

Supplementary Information

Truncated yet functional viral protein produced *via* RNA polymerase slippage implies underestimated coding capacity of RNA viruses

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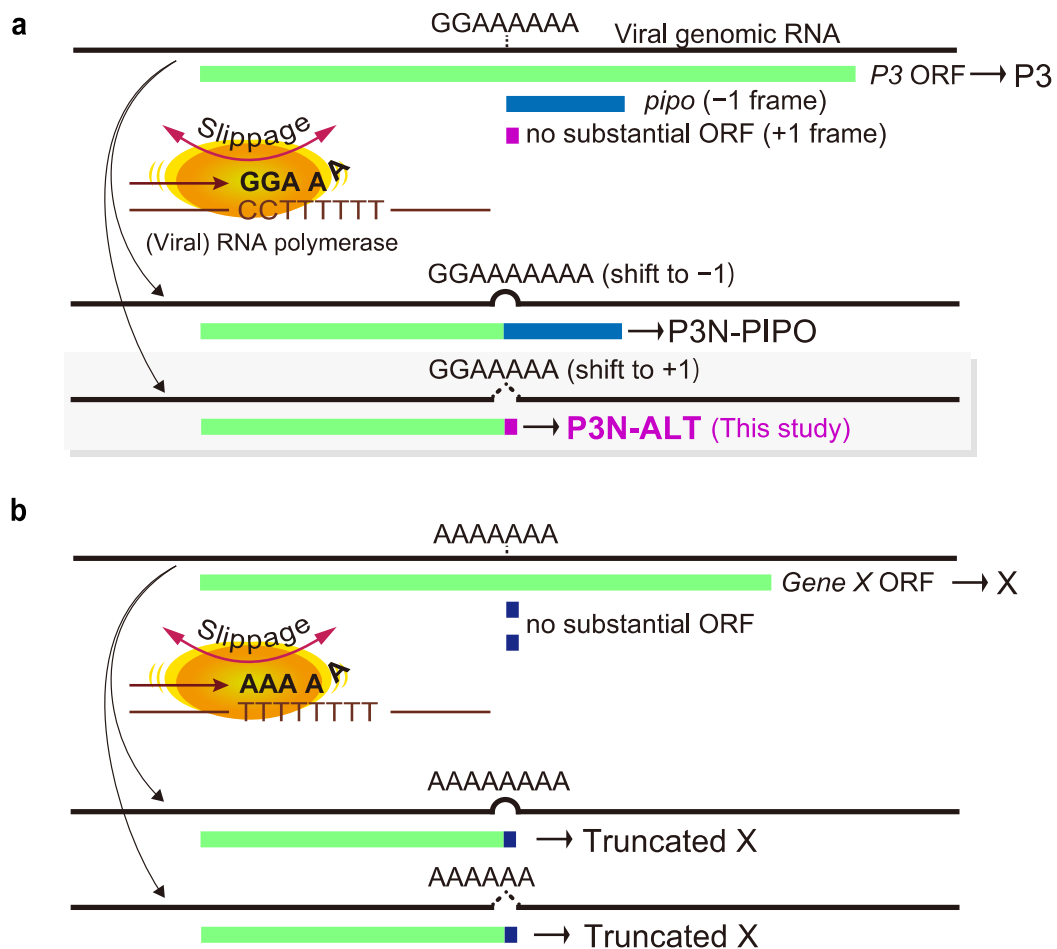
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Supplementary Methods

Supplementary References



Supplementary Figure 1. Schematic model of the production of viral protein truncations via transcriptional slippage (TS) by viral RNA polymerases. (a) A small ORF exists in the *P3* cistron at the -1 reading frame in the large ORF that encodes the potyvirus polyprotein. Potyviruses produce the P3N-PIPO protein *via* TS by viral RNA polymerase Nib at the $G_{1-2}A_{6-7}$ motif. This study revealed that TS also causes frameshift to the $+1$ reading frame to produce P3N-ALT. Although P3N-ALT is considered a truncation of P3 because there is no substantial ORF at the $+1$ reading frame following to the motif, it contributes to the cell-to-cell movement of CIYVV in infected plants. (b) The findings in this study imply that truncated, yet functional, proteins from a certain gene *X* can be produced *via* TS, even without a substantial ORF in alternative reading frames of the RNA genomes of other viruses. In fact, as unveiled in this study, a number of $G_{1-2}A_{6-7}$ and G_0A_{6-7} motifs with no substantial ORFs in one or two alternative frames are found in a diverse range of RNA viruses. Our study implied that not only a fusion protein such as P3N-PIPO shown in panel (a), but also a truncated protein as P3N-ALT could be produced *via* TS that causes a frameshift to an alternative frame where no substantial ORF exists.

RB-P3(PIPO:FLAG⁻¹)

P3N' (34.3 kDa)

MGKSLTGQVIQFDTKMLISSIYRPRQMEMIINEEPFVLVLMQSPSVLLALFNSASLEKAVEVWLHKDMRVSHVMTMLALLAAKVSAAKMVNLMQMEIEEA
SASHFLAAMDTHHKPMHSINTANIFLMNLEEGRSTDRITDELGFHSLKSSQVLMKEIWAEDLEQQWGLRLSQKPYLIRQSWKQRAKYSKILAQRDELG
ASDKFASLRLSATSIKNQAIISCRKRMVATRLVVEVEVVVEVVWTTKMTMTVIKIMTSTRIMMGKLDLDDPGLPAFVRIIGFDNVRQVQCISFIAHTPE
SY*

P3N-PIPO (29.8 kDa)

MGKSLTGQVIQFDTKMLISSIYRPRQMEMIINEEPFVLVLMQSPSVLLALFNSASLEKAVEVWLHKDMRVSHVMTMLALLAAKVSAAKMVNLMQMEIEEA
SASHFLAAMDTHHKPMHSINTANIFLMNLEEGRSTDRITDELGFHSLKSSQVLMKEK**DLGRGFRAAMARFKIVTKVLFNKAIVMEAGKVFQNI**SPERRAR
CQRQVQRITQIVSNKHKAPGPNQEQENGRNKTSGGSGGSGGSGSMDYKDHDGDYKDHDIDYKDHDG*

P3N-ALTy (18.2 kDa)

MGKSLTGQVIQFDTKMLISSIYRPRQMEMIINEEPFVLVLMQSPSVLLALFNSASLEKAVEVWLHKDMRVSHVMTMLALLAAKVSAAKMVNLMQMEIEEA
SASHFLAAMDTHHKPMHSINTANIFLMNLEEGRSTDRITDELGFHSLKSSQVLMKE**FGQRI***

C130-P3(PIPO:FLAG⁻¹)

P3N' (32.5 kDa)

MGKSLTGQVIQFDTKMLISSIYRPRQMEKIINEEPFVLVLMQSPSVLLALFNSASLEKAVEVWLHKDMRVSHVMTMLALLAAKVSAAKMVNLMQMEIEEA
SAGHFLAAMDTHHKPMHSINTANIFLMNLEEGRSTDRITDELGFHSLKSSQVLMKEIWAEDLEQQWGLRLSQKPYLIRQSWKQRAKYSKILAQRDELG
ASDKFASLRLSVTSILVVEVEVVVEVVWTTKMTMTVIKIMTSTRIMMGKLDLDDPGLPAFVRIIGFDNVRQVQCISFIAHTPE**SY***

P3N-PIPO (28.0 kDa)

MGKSLTGQVIQFDTKMLISSIYRPRQMEKIINEEPFVLVLMQSPSVLLALFNSASLEKAVEVWLHKDMRVSHVMTMLALLAAKVSAAKMVNLMQMEIEEA
SAGHFLAAMDTHHKPMHSINTANIFLMNLEEGRSTDRITDELGFHSLKSSQVLMKE**DLGRGFRAAMARFKIVTKVLFNKAIVMEAGKVFQNT**SPERRAR
CQRQVQRITQIVSNKHTSGGSGGSGGSGSMDYKDHDGDYKDHDIDYKDHDG*

P3N-ALT (18.1 kDa)

MGKSLTGQVIQFDTKMLISSIYRPRQMEKIINEEPFVLVLMQSPSVLLALFNSASLEKAVEVWLHKDMRVSHVMTMLALLAAKVSAAKMVNLMQMEIEEA
SAGHFLAAMDTHHKPMHSINTANIFLMNLEEGRSTDRITDELGFHSLKSSQVLMKE**SGQRI***

CS-P3(PIPO:FLAG⁻¹)

P3N' (35.3 kDa)

MGNQSVGSHRIDTELLVKSVMYKPELMAHIEQEFPFLLVLMQSPATLMALFNSSSLEKAVQYWLHKDMQVSHIMTMLAVLASNVSAKLLTTQFEVIEEA
SAPQILAAAMDHVYKPMHSINTANTFLMNLNESRETDKIDELGFYSPFKKSTRILMEK**TLMDLQWQELGLLERFSLIKRSWQVRAKYSSFAIQREEKG**
ITDKFTTSLRSGAQIKHQALAKRDQIVRFAERRVERTLVVEVEVVVEVVWTTKMTMTVIKIMTSTRIMMGKLDLDDPGLPAFVRIIGFDNVRQVQCIS
FIAHTPE**SY***

P3N-PIPO (30.6 kDa)

MGNQSVGSHRIDTELLVKSVMYKPELMAHIEQEFPFLLVLMQSPATLMALFNSSSLEKAVQYWLHKDMQVSHIMTMLAVLASNVSAKLLTTQFEVIEEA
SAPQILAAAMDHVYKPMHSINTANTFLMNLNESRETDKIDELGFYSPFKKSTRILMEK**DLGGLRSTMARIRVKGILFNKAIVASAKVFKFCNSERRER**
YHRQVHNLTVIRGTNKAAPGQERSNCAICREKSRREDTSGGSGGSGGSGSMDYKDHDGDYKDHDIDYKDHDG*

P3N-ALT (17.8 kDa)

MGNQSVGSHRIDTELLVKSVMYKPELMAHIEQEFPFLLVLMQSPATLMALFNSSSLEKAVQYWLHKDMQVSHIMTMLAVLASNVSAKLLTTQFEVIEEA
SAPQILAAAMDHVYKPMHSINTANTFLMNLNESRETDKIDELGFYSPFKKSTRILMEK**P***

RB-P3(ALT:FLAG⁺¹)

P3N' (18.4 kDa)

MGKSLTGQVIQFDTKMLISSIYRPRQMEMIINEEPFVLVLMQSPSVLLALFNSASLEKAVEVWLHKDMRVSHVMTMLALLAAKVSAAKMVNLMQMEIEEA
SASHFLAAMDTHHKPMHSINTANIFLMNLEEGRSTDRITDELGFHSLKSSQVLMKEIWAEDY*

P3N-PIPO' (26.2 kDa)

MGKSLTGQVIQFDTKMLISSIYRPRQMEMIINEEPFVLVLMQSPSVLLALFNSASLEKAVEVWLHKDMRVSHVMTMLALLAAKVSAAKMVNLMQMEIEEA
SASHFLAAMDTHHKPMHSINTANIFLMNLEEGRSTDRITDELGFHSLKSSQVLMKE**DLGRGLLVVEVEVVVEVVWTTKMTMTVIKIMTSTRIMMGK**
LDDPGLPAFVRIIGFDNVRQVQCISFIAHTPESY*

P3N-ALT:FLAG (21.9 kDa)

MGKSLTGQVIQFDTKMLISSIYRPRQMEMIINEEPFVLVLMQSPSVLLALFNSASLEKAVEVWLHKDMRVSHVMTMLALLAAKVSAAKMVNLMQMEIEEA
SASHFLAAMDTHHKPMHSINTANIFLMNLEEGRSTDRITDELGFHSLKSSQVLMKE**FGQRI**TSGGSGGSGGSGSM**DYKDHDGDYKDHDIDYKDHDG***

C130-P3(ALT:FLAG⁺¹)

P3N' (18.4 kDa)

MGKSLTGQVIQFDTKMLISSIYRPRQMEKIINEEPFVLVLMQSPSVLLALFNSASLEKAVEVWLHKDMRVSHVMTMLALLAAKVSAAKMVNLMQMEIEEA
SAGHFLAAMDTHHKPMHSINTANIFLMNLEEGRSTDRITDELGFHSLKSSQVLMKEIWAEDY*

P3N-PIPO' (26.2 kDa)

MGKSLTGQVIQFDTKMLISSIYRPRQMEKIINEEPFVLVLMQSPSVLLALFNSASLEKAVEVWLHKDMRVSHVMTMLALLAAKVSAAKMVNLMQMEIEEA
SAGHFLAAMDTHHKPMHSINTANIFLMNLEEGRSTDRITDELGFHSLKSSQVLMKE**DLGRGLLVVEVEVVVEVVWTTKMTMTVIKIMTSTRIMMGK**
LDDPGLPAFVRIIGFDNVRQVQCISFIAHTPESY*

P3N-ALT:FLAG (21.8 kDa)

MGKSLTGQVIQFDTKMLISSIYRPRQMEKIINEEPFVLVLMQSPSVLLALFNSASLEKAVEVWLHKDMRVSHVMTMLALLAAKVSAAKMVNLMQMEIEEA
SAGHFLAAMDTHHKPMHSINTANIFLMNLEEGRSTDRITDELGFHSLKSSQVLMKE**SGQRI**TSGGSGGSGGSGSM**DYKDHDGDYKDHDIDYKDHDG***

CS-P3(ALT:FLAG⁺¹)

P3N' (18.0 kDa)

MGNQSVGSHRIDTELLVKSVMYKPELMAHIEQEFPFLLVLMQSPATLMALFNSSSLEKAVQYWLHKDMQVSHIMTMLAVLASNVSAKLLTTQFEVIEEA
SAPQILAAAMDHVYKPMHSINTANTFLMNLNESRETDKIDELGFYSPFKKSTRILMEK**TY***

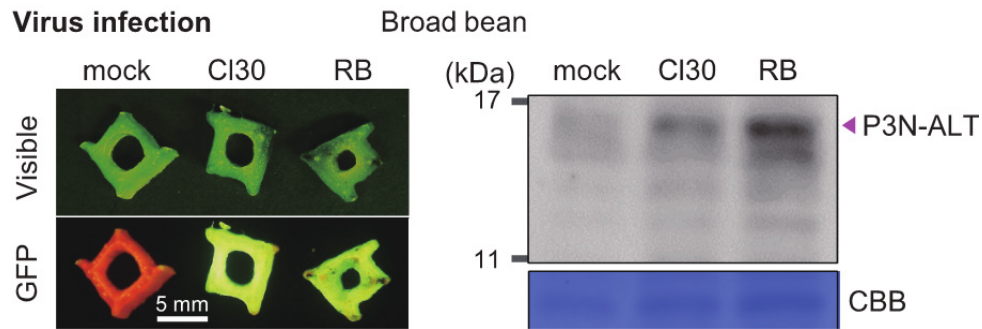
P3N-PIPO' (25.9 kDa)

MGNQSVGSHRIDTELLVKSVMYKPELMAHIEQEFPFLLVLMQSPATLMALFNSSSLEKAVQYWLHKDMQVSHIMTMLAVLASNVSAKLLTTQFEVIEEA
SAPQILAAAMDHVYKPMHSINTANTFLMNLNESRETDKIDELGFYSPFKKSTRILMEK**DLVVEVEVVVEVVWTTKMTMTVIKIMTSTRIMMGKLDLDDP**
GLPAFVRIIGFDNVRQVQCISFIAHTPESY*

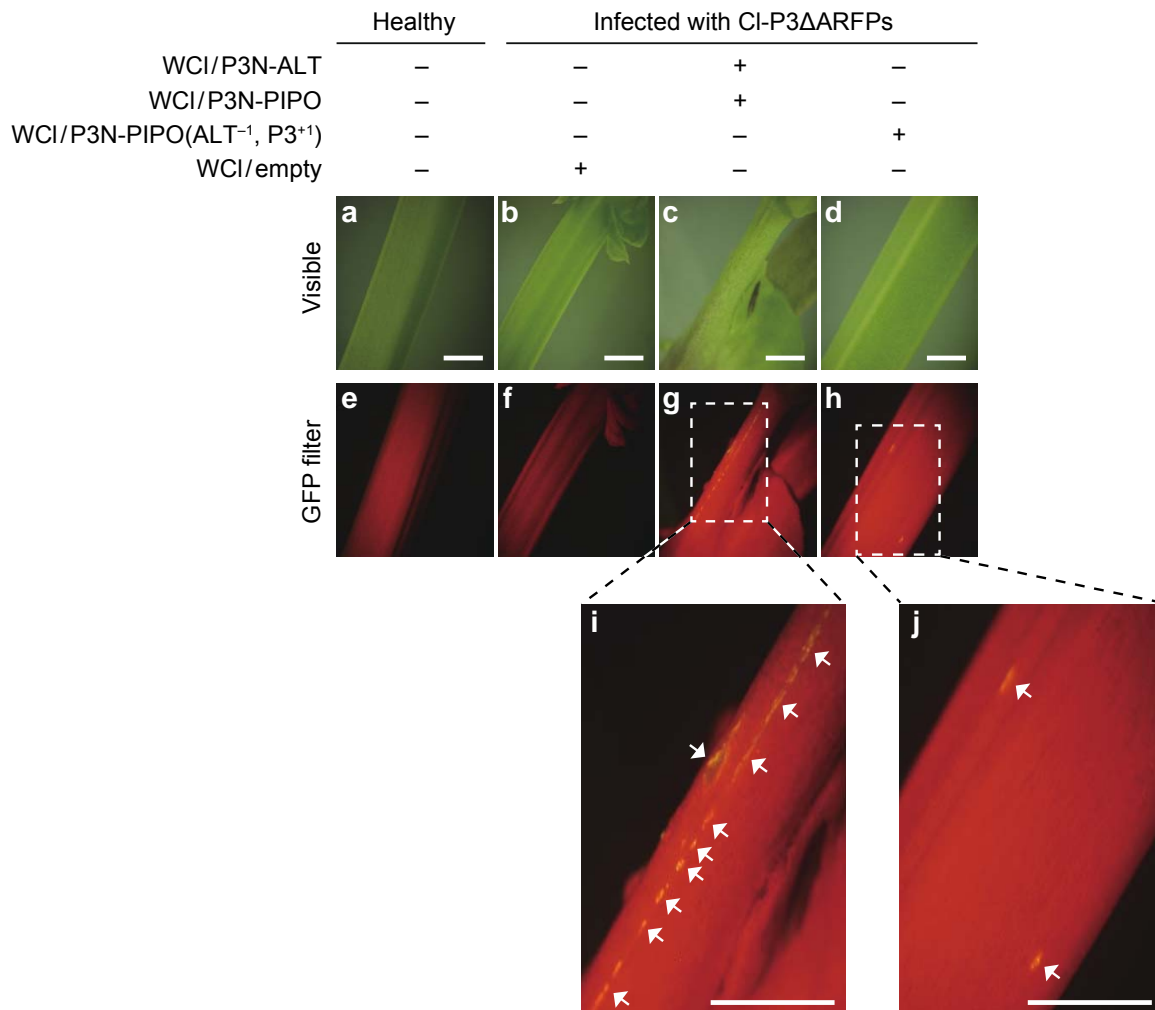
P3N-ALT:FLAG (21.5 kDa)

MGNQSVGSHRIDTELLVKSVMYKPELMAHIEQEFPFLLVLMQSPATLMALFNSSSLEKAVQYWLHKDMQVSHIMTMLAVLASNVSAKLLTTQFEVIEEA
SAPQILAAAMDHVYKPMHSINTANTFLMNLNESRETDKIDELGFYSPFKKSTRILMEK**PTSGGSGGSGGSGSM**DYKDHDGDYKDHDIDYKDHDG*

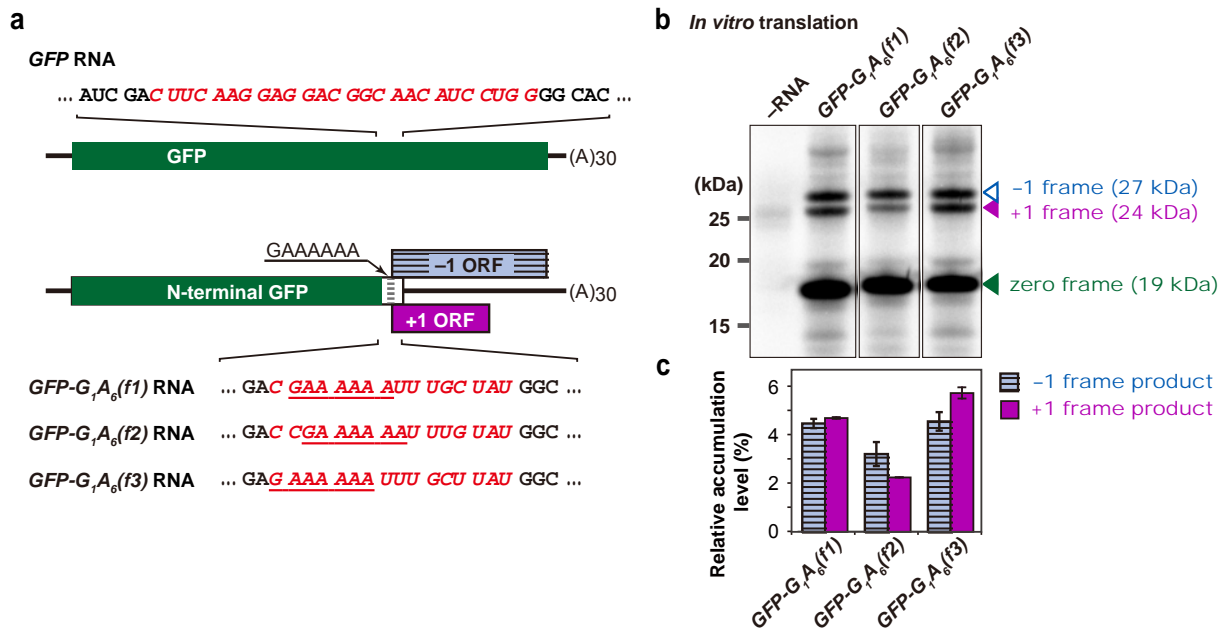
Supplementary Figure 2. Amino acid sequence of the constructs used in Fig. 2. Amino acid sequences of the three alternative reading frame products are shown for each of *RB-P3(PIPO:FLAG⁻¹)*, *C130-P3(PIPO:FLAG⁻¹)*, *CS-P3(PIPO:FLAG⁻¹)*, *RB-P3(ALT:FLAG⁺¹)*, *RB-P3(ALT:FLAG⁺¹)* and *CS-P3(ALT:FLAG⁺¹)* RNAs. For P3N' and P3N-PIPO', the C-terminal regions are different among the virus constructs. P3 and P3N sequences are shown in green, PIPO sequences are in blue (underlined), ALT sequences are in magenta (underlined), and the FLAG-tag sequence is shown in black (bold).



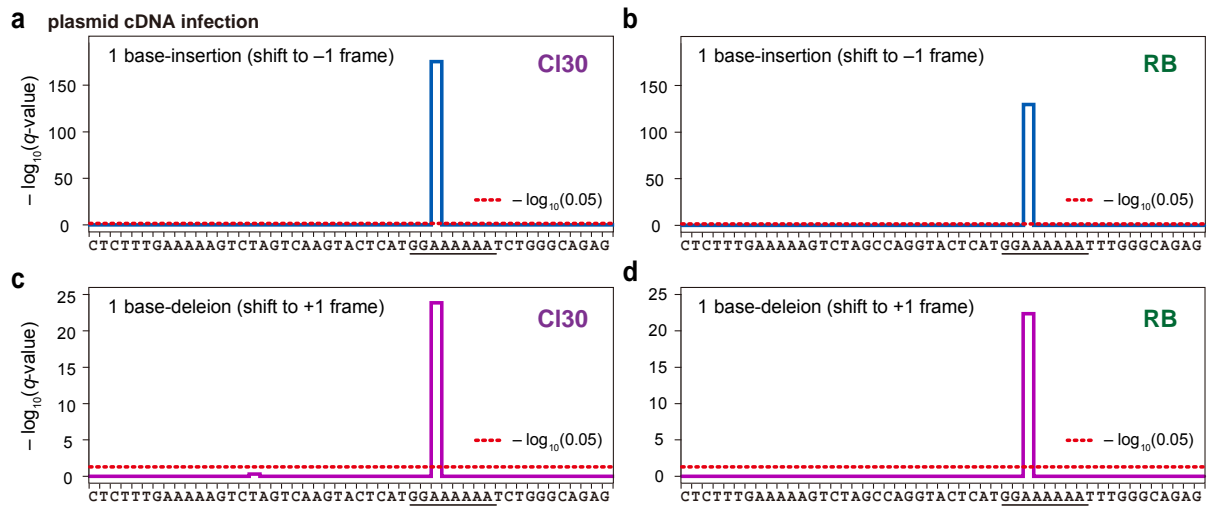
Supplementary Figure 3. Detection of P3N-ALT accumulation in CIYVV-infected broad bean stems. The stem tissues systemically infected with CI30 and RB as in Fig. 3 were harvested at 10 days post-inoculation. The infected areas were confirmed by virus-derived GFP fluorescence (left panel). P3N-ALT was detected by western blotting using a polyclonal antibody raised against the N-terminal region of P3. CBB-stained gel image is shown at the bottom of the western blot panel as a loading control. The position of P3N-ALT is marked with an arrowhead. Positions of molecular mass markers (kDa) are indicated on the left side of the panel.



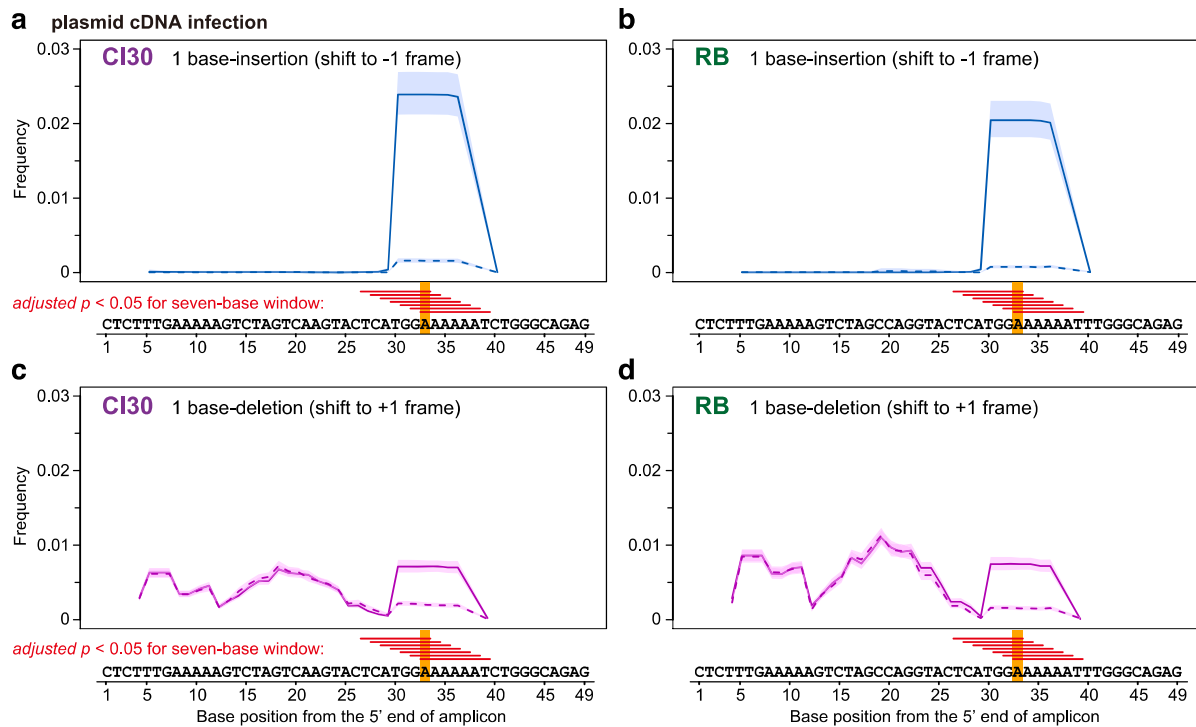
Supplementary Figure 4. Systemic infection of broad bean plants with CIYVV requires P3N-ALT and P3N-PIPO. Broad bean leaves were biolistically co-inoculated with CI-P3ΔARFPs (Fig.4a) and WCIMV vectors, WCI/P3N-ALT expressing P3N-ALT, WCI/P3N-PIPO expressing P3N-PIPO, WCI/P3N-PIPO(ALT⁻¹, P3⁺¹) expressing both P3N-PIPO and P3N-ALT, or an empty vector WCI/empty as indicated. Images of a healthy plant (**a**, **e**) are shown for comparison. Systemic movement of CI-P3ΔARFPs was examined by monitoring GFP fluorescence derived from CI-P3ΔARFPs. Visible images (**a–d**) and GFP fluorescence images in stems at 7 or 8 days post-inoculation (**e–j**) are shown. When co-inoculated with CI-P3ΔARFPs and WCI/empty, no GFP signal was detected in any of the non-inoculated parts of the plants (**f**), whereas sporadic GFP signals (arrows) were detected in systemic stems of plants that were co-inoculated with WCI/P3N-ALT plus WCI/P3N-PIPO (**g**, **i**) or WCI/P3N-PIPO(ALT⁻¹, P3⁺¹) (**h**, **j**). This suggests that expression of P3N-ALT and P3N-PIPO *in trans* using WCIMV vectors at least partly compensated for the defect of CI-P3ΔARFPs to enable systemic spread of the virus in the inoculated broad bean plants. The test was repeated twice, and representative images are shown. Scale bar = 5 mm.



Supplementary Figure 5. Alternative reading frame proteins are produced regardless of the original reading frame *in vitro*. (a) Schematic diagram of RNA used for *in vitro* translation analysis. The nucleotide sequences around the G₂A₆ motif derived from RB were replaced with *GFP* RNA sequence (red italic letters) in three different reading frames to generate *GFP-G₁A₆(f1)*, *GFP-G₁A₆(f2)* and *GFP-G₁A₆(f3)* RNAs. The replaced sequences are marked in red italic letters. Spaces denote the zero reading frame. The G₂A₆ motif is underlined. (b) *In vitro* translation analysis using WGE. RNA prepared *in vitro* were translated in the presence of [³⁵S]methionine. The translation products were separated by SDS-PAGE, and the signals were visualized by autoradiography. The positions of zero frame, +1 frame, and -1 frame products are marked on the right side of the panel. Positions of molecular mass markers (kDa) are indicated on the left. (c) The accumulated levels of -1 and +1 frame proteins produced in each construct. In the calculation, the number of methionine codons present in each product was taken into account. The value for each product was obtained from at least two independent translation assays. The means and standard deviations are shown.



Supplementary Figure 6. Results of statistical tests for enrichment of indels in systemically infected viral cDNA. (a–d) Solid lines indicate q -values for enrichment of one base insertion (a,b) and one base deletion (c,d) in viral cDNAs from CI30 (a,c) and RB (b,d), which are shown in Fig. 5b,c. Fisher’s exact test was performed for the indel counts observed at each position in the amplicons from systemically infected viral RNA and control plasmids used for inoculation (Supplementary Methods). The q -values were obtained by multiple test correction of p -values calculated by Fisher’s exact test using Storey’s method. The vertical axis represents negative \log_{10} -transformed q -values. The red dashed lines indicate a statistical cut-off (q -value = 0.05).



Supplementary Figure 7. Frequency of transcriptional slippage at CIYVV P3 region estimated using a negative binomial regression. Frequencies of one base insertions in CI30 (a) and RB (b), and that of one base deletions in CI30 (c) and RB (d), are shown. Sites and distribution of indels at the CIYVV P3 region were measured by amplicon sequencing of cDNA prepared from total RNA of CI30 or RB-infected broad bean leaves. As controls, plasmids used for inoculation were subjected to sequencing. The frequency of insertions and deletions per base was estimated using a negative binomial regression as a ratio to the total number of reads obtained from respective samples. Solid and dashed lines indicate estimated indel frequencies in systemically propagated viral sample and inoculated plasmid sample, respectively. The indicated frequency was estimated in a window size of seven bases (Supplementary Table 2), with a step size of one base. Standard errors of the estimated frequencies are shown as shaded area. Numbers at the bottom indicate the position in the amplicons (numbered from the 5' end). Seven base-windows in which indel frequencies are statistically significantly different between viral RNA and control plasmid are shown with red bars under the plot (adjusted $p < 0.05$). The adjusted p -values were obtained by multiple test correction using Holm's method. The 33rd A, which is the common site among all the significant windows is highlighted by orange bars. Estimated indel frequencies obtained using different window sizes are shown in Supplementary Table 2.

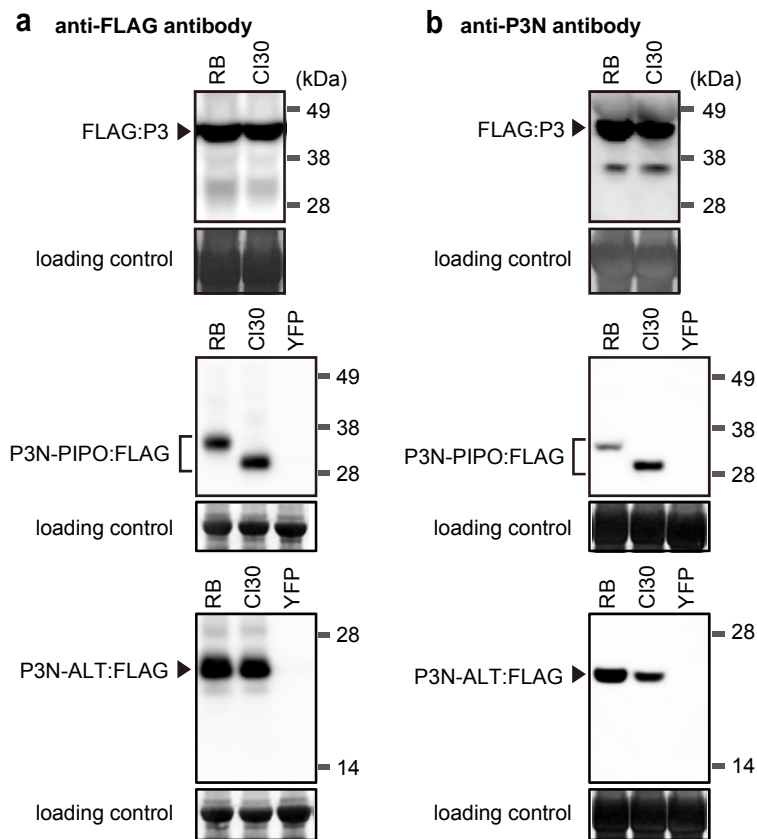
Supplementary Figure 8. Alignment of the P3N-ALT amino acid sequences of potyviruses. Potyviral ORF sequences were retrieved from GenBank using the queries “potyvirus” and “complete genome”. These entries were further filtered by selecting for those with annotation for the *P3* cistron. The *P3* cistron sequences of the resultant 197 potyvirus genomes were obtained and one base deletion at the $G_{1-2}A_{6+}$ or G_0A_{6+} motif was simulated using a custom Perl script. The simulated sequences were aligned using Muscle v3.8.31 software. For those entries that gave identical P3N-ALT amino acid sequence, only one entry is shown. These include (the omitted Accession Nos. are listed in the parentheses), plum pox virus: AB576046 (AB576047, AB576048, AB576049, AB576050, AB576051, AB576052, AB576054, AB576055, AB576056, AB576057, AB576058, AB576059, AB576061, AB576062, AB576063, AB576064, AB576065, AB576066, AB576067, AB576068, AB576069, AB576070, AB576073, AB576074, AB576075, AB576076, AB576077, AB576078, AB576079, AB576080); potato virus Y: AB711153 (AB711154, AB711155, AB714134); AB711143 (AB711152); AB711147 (AB711148); AB711145 (AB711146); bean yellow mosaic virus: AB079886 (AB079887); konjac mosaic virus: NC_007913 (AB219545); papaya leaf distortion mosaic virus: AB088221 (NC_005028); soybean mosaic virus: AY294044 (AJ312439); zucchini yellow mosaic virus: AB188115 (AB188116); Algerian watermelon mosaic virus: EU410442 (NC_010736); Moroccan watermelon mosaic virus: EF579955 (NC_009995); sugarcane mosaic virus: JX237862 (JX237863); peanut mottle virus: AF023848 (NC_002600); turnip mosaic virus: AB701740 (AB701741); AB701702 (AB701728, AB701731, AB701732); AB701700 (AB701719); AB701712 (AB701714); AB194790 (AB194791); AB194785 (AB194786, AB194787, AB194788, AB194789, AB194792, AB194793, AB194794, AB194795, AB194796, AB194797, AB194798, AB194799, AB194800, AB194801, AB194802); AB701733 (AB701738); AB701734 (AB701735); AB701690 (AB701691); Habeneria mosaic virus: AB818538 (NC_021786); chilli veinal mottle virus: AJ972878 (AM909717). As a result, 125 entries are shown. Lupine mosaic virus (LuMV; Accession No. NC_014898) was recently isolated⁶⁸ and is distantly related to other potyviruses⁴⁹. LuMV does not carry the $G_{1-2}A_{6-7}$ motif⁶⁷, but carries G_0A_6 within the *P3* cistron at a similar position to other potyviruses. Amino acid sequences of the ALT region were aligned manually. LuMV was placed at the bottom of the figure. Number of amino acid residues from the N-terminus of P3N is indicated. The amino acid sequence EK, which is highlighted in yellow, is of the expected slippage site of $G_{1-2}A_{6-7}$ motif in P3. P3N and ALT regions are marked in green and magenta, respectively.

Accession	P3N		Viral species
AB576053	G	-----LEVD	KCDEFKN - VKLLIRSIYKPKIMEQVLKEEYLLLMSVLS PGV 46 plum pox virus
AB576046	G	-----LEVD	KCDEFKN - VKLLIRSIYKPKIMEQVLKEEYLLLMSVLS PGV 46 plum pox virus
AB576072	G	-----LEVD	RCDEFKN - VKLLIRSIYKPKIMEQVLKEEYLLLMSVLS PGV 46 plum pox virus
AB576071	G	-----LEVD	KCDEFKN - VKLLIRSIYKPKIMEQVLKEEYLLLMSVLS PGV 46 plum pox virus
AB594926	G	-----LEVD	KCDEFKN - VKLLIRSIYKPKIMEQVLKEEYLLLMSVLS PGV 46 plum pox virus
AB576045	G	-----LEVD	KCDEFKN - VKLLIRSIYKPKIMEQVLKEEYLLLMSVLS PGV 46 plum pox virus
AB576069	G	-----LEVD	KCDEFKN - VKLLIRSIYKPKIMEQVLKEEYLLLMSVLS PGV 46 plum pox virus
NC_001445	G	-----LEVD	KCDEFKN - VKLLIRSIYKPKIMEQVLKEEYLLLMSVLS PGV 46 plum pox virus
NC_004752	G	-----LVHH	SEHDSRS - VKLLIKSVYKPKLMEQVLMEEYLLLVFAMVSPCV 46 Yam mosaic virus
AB711144	G	- I P G A C P E L G S T I S P F R E G G I -	IMSESAA - LKLLKGFIFRPKVMRQLLDEPYLLLSILSPGI 62 potato virus Y
AB714135	G	- I P G A C P E L G S T I S P F R E G G I -	IMSESAA - LKLLKGFIFRPKVMRQLLDEPYLLLSILSPGI 62 potato virus Y
AB711153	G	- V P N A C P E L G S T I S P F R E G G V -	IMSESAA - LKLLKGFIFRPKVMRQLLDEPYLLLSILSPGI 62 potato virus Y
NC_001616	G	- V P N A C P E L G S T I S P F R E G G V -	IMSESAA - LKLLKGFIFRPKVMRQLLDEPYLLLSILSPGI 62 potato virus Y
AM113988	G	- V P N A C P E L G S T I S P F R E G G V -	IMSESAA - LKLLKGFIFRPKVMRQLLDEPYLLLSILSPGI 62 potato virus Y
AB711143	G	- V P N A C P E L G S T I S P F R E G G V -	IMSESAA - LKLLKGFIFRPKVMRQLLDEPYLLLSILSPGI 62 potato virus Y
AB711151	G	- V P N A C P E L G S T I S P F R E G G V -	IMSESAA - LKLLKGFIFRPKVMRQLLDEPYLLLSILSPGI 62 potato virus Y
AB711149	G	- V P N A C P E L G S T I S P F R E G G V -	IMSESAA - LKLLKGFIFRPKVMRQLLDEPYLLLSILSPGI 62 potato virus Y
AB711147	G	- V P N A C P E L G S T I S P F R E G G V -	IMSESAA - LKLLKGFIFRPKVMRQLLDEPYLLLSILSPGI 62 potato virus Y
AB711145	G	- V P N A C P E L G S T I S P F R E G G V -	IMSESAA - LKLLKGFIFRPKVMRQLLDEPYLLLSILSPGI 62 potato virus Y
AB711150	G	- V P N A C P E L G S T I S P F R E G G V -	IMSESAA - LKLLKGFIFRPKVMRQLLDEPYLLLSILSPGI 62 potato virus Y
FJ214726	G	- I P S A C P E L G S T R S P F R E G G V -	IMSESEA - LKLLKGFIFRPKVMRQLLDEPYLLLSILSPGI 62 potato virus Y
AB126033	G	-----T V N H K V K I D D Q P G R C G -	VSEFHA - IRMLIKGIVYRPSVMYEELSEEYLLLVFSLSPSI 58 pepper mottle virus
AF501591	G	IVENHKVQIDS-----QPSSR	GVSEFHA - RMLIKGIVYRPSVMYEELSEEYLLLVFSLSPSI 58 pepper mottle virus
NC_001517	G	-----IVENHKVQIDNQPSSR	GVSEFHA - RMLIKGIVYRPSVMYEELSEEYLLLVFSLSPSI 58 pepper mottle virus
AB570195	G	-----KK	FTVEYEA - VKLLIKAIYRPKLMRTLMEDEPYLLCLAMCSPGV 44 lily mottle virus
AM048875	G	-----RK	FTVEYEA - VKLLIKAIYRPKLMRTLMEDEPYLLCLAMCSPGV 44 lily mottle virus
JN127341	G	-----QK	FTVEYEA - VKLLIKAIYRPKLMRTLMEDEPYLLCLAMCSPGV 44 lily mottle virus
NC_003492	G	-----DWN	FGNRVRT - DTKFLKLSIYRDPDLLEIEHEPFVLLVLAQSPAV 46 bean yellow mosaic virus
AB079888	G	-----DWN	FGNRVRT - DTKFLKLSIYRDPDLLEIEHEPFVLLVLAQSPAV 46 bean yellow mosaic virus
AB079886	G	-----DYN	FGNRVRT - DTKFLKLSIYRDPDLLEIEHEPFVLLVLAQSPAV 46 bean yellow mosaic virus
AB011819	G	-----KSLT	QVYIQFD - TKMLISSIYRPRQMEKINEEYVLLVLAQSPAV 46 clover yellow vein virus
NC_007913	G	-----TANQ	LLMPQNA - FSLVKGIVYRPEVMLQILEEYVLLVLAQSPAV 46 konjac mosaic virus
AJ885005	G	-----KFVA	SHLVMTN - FNTLTKAIYRADLMRDLTHEPVLLVLAQSPAV 46 thunberg fritillary virus
AB088221	G	-----DLHS	KQAPQCS - KLLCKIYRPKLMRQCIEEYVLLVLAQSPAV 46 papaya leaf distortion mosaic virus
NC_001768	G	-----LVYS	ENNDASA - VKALTQAIYRPDVLSLEIEEYVLLVLAQSPAV 46 tobacco vein mottling virus
AJ131401	G	-----TSNS	QINEYAT - IKMLAKAVYRPKLMKEIEHEQPYVLMVLSLSPGI 46 potato virus A
NC_004039	G	-----TSNS	QINEYAT - IKMLAKAVYRPKLMKEIEHEQPYVLMVLSLSPGI 46 potato virus A
AJ131400	G	-----TPNS	QINGYAT - IKMLAKAVYRPKLMREIEHEQPYVLMVLSLSPGI 46 potato virus A
AJ131402	G	-----TPNS	QINGYAT - IKMLAKAVYRPKLMREIEHEQPYVLMVLSLSPGI 46 potato virus A
AJ131403	G	-----TSNS	QINGYST - IKMLAKAVYRPKLMREIEHEQPYVLMVLSLSPGI 46 potato virus A
NC_007728	G	-----VME	QRMKC - ETALISSIFKPKRMIQILEDDPYLLVLAQSPAV 43 east Asian passiflora virus
AB046110	G	-----E	VEQRMC - ETALISSIFKPKRMIQILEDDPYLLVLAQSPAV 43 east Asian passiflora virus
DQ399708	G	-----E	AQRMKC - ETALIKSIFKPKRMIQILEDDPYLLVLAQSPAV 43 watermelon mosaic virus
AB218280	G	-----E	AQRMKC - ETALIKSIFKPKRMIQILEDDPYLLVLAQSPAV 43 watermelon mosaic virus
KC292915	G	-----V	AQRMKC - ETALITSIFKPKRMIHILEDDPYLLVLAQSPAV 43 watermelon mosaic virus
AY294044	G	-----E	VQRMKC - ETALITSIFKPKRMIQILEDDPYLLVLAQSPAV 43 soybean mosaic virus
D00507	G	-----E	VQRMKC - ETALITSIFKPKRMIQILEDDPYLLVLAQSPAV 43 soybean mosaic virus
AY294045	G	-----E	VQRMKC - ETALITSIFKPKRMIQILEDDPYLLVLAQSPAV 43 soybean mosaic virus
AY216010	G	-----E	VQRMKC - ETALITSIFKPKIMVQILEDDPYLLVLAQSPAV 43 soybean mosaic virus
AY216987	G	-----E	VQRMKC - ETALITSIFKPKIMVQILEDDPYLLVLAQSPAV 43 soybean mosaic virus
AY278998	G	-----T	PTQRIKL - EEQLIKGIFKPKLMMQLLHDDPYLLVLAQSPAV 43 zucchini yellow mosaic virus
AB188115	G	-----T	PTQRIKL - EEQLIKGIFKPKLMMQLLHDDPYLLVLAQSPAV 43 zucchini yellow mosaic virus
AY278999	G	-----A	PTQRIKL - EEQLIKGIFKPKLMMQLLHDDPYLLVLAQSPAV 43 zucchini yellow mosaic virus
AY279000	G	-----A	PTQRIKL - EEQLIKGIFKPKLMMQLLHDDPYLLVLAQSPAV 43 zucchini yellow mosaic virus
AF014611	G	-----T	PTQRIKL - EEQLIKGIFKPKLMMQLLHDDPYLLVLAQSPAV 43 zucchini yellow mosaic virus
EU410442	G	-----T	PTQRIKL - EEQLIKGIFKPKLMMQLLHDDPYLLVLAQSPAV 43 zucchini yellow mosaic virus
EF579555	G	-----NPD	EAPVMPD - IRLIKGIVYRPKLMELELLNPFVLAQSPAV 45 Algerian watermelon mosaic virus
AJ278405	G	-----TLT	QTFNTL - LKSLTKNMFKPERIKQIEEYVLLVLAQSPAV 45 Moroccan watermelon mosaic virus
BJ237862	G	-----TLT	QTFNTL - LKSLTKNMFKPERIKQIEEYVLLVLAQSPAV 45 sugarcane mosaic virus
AJ297628	G	-----TLT	QTFNTL - LKSLTKNMFKPERIKQIEEYVLLVLAQSPAV 45 sugarcane mosaic virus
U57358	G	-----TLT	HNTFSSL - MKLLTKNMFKPERIKQIEEYVLLVLAQSPAV 45 sugarcane mosaic virus
NC_003377	G	-----TIT	HKFSFSL - ISHLTKNMFKPERIKQIEEYVLLVLAQSPAV 45 maize dwarf mosaic virus
AB465608	G	-----VTAL	PDQERC - IRLIKGIVYRPKLMELELLNPFVLAQSPAV 46 sweet potato feathery mottle virus
AB439206	G	-----VTAL	PDQERC - IRLIKGIVYRPKLMELELLNPFVLAQSPAV 46 sweet potato feathery mottle virus
NC_001841	G	-----VTAL	PDQERC - IRLIKGIVYRPKLMELELLNPFVLAQSPAV 46 sweet potato feathery mottle virus
NC_001671	G	-----TQKMEINM	CCCQRKNLLKQLRAIYRPKLMELEETEPVLAQSPAV 52 pea seedborne mosaic virus
EU119422	G	-----TLE	NLSVMA - VQVLLKSLWRPKLMLNLVTQEPFLVLAQSPAV 45 cocksfoot streak virus
GU214748	G	-----DAN	SRRMRM - ERALKGIVYRPKLMLMHIEEDPYLLVLAQSPAV 45 freesia mosaic virus
AF023848	G	-----EAP	HARRMR - EKALIKGIVYRPKLQVLYLIEEDPYLLVLAQSPAV 45 peanut mottle virus
NC_000947	G	-----PILNNEIDPTE	YRTPSWH - LRLLIKGIVYRPKLQVLLDDIRDRYVLAQSPAV 54 Japanese Yam mosaic virus
NC_011541	G	-----DPLAEETEQI	LSDPNWN - LRLLIKGIVYRPKLQVLLDDIRDRYVLAQSPAV 54 narcissus yellow stripe virus
AB701695	G	-----TKWEDAHGTSN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701694	G	-----TKWEDAHGTSN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701737	G	-----TKWEDAHGANN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701720	G	-----TKWEDAHGANN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701726	G	-----TKWEDAHGASN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701705	G	-----TKWEDAHGASN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701696	G	-----TKWEDAHGASN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701706	G	-----TKWEDAHGASN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701707	G	-----TKWEDAHGASN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701721	G	-----TKWEDAHGASN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701697	G	-----TKWEDTHGVSN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701725	G	-----TKWEDTHGVSN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701701	G	-----TKWEDTHGVSN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701740	G	-----TKWEDTHGVSN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701702	G	-----TKWEDTHGVSN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701710	G	-----TKWEDTHGVSN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701736	G	-----TKWEDTHGVSN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701708	G	-----TKWEDTHGVSN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701703	G	-----TKWEDTHGVSN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701699	G	-----TKWEDTHGVSN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701729	G	-----TKWEDTHGVSN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701716	G	-----TKWEDTHGVSN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701717	G	-----TKWEDTHGVSN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701739	G	-----TKWEDTHGVSN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701700	G	-----TKWEDTHGVSN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701727	G	-----TKWEDTHGVSN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701742	G	-----TKWEDTHGVSN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701711	G	-----TKWEDTHGVSN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB362512	G	-----TKWEDTHGVSN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701712	G	-----TKWEDTHGVSN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701718	G	-----TKWEDTHGVSN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701715	G	-----TKWEDTHGVSN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701713	G	-----TKWEDTHGVSN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB194790	G	-----TKWEDTHGVSN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB194785	G	-----TKWEDTHGVSN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701730	G	-----TKWEDTHGVSN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701733	G	-----TKWEDTHGVSN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701709	G	-----TKWEDTHGVSN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701704	G	-----TKWEDTHGVSN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB362513	G	-----TAWEAAGFN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701722	G	-----TKWEDVHGTSN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701723	G	-----TKWEDVHGTSN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701724	G	-----TKWEDVHGTSN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB440238	G	-----TKWEDVHGTSN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB440239	G	-----TKWEDVHGTSN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701734	G	-----TRWEDVHGTSN	VDDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701698	G	-----TKWADTHGATN	INDPQWC - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701690	G	-----TILEDAYGAEN	IADPQWN - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701692	G	-----TILEDAYGAEN	IADPQWN - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB701693	G	-----TILEDAYGAEN	IADPQWN - IRLIKGIVYRPKLQKEDMLNPFVLAQSPAV 53 turnip mosaic virus
AB818538	G	-----TNYGTDHTRS	LKQVIRGIVYRPNELRSILSHDYLLVLAQSPAV 47 Habeneria mosaic virus
NC_005778	G	-----ETQ	SFGFT - RLMRVYRPSVFEQLMIDEPLTLVLAQSPAV 44 chili vein mottle virus
AJ972879	G	-----EEMO	SLPGFT - RLMRVYRPSVFEQLMIDEPLTLVLAQSPAV 44 chili vein mottle virus
NC_014898	G	-----RTDNLDPALGN	GNNRTNI - MSQLIRSIYRPNEMHRMLTEHPYVLAQSPAV 54 lupine mosaic virus

Supplementary Figure 8 (part 1/3)

Table with columns: Accession, P3N, ALT, and Viral species. Contains a list of genetic sequences and their corresponding viral species, such as plum pox virus, pepper mottle virus, and various mosaic viruses.

Supplementary Figure 8 (part 3/3)



Supplementary Figure 9. Western blotting to confirm specific binding of the anti-P3N peptide antibody to the FLAG-tagged P3 products of C130, P3, P3N-PIPO and P3N-ALT. RNAs that produce FLAG:P3, P3N-PIPO:FLAG and P3N-ALT proteins (Supplementary Methods) were transiently expressed in *N. benthamiana* leaves by agroinfiltration, as in Fig. 2. The products were detected by western blotting using an anti-FLAG monoclonal antibody (**a**) or the anti-P3N peptide polyclonal antibody (**b**). Loading controls are the part of the PVDF membranes covering the RubisCO large subunit band that were stained with Amido Black after western transfer. The membranes were stained with 0.1% Amido Black in 45% methanol and 10% acetic acid followed by destaining in 90% methanol and 2% acetic acid.

Supplementary Table 1. Occurrence of G₀A₆₊ and G₁₋₂A₆₊ motifs in GenBank/EMBL/DDBJ accessions.

Family	Genus	number of accessions			% found	
		total ^a	G ₀ A ₆₊ ^b	G ₁₋₂ A ₆₊ ^b	G ₀ A ₆₊	G ₁₋₂ A ₆₊
Alphaflexiviridae		132	86	20	65.2	15.2
	<i>Potexvirus</i>	132	86	20	65.2	15.2
Alphatetraviridae		1	0	0	0.0	0.0
	<i>Betatetravirus</i>	1	0	0	0.0	0.0
Arteriviridae		465	379	291	81.5	62.6
	<i>Arterivirus</i>	465	379	291	81.5	62.6
Astroviridae		14	14	14	100.0	100.0
	<i>Avastrovirus</i>	14	14	14	100.0	100.0
Bornaviridae		12	4	0	33.3	0.0
	<i>Bornavirus</i>	12	4	0	33.3	0.0
Betaflexiviridae		48	41	14	85.4	29.2
	<i>Capillovirus</i>	17	17	7	100.0	41.2
	<i>Carlavirus</i>	2	1	0	50.0	0.0
	<i>Foveavirus</i>	29	23	7	79.3	24.1
Betaflexiviridae		45	35	9	77.8	20.0
	<i>Trichovirus</i>	26	26	8	100.0	30.8
	<i>Vitivirus</i>	19	9	1	47.4	5.3
Caliciviridae		81	23	6	28.4	7.4
	<i>Lagovirus</i>	49	22	6	44.9	12.2
	<i>Norovirus</i>	22	0	0	0.0	0.0
	<i>Sapovirus</i>	10	1	0	10.0	0.0
Closteroviridae		118	72	35	61.0	29.7
	<i>Closterovirus</i>	75	71	34	94.7	45.3
	<i>Vesivirus</i>	43	1	1	2.3	2.3
Coronaviridae		535	442	212	82.6	39.6
	<i>Alphacoronavirus</i>	191	149	18	78.0	9.4
	<i>Betacoronavirus</i>	336	287	194	85.4	57.7
	<i>Bafinivirus</i>	1	1	0	100.0	0.0
	<i>Deltacoronavirus</i>	4	2	0	50.0	0.0
	<i>Gammacoronavirus</i>	1	1	0	100.0	0.0
	<i>Torovirus</i>	2	2	0	100.0	0.0
Filoviridae		162	162	47	100.0	29.0
	<i>Ebolavirus</i>	162	162	47	100.0	29.0
Flaviviridae		5,990	5,351	2,138	89.3	35.7
	<i>Flavivirus</i>	4,765	4,761	1,873	99.9	39.3
	<i>Hepacivirus</i>	1,035	400	139	38.6	13.4
	<i>Pestivirus</i>	190	190	126	100.0	66.3
Hepeviridae		35	0	0	0.0	0.0
	<i>Hepevirus</i>	35	0	0	0.0	0.0
Hypoviridae		7	2	2	28.6	28.6
	<i>Hypovirus</i>	7	2	2	28.6	28.6
Luteoviridae		215	16	3	7.4	1.4
	<i>Enamovirus</i>	3	0	0	0.0	0.0
	<i>Luteovirus</i>	101	13	2	12.9	2.0
	<i>Polerovirus</i>	111	3	1	2.7	0.9
Narnaviridae		12	2	1	16.7	8.3
	<i>Mitovirus</i>	9	2	1	22.2	11.1
	<i>Narnavirus</i>	3	0	0	0.0	0.0
Paramyxoviridae		1,269	1,244	42	98.0	3.3
	<i>Avulavirus</i>	324	303	25	93.5	7.7
	<i>Morbillivirus</i>	84	81	6	96.4	7.1
	<i>Pneumovirus</i>	593	592	3	99.8	0.5
	<i>Respirovirus</i>	190	190	5	100.0	2.6
	<i>Rubulavirus</i>	78	78	3	100.0	3.8
Picornaviridae		1,135	415	143	36.6	12.6
	<i>Aphthovirus</i>	374	37	13	9.9	3.5
	<i>Cardiovirus</i>	43	5	1	11.6	2.3
	<i>Enterovirus</i>	436	192	45	44.0	10.3
	<i>Erbovirus</i>	2	2	2	100.0	100.0
	<i>Hepatovirus</i>	153	151	77	98.7	50.3

	<i>Kobuvirus</i>	33	2	1	6.1	3.0
	<i>Parechovirus</i>	46	24	3	52.2	6.5
	<i>Senecavirus</i>	3	0	0	0.0	0.0
	<i>Teschovirus</i>	45	2	1	4.4	2.2
Potyviridae		116	105	86	90.5	74.1
	<i>Bymovirus</i>	64	53	38	82.8	59.4
	<i>Ipomovirus</i>	3	3	2	100.0	66.7
	<i>Potyvirus</i>	46	46	43	100.0	93.5
	<i>Rymovirus</i>	2	2	2	100.0	100.0
	<i>Tritimovirus</i>	1	1	1	100.0	100.0
Retroviridae		2,485	2,423	1,862	97.5	74.9
	<i>Alpharetrovirus</i>	104	93	92	89.4	88.5
	<i>Betaretrovirus</i>	126	109	59	86.5	46.8
	<i>Deltaretrovirus</i>	128	127	21	99.2	16.4
	<i>Epsilonretrovirus</i>	5	5	1	100.0	20.0
	<i>Gammaretrovirus</i>	28	10	1	35.7	3.6
	<i>Lentivirus</i>	2,059	2,045	1,671	99.3	81.2
	<i>Spumavirus</i>	35	34	17	97.1	48.6
Rhabdoviridae		408	298	58	73.0	14.2
	<i>Cytorhabdovirus</i>	4	3	1	75.0	25.0
	<i>Ephemerovirus</i>	7	7	4	100.0	57.1
	<i>Lyssavirus</i>	295	190	21	64.4	7.1
	<i>Novirhabdovirus</i>	35	33	12	94.3	34.3
	<i>Nucleorhabdovirus</i>	12	10	4	83.3	33.3
	<i>Sigmavirus</i>	1	1	0	100.0	0.0
	<i>Tibrovirus</i>	2	2	0	100.0	0.0
	<i>Tupavirus</i>	1	1	0	100.0	0.0
	<i>Vesiculovirus</i>	51	51	16	100.0	31.4
Secoviridae		8	4	4	50.0	50.0
	<i>Sequivirus</i>	2	2	2	100.0	100.0
	<i>Waikavirus</i>	6	2	2	33.3	33.3
Togaviridae		505	372	52	73.7	10.3
	<i>Alphavirus</i>	456	372	52	81.6	11.4
	<i>Rubivirus</i>	49	0	0	0.0	0.0
Tombusviridae		132	5	1	3.8	0.8
	<i>Alphanecrovirus</i>	10	0	0	0.0	0.0
	<i>Aureusvirus</i>	7	0	0	0.0	0.0
	<i>Avenavirus</i>	1	0	0	0.0	0.0
	<i>Betanecrovirus</i>	20	0	0	0.0	0.0
	<i>Carmovirus</i>	44	2	1	4.5	2.3
	<i>Dianthovirus</i>	9	2	0	22.2	0.0
	<i>Gallantivirus</i>	1	0	0	0.0	0.0
	<i>Macanavirus</i>	1	0	0	0.0	0.0
	<i>Machlomovirus</i>	13	0	0	0.0	0.0
	<i>Panicovirus</i>	3	1	0	33.3	0.0
	<i>Tombusvirus</i>	22	0	0	0.0	0.0
	<i>Zeavirus</i>	1	0	0	0.0	0.0
Totiviridae		15	10	3	66.7	20.0
	<i>Giardiavirus</i>	3	2	0	66.7	0.0
	<i>Leishmaniavirus</i>	6	6	3	100.0	50.0
	<i>Totivirus</i>	6	2	0	33.3	0.0
Tymoviridae		10	3	3	30.0	30.0
	<i>Marafivirus</i>	10	3	3	30.0	30.0
Virgaviridae		125	115	87	92.0	69.6
	<i>Tobamovirus</i>	125	115	87	92.0	69.6
unassigned					0.0	0.0
	<i>Sobemovirus</i>	64	1	0	1.6	0.0
	<i>Umbravirus</i>	5	0	0	0.0	0.0
Total		14,149	11,624	5,133	82.2	36.3

^a Number of accessions in GenBank/EMBL/DDBJ.

^b Number of accessions containing the indicated motif at least once.

Supplementary Table 2. Statistical analysis of the indel frequency observed in the amplicon encompassing G₂A₆ motif.

Window	Geno- type	log _e -transformed estimate ± SE ^a		Adjusted p-value ^b	Geno- type	log _e -transformed estimate ± SE ^a		Adjusted p-value ^b
		Virus	Plasmid			Virus	Plasmid	
Window size = 3 base								
Indel = +1 base								
29 - 31	CI30	-9.119 ± 0.866	-10.028 ± 1.118	1	RB	-9.259 ± 0.866	-10.212 ± 1.225	1
31 - 33	CI30	-3.889 ± 0.119	-6.446 ± 0.178	8.20E-50*	RB	-3.736 ± 0.119	-7.163 ± 0.241	2.47E-39*
32 - 34	CI30	-3.893 ± 0.119	-6.446 ± 0.178	9.88E-50*	RB	-3.736 ± 0.119	-7.208 ± 0.246	6.29E-39*
33 - 35	CI30	-3.910 ± 0.119	-6.446 ± 0.178	2.29E-49*	RB	-3.748 ± 0.119	-7.208 ± 0.246	1.49E-38*
Indel = -1 base								
2 - 4	CI30	-6.020 ± 0.165	-5.897 ± 0.138	1	RB	-5.863 ± 0.165	-6.180 ± 0.191	1
3 - 5	CI30	-6.000 ± 0.164	-5.897 ± 0.138	1	RB	-5.863 ± 0.164	-6.164 ± 0.189	1
4 - 6	CI30	-6.000 ± 0.164	-5.897 ± 0.138	1	RB	-5.863 ± 0.164	-6.164 ± 0.189	1
5 - 7	CI30	-7.735 ± 0.527	-8.965 ± 0.646	1	RB	-8.991 ± 0.527	-8.345 ± 0.504	1
6 - 8	CI30	-5.096 ± 0.118	-5.679 ± 0.124	1	RB	-5.665 ± 0.118	-5.060 ± 0.115	1
7 - 9	CI30	-5.096 ± 0.118	-5.679 ± 0.124	1	RB	-5.665 ± 0.118	-5.060 ± 0.115	1
8 - 10	CI30	-5.162 ± 0.121	-5.717 ± 0.126	1	RB	-5.702 ± 0.121	-5.093 ± 0.118	1
11 - 13	CI30	-7.160 ± 0.306	-7.519 ± 0.300	1	RB	-7.317 ± 0.306	-7.726 ± 0.373	1
12 - 14	CI30	-6.636 ± 0.233	-6.951 ± 0.225	1	RB	-6.722 ± 0.233	-7.156 ± 0.284	1
13 - 15	CI30	-6.269 ± 0.195	-6.363 ± 0.175	1	RB	-6.376 ± 0.195	-6.577 ± 0.224	1
14 - 16	CI30	-6.000 ± 0.175	-6.193 ± 0.163	1	RB	-6.272 ± 0.175	-5.837 ± 0.177	1
15 - 17	CI30	-5.656 ± 0.155	-5.852 ± 0.146	1	RB	-6.188 ± 0.155	-5.609 ± 0.152	1
16 - 18	CI30	-5.538 ± 0.142	-5.798 ± 0.136	1	RB	-5.939 ± 0.142	-5.471 ± 0.143	1
17 - 19	CI30	-5.288 ± 0.132	-5.830 ± 0.137	1	RB	-5.947 ± 0.132	-5.267 ± 0.127	1
18 - 20	CI30	-5.641 ± 0.170	-6.019 ± 0.167	1	RB	-5.977 ± 0.170	-5.495 ± 0.170	1
19 - 21	CI30	-5.368 ± 0.127	-5.670 ± 0.122	1	RB	-5.618 ± 0.127	-5.321 ± 0.132	1
20 - 22	CI30	-5.378 ± 0.134	-5.921 ± 0.139	1	RB	-5.878 ± 0.134	-5.479 ± 0.137	1
21 - 23	CI30	-5.378 ± 0.134	-5.933 ± 0.139	1	RB	-5.863 ± 0.134	-5.479 ± 0.137	1
22 - 24	CI30	-5.684 ± 0.174	-6.688 ± 0.204	1	RB	-6.656 ± 0.174	-5.748 ± 0.158	1
23 - 25	CI30	-6.132 ± 0.215	-6.355 ± 0.204	1	RB	-6.444 ± 0.215	-6.374 ± 0.230	1
24 - 26	CI30	-6.061 ± 0.198	-6.591 ± 0.204	1	RB	-6.776 ± 0.198	-6.321 ± 0.199	1
25 - 27	CI30	-6.600 ± 0.265	-7.056 ± 0.267	1	RB	-7.416 ± 0.265	-7.113 ± 0.278	1
26 - 28	CI30	-8.834 ± 0.626	-7.713 ± 0.361	1	RB	-7.950 ± 0.626	-9.598 ± 0.913	1
27 - 29	CI30	-9.813 ± 1.414	-8.236 ± 1.080	1	RB	-10.645 ± 1.414	-9.519 ± 1.225	1
28 - 30	CI30	-9.813 ± 1.414	-8.082 ± 1.069	1	RB	-10.645 ± 1.414	-9.519 ± 1.225	1
29 - 31	CI30	-8.141 ± 0.556	-8.560 ± 0.556	1	RB	-8.837 ± 0.556	-8.905 ± 0.646	1
30 - 32	CI30	-8.323 ± 0.586	-9.659 ± 0.802	1	RB	-8.837 ± 0.586	-9.598 ± 0.837	1
31 - 33	CI30	-4.899 ± 0.108	-6.242 ± 0.146	2.43E-16*	RB	-4.942 ± 0.108	-6.486 ± 0.176	7.04E-17*
32 - 34	CI30	-4.933 ± 0.112	-6.259 ± 0.149	1.16E-15*	RB	-4.960 ± 0.112	-6.531 ± 0.181	3.88E-16*
33 - 35	CI30	-4.933 ± 0.111	-6.259 ± 0.148	8.17E-16*	RB	-4.962 ± 0.111	-6.531 ± 0.180	2.65E-16*
40 - 42	CI30	-9.239 ± 0.791	-9.253 ± 0.677	1	RB	-8.703 ± 0.791	-9.598 ± 1.000	1
Window size = 5 base								
Indel = +1 base								
4 - 8	CI30	-9.932 ± 1.118	-10.352 ± 1.118	1	RB	-9.397 ± 1.118	-9.598 ± 1.225	1
17 - 21	CI30	-9.932 ± 1.155	-9.659 ± 0.913	1	RB	-9.684 ± 1.155	-10.291 ± 1.414	1
18 - 22	CI30	-9.932 ± 1.225	-10.352 ± 1.225	1	RB	-10.090 ± 1.225	-8.499 ± 1.080	1
19 - 23	CI30	-9.932 ± 1.414	-10.352 ± 1.414	1	RB	-10.783 ± 1.414	-8.682 ± 1.095	1
20 - 24	CI30	-9.932 ± 1.414	-10.352 ± 1.414	1	RB	-10.783 ± 1.414	-8.682 ± 1.095	1
21 - 25	CI30	-9.932 ± 1.414	-10.352 ± 1.414	1	RB	-10.783 ± 1.414	-8.682 ± 1.095	1
27 - 31	CI30	-9.119 ± 0.866	-10.028 ± 1.118	1	RB	-9.259 ± 0.866	-10.212 ± 1.225	1
28 - 32	CI30	-7.735 ± 0.401	-10.352 ± 1.025	1	RB	-7.787 ± 0.401	-10.291 ± 1.054	1
29 - 33	CI30	-3.889 ± 0.119	-6.428 ± 0.177	9.88E-50*	RB	-3.734 ± 0.119	-7.163 ± 0.242	2.56E-39*
30 - 34	CI30	-3.889 ± 0.119	-6.446 ± 0.178	6.12E-50*	RB	-3.734 ± 0.119	-7.163 ± 0.241	2.30E-39*
31 - 35	CI30	-3.889 ± 0.119	-6.446 ± 0.178	7.43E-50*	RB	-3.735 ± 0.119	-7.163 ± 0.241	2.41E-39*
32 - 36	CI30	-3.893 ± 0.119	-6.446 ± 0.178	9.88E-50*	RB	-3.736 ± 0.119	-7.208 ± 0.246	6.29E-39*
33 - 37	CI30	-3.910 ± 0.119	-6.446 ± 0.178	2.29E-49*	RB	-3.748 ± 0.119	-7.208 ± 0.246	1.49E-38*
Indel = -1 base								
0 - 4	CI30	-6.020 ± 0.165	-5.897 ± 0.138	1	RB	-5.863 ± 0.165	-6.180 ± 0.191	1
1 - 5	CI30	-6.000 ± 0.164	-5.897 ± 0.138	1	RB	-5.863 ± 0.164	-6.164 ± 0.189	1
2 - 6	CI30	-6.000 ± 0.164	-5.897 ± 0.138	1	RB	-5.863 ± 0.164	-6.164 ± 0.189	1
3 - 7	CI30	-5.855 ± 0.155	-5.852 ± 0.135	1	RB	-5.820 ± 0.155	-6.072 ± 0.178	1
4 - 8	CI30	-4.756 ± 0.095	-5.089 ± 0.092	1	RB	-5.066 ± 0.095	-4.774 ± 0.098	1
5 - 9	CI30	-5.088 ± 0.118	-5.679 ± 0.124	1	RB	-5.665 ± 0.118	-5.055 ± 0.115	1
6 - 10	CI30	-5.096 ± 0.118	-5.679 ± 0.124	1	RB	-5.665 ± 0.118	-5.060 ± 0.115	1
7 - 11	CI30	-5.096 ± 0.118	-5.679 ± 0.124	1	RB	-5.665 ± 0.118	-5.060 ± 0.115	1
8 - 12	CI30	-5.162 ± 0.121	-5.717 ± 0.126	1	RB	-5.702 ± 0.121	-5.093 ± 0.118	1
9 - 13	CI30	-7.160 ± 0.306	-7.519 ± 0.300	1	RB	-7.317 ± 0.306	-7.726 ± 0.373	1
10 - 14	CI30	-6.636 ± 0.233	-6.951 ± 0.225	1	RB	-6.722 ± 0.233	-7.156 ± 0.284	1
11 - 15	CI30	-6.269 ± 0.195	-6.363 ± 0.175	1	RB	-6.376 ± 0.195	-6.577 ± 0.224	1
12 - 16	CI30	-5.728 ± 0.152	-5.957 ± 0.143	1	RB	-5.971 ± 0.152	-5.696 ± 0.158	1
13 - 17	CI30	-5.337 ± 0.128	-5.564 ± 0.121	1	RB	-5.727 ± 0.128	-5.416 ± 0.133	1

14 - 18	CI30	-5.288 ± 0.124	-5.469 ± 0.115	1	RB	-5.607 ± 0.124	-5.267 ± 0.127	1
15 - 19	CI30	-4.962 ± 0.110	-5.389 ± 0.111	1	RB	-5.531 ± 0.110	-4.862 ± 0.106	1
16 - 20	CI30	-5.049 ± 0.112	-5.389 ± 0.109	1	RB	-5.480 ± 0.112	-4.944 ± 0.111	1
17 - 21	CI30	-4.928 ± 0.103	-5.181 ± 0.098	1	RB	-5.218 ± 0.103	-4.944 ± 0.107	1
18 - 22	CI30	-4.814 ± 0.102	-5.432 ± 0.108	1	RB	-5.358 ± 0.102	-4.798 ± 0.101	1
19 - 23	CI30	-4.949 ± 0.108	-5.477 ± 0.111	1	RB	-5.431 ± 0.108	-4.949 ± 0.108	1
20 - 24	CI30	-5.187 ± 0.117	-5.540 ± 0.115	1	RB	-5.500 ± 0.117	-5.267 ± 0.124	1
21 - 25	CI30	-4.991 ± 0.113	-5.616 ± 0.120	1	RB	-5.618 ± 0.113	-5.132 ± 0.114	1
22 - 26	CI30	-5.347 ± 0.146	-6.162 ± 0.162	1	RB	-6.272 ± 0.146	-5.520 ± 0.137	1
23 - 27	CI30	-6.061 ± 0.178	-6.162 ± 0.162	1	RB	-6.272 ± 0.178	-6.321 ± 0.199	1
24 - 28	CI30	-6.061 ± 0.198	-6.545 ± 0.201	1	RB	-6.776 ± 0.198	-6.321 ± 0.199	1
25 - 29	CI30	-6.565 ± 0.260	-6.855 ± 0.252	1	RB	-7.382 ± 0.260	-7.033 ± 0.270	1
26 - 30	CI30	-8.546 ± 0.553	-7.407 ± 0.329	1	RB	-7.893 ± 0.553	-8.905 ± 0.707	1
27 - 31	CI30	-8.141 ± 0.556	-8.272 ± 0.518	1	RB	-8.837 ± 0.556	-8.905 ± 0.646	1
28 - 32	CI30	-8.141 ± 0.540	-8.272 ± 0.500	1	RB	-8.703 ± 0.540	-8.905 ± 0.646	1
29 - 33	CI30	-4.896 ± 0.117	-6.165 ± 0.149	7.78E-14*	RB	-4.945 ± 0.117	-6.444 ± 0.179	1.99E-15*
30 - 34	CI30	-4.899 ± 0.109	-6.226 ± 0.146	5.08E-16*	RB	-4.943 ± 0.109	-6.486 ± 0.177	8.48E-17*
31 - 35	CI30	-4.899 ± 0.108	-6.242 ± 0.146	2.43E-16*	RB	-4.942 ± 0.108	-6.486 ± 0.176	7.04E-17*
32 - 36	CI30	-4.933 ± 0.112	-6.259 ± 0.149	1.16E-15*	RB	-4.960 ± 0.112	-6.531 ± 0.181	3.88E-16*
33 - 37	CI30	-4.933 ± 0.111	-6.259 ± 0.148	8.17E-16*	RB	-4.962 ± 0.111	-6.531 ± 0.180	2.65E-16*

Window size = 7 base

Indel = +1 base

2 - 8	CI30	-9.932 ± 1.069	-10.352 ± 1.069	1	RB	-8.837 ± 1.069	-9.598 ± 1.225	1
3 - 9	CI30	-9.932 ± 1.095	-10.352 ± 1.095	1	RB	-9.173 ± 1.095	-9.598 ± 1.225	1
4 - 10	CI30	-9.932 ± 1.118	-10.352 ± 1.118	1	RB	-9.397 ± 1.118	-9.598 ± 1.225	1
15 - 21	CI30	-9.932 ± 1.118	-9.659 ± 0.866	1	RB	-9.397 ± 1.118	-9.598 ± 1.225	1
16 - 22	CI30	-9.932 ± 1.155	-9.659 ± 0.913	1	RB	-9.684 ± 1.155	-8.499 ± 1.080	1
17 - 23	CI30	-9.932 ± 1.155	-9.253 ± 0.817	1	RB	-9.684 ± 1.155	-8.499 ± 1.080	1
18 - 24	CI30	-9.932 ± 1.225	-9.659 ± 1.000	1	RB	-10.090 ± 1.225	-8.499 ± 1.080	1
19 - 25	CI30	-9.932 ± 1.225	-10.352 ± 1.225	1	RB	-10.090 ± 1.225	-8.682 ± 1.095	1
20 - 26	CI30	-9.932 ± 1.225	-10.352 ± 1.225	1	RB	-10.090 ± 1.225	-8.682 ± 1.095	1
21 - 27	CI30	-9.932 ± 1.414	-10.352 ± 1.414	1	RB	-10.783 ± 1.414	-8.682 ± 1.095	1
25 - 31	CI30	-9.119 ± 0.837	-10.028 ± 1.095	1	RB	-9.035 ± 0.837	-10.212 ± 1.225	1
26 - 32	CI30	-7.735 ± 0.401	-10.352 ± 1.025	1	RB	-7.787 ± 0.401	-10.291 ± 1.054	1
27 - 33	CI30	-3.889 ± 0.119	-6.428 ± 0.177	9.88E-50*	RB	-3.734 ± 0.119	-7.163 ± 0.242	2.56E-39*
28 - 34	CI30	-3.889 ± 0.119	-6.428 ± 0.177	9.12E-50*	RB	-3.734 ± 0.119	-7.163 ± 0.241	2.54E-39*
29 - 35	CI30	-3.889 ± 0.119	-6.428 ± 0.177	9.12E-50*	RB	-3.734 ± 0.119	-7.163 ± 0.241	2.54E-39*
30 - 36	CI30	-3.889 ± 0.119	-6.446 ± 0.178	6.12E-50*	RB	-3.734 ± 0.119	-7.163 ± 0.241	2.30E-39*
31 - 37	CI30	-3.889 ± 0.119	-6.446 ± 0.178	7.43E-50*	RB	-3.735 ± 0.119	-7.163 ± 0.241	2.41E-39*
32 - 38	CI30	-3.893 ± 0.119	-6.446 ± 0.178	9.88E-50*	RB	-3.736 ± 0.119	-7.208 ± 0.246	6.29E-39*
33 - 39	CI30	-3.906 ± 0.120	-6.447 ± 0.179	4.85E-49*	RB	-3.747 ± 0.120	-7.117 ± 0.238	6.13E-39*
37 - 43	CI30	-9.239 ± 0.913	-10.352 ± 1.155	1	RB	-9.684 ± 0.913	-9.598 ± 1.000	1

Indel = -1 base

1 - 7	CI30	-5.855 ± 0.155	-5.852 ± 0.135	1	RB	-5.820 ± 0.155	-6.072 ± 0.178	1
2 - 8	CI30	-4.756 ± 0.095	-5.089 ± 0.092	1	RB	-5.066 ± 0.095	-4.774 ± 0.098	1
3 - 9	CI30	-4.756 ± 0.095	-5.089 ± 0.092	1	RB	-5.066 ± 0.095	-4.774 ± 0.098	1
4 - 10	CI30	-4.756 ± 0.095	-5.089 ± 0.092	1	RB	-5.066 ± 0.095	-4.774 ± 0.098	1
5 - 11	CI30	-5.088 ± 0.118	-5.679 ± 0.124	1	RB	-5.665 ± 0.118	-5.055 ± 0.115	1
6 - 12	CI30	-5.096 ± 0.118	-5.679 ± 0.124	1	RB	-5.665 ± 0.118	-5.060 ± 0.115	1
7 - 13	CI30	-4.976 ± 0.110	-5.532 ± 0.114	1	RB	-5.490 ± 0.110	-4.993 ± 0.110	1
8 - 14	CI30	-4.956 ± 0.107	-5.461 ± 0.110	1	RB	-5.394 ± 0.107	-4.973 ± 0.109	1
9 - 15	CI30	-6.269 ± 0.195	-6.363 ± 0.175	1	RB	-6.376 ± 0.195	-6.577 ± 0.224	1
10 - 16	CI30	-5.728 ± 0.152	-5.957 ± 0.143	1	RB	-5.971 ± 0.152	-5.696 ± 0.158	1
11 - 17	CI30	-5.337 ± 0.128	-5.564 ± 0.121	1	RB	-5.727 ± 0.128	-5.416 ± 0.133	1
12 - 18	CI30	-5.145 ± 0.115	-5.348 ± 0.107	1	RB	-5.441 ± 0.115	-5.185 ± 0.120	1
13 - 19	CI30	-4.791 ± 0.099	-5.198 ± 0.099	1	RB	-5.265 ± 0.099	-4.766 ± 0.099	1
14 - 20	CI30	-4.889 ± 0.102	-5.159 ± 0.098	1	RB	-5.258 ± 0.102	-4.819 ± 0.103	1
15 - 21	CI30	-4.691 ± 0.091	-4.927 ± 0.086	1	RB	-4.996 ± 0.091	-4.635 ± 0.094	1
16 - 22	CI30	-4.507 ± 0.088	-5.039 ± 0.091	1	RB	-5.073 ± 0.088	-4.483 ± 0.086	1
17 - 23	CI30	-4.639 ± 0.092	-5.058 ± 0.092	1	RB	-5.089 ± 0.092	-4.674 ± 0.093	1
18 - 24	CI30	-4.701 ± 0.094	-5.181 ± 0.096	1	RB	-5.117 ± 0.094	-4.685 ± 0.095	1
19 - 25	CI30	-4.680 ± 0.096	-5.264 ± 0.101	1	RB	-5.265 ± 0.096	-4.730 ± 0.095	1
20 - 26	CI30	-4.969 ± 0.107	-5.341 ± 0.105	1	RB	-5.362 ± 0.107	-5.121 ± 0.113	1
21 - 27	CI30	-4.969 ± 0.110	-5.516 ± 0.115	1	RB	-5.525 ± 0.110	-5.121 ± 0.113	1
22 - 28	CI30	-5.347 ± 0.146	-6.132 ± 0.160	1	RB	-6.272 ± 0.146	-5.520 ± 0.137	1
23 - 29	CI30	-6.040 ± 0.177	-6.075 ± 0.157	1	RB	-6.261 ± 0.177	-6.284 ± 0.196	1
24 - 30	CI30	-6.040 ± 0.196	-6.440 ± 0.195	1	RB	-6.758 ± 0.196	-6.284 ± 0.196	1
25 - 31	CI30	-6.406 ± 0.239	-6.796 ± 0.237	1	RB	-7.199 ± 0.239	-6.959 ± 0.255	1
26 - 32	CI30	-7.735 ± 0.389	-7.356 ± 0.300	1	RB	-7.564 ± 0.389	-8.499 ± 0.527	1
27 - 33	CI30	-4.896 ± 0.119	-6.136 ± 0.149	4.47E-13*	RB	-4.946 ± 0.119	-6.444 ± 0.181	3.81E-15*
28 - 34	CI30	-4.896 ± 0.119	-6.136 ± 0.149	4.47E-13*	RB	-4.946 ± 0.119	-6.444 ± 0.181	3.81E-15*
29 - 35	CI30	-4.896 ± 0.117	-6.165 ± 0.149	7.78E-14*	RB	-4.945 ± 0.117	-6.444 ± 0.179	1.99E-15*
30 - 36	CI30	-4.899 ± 0.109	-6.226 ± 0.146	5.08E-16*	RB	-4.943 ± 0.109	-6.486 ± 0.177	8.48E-17*
31 - 37	CI30	-4.899 ± 0.108	-6.242 ± 0.146	2.43E-16*	RB	-4.942 ± 0.108	-6.486 ± 0.176	7.04E-17*

32 - 38	CI30	-4.933 ± 0.112	-6.259 ± 0.149	1.16E-15*	RB	-4.960 ± 0.112	-6.531 ± 0.181	3.88E-16*
33 - 39	CI30	-4.935 ± 0.117	-6.259 ± 0.153	6.14E-15*	RB	-4.960 ± 0.117	-6.466 ± 0.180	6.63E-15*
36 - 42	CI30	-9.239 ± 0.775	-9.253 ± 0.658	1	RB	-8.480 ± 0.775	-8.682 ± 0.837	1
Window size = 9 base								
Indel = +1 base								
0 - 8	CI30	-9.932 ± 1.069	-10.352 ± 1.069	1	RB	-8.837 ± 1.069	-9.598 ± 1.225	1
1 - 9	CI30	-9.932 ± 1.069	-10.352 ± 1.069	1	RB	-8.837 ± 1.069	-9.598 ± 1.225	1
2 - 10	CI30	-9.932 ± 1.069	-10.352 ± 1.069	1	RB	-8.837 ± 1.069	-9.598 ± 1.225	1
3 - 11	CI30	-9.932 ± 1.095	-10.352 ± 1.095	1	RB	-9.173 ± 1.095	-9.598 ± 1.225	1
4 - 12	CI30	-9.932 ± 1.118	-10.352 ± 1.118	1	RB	-9.397 ± 1.118	-9.598 ± 1.225	1
13 - 21	CI30	-9.932 ± 1.118	-9.659 ± 0.866	1	RB	-9.397 ± 1.118	-9.598 ± 1.225	1
14 - 22	CI30	-9.932 ± 1.118	-9.659 ± 0.866	1	RB	-9.397 ± 1.118	-8.345 ± 1.069	1
15 - 23	CI30	-9.932 ± 1.118	-9.253 ± 0.764	1	RB	-9.397 ± 1.118	-8.345 ± 1.069	1
16 - 24	CI30	-9.932 ± 1.155	-9.253 ± 0.817	1	RB	-9.684 ± 1.155	-8.499 ± 1.080	1
17 - 25	CI30	-9.932 ± 1.118	-9.253 ± 0.764	1	RB	-9.397 ± 1.118	-8.499 ± 1.080	1
18 - 26	CI30	-9.932 ± 1.155	-9.659 ± 0.913	1	RB	-9.684 ± 1.155	-8.499 ± 1.080	1
19 - 27	CI30	-9.932 ± 1.225	-10.352 ± 1.225	1	RB	-10.090 ± 1.225	-8.682 ± 1.095	1
20 - 28	CI30	-9.932 ± 1.225	-10.352 ± 1.225	1	RB	-10.090 ± 1.225	-8.682 ± 1.095	1
21 - 29	CI30	-9.932 ± 1.225	-9.659 ± 1.000	1	RB	-10.090 ± 1.225	-8.682 ± 1.095	1
23 - 31	CI30	-9.239 ± 0.837	-9.659 ± 0.837	1	RB	-9.174 ± 0.837	-10.291 ± 1.225	1
24 - 32	CI30	-7.735 ± 0.398	-10.352 ± 1.024	1	RB	-7.738 ± 0.398	-10.291 ± 1.054	1
25 - 33	CI30	-3.889 ± 0.119	-6.428 ± 0.177	9.12E-50*	RB	-3.734 ± 0.119	-7.163 ± 0.241	2.54E-39*
26 - 34	CI30	-3.889 ± 0.119	-6.428 ± 0.177	9.12E-50*	RB	-3.734 ± 0.119	-7.163 ± 0.241	2.54E-39*
27 - 35	CI30	-3.889 ± 0.119	-6.428 ± 0.177	9.12E-50*	RB	-3.734 ± 0.119	-7.163 ± 0.241	2.54E-39*
28 - 36	CI30	-3.889 ± 0.119	-6.428 ± 0.177	9.12E-50*	RB	-3.734 ± 0.119	-7.163 ± 0.241	2.54E-39*
29 - 37	CI30	-3.889 ± 0.119	-6.428 ± 0.177	9.12E-50*	RB	-3.734 ± 0.119	-7.163 ± 0.241	2.54E-39*
30 - 38	CI30	-3.889 ± 0.119	-6.446 ± 0.178	6.12E-50*	RB	-3.734 ± 0.119	-7.163 ± 0.241	2.30E-39*
31 - 39	CI30	-3.885 ± 0.120	-6.447 ± 0.179	1.35E-49*	RB	-3.733 ± 0.120	-7.076 ± 0.234	1.06E-39*
32 - 40	CI30	-3.889 ± 0.120	-6.447 ± 0.179	2.03E-49*	RB	-3.734 ± 0.120	-7.117 ± 0.238	2.53E-39*
33 - 41	CI30	-3.906 ± 0.120	-6.447 ± 0.179	4.85E-49*	RB	-3.747 ± 0.120	-7.117 ± 0.238	6.13E-39*
Indel = -1 base								
0 - 8	CI30	-4.756 ± 0.095	-5.089 ± 0.092	1	RB	-5.066 ± 0.095	-4.774 ± 0.098	1
1 - 9	CI30	-4.756 ± 0.095	-5.089 ± 0.092	1	RB	-5.066 ± 0.095	-4.774 ± 0.098	1
2 - 10	CI30	-4.756 ± 0.095	-5.089 ± 0.092	1	RB	-5.066 ± 0.095	-4.774 ± 0.098	1
3 - 11	CI30	-4.756 ± 0.095	-5.089 ± 0.092	1	RB	-5.066 ± 0.095	-4.774 ± 0.098	1
4 - 12	CI30	-4.756 ± 0.095	-5.089 ± 0.092	1	RB	-5.066 ± 0.095	-4.774 ± 0.098	1
5 - 13	CI30	-4.969 ± 0.110	-5.532 ± 0.114	1	RB	-5.490 ± 0.110	-4.988 ± 0.109	1
6 - 14	CI30	-4.902 ± 0.105	-5.432 ± 0.108	1	RB	-5.367 ± 0.105	-4.944 ± 0.106	1
7 - 15	CI30	-4.826 ± 0.100	-5.270 ± 0.101	1	RB	-5.265 ± 0.100	-4.862 ± 0.102	1
8 - 16	CI30	-4.712 ± 0.094	-5.137 ± 0.095	1	RB	-5.134 ± 0.094	-4.656 ± 0.095	1
9 - 17	CI30	-5.337 ± 0.128	-5.564 ± 0.121	1	RB	-5.727 ± 0.128	-5.416 ± 0.133	1
10 - 18	CI30	-5.145 ± 0.115	-5.348 ± 0.107	1	RB	-5.441 ± 0.115	-5.185 ± 0.120	1
11 - 19	CI30	-4.791 ± 0.099	-5.198 ± 0.099	1	RB	-5.265 ± 0.099	-4.766 ± 0.099	1
12 - 20	CI30	-4.791 ± 0.097	-5.069 ± 0.093	1	RB	-5.137 ± 0.097	-4.766 ± 0.099	1
13 - 21	CI30	-4.557 ± 0.085	-4.803 ± 0.081	1	RB	-4.832 ± 0.085	-4.558 ± 0.089	1
14 - 22	CI30	-4.411 ± 0.083	-4.871 ± 0.084	1	RB	-4.919 ± 0.083	-4.402 ± 0.082	1
15 - 23	CI30	-4.456 ± 0.083	-4.830 ± 0.082	1	RB	-4.891 ± 0.083	-4.430 ± 0.084	1
16 - 24	CI30	-4.423 ± 0.082	-4.863 ± 0.083	1	RB	-4.886 ± 0.082	-4.399 ± 0.083	1
17 - 25	CI30	-4.435 ± 0.084	-4.914 ± 0.086	1	RB	-4.969 ± 0.084	-4.504 ± 0.085	1
18 - 26	CI30	-4.562 ± 0.088	-5.039 ± 0.090	1	RB	-5.021 ± 0.088	-4.601 ± 0.090	1
19 - 27	CI30	-4.664 ± 0.094	-5.193 ± 0.098	1	RB	-5.199 ± 0.094	-4.723 ± 0.095	1
20 - 28	CI30	-4.969 ± 0.107	-5.328 ± 0.105	1	RB	-5.362 ± 0.107	-5.121 ± 0.113	1
21 - 29	CI30	-4.962 ± 0.110	-5.469 ± 0.113	1	RB	-5.520 ± 0.110	-5.109 ± 0.112	1
22 - 30	CI30	-5.337 ± 0.145	-6.061 ± 0.157	1	RB	-6.261 ± 0.145	-5.504 ± 0.136	1
23 - 31	CI30	-5.943 ± 0.169	-6.048 ± 0.154	1	RB	-6.198 ± 0.169	-6.248 ± 0.190	1
24 - 32	CI30	-5.943 ± 0.185	-6.420 ± 0.188	1	RB	-6.640 ± 0.185	-6.248 ± 0.190	1
25 - 33	CI30	-4.729 ± 0.109	-5.802 ± 0.129	8.22E-11*	RB	-4.858 ± 0.109	-6.032 ± 0.153	4.29E-15*
26 - 34	CI30	-4.876 ± 0.117	-5.973 ± 0.140	5.39E-12*	RB	-4.898 ± 0.117	-6.402 ± 0.178	2.59E-15*
27 - 35	CI30	-4.896 ± 0.119	-6.136 ± 0.149	4.47E-13*	RB	-4.946 ± 0.119	-6.444 ± 0.181	3.81E-15*
28 - 36	CI30	-4.896 ± 0.119	-6.136 ± 0.149	4.47E-13*	RB	-4.946 ± 0.119	-6.444 ± 0.181	3.81E-15*
29 - 37	CI30	-4.896 ± 0.117	-6.165 ± 0.149	7.78E-14*	RB	-4.945 ± 0.117	-6.444 ± 0.179	1.99E-15*
30 - 38	CI30	-4.899 ± 0.109	-6.226 ± 0.146	5.08E-16*	RB	-4.943 ± 0.109	-6.486 ± 0.177	8.48E-17*
31 - 39	CI30	-4.901 ± 0.113	-6.242 ± 0.150	1.33E-15*	RB	-4.940 ± 0.113	-6.423 ± 0.176	1.38E-15*
32 - 40	CI30	-4.936 ± 0.119	-6.259 ± 0.154	1.03E-14*	RB	-4.957 ± 0.119	-6.466 ± 0.182	1.25E-14*
33 - 41	CI30	-4.936 ± 0.118	-6.259 ± 0.154	7.98E-15*	RB	-4.959 ± 0.118	-6.466 ± 0.181	9.13E-15*

^a Indel frequencies were estimated by fitting the negative binomial model to observed counts of the reads with indels obtained by amplicon sequencing. SE, standard error of estimated mean.

^b *p*-values for differences between the estimated indel frequencies between inoculated plasmid and systemically propagated virus were obtained by the model fitting and were corrected for multiple tests by Holm's method.

Supplementary Table 3. Primers used in this study.

Name	Sequence (5'–3')
796	AGCTACTAGTCTAAGCCTTGTACAGCTCGTC
797	AGCTCTCGAGATGGTGAGCAAGGGCGAGGAGCTG
1007	AGCTACTAGTTTATTCCATGACAAACCACTTTG
1009	AGTCCTCGAGATGGGCAAATCATTGACAGG
1010	AGTCCTCGAGATGGGCAACCAATCTGTTGG
1011	AGCTACTAGTCTTGTGCGACCATTCTCTTC
1012	AGCTACTAGTATGCTTGTTACTGACAATCTG
1013	AGCTACTAGTGTCTCTCGACTCTTCTCTC
1049	AGCTACTAGTTTACTCCATGACAAACCACTTTGG
1123	AGCTACTAGTAATCCTCTGCCCAAATTTTTTCC
1124	AGCTACTAGTAATCCTCTGCCCAGATTTTTTCC
1125	AGCTACTAGTAGTTTTTTTCCATGAGAATTC
1349	GCATACTAGTAATCCTCTGCCCAAATTTCTCCATGAGTACCTGGCTAGAC
1350	GCATACTAGTAATCCTCTGCCCAGATTTCTCCATGAGTACTTGACTAG
1351	GCATACTAGTAGTTTTCTCCATGAGAATTCGAGTTGAC
3668	GGAGAAAAACCTAGATGGCGGACTTAGATC
3669	GATCTAAGTCCGCCATCTAGGTTTTTCTCC
3879	GGAGATCTCTCTTTGAAAAAGTCTAGTCAAGTACTCA
3880	GGAGATCTCTCTTTGAAAAAGTCTAGCCAGGACTCA
3881	GGCATATGTTGTTACTGACAATCTGAGTGATGC
3882	GGCATATGTTGTTGCTGACAATCTGAGTGATGC
afs_as	CTAAATCCTCTGCCCAGATTTCTCC
afs_s	GGAGAAATCTGGGCAGAGGATTTAG
BamHI-kzk-GFP-F	TTTGGATCCAACAATGGTGAGCAAGGGCGAG
delPIPO_s	GGAGAAAATCTGGGCTGAGGATTTAG
delPIPO_as	ATGAGTACTTGACTAGACTTTTTTC
GFP-SmaI-SacI-R	AAAGAGCTCGCCCGGGTTACTTGTACAGCTCGTCCATGCCG
P3s3	GGAGAAAATTTAGGCAGAGGATTT
P3as3	AAATCCTCTGCCTAAATTTTTCTCC
sGFP-387-R	GATGCCCTTCAGCTCGATGCGGTTTAC
sGFP6421-R	TCGTTGGGGTCTTTGCTCAGGGCG
sGFP642-stop-F	TAAGCGGATCACATGGTCCTGCT
TTTTf1	GACGAAAAAATTTGCTATGGCACAAGCTGGAGTACA ACTACA
TTTTf2	GACCGAAAAAATTTGTATGGCACAAGCTGGAGTACA ACTACA
TTTTf3	GAGAAAAAATTTGCTTATGGCACAAGCTGGAGTACA ACTACA

Supplementary Methods

Plasmids used for Supplementary Fig. 5

To construct pSP/GFP-f1 [to prepare *GFP-G₁A₆(f1)* RNA], pSP/GFP-f2 [to prepare *GFP-G₁A₆(f2)* RNA] and pSP/GFP-f3 [to prepare *GFP-G₁A₆(f3)* RNA], a template plasmid pSP/GFPstp was generated. The sGFP(S65T) sequence present in the 35Somega-sGFP(S65T) plasmid⁶⁹ was PCR amplified using primers BamHI-kzk-GFP-F and GFP-SmaI-SacI-R (Supplementary Table 3). The PCR product was inserted into pSP64 Poly(A) vector using the *Bam*HI and *Sac*I sites. The resulting plasmid was used as a template for inverse PCR with primers sGFP642-stop-F and sGFP6421-R (Supplementary Table 3) to introduce a stop codon with the +1 reading frame. The PCR product was phosphorylated and circularized to produce pSP-GFPstp. Inverse PCR was further conducted using pSP/GFPstp as a template, reverse primer sGFP-387-R (Supplementary Table 3) and one of the following forward primers; TTTf1 for pSP/GFP-f1; TTTf2 for pSP/GFP-f2; and TTTf3 for pSP/GFP-f3 (Supplementary Table 3). The PCR product was phosphorylated and circularized.

Indel enrichment analysis for Supplementary Fig. 6

Enrichment of indels at each position in the amplicons was analysed using Fisher's exact test. Briefly, a 2 × 2 contingency table comprising counts of reads with or without indels in viral or plasmid samples was prepared for each position and each genotype. The ratios of counts of reads with or without indels for each position were used as null hypotheses and *p*-values for enrichment of indels in viral samples were obtained. Storey's *q*-values were calculated from the *p*-values. These were performed using R software (version 3.1.2) with the *qvalue* package (version 1.43).

Plasmids used for Supplementary Fig. 9

P3 of RB and Cl30 were amplified with primers 1009/1049 and 1009/1007 (Supplementary Table 3) from P3^{+P3N-PIPO} and pCl30, respectively. Each fragment was digested with *Xho*I and *Spe*I, and introduced into the pTA/3FLAG-XhSp vector to obtain pTA/FLAG-P3-RB and pTA/FLAG-P3-Cl30, which produce P3 proteins tagged with 3×FLAG at the N-terminus. The pTA/3FLAG-XhSp vector was created by introducing 3×FLAG tag + Gly/Ser linker + *Xho*I + *Spe*I fragment with *Sa*II site at the 5' terminus (gtcgacATGGACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGATTACAAGGATCATGATGGGggtggaagtggaggtagtggtggaagtggaggtagtagtCTCGAGatggccACTAGT) into the cloning site using the *Xho*I and *Spe*I sites of pTA7001.

Supplementary References

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