A programmable pAgo nuclease with universal guide and target specificity from the mesophilic bacterium *Kurthia massiliensis*

Ekaterina Kropocheva, Anton Kuzmenko, Alexei A. Aravin, Daria Esyunina, Andrey Kulbachinskiy

Supplementary Information

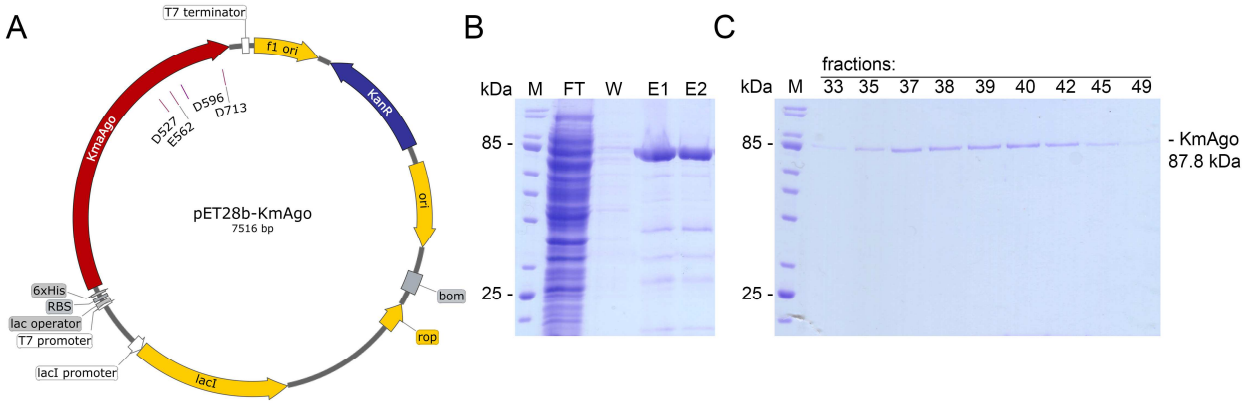

Fig. S1. Purification of KmAgo from *E. coli* cells. (A) Scheme of the expression plasmid. Positions of the catalytic tetrad residues in KmAgo are shown. (B) The first purification step by Co^{2+}-affinity chromatography. M, molecular weight marker; FT, flowthrough fraction; W, washing step with buffer containing 20 mM imidazole; E1 and E2 – elution fractions. (C) The second purification step by Heparin affinity chromatography (Coumassie staining). Fraction numbers after elution with a 0.1-1.0 M NaCl gradient. The position of KmAgo is indicated.

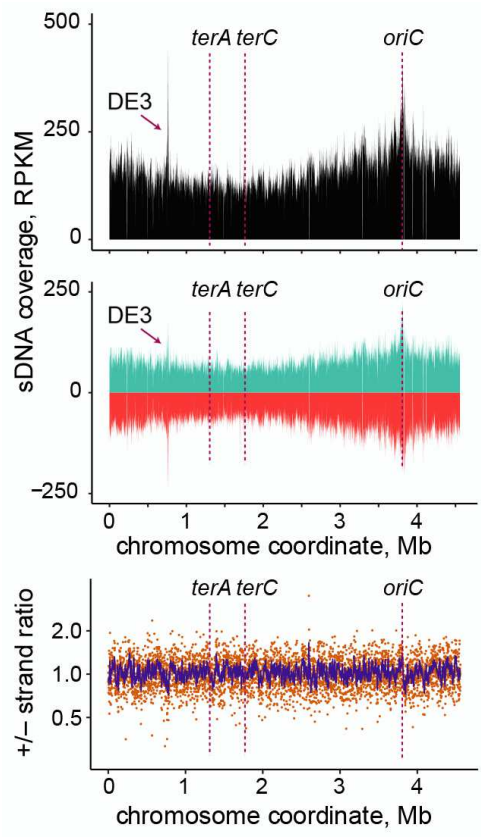

Fig. S2. Second replica of KmAgo-associated smDNAs mapping to the chromosome of *E. coli* BL21(DE3). Positions of the origin of replication (*oriC*) and replication termination sites (terA and terC) are indicated along the chromosomal coordinate. SmDNA coverage is shown in RPKM.

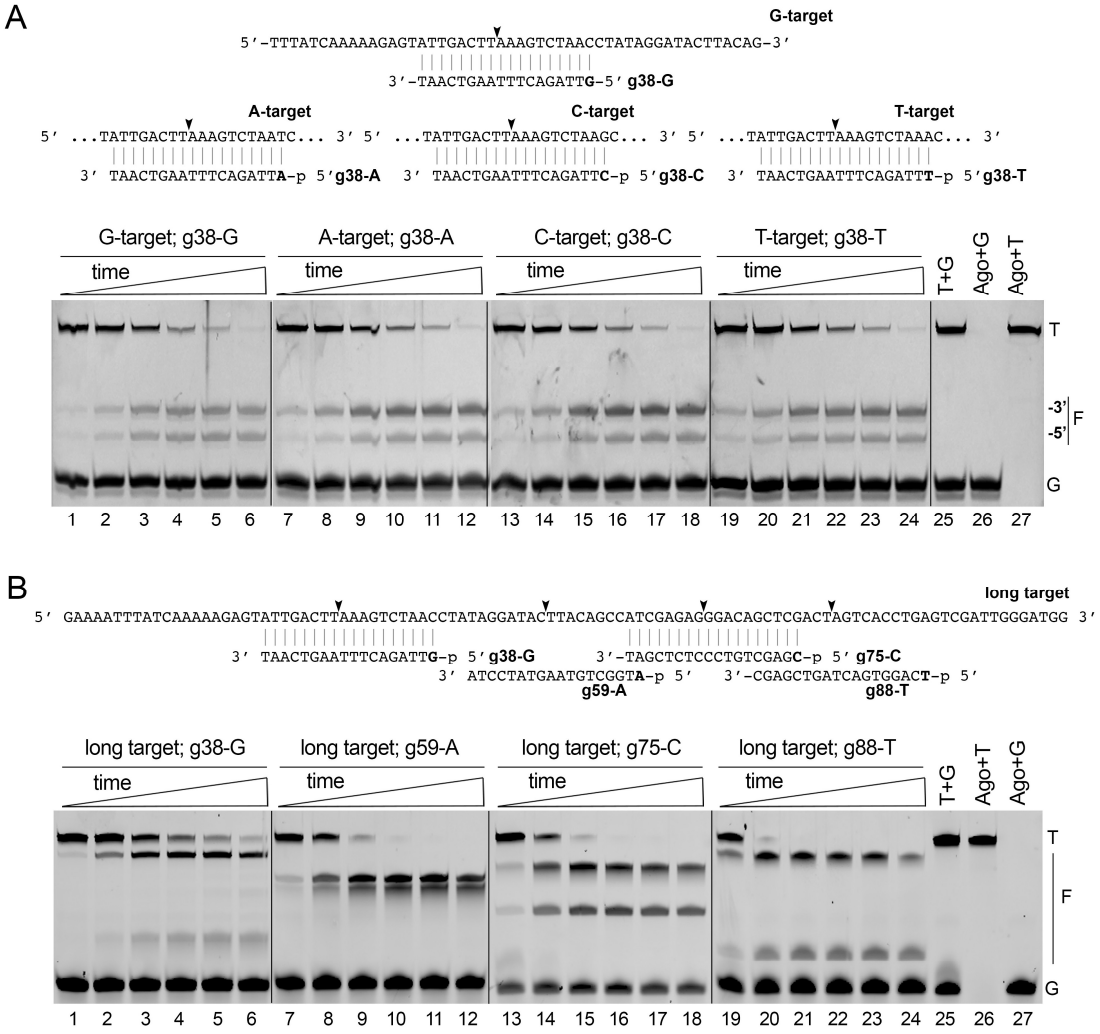

Fig. S3. Analysis of single-stranded DNA cleavage by KmAgo loaded with various guide DNA sequences. (A) Kinetics of cleavage of the 50 nt target by KmAgo loaded with guide DNAs containing different 5’-nucleotides (G, A, C, and T) and corresponding to the same target site. The scheme of the target and guide DNAs is shown on the top. (B) Kinetics of cleavage of a longer target variant (shown on the top) with guide DNAs containing different 5’-nucleotides and corresponding to different target sites. Note that the rate of the reaction with the ‘-38’ guide DNA is somewhat lower than in the case of other guide molecules. Positions of the target (T), guide (G) molecules and the cleavage fragments (F) are indicated.

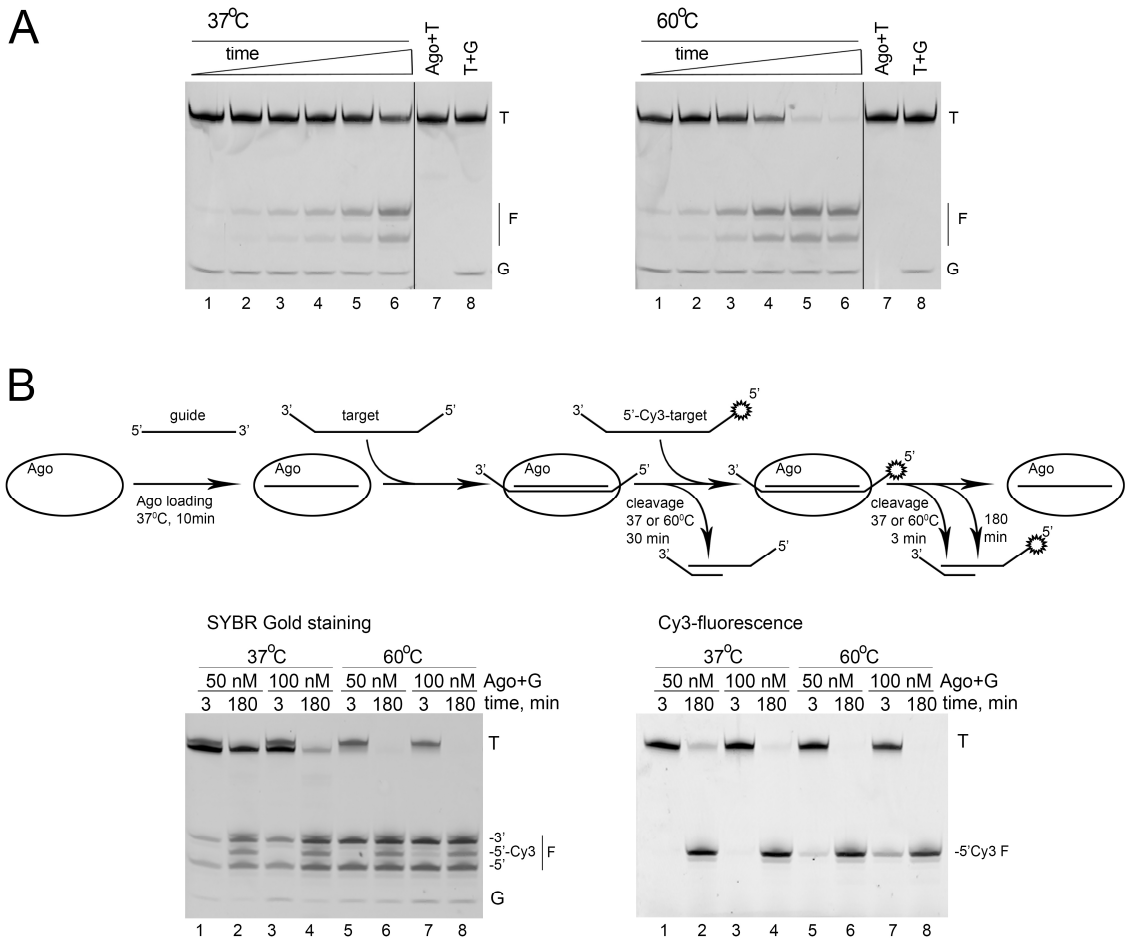

Fig. S4. Multiple turnover DNA cleavage by KmAgo. (A) Comparison of the kinetics of target DNA cleavage by KmAgo at different temperatures. KmAgo (100 nM) pre-loaded with guide DNA (100 nM) was incubated with target DNA (200 nM) at 37 °C (left panel) or 60 °C (right panel) for indicated time intervals. (B) Experiments on two-step target DNA cleavage. KmAgo (50 nM or 100 nM) pre-loaded with equimolar amounts of guide DNA was incubated with unlabeled target DNA (200 nM) for 30 min at 37 °C, followed by the addition of identical 3'-Cy3-labeled target DNA (200 nM). The reactions were stopped after 3 minutes or 3 hours, separated by denaturing PAGE and visualized by SYBR Gold staining (left) or Cy3 fluorescence scanning (right). Positions of the target (T), guide (G) molecules and cleavage fragments (F) are indicated. After 3 minutes at 37 °C, only the first unlabeled DNA target is cleaved (lanes 1,3, with no Cy3-labeled products visible on lanes 9,11). At 60 °C initial cleavage of the Cy3-labeled target is also visible (lanes 13,15). After 3 hours, the Cy3-containing target is cleaved with high efficiency at both temperatures (lanes 10,12,14 and 16).

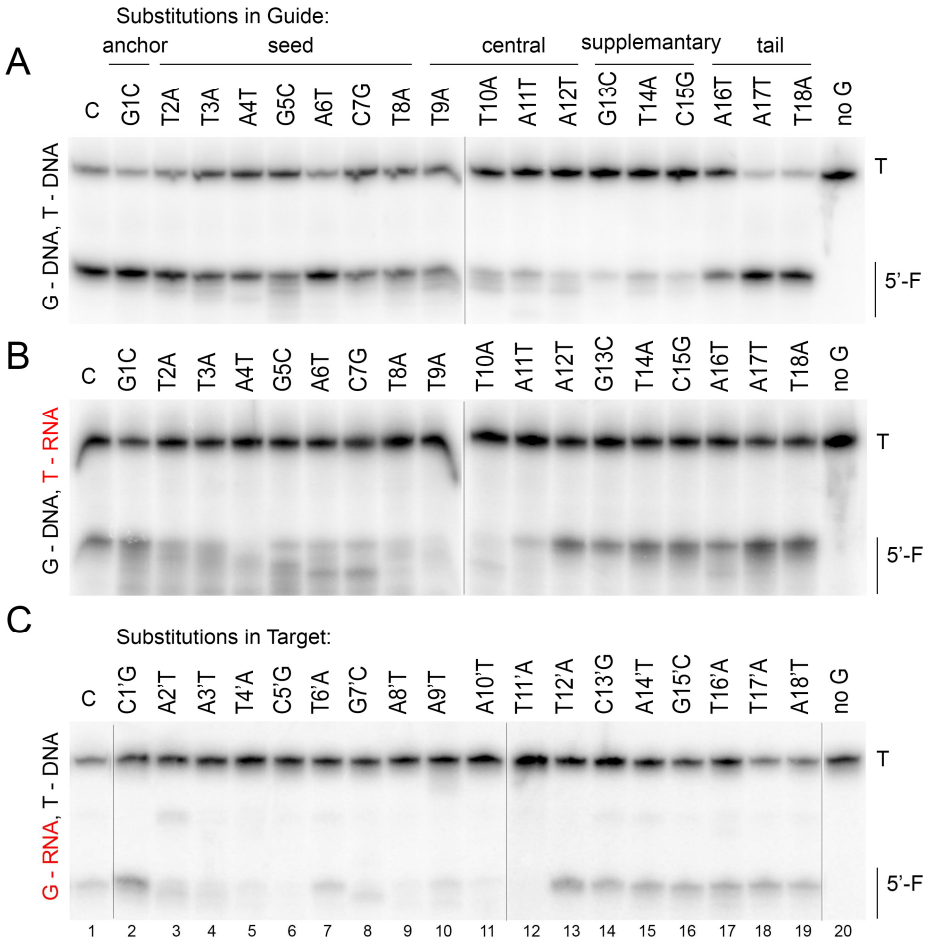

Fig. S5. Reproducibility of the target DNA or RNA cleavage by KmAgo with mismatched guide molecules. A replica of the experiment from Fig. 4 is shown.

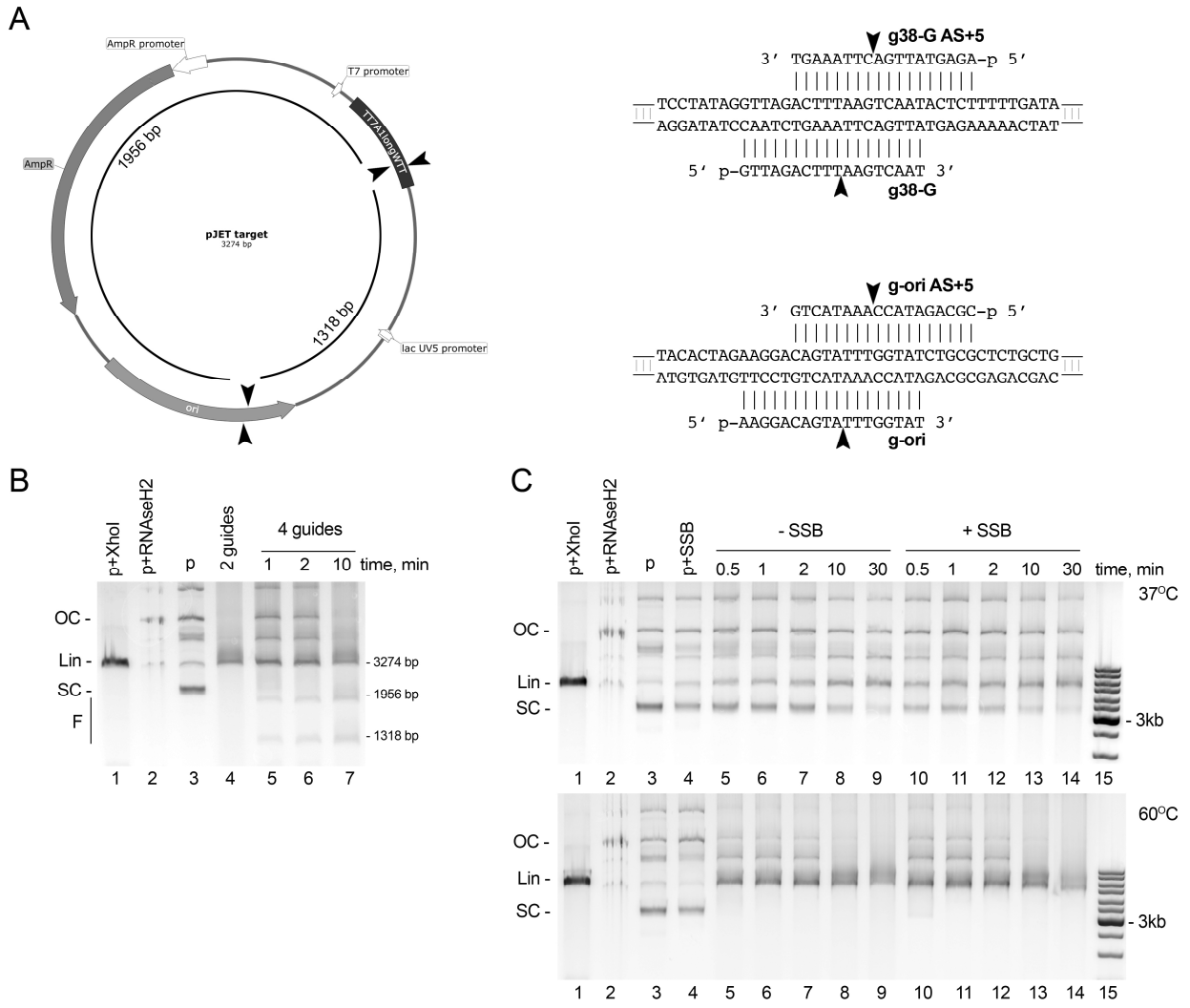

Fig. S6. Cleavage of plasmid DNA by KmAgo. (A) Scheme of the pJET1.2 target plasmid used in the cleavage assay. The plasmid contained a 300 bp insert shown in black (insert 2 in Table S1). The sites of cleavage in each strand and the lengths of resulting fragments are indicated. The sequences of the target regions and corresponding pairs of guides are shown on the right. (B) Plasmid cleavage with one or two pairs of guides corresponding to the two target sites in the plasmid. Positions of supercoiled (SC), relaxed open circle (OC), linear (Lin) plasmid forms, and the cleavage fragments (F; 1956 bp and 1318 bp) are indicated. (C) Effects of SSB on plasmid cleavage by KmAgo at 37 °C (top) and 60 °C (bottom). The reactions were performed with two DNA guides, gS0 and gAS+5, corresponding to the Amp resistance gene (see Fig. 5A and Table S1). The kinetics of cleavage was measured either in the absence (lanes 5-9) or in the presence (lanes 10-14) of SSB proteins. *E. coli* SSB (top) or thermostable ET SSB (bottom) were first incubated at 10 μM concentration with 20 nM target plasmid for 10 minutes at corresponding temperatures. Then the samples were mixed with guide-loaded KmAgo to the 1 μM, 2 nM and 500 nM final concentrations of SSB, plasmid and guides/KmAgo, respectively, and incubated for indicated time intervals at the same temperatures.

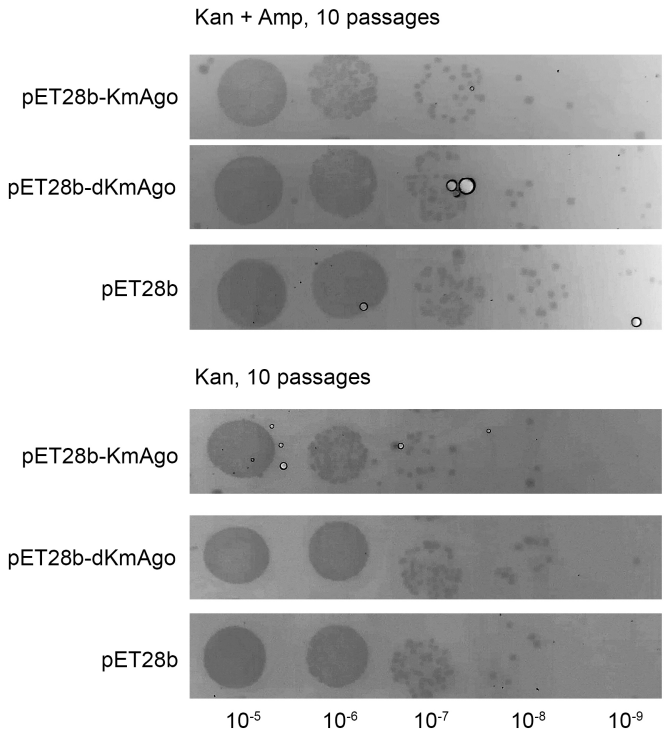

Fig. S7. Effects of KmAgo on plasmid loss. *E. coli* BL21(DE3) were co-transformed with pSRKAmp (Amp^R) and pET28b-KmAgo or pET28b-dKmAgo (catalytically dead Ago) or empty vector pET28b (Kan^R). The cells were cultivated in the LB medium with 50 μg/ml kanamycin and 0.2 mM IPTG in the absence of ampicillin (to allow the loss of the pSRKAmp plasmid) or with kanamycin, IPTG and 200 μg/ml ampicillin (for control) at 18°C during 10 passages. The duration of each passage was 24 h; after 24 h the cells were diluted 1:100 in the fresh LB medium with IPTG and antibiotics. After the final passage, serial dilutions of bacterial cultures were plated on agar LB dishes with kanamycin and ampicillin to compare the number of cells containing both plasmids and incubated at 37 °C overnight. No differences could be seen between the cultures that were grown with and without ampicillin.

Table S1. Sequences of oligonucleotides.

Oligonucleotide name	Sequence (5'-3')	Description
1. g38-G	GTTAGACTTTAAGTCAAT	18 nt guide, forms 5'-G pair with G-target
2. G-target	TTTATCAAAAAGAGTATTGACTTAAAG TCTAACCTATAGGATACTTACAG	50 nt target DNA for g38-G
3. g38-G-RNA	GUUAGACUUUAAGUCAAU	18 nt RNA guide for the guide/target specificity assay
4. G-target-RNA	UUUAUCAAAAAGAGUAUUGACUUAA AGUCUAACCUAUAGGAUACUUACAG	50 nt RNA target for the guide/target specificity assay
5. g38-10nt	GTTAGACTTT	10 nt guide, forms 5'-G pair with G-target
6. g38-12nt	GTTAGACTTTAA	12 nt guide, forms 5'-G pair with G-target
7. g38-14nt	GTTAGACTTTAAGT	14 nt guide, forms 5'-G pair with G-target
8. g38-16nt	GTTAGACTTTAAGTCA	16 nt guide, forms 5'-G pair with G-target
9. g38-20nt	GTTAGACTTTAAGTCAATAC	20 nt guide, forms 5'-G pair with G-target
10. g38-22nt	GTTAGACTTTAAGTCAATACTC	22 nt guide, forms 5'-G pair with G-target
11. g38_mm1	CTTAGACTTTAAGTCAAT	guide forms mismatched pair in position 1 with G-target
12. g38_mm2	GATAGACTTTAAGTCAAT	guide forms mismatched pair in position 2 with G-target
13. g38_mm3	GTAAGACTTTAAGTCAAT	guide forms mismatched pair in position 3 with G-target
14. g38_mm4	GTTTGACTTTAAGTCAAT	guide forms mismatched pair in position 4 with G-target
15. g38_mm5	GTTACACTTTAAGTCAAT	guide forms mismatched pair in position 5 with G-target
16. g38_mm6	GTTAGTCTTTAAGTCAAT	guide forms mismatched pair in position 6 with G-target
17. g38_mm7	GTTAGAGTTTAAGTCAAT	guide forms mismatched pair in position 7 with G-target
18. g38_mm8	GTTAGACATTAAGTCAAT	guide forms mismatched pair in position 8 with G-target
19. g38_mm9	GTTAGACTATAAGTCAAT	guide forms mismatched pair in position 9 with G-target
20. g38_mm10	GTTAGACTTAAAGTCAAT	guide forms mismatched pair in position 10 with G-target
21. g38_mm11	GTTAGACTTTTAGTCAAT	guide forms mismatched pair in position 11 with G-target
22. g38_mm12	GTTAGACTTTATGTCAAT	guide forms mismatched pair in position 12 with G-target
23. g38_mm13	GTTAGACTTTAACTCAAT	guide forms mismatched pair in position 13 with G-target
24. g38_mm14	GTTAGACTTTAAGACAAT	guide forms mismatched pair in position 14 with G-target
25. g38_mm15	GTTAGACTTTAAGTGAAT	guide forms mismatched pair in position 15 with G-target

26. g38_mm16	GTTAGACTTTAAGTCTAT	guide forms mismatched pair in position 16 with G-target
27. g38_mm17	GTTAGACTTTAAGTCATT	guide forms mismatched pair in position 17 with G-target
28. g38_mm18	GTTAGACTTTAAGTCAAA	guide forms mismatched pair in position 18 with G-target
29. G-target_mm1'	TTTATCAAAAAGAGTATTGACTTAAAG TCTAAGCTATAGGATACTTACAG	target forms mismatched pair in position 1 of g38 guide
30. G-target_mm2'	TTTATCAAAAAGAGTATTGACTTAAAG TCTATCCTATAGGATACTTACAG	target forms mismatched pair in position 2 of g38 guide
31. G-target_mm3'	TTTATCAAAAAGAGTATTGACTTAAAG TCTTACCTATAGGATACTTACAG	target forms mismatched pair in position 3 of g38 guide
32. G-target_mm4'	TTTATCAAAAAGAGTATTGACTTAAAG TCAAACCTATAGGATACTTACAG	target forms mismatched pair in position 4 of g38 guide
33. G-target_mm5'	TTTATCAAAAAGAGTATTGACTTAAAG TGTAACCTATAGGATACTTACAG	target forms mismatched pair in position 5 of g38 guide
34. G-target_mm6'	TTTATCAAAAAGAGTATTGACTTAAAG ACTAACCTATAGGATACTTACAG	target forms mismatched pair in position 6 of g38 guide
35. G-target_mm7'	TTTATCAAAAAGAGTATTGACTTAAAC TCTAACCTATAGGATACTTACAG	target forms mismatched pair in position 7 of g38 guide
36. G-target_mm8'	TTTATCAAAAAGAGTATTGACTTAATG TCTAACCTATAGGATACTTACAG	target forms mismatched pair in position 8 of g38 guide
37. G-target_mm9'	TTTATCAAAAAGAGTATTGACTTATAG TCTAACCTATAGGATACTTACAG	target forms mismatched pair in position 9 of g38 guide
38. G-target_mm10'	TTTATCAAAAAGAGTATTGACTTTAAG TCTAACCTATAGGATACTTACAG	target forms mismatched pair in position 10 of g38 guide
39. G-target_mm11'	TTTATCAAAAAGAGTATTGACTAAAAG TCTAACCTATAGGATACTTACAG	target forms mismatched pair in position 11 of g38 guide
40. G-target_mm12'	TTTATCAAAAAGAGTATTGACATAAAG TCTAACCTATAGGATACTTACAG	target forms mismatched pair in position 12 of g38 guide
41. G-target_mm13'	TTTATCAAAAAGAGTATTGAGTTAAAG TCTAACCTATAGGATACTTACAG	target forms mismatched pair in position 13 of g38 guide
42. G-target_mm14'	TTTATCAAAAAGAGTATTGTCTTAAAG TCTAACCTATAGGATACTTACAG	target forms mismatched pair in position 14 of g38 guide
43. G-target_mm15'	TTTATCAAAAAGAGTATTCACTTAAAG TCTAACCTATAGGATACTTACAG	target forms mismatched pair in position 15 of g38 guide
44. G-target_mm16'	TTTATCAAAAAGAGTATAGACTTAAAG TCTAACCTATAGGATACTTACAG	target forms mismatched pair in position 16 of g38 guide
45. G-target_mm17'	TTTATCAAAAAGAGTAATGACTTAAAG TCTAACCTATAGGATACTTACAG	target forms mismatched pair in position 17 of g38 guide
46. G-target_mm18'	TTTATCAAAAAGAGTTTTGACTTAAAG TCTAACCTATAGGATACTTACAG	target forms mismatched pair in position 18 of g38 guide
47. gAS-45	TGATAACACTGCGGCCAA	guide for plasmid pJET cleavage
48. gAS-25	AGTGCTGCCATAACCATG	guide for plasmid pJET cleavage
49. gAS-20	TATGCAGTGCTGCCATAA	guide for plasmid pJET cleavage
50. gAS-15	AGAATTATGCAGTGCTGC	guide for plasmid pJET cleavage
51. gAS-10	ACTGCATAATTCTCTTAC	guide for plasmid pJET cleavage
52. gAS-5	TGACAGTAAGAGAATTAT	guide for plasmid pJET cleavage

53. gS0	TCTCTTACTGTCATGCCA	guide for plasmid pJET cleavage
54. gAS0	TGGCATGACAGTAAGAGA	guide for plasmid pJET cleavage
55. gAS+5	ACGGATGGCATGACAGTA	guide for plasmid pJET cleavage
56. gAS+10	ATCTTACGGATGGCATGA	guide for plasmid pJET cleavage
57. gAS+15	AAAGCATCTTACGGATGG	guide for plasmid pJET cleavage
58. gAS+20	ACAGAAAAGCATCTTACG	guide for plasmid pJET cleavage
59. gAS+25	CAGTCACAGAAAAGCATC	guide for plasmid pJET cleavage
60. gAS+45	ATACACTATTCTCAGAAT	guide for plasmid pJET cleavage
61. 6S-1	GCGAACATCTCAGAGAA	guide for 6S RNA cleavage
62. 6S-2	ATGAAATATCGGCTCAG	guide for 6S RNA cleavage
63. 6S-3	TTGTGGTATGAAATATC	guide for 6S RNA cleavage
64. 6S-4	CACATTCTTGTGGTATG	guide for 6S RNA cleavage
65. 6S-5	TCTCGGACGGACCGAGC	guide for 6S RNA cleavage
66. 6S-6	AATGTGTCGTCGCAGTT	guide for 6S RNA cleavage
67. 6S-7	TCAAGGTGAATGTGTCG	guide for 6S RNA cleavage
68. 6S-8	GCTGTAACCCTTGAACC	guide for 6S RNA cleavage
69. 6S-9	CGCAGGCTGTAACCCTT	guide for 6S RNA cleavage
70. 6S-10	CGCCGCAGGCTGTAACC	guide for 6S RNA cleavage
71. KmAgo-NdeI	GCGCCCGCTAGCATGGAAGCTTATATAACAGAAATGGTCTCAC	primer for cloning the KmAgo gene into pET28b
72. KmAgo-XhoI	GCGCCCCTCGAGTTAAACGAATGGCAAGTTCGTCTGATT	primer for cloning the KmAgo gene into pET28b
73. T-target_Cy3	Cy3-TTTATCAAAAAGAGTATTGACTTAAAGTCTAAACTATAGGATACTTACAG	50nt fluorescently labelled target DNA for g38-T guide
74. long target	GAAAATTTATCAAAAAGAGTATTGACTTAAAGTCTAACCTATAGGATACTTACAGCCATCGAGAGGGACAGCTCGACTAGTCACCTGAGTCGATTGGGATGG	102 nt target for g38-G, g59-A, g75-C, g88-T guides
75. g59-A	ATGGCTGTAAGTATCCTA	5'-A guide for long target
76. g75-C	CGAGCTGTCCCTCTCGAT	5'-C guide for long target
77. g88-T	CGAGCTGTCCCTCTCGAT	5'-T guide for long target
78. g38-C	CTTAGACTTTAAGTCAAT	5'-C guide for C-target
79. g38-A	ATTAGACTTTAAGTCAAT	5'-A guide for A-target
80. g38-T	TTTAGACTTTAAGTCAAT	5'-T guide for T-target
81. C-target	TTTATCAAAAAGAGTATTGACTTAAAGTCTAAGCTATAGGATACTTACAG	50 nt target DNA for g38-C guide
82. A-target	TTTATCAAAAAGAGTATTGACTTAAAGTCTAATCTATAGGATACTTACAG	50 nt target DNA for g38-A guide

79. T-target	TTTATCAAAAAGAGTATTGACTTAAAG TCTAAACTATAGGATACTTACAG	50nt target DNA for g38-T guide
80. g38-G AS+5	AGAGTATTGACTTAAAGT	guide for plasmid pJET-target cleavage
81. g-ori	AAGGACAGTATTTGGTAT	guide for plasmid pJET-target cleavage
82. g-ori AS+5	CGCAGATACCAAATACTG	guide for plasmid pJET-target cleavage
83. pJET insert 1	GATCTTTAAGAAGGAGATATACCATG CACCACCACCATCATCATCACCACGG TGGAGGTAGCGGAGGAGGTAGCTGG AACTGCA	sequence of the insert in pJET1.2 (CloneJET PCR Cloning Kit, Thermo Fisher Scientific) used in experiments in Fig. 5
84. pJET insert 2	AAAAAAAAAACCCCGCCCTGTCAGG GGCGGGGTTTTTTTTTGTAAAACGAC GGCCAGTGAAGCTTCGAGCTCGGTA CCGACTCAGGTGACTAGTCGAGCGA TTACCAGCAGGCCTGTTATTAGCGAT CCAGATCCAGAACCCGGACCCCGGT GTCGATTGGGATGGCTATTCGCCGT GTCCCTCTCGATGGCTGTAAGTATCC TATAGGTTAGACTTTAAGTCAATACTC TTTTTGATAAATTTTCGGGATCTGGAT CCAAATAGAATTCACTGAAAAAAAAA CCCCGCCGAAGCGGGGT	sequence of the insert in pJET1.2 used in experiments in Fig. S6