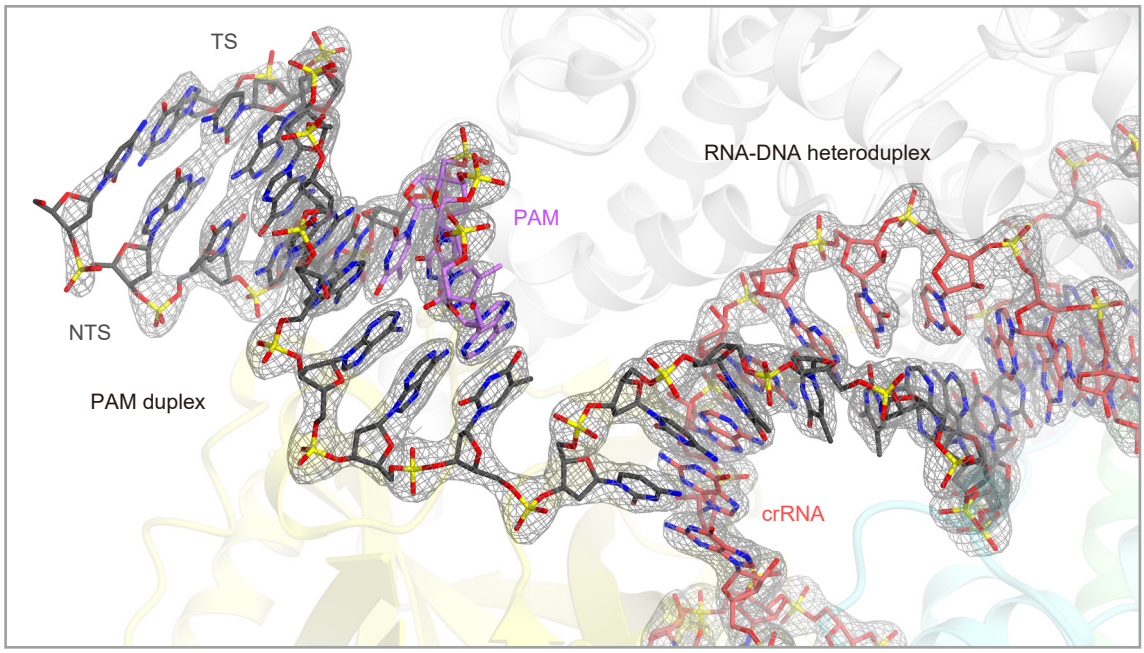

Figure S1. Electron Density Map, Related to Figure 2

The $2mF_O - DF_C$ electron density map for the nucleic acid molecules (contoured at 2σ).

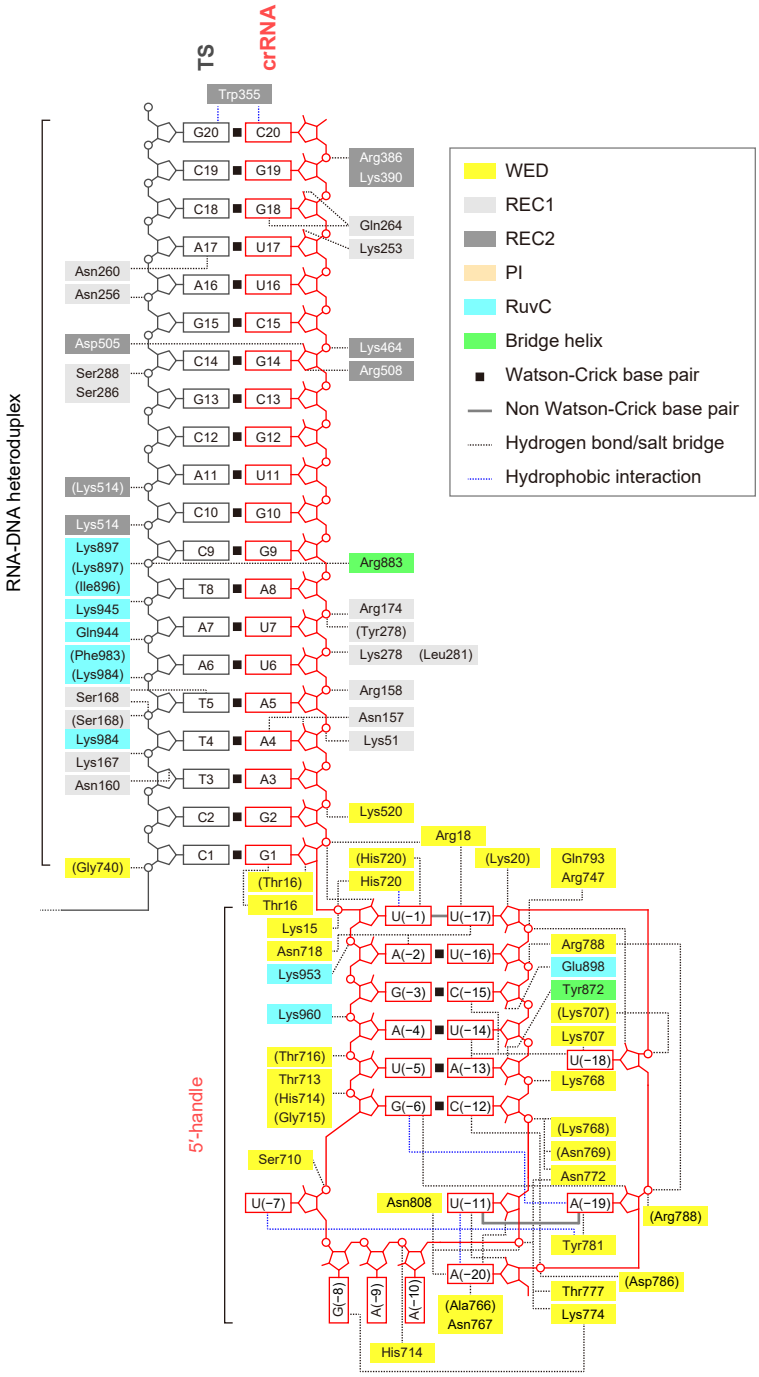

Figure S2. Schematic of the Nucleic Acid Recognition by LbCpf1, Related to Figure 2

Residues that interact with the crRNA and the target DNA via their main chain are shown in parentheses. Water-mediated hydrogen-bonding interactions are omitted for clarity.

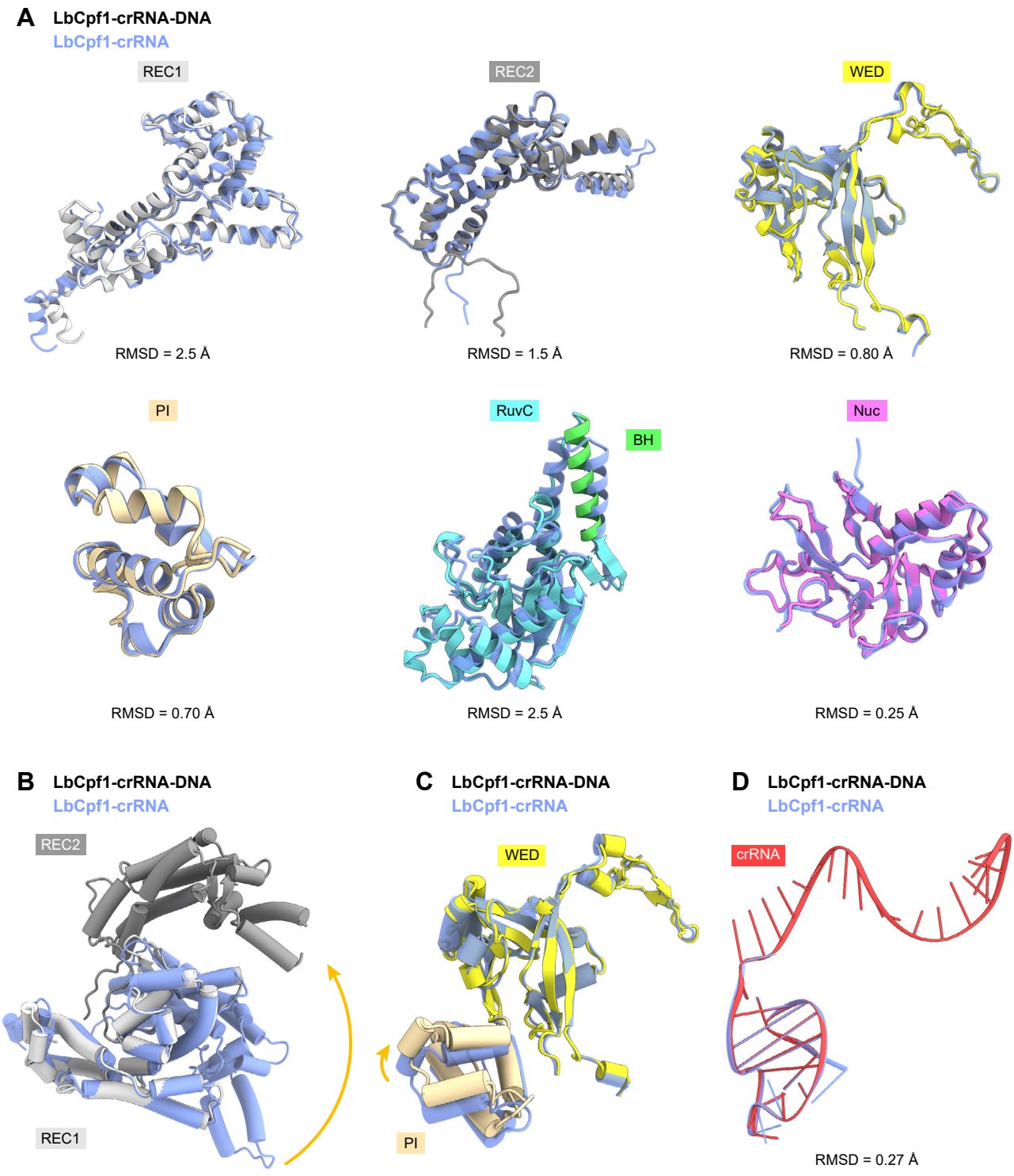

Figure S3. Structural Comparison between the Binary and Ternary Complexes of LbCpf1, Related to Figure 3

(A) Superimposition of the individual domains in the binary (PDB: 5ID6) (Dong et al., 2016) (blue) and ternary (colored) complexes of LbCpf1. The RMSD values for equivalent Cα atoms are shown below the structures.

(B and C) Superimposition of the REC1-REC2 (B) and WED-PI (C) domains in the binary (blue) and ternary (colored) complexes of LbCpf1, based on their REC1 and WED domains, respectively. Structural changes are indicated by orange arrows.

(D) Superimposition of the crRNA in the binary (blue) and ternary (colored) complexes of LbCpf1. The RMSD value for equivalent phosphorus atoms is shown below the structure.

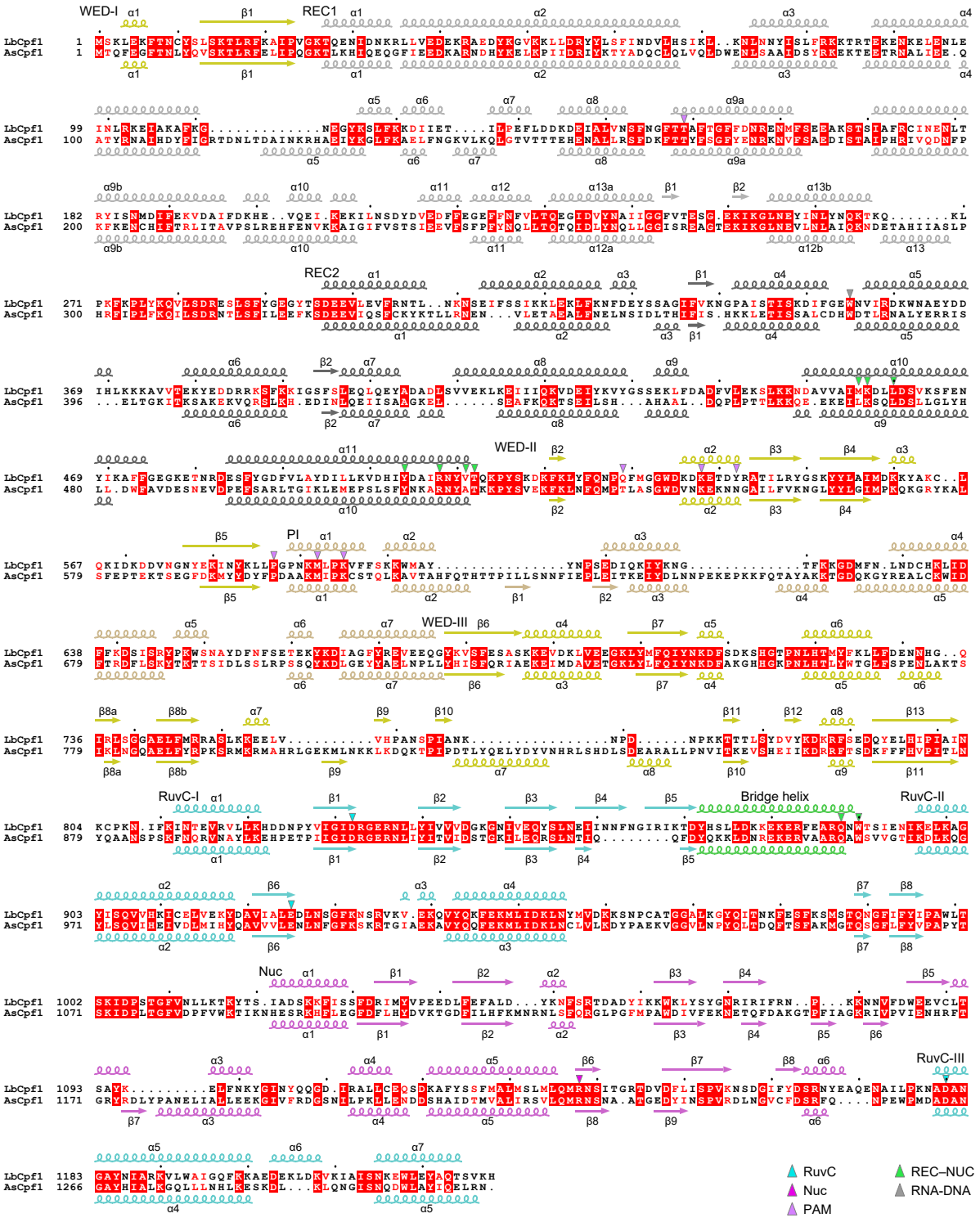

Figure S4. Sequence Alignment of LbCpf1 and AsCpf1, Related to Figure 4

Key residues are indicated by triangles. The figure was prepared using Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo) and ESPript (http://espript.ibcp.fr/ESPript/ESPript). As, *Acidaminococcus sp. BV3L6*; Lb, *Lachnospiraceae bacterium ND2006*.

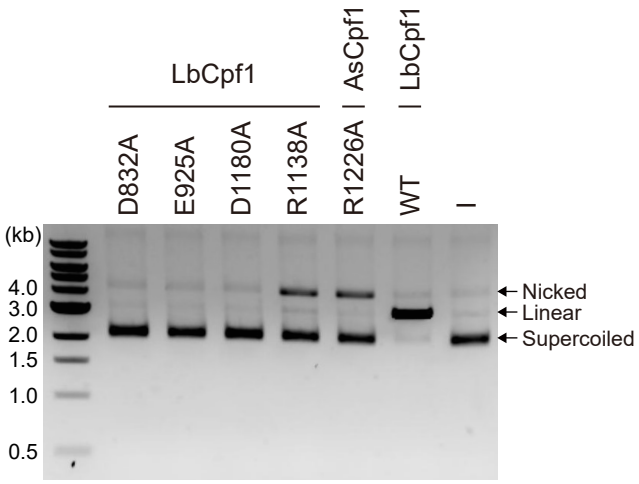

Figure S5. Mutational Analysis of the Nuclease Domains, Related to Figure 4

In vitro cleavage activities of the wild-type or mutants of Cpf1 proteins. The Cpf1-crRNA complex (100 nM) was incubated at 37°C for 20 min with a circular plasmid target with the TTTA PAM.

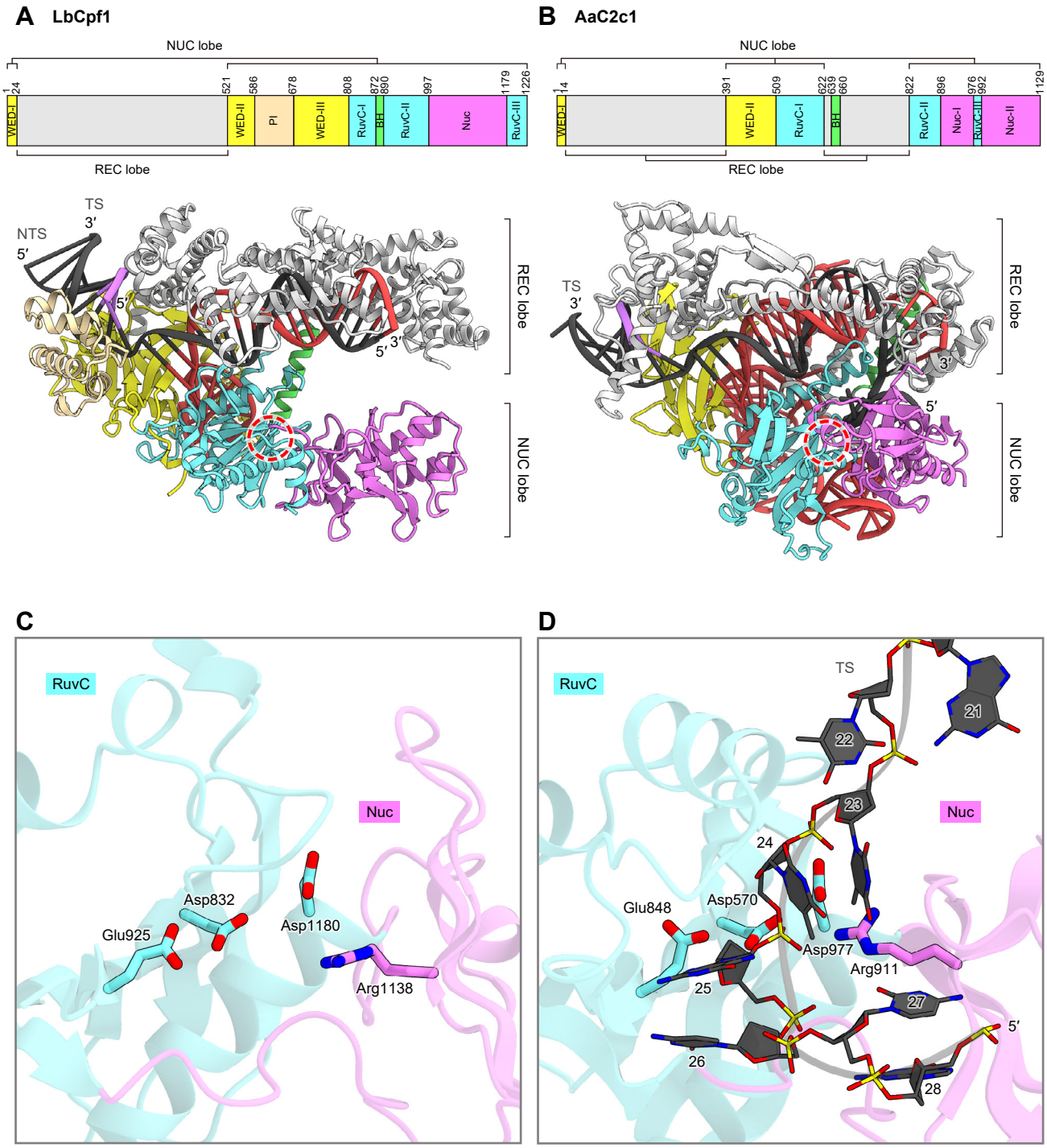

Figure S6. Structural Comparison between LbCpf1 and AaC2c1, Related to Figure 4

(A and B) Overall structures of LbCpf1 (A) and AaC2c1 (PDB: 5U30) (Yang et al., 2016) (B). The RuvC active sites are indicated by red circles.

(C and D) Active sites of LbCpf1 (C) and AaC2c1 (D). In (D), the side chains of Asp570, Glu848 and Asp977 in AaC2c1 are modeled, since these residues were mutated to alanine for crystallization.

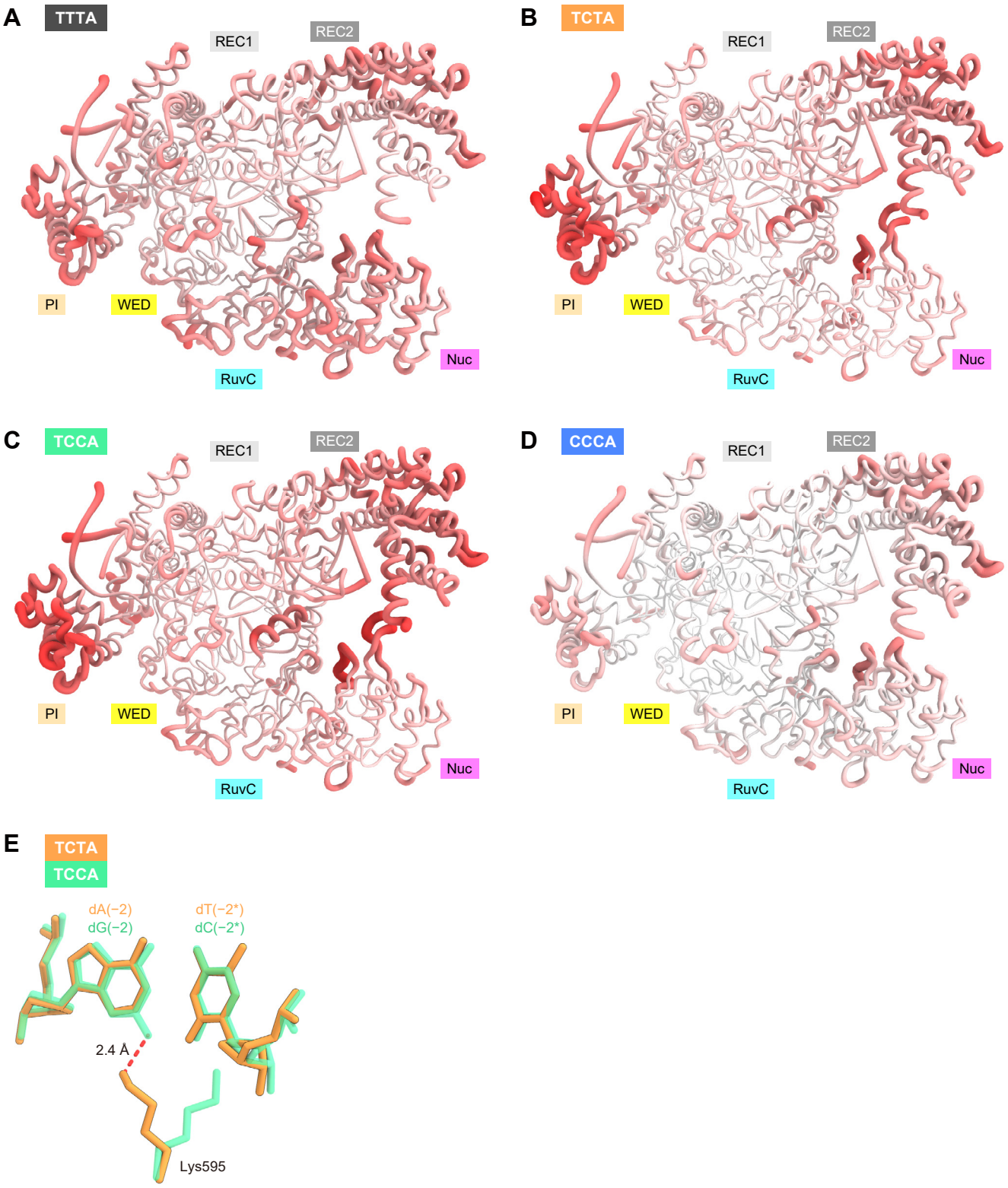

Figure S7. Flexibility of the PI Domain, Related to Figures 6 and 7

(A–D) *B*-factor distributions of the TTTA (A), TCTA (B), TCCA (C) and CCCA (D) complexes. The *B*-factor values are colored from white (40 Å^2) to red (200 Å^2). Note that the CCCA complex exhibits lower *B*-factor values, due to the different crystal packing interactions.

(E) Superimposition of the TCTA (orange) and TCCA (green) complexes.

Table S1. Oligonucleotides, Related to STAR Methods

Oligonucleotides used to generate the LbCpf1 expression vector (pE-SUMO-LbCpf1, with the TEV recognition site between His_6-SUMO and LbCpf1)		
PCR template	Forward primer	Reverse primer
pcDNA3.1-LbCpf1	CCCGGGGCATATGAGCAAGCTGGAGAAGTTTACAAAC	GGGGCTCGAGTTAGTGCTTCACGCTGGTCTGGGCGTAC
Oligonucleotides used to introduce the Cpf1 mutations (pE-SUMO-LbCpf1 or pE-SUMO-AsCpf1)		
Mutation	Forward primer	Reverse primer
LbCpf1-D832A	GCTAGGGGCGAGCGCAATCTGCTGTATA	GATGCCGATCACATAGGGGTTATCG
LbCpf1-E925A	GCGGACCTGAACTCTGGCTTTAAGAATA	CAGGGCGATCACGGCATCGTACTTC
LbCpf1-D1180A	GCCGCCAATGGCGCCTATAACATCGCCA	GGCGTTCTTTGGCAGGATGGCATTC
LbCpf1-R1138A	GCTAACAGCATCACAGGCCGC	CATCTGCAGCATCAGGCTCATCAG
AsCpf1-R1226A	GCTAACTCCAATGCCGCCACA	CATCTGCAGCACGCTGCGGATCAG
DNA oligonucleotides used for crystallization		
PAM	Target DNA strand	Non-target DNA strand
TTTA	GCCAAGCGCACCTAATTTCCTAAAGGACG	CGTCCTTTA
TCTA	GCCAAGCGCACCTAATTTCCTAGAGGACG	CGTCCTCTA
TCCA	GCCAAGCGCACCTAATTTCCTGGAGGACG	CGTCCTCCA
CCCA	GCCAAGCGCACCTAATTTCCTGGGGGACG	CGTCCCCCA
crRNA used for crystallization		
LbCpf1 crRNA 20	AAUUUCUACUAAGUGUAGAUGGAAAUUAGGUGCGCUUGGC	
crRNA used for cleavage assays		
LbCpf1 crRNA 24	AAUUUCUACUAAGUGUAGAUGGAAAUUAGGUGCGCUUGGCAACC	
AsCpf1 crRNA 24	AAUUUCUACUCUUGUAGAUGGAAAUUAGGUGCGCUUGGCAACC	
Oligonucleotides used to introduce the PAM sequences into the target plasmids (pUC119)		
Mutation	Forward primer	Reverse primer
TTTA	TTTAGGAAATTAGGTGCGCTTGGCAACC	GTATTTAGAAAAATAAACAAATAGGG
CTTA	CTTAGGAAATTAGGTGCGCTTGGCAACC	
TCTA	TCTAGGAAATTAGGTGCGCTTGGCAACC	
TTCA	TTCAGGAAATTAGGTGCGCTTGGCAACC	
CCTA	CCTAGGAAATTAGGTGCGCTTGGCAACC	
TCCA	TCCAGGAAATTAGGTGCGCTTGGCAACC	
CCCA	CCCAGGAAATTAGGTGCGCTTGGCAACC	
Oligonucleotides used for surveyor assays		
Locus	Forward primer	Reverse primer
DNMT1	CTGGGACTCAGGCGGGTCAC	CCTCACACAACAGCTTCATGTCAGC
EMX1	CCATCCCCTTCTGTGAATGT	GGAGATTGGAGACACGGAGA

Table S2. Target Sequences for the *In Vivo* Cleavage Assays, Related to STAR Methods

Locus	Site	PAM	Target sequence
DNMT1	1	CTTA	GAGCAGGCGTGCTGCACACAGCA
	2	CTTG	AGCCTCTGGGTCTAGAACCCTCT
	3	CTTC	AGCTAAAATAAAGGAGGAGGAAG
	4	TCTG	GGTCTAGAACCCTCTGGGGACCG
	5	TCTC	CGTGAACGTTCCCTTAGCACTCT
	6	TCTG	TTACTCGCCTGTCAAGTGGCGTG
	7	TTCA	GTCTCCGTGAACGTTCCCTTAGC
	8	TTCC	CCAGAGTGACTTTTCCTTTTATT
	9	TTCC	CTTAGCACTCTGCCACTTATTGG
	10	CCTG	GGGCCGTTTCCCTCACTCCTGCT
	11	CCTG	CCTCAGCTGCTCACTTGAGCCTC
	12	CCTG	TCAAGTGGCGTGACACCGGGCGT
	13	TCCC	TCACTCCTGCTCGGTGAATTTGG
	14	TCCG	TGAACGTTCCCTTAGCACTCTGC
	15	TCCA	TGTCTGTTACTCGCCTGTCAAGT
	16	CCCA	GAGTGACTTTTCCTTTTATTTCC
	17	CCCG	TCACCCCTGTTTCTGGCACCAGG
	18	CCCA	TAAGTGGCAGAGTGCTAAGGGAA
	19	TTTC	CCTCACTCCTGCTCGGTGAATTT
	20	TTTC	CTGATGGTCCATGTCTGTTACTC
	21	TTTC	TGGCACCAGGAATCCCCAACATG
EMX1	1	CTTG	CTGCTGGCCAGGCCCCTGCGTGG
	2	CTTG	AAGCCCGGGGCCGCCATTGACAG
	3	CTTC	CGGAGGACAAAGTACAAACGGCA
	4	TCTC	AGCTCAGCCTGAGTGTTGAGGCC
	5	TCTG	GGGGCCTCCTGAGTTTCTCATCT
	6	TCTG	TGCCCCTCCCTCCCTGGCCCAGG
	7	TTCC	AGAACCGGAGGACAAAGTACAAA
	8	TTCC	TCCTCCAGCTTCTGCCGTTTGTA
	9	TTCG	TGGCAATGCGCCACCGGTTGATG
	10	CCTG	AGTTTCTCATCTGTGCCCCTCCC
	11	CCTC	CAATGACTAGGGTGGGCAACCAC
	12	CCTG	CGTGGGCCCAAGCTGGACTCTGG
	13	TCCC	TCCCTGGCCCAGGTGAAGGTGTG
	14	TCCA	GAACCGGAGGACAAAGTACAAAC
	15	TCCG	AGCAGAAGAAGAAGGGCTCCCAT
	16	CCCA	TCAGGCTCTCAGCTCAGCCTGAG
	17	CCCA	GGTGAAGGTGTGGTTCCAGAACC
	18	CCCA	CGAGGGCAGAGTGCTGCTTGCTG
	19	TTTC	TCCTCCGGTTCTGGAACCACACC
	20	TTTG	TACTTTGTCCTCCGGTTCTGGAA
	21	TTTG	TGGTTGCCCACCCTAGTCATTGG