SUPPORTING INFORMATION AVAILABLE

Comparison of the 2D $^{1}H$-$^{15}N$ HSQC spectra of M-Rep$_{2-95}$, Tag$^{21}$-M-Rep$_{1-117}$, and Tag$^{21}$-M-Rep$_{1-124}$ (Supplementary Figure S1). Schematic representation of the five β-strands in M-Rep$_{2-95}$, indicating inter-strand NOEs between backbone protons that allow to establish the topology and register of β-strands in the 5-stranded antiparallel β-sheet, as well as backbone amides resistant to $D_2O$ exchange (Supplementary Figure S2). Consensus chemical shift differences and ribbon representation of the M-Rep$_{2-95}$ structure, indicating those residues that experience large differences in chemical shifts in the extension construct Tag$^{21}$-M-Rep$_{1-117}$ with respect to M-Rep$_{2-95}$ (Supplementary Figure S3). Histograms depicting intensity changes observed for the backbone amide and side chain amide resonances the $^{1}H$-$^{15}N$ HSQC spectrum of M-Rep$_{2-95}$ for increasing amounts of $MnCl_{2.}$ with final protein:$MnCl_2$ molar ratios of 1:0.4, 1:0.8, and 1:1.6 (Supplementary Figure S4). DALI structural comparison among the endonuclease domains from viral RCR Rep proteins and with those from the relaxases of bacterial conjugative plasmids (Supplementary Table S1). Atomic coordinate differences of common structural elements in the endonuclease domains of viral RCR Rep proteins and from the relaxases of bacterial conjugative plasmids (Supplementary Table S2). This material is available free of charge via the Internet at http://pubs.acs.org.

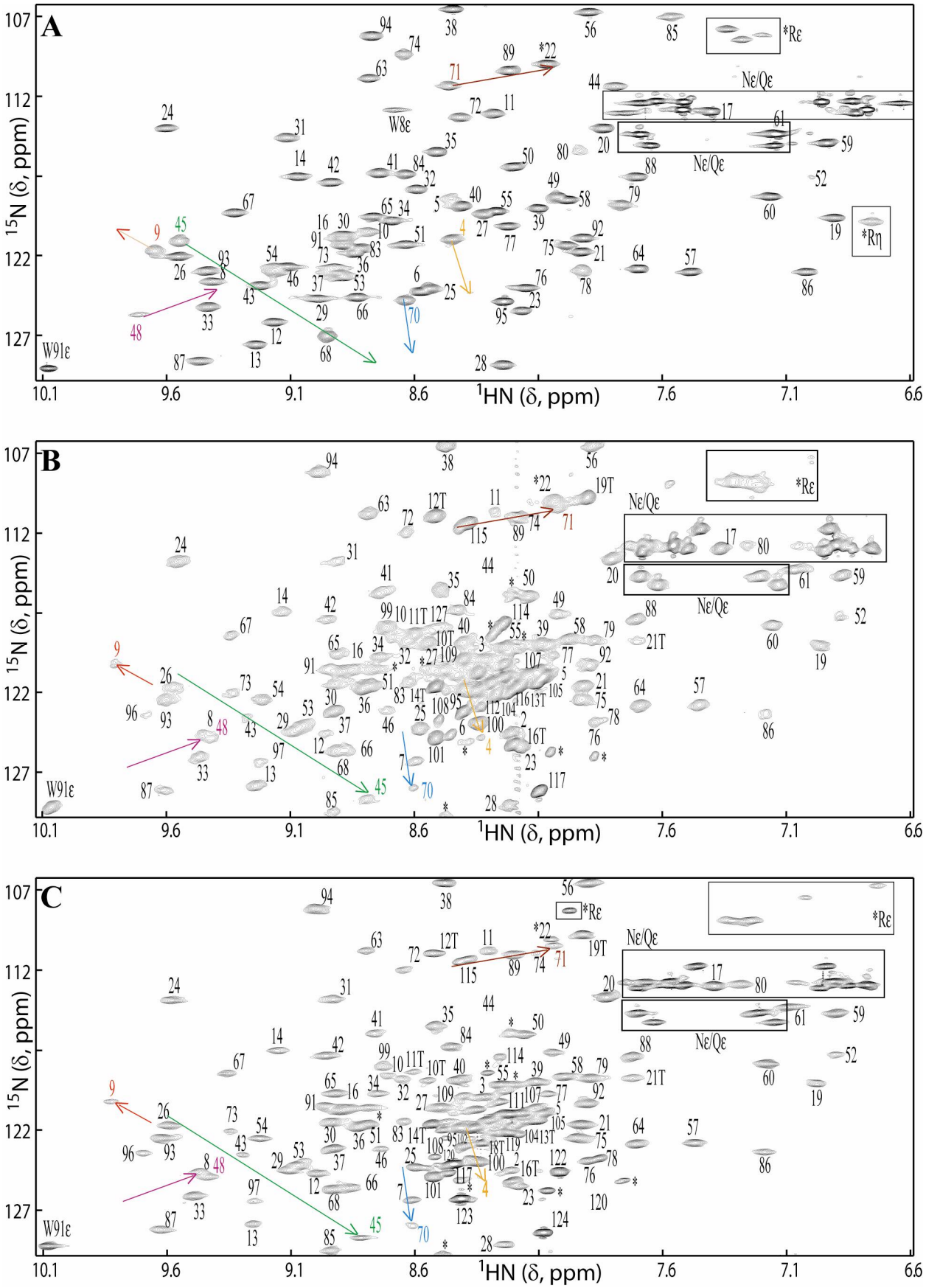
A
B
C
$^{15}$N (δ, ppm)
$^{1}$HN (δ, ppm)
107
112
122
127
10.1
9.6
9.1
8.6
7.6
7.1
6.6
W91ε
W8ε
*Rε
Nε/Qε
*Rη

**Figure S1.** Comparison of the 2D $^{1}H$-$^{15}N$ HSQC spectra of FBNYV M-Rep$_{2\text{-}95}$ (A), Tag$^{21}$-M-Rep$_{1\text{-}117}$ (B), and Tag$^{21}$-M-Rep$_{1\text{-}124}$ (C). Backbone amide resonances are labeled (except those in the central, overlapped region in B and C) according to residue position in the protein sequence and the side chain $NH_2$ signals of Asn and Gln, as well as those in Arg side chains are boxed. Folded signals in the $^{15}N$ dimension are marked with asterisks before the residue number, and those marked only with an asterisk remain unassigned. The backbone amide signals from residues in the Tag$^{21}$ sequence are numbered from 1 to 21 followed by "T". Representative resonances that are significantly different between M-Rep$_{2\text{-}95}$ and Tag$^{21}$-M-Rep$_{1\text{-}117}$ (or Tag$^{21}$-M-Rep$_{1\text{-}124}$) are indicated in the three spectra by colored residue numbers and their change in position is represented by an arrow of the corresponding color.

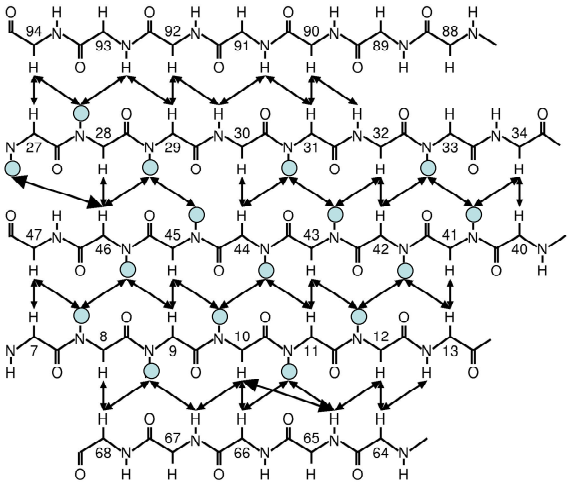

**Figure S2.** Schematic representation of the β-sheet region in M-Rep$_{2\text{-}95}$. Inter-strand NOEs between backbone protons that establishes the topology and register of the β-strands in the 5-stranded antiparallel β-sheet are shown by arrows, and slowly exchanging backbone amide protons are depicted by blue circles.

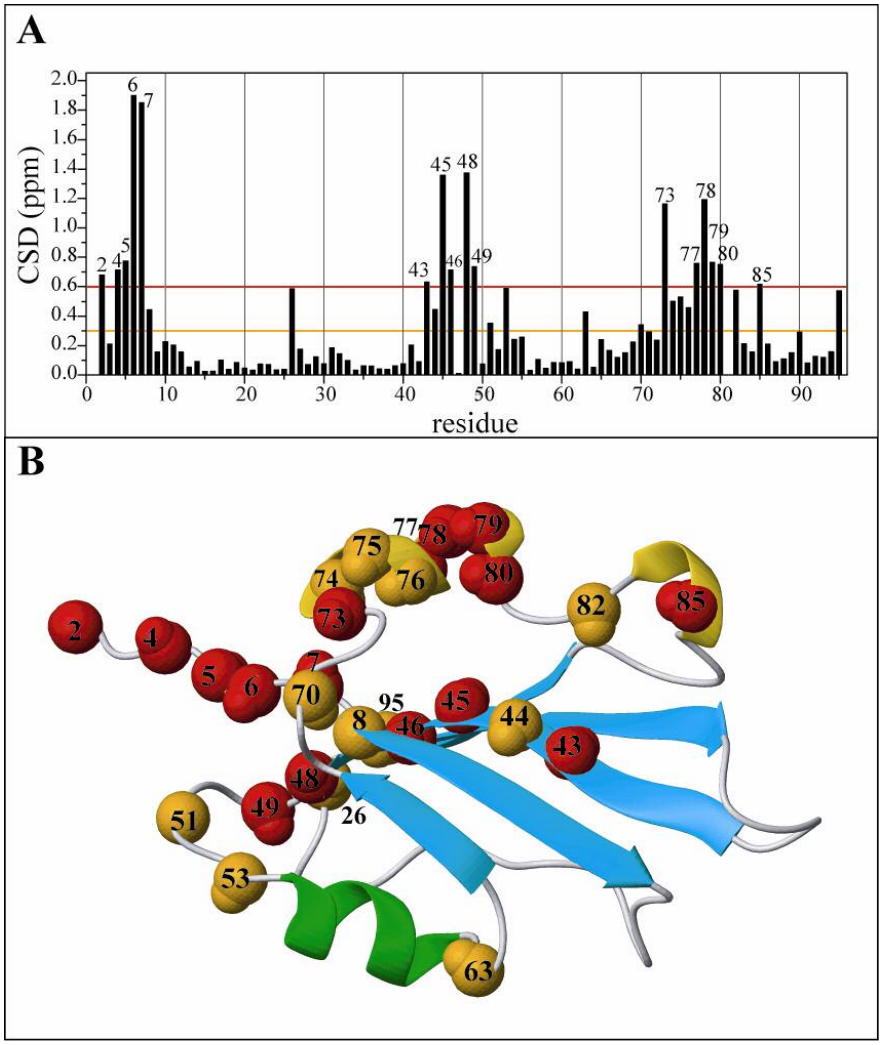


**Figure S3.** (A) Consensus chemical shift differences (CSD=(( $(\Delta\delta_{HN})^2 + (\Delta\delta_{H\alpha})^2 + (\Delta\delta_N/5)^2 + (\Delta\delta_{C\alpha}/2)^2 + (\Delta\delta_{CO}/2)^2 + (\Delta\delta_{C\beta}/2)^2$ )/6)$^{1/2}$) between the resonances of M-Rep$_{2\text{-}95}$ and those of Tag$^{21}$-M-Rep$_{1\text{-}117}$, represented against the protein sequence. The horizontal lines indicate the cut-off for selection of significantly affected residues (CSD>0.6, in red, with residues in this class labelled according to sequence position above the bars; and 0.6>CSD>0.3 in orange). (B) Ribbon representation of the M-Rep$_{2\text{-}95}$ structure indicating with colored spheres at the NH positions the residues experiencing a large difference in chemical shifts (CSD>0.6 in red, 0.6>CSD>0.3 in orange) in the extension construct Tag$^{21}$-M-Rep$_{1\text{-}117}$ with respect to M-Rep$_{2\text{-}95}$.

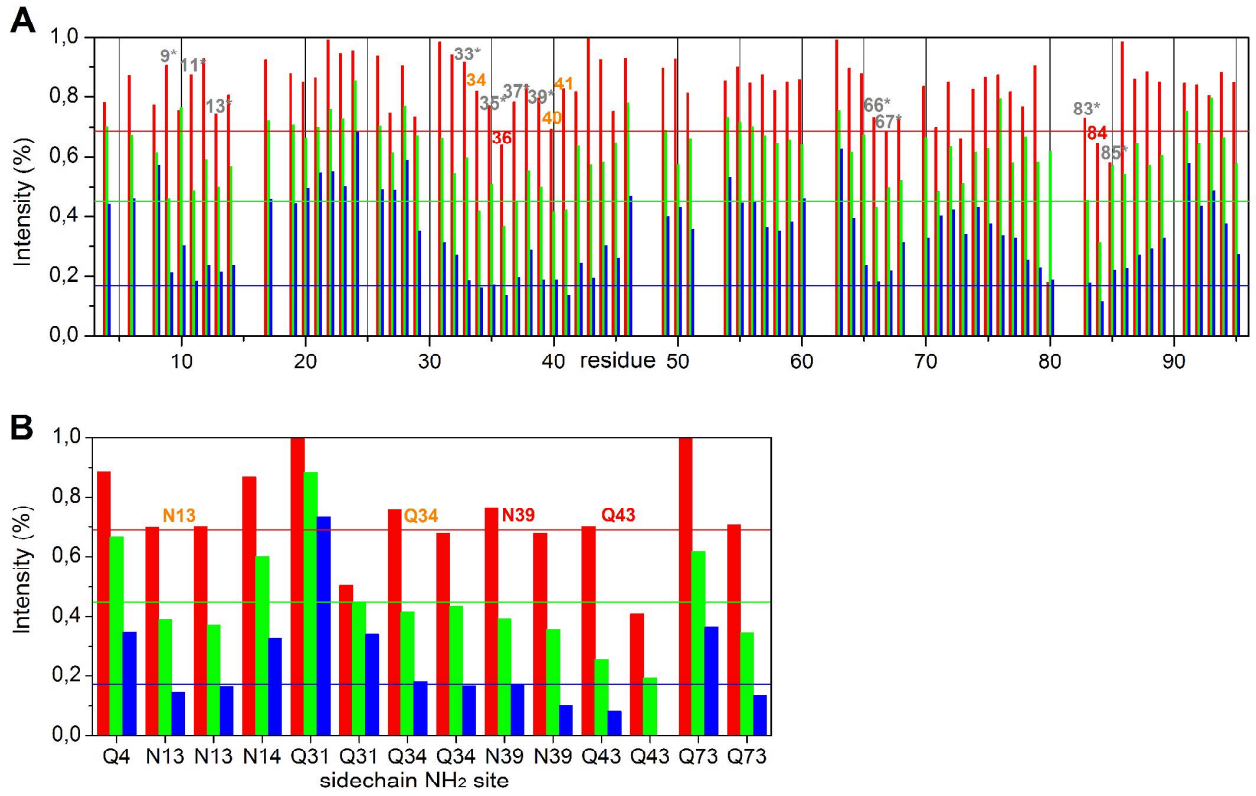


**Figure S4.** Histograms showing the intensity changes due to paramagnetic broadening upon $MnCl_2$ titration. Backbone amide (A) and side chain amide (B) intensities extracted from $^{1}H$-$^{15}N$ HSQC spectra of M-$Rep_{2\text{-}95}$ for protein:$MnCl_2$ molar ratios of 1:0.4 (red), 1:0.8 (green), and 1:1.6 (blue) are represented. Data were normalized with respect to the number of scans and intensites in the reference spectrum without metal. No data is shown if resonances were absent or overlapped. The cut-off for selecting affected residues (see text) is indicated by the three horizontal lines, color coded according to the molar ratios. Strongly affected residues are labelled in red, less affected in orange, and borderline ones in grey and marked with an asterisk.

Table S1. DALI structural comparison among the endonuclease domains from Rep proteins of nanovirus (FBNYV), circovirus (PCV2), geminivirus (TYLCSV), and adeno-associated virus (AAV5) and with the corresponding domains from the relaxases (TraI and TrwC) of bacterial conjugative plasmids[a].

| | M-$Rep_{2-95}$ | $Rep_{2-116}^{PCV2}$ | $Rep_{4-121}^{TYLCSV}$ | $Rep_{1-197}^{AAV5}$ | $TraI_{1-330}$ | $TrwC_{1-293}$ |
|---|---|---|---|---|---|---|
| M-$Rep_{2-95}$ | 2HWT | 7.0 | 5.7 | 4.7 | 0.8 | 0.7 |
| $Rep_{2-116}^{PCV2}$ | 3.0 (84) | 2HWO | 7.9 | 3.8 | 0.8 | 0.8 |
| $Rep_{4-121}^{TYLCSV}$ | 2.9 (80) | 3.9 (100) | 1L2M | 5.1 | 1.6 | 1.8 |
| $Rep_{1-197}^{AAV5}$ | 2.9 (84) | 3.1 (85) | 3.0 (98) | 1M55 | 3.9 | 4.2 |
| $TraI_{1-330}$ | 3.8 (55) | 3.9 (60) | 3.1 (68) | 3.2 (91) | 1P4D | 28.0 |
| $TrwC_{1-293}$ | 2.5 (52) | 4.6 (70) | 2.4 (67) | 3.7 (97) | 2.4 (236) | 1OMH |

[a]The PDB codes for the structures considered are given in the diagonal. DALI Z-scores *(44)* and the corresponding rmsd (in Å) of Cα atoms after superimposition are given above and below the diagonal, respectively. Values in parentheses indicate the number of residues considered in each comparison. Average NMR structures are used for comparison, and when several non-identical chains are available in X-ray structures the most similar one/s is/are selected.

Table S2. Atomic coordinate differences of common structural elements in the endonuclease domains of the Rep proteins from the nanovirus FBNYV, the circovirus PCV2, the geminivirus TYLCSV, the adeno-associated virus AAV5, and the relaxases of bacterial plasmids TraI and TrwC[a].

| | M-Rep$_{2\text{-}95}$ | Rep$_{2\text{-}116}^{PCV2}$ | Rep$_{4\text{-}121}^{TYLCSV}$ | Rep$_{1\text{-}197}^{AAV5}$ | TraI$_{1\text{-}330}$ | TrwC$_{1\text{-}293}$ |
|---|---|---|---|---|---|---|
| M-Rep$_{2\text{-}95}$ | 0.46 (33) | 1.86 (30) | 1.50 (32) | 1.50 (33) | 1.64 (33) | 1.56 (33) |
| Rep$_{2\text{-}116}^{PCV2}$ | 2.00 (32) | 0.22 (35) | 1.48 (34) | 1.95 (33) | 1.99 (33) | 1.98 (33) |
| Rep$_{4\text{-}121}^{TYLCSV}$ | 2.19 (34) | 1.80 (36) | 0.41 (38) | 1.61 (33) | 1.65 (33) | 1.39 (33) |
| Rep$_{1\text{-}197}^{AAV5}$ | 1.71 (35) | 2.06 (35) | 2.06 (35) | - | 2.03 (33) | 1.17 (33) |
| TraI$_{1\text{-}330}$ | 1.96 (35) | 2.17 (35) | 2.56 (35) | 2.08 (35) | - | 1.20 (121) |
| TrwC$_{1\text{-}293}$ | 1.77 (34) | 2.01 (34) | 1.63 (34) | 1.35 (34) | 1.24 (122) | - |

[a] Root-mean-squared deviations (rmsds, in Å) of the backbone heavy atoms between the average structures of the domains considering the five β-strands of the central conserved β-sheet alone and together with the residues Y and K of the YXXK conserved motif are given above and below the diagonal, respectively. In the comparison of the viral Rep domains with those of the relaxases the residues Y16/K20 and Y18 of TraI and TrwC are considered, respectively. The diagonal values are the coordinate average pairwise rmsds between backbone heavy atoms of the NMR conformer ensembles considering the central 5-stranded β-sheet plus the Y and K regions of the domains. Values in parentheses indicate the number of residues considered in each comparison. Structural superpositions were carried out with SuperPose (*34*) using the same structures as those in Table S1.