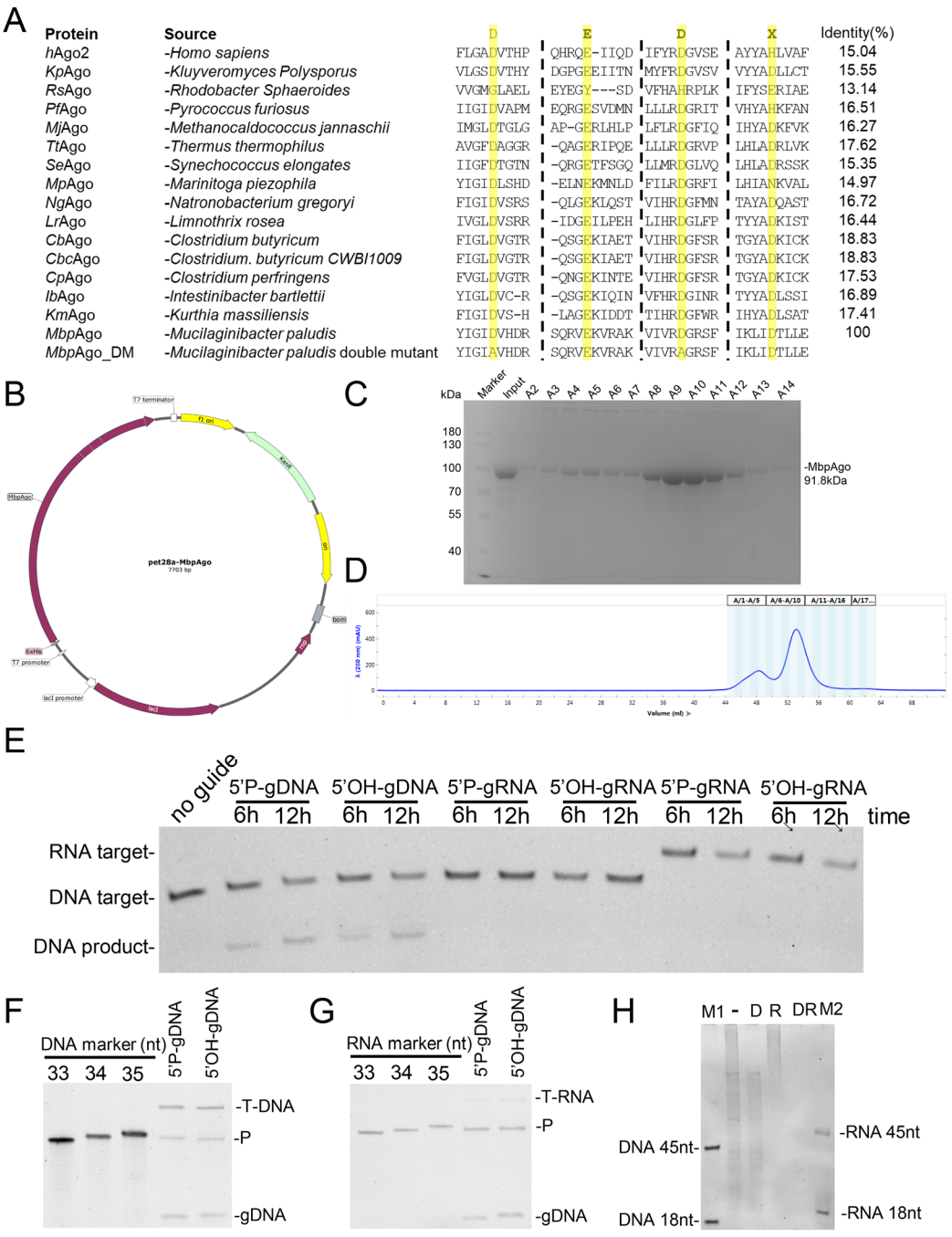
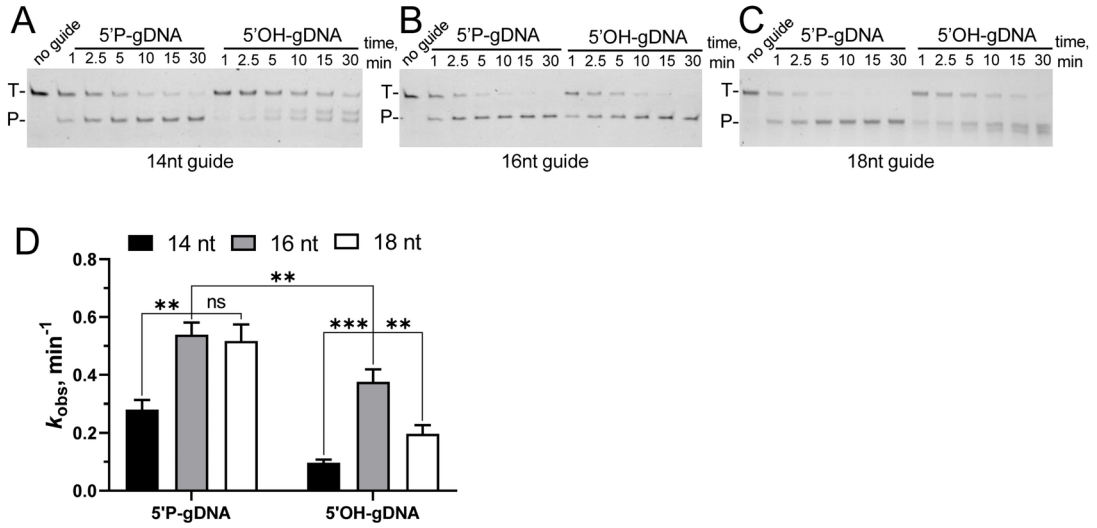
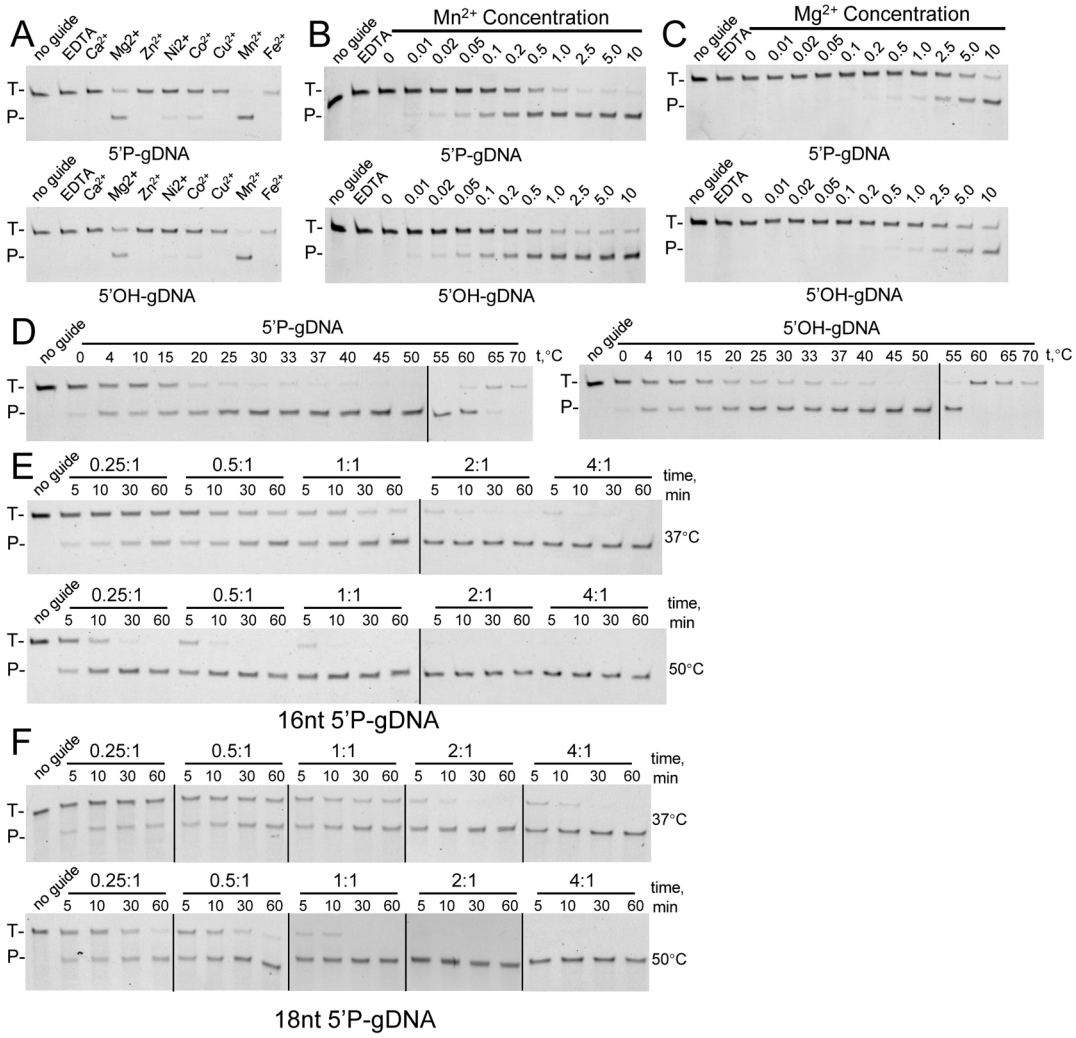


Supplementary Figure S1. *Mbp*Ago harbors a catalytically DEDD tetrad. (A) Multiple sequence alignment of a part of the PIWI domain from the *Mbp*Ago with several other characterized Ago proteins. (B) Schematic representation of the pET28a based expression vector of *Mbp*Ago. (C) The purity of the purified *Mpb*Ago was determined using SDS-PAGE. (D) Size-exclusion chromatography diagram showing the elution peak of *Mbp*Ago. (E) *Mbp*Ago shows DNA cleavage activity at 37°C if incubation is extended. (F) Determining the DNA cleavage site with non-labeled T-DNA. DNA marker (33, 34, 35 nt) were partially hydrolyzed T-DNA. The

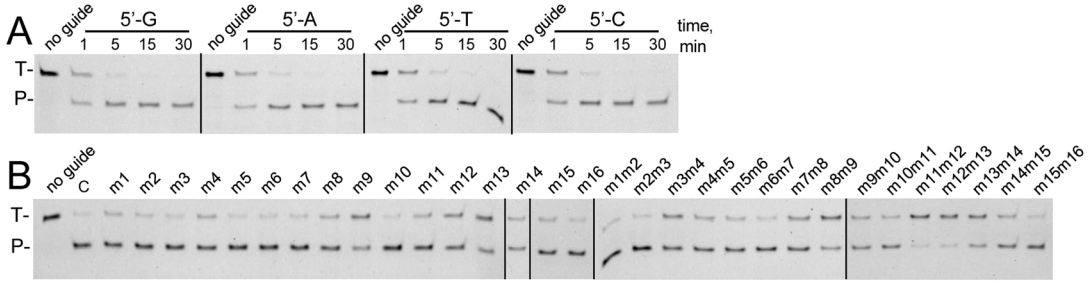
experiments were performed at the 4:2:1 *Mbp*Ago:guide:target molar ratio for 12 h at 37 °C. Positions of the cleavage products (P) are indicated on the right of the gels. (G) Determining the RNA cleavage site with non-labeled tRNA. RNA marker (33, 34, 35 nt) were partially hydrolyzed tRNA. The experiments were performed at the 4:2:1 *Mbp*Ago:guide:target molar ratio for 30 min at 37°C. Positions of the cleavage products (P) are indicated on the right of the gels. (H) Purification of *Mbp*Ago-associated nucleic acids after its expression in E. coli. Nucleic acids were treated with DNase I (D), RNase A (R), both nucleases (DR), or left untreated (-), separated by denaturing PAGE and stained with SYBR Gold. M1, DNA length markers; M2, RNA length markers.



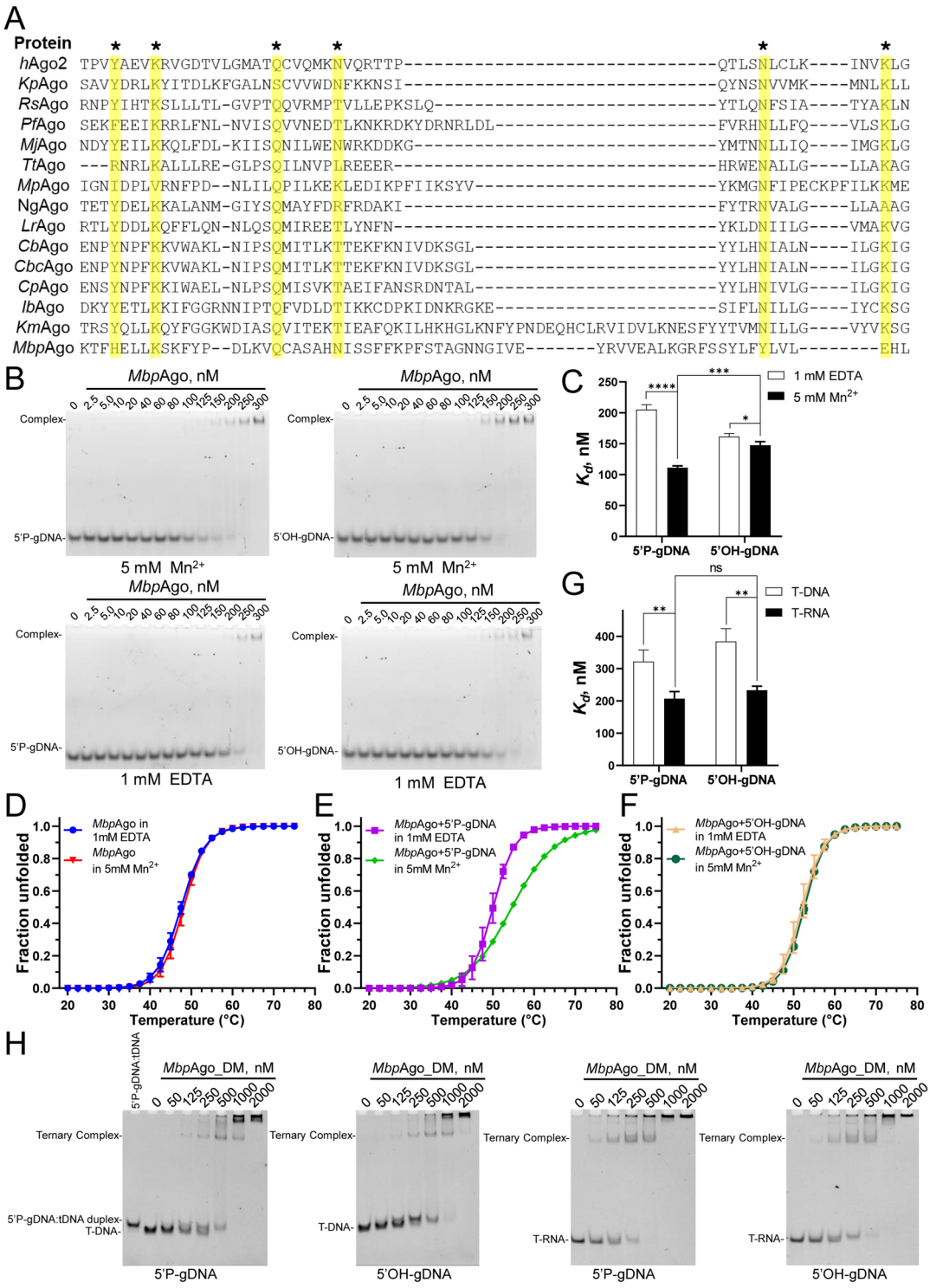
Supplementary Figure S2. Both the length and the 5'-phosphate of guide affect the cleavage efficiency and precision. (A-C) Representative denaturing PAGEs from three independent measurements showing kinetics analysis of RNA cleavage by *Mbp*Ago with 14 nt, 16 nt and 18 nt gDNAs, respectively. Positions of the cleavage products (P) and targets (T) are indicated on the left of the gels. (D) Effects of the guide length on the cleavage efficiency of *Mbp*Ago. The k_{obs} values were determined from the single-exponential fits of the data in Figure 2C-E. Means and standard deviations were from three independent measurements. P-values for all comparisons of k_{obs} values were calculated using the Student's *t*-test. $^{ns}P > 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.



Supplementary Figure S3. Characteristics of nuclease activity of *Mbp*Ago. (A) Representative denaturing PAGEs showing 5'P-gDNA-mediated RNA cleavage (Upper panel) and 5'OH-gDNA-mediated RNA cleavage by *Mbp*Ago with various divalent cations. Effects of Mn^{2+} (B) and Mg^{2+} (C) concentration on RNA cleavage activity mediated by 5'P-gDNA (Upper panel) and 5'OH-gDNA (Lower panel). (D) (Left panel) Representative denaturing PAGEs showing temperature dependence of 5'P-gDNA-mediated RNA cleavage by *Mbp*Ago. (Right panel) Representative denaturing PAGEs showing temperature dependence of 5'OH-gDNA-mediated RNA cleavage by *Mbp*Ago. (E) Representative denaturing PAGEs of the *Mbp*Ago-mediated 16 nt 5'P-DNA-guided RNA cleavage turnover experiments at 37°C (upper panel) and 50°C (lower panel), respectively. (F) Representative denaturing PAGEs of the *Mbp*Ago-mediated 18 nt 5'P-DNA-guided RNA cleavage turnover experiments at 37°C (upper panel) and 50°C (lower panel), respectively. Positions of the cleavage products (P) and targets (T) are indicated on the left of the gels.

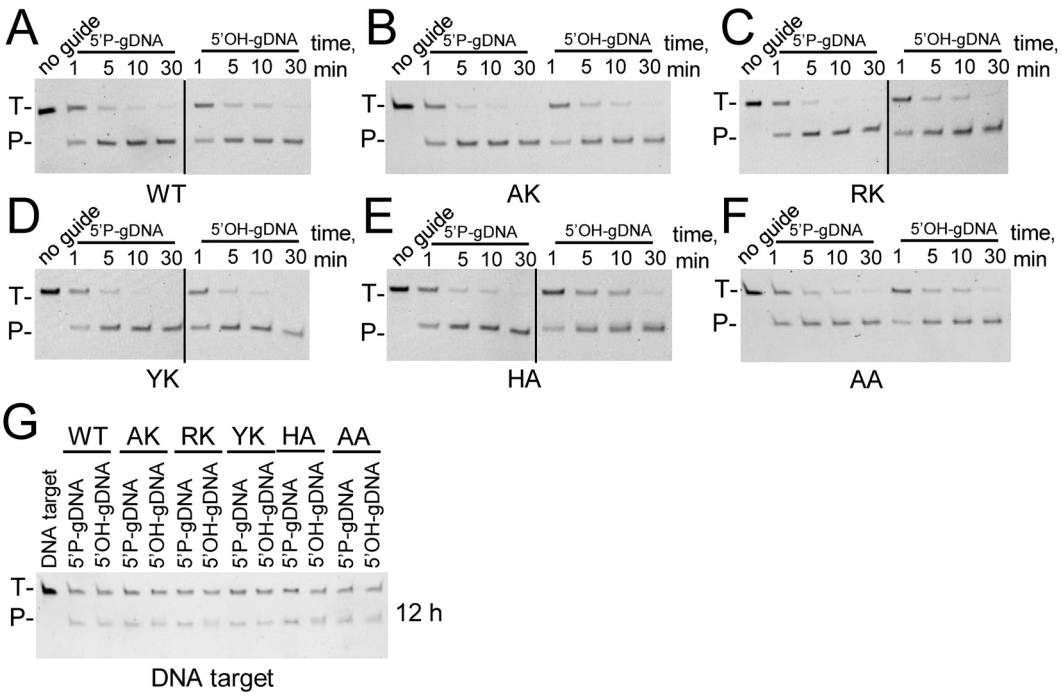


Supplementary Figure S4. Representative denaturing PAGE showing the results in Figure 4. (A) Representative denaturing PAGE showing the preferences for the 5'end nucleotide. (B) Representative denaturing PAGE showing effects of mismatches in the guide-target duplex on the slicing activity of *Mbp*Ago. Positions of the cleavage products (P) and targets (T) are indicated on the left of the gels.

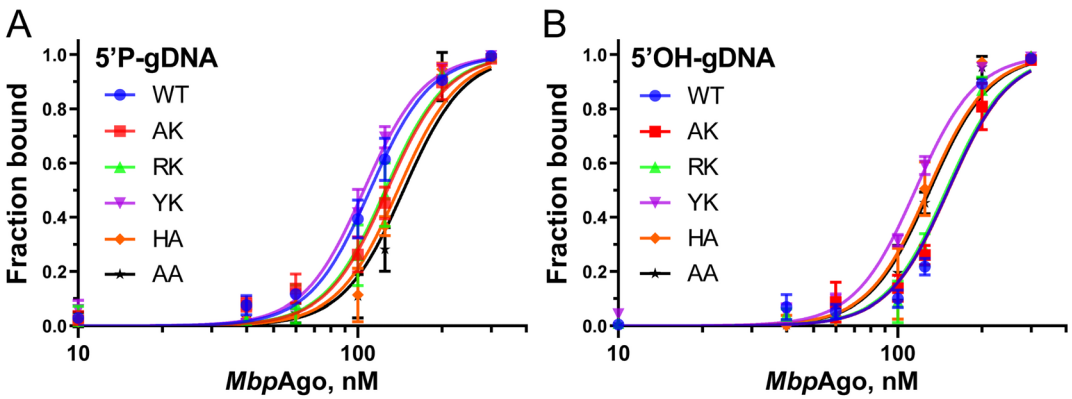


Supplementary Figure S5. Electrophoresis mobility shift assay (EMSA) of the binding of the *Mbp*Ago to guides and *Mbp*Ago-gDNA complex to target. (A) Multiple sequence alignment of the 5'end guide binding pocket of the MID domain from *Mbp*Ago with several other characterized Ago proteins. Black asterisks are the positions of amino acid residues involved in the binding of the 5'end of a guide. (B) Representative native gel images (one of three independent experiments) of binding reactions in Figure 5B with various ratios between

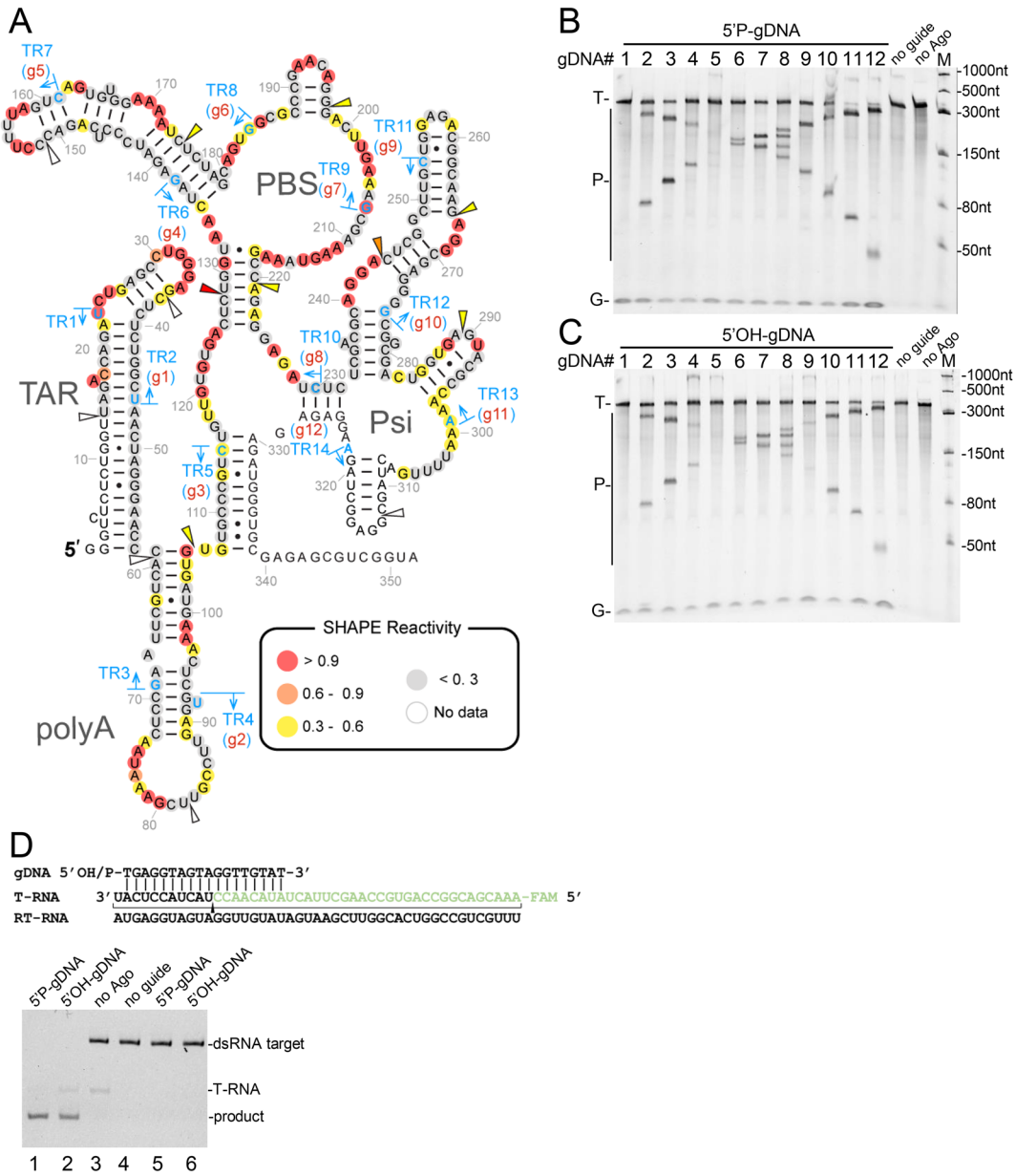
*Mbp*Ago and guide. (C) The K_d values were determined from the data in Figure 5B. Means and standard deviations were from three independent measurements. P-values for all comparisons of K_d values were calculated using the Student's *t*-test. *P<0.05, ***P<0.001 and ****P<0.0001. (D), (E) and (F) Thermal unfolding transition curves of *Mbp*Ago and *Mbp*Ago-gDNA Complex followed by circular dichroism. Measures were performed in duplicate. (G) The K_d values were determined from the data in Figure 5D. Means and standard deviations were from three independent measurements. P-values for all comparisons of K_d values were calculated using the Student's *t*-test. ^{ns}P>0.05, and **P<0.01. (H) Representative native gel images (one of three independent experiments) of binding reactions in Figure 5D with various ratios between *Mbp*Ago-gDNA complex: target.



Supplementary Figure S6. Representative denaturing PAGE showing the results in Figure 6. (A)-(F) Representative denaturing PAGE showing kinetics analysis of RNA cleavage by WT, AK variant, RK variant, YK variant, HA variant and AA variant. (G) Cleavage analysis of DNA cleavage by WT, AK variant, RK variant, YK variant, HA variant and AA variant. The reaction was performed at 37°C for 12 h. Positions of the cleavage products (P) and targets (T) are indicated on the left of the gels.



Supplementary Figure S7. (A) and (B) Binding of 16 nt 5'P-gDNA and 5'OH-gDNA by *Mbp*Ago variants with 5 mM Mn^{2+}. The fraction of bound guides was plotted against protein concentration and fitted using the model of specific binding with the Hill slope. Results for WT and its binding analysis of guides are from Figure 5B. Data are represented as the mean ± SD from three independent experiments.



Supplementary Figure S8. Cleavage of highly structured HIV-1 ΔDIS 5′UTR RNA by *Mbp*Ago-gDNA complex with 5 mM Mg^{2+}. (A) Secondary structure of HIV-1 ΔDIS 5′UTR predicted by SHAPE, which is copied from Figure 3C of Dayeh et al (1). The highly structured RNA is composed of several structured sub-domains; the transactivation response (TAR; 1–57 nt) element, polyadenylation signal (poly(A); 58–104 nt), primer-binding site (PBS; 125–223 nt), and genomic RNA packaging domain (Psi, 228–334 nt). Designed guide DNAs targeting the different target regions (TRs) of the HIV transcript are marked with red g1–g12. (B) Analysis of the cleavage products obtained after incubation of 5'P-gDNA-*Mbp*Ago complex with HIV-1 ΔDIS 5′UTR RNA. (C) Analysis of the cleavage products obtained after incubation of 5'OH-

gDNA-*Mbp*Ago complex with HIV-1 ΔDIS 5′UTR RNA. Experiments in (B) and (C) were carried out with 5 mM Mg^{2+} at 37°C for 30 min. Positions of the targets (T), gDNAs (G) and cleavage products (P) are indicated on the left of the gels. M, RNA marker. (D) Cleavage analysis of double-stranded RNA (dsRNA) target by *Mbp*Ago. lane 1 and 2, ssRNA target; lane 3-6, dsRNA target. FAM-labled T-RNA and RT-RNA (reverse T-RNA) were denatured for 5 min at 95°C, and temperature was lowered at a rate of 0.1°C/s until 25°C was reached. *Mbp*Ago, guide and target (dsRNA or T-RNA) were mixed in a 4:2:1 molar ratio (800 nM MbpAgo preloaded with 400 nM guide, plus 200 nM target) and incubated at 37°C for 30 min.

Supplementary tables

Table S1. Primers used in constructing mutant

Primer	Sequence (5'-3')	template	Mutant
H482A-F/ H482A-R	TGAAAACCTTCGCAGAGCTGCTGAAGTCTAAGTTC/ CAGCTCTGCGAAGGTTTTCAGGAAGGCGGGGC	pET28a-MbpAgo	AK
H482R-F/ H482R-R	TGAAAACCTTCAGAGAGCTGCTGAAGTCTAAGTTC/ CAGCTCTCTGAAGGTTTTCAGGAAGGCGGGGC	pET28a-MbpAgo	RK
H482Y-F/ H482Y-R	TGAAAACCTTCTACGAGCTGCTGAAGTCTAAGTTC/ CAGCTCGTAGAAGGTTTTCAGGAAGGCGGGGC	pET28a-MbpAgo	YK
K486A-F/ K486A-R	GCTGCTGGCGTCTAAGTTCTACCCCGATCTGAAGG/ AGAACTTAGACGCCAGCAGCTCGTGGAAGGTTTTC	pET28a-MbpAgo	HA
K486A-F/ H482AK486A-R	GCTGCTGGCGTCTAAGTTCTACCCCGATCTGAAGG/ AGAACTTAGACGCCAGCAGCTCTGCGAAGGTTTTC	pET28a-AK	AA

Table S2. List of let-7-derived sequences used in this study.

Oligonucleotide name	Sequence (5'-3')	Description	Used in figure
FAM-T-tDNA	FAM-AAACGACGGCCAGTGCCAAGCTTACTATACAACCTACTACCTCAT	5' FAM labeled T-tDNA	1D, S1J, S6G
M2	FAM-AAACGACGGCCAGTGCCAAGCTTACTATACAACC	5' FAM labeled 34 nt DNA	1D
FAM-U-tRNA	FAM-AAACGACGGCCAGUGCCAAGCUUACUAUACAACCUACUACCUCAU	5' FAM labeled U-tRNA	1C, S1J, 2A-2E, S2A-S2C, 3A-3F, S3A-S3F, 4A, 4C, S4A-S4B, 5D, S5H, 6A, 6D 6G, S6A-S6F, S8D
M1	FAM-AAACGACGGCCAGUGCCAAGCUUACUAUACAACC	5' FAM labeled 34 nt RNA	1C
T-gDNA	TGAGGTAGTAGGTTGT	16 nt guide forms 5'-T pair with T-tDNA/T-tRNA	1C-1D, S1H-S1J, 2D, S2B, 3A-3C, 3E, S3A-

			3E, S4A-S4B, 5C-5D, S5D-5H, 6A, 6D, 6G, S6A-6G, S8D
14nt T-gDNA	TGAGGTAGTAGGTT	14 nt guide pair with U-tRNA	2C, S2A, 6G, S6G
18nt T-gDNA	TGAGGTAGTAGGTTGTAT	18 nt guide pair with U-tRNA	S1E, 2E, S2C, 3D, 3F, S3F, 6G, S6G
33nt DNA product	AAACGACGGCCAGTGCCAAGCTTACTATACAAC	33 nt DNA marker	S1H
34nt DNA product	AAACGACGGCCAGTGCCAAGCTTACTATACAACC	34 nt DNA marker	
35nt DNA product	AAACGACGGCCAGTGCCAAGCTTACTATACAACCT	35 nt DNA marker	
T-tDNA	AAACGACGGCCAGTGCCAAGCTTACTATACAACCTACTACCTCAT	let-7 based 45 nt DNA target for T-gDNA/U-gRNA	S1E, S1H
U-gRNA	UGAGGUAGUAGGUUGU	guide forms 5'-U pair with T-tDNA/T-tRNA	1C, 1D, S1J
18 nt RNA	UGAGGUAGUAGGUUGUAU	18 nt RNA marker	S1E
U-tRNA	AAACGACGGCCAGUGCCAAGCUUACUAUACAACCUACUACCUCAU	let-7 based 45 nt RNA target for T-gDNA/U-gRNA	S1E, S1I
33nt RNA product	AAACGACGGCCAGUGCCAAGCUUACUAUACAAC	33 nt RNA marker	S1I
34nt RNA product	AAACGACGGCCAGUGCCAAGCUUACUAUACAACC	34 nt RNA marker	
35nt RNA product	AAACGACGGCCAGUGCCAAGCUUACUAUACAACCU	35 nt RNA marker	
8nt T-gDNA	TGAGGTAG	8 nt guide pair with U-tRNA	2A, 2B
9nt T-gDNA	TGAGGTAGT	9 nt guide pair with U-tRNA	
10nt T-gDNA	TGAGGTAGTA	10 nt guide pair with U-tRNA	
11nt T-gDNA	TGAGGTAGTAG	11 nt guide pair with U-tRNA	
12nt T-gDNA	TGAGGTAGTAGG	12 nt guide pair with U-tRNA	

13nt T-gDNA	TGAGGTAGTAGGT	13 nt guide pair with U-tRNA	
14nt T-gDNA	TGAGGTAGTAGGTT	14 nt guide pair with U-tRNA	
15nt T-gDNA	TGAGGTAGTAGGTTG	15 nt guide pair with U-tRNA	
16nt T-gDNA	TGAGGTAGTAGGTTGT	guide forms 5'-T pair with T-tDNA/T-tRNA	2A, 2B, 4A, 4C, S4A-4B
17nt T-gDNA	TGAGGTAGTAGGTTGTA	17 nt guide pair with U-tRNA	2A, 2B
18nt T-gDNA	TGAGGTAGTAGGTTGTAT	18 nt guide pair with U-tRNA	
19nt T-gDNA	TGAGGTAGTAGGTTGTATA	19 nt guide pair with U-tRNA	
20nt T-gDNA	TGAGGTAGTAGGTTGTATAG	20 nt guide pair with U-tRNA	
21nt T-gDNA	TGAGGTAGTAGGTTGTATAGT	21 nt guide pair with U-tRNA	
25nt T-gDNA	TGAGGTAGTAGGTTGTATAGTAAGC	25 nt guide pair with U-tRNA	
30nt T-gDNA	TGAGGTAGTAGGTTGTATAGTAAGCTTGGC	30 nt guide pair with U-tRNA	
40nt T-gDNA	TGAGGTAGTAGGTTGTATAGTAAGCTTGGCACTGGCCGTC	40 nt guide pair with U-tRNA	
gDNA_mm1	AGAGGTAGTAGGTTGT	guide forms mismatched pair in position 1 with U-tRNA	4C, S4B
gDNA_mm2	TCAGGTAGTAGGTTGT	guide forms mismatched pair in position 2 with U-tRNA	
gDNA_mm3	TGTGGTAGTAGGTTGT	guide forms mismatched pair in position 3 with U-tRNA	
gDNA_mm4	TGACGTAGTAGGTTGT	guide forms mismatched pair in position 4 with U-tRNA	
gDNA_mm5	TGAGCTAGTAGGTTGT	guide forms mismatched pair in position 5 with U-tDNA	
gDNA_mm6	TGAGGAAGTAGGTTGT	guide forms mismatched pair in position 6 with U-tRNA	

gDNA_mm7	TGAGGTTGTAGGTTGT	guide forms mismatched pair in position 7 with U-tRNA	
gDNA_mm8	TGAGGTACTAGGTTGT	guide forms mismatched pair in position 8 with U-tRNA	
gDNA_mm9	TGAGGTAGAAGGTTGT	guide forms mismatched pair in position 9 with U-tRNA	
gDNA_mm10	TGAGGTAGTTGGTTGT	guide forms mismatched pair in position 10 with U-tRNA	
gDNA_mm11	TGAGGTAGTACGTTGT	guide forms mismatched pair in position 11 with U-tRNA	
gDNA_mm12	TGAGGTAGTAGCTTGT	guide forms mismatched pair in position 12 with U-tRNA	
gDNA_mm13	TGAGGTAGTAGGATGT	guide forms mismatched pair in position 13 with U-tRNA	
gDNA_mm14	TGAGGTAGTAGGTAGT	guide forms mismatched pair in position 14 with U-tRNA	
gDNA_mm15	TGAGGTAGTAGGTTCT	guide forms mismatched pair in position 15 with U-tRNA	
gDNA_mm16	TGAGGTAGTAGGTTGA	guide forms mismatched pair in position 16 with U-tRNA	
gDNA_m1m2	ACAGGTAGTAGGTTGT	guide forms mismatched pair in position 1 and 2 with U-tRNA	
gDNA_m2m3	TCTGGTAGTAGGTTGT	guide forms mismatched pair in position 2 and 3 with U-tRNA	
gDNA_m3m4	TGTCGTAGTAGGTTGT	guide forms mismatched pair in position 3 and 4 with U-tRNA	
gDNA_m4m5	TGACCTAGTAGGTTGT	guide forms mismatched pair in position 4 and 5 with U-tRNA	
gDNA_m5m6	TGAGCAAGTAGGTTGT	guide forms mismatched pair in position 5 and 6 with	

		U-tRNA	
gDNA_m6m7	TGAGGATGTAGGTTGT	guide forms mismatched pair in position 6 and 7 with U-tRNA	
gDNA_m7m8	TGAGGTTCTAGGTTGT	guide forms mismatched pair in position 7 and 8 with U-tRNA	
gDNA_m8m9	TGAGGTACAAGGTTGT	guide forms mismatched pair in position 8 and 9 with U-tRNA	
gDNA_m9m10	TGAGGTAGATGGTTGT	guide forms mismatched pair in position 9 and 10 with U-tRNA	
gDNA_m10m11	TGAGGTAGTTCGTTGT	guide forms mismatched pair in position 10 and 11 with U-tRNA	
gDNA_m11m12	TGAGGTAGTACCTTGT	guide forms mismatched pair in position 11 and 12 with U-tRNA	
gDNA_m12m13	TGAGGTAGTAGCATGT	guide forms mismatched pair in position 12 and 13 with U-tRNA	
gDNA_m13m14	TGAGGTAGTAGGAAGT	guide forms mismatched pair in position 13 and 14 with U-tRNA	
gDNA_m14m15	TGAGGTAGTAGGTACT	guide forms mismatched pair in position 14 and 15 with U-tRNA	
gDNA_m15m16	TGAGGTAGTAGGTTCA	guide forms mismatched pair in position 15 and 16 with U-tRNA	
C-gDNA	CGAGGTAGTAGGTTGT	guide forms 5'-C pair with C-tRNA	4A, S4A
FAM-C-tRNA	FAM-AAACGACGGCCAGUGCCAAGCUUACUAUACAACCUACUACCUCGU	5’ FAM labeled C-tRNA	
A-gDNA	AGAGGTAGTAGGTTGT	guide forms 5'-A pair with A-tRNA	
FAM-A-tRNA	FAM-AAACGACGGCCAGUGCCAAGCUUACUAUACAACCUACUACCUCUU	5’ FAM labeled A-tRNA	
G-gDNA	GGAGGTAGTAGGTTGT	guide forms 5'-G pair with	

		G-tRNA	
FAM-G-tRNA	FAM-AAACGACGGCCAGUGCCAAGCUUACUAUACAACCUACUACCUCCU	5’ FAM labeled G-tRNA	
RT-RNA	AUGAGGUAGUAGGUUGUAUAGUAAGCUUGGCACUGGCCGUCGUUU	45 nt RNA complementary to U-tRNA	S8D
T-gDNA iFAM	TGAGGTAGTAGGTT(FAM)GT	internally-labeled T-gDNA	5B, S5B, 6C, 6F, S7A-S7B

Table S3. List of gDNAs targeting HIV-1 ΔDIS 5′UTR RNA

gDNA#	Sequence (5′-3′)	Target region	5′ product length (nt)
gDNA_1	AGCCAGAGAGCTCCCA	31-46	36
gDNA_2	ACTCAAGGCAAGCTTT	77-92	82
gDNA_3	GACGGGCACACACTAC	100-115	105
gDNA_4	CTAGTTACCAGAGTCA	123-138	128
gDNA_5	GACTAAAAGGGTCTGA	146-161	151
gDNA_6	CACTGCTAGAGATTTT	169-184	174
gDNA_7	CTTTCAAGTCCCTGTT	192-207	197
gDNA_8	GATCTCCTCTGGCTTT	215-230	220
gDNA_9	GCAAGCCGAGTCCTGC	238-253	243
gDNA_10	CCCCTCGCCTCTTGCC	261-276	266
gDNA_11	TTTGGCGTACTCACCA	284-299	289
gDNA_12	TCTAGCCTCCGCTAGT	307-322	312

Table S4. The sequence of *Mbp*Ago expression cassete

taatacgactcactatagggaattgtgagcggataacaattcccctctagaaataattttgtttaactttaagaaggagatataccat

gggcagcagccatcatcatcatcatcacagcagcggcATGAAGGACCACATCCTGAACCTGTACCGGATC

GACAACCTGAGCGAGCTGGACTTCAGCTACAAGCTGATCGACTTCGACCTGAGCTTTATC

GCCGGCAAAGAGGAACTGCTGAACAAGCAGCTGCAGAAAATCGCCGAAGAGGTGTCCA

GCGTGACAAAGGGACCTACCGCCGTGCTGAAGCGGAACCAGAGATTCTTTGTGGCCGT

GCCTGCCGACAAGCAGATGGAAGATCGGTCTATCGACGGCATCCCCTTCAGCATCCCCA

TCAAGCTGCTGCCCGAGGTGTACAGAATCGACAGCAAGGACATCCAGGGCCACCAGCT

GGACGTGGTGTACAAGTTCCTGGACTACGAGATCAGACGGCAGCTGGGCCAGCACAGA

GATCTGTGGAAGCTGAACACCCACCAGTTCTTTCTGCGCGAGCCCATGAAGGGAATCCA

GGGCAGCATCAACGTGTTCGAGGGCTTCACATACAAGCTGGCCAGACTGGCCGACGGC

CACTTTTACGTGACACTGGACCTGAGCACCAAGTACATCGACAAGTACTGCCTGAGCCAC

TACATCAACGAGGGCAACGTGCGGACCTTCGAGAACAACTACAAGGGCAGAAGATTCCT

GTACCTGAACGGCGACAACTGGTACACCATCGAGCTGCTCGGCTTCGGCAAGAGCGTGA

AAGAGCAGGACTTCATCAGAGAGGGCACCACCTACAACGTGCTGAATTACATCACCGAG

AAGATCGAGCACAGCCGGACCGACCTGAAGAGATACGTGAAGCCCAACGACCTGTCCAT

GAGCTACACATACCCCGGCAGAACAATGGACCCTCACAGCGGAGCTACATCCCTGGCCA

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TTCTGGATTCCTGAGCTGCTGTTCAACAACAACCGGCGGCTGAAGATCACCGGCTTCAA

CAGCGGCATGCGGGACTTCGCCTACCAGAGAAAGCAGCTGATTAAGAACAACGGGGTG

CTGAACAGGACCAGCTTCGACGTGCAGTACCTGCTGGTGCCCGACGAGCAGTACATGGA

CGCCAATCTGGTGGAAGGGTTCAAGAACAATGCCGAGTTCCTGATCAAGAAGCTGGCCC

CTGCCTTCGACAAGTTCATCATCATCAGATACCCCGTCAAGAGCTGCACCAGCGCCAGC

GTGCAGATCCAAGAGATCGAGAAGGTGCTGCACAGACGGAATGCCCTGCACGGATTTGC

TCTGGTGGTGCTGCCTGACCTGGACGCTTTTAGCCCCGCCTTCCTGAAAACCTTCCACG

AGCTGCTGAAGTCTAAGTTCTACCCCGATCTGAAGGTGCAGTGCGCCAGCGCTCACAAC

ATCTCTAGCTTTTTCAAGCCCTTCTCCACCGCCGGCAACAACGGCATCGTCGAGTACAGA

GTGGTGGAAGCCCTGAAGGGCAGATTCAGCTCCTACCTGTTCTACCTGGTGCTGGAACA

CCTGATCGTGAACCGGAAGTGGCCTTACGCTCTGGCCAAGAATCTGTTCTACGACATCTA

CATCGGCATCGATGTGCACGACCGGCACGCCGGCTTTACATTCTTCTTCAAAAACGGCGA

ACAGATCATCTTTCACCCCGAGGAAGTGCCCCAGAAAACAAACAGCCAGCGCGTGGAAA

AAGTGCGGGCCAAGACACTGAACAAAGTGATCTACGAGAAGCTGAAGCTGTACATCCCA

CTGTTCGCCCCTAATCCTAATGGCATCGTGATCGTCCGCGACGGCAGAAGCTTTGGCGT

CGAGTATAAGGCCCTGCAGGCCGCCATCAATACTCTGGCCGCTGAGGGAATCGTGAACA

AGGACACCGTGAAGTACGGCGTGGTGGACCTGCACAAGCAGAGCAGCGTGCCAATCAG

AATCGCCGCCAAGACCAACAGCTACGATCAGCTGGAAAACCCCGTGGCCGGCTCCTATA

AGCTGGTGTCTCCTAAAGAGGGCTTTATCTTCAGCACAGGCTACCCCTTCGACATCAAGG

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cgaaaggaagctgagttggctgctgccaccgctgagcaataactagcataaccccttggggcctctaaacgggtcttgaggggttt
tttg

T7 promoter is underlined, Translation initiation codon is marked cyan, His_6 tag is marked yellow, the uppercase letter represents the human codon-optimized sequence of *Mbp*Ago gene, T7 terminator is marked with grey.

REFERENCES

1. Dayeh,D.M., Cantara,W.A., Kitzrow,J.P., Musier-Forsyth,K. and Nakanishi,K. (2018) Argonaute-based programmable RNase as a tool for cleavage of highly-structured RNA. Nucleic Acids Res., 46, e98.