCACCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGAT
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ATGTTGAATACTCATACTCTTCTTTTTCAATATTATTGAAGCATTATCAGGGTTATTGTCTCATGGTCTCGG

TMV-MK087763 genome fragment is in bold. The ATG to AGA mutation of the TMV CP initiation codon is indicated. Unique restriction sites *NcoI*, *Pfl23II* and *CpoI* are on green

background. A polylinker is inserted between TMV positions 5756 and 6177. The polylinker consists of two unique restriction sites **AgeI** and **XhoI** (yellow background) separated by a blue-white *LacZ* marker (in blue and italics). 3' fragment of tomato mosaic virus (ToMV) genome (CP promoter, CP ORF and 3' UTR) is on gray background (**transcription initiation** and **start** and **stop** codons are indicated). **HDV-derived ribozyme** is in red. Two **BsaI** recognition sites to release the manipulated TMV fragment are indicated on blue background. Hybridization sites for PI and PIII oligonucleotides to alternatively produce the manipulated TMV fragment by PCR are doubly underlined. Plasmid also contains pUC replication origin (in gray) and ampicillin resistance marker (on gray background).

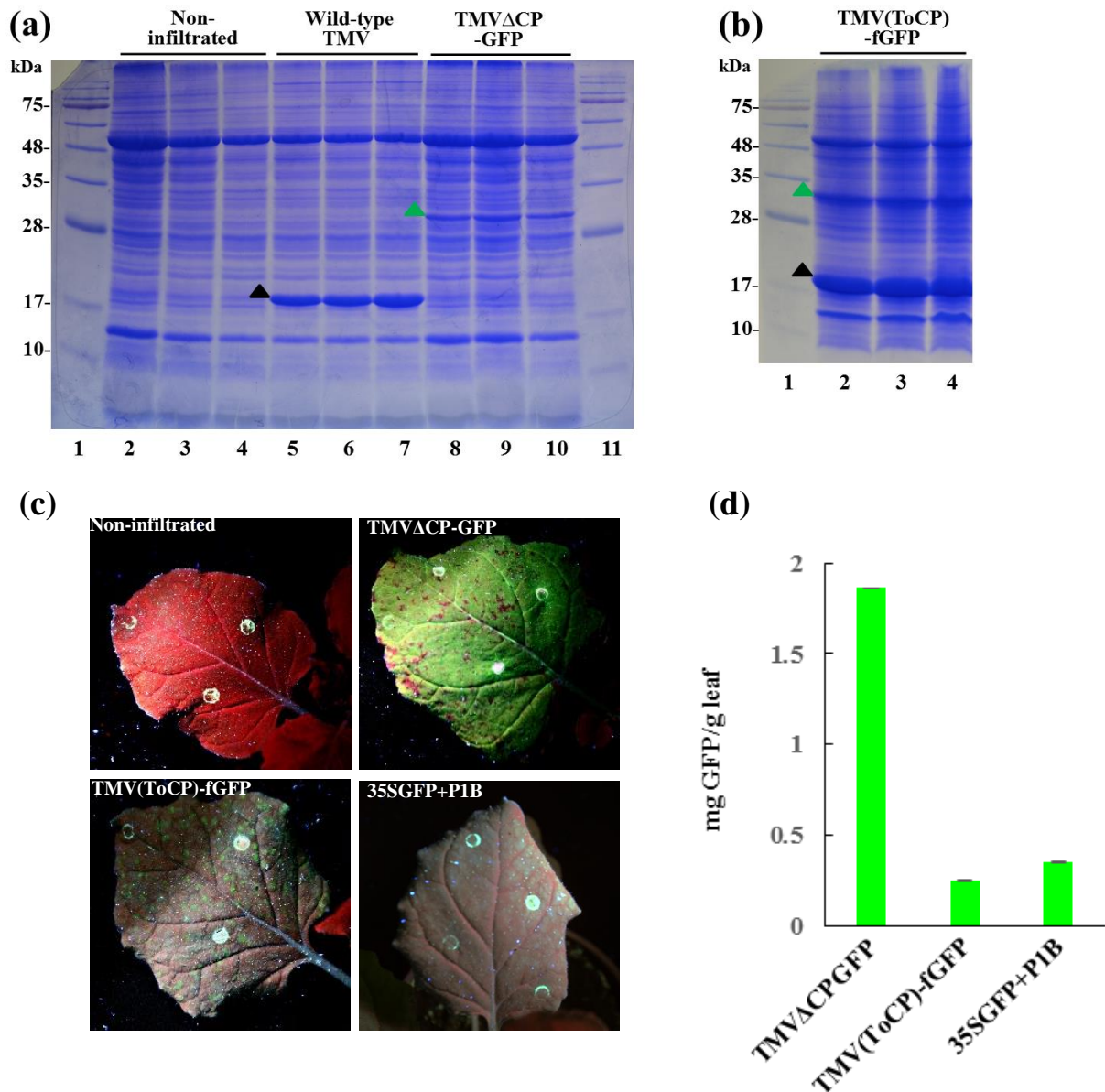


Figure S3. Analysis of GFP produced in *N. benthamiana* plants using the TMV vector system. Total proteins from plant leaves were separated by SDS-PAGE in 12.5% polyacrylamide gels and stained with Coomassie blue. (a) Lanes 1 and 11, and (b) lane 1, marker proteins with the sizes in kDa on the left. (a) Proteins from tissues of three independent plants non-infiltrated (lanes 2 to 4), or infiltrated with wild-type TMV (lanes 5 to 7) or TMV Δ CP-GFP (lanes 8 to 10). Tissues were collected 7 dpi. (b) Proteins from upper non-inoculated tissues from three independent plants agroinoculated with TMV(ToCP)-fGFP (lanes 2 to 4). Tissues were collected 13 dpi. Green and black arrowheads points to GFP and TMV CP. (c) Appearance of *N. benthamiana* leaves agroinfiltrated with the indicated TMV vectors, or the binary vector pGWB5 along with the silencing suppressor P1b from *Cucumber vein yellowing virus* (CMYV) at 7 dpi under UV light. (d) Comparative analysis of GFP production in the agroinfiltrated leaves with the two viral vectors and a simple 35S promoter using the P1b silencing suppressor at 7 dpi.

Figure S4. Sequence of TMV-derived recombinant viruses TMV Δ CP-GFP, TMV Δ CP-AfpB, TMV Δ CP-AfpBKDEL, TMV Δ CP-UP, TMV Δ CP-UPKDEL, TMV Δ CP-UPVS, TMV Δ CP-AgAFP and TMV(ToCP)-fGFP.

>TMV (with the ATG to AGA mutation of CP start codon)

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>TMVΔCP-GFP (sequence that replaces nucleotides 5757 to 6176 in TMV)

ATGGTGAGCAAGGGCGAGGAGCTGTTACCCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGC
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ACCGGCAAGCTGCCCCTGCCCTGGCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTAC
CCCGACCACATGAAGCAGCAGCACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTC
TTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAG
CTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACAGCCACAAC
GTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGC
AGCGTGCAGCTCGCCGACCCTACCAGCAGAACACCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCAC
TACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCTGCTGGAGTTCGTG
ACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTGA

>TMVΔCP-AfpB (sequence that replaces nucleotides 5757 to 6176 in TMV; AP24 is in blue)

*ATGTCCAACAACATGGGCAACCTCAGGTCCCTCCTTCGTCTTCTTCTCCTGGCCCTGGTGACCTTACTTTATGCA
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AACTGCGGTTTCGGCTGCCAATAAGAGGTGCAAGTCTGATCGCCACCCTGTGAATACGATGAGCACCACAGGAGG
GTTGACTGCCAGACTCCAGTTTTGA*

>TMVΔCP-AfpBKDEL (sequence that replaces nucleotides 5757 to 6176 in TMV; AP24 is in blue; KDEL is in green)

*ATGTCCAACAACATGGGCAACCTCAGGTCCCTCCTTCGTCTTCTTCTCCTGGCCCTGGTGACCTTACTTTATGCA
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AACTGCGGTTTCGGCTGCCAATAAGAGGTGCAAGTCTGATCGCCACCCTGTGAATACGATGAGCACCACAGGAGG
GTTGACTGCCAGACTCCAGTTAAGGACGAACTCTGA*

>TMVΔCP-UP (sequence that replaces nucleotides 5757 to 6176 in TMV; AP24 is in blue)

ATGTCCAACAACATGGGCAACCTCAGGTCTCCTTCGTCTTCTTCTCCTCCTGGCCCTGGTGACCTTACACTTATGCA
GTTGACAGCCCTCAGTGGACCATCCAGAACGCCAGCGTGTCTGCAACCCCAAGACACCTCCTGCACCTGGACC
TTCAGCATCTACCCTGGCGCCGGTGCCGCCACTCCCTGCACCTACGTGGTTGAAGGCAGCCCTGCCTCCCAGGCC
AACGGTGGCCCCGTACCTGCGGGCATAACCGTCACTTCTGGCTGGAGCGGCCAGTTTCGGTGCAGGACAATGGC
TTCACCACTCTCTGTGTCGACAACAACCTCTCGCCAGATCATCTGGCCTGCTTATACCGATAAGCAGCTCGCT
GGCGGTGCGGTTGTCAAGCCTGACCAGAGCTACGCCCTGCTGCTCTCCCTTGA

>TMVΔCP-UPKDEL (sequence that replaces nucleotides 5757 to 6176 in TMV; AP24 is in blue; KDEL is in green)

ATGTCCAACAACATGGGCAACCTCAGGTCTCCTTCGTCTTCTTCTCCTCCTGGCCCTGGTGACCTTACACTTATGCA
GTTGACAGCCCTCAGTGGACCATCCAGAACGCCAGCGTGTCTGCAACCCCAAGACACCTCCTGCACCTGGACC
TTCAGCATCTACCCTGGCGCCGGTGCCGCCACTCCCTGCACCTACGTGGTTGAAGGCAGCCCTGCCTCCCAGGCC
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>TMVΔCP-UPVS (sequence that replaces nucleotides 5757 to 6176 in TMV; AP24 is in blue; VS is in red)

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GTTGACAGCCCTCAGTGGACCATCCAGAACGCCAGCGTGTCTGCAACCCCAAGACACCTCCTGCACCTGGACC
TTCAGCATCTACCCTGGCGCCGGTGCCGCCACTCCCTGCACCTACGTGGTTGAAGGCAGCCCTGCCTCCCAGGCC
AACGGTGGCCCCGTACCTGCGGGCATAACCGTCACTTCTGGCTGGAGCGGCCAGTTTCGGTGCAGGACAATGGC
TTCACCACTCTCTGTGTCGACAACAACCTCTCGCCAGATCATCTGGCCTGCTTATACCGATAAGCAGCTCGCT
GGCGGTGCGGTTGTCAAGCCTGACCAGAGCTACGCCCTGCTGCTCTCCCTGGCAACGGCCTCCTGGTTCGACACC
ATGTGA

>TMVΔCP-AgAFP (sequence that replaces nucleotides 5757 to 6176 in TMV; AP24 is in blue)

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GCCACATACAATGGCAAATGCTACAAGAAGGATAATATCTGCAAGTACAAGGCACAGAGCGGCAAGACTGCCATT
TGCAAGTGCTATGTCAAAAAGTGCCCCCGGACGGCGGCAAAATGCGAGTTTGACAGCTACAAGGGGAAGTGCTAC
TGCTGA

>TMV (ToCP) -fGFP

(sequence that replaces nucleotides 5757 to 6176 in TMV; FLAG epitope in purple; linker in gray)

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GAGAAGCGGATCACATGGTCTGCTGGAGTTCGTGACCCGCCCGGGATCACTCTCGGCATGGACGAGCTGTAC
AAGTGA

(ToMV sequence that replaces nucleotides 6366 to 6395 in TMV)

GAACCAAATCCTCAAAAAGAGGTCCGAAAAATAATAAATTTAGGTAAGGGCGTTCAGGCGGAAGGCCCTAAAC
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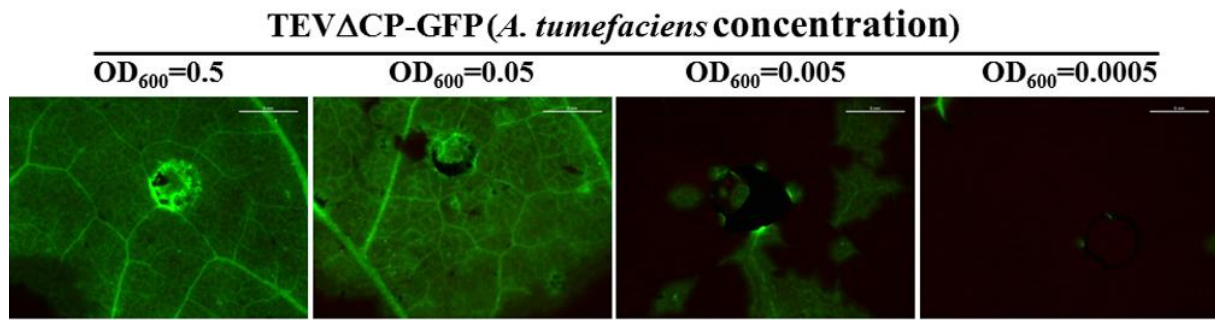


Figure S5. Dilution analysis of TMVΔCP-GFP infectivity in *N. benthamiana*. *A. tumefaciens* cultures to express TMVΔCP-GFP at the indicated OD₆₀₀ were used to infiltrate *N. benthamiana* leaves. GFP fluorescence in the agroinfiltrated areas was detected at 7 dpi using a stereomicroscope. Representative pictures of three biological replicates are shown. Bars represent 5 mm.

Table S1. Primers used in this work.

Primer name	Sequence
PI	5'-TTACAAACGTGAGAGACGGAGGGCC-3'
PII	5'-GGAGGGAAAAACACTATGCGTTATC-3'
PIII	5'-GTAGAGAGAGACTGGTGATTTTCAGC-3'
CP:AP24_fwd	5'-CTCAGTTCGTGTTCTTGTC AATGTCCAACAACATGGGC-3'
AP24AfpB_rev	5' -TCCTCCGTATTTACTTGCATAAGTGTAGGT-3'
AP24AfpB_fwd	5' -CACTTATGCAAGTAAATACGGAGGACAATGC-3'
AfpB:CP_rev	5'-CTACCTCAAGTTGCAGGACCTCAAAC TGGAGTCTGGCAG-3'
AfpBKDEL_rev	5'-CCCGGGTTACTAGAGTTCGTCCTTAACTGGAGTCTGGCAGTC-3'
KDEL:CP_rev	5'-CTACCTCAAGTTGCAGGACCTCAGAGTTCGTCCTTAACTG-3'
AfpD:CP_rev	5'-CTACCTCAAGTTGCAGGACCTCAAGGGAGAGCAGCAGG-3'
AfpDKDEL_rev	5'-CCCGGGTTACTAGAGTTCGTCCTTAGGGAGAGCAGCAGGGGC-3'
AfpDVS_rev	5'-TCACATGGTGTGCGACCAGGAGCCGTTGCCAGGGAGAGCAGCAGGGGC- 3'
VS:CP_rev	5'-CTACCTCAAGTTGCAGGACCTCACATGGTGTGCGACCAG-3'
AgAFP:CP_rv	5'-CTACCTCAAGTTGCAGGACCTCAGCAGTAGCACTTCCC-3'