Supplementary Information

Mechanistic insights into target searching, de-dimerization, and activation of *Mucilaginibacter paludis* Argonaute

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Supplementary figures

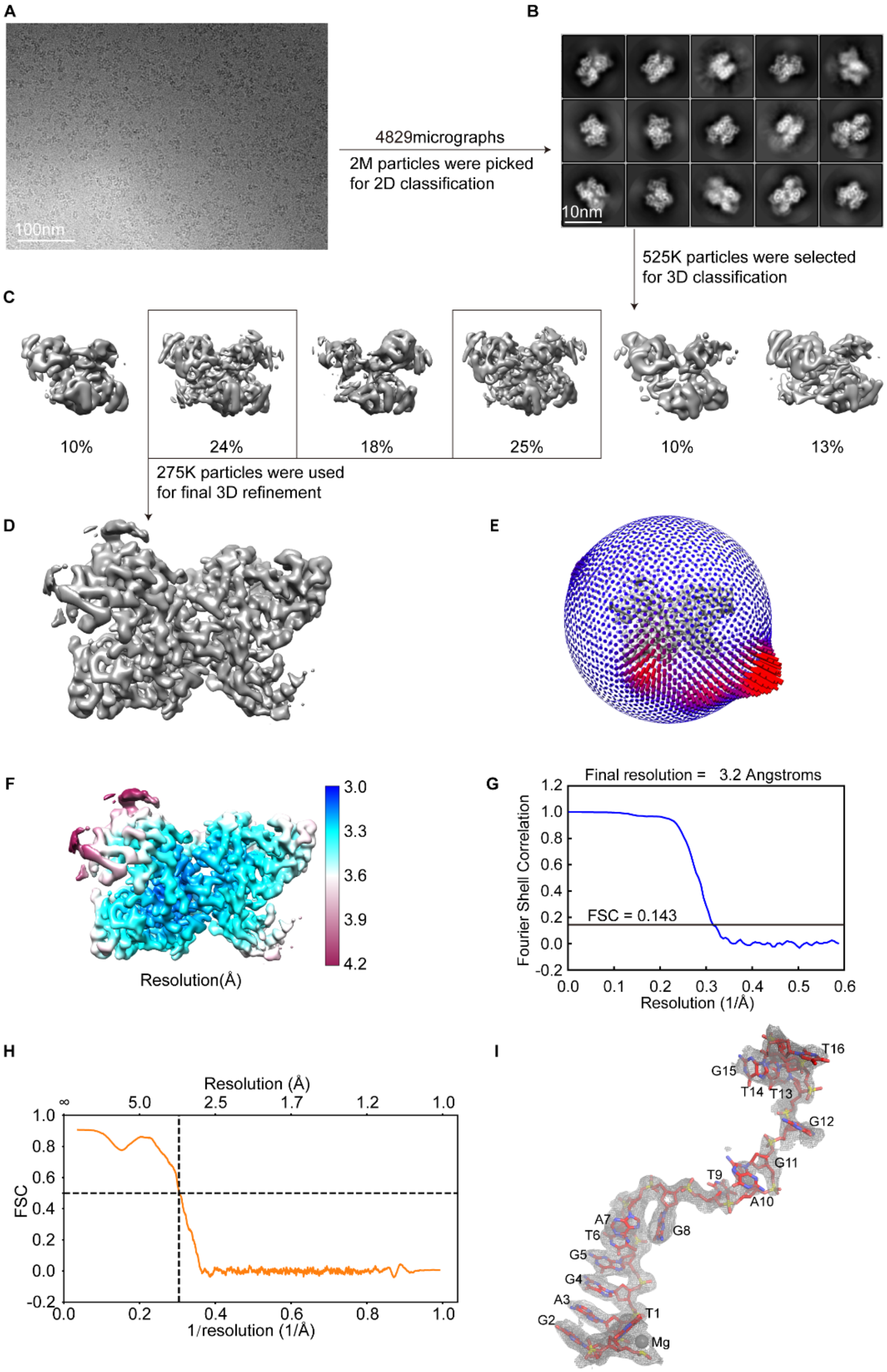

Figure S1. Cryo-EM of MbpAgo-guide DNA-target DNA binary complex. (A) Cryo-EM micrograph. **(B)** Representative 2D class averages. **(C)** Heterogeneous refinement with particle distribution. **(D)** Final reconstruction. **(E)** Angular distribution of the reconstruction shown in D. **(F)** Local resolution estimation on the reconstruction in D. **(G)** Global half-map FSC plot of the reconstruction shown in D. **(H)** Map-to-model FSC curve. **(I)** Local cryo-EM density of guide DNA.

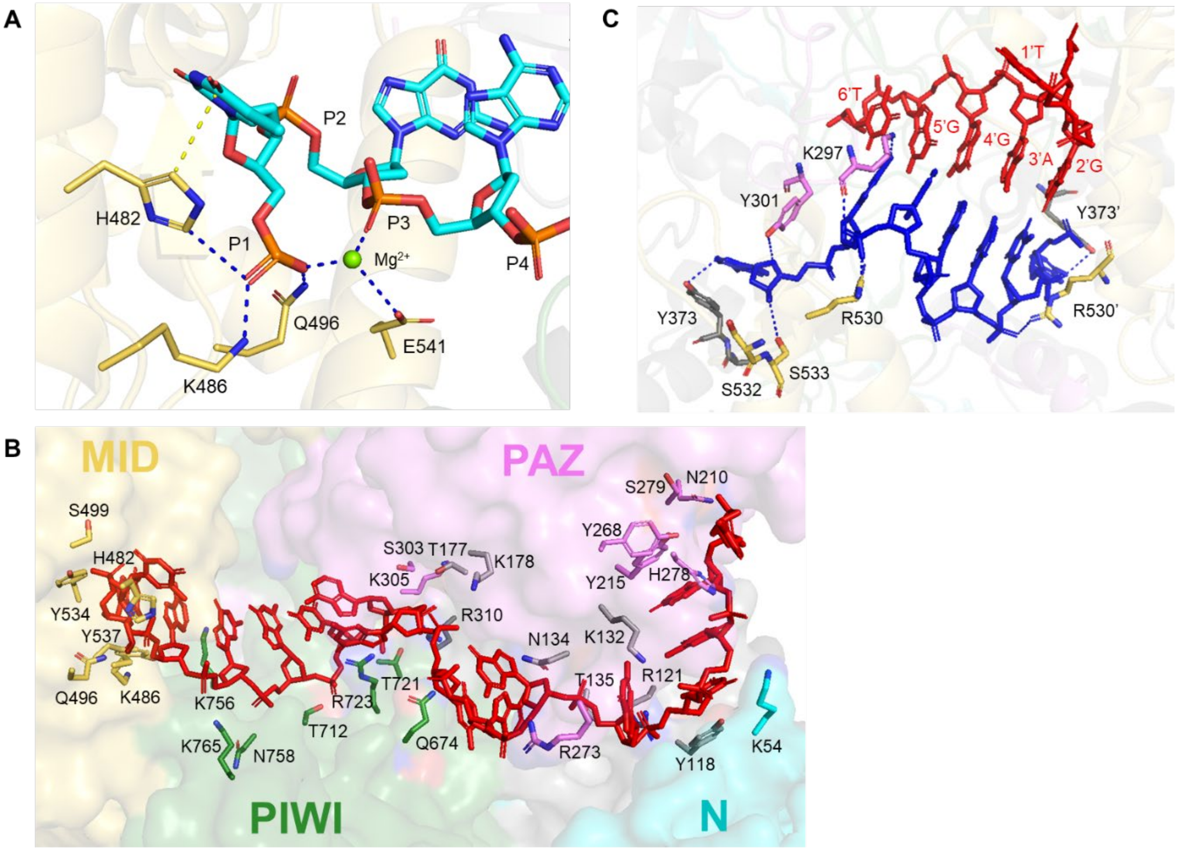

Figure S2. The interactions of MbpAgo with guide DNA. (A) Interactions of the MID domain (yellow ribbon) with the 5'-phosphate of gDNA (stick representation) in MbpAgo-gDNA complex. Salt bridges between amino acid residues and phosphate groups, as well as coordination bonds of Mg^{2+}, are shown as blue dashed lines. The stacking histidine residue H482 with the 5'-end nucleotide of the gDNA is shown as yellow dashed lines. **(B)** A magnified view of the MbpAgo- gDNA interactions. MbpAgo is depicted as a partially transparent surface. DNA nucleotides and amino acid residues responsible for guide positioning are shown as stick models and labeled. **(C)** The binding and recognition candidate target DNA by MbpAgo. The view is the same as Figure 1A.

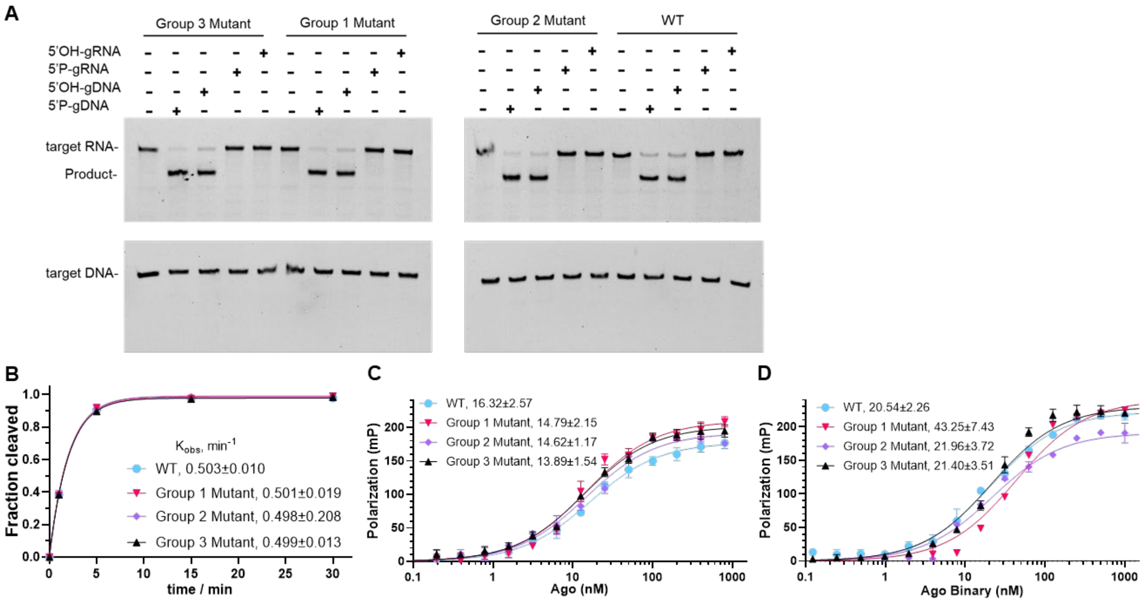

Figure S3. Target cleavage assay with wild-type MbpAgo and mutant MbpAgo (Group 1, Group 2 and Group 3). (A) Mutant MbpAgo (Group 1, Group 2 and Group 3) exhibits a preference for cleaving RNA consistent with the wild-type. **(B)** Target RNA cleavage assay with wild-type MbpAgo and mutant MbpAgo (Group 1, Group 2 and Group 3). **(C)** Binding of wild-type MbpAgo and mutant MbpAgo (Group 1, Group 2 and Group 3) to 16 nt gDNA by fluorescence polarization assay. **(D)** Binding of wild-type MbpAgo and mutant MbpAgo (Group 1, Group 2 and Group 3) binary complex to 8 nt target DNA by fluorescence polarization assay. Data are represented as the mean ± standard deviation (SD) from three independent experiments.

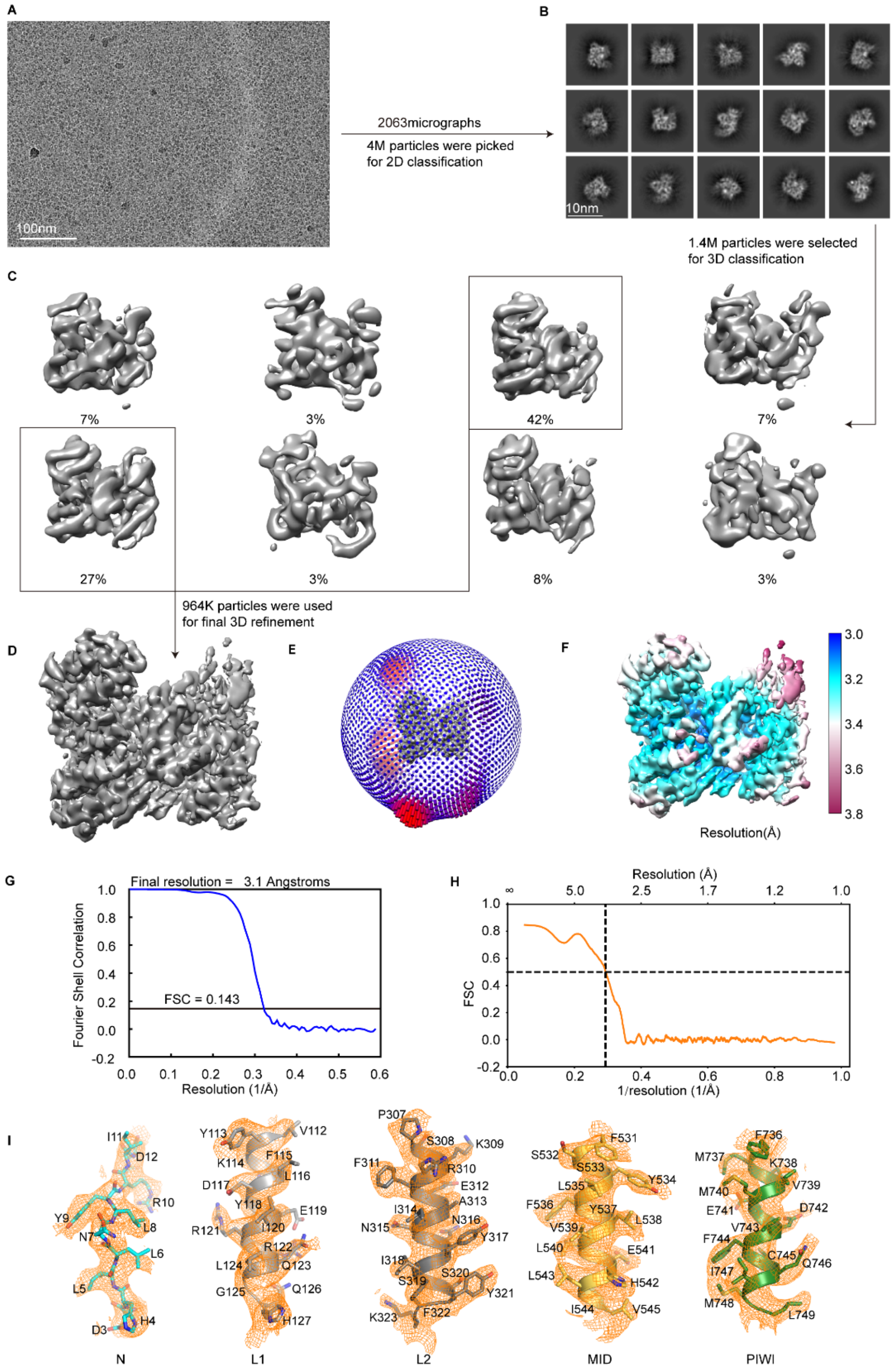

Figure S4. Cryo-EM of MbpAgo-guide DNA-target DNA ternary complex. (A) Cryo-EM micrograph. **(B)** Representative 2D class averages. **(C)** Heterogeneous refinement with particle distribution. **(D)** Final reconstruction. **(E)** Angular distribution of the reconstruction shown in D. **(F)** Local resolution estimation on the reconstruction in D. **(G)** Global half-map FSC plot of the reconstruction shown in D. **(H)** Map-to-model FSC curve. **(I)** Local cryo-EM density of representative helices from the individual domain of MbpAgo.

Supplementary tables

Table S1. List of oligonucleotides used in this study.

Oligonucleotide name		Sequence (5'-3')	Description	Used in figure
1.	T-gDNA	TGAGGTAGTAGGTTGT	16 nt guide DNA pair with tDNA/ tRNA	Fig. 1-4; Fig. S3
2.	45-nt tDNA	FAM-AAACGACGGCCAGTGCCAAGC TTACTATACAACCTACTACCTCAT	5’ FAM labeled 45-nt target DNA	Fig. S3A
3.	45-nt tRNA	FAM-AAACGACGGCCAGUGCCAAGC UUACUAUACAACCUACUACCUCAU	5’ FAM labeled 45-nt target RNA	Fig. 3E-G; Fig. S3A, B
4.	gRNA	UGAGGUAGUAGGUUGU	16 nt guide RNA pair with tDNA/ tRNA	Fig. S3A
5.	gDNA iFAM	TGAGGTAGTAGGTT(FAM)GT	internally-labeled gDNA	Fig. S3C
6.	9 nt-TD	CTACCTCAT-FAM	3’ FAM labeled 9-nt target DNA pair with gDNA form 8 bp duplex	Fig. S3D
7.	18-nt TD	TACAACCTACTACCTCAT	18-nt target DNA pair with gDNA form 16 bp duplex	Fig. 3A; Fig. 4A, D
8.	8-nt-TD	TACCTCAT	8-nt target DNA pair with gDNA form 7 bp duplex	Fig. 4A
9.	9 nt-TD	CTACCTCAT	9-nt target DNA pair with gDNA form 8 bp duplex	
10.	11 nt-TD	TACTACCTCAT	11-nt target DNA pair with gDNA form 10 bp duplex	
11.	13nt-TD	CCTACTACCTCAT	13-nt target DNA pair with gDNA form 12 bp duplex	
12.	15 nt-TD	AACCTACTACCTCAT	15-nt target DNA pair with gDNA form 14 bp duplex	
13.	8 nt-TD+10NT	ATGTTGGATGTACCTCAT	18-nt target DNA pair with gDNA form 7 bp matched duplex	Fig. 4B
14.	9 nt-TD+9NT	ATGTTGGATCTACCTCAT	18-nt target DNA pair with gDNA form 8 bp matched duplex	
15.	11 nt-TD+7NT	ATGTTGGTACTACCTCAT	18-nt target DNA pair with gDNA form 10 bp matched duplex	
16.	13 nt-TD+5NT	ATGTTCCTACTACCTCAT	18-nt target DNA pair with gDNA form 12 bp matched duplex	
17.	15 nt-TD+3NT	ATGAACCTACTACCTCAT	18-nt target DNA pair with gDNA form 14 bp matched duplex	
18.	8-nt-TR	UACCUCAU	8-nt target RNA pair with gDNA form 7 bp duplex	Fig. 4C
19.	18-nt TR	UACAACCUACUACCUCAU	18-nt target RNA pair with gDNA form 17 bp duplex	
20.	3’-Cy3 gDNA	TGAGGTAGTAGGTTGT-Cy3	3’ Cy3 labeled guide DNA	Fig. 4D
21.	3’-Cy5 gDNA	TGAGGTAGTAGGTTGT-Cy5	5’ Cy3 labeled guide DNA	

Table S2 Primers used in constructing MbpAgo mutant.

Primer		Sequence (5'-3')	template
Constructing Group 1 mutant			
22.	R530A-F	TGAAGGGCgcATTCAGCTCCTACCTGTTCTAC	pET28a-MbpAgo
23.	R530A-R	AGCTGAATgcGCCCTTCAGGGCTTCCACC	
24.	Y301A-F	CCCTGCACgcCCTGTCTATCAAGGGCCCC	
25.	Y301A-R	TAGACAGGgcGTGCAGGGACTTCACTCTC	
26.	Y373A-F	GACTTCGCCgcCCAGAGAAAGCAGCTGATTAAGAAC	
27.	Y373A-R	CTTTCTCTGGgcGGCGAAGTCCCGCATGCC	
Constructing Group 2 mutant			
28.	Y364AR369A-F	CgcCAACAGCGGCATGgcGGACTTCGCCTACC	pET28a-MbpAgo
29.	Y364AR369A-R	CgcCATGCCGCTGTTGgcGCCGGTGATCTTC	
30.	E312A-F	AGAGATTCgcGGCCATCAACAATTACATCTCC	
31.	E312A-R	GTTGATGGCCgCGAATCTCTTGCTGGGGCC	
32.	Y688AV695A-F	CGATCAGCTGGAAAACCCCGcGGCCGGCTCCTATAAG	
33.	Y688AV695A-R	GGGGTTTTCCAGCTGATCGgcGCTGTTGGTCTTGGCGGC	
34.	K2A-F	gcggcATGgcGGACCACATCCTGAACCTG	
35.	K2A-R	TGTGGTCCgcCATgccgctgctgtgatg	
36.	Y714A-F	GCACAGGCgcCCCCTTCGACATCAAGGG	
37.	Y714A-R	CGAAGGGGgcGCCTGTGCTGAAGATAA	
38.	Constructing Group 3 mutant		
39.	684-690del-F	CGCCGCCCTGGAAAACCCCGTGGCCGGCTC	pET28a-MbpAgo
40.	684-690del-R	GGGTTTTCCAGGGCGGCGATTCTGATTGG	
41.	684-690GG-F	CGCCggcggtCTGGAAAACCCCGTGGCCGGCTC	
42.	684-690GG-R	TTTTCCAGaccgccGGCGGCGATTCTGATTGG	
43.	Constructing N686C mutant		
44.	N686C-F	GCCAAGACCTGCAGCTACGATCAGCTGG	pET28a-MbpAgo
45.	N686C-R	GTAGCTGCAGGTCTTGGCGGCGATTC	