

Supplementary figures

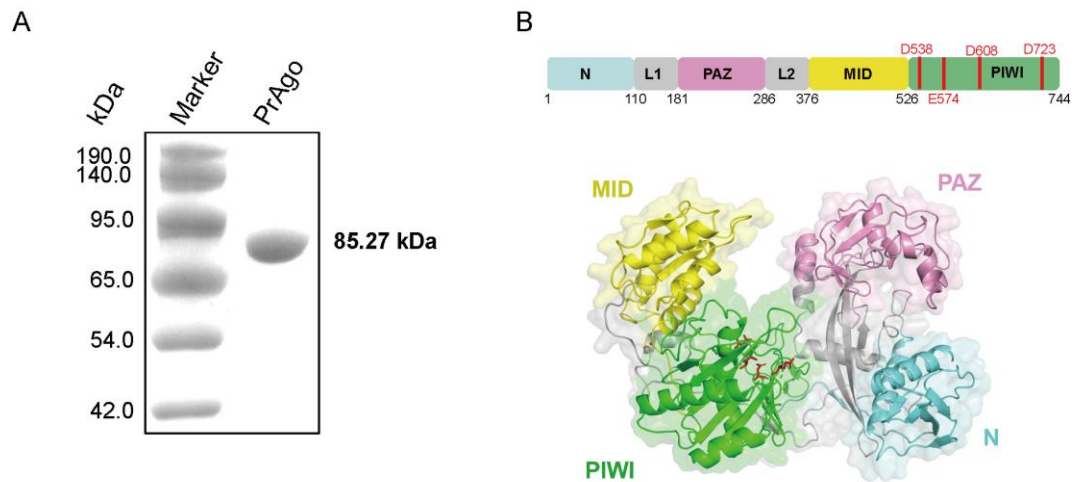


Figure S1 The three-dimensional structure model of *PrAgo* (A) The purity of *PrAgo* was determined using SDS-PAGE. (B) Upper panel: Schematic diagram of the domain organization of *PrAgo*. L1 and L2 are linker domains. Red mark means the key catalytic residues. Lower panel: Overall 3D structure prediction of the *PrAgo* domains are coloured according to the colour scheme in panel A.

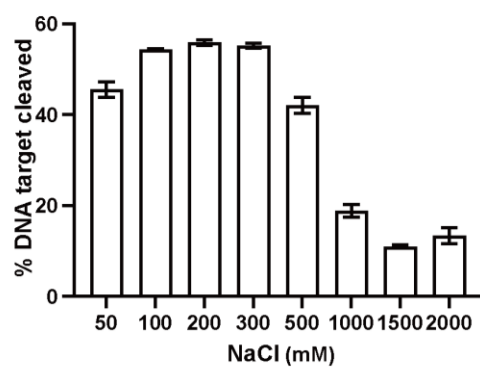
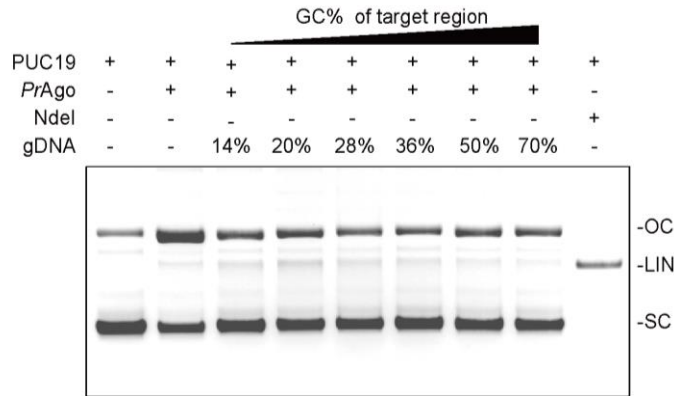


Figure S2 Effect of NaCl on *PrAgo* activity mediated by 5'P gDNA. Error bars

1 represent the SDs of three independent experiments.

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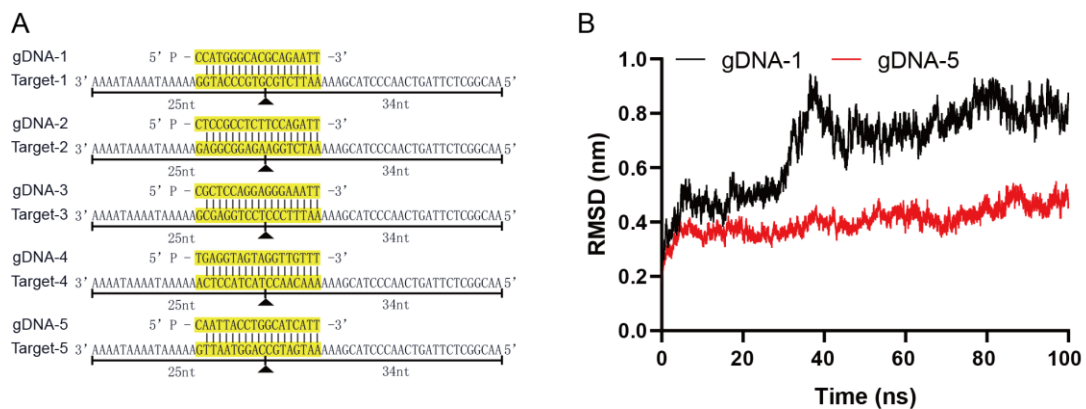


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4 **Figure S3 *PrAgo* cleaves pUC19 at 37 °C guided by a pair of 5'-P gDNAs. OC,**
5 **open circular plasmid; Lin, linearized plasmid; SC, supercoiled plasmid.**

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9 **Figure S4 Molecular dynamics simulation analysis of binding differences**

10 **between *PrAgo* and gDNA-1/5 (A) Schematic of different gDNAs and targets.**

11 Yellow markings indicate different sequences. The cleavage positions are indicated

12 with a black triangle, black lines indicating the predicted 25- and 34-nt cleavage

13 products. (B) RMSD analysis for gDNA 1/5 -*PrAgo* complexes.

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2 **Supplementary tables**

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4 **Table S1 Nucleic acids used in this study**

Name	Sequence(5'-3')
5'P-gDNA -5(18nt)	P -CAATTACCTGGCATCATT
5'OH-gDNA -5(18nt)	OH -CAATTACCTGGCATCATT
5'P-gRNA -5(18nt)	P -CAAUUACCUGGCAUCAUU
5'OH-gRNA -5(18nt)	OH -CAAUUACCUGGCAUCAUU
DNA -target-5(59nt)	AACGGCTCTTAGTCAACCCTACGAAAAATGATGCCAGGT AATTGAAAAATAAAATAAAA
RNA -target-5(59nt)	AACGGCUCUUAGUCAACCCUACGAAAAAUGAUGCCAG GUAAUUGAAAAAUAAAAUAAAA
5'P-gDNA -1(18nt)	P -CCATGGGCACGCAGAATT
DNA -target-1(86nt)	GGCGACGCATCTAACGGCTCTTAGTCAACCCTACGAAAA ATTCTGCGTGCCCATGGAAAAATAAAATAAAATAAAATAA AATAAAA
5'P-gDNA -2(18nt)	P -CTCCGCCTCTTCCAGATT
DNA -target-2(86nt)	GGCGACGCATCTAACGGCTCTTAGTCAACCCTACGAAAA ATCTGGAAGAGGCGGAGAAAAATAAAATAAAATAAAATA AAATAAAA
5'P-gDNA -3(18nt)	P -CGCTCCAGGAGGGAAATT
DNA -target-3(86nt)	GGCGACGCATCTAACGGCTCTTAGTCAACCCTACGAAAA ATTTCCCTCTGGAGCGAAAAATAAAATAAAATAAAATAA AATAAAA
5'P-gDNA -4(18nt)	P -TGAGGTAGTAGGTTGTTT
DNA -target-4(86nt)	GGCGACGCATCTAACGGCTCTTAGTCAACCCTACGAAAA AACAACCTACTACCTCAAAAAATAAAATAAAATAAAATAA AATAAAA
DNA -target-5(86nt)	GGCGACGCATCTAACGGCTCTTAGTCAACCCTACGAAAA ATGATGCCAGGTAATTGAAAAATAAAATAAAATAAAATAA AATAAAA
5'P-gDNA -6(35 nt)	P-CGAGGTAGTAGGTTGTATAGTATATTAAATTATTT
5'P-gDNA -6(30 nt)	P-CGAGGTAGTAGGTTGTATAGTATATTAAAT
5'P-gDNA -6(25 nt)	P-CGAGGTAGTAGGTTGTATAGTATAT
5'P-gDNA -6(21 nt)	P-CGAGGTAGTAGGTTGTATAGT
5'P-gDNA -6(18 nt)	P-CGAGGTAGTAGGTTGTAT
5'P-gDNA -6(16 nt)	P-CGAGGTAGTAGGTTGT
5'P-gDNA -6(15 nt)	P-CGAGGTAGTAGGTTG
5'P-gDNA -6(14 nt)	P-CGAGGTAGTAGGTT
5'P-gDNA -6(13 nt)	P-CGAGGTAGTAGGT

5'P-gDNA -6(12 nt)	P-CGAGGTAGTAGG
5'P-gDNA -6(10 nt)	P-CGAGGTAGTA
5'P-gDNA -6(8 nt)	P-CGAGGTAG
5'P-gDNA -5-mis-1-A	P -AAATTACCTGGCATCATT
5'P-gDNA -5-mis-1-T	P -TAATTACCTGGCATCATT
5'P-gDNA -5-mis-1	P -GAATTACCTGGCATCATT
5'P-gDNA -5-mis-2	P -CTATTACCTGGCATCATT
5'P-gDNA -5-mis-3	P -CATTTACCTGGCATCATT
5'P-gDNA -5-mis-4	P -CAAATACCTGGCATCATT
5'P-gDNA -5-mis-5	P -CAATAACCTGGCATCATT
5'P-gDNA -5-mis-6	P -CAATTCCTGGCATCATT
5'P-gDNA -5-mis-7	P -CAATTAGCTGGCATCATT
5'P-gDNA -5-mis-8	P -CAATTACGTGGCATCATT
5'P-gDNA -5-mis-9	P -CAATTACCAAGGCATCATT
5'P-gDNA -5-mis-10	P -CAATTACCTCGCATCATT
5'P-gDNA -5-mis-11	P -CAATTACCTGCCATCATT
5'P-gDNA -5-mis-12	P -CAATTACCTGGGATCATT
5'P-gDNA -5-mis-13	P -CAATTACCTGGCTTCATT
5'P-gDNA -5-mis-14	P -CAATTACCTGGCAACATT
5'P-gDNA -5-mis-15	P -CAATTACCTGGCATGATT
5'P-gDNA -5-mis-16	P -CAATTACCTGGCATCTTT
5'P-gDNA -5-mis-17	P -CAATTACCTGGCATCAAT
5'P-gDNA -5-mis-18	P -CAATTACCTGGCATCATAA
5'P-gDNA -5-doub-mis-1,2	P -GTATTACCTGGCATCATT
5'P-gDNA -5-doub-mis-2,3	P -CTTTTACCTGGCATCATT
5'P-gDNA -5-doub-mis-3,4	P -CATATACCTGGCATCATT
5'P-gDNA -5-doub-mis-4,5	P -CAAACCTGGCATCATT
5'P-gDNA -5-doub-mis-5,6	P -CAATATCCTGGCATCATT
5'P-gDNA -5-doub-mis-6,7	P -CAATTTGCTGGCATCATT
5'P-gDNA -5-doub-mis-7,8	P -CAATTAGGTGGCATCATT
5'P-gDNA -5-doub-mis-8,9	P -CAATTACGAGGCATCATT
5'P-gDNA -5-doub-mis-9,10	P -CAATTACCAACGCATCATT
5'P-gDNA -5-doub-mis-10,11	P -CAATTACCTCCCATCATT
5'P-gDNA -5-doub-mis-11,12	P -CAATTACCTGCGATCATT
5'P-gDNA -5-doub-mis-12,13	P -CAATTACCTGGGTTTCATT
5'P-gDNA -5-doub-mis-13,14	P -CAATTACCTGGCTACATT
5'P-gDNA -5-doub-mis-14,15	P -CAATTACCTGGCAAGATT
5'P-gDNA -5-doub-mis-15,16	P -CAATTACCTGGCATGTTT
5'P-gDNA -5-doub-mis-16,17	P -CAATTACCTGGCATCTAT
5'P-gDNA -5-doub-mis-17,18	P -CAATTACCTGGCATCAAA
FW 5'P gDNA (14% GC of target region)	P-TTAGATTGATTTAAACTTCA
RV 5'P gDNA (14% GC of target region)	P-TGAAGTTTTAAATCAATCTAA
FW 5'P gDNA (20% GC of target region)	P-TTAAACTTCATTTTAAATT
RV 5'P gDNA (20% GC of target region)	P-AAATTAAAAATGAAGTTTAA

FW 5'P gDNA (28% GC of target region)	P-TTACTCATATATACTTTAGAT
RV 5'P gDNA (28% GC of target region)	P-ATCTAAAGTATATATGAGTAA
FW 5'P gDNA (36% GC of target region)	P-TCTCATGACCAAAATCCCTTA
RV 5'P gDNA (36% GC of target region)	P-TAAGGGATTTTGGTCATGAGA
FW 5'P gDNA (50% GC of target region)	P-CGTGAGTTTTCGTTCCACTGA
RV 5'P gDNA (50% GC of target region)	P-TCAGTGGAACGAAAACACG
FW 5'P gDNA (70% GC of target region)	P-CGCCCTGACGGGCTTGTCTGC
RV 5'P gDNA (70% GC of target region)	P-GCAGACAAGCCCGTCAGGGCG

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