

SUPPLEMENTARY INFORMATION

CRISPR/Cas9-Mediated Immunity to Geminiviruses: Differential Interference and Evasion

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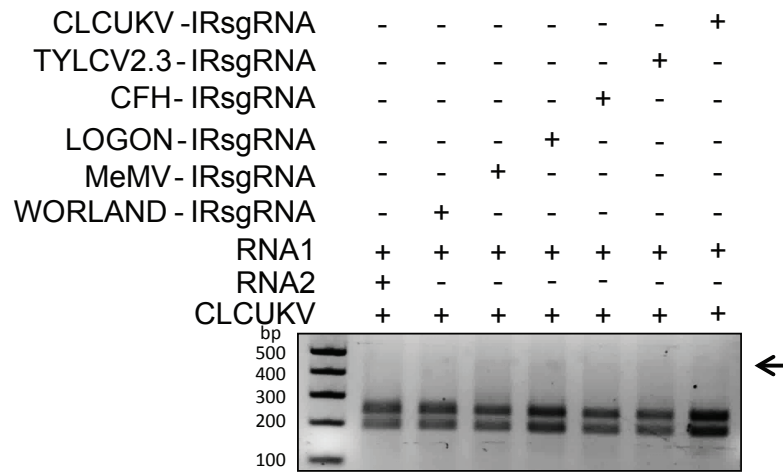
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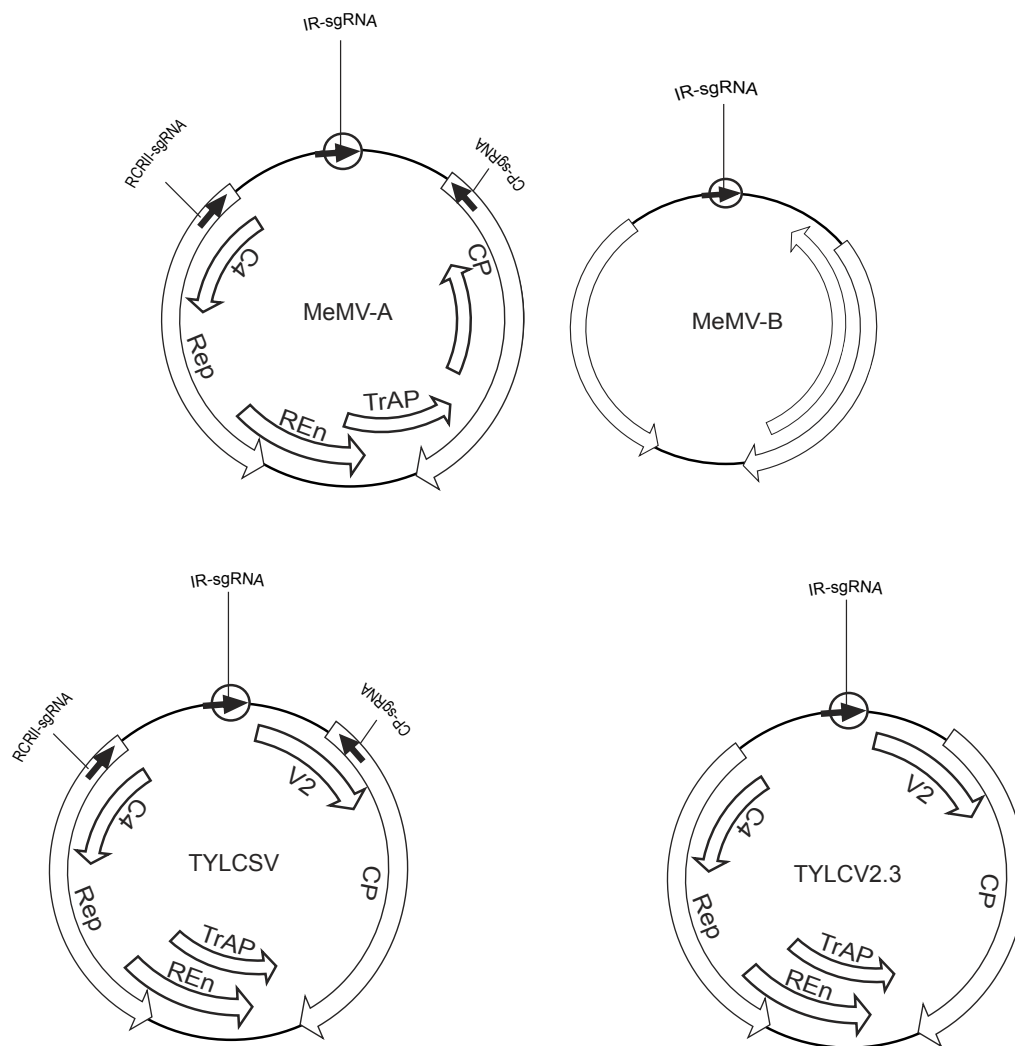
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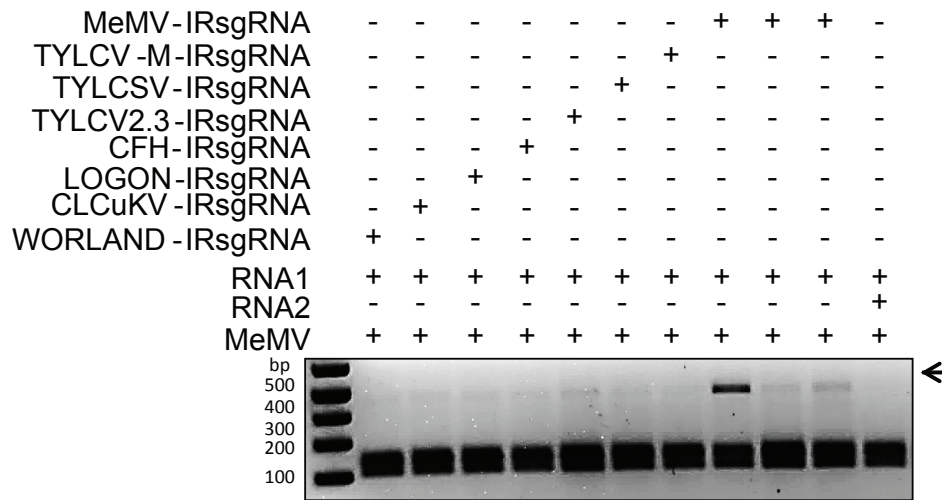
Supplementary Figure 2. Diagrammatic representation of genomes of different Geminiviruses.

Genome organization of the three selected viruses, bipartite (MeMV-A and MeMV-B), monopartite (TYLCSV and TYLCV2.3) are represented with targets (filled arrows, one in non-coding *IR*, one each in coding *CP*, or in *Rep* *RCRII* domain), *IR* is represented with open box and stem loop of nonanucleotide is represented by small circle, bidirectional ORFs (overlapping on inside) are represented by open arrows.

G12	CGGGGGAA--63Δ---ACCGGATG
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A06	TAAGGGAT--284Δ---CGTTTAA

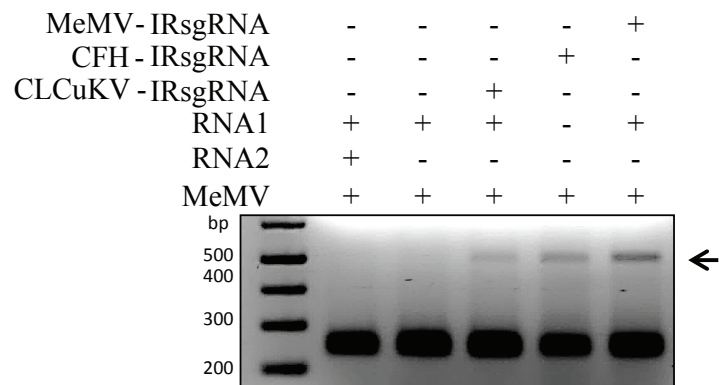
Supplementary Figure 3. Long deletions at the IR region of MeMV.

Sanger sequencing of the IR of MeMV-A and MeMV-B, showing long deletions at the IR site. After targeting the IR region is PCR amplified and cloned into pJet2,1 and were sanger sequenced. Specific deletions are represented at their respective sites.



Supplementary Figure 4. *SspI*-recognition site loss assay of MeMV targeted with variant IR-sgRNAs.

IR sequence targeting and NHEJ repair in MeMV-A. The *SspI*-resistant IR fragment (446 bp) was analyzed for the loss of the *SspI* recognition. MeMV-A genome targeted (with different variants of IR-sgRNAs including the native MeMV-IRsgRNA) used was able to repair by NHEJ. Arrow indicating the expected *SspI* resistant 446 bp DNA fragments in all of the samples expressing an invariant IR-sgRNA. DNA fragments were resolved on 2% agarose gel premixed with Ethidium Bromide stain.



Supplementary Figure 5. *SspI*-recognition site loss assay for IR sequence modification of MeMV-B genome.

IR sequence targeting and NHEJ repair in MeMV-B. The *SspI*-resistant IR fragment (496 bp) was analyzed for the loss of the *SspI* recognition. MeMV-B genome targeted with MeMV-IRsgRNA used was able to repair by NHEJ. Arrow indicating the expected *SspI* resistant 496 bp DNA fragments in all of the samples compared to TRV alone. DNA fragments were resolved on 2% agarose gel premixed with Ethidium Bromide stain.

Supplementary Figure 6. Stem loop sequence in the IR of available geminiviruses.

The conserved nonanucleotide shown in Red, and flanking complementary sequence making the stem loop of 143 Begomoviruses. Each sequence is represented with its accession number.

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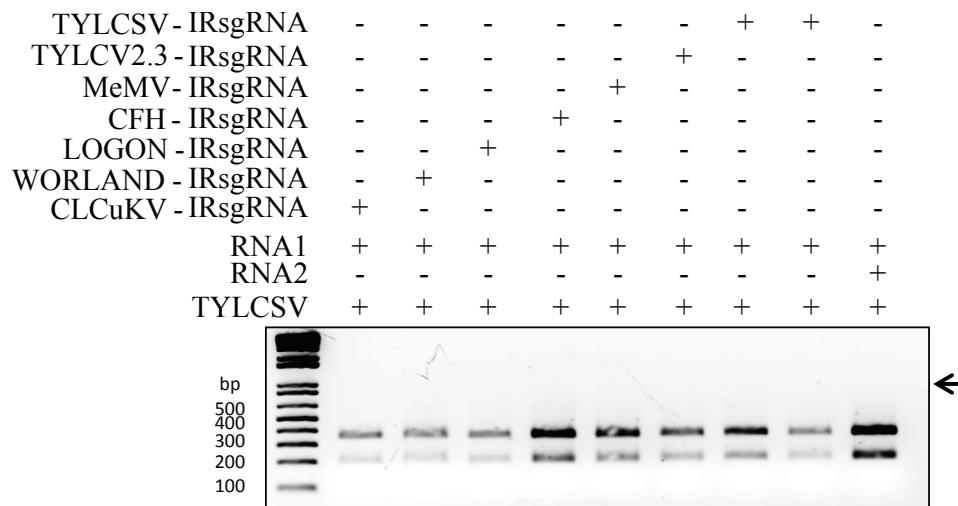
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Supplementary Figure 7. *SspI*-recognition site loss assay of TYLCSV targeted with variant IR-sgRNAs.

NHEJ repair analysis at the IR sequence of TYLCSV. The TYLCSV IR region (562 bp) was analyzed for the loss of the *SspI* recognition site through NHEJ (Indels). Different variants of IR-sgRNAs were used to target IR of TYLCSV. Like TYLCSV-IRsgRNA, none of the variant IRsgRNAs targeting of CLCuKV IR sequence results in NHEJ repair. Arrow indicating the expected *SspI* resistant 562 bp DNA fragments. DNA fragments were resolved on 2% agarose gel premixed with Ethidium Bromide stain.

A

PAM

```

WT  ACGAGACCGAAGGCCGTATGGACTA-TGGATTTTGGTC
B01 ACGAGACCGAAGGCCG-----CTA-TGGATTTTGGTC  -13
C02 ACGAGACCGAAGGCCGTATGGA-----CTA-TGGATTTTGGTC  -07
E02 ACGAGACCGAAGGCCGTATGGACTA-----TGGATTTTGGTC  -06
H05 ACGAGACCGAAGGCCGTATGGACTAA-----CTA-TGGATTTTGGTC  -03+1
C01 ACGAGACCGAAGGCCGTATGGACTAA-----CTATGGATTTTGGTC  +01

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B

PAM

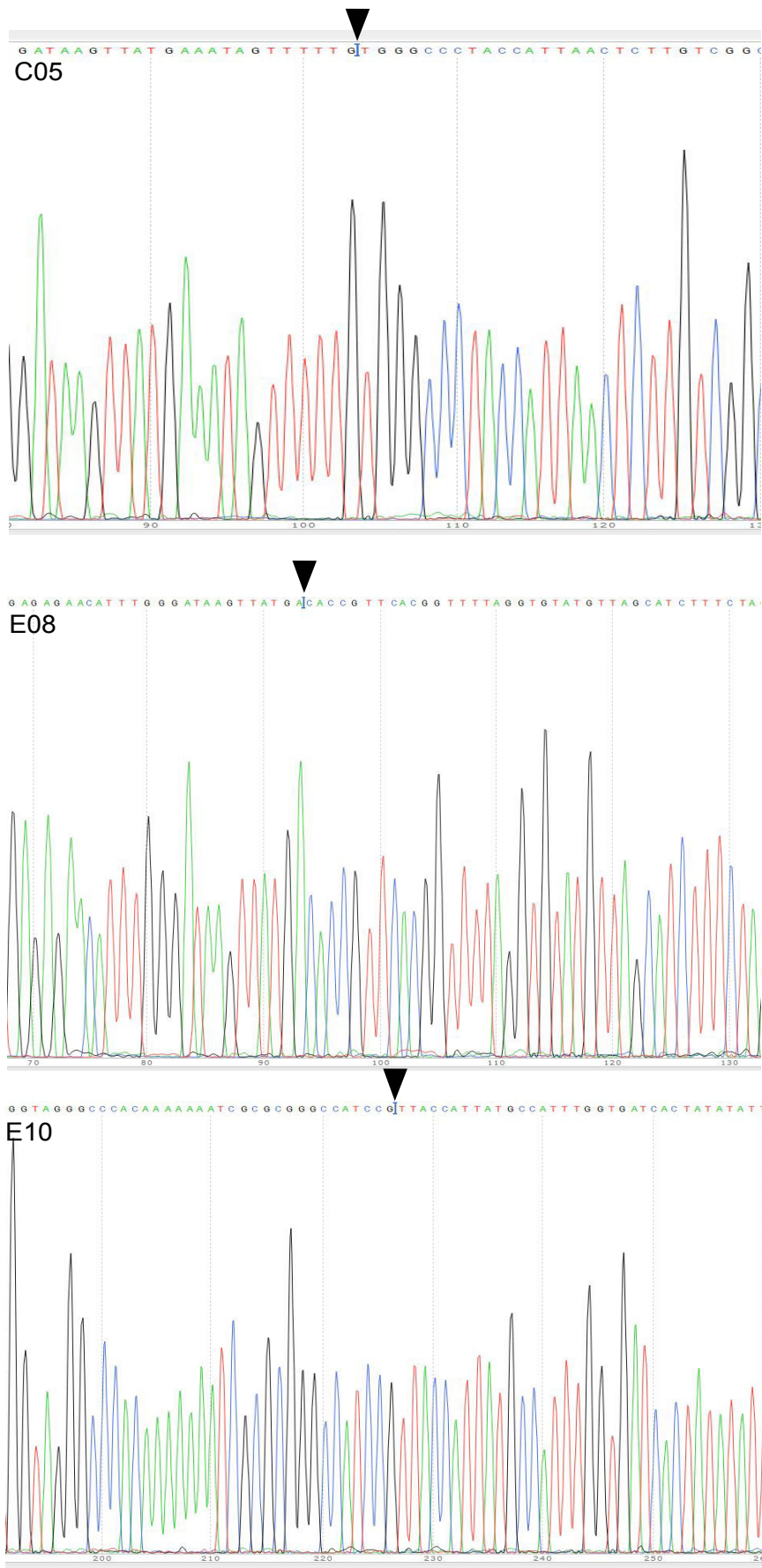
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WT  TTTACCCTCGAACTGAATGAGCATGTGGAG-ATGAGGTGCCCATCT
G07 TTTACCCTCGAACTGAATGAG-----ATGAGGTGCCCATCT  -09
B11 TTTACCCTCGAACTGAATGAGCA-----ATGAGGTGCCCATCT  -07
A08 TTTACCCTCGAACTGAATGAGCATGTGGAGATGAGGTGCCCATCT  +01
E11 TTTACCCTCGAACTGAATGAGCATGTGGAGATGAGGTGCCCATCT  +01

```

Supplementary Figure 8. Alignment of Sanger sequence reads for Indels in the CP and RCRII sequences of TYLCSV.

Alignment of Sanger sequence reads of PCR amplicons encompassing the CP (A) and RCRII (B) target for Indels sequence confirmation. The wild-type (WT) sequences are shown at the top (target sequence is shown in red; the protospacer-associated motif [PAM] is indicated by green, followed by the various indels formed, indicated by numbers to the right of the sequence [-, deletion of x nucleotides; +, insertion of x nucleotides; and >, change of x nucleotides to y nucleotides].



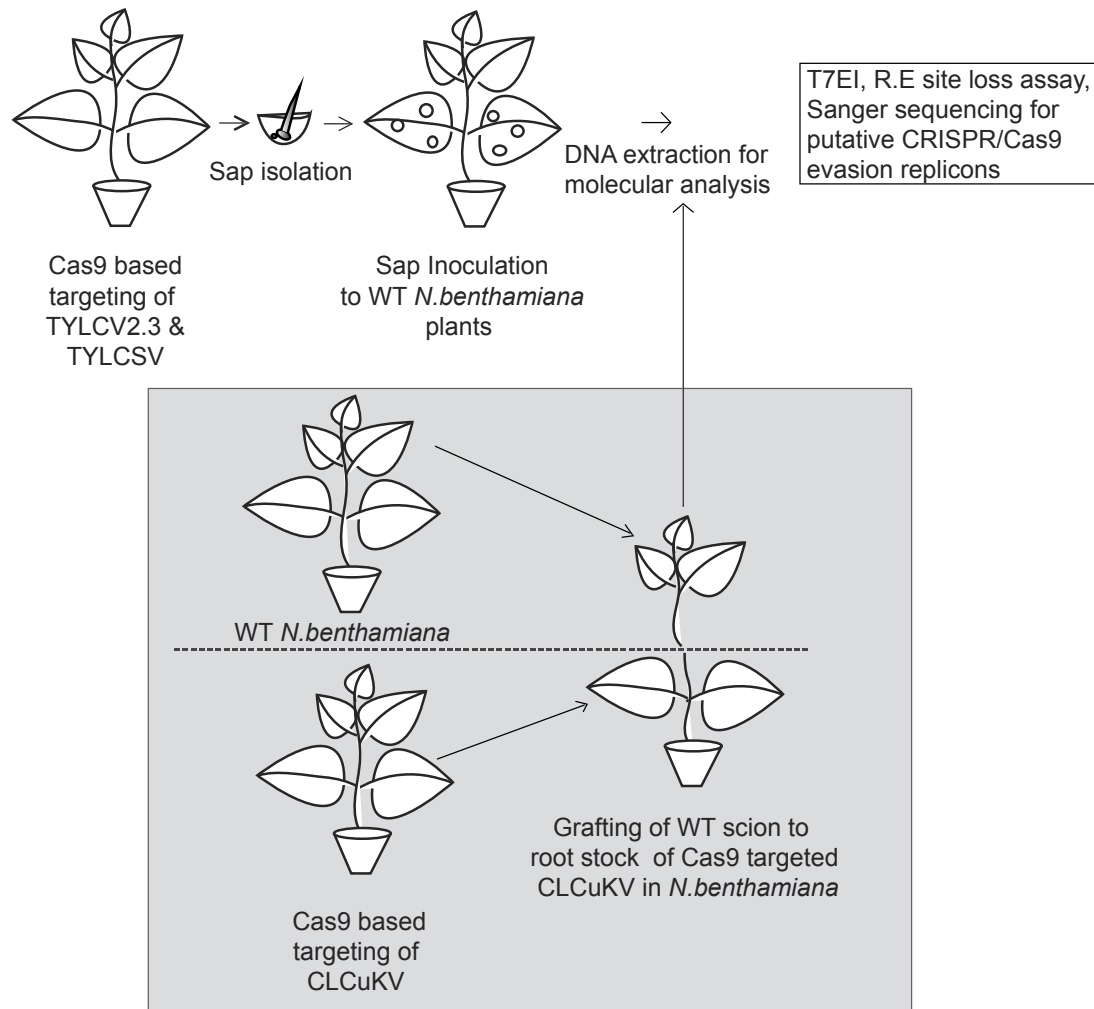
Supplementary Figure 9. Chromatogram of Sanger sequence reads for Indels in the IR of CLCuKV.

Sanger-sequencing chromatograms of PCR amplicons of IR region of CLCuKV targeted with IR-sgRNA. PCR amplicons show the respective sequence long deletions after targeting by CRISPR/Cas9.

```
G04  ACCGATTGACC--128Δ---TACCGGATGG
F11  TCGGTGATCAC--199Δ---TCGTTGCTAA
F03  TGTCGGCCAATC--62Δ---CTTGTCGGCC
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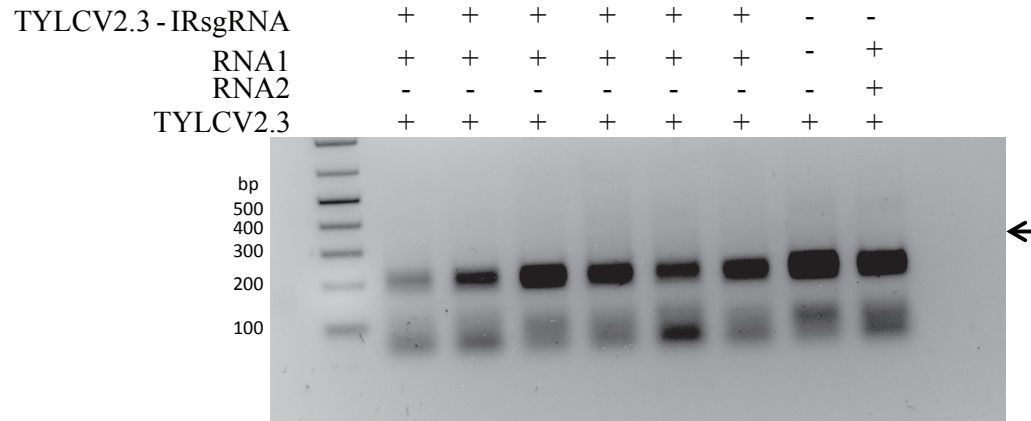
Supplementary Figure 10. Alignment of Sanger sequence reads for Indels in the IR of CLCuKoV.

Alignment of Sanger-sequencing reads of PCR amplicons of IR region of CLCuKV targeted with an invariant IR-sgRNA. Total DNA was extracted and the PCR amplicons were cloned and sanger sequenced. PCR amplicons show the respective sequence mutation at CRISPR/Cas9 targeting site.



Supplementary Figure 11. Diagrammatic representation of the sap inoculation methods.

Sap was isolated from plants in which TYLCV2.3 was targeted with CRISPR/Cas9 at CP or IR. The extracted sap was rub inoculated with Carborundum, injected to the leaf pedicels and main stem directly. Alternatively for CLCuKoV (in gray box), a scion from the wild *N benthamiana* plant was grafted to a CRISPR/Cas9 targeted CLCuKoV plants. Collected samples were used for different molecular assays.



Supplementary Figure 12. *SspI*-recognition site loss assay of TYLCV2.3 targeted with IR-sgRNAs.

NHEJ repair analysis at the IR sequence of TYLCV2.3. The IR region was analyzed for the loss of the *SspI* recognition site through NHEJ (Indels). None of sample show any *SspI* resistant DNA fragment. Arrow indicates the expected *SspI* resistant DNA fragments. DNA fragments were resolved on 2% agarose gel premixed with Ethidium Bromide stain.

PAM

```

WT      AGCAGCACGGCTCACATATGGGCTGTCGAAGTTG-AGACGGCGGTACCTTCGAA
ESC1    AGCAGCACGGCTCACATATGGGCTGTCGAA---G-AGACGGCGGTACCTTCGAA  -03
ESC2    AGCAGCACGGCTCACATATGGGCTGTGGA---G-AGACGGCGGTACCTTCGAA  -03, C>G
ESC3    AGCAGCACGGCTCACATATGGGCTGTAAAGTTG-AGACGGCGGTACCTTCGAA  -G>A
ESC4    AGCAGCACGGCTCACATATGGGCTGTGGAAGTTGCAGACGGCGGTACCTTCGAA  +01

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Supplementary Figure 13. Alignment of Sanger sequence reads for Indels in the CLCuKoV escapees.

Alignment of Sanger-sequencing reads of PCR amplicons of CP region of CLCuKV. Total DNA was extracted from the WT scion grafted to virus (CLCuKV and CLCuMβ) inoculated and CRISPR/Cas9 machinery expressing plants. Reads of the PCR amplicons show the respective sequence mutation at CRISPR/Cas9 targeting site. The wild-type (WT) sequences is shown at the top (target sequence is shown in red; and the protospacer-associated motif [PAM] is indicated by green, the various indels formed are shown enlarge, bold and in blue at their respective sites.

A

WT	Ile	Ser	Thr	Pro	Val	Ser	lys	Val	Val	Arg	Arg	Arg	Leu	Asn	Phe	Asp
C03	Ile	Ser	Thr	Pro	Ala	Ser	lys	Val	Val	Arg	Arg	Arg	Leu	Asn	Phe	Asp
E06	Ile	Ser	Thr	Pro	Ile	Ser	lys	Val	Val	Arg	Gly	Arg	Leu	Asn	Phe	Asp
F12	Ile	Ser	Thr	Pro	Val	***	lys	Val	Val	Arg	Arg	Arg	Leu	Asn	Phe	Asp
G06	Ile	Ser	Thr	Pro	Val	***	lys	Val	Val	Arg	Arg	Arg	Leu	Asn	Phe	Asp
D12	Ile	Ser	Thr	Pro	Ala	***	lys	Val	Val	Arg	Arg	Arg	Leu	Asn	Phe	Asp

B

WT	Val	Arg	Arg	Arg	Leu	Asn	Phe	Asp	Ser	Pro	Tyr	Val	Ser	Arg	Ala
ESC1	Val	Arg	Arg	Arg	Leu	---	Phe	Asp	Ser	Pro	Tyr	Val	Ser	Arg	Ala
ESC2	Val	Arg	Arg	Arg	Leu	---	Phe	His	Ser	Pro	Tyr	Val	Ser	Arg	Ala
ESC3	Val	Arg	Arg	Arg	Leu	Asn	Phe	Asp	Ser	Pro	Tyr	Val	Ser	Arg	Ala
ESC4	Val	Arg	Arg	Arg	Gln	Leu	***	Gln	Pro	Ile	Cys	Glu	Pro	Cys	Cys

Supplementary Figure 14. Alignment of deduced amino acid sequences of CLCuKoV CP escapees.

Amino acid sequence alignment of CP region of CLCuKoV. Targeted CP region Sanger sequencing reads were converted to amino acid sequence. A) TYLCV2.3, B) CLCuKoV. Missense mutations are shown in blue, deletions with blue dots, nonsense with asterisks and frame shift resulting in premature stop with green. The wild-type (WT) amino acids sequences is shown at the top (amino acids in the target sequence are shown bold and in bigger font)

Supplementary Sequence 1; *Cotton Leaf Curl Kokhran virus* (CLCuKV; AJ496286)

ACCGGATGGCCGCGCATTTTTTTGTGGGCCCTACCATTAACCTTGTGCGGCCAATCATATGACGCGCTCAAAGCTT
AAATAATTCTCCCGCTTATTATAAGTACTTCGTTGCTAAGTATGCGTTTGAAAAATGTGGGATCCACTGTAAATGA
GTTCCCCGACACCGTTCACGGTTTTAGGTGTATGTTAGCAGTTAAATATTTGCAGTTAGTACAGAAAACCTACTCTC
CTGATACATTGGGTTACGATTTGATAAGGGATTTAATCCTGGTAATAAGGGCTAGGAATTATGTCGAAGCGACCA
GCAGATATAATCATTTCCACGCCCCGCTTCGAAGGTACGCCGCCGTCTCAACTTCGACAGCCCATATGTGAGCCGTG
CTGCTGCCCCATTGTCCGCGTCACCAAAGCAAAAGCATGGGCGAACAGGCCCATGAACAGAAAGCCCAGGATGT
ACAGGATGTACAGAAGTCCAGATGTTCTAGAGGATGTGAAGGTCCATGTAAGGTTCACTCGTTTGAGTCCAGAC
ATGATATTCAGCATATAGGTAAAGTAATGTGTGTTAGTGATGTTACTCGTGGTACTGGGCTGACCCATAGAGTTGG
TAAGAGATTTTGTGTTAAGTCTGTTTATGTGTTGGGTAAGATCTGGATGGATGAGAACATTAAGACGAAGAATCAC
ACGAATAGTGTGATGTTTTCTTGTTAGAGATCGTAGACCTGTTGATAAACCTCAAGATTTTGGAGAGGTATTTA
ATATGTTTGATAATGAGCCCAGTACGGCGACTGTGAAGAATGTTTCATCGTGATAGGTATCAAGTTCTGCGCAAATG
GTATGCAACTGTCACCGGTGGACAATACGCTTCAAAGGAACAAGCTCTCGTGAAGAAATTTATTAGAGTTAATAAT
TATGTTGTGTATAACCAGCAGGAAGCTGGCAAGTATGAGAATCATTCTGAGAATGCTTTAATGTTGTATATGGCGT
GTACTCACGCCTCTAACCCAGTGTATGCTACCTTGAAGATACGGATCTACTTCTATGATTCCGTGACAAATTAATAG
ATATTGAATTTTATTGAAGATGATTGGTCTACAAATACAACATGTTGTAATACATTCCATAATACATGATCAACTGC
TCTAACTACATTATTAATACTGACAATTCCTAAGTTATTTAAATATTTAAGCACTTGAGTCCTAAAGACCCCTAAGAA
ACGACCAGTCGGAGGCTGTGAGGTCATCCAGATTTCGGAAAGCTATGAAACATTTGTGTATCCCCAACGCTTTCCTC
AGGTTGTGATTGAACTGTATCTGGACGGTGATGATGTCTCTGTTTCATTAGGAATGCTCGATTGTGGTGCTCTGTTA
TCTTGAAATACAGGGGATTTTGAATCTCCAGATAAACACGCCATTCTCTGCTTGAGCTGCAGTGATGAGTTCCCT
GTGCGTGAATCCATGGTTGTGGCAGGCTAATGCTATGAAGTACGAACACCCACAAGGGAGATCAACTCTCCGACG
TCTGGTCCCCTTCTTGCTAGCCTGTGCTGCACTTTGATTGGAACCTGAGTAGAGTGGGCTCTCGAGGGTGATGAA
GATTGCATTCTTTAAAGCCCAATTTTTGAGTGCAGAATTCTTCTCTCATCCAAAACTCTTTATAGCTTGAATTGGG
TCCTGGATTGCAGAGGAAGATAGTGGGAATTCCGCCTTTAATTTGAACTGGCTTCCCGTATTTTGTATTTGATTGCC
AGTCCTTTTGGGCCCCCATGAACTCCTTAAAGTGCTTTAGGTAATGCGGGTCGACGTCATCAATGACGTTAAACCA
GGCGTCATTACTGTATACCTTGGGCTCAGATCTAGATGTCCACACAGATAATTATGTGGACCTAATGATCTGGCC
CACATCGTCTTCCCCGTCTACTGTCACCCTCAATCACTACACTTATTGGTCTATTGGCCCCGCGCAGCGGCACTGAC
GACGTTCTCGGCAGCCACACTTCAAGTTCTTCTGGAACCTTGATCGAAAGAAGAAGAGGAAAAAGGAGAAACATA
AGGAGCTGGTGGCTCCTGAAAGATTCTGTCTAGATTTGCATTTAAATTATGAAATTGCAGTACAAAATCCTTAGGA
GCTAGTTCCTTAATGACTCTAAGAGCCTCCGACTTACTTCCGCGTTAAGTGCTGCGGCGTAAGCGTCATTGGCTGT
CTGTTGCCCTCCTCTTGCTGACCTTCCGTCGATCTGAAATTGCCCCAGTCGAGAATGTCCCCGTCTTCTCGATGTA
GGATTTGACGTCGGAGCTGGATTTAGCTCCCTGTATGTTGCGATGGAAATGTGCTGACCTGCTTGGGGAGACCAA
GTCGAAGAATCGCATATTCTGGCACTTGAATTTCCCTTCGAACTGGATGAGAACATGCAAGTGAGGAGTCCCATCT
TCGTGAAGCTCTCTGCAGATTCTAATATATTTTTTTGAAGTTGGGGTTTGTATATTTAATAATTGGGAAAGTGCTTC
CTCTTTGGTGAGAGAACATTTGGGATAAGTTATGAAATAGTTTTTGGAAATAATACCGTTCCGCTTTGGAGGCATG
TTGACTAAAATTGAATCACCGATTGACCGTTCTTGCAAACCTCTCCCCGGTATATCGGTGATCAATATATAGTGATCA
CCAAATGGCATAATGGTAATAAAAAAACTTTAATTTGAAATTCAAACCAAAGGCTAAAGCGGCCATCCGTTTAAT
ATT

Target sequences are shown in gray.

Cotton leaf curl Multan Betasatellite (CLCuMB; AJ298903)

accgtggcgagcgggtgccgatggtttcttggtgggtcccattgctggtattgacttgattgacttatattgggccaattaatgggttgaaaatgtttg
ggcctttggaagagggtcttttatggaatagggtctgtatattgttggtgatattgtgttaaataatgcattgctggtttgtgttggaatttaaagggtg
aactttttattgaatacgtacggttcgtttacatccattccaatatctctgggttttcaagtacaagtatatcaagtctgtgaactatatcttctatctcga
tctcttctatctttgccccgttgatgcgaataggaaattcgctatgatgctccctcaaagccgttgaagtcgaatggaacgtgtatgtcttcgtacgtgt
actggacgatcccttcatacttgattagcgatggtagcttttggtatagatcctcatgtgaatgaagatcttcatattctcatgatgcgaacgtcgact
atgaacctgactccctccttgtttgttcgctcgggtgctatttctgcttatttgatggaaatgtttagtgtgcatgcatttatagacttaataacagatattt
tgtgtagtgtgtggttgtagtgatttattatgtgattgtccattaaagggataaagtgtatgatggagacgtattacacgtgttgcatgttggttg
aaatctttatacatgggtttgtgccgtatacctatatatacggatagaaaaacggatgagaaaaaggaaaaacggaactgaagaggaaaaaacaaga
aaagaaacaaggatatattttatgaagaaatgggagcgcagcgaatcgaacaggaaaaaccaaggaaagagaaaaaattaaagtaaaga
gaaaaaaaattcaaattcgaacgtcatcgtttgagagtaataaaaaaaagaaaaaacaacatatccgaaaacgtgtcgttgaaggtgt
ctgtgtggttttaccatttactgtgtgtaaatggtaagtgtgaagaatagttaaaaaatggaggcccgataggtaaattgtacccaatatattggg
gttcaattggggactcataaattgcctttcctaaaataccccgcctttgtgtctaagaggcgctcgagtgcgctataaagttaacattctctcct
ctttgatctcaatacaatttccgggtgatcgagtcgaattttccgacacgcgcggcggtgtgtacccctgggagggttaggtaccactacgctacgca
gcagccttagctacgccggagcttagctcgccacgttctaatt

Supplementary Sequence 2;

Merremia Mosaic Virus (MeMV-A)

ACCGGATGGCCGCCCGCCGCGCCCCCCTGGGCCACATATTAAGCCGTCCAATCACAAAGCGTCCTGGAAGTCT
AATTGTTTAAATAAGCCTATAAATACATTGGAGTCCGTCTATACCCACCAACTTTAATTTAAATGGTTAAGAGG
GACGCCCCATGGCGTTTAATGGCGGGGACCACTAAAGTTAGTCGCAACGCCAATTTCTCGCCACGTGGAGGTATG
GGCCCTAAGGCCGCTGCTTGGGTTAACAGGCCCATGTACAGGAAGCCCAGAATTTATCGCACTTTGAGAGGGCCT
GATGTTCTAAAGGTTGTGAAGGCCCATGTAAGGTACAGTCTTTCGAGCAGCGTCATGATATTTCTCATGTTGGTA
AGGTAATCTGTATATCCGATGTAACCTCGTGGTAACGGTATTACTACCGTGTTGGCAAGCGTTTTTGTGTGAAGTCT
GTGTATATTCTAGGTAAAATATGGATGGATGAGAACATAAAGCTGAAGAACCACACGAACAGCGTCATGTTTTGG
TTGATTCGTGACAGGAGACCCTATGGTACCCCTATGGATTTTGGTCAGGTGTTTAACATGTATGACAATGAGCCGA
GTACTGCTACCGTCAAGAACGATCTTCGCGATCGATTTCAAGTCATGCATAGGTTCTATGCCAAAGTAACTGGTGG
TCAGTATGCCAGTAACGAGCAGGCATTGGTTCCGCGATTTTGAAGGTTAACTACGTCGTGTATAACCATCAG
GAAGCAGGAAAATACGAGAATCACACGGAGAATGCTCTGTTATTGTATATGGCATGTACTCATGCTTCTAATCCTG
TGTATGCTACCTTGAAAATTCGTAGTTATTTTTATGACTCCATTTGAAATTAATAAAGATTAAATTTTATTGAATGTC
TTTCGAGCACACAATTTACATATGGTTTATCCGTTGCGAAACGAACAGCTCTAATGACATTGTTAAGCGAAACAAC
ACCTAATTGATCTAAATACATTAATACTAAATCTTTAAATCTATTTAAATATGTCGTCCAGAAAGCTTGAAGTATGT
CGTCCAGATTTGGAAGTTCAGGTATGCTTTGTGTAGACTCAACGCCTTCCTCAGGTTGTAGTTGAACCGTATTTGGA
TGGTGTATATTCTGGTTGTGGTGTATATTGGCTCCTCCACTTGGATTATCTTGAAATAGAGGGGATTTGGAACCTCC
CAGATAAAACGCCATTCATTGCTTGATGAGCAGTGATGGGTTCCCTGTGCGTGAATCCATGGTTTCTGCAGTTG
ATGTGTACGTAAATTGAACAGCCACAGTCCAGGTCTAACCTTCTCCGTCTAATGACAGATTTGGATCTCTTCGCTCT
CCTGTGCTGTGCTTTGATAGAGGGGGGAGTTGAGGAAGATGAATTTGCATTGTGGATCGTCCACGCTCTGAGAG
ATGCGTTTTTCATCTTTATCGAGGAAGTCTTTATAGCTAGCCCCCTCTCCTGGATTGCACAGCACGATTGAGGGTATT
CCTCCTTAATTTGAACTGGCTTCCCGTATTTACAGTTGGACTGCCAGTCCTTTTGGGCCCTATCAATTTCTTCCAA
TGCTTTAATTTCAAATAATTAGGGCTTATGTCATCAATGACGTTATATTGACGTCATTGGAATAGACTCTGCCATTA
AAGTCAAGATGTCCACTAAGATAATTATGTTTACCTAATGAACGGGCCACATTGTTTTCCAGTTCGACTATCTCC

TTGATGATGATACTTATCGGTCTATCTGCCCCGCGCAGCGGCACCCCTCCCAAAGTAGTTGTCTGCCATTCTTGCA
TCTCATCTGGAACGTTCTGTGAAGGAGGAGAGTTGAAACGGAGGAACCCATGGTTCTGGAGACTTCTGAAATATTC
TTGTTGCGTTCGCAACGAGATTGTGATGTTGAAGAAAGAAGTGTGTTGTTCTCTTATTATTGTCAGTGCT
TCCTCTGCAGAAGCTGCGTTTAACGCCTTTGCGTATGTATCGTTAGAAGACTGCTGACCTCCTAGCAGATCTTCC
GTCGATCTGGAATTGTCCCCATTCAATTGTATCTCCGTCCTTGTGATGTAGGACTTGACATCGGAGCTGGATTAG
CTCCCTGAATGTTTCGGATGGAAATGTGCTGACCTGGTTGGGGATACCAAATCGAAGAATCTGTTATTCGTGCAGTT
GTATTTTCCTTCGAACTGGATAAGCACATGGAGATGAGGTTCCCATTTATCGTGAAGCTCTCTACAGATCTTGATGA
ATTTTTGTTTACAGGGGTGTGCAGAGCTTTGATTTGGGACAGTGCTTCTTTGGCTAATGAACATATAGGGTAT
GTGAGGAAATAGTTTTGGCTTTTATTGAGAATGAACCCTCCGTGGCATTTTTGTAAATAAGGGATGTTCCCCCAAT
TGCTCCGCTCTCAAACTCTATATGAATCGGGGAACTGGGGGTACATTTATACTAGAACTCTCATTAAAGGGATT
TGCAACACGTGGCGGCCATCCGCTATAATATT

Target sequences are shown in gray.

Merremia Mosaic Virus (MeMV-B)

ACCGGATGGCCGCCGCCGCCGCCCTGGCCCGCCACCGCGCCCTTGACCCACATGGTGCCACCAGCTGT
GATGCACTTGTAAAGGTAATACGTGGAACGCTGTATGCATTCATTTGAACTAACTGTAACTTCTCTTTAATTT
GAATTATTGTCGCGCTACTATGAAAGGTTGGGTCATACTACAATGATTAATTTGTTAACGTAATGTACGATTGG
AGACGTGGACCAGTTATAACCATTATACATCGTCAAATTAGTTTGCGTCTCTTTAAGATCTATATATATATGCTT
GAACGTGGAGTTATGTAAACATATATTTTATTAATATCTATTACTAGAATATGTATTCTTTAGATATAGACCGTATC
ATTTAATTATCGGAAACGATTTTACACACGTACGCAAGTGTCTAAACGGGTAGCCCCGTTTAAACGAGCTGATGT
GAAATGTGCAACGAGGCAGACGACTATCGTTCATGATGAGACTAAGATGTCTTCGACGCGCATTGATGAGAACCA
ATTTGGTCCAGAGTTTGTGATGACACATAACTCCGCCATATCCACTTTCATTAAATTATCCTACTTTGAGTAAGACCG
AGCCTAACAGAAGCAGATCTTACATTAAAGTTGAAACGCTTGCAGTTTAAAGGGAAGTGTAAATTTGAGCGTGTGT
ATGCGGATATGAACATGGATGGTTTGAACCCTAAAGTTGAAGGGGTATTCACTCTTGCTGTTGTAGTTGACCGAAA
ACCTCATTTAAAGCCATCTGGATGTCTGCATACATTTGATGAGGTATTTGGTGACGGATTCACAGTCATGGTACGT
TAGCCATTACTCCGTCACTGACAGATCGTTACTACATTCGCCATGTGTTTAAACGTGTAATGTCTGTTGAGAAGGAT
ACTGCCATGGTTGATGTGGAAGGATCGATGTCTCTCTCTAATAAGCGTTTAAATTTGTTGGGCTACGTTTAAAGGATCT
TGATCATGAATCTTGTAAGGGTGTATGACAATATTAGCAAAAACGCCTTGTTAATTTATTATTGTTGGATGTCTG
ATGTACCATCTAAGGCATCGTCATTTGTATCATTTGATTTGGATTATGTTGGCTAAATAAAAATATGTAATTTCTGTT
TTAAATAAATAACTGTGTATTGAACAATAAATTCTATTTTAAAGATTTCGGCTGAGCCGGTGACAATTACTATTAA
TACACTCATGGACCGTTGATCGTACAAGATCATTTAATTGGGCCATTGACATTGTTATATTGGATTGGGCCCTTGAA
GCCCCTACGATTGAAGCAGAATCACCTGGATCCAACGCACTGGTTCCAGACGATTGAGTTGCCTGTATGGGTGTA
TTGCATTTTCCAGTTCCGAATCTGTGTCCGTTTGGCCTATACCGATTGTGCTTCTTGAGGCCCATGACTCGCCTGGT
AATAATTCTATTGGGCTTGGTAGCCCAATTCTTGCCATTGAGATTGACCGGATCATTTTTCTTTCCCATCTCCCGTAG
CCCACATGTGAGAAATCGACATCCTTCTCGGAAAATTGCTTAGACAGGATCTTCACCGCCGGAGCTCTGAATGGGA
TATCTACAGAATGTTTCGCTGTGACAGTTTTAGTTTCCCTTTGAACTTGCGGAAATGTGTTGTTGGTGTACGTTT
GAATCACAGACTCTGTAGTATAGTTTCCACGGGATTGGGTCTTTCAGTGAGAAGAAAGACGAGGAAAAAGTAATGA
AGGTCTATGTTGCATCTGATTGGGAACGTCCATGATGCTTGTAAATGATTTCGTTATCCGTCATCCTTCTGTCTGGAT
CTCCACAATTACGTACCTGTTGCGTTTATCGGTACCTGTTGCCTGTATTCTATGACGCAGTGATCTATTTTCATACA
ACTGCGACTTAATCTTGCTGATAATTGAGCCGCTGCTGACGGAAAGTGCAGTATTATCTCAGTTAAATCATGAGAT
AGCTGATATTCGTCTCTGTGAGATTCTATATAATTAAGGCCACTTGGAGGATTAACCAATTGAGCATCCATATAGC

AAAATTAGCCCGCGCAGCGGAATGGATCTAACTGAGAGACGTCGTGGTGATTAATATAGGATGTTTTCTTGAAGA
AAAGAAGATGATATTTGGGAGAGTAGATCTGGATGTTTGTGAGGTTTTTGTGTGAAGAAGTATATGTGGTTCTGT
CTATATATAGACTTTATTAATGTATTGGAATAAACTAATAACAATTGCTTCATTGACACGTTTATGGTTGTACTATG
TCTCTACTGTTGCTAAAGAAGTTTAAGATCTATACTGGAAGCACTTAGTGGCATTGTGTGAATAAGGGATGTTCCC
CCAATTGCTAAGGGgTGTTCCCCCAATTGCTCCGCTCTCAAACTCTCTATGAATTGGGGAACTGGGGGTACATT
ATACTAGAACTCTCATTAAGGGATTGCAACACGTGGCGGCCATCCGCTATAATATT

Target sequences are shown in gray.

Tomato yellow leaf curl Sardinia virus; (TYLCSV; L27708)

TAATATTACCGGATGGCCGCGCTCCCCGATAAAGTAGTGGGCCCTACGCACTAATTTGTGTCGACCAATGAAAATG
CAGCCTCAAAGCTTAAATAATGATTTACTTTGTTATAAACTTGGCTTCTAAGTTTTGAAAAACCATCAATATGTG
GGATCCTCTATTAATGAATTTCCAGATTCAGTTCATGGTCTCCGTTGTATGCTTGCAATAAAATATTTGCAGCTAG
TTGAAGAAACCTATGAACCCAATACTCTGGGTACGATCTAATTAGGGATCTCATTTCCGTCATTCTGTGCTCGTGAC
TATGCCGAAGCGAACAGGCGATATACTAATGTCAAGCCCCTCTCGAAGTTTCGTCGAAAACCTGAACTTCGACAGC
CCGTATACCAGCCGTGCTGCTGCCCCACTGTCCAAGGCATCAAGCGTCGATCATGGACTTACAGGCCCATGTATC
GAAAGCCGCGGATGTACAGAATGTACAGAAGCCCTGATGTCCCGTTTGGTTGTGAAGGTCCTGTAAAGTCCAGT
CGTATGAGCAGCGTGACGACGTCAAGCATACCGGTGTTGTTGTTAGTGATGTAAGTGGGGTTCTGGTAT
TACACATAGAGTAGGTAAACGGTTTTGTATTAAGTCAATCTATATTTAGGGAAGATTGGATGGATGAAAAATATA
AAAAACAAAATCATACTAACCAGGTCATGTTCTTTTTAGTACGAGACCGAAGGCCGTATGGAACCTAGTCTATGG
ATTTTGGTCAAGTTTTTAACATGTTTGATAATGAACCTAGTACGGCTACTGTGAAGAACGATTTAAGGGATAGGTA
CCAAGTAATGAGGAAGTTCCATGCCACGGTGGTAGGTGGTCCGTCAGGGATGAAGGAGCAGTGTCTGTTGAAGA
GGTTTTTTAAAGTTAATACCCATGTAGTTTATAATCATCAAGAGCAGGCGAAGTATGAAAACCATACTGAGAATGC
GTTGTTGTTGTATATGGCATGTACTCATGCTTCTAACCAGTGTATGCTACGTTGAAAATACGTATCTATTTTTATGA
TGCTGTAACAAATTAATAAAGATTGTATTTATTTTATGTTGTTCAATTACATCTATTGTGTTCTCAAATACATCGAA
TAAGACATAATCAACTGCTCTAATAACATTGTTTAATGAAATTACACCTATATTATTCAAATACTTATACACTTGAA
CCTAAAGACTCTTAAAAAATGACCAGTCGGAGACTGTAAGGTGCTCCAGATGCGGAAGTTGAGAAAACATTTGTG
AATCCCCAGTGCCTTCCTCAAGTTGTGGTTGAACCTGATTTGAAATGTTAGTAAATTGTATTTGCTGTTGAATGGTT
GTTGGTTGTGTCTGGTTATCGTGAAATATAGGGGATTTGTTATCTCGAAGGTATAAACGCCACTCGTTGCTTGATG
CGCAGTGATGTACTCCCTGTGCGTAAATCCATGGTTTATGCAGTCTAAATGTATATAGTATGAACAGCCACAGTC
CAGATCTACCCTTCTACGCCTCACTTGTCTCTTGGCGATGTGGTGTGGATCTTGATTGGTATTTGTGAACAAT
GGCTGGTGGATGGTGACGAAGATTGCATTTTTATGGCCCAATTTTTAATGTTTGATTTTTCTTCGTCGAGGTA
TTCTTTAAATGATGATTGTGGGCCTGGATTGCAGAGGAAGATAGTGGGTATGCCTCCTTTAATTTGAATGGGCTC
CCATACTTTGTGTTGCTTTGCCAGTCCCTTTGGGACCCCATGAATCTTTAAAGTGTTTAAATAATGCGGGTCTAC
GTCATCAATGACGTTGTACCAAGCATTATTGCTATATACTTTTTGACTGAGGTCAAGATGTCCGCACAAATAATTAT
GTGGGCCTAGGGAACGGGCCACATAGTCTTTCTGTCCGGCTGTCAACCTCAATCACTATACTACCGGCCTCCA
AGGCCGCGCAGCGACATCCATGACGTTCTCGGAAACCCAGTGTCAAGTTCATCCGGAACCTGATCAAAAGAAGA
AGATAAAAAAGGAGAAACATATGGTGCCGGAGGCACCTGAAAAACCATATTTAAATTACTATTTATATTATGAAAA
TGTAATGTAATCTCTAGGGGCTAATCTTTAATTACATCAAGAGCCTCCGACTTACGTCCTGCGTTAATTGCCTT
GGCGTAAGCGTCATTGGCTGTCTGCTGTCTCCCTTGCAGATCGTCCGTCGATCTGGAAAGTACCCATTCAAGA
ACATCTCCGTCCTTGTGATGTAGGACTTGACGTGCGAGCTTGATTAGCTCCCTGAATGTTCCGATGGAAATGTG

CTGATCTGGTTGGGGATACCAGATCGAAGAATCTGTTATTTTTGCAGTTGAATTTACCCTCGAAC**TGAATGAGCAT**
GTGGAGATGAGGTTGCCCATCTTCGTGTAATTCTCTGCAAATCTTAATGTATTTTTATTGTTGGTGTGTTGTAGTTG
AAGTAGTTGTTCTAGGGCTTCTTCTTTGGAGAGAGAACATTTGGGAAAGTTAGAAAATAATGTTTTGCATTTATTT
GAAAACGCTTAGGCTGAGCCATTTGGTCAATGGGTACCAATTGACCTCAGATTCATTTTATTCCATGTATTGGTAGA
TTGGTAGCTCTTATATACCTGGGTACTAAATGGCATGTATGTAAATATGTTAAGTTATTTTTTAATTTATTTTAATTT
TTGGAATTTTAGC**GGCCATCCGTT**

Target sequences are shown in gray.

Tomato yellow leaf curl virus (TYLCV2.3)

ACCGGATGGCCGCGAATTTTGTGTGGGCCCCCTCAACGCACTAACTGACAAGGACATGCGAACCAATCAAATTGCA
TCCTCAAACGTTAGATAAGTGTTCAATTTGTcTTTATATACTTGGTCCCCAAGTATTTTGTCTTGCAATATGTGGGACC
CACTTCTAAATGAATTTCTGAATCTGTTACGGATTTCTGTTGTATGTTAGCTATTAATATTTGCAGGCTGTTGAG
GAAACTTACGAGCCCAATACATTGGGCCACGATTTAATTAGGGATCTTATATCTGTTGTAAGGGCCCGTGACTATG
TCGAAGCGACCAGGCGATATAATCATTTCCACGCCCGTCTCGAAGGTTGCGCGAAGGCTGAACTTCGACAGCCCAT
ACAGCAGCCGTGCTGCTGTCCCCATTGTCCAAGGCACAAACAAGCGCAGCATCATGGACGTACAGGCCCATGTACC
GAAAGCCCAGAATATACAGAATGTATCGAAGCCCTGATGTTCCCCGTGGATGTGAAGGCCCATGTAAAGTCCAGT
CTTATGAGCAACGGGATGATATTAAGCATACTGGTATTGTTCTGTTGTGTTAGTGATGTTACTCGTGGATCTGGAAT
TACTCACAGAGTGGGTAAGAGGTTCTGTGTTAAATCGATATATTTTTAGGTAAAGTCTGGATGGATGAAAATATC
AAGAAGCAGAATCACACTAATCAGGTCATGTTCTTCTTGGTCCGTGATAGAAGGCCTTATGGAAGCAGCCCAATG
GATTTTGGACAGGTTTTTAATATGTTGATAATGAGCCCAGTACCGCAACCGTGAAGAATGATTTGCGGGATAGGT
TTCAAGTGATAAGGAAATTTGCTACAGTTATTGGTGGGCCCTCTGGAATGAAGGAACAGGCATTAGTTAAGA
GATTTTTTAGAATTAACAGTCATGTAACCTATAATCATCAGGAGGCAGCCAAGTATGAGAACCATACTGAGAACGC
CTTGTTATTGTATATGGCATGTACGCATGCCTCTAATCCAGTGTATGCAACTATGAAAATACGCATCTATTTCTATG
ATTCAATATCAAATTAATAAATTTTATATTTTATATCATGACTTTCTGTTACATTTATTGTGTTTTCAAGTACATCATA
CAATACATGATCAACTGCTCTGATTACATTGTTAATGGAAATTACACCAAGACTATCTAAATACTTAAGAACTTGAT
ATCTAAATACTCTTAAGAAACGACCAGTCTGAGGCCGTAAGGTCGTCCAGATTTGGAAGTTGAGATAACATTTGTG
AATCCCCAGTACCTTCCTGATATTGTGATTGAATCTTATCTGTATTGAAATGATGTCGTGGCTCATTAGAAATGGCC
TCTCGTCGTGGTTGGTGATCTTGAAATATAGGGGATTTTCTATCTCCCATATAAAAACGCCATTCTGGGCTTGATGA
GCAGTGATGAGTTCCCCGGTGCGTGAATCCATGATTGATGCAGTTGATGTGGAGGTAATATGAGCATCCGCGAGTC
GAGGTCTATGCGCTTACGTCTGACTGGCTTAGTCTTCGCTATGCGGTGTTGGATTTTGATTGGCACTTGAGAACAG
TGGCTCGTAGAGGGTGACGAAGGTTGCATTCTTGAGAGCCCAATTTTTCAAGGATATGTTTTTTCTTCGTCTAGAT
ATTCCCTATATGATGAGGTAGGTCCTGGATTGCAGAGGAAGATAGTGGGAATCCCCCTTAATTTGAATCGGCTT
CCCGTACTTTGTGTTGCTTTGCCAGTCCCTCTGGGCCCCCATGAATTCCTTGAAGTGCTTTAAATAATGCGGGTctac
gtcatcgatgacgtgtaccacgcatcattactgtacaccttggacttaggtctagatgtccacataaataattatgtgggcctagagacctggcccac
attgtcttccccgttctgctatcaccctcgatgacaatactattaggtctCCATGGCCGCGCAGCGGAAGACATGACGTTCTCGGACA
CCCATACTTCAAGTTCATCTGGAACCTGATTAAAAGATGAAGATAAAAAGGGAGAAAATATAAGGAGCCGGAGGCT
CCTGAAAAATTCTATCTAAATTTGAATTTAAATTATGAAATTGAAGTATAAAGTCTCTAGGAGCTTTCTCCTTCAGTA
TATTGAGGGCCTGAGCTTTGGACCCTGAATTGATTGCCTCGGCATATGCGTCGTTGGCAGATTGGCAACCTCCTCT
AGCTGATCGTCCATCGACTTGGAAAACCTCATGATCAATGACGTCTCCGTCTTTTCCATATAGGATTTGACATCGC
TTGAACTCTTAGCTCCCTGAATGTTCCGATGGAAATGTGCTGACCTGGTTGGGGATGTGAGGTGCAAGAATCTGTT

GTTTTGCACTGGAACCTTCCTTCGAACTGGATGAGCACATGCAAGTGAGGAGTCCCATCTTCATGAAGCTCTCTGC
AGATTCTAATGAATTTTTGGAAGTGGGTGTTGTATATTTAATAATTGGGAAAGTGCTTCCTCTTTAGTTAGAGAG
CATTGGGATAAGTGAGAAAATAATTTTTGGCATTATTTAAACCGATTGGGGGCTGCCATATTGACTTGGTCAAT
CGGAGTCTCTCAACTCTTCTATGTATTGGTGTATTGGAGTCCTATATATATGGAGACTCCAATGGCATATATGTAA
ATATTGTACTTTAATTCAAAATCATCACGCTCCAAAAAGCGGCCATCCGTATAATATT

Target sequences are shown in gray.

Supplementary Table 1. Primers used in this study.

primers name	sequence (5' ---- 3')	Usage
TYLCV2.3-IR-T-F	AATTGGGAAAGTGCTTCCTCT	TYLCV IR flanking region
TYLCV2.3-IR-T-R	ATAGTCACGGGCCCTTACAACA	
TYLCV-IR-T1	CGAGTCTAGAGGCCATCCGTATAATATTA CGTTTTAGAGCTAGAAATAGCAAG	To clone TYLCV IR-sgRNA
SPDK-gRNA-R	acatGCCCCGgAAAAAAGCACCGACTCGG	To clone all sgRNA
TYLCSV-IR-F	CGAACTGAATGAGCATGTGG	To amplify IR region for T7EI and SspI assay And probe synthesis
TYLCSV-IR-R	TGCAAGCATACAACGGAGAC	
TYLCSV-IR-T CLCUKV-IR-T	CGAGTCTAGAGGCCATCCGTTTAATATTA CGTTTTAGAGCTAGAAATAGCAAG	To clone TYLCSV and CLCuKoV IR-sgRNA
TYLCSV-RCR11-T	CGAGTCTAGAGTGAATGAGCATGTGGAG ATGGTTTTAGAGCTAGAAATAGCAAG	To clone TYLCSV-RCR11- sgRNA
TYLCSV-RCR11-R	CATGCCATTTAGTACCCAGGT	To amplify RCR11 region for T7EI and RSL assay
TYLCSV-RCR11-F	TCCTGCGTTAATTGCCTTGG	
TYLCSV-CP-F	AAGCCGCGGATGTACAGAAT	To amplify CP region for T7EI and RSL assay
TYLCSV-CP-R	ACTTCGCCTGCTCTTGATGA	
TYLCSV-CP-T	CGAGTCTAGAGCCGTATGGAAGTAGTCCT AGTTTTAGAGCTAGAAATAGCAAG	To clone TYLCSV-CP- sgRNA
CLCUKV-CP-F	GTAATTCGTTGCTAAGTATGCGTTT	To amplify CP region for T7EI and RSL assay
CLCUKV-CP-R	TATGCTGAATATCATGTCTGGACTC	
CLCUKV-CP-T	CGAGTCTAGAGATGGGCTGTGCAAGTTGA GAGTTTTAGAGCTAGAAATAGCAAG	To clone CLCuKoV-CP- sgRNA
CLCUKV-RCR11-F	GACCTTCCGTCGATCTGAAAT	To amplify RCR11 region for T7EI and RSL assay
CLCUKV-RCR11-F	AGAGTTTGCAAGAACGGTCAAT	
CLCUKV-RCR11-T	CGAGTCTAGAGCTGGATGAGAACATGCA AGTGGTTTTAGAGCTAGAAATAGCAAG	To clone CLCuKoV-RCR11- sgRNA
MeMV-IR-T-F	TGTGCAGAGCTTTGATTTGG	To amplify IR region for T7EI and RSL assay
MeMV-IR-T-R	AAATTGGCGTTGCGACTAAC	
MeMV-IR-T	CGAGTCTAGAGCCATCCGCTATAATATTACGTT TAGAGCTAGAAATAGCAAG	To clone MeMV-IR-sgRNA
MeMV-RCR11-T	CGAGTCTAGAGTGGATAAGCACATGGAGATG GTTTTAGAGCTAGAAATAGCAAG	To clone MeMV-RCR11- sgRNA
MeMV-RCR11-T-F	CGTTTAACGCCTTTGCGTAT	To amplify RCR11 region for T7EI and RSL assay
MeMV-RCR11-T-R	AGAGTTTTGAGAGCGGAGCA	
MeMV-CP-T	CGAGTCTAGAGACGTGGAGGTATGGGCCCTA GTTTTAGAGCTAGAAATAGCAAG	To clone MeMV-CP-sgRNA
MeMV-CP-T-F	AAAGCCGTCCAATCACAAAG	To amplify CP region for T7EI and RSL assay
MeMV-CP-T-R	TCCTGTACGAATCAACCA	
MeMVB-IR-F	GCTTCATTGACACGTTTATGGTT	To amplify IR region of MeMV for T7EI and RSL assay
MeMVB-IR-R	TGGTTATAACTGGTCCACGTCTC	