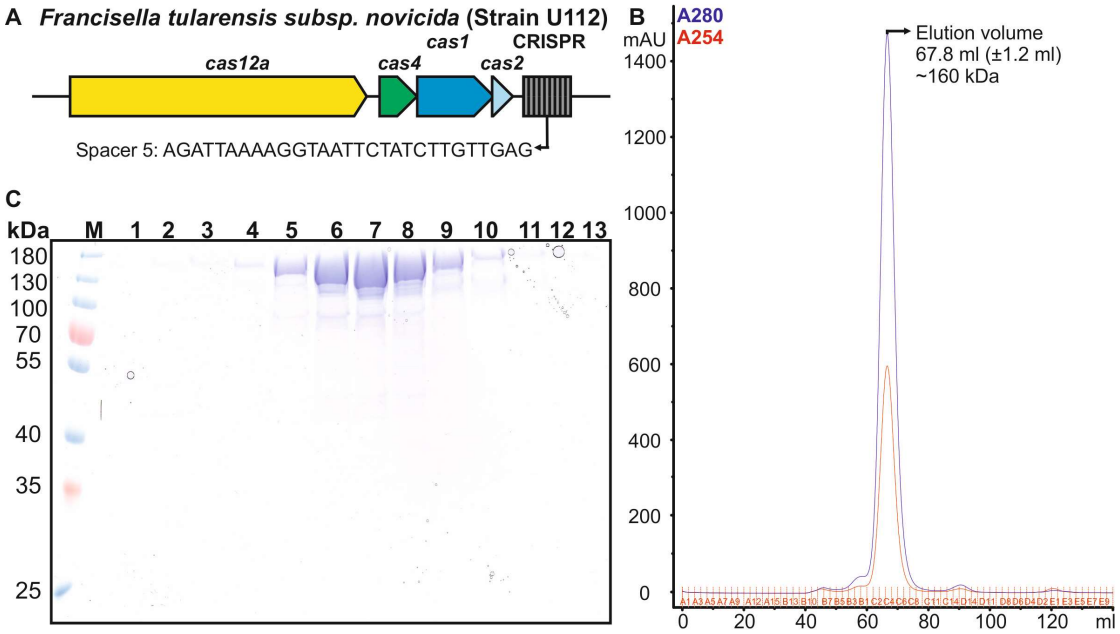


**Figure S1. Genomic context and purification details of FnCas12a, Related to Figures 1 and 3.**
(A) Schematic scaled representation of the genomic context of the *cas12a* (FTN_1397) gene, encoding FnCas12a. Spacers in the CRISPR locus are indicated as grey bars. The sequence of spacer 5 is used as guide sequence for the crRNA1 used throughout this study.
(B) Typical size exclusion purification chromatogram of apo-FnCas12a. Size exclusion purification is the last step of the FnCas12a purification process. The retention volume correlates with a molecular weight (MW) of ~160 kDa, indicating that apo-FnCas12a (152 kDa) is monomeric.
(C) SDS-PAGE analysis of elution fractions from the size exclusion purification step.

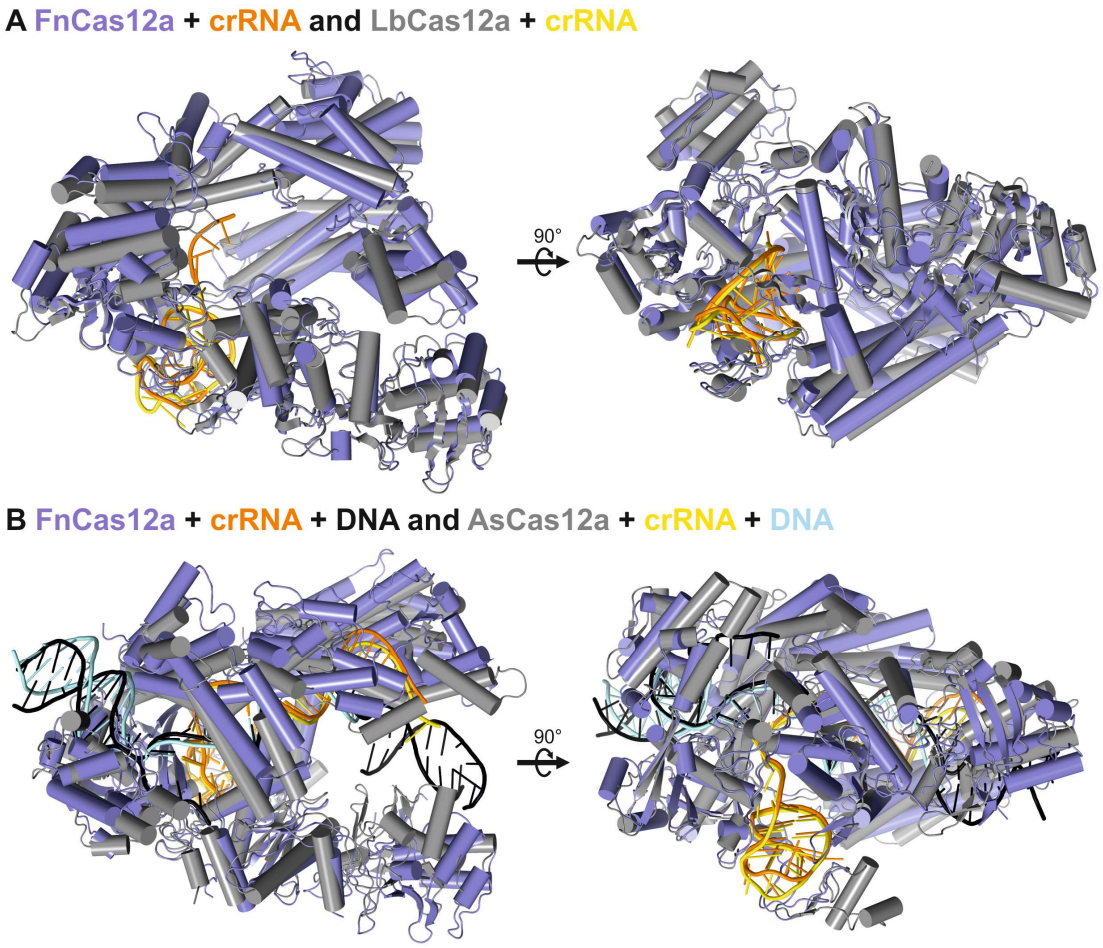


**Figure S2. Structural alignments of Cas12a orthologs, Related to Figures 1 and 3.**
(A) Structural alignment of the structure of the binary FnCas12a-crRNA complex, aligned to the structure of the binary LbCas12a-crRNA complex (PDB: 5ID6).
(B) Structural alignment of the structure of the ternary FnCas12a-crRNA-DNA complex, aligned to the structure of the ternary AsCas12a-crRNA-DNA complex (PDB accession code: 5B43).

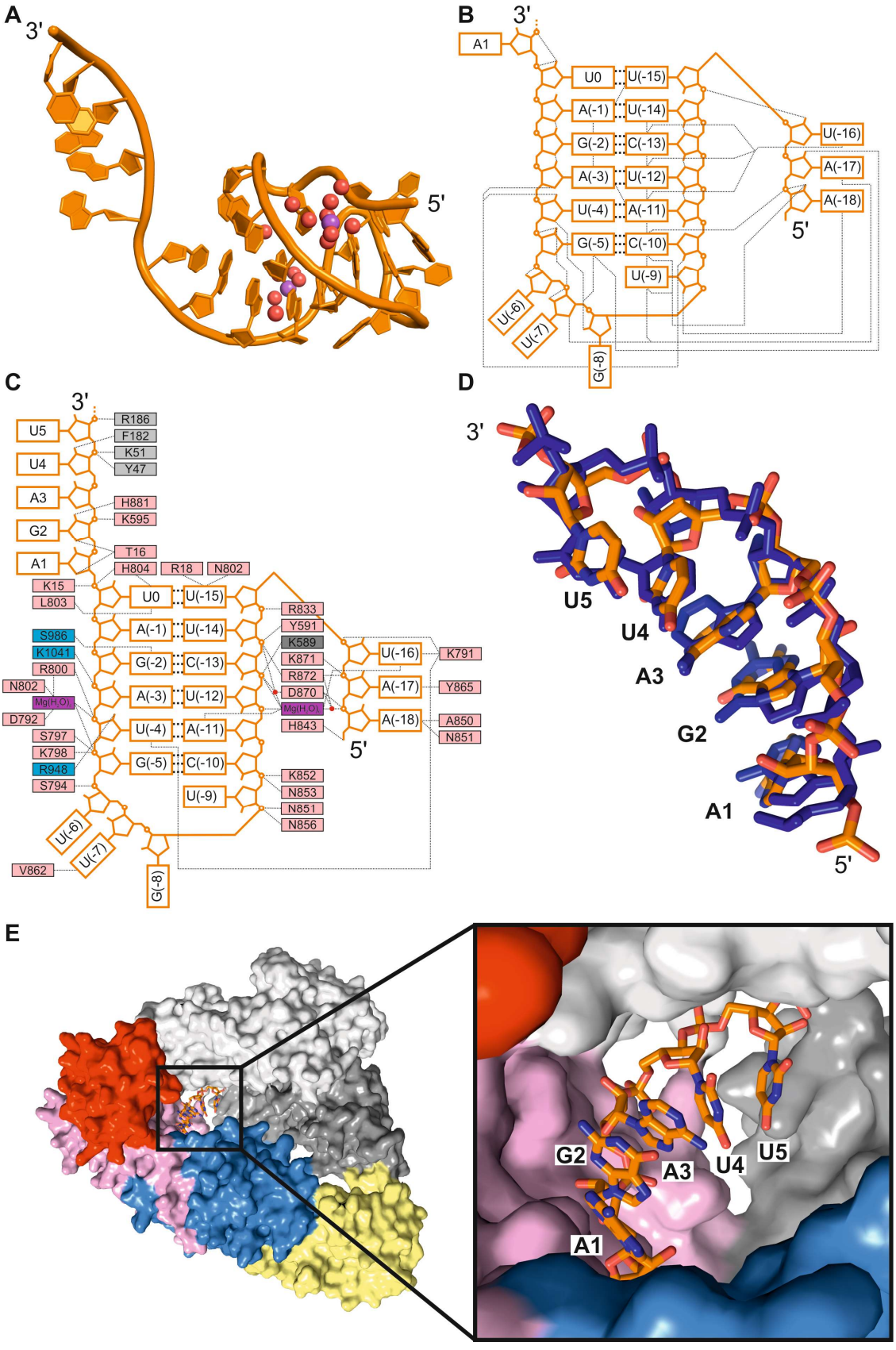
A
3'
5'
B
3'
A1
U0
U(-15)
A(-1)
U(-14)
G(-2)
C(-13)
A(-3)
U(-12)
U(-4)
A(-11)
G(-5)
C(-10)
U(-9)
U(-16)
A(-17)
A(-18)
5'
U(-6)
U(-7)
G(-8)
C
3'
U5
U4
A3
G2
A1
R186
F182
K51
Y47
H881
K595
T16
H804
R18
N802
K15
L803
S986
K1041
R800
N802
Mg(H2O)
D792
S797
K798
R948
S794
V862
R833
Y591
K589
K871
R872
D870
Mg(H2O)
H843
K852
N853
N851
N856
K791
Y865
A850
N851
5'
D
3'
U5
U4
A3
G2
A1
5'
E
G2
A3
U4
U5
A1

**Figure S3. Details of FnCas12a-crRNA binding interactions, Related to Figure 1.**
(A) Structure of the crRNA pseudoknot and crRNA seed segment in the binary FnCas12a-crRNA complex. Water molecules are depicted as red spheres. $Mg^{2+}$ ions are depicted as magenta spheres.
(B) Schematic representation of intra-pseudoknot hydrogen bonds. Base pairs are indicated with thick dashed lines, while other hydrogen bonding interactions are indicated with thin dashed lines.
(C) Schematic representation of hydrogen bonding contacts formed between FnCas12a, the crRNA, and the hydrated divalent cations. FnCas12a residues are colored according to their domains (see **Figure 1B**). Base pairs are indicated with thick dashed lines, while other hydrogen bonds are indicated with thin dashed lines. Red circles indicate water-mediated hydrogen bonding. Nucleotides 6–24 of the crRNA are not ordered in the structure.
(D) Structure of the nucleotides 1–5 of the RNA, spanning the crRNA seed sequence, superimposed on a model of the same sequence adopting perfect A-helix geometry.
(E) The crRNA seed sequence is solvent exposed. FnCas12a is shown in surface representation and the crRNA in stick format. The right panel is a close-up view of the seed sequence bases.

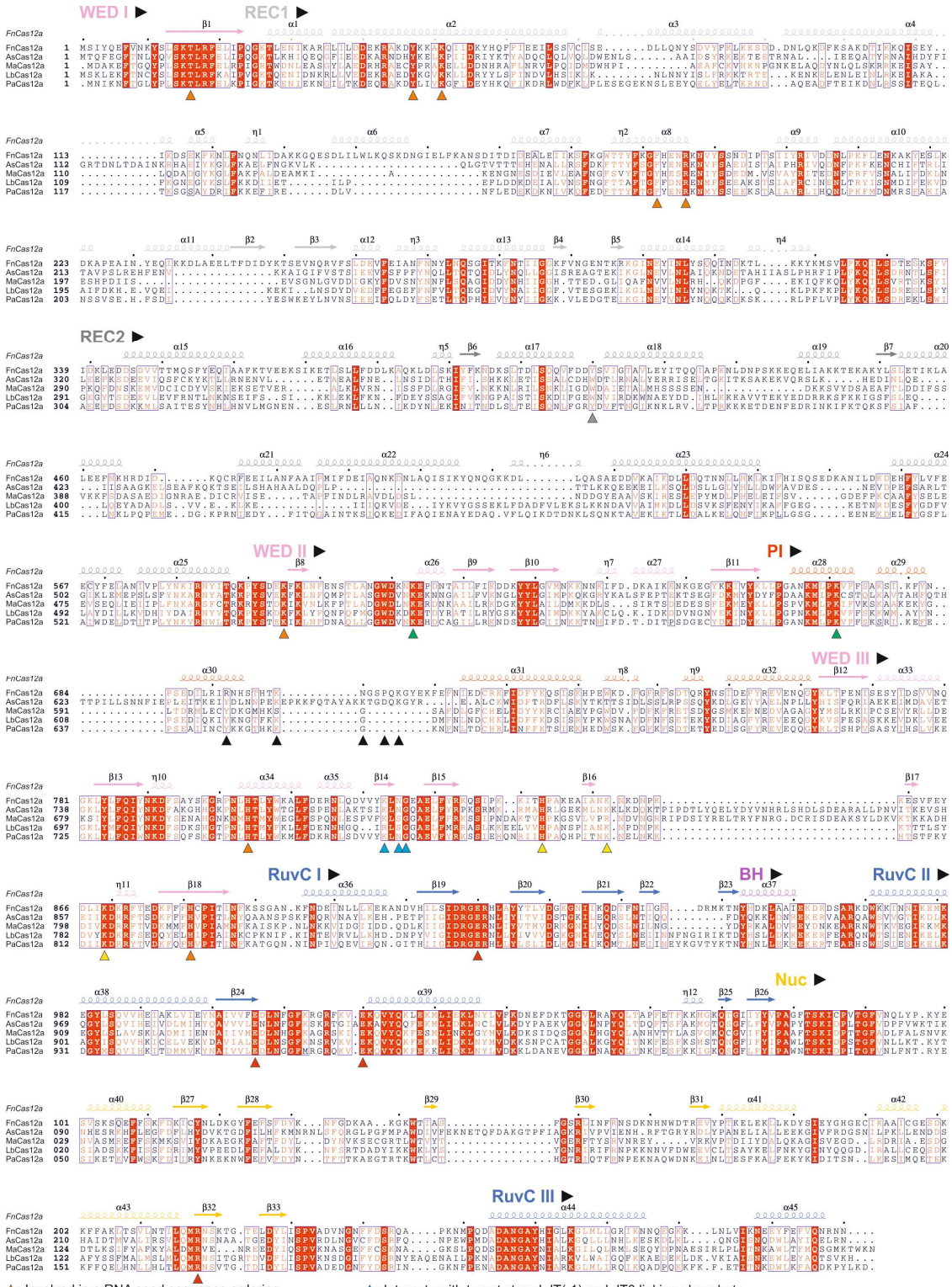
WED I
REC1
REC2
WED II
PI
WED III
RuvC I
BH
RuvC II
Nuc
RuvC III
FnCas12a
AsCas12a
MaCas12a
LbCas12a
PaCas12a
▲: Involved in crRNA seed sequence ordering
▲: Aromatic residue interupting RNA-DNA heteroduplex
▲: Stabilizes the displaced non-target strand in FnCas12a
▲: Involved in PAM base readout
▲: Interacts with target strand dT(-1) and dT0 linking phosphate
▲: Catalytic residue involved in pre-crRNA processing
▲: Catalytic residue involved in DNA cleavage

**Figure S4. Multiple sequence alignment of FnCas12a orthologs, Related to Figure 1 and 3.** Clustal Omega (Sievers et al., 2011) was used to generate a Multiple sequence alignment of Cas12a protein sequences of *Francisella novicida* U122 (FnCas12a), *Acidaminococcus sp.* BV3L6 (AsCas12a), *Methanomethylophilus alvus* Mx1201 (MaCas12a), *Lachnospiraceae bacterium* ND2006 (LbCas12a), and *Prevotella albensis* DSM11370 (PaCas12a). The Clustal Omega sequence alignment and the structural information from the structure of the binary FnCas12a-crRNA complex were used as input for ESPript 3.0 (http://espript.ibcp.fr) (Robert and Gouet, 2014) to align secondary structure features to the sequence alignment. Residues important for specific FnCas12a functions are indicated with colored.

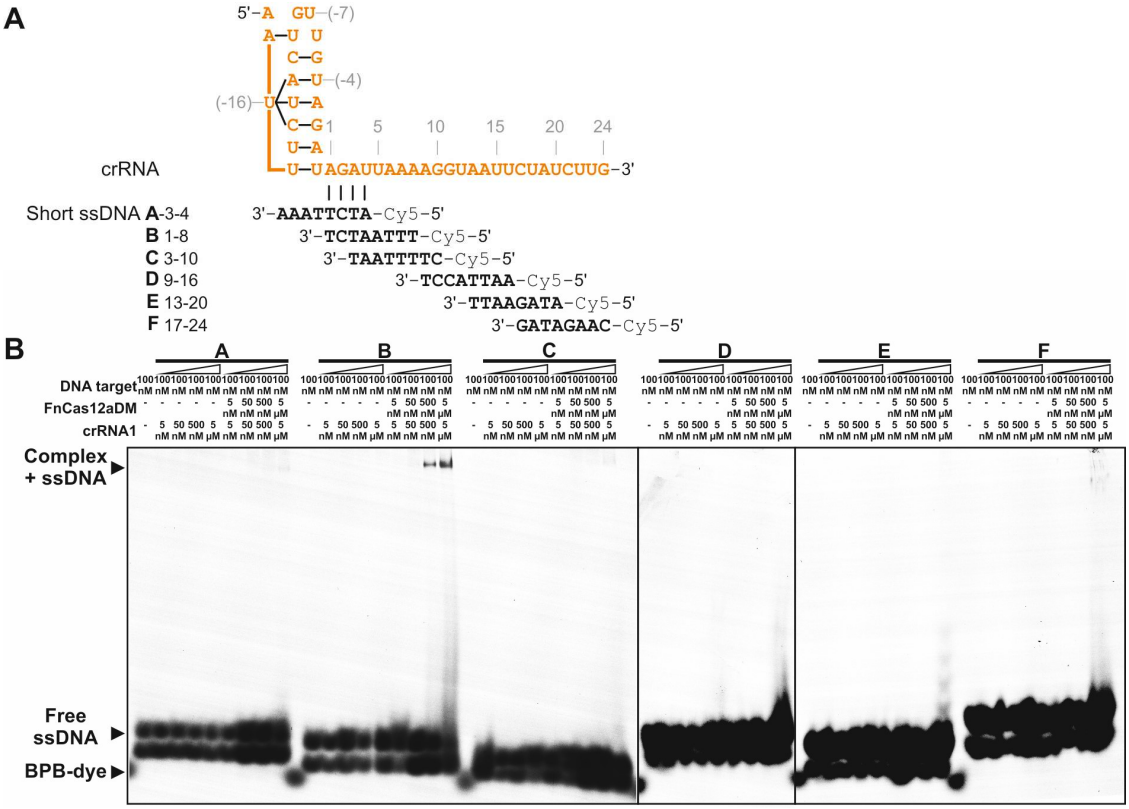


**Figure S5. EMSA binding assay of single stranded DNA targets with FnCas12aDM-crRNA complexes, Related to Figure 1.**

(A) Schematic representation of the crRNA and short single-stranded DNA targets.

(B) Pre-ordering of the crRNA seed segment increases the binding affinity to target DNA. crRNA alone (negative control) and FnCas12aDM-crRNA complexes were incubated with 5'-end Cy5 labeled single-stranded target DNA complementary to segments of the crRNA, and resolved on native 8% polyacrylamide gels. BPB-dye: Bromophenol blue dye used to indicate empty lanes.

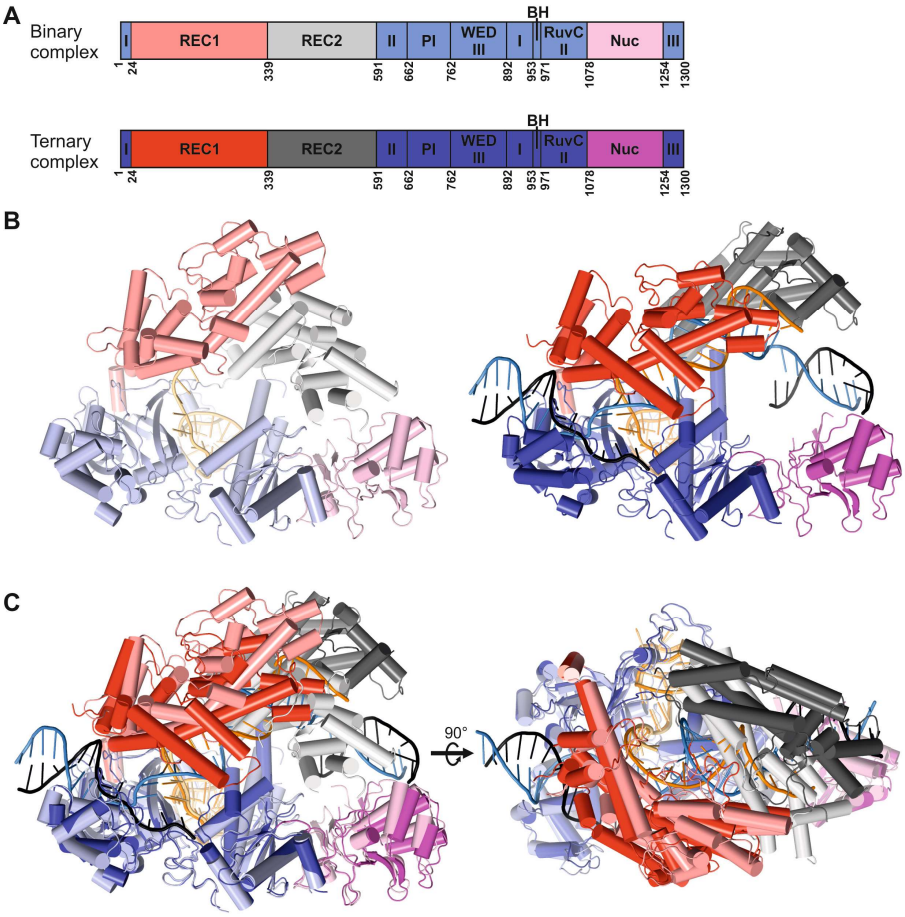


**Figure S6. Conformational rearrangements in FnCas12a upon transition from the binary FnCas12a-crRNA to the ternary FnCas12a-crRNA-DNA complex, Related to Figure 1 and 3.**
(A) Schematic diagram of FnCas12a domains and their color coding in this figure.
(B) Side-by-side comparison of the binary (left, pale) and ternary (right, bright) complexes.
(C) Superposition of the structures of the binary and ternary complexes. The NUC lobes of both structures were aligned using least-squares alignment in COOT. The WED, PI and RuvC domains within the NUC lobe do not substantially change their conformations, while the Nuc domain rotates by 22° relative to the rest of the NUC lobe. This movement does not appear to affect the position of the putative catalytic residue (Arg1218) in the Nuc domain relative to the RuvC domain catalytic residues Asp917, Glu1006, and Glu1020. No major rearrangements are observed in the crRNA pseudoknot, which is mostly coordinated by the rigid NUC lobe domains. The PAM recognition site is fully formed in the binary complex, and PAM binding results in only a minor narrowing (~5 Å) of the PAM binding cleft. Unlike the NUC lobe, the REC lobe undergoes substantial rearrangement upon target binding. The REC1 domain rotates by approximately 27° relative to the NUC lobe, resulting in a small shift of the crRNA nucleotides comprising the seed sequence. The REC2 domain undergoes a rotation of ~50° combined with a translation of 6 Å. The restructuring of the REC lobe generates the binding surface for the crRNA-target DNA heteroduplex in the central channel of Cas12a, establishing numerous hydrogen bonding and salt bridge interactions (see **Figure S7**).

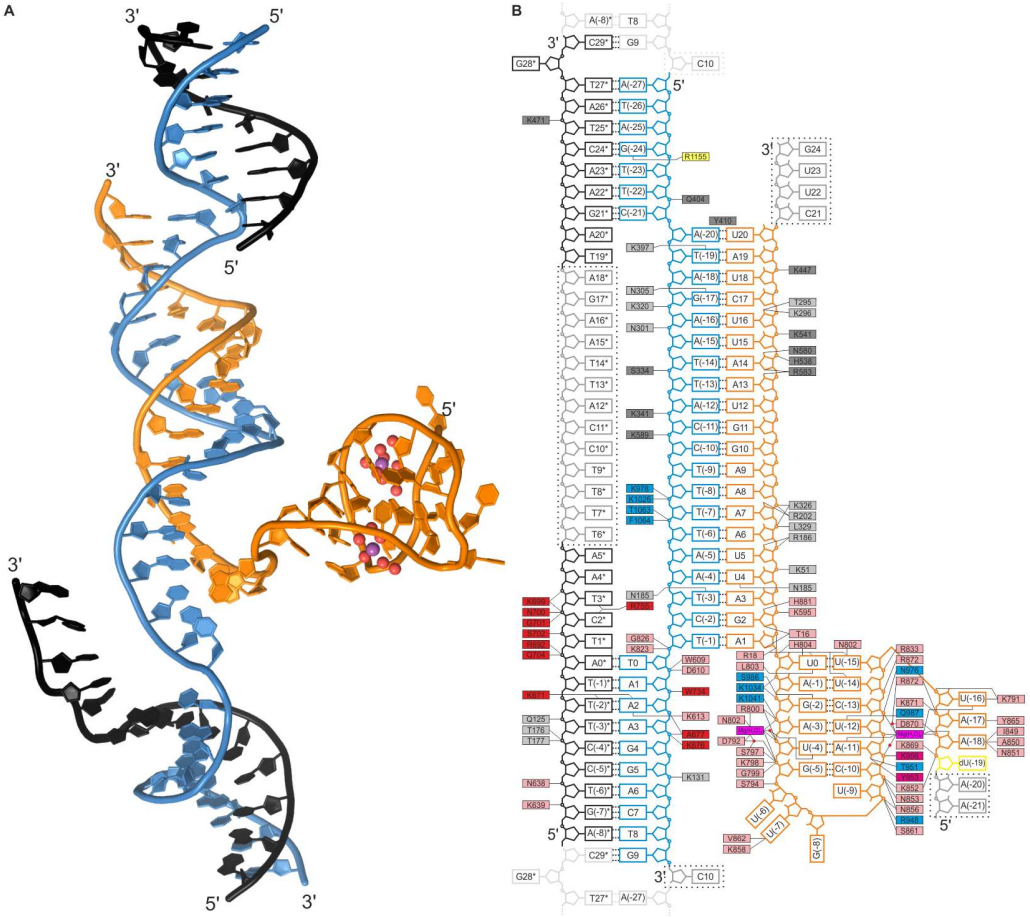


**Figure S7. Details of FnCas12a-crRNA-DNA interactions, Related to Figure 3 and 4.**

(A) Structure of nucleic acids in the structure of FnCas12a-crRNA complex bound to a dsDNA target. Water molecules are depicted as red spheres, while $Mg^{2+}$ ions are depicted as magenta spheres.

(B) Schematic representation of hydrogen bonding interactions between FnCas12a, nucleic acids, and divalent cations in the ternary structure. FnCas12a residues are colored according to their domains (see **Figure 1B**). Nucleotides colored in dark grey are not ordered in the structure, while light grey nucleotides represent crystal-contact forming residues in symmetry-related molecules. Base pairs are indicated with thick dashed lines, while other hydrogen bonds are indicated with thin dashed lines. Red circles indicate water-mediated hydrogen bonding. Intra-crRNA pseudoknot hydrogen bonds in the crRNA are not displayed for clarity.

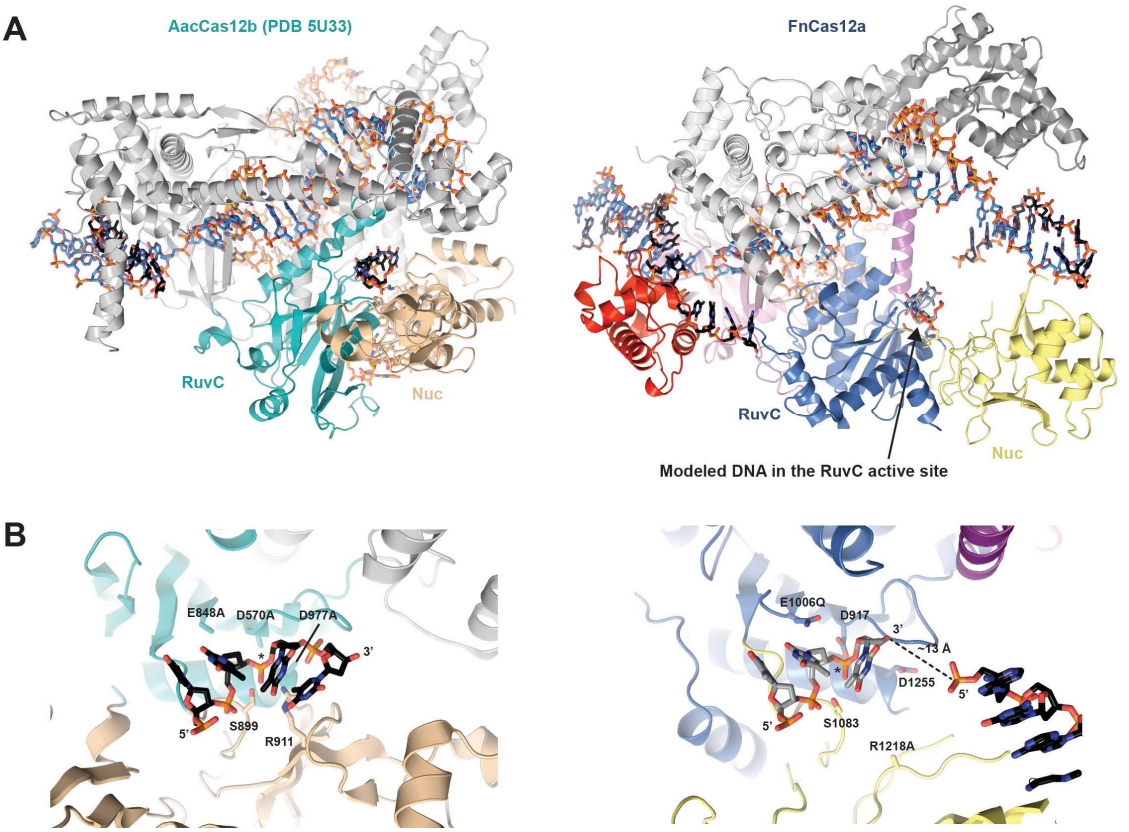


**Figure S8. A modeled non-target strand in the RuvC catalytic site of FnCas12a, Related to Figure 5.**

(A) Structures of AacCas12b (left panel, PDB accession code: 5U33) and the FnCas12a R-loop structure with three modeled non-target strand nucleotides bound in the RuvC catalytic site (right panel; the modeled DNA fragment is colored grey).

(B) RuvC catalytic site residues of AacCas12b (left panel) and FnCas12a (right panel) are located in the vicinity of the (modeled) scissile phosphate (indicated with an *). Dashed line indicates the distance between the 3' end of the modeled DNA and the 5' phosphate group of nucleotide A20 in the non-target strand in the PAM-distal DNA duplex.

**Table S1. Oligonucleotides used in this study, Related to Figure 1-5.**

| | Oligo | Sequence (5'-3') | Description |
|---|---|---|---|
| TS/NTS oligonucleotides | oDS047 | AATTCTAATAATTTAAGATTAAAAGGTAATTCTATCTTGTTGAGATCTGAGCTTA | Target insert FW |
| | oDS048 | AGCTTAAGCTCAGATCTCAACAAGATAGAATTACCTTTTAATCTTAAATTATTAG | Target insert RV |
| | oDS074 | CAGATCTCAACAAGATAGAATTACCTTTTAATCTTAAATTATTAGAA | TS |
| | oDS079 | TTCTAATAATTTAAGATTAAAAGGTAATTCTATCTTGTTGAGATCTG | NTS |
| | oDS141 | AGTCCTTTATCTAATTTTCCATTAAGATAGAACTATGC | NTS Crystal |
| | oDS142 | ATAGTTCATAGAATTACCTTTTAATCTTAAAGGACTGC | TS Crystal |
| EMSA | oDS205 | Cy5-ATCTTAAA | -4-4 |
| | oDS206 | Cy5-TTTAATCT | 1-8 |
| | oDS207 | Cy5-CTTTTAAT | 3-10 |
| | oDS209 | Cy5-AATTACCT | 9-16 |
| | oDS210 | Cy5-ATAGAATT | 13-20 |
| | oDS211 | Cy5-CAAGATAG | 17-24 |
| Synthetic crRNA | crRNA1 | AAUUUCUACUGUUGUAGAUAGAUUAAAAGGUAAUUCUAUCUUG | crRNA |
| | Pre-crRNA1 | AAUAAUUUCUACUGUUGUAGAUAGAUUAAAAGGUAAUUCUAUCUUG | Pre-crRNA |
| | Pre-crRNAX | AAdUAAUUUCUACUGUUGUAGAUAGAUUAAAAGGUAAUUCUAUCUUG | Mimic pre-crRNA |
| | Cy5-pre-crRNA | Cy5-UUUAAAUAAUUUCUACUGUUGUAGAU | Truncated pre-crRNA 5' Cy5 |
| | crRNAλ | AAUUUCUACUGUUGUAGAUGUGAUAAGUGGAAUGCCAUGUGGG | crRNA |
| Labeled DNA targets | oDS073 | CAGATCTCAACAAGATAGAATTACCTTTTAATCTTAAATTATTAGAA-ATTO532 | TS 3' ATTO532 |
| | oDS078 | TTCTAATAATTTAAGATTAAAAGGTAATTCTATCTTGTTGAGATCTG-ATTO532 | NTS 3' ATTO532 |
| | oDS203 | Cy5-CAGATCTCAACAAGATAGAATTACCTTTTAATCTTAAATTATTAGAA | TS 5' Cy5 |
| | oDS204 | Cy5-TTCTAATAATTTAAGATTAAAAGGTAATTCTATCTTGTTGAGATCTG | NTS 5' Cy5 |
| | oDS270 | Cy5-ACTCAATTTTGACAGCCCACATGGCATTCCACTTATCACTAAAGGCATCCTTCCACGT | λ TS 5' Cy5 |
| | oDS271 | Cy5-ACGTGGAAGGATGCCTTTAGTGATAAGTGGAATGCCATGTGGGCTGTCAAAATTGAGT | λ NTS 5' Cy5 |
| | oDS272 | ACTCAATTTTGACAGCCCACATGGCATTCCACTTATCACTAAAGGCATCCTTCCACGT-ATTO532 | λ TS 3' ATTO532 |
| | oDS273 | ACGTGGAAGGATGCCTTTAGTGATAAGTGGAATGCCATGTGGGCTGTCAAAATTGAGT-ATTO532 | λ NTS 3' ATTO532 |
| Exonuclease III experiments | TSλ* | ATTO532-ACTCAATTTTGACAGCCCACATGGCATTCCACTTATCACTAAAGGCATCCTTCCACGT | λ TS 5' ATTO532 |
| | NTSλ* | ATTO532-ACGTGGAAGGATGCCTTTAGTGATAAGTGGAATGCCATGTGGGCTGTCAAAATTGAGT | λ NTS 5' ATTO532 |
| | TSλ | ACTCAATTTTGACAGCCCACATGGCATTCCACTTATCACTAAAGGCATCCTTCCACGT | λ TS |
| | NTSλ | ACGTGGAAGGATGCCTTTAGTGATAAGTGGAATGCCATGTGGGCTGTCAAAATTGAGT | λ NTS |

Nucleotide mismatched in dsDNA targets are colored red. Nucleotides used for cloning are underlined.
Cy5: Fluorescent label Cyanine 5. ATTO532: Fluorescent label ATTO532.

**Table S1 (continued). Oligonucleotides used in this study, Related to Figure 1-5.**

| | Oligo | Sequence (5'-3') | Description |
|---|---|---|---|
| Site Directed Mutagenesis of target plasmid | oDS171 | CAGATCTCAACAAGATAGAATTACCTTTTAATCATAAATTATTAGAA | pDS074 FW |
| | oDS172 | TTCTAATAATTTATGATTAAAAGGTAATTCTATCTTGTTGAGATCTG | pDS074 RV |
| | oDS173 | CAGATCTCAACAAGATAGAATTACCTTTTAATGTTAAATTATTAGAA | pDS075 FW |
| | oDS174 | TTCTAATAATTTAACATTAAAAGGTAATTCTATCTTGTTGAGATCTG | pDS075 RV |
| | oDS175 | CAGATCTCAACAAGATAGAATTACCTTTTAAACTTAAATTATTAGAA | pDS076 FW |
| | oDS176 | TTCTAATAATTTAAGTTTAAAAGGTAATTCTATCTTGTTGAGATCTG | pDS076 RV |
| | oDS177 | CAGATCTCAACAAGATAGAATTACCTTTTATTCTTAAATTATTAGAA | pDS077 FW |
| | oDS178 | TTCTAATAATTTAAGAATAAAAGGTAATTCTATCTTGTTGAGATCTG | pDS077 RV |
| | oDS179 | CAGATCTCAACAAGATAGAATTACCTTTTTATCTTAAATTATTAGAA | pDS078 FW |
| | oDS180 | TTCTAATAATTTAAGATAAAAAGGTAATTCTATCTTGTTGAGATCTG | pDS078 RV |
| | oDS181 | CAGATCTCAACAAGATAGAATTACCTTTAAATCTTAAATTATTAGAA | pDS079 FW |
| | oDS182 | TTCTAATAATTTAAGATTTAAAGGTAATTCTATCTTGTTGAGATCTG | pDS079 RV |
| | oDS183 | CAGATCTCAACAAGATAGAATTACGTTTTAATCTTAAATTATTAGAA | pDS080 FW |
| | oDS184 | TTCTAATAATTTAAGATTAAAACGTAATTCTATCTTGTTGAGATCTG | pDS080 RV |
| | oDS185 | CAGATCTCAACAAGATAGAAATACCTTTTAATCTTAAATTATTAGAA | pDS081 FW |
| | oDS186 | TTCTAATAATTTAAGATTAAAAGGTATTTCTATCTTGTTGAGATCTG | pDS081 RV |
| | oDS187 | CAGATCTCAACAAGATTGAATTACCTTTTAATCTTAAATTATTAGAA | pDS082 FW |
| | oDS188 | TTCTAATAATTTAAGATTAAAAGGTAATTCAATCTTGTTGAGATCTG | pDS082 RV |
| | oDS189 | CAGATCTCAACAAGAAAGAATTACCTTTTAATCTTAAATTATTAGAA | pDS083 FW |
| | oDS190 | TTCTAATAATTTAAGATTAAAAGGTAATTCTTTCTTGTTGAGATCTG | pDS083 RV |
| | oDS191 | CAGATCTCAACAAGTTAGAATTACCTTTTAATCTTAAATTATTAGAA | pDS084 FW |
| | oDS192 | TTCTAATAATTTAAGATTAAAAGGTAATTCTAACTTGTTGAGATCTG | pDS084 RV |
| | oDS193 | CAGATCTCAACAACATAGAATTACCTTTTAATCTTAAATTATTAGAA | pDS085 FW |
| | oDS194 | TTCTAATAATTTAAGATTAAAAGGTAATTCTATGTTGTTGAGATCTG | pDS085 RV |
| | oDS195 | CAGATCTCAACATGATAGAATTACCTTTTAATCTTAAATTATTAGAA | pDS086 FW |
| | oDS196 | TTCTAATAATTTAAGATTAAAAGGTAATTCTATCATGTTGAGATCTG | pDS086 RV |
| | oDS197 | CAGATCTCAACTAGATAGAATTACCTTTTAATCTTAAATTATTAGAA | pDS087 FW |
| | oDS198 | TTCTAATAATTTAAGATTAAAAGGTAATTCTATCTAGTTGAGATCTG | pDS087 RV |
| | oDS199 | CAGATCTCAAGAAGATAGAATTACCTTTTAATCTTAAATTATTAGAA | pDS088 FW |
| | oDS200 | TTCTAATAATTTAAGATTAAAAGGTAATTCTATCTTCTTGAGATCTG | pDS088 RV |
| | oDS201 | CAGATCTCAAGTTCATAGAATTACCTTTTAATCTTAAATTATTAGAA | pDS089 FW |
| | oDS202 | TTCTAATAATTTAAGATTAAAAGGTAATTCTATGAACTTGAGATCTG | pDS089 RV |
| | oDS251 | CAGATCTCAACAAGATAGAATTACCTTATAATCTTAAATTATTAGAA | pDS103 FW |
| | oDS252 | TTCTAATAATTTAAGATTATAAGGTAATTCTATCTTGTTGAGATCTG | pDS103 RV |
| | oDS253 | CAGATCTCAACAAGATAGAATTACCTATTAATCTTAAATTATTAGAA | pDS104 FW |
| | oDS254 | TTCTAATAATTTAAGATTAATAGGTAATTCTATCTTGTTGAGATCTG | pDS104 RV |
| | oDS255 | CAGATCTCAACAAGATAGAATTACCATTTAATCTTAAATTATTAGAA | pDS105 FW |
| | oDS256 | TTCTAATAATTTAAGATTAAATGGTAATTCTATCTTGTTGAGATCTG | pDS105 RV |
| | oDS257 | CAGATCTCAACAAGATAGAATTAGCTTTTAATCTTAAATTATTAGAA | pDS106 FW |
| | oDS258 | TTCTAATAATTTAAGATTAAAAGCTAATTCTATCTTGTTGAGATCTG | pDS106 RV |
| | oDS259 | CAGATCTCAACAAGATAGAATTTCCTTTTAATCTTAAATTATTAGAA | pDS107 FW |
| | oDS260 | TTCTAATAATTTAAGATTAAAAGGAAATTCTATCTTGTTGAGATCTG | pDS107 RV |
| | oDS261 | CAGATCTCAACAAGATAGAATAACCTTTTAATCTTAAATTATTAGAA | pDS108 FW |
| | oDS262 | TTCTAATAATTTAAGATTAAAAGGTTATTCTATCTTGTTGAGATCTG | pDS108 RV |
| | oDS263 | CAGATCTCAACAAGATAGATTTACCTTTTAATCTTAAATTATTAGAA | pDS109 FW |
| | oDS264 | TTCTAATAATTTAAGATTAAAAGGTAAATCTATCTTGTTGAGATCTG | pDS109 RV |
| | oDS265 | CAGATCTCAACAAGATAGTATTACCTTTTAATCTTAAATTATTAGAA | pDS110 FW |
| | oDS266 | TTCTAATAATTTAAGATTAAAAGGTAATACTATCTTGTTGAGATCTG | pDS110 RV |
| | oDS267 | CAGATCTCAACAAGATACAATTACCTTTTAATCTTAAATTATTAGAA | pDS111 FW |
| | oDS268 | TTCTAATAATTTAAGATTAAAAGGTAATTGTATCTTGTTGAGATCTG | pDS111 RV |

Nucleotide mismatched in dsDNA targets are colored red. Nucleotides used for cloning are underlined.
Cy5: Fluorescent label Cyanine 5. ATTO532: Fluorescent label ATTO532.

**Table S1 (continued). Oligonucleotides used in this study, Related to Figure 1-5.**

| | Oligo | Sequence (5'-3') | Description |
|---|---|---|---|
| Site Directed Mutagenesis/inverse PCR of FnCas12a expression plasmid | oDS094 | CATATTCTGAGCATTGCTCGTGGTGAACGTCATC | D917A FW |
| | oDS095 | GATGACGTTCACCACGAGCAATGCTCAGAATATG | D917A RV |
| | oDS096 | GCAATTGTAGTTTTTGCGGATCTGAATTTTGGG | E1006A FW |
| | oDS097 | CCCAAAATTCAGATCCGCAAAAACTACAATTGC | E1006A RV |
| | oDS098 | GCCGCAGGATGCAGCTGCTAATGGTGCATATC | D1255A FW |
| | oDS099 | GATATGCACCATTAGCAGCTGCATCCTGCGGC | D1255A RV |
| | oDS144 | GAGCATTGATCGTGGTGCACGTCATCTGGCATAC | E920A FW |
| | oDS145 | GTATGCCAGATGACGTGCACCACGATCAATGCTC | E920A RV |
| | oDS146 | GTGGGAGATTCAAGGTCGCGAAGCAAGTATATCAGAAG | E1020A FW |
| | oDS147 | CTTCTGATATACTTGCTTCGCGACCTTGAATCTCCCAC | E1020A RV |
| | oDS153 | GCAATTGTAGTTTTTCAGGATCTGAATTTTGGG | E1006Q FW |
| | oDS154 | CCCAAAATTCAGATCCTGAAAAACTACAATTGC | E1006Q RV |
| | oDS157 | CCATACTGCAAATGGCAAACAGCAAAACAGGTACC | R1218A FW |
| | oDS158 | GGTACCTGTTTTGCTGTTTGCCATTTGCAGTATGG | R1218A RV |
| | oDS216 | GCCGCAGGATGCAGCTGCTAATGGTGCATATC | D1255A FW |
| | oDS217 | GATATGCACCATTAGCAGCTGCATCCTGCGGC | D1255A RV |
| | oDS218 | GCCGCAGGATGCAAACGCTAATGGTGCATATC | D1255N FW |
| | oDS219 | GATATGCACCATTAGCGTTTGCATCCTGCGGC | D1255N RV |
| | oDS220 | CAGGTACCGAGCTGGCTTATTTAATTAGCCCG | D1227A FW |
| | oDS221 | CGGGCTAATTAAATAAGCCAGCTCGGTACCTG | D1227A RV |
| | oDS222 | CAAAACAGGTACCGAGCTGAACTATTTAATTAGCCCGGTCG | D1227N FW |
| | oDS223 | CGACCGGGCTAATTAAATAGTTCAGCTCGGTACCTGTTTTG | D1227N RV |
| | oDS226 | CAAAAAATGGTAGCCCGGCGAAAGGGTATGAAAAATTTG | Q704A FW |
| | oDS227 | CAAATTTTTCATACCCTTTCGCCGGGCTACCATTTTTTG | Q704A RV |
| | oDS228 | CCGAGCGAAGATATTTTACGTATTGCTAATCATTCGACAC | R692A FW |
| | oDS229 | GTGTCGAATGATTAGCAATACGTAAAATATCTTCGCTCGG | R692A RV |
| | oDS230 | GCTGGTGGTGGTCCGCAGAAAGGGTATGAAAAATTTG | PI deletion |
| | oDS231 | ATGTGTCGAATGATTACGAATACG | PI deletion |
| | oDS245 | GTCCCGGCAGGTTTTACCGCCAAAATTTGTCCGGTCACC | S1083A FW |
| | oDS246 | GGTGACCGGACAAATTTTGGCGGTAAAACCTGCCGGGAC | S1083A RV |
| | oDS247 | GAGGATCTGAATTTTGGGGCTAAACGTGGGAGATTCAAG | F1012A FW |
| | oDS248 | CTTGAATCTCCCACGTTTAGCCCCAAAATTCAGATCCTC | F1012A RV |

Nucleotide mismatched in dsDNA targets are colored red. Nucleotides used for cloning are underlined.
Cy5: Fluorescent label Cyanine 5. ATTO532: Fluorescent label ATTO532.

**Table S2. Plasmids used in this study, Related to Figures 1 and 3.**

| Plasmid | Description | Restriction sites used | Primers | Source |
|---|---|---|---|---|
| **pRARE** | *E. coli* Rosetta™ (DE3) plasmid, encodes rare tRNAs, $Cam^R$ | - | - | EMD Millipore |
| **pML-1B** | T7 RNA polymerase based expression vector, $Kan^R$ | - | - | Macrolab, AddGene |
| **pDS015** | *F. novicida cas12a* with N-term. His-tag and TEV cleavage site in pML-1B. Expression vector for FnCas12a. | SspI and Ligase Independent cloning | oDS027 oDS028 | This study |
| **pDS054 pDS066-pDS073 pDS090-pDS101** | Like pDS015, with introduced mutations, for expression of *F. novicida cas12a* mutants | Site Directed Mutagenesis or inverse PCR | See Table S1 | This study |
| **pUC19** | High copy number cloning vector, $Amp^R$ | - | - | New England Biolabs |
| **pDS027** | Target sequence in pUC19 vector | EcoRI HindIII | oDS047 oDS048 | This study |
| **pDS074-088** | Like pDS027, but with introduced mutations | Site Directed Mutagenesis | oDS171-oDS202 | This study |
| **pDS103-pDS111** | Like pDS027, but with introduced mutations | Site Directed Mutagenesis | oDS251-oDS268 | This study |