Supplementary data to

A type III-B CRISPR–Cas effector complex mediating massive target DNA destruction

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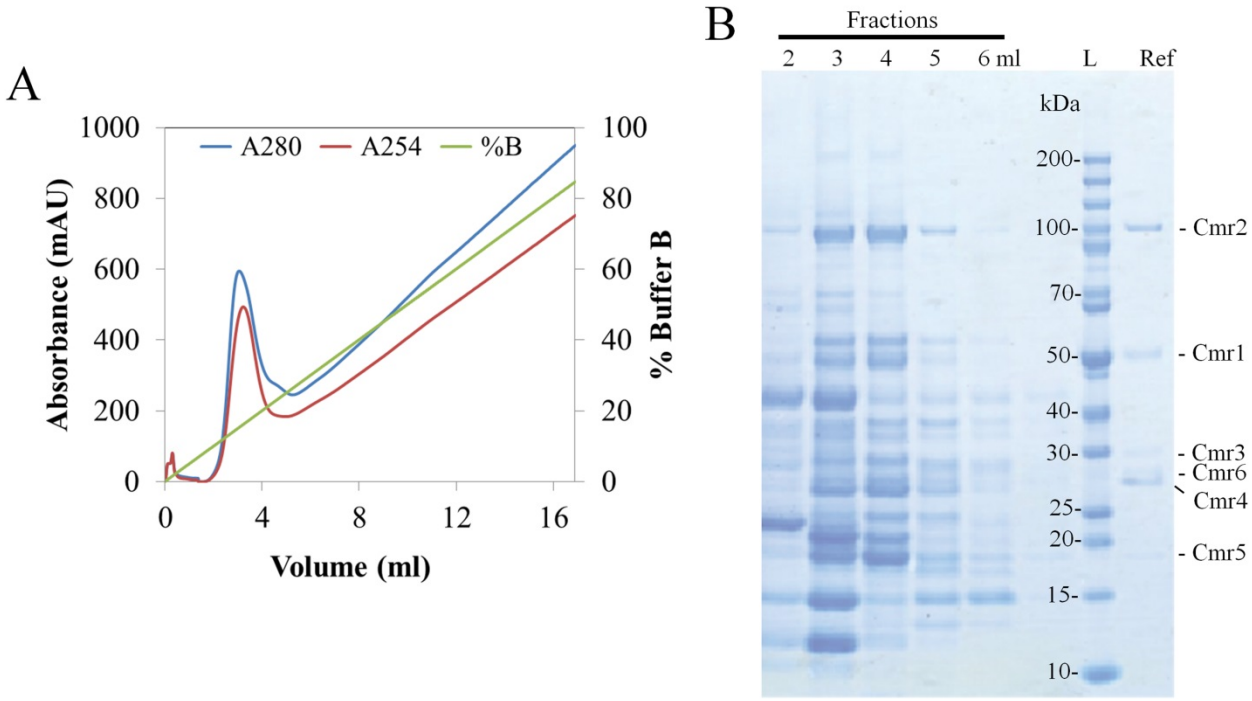
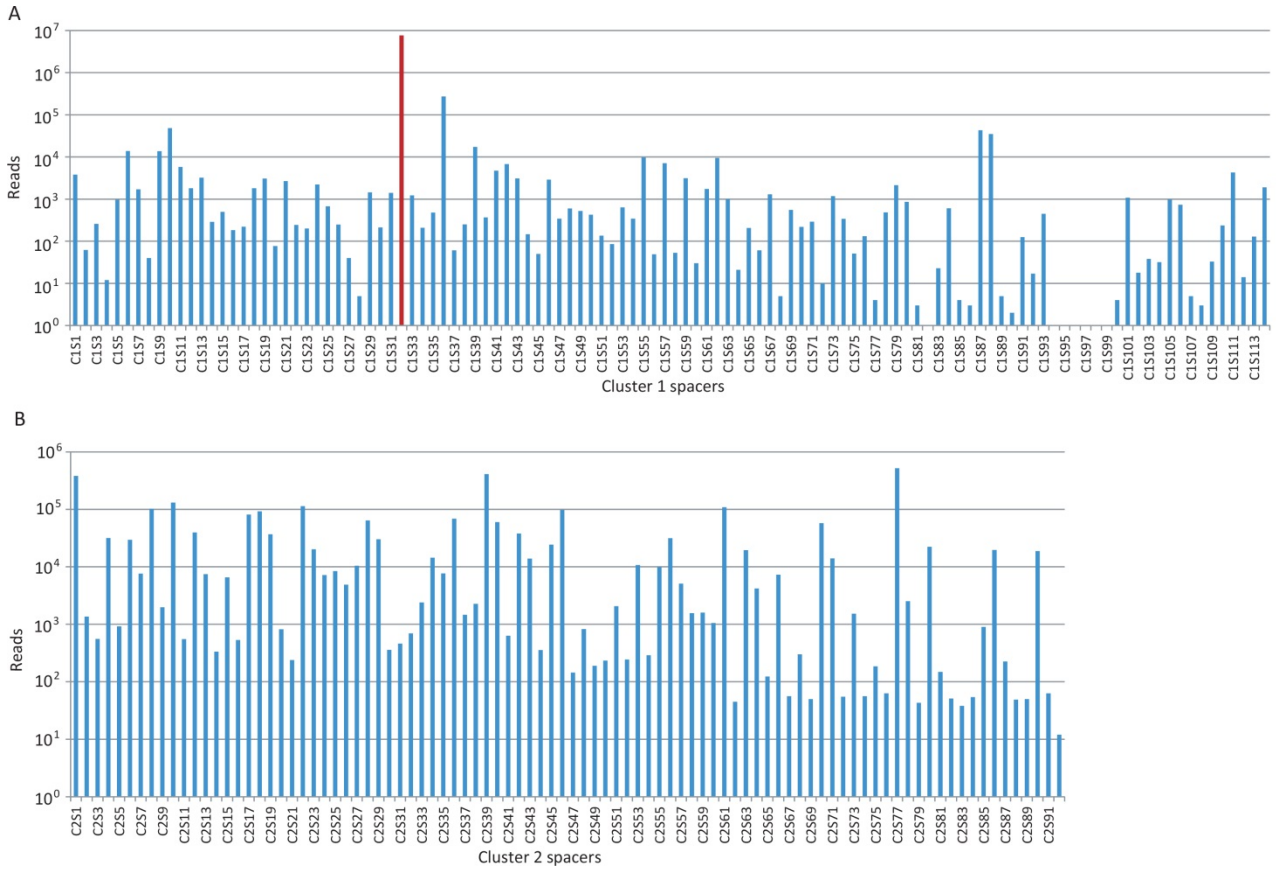
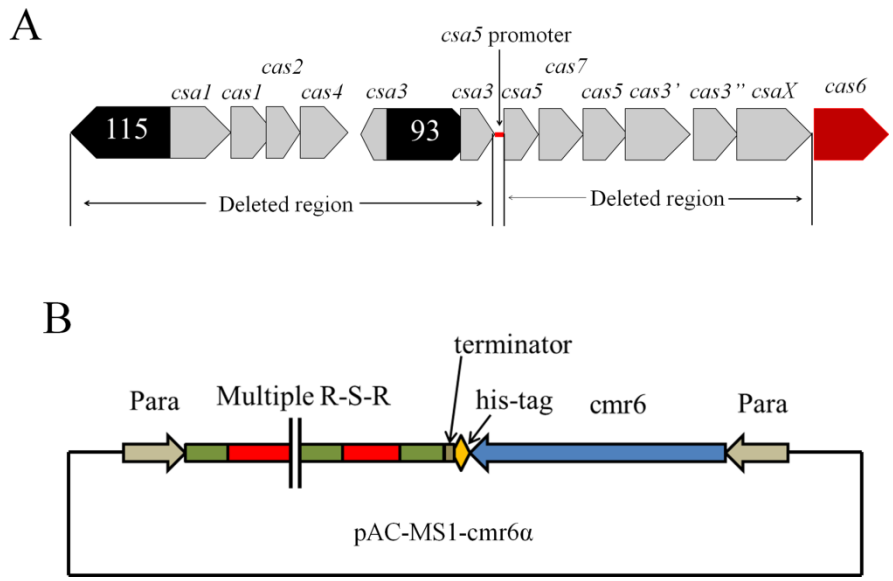
Supplementary figures and tables



Supplementary figure S1. (A) UV-Vis spectrograms of the Ni-sepharose affinity chromatography of the His-tagged Cmr-α complex. Depicted is the absorbance at 280 nm (blue), 254 nm (red) as well as the elution buffer gradient (green). (B) SDS-PAGE of the selected peak fractions after the HisTrap purification. Ref represents purified Cmr-α complex.



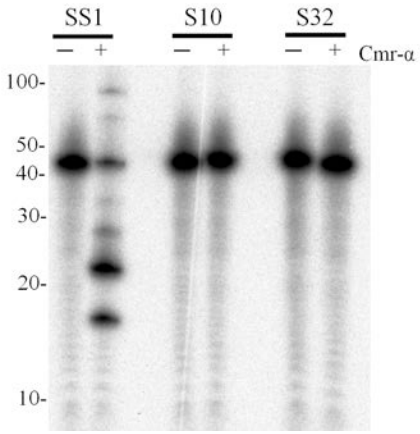
Supplementary figure S2. Distribution of Cmr-α mature crRNAs on the chromosomal CRISPR loci revealed from RNA sequencing of crRNAs extracted from the Cmr-α complex. Reads number matching each spacer is plotted to the position corresponding spacer in the CRISPR locus 1 (A) and 2 (B) of the *S. islandicus* REY15A chromosome



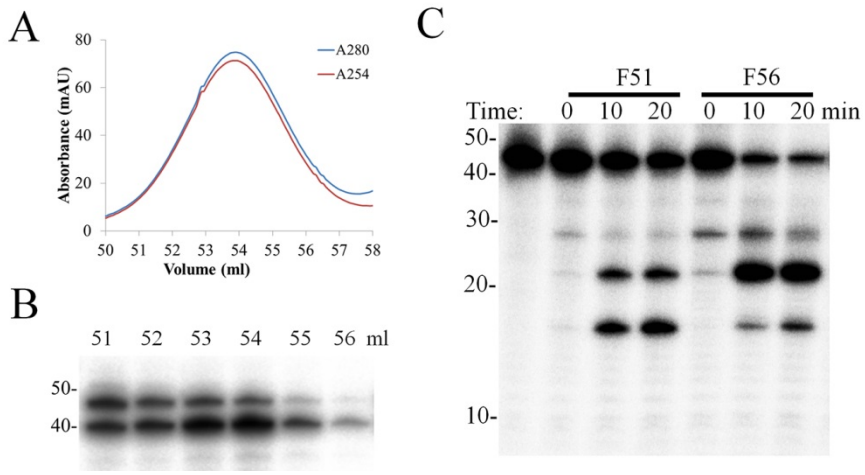
Supplementary figure S3.

(A) Schematic of deleted regions in the *S. islandicus* MF1 strain. The first deleted region contains two CRISPR loci (indicated in black) and all type I-A adaptation *cas* genes plus a regulatory gene *csa3b* (indicated in gray). The second region includes all type I-A interference genes except *cas6*. An active cas6 gene is generated by the fusion of the promoter of *csa5* and the coding sequence of *cas6* (shown in red) by SOE-PCR.

(B) Schematic of the pAC-MS1-cmr6α plasmid employed for purifying a native Cmr-α complex harboring a single type of crRNA. The expression of the artificial CRISPR array consisting of 10 repeat-spacer units (Multiple R-S-R) and the gene coding for the 6xHis-Cmr6-α is under the control of the promoter of the arabinose-inducible *araS* gene coding for a arabinose-binding protein. terminator: transcriptional terminator signal.

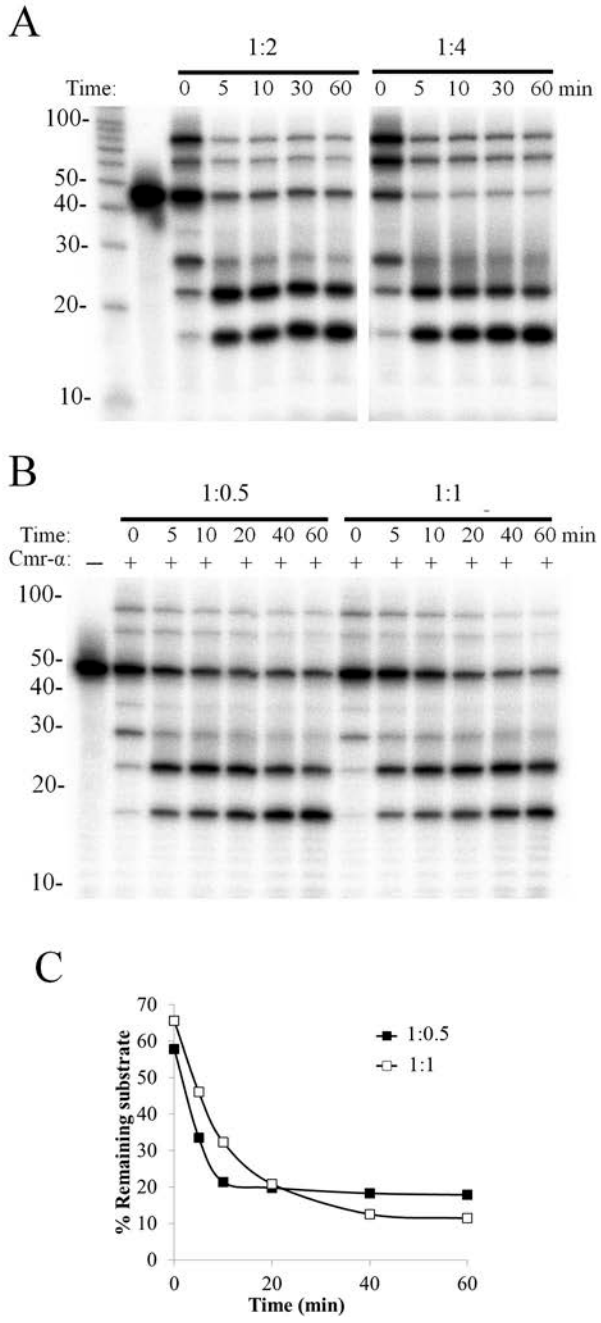


Supplementary figure S4. Cmr-α-SS1 does not cleave S10 or S32 RNA substrate. Twenty five nM of labelled S10 and S32 RNA substrate was incubated with 25 nM Cmr-α-SS1 for 20 min and resolved on a denaturing polyacrylamide gel.



Supplementary figure S5. RNA cleavage sites correlate with the length of crRNAs present in the Cmr-α complex.

(A) UV spectrum of gel filtration chromatography of the His-tagged Cmr-α-SS1. Only the Cmr-α region (fraction no. 50-58 ml) is shown. Blue: UV absorbance at 280 nm; red: UV absorbance at 254 nm. (B) Denaturing gel electrophoresis analysis of crRNA extracted from different fractions in (A). The RNA samples were labelled with T4 polynucleotide kinase using ^{32}P-γ-ATP. (C) RNA cleavage by the 51-ml fraction (F51) and 56-ml fraction (F56), respectively. 5'-labeled SS1 RNA substrate was incubated with 1.5 µl of F51 or 7.5 µl of F56 for indicated times, and then analyzed by denaturing gel electrophoresis.

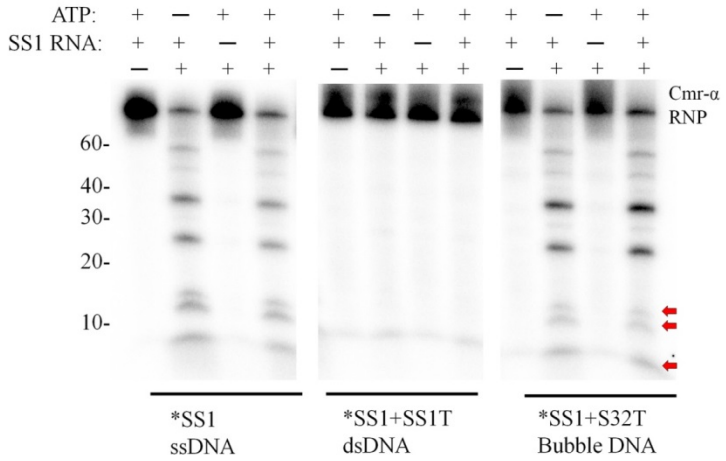


Supplementary figure S6. Analysis of RNA cleavage with altering radios between Cmr-α complex and target RNA

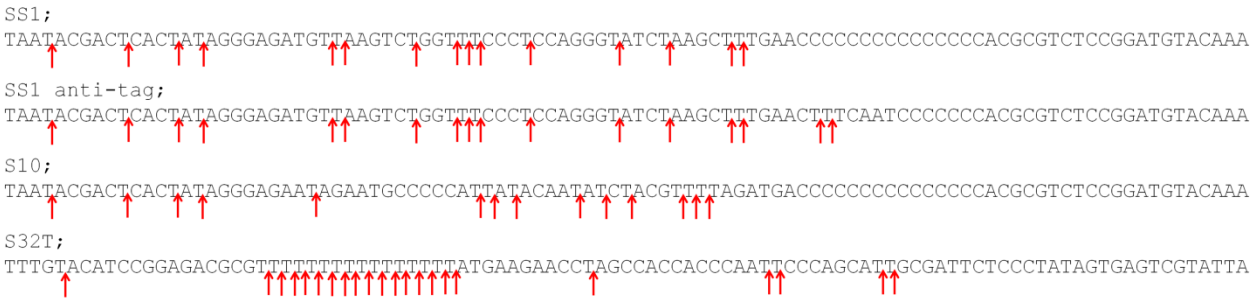
(A) Twenty five nM of labelled SS1 RNA was incubated with 50 nM or 100 nM Cmr-α-SS1 for indicated time, respectively. Then, the samples were analyzed by denaturing gel. (B) Twenty five nM Cmr-α-SS1 was incubated with 25 nM labelled SS1 RNA or 25 nM labelled SS1 RNA and 25 nM cold SS1 RNA (50 nM in total) for indicated time points. Then, the samples were analyzed by denaturing PAGE. (D) Quantification of remaining RNA

substrate from 4B. The percentage was normalized by the amount of SS1 RNA without Cmr-α-SS1.

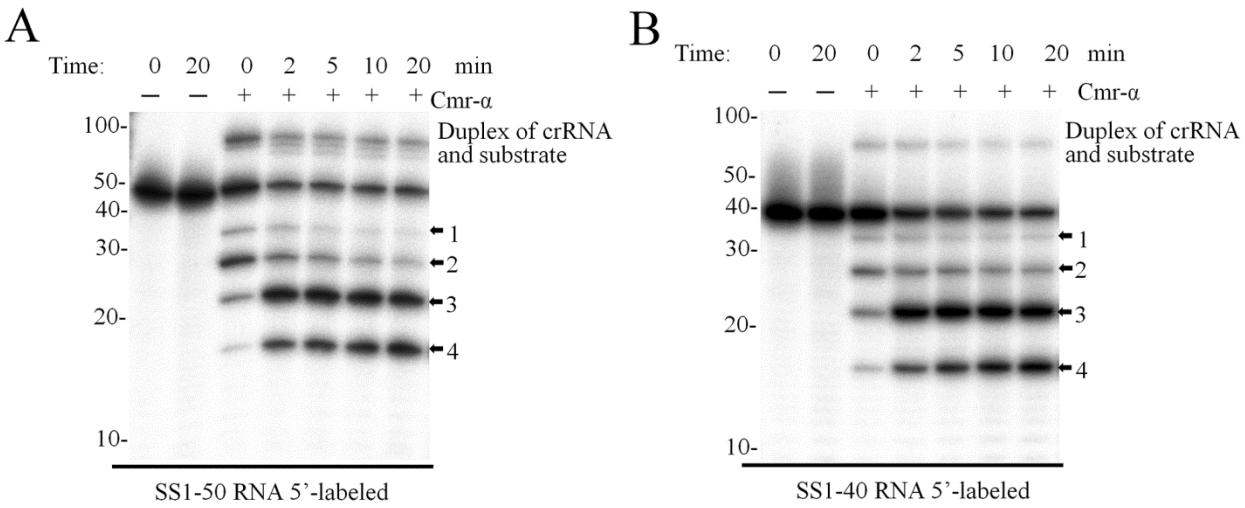
Supplementary figure S7. Schematic map of bubble DNA and R-loop DNA used in Fig. 6A. Bubble DNA was produced by annealing of SS1 and S32T ssDNA, while R-loop DNA was yielded by annealing of S32* RNA and S32T ssDNA followed by SS1 ssDNA.



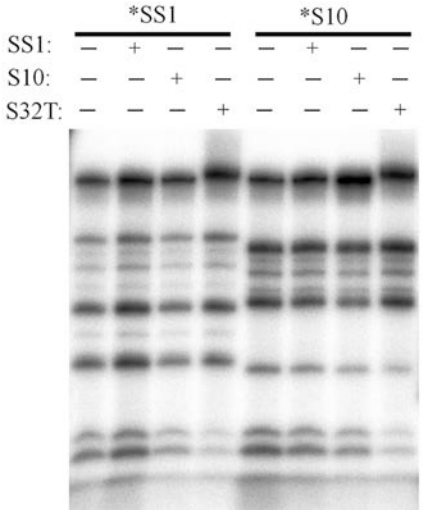
Supplementary figure S8. Twenty five nM of ssDNA (labelled SS1), dsDNA (labelled SS1 + unlabeled SS1T) and bubble DNA (labelled SS1 + unlabeled S32T) were incubated with (+) without (-) 50 nM Cmr-α-SS1, 200 nM SS1 RNA and 1 mM ATP for 1 h, and then the samples were analyzed by denaturing PAGE. The three red arrows indicate that the cleavage sites are located in the double strand region of the bubble DNA.



Supplementary figure S9. Schematic depicting the cleavage sites on the ssDNA substrates.



Supplementary figure S10. Cleavage of SS1-50 (A) and SS1-40 (B) by Cmr-α-SS1.Tweenty five nM of the labelled substrates were incubated with (+) or without (−) 25 nM of Cmr-α-SS1 for indicated time and then analyzed by denaturing PAGE.



Supplementary figure S11. Additional 10 times of unlabeled ssDNA substrate does not affect the cleavage of labeled substrate by Cmr-α. Twenty five nM labeled SS1 and S10 ssDNA substrate were incubated with 50 nM Cmr-α-SS1 and 200 nM SS1 RNA in the presence of 250 nM of unlabeled SS1, S10 or S32T ssDNA as indicated. After 1 h's incubation, the samples were analyzed by denaturing PAGE.

Supplementary Table 1. *Sulfolobus* strains used in this work

Strains	Genotype and features	Reference
S. islandicus E233S1	Δ*pyrEF*Δ*lacS*	(1)
S. islandicus MF1	Derived from E233S1. The genes for CRISPR cascade and the two CRISPR modules are deleted.	This work
S. islandicus Δcmr-β	Derived from E233, carrying deletion of IIIB Cmr-β locus including 7 *cmr*-β genes	(2)
S. islandicus Cmr-2αHD-M1	Derived from *S. islandicus* Δcmr-β, carrying a double mutation in the HD motif (H14N, D15N) of Cmr-2α	(2)
S. islandicus Cmr-2αHD-M2	Derived from *S. islandicus* Δcmr-β, carrying a quadruple mutation in the HD domain (H14N, D15N, K19A and I23A) of Cmr-2α	(2)
S. islandicus Cmr-2αPalm-M1	Derived from *S. islandicus* Δcmr-β, carrying a double mutation in the GGDD motif (G666K, D667K) of Cmr-2α	This work
S. islandicus Cmr-2αPalm-M2	Derived from *S. islandicus* Δcmr-β, carrying an octal mutation in the Palm domain (from IYlGGDDiLA to AAlAAAAiAS) of Cmr-2α	This work

Supplementary Table 2. Plasmids used in this work

Plasmids	Genotype and features	Reference
pSeSD1	A *Sulfolobus-E. coli* shuttle vector with an expression cassette controlled under ParaS-SD promoter	(3)
pSe-Rp	A cloning vector for constructing mini-CRISPR arrays for *Sulfolobus*	(4)
pAC-MS1	Derived from pSeSD1, carrying an artificial CRISPR locus with 10 copies of 43 nt SS1 spacer.	This work
pcmr6α	Derived from pSeSD1, expressing His-tagged Cmr6	This work
pAC-MS1-cmr6α	Derived from pAC-MS1, both carrying an artificial CRISPR locus with 10 copies of SS1 spacer and expressing His-tagged Cmr6	This work

Supplementary Table S3. Primers used in this work

Name	Sequence (5'~3')
SS1- fwd	GCTAATCTACTATAGAATTGAAAGTTCAAAGCTTAGATACCCTGGAGGGAAACCAGACTT
SS1- rev	CGCAGTCGACTTTCAATTCTATAGTAGATTAGCTGGTGTTAAGTCTGGTTTCCCTCCAGG
Cmr6α fwd	TTTTTTTCATATGCAAGGTAATCGGATTCTTAATG
Cmr6α fwd	TTTTAGGCCTAGAACTCGGACATTTTAATTCCACC
MCS-fwd	ATGCCCCGGGATGTTAAACAAGTTAGG
MCS-rev	GGCACTCGAGAAAAAAAAGATTTTGCTTAATGGTG
IA-L-F_SalI	TTACGC GTCGAC ATTGCAGAGAGGCCTAAGAGGACCA
IA-SOE-R	AATATATAAAACTTTCTATTTCTTGTGAACGAAAAGATTTTCATACGTTTCCCCGACT
IA-SOE-F	AATAGAAAGTTTTATATATTGGGGTAGTAAAGAGAATAATGTGCCATTAATTTTCAAG
IA-R-R_NotI	AAGGAAAAAA GCGGCCGC CAATGTAGGGGAAACAAACCTCACT
IAspacer1F	aaagAAGGATATACATTTTGATGGAAGAGATACGGACTCGGCTG
IAspacer1R	tagcCAGCCGAGTCCGTATCTCTTCCATCAAAATGTATATCCTT
2α-GD-SpF	aagCCACCCAAATATATAGCTACAGCATTATTATCGCGTAATA
2α-GD-SpR	agcTATTACGCGATAATAATGCTGTAGCTATATATTTGGGTGG
2α-GD-SOEF	CTGTAGCTATATATTTGGGTAAGAAAGACATATTGG
2α-GD-SOER	ACCCAAATATATAGCTACAG
2α-I-A-SpF	aaagATATGTCGTCACCACCCAAATATATAGCTACAGCATTATT
2α-I-A-SpR	tagcAATAATGCTGTAGCTATATATTTGGGTGGTGACGACATAT
2α-I-A-SOEF	GCAGCTTTGGCTGCTAACAACATAGCTTCGTTATCGCCTATAAAGTTTAACG
2α-I-A-SOER	AAGCTATGTTGTTAGCAGCCAAAGCTGCAGCTACAGCATTATTATCGCGTAAT
2α-Palm-SalIF	ACGCGTCGACTACTGTGCAATCAAACGCG
2α-Palm-NotIR	ATAAGAATGCGGCCGCCCTTAAGTAAGATACTG

Supplementary Table S4. Nucleic acid substrates used in this work

Name	Sequence (5'~3')	Size (mer)
RNA		
SS1	UGUUAAGUCUGGUUUCCCUCCAGGGUAUCUAAGCUUUGAAAAAAAA	46
SS1-50	UGUUAAGUCUGGUUUCCCUCCAGGGUAUCUAAGCUUUGAACTTTCAATAA	50
SS1-40	UGUUAAGUCUGGUUUCCCUCCAGGGUAUCUAAGCUUUGAA	40
S10	AUAGAAUGCCCCCAUUAUACAAUAUCUACGUUUUAGAUGAAAAAAA	46
S32	AUCGCAAUGCUGGGAAUUGGGUGGUGGCUAGGUUCUUCAUUAAAAAA	46
S32*	AUCGCAAUGCUGGGAAUUGGGUGGUGGCUAGGUUCUUCAUUAAAAAAAAAAAAAA	55
DNA		
SS1	TAATACGACTCACTATAGGGAGaTGTTAAGTCTGGTTTCCCTCCAGGGTATCTAAGC TTTGAAccccccccccccccccACGCGTCTCCGGATGTACAAA	99
SS1T	TTTGTACATCCGGAGACGCGTgggggggggggggggTTCAAAGCTTAGATACCCTGGAG GGAAACCAGACTTAACAtCTCCCTATAGTGAGTCGTATTA	99
SS1 anti-tag	TAATACGACTCACTATAGGGAGaTGTTAAGTCTGGTTTCCCTCCAGGGTATCTAAGC TTTGAACTTTCAATcccccccACGCGTCTCCGGATGTACAAA	99
S10	TAATACGACTCACTATAGGGAGaATAGAATGCCCCCATTATACAATATCTACGTTTTA GATGAccccccccccccccccACGCGTCTCCGGATGTACAAA	99
S32T	TTTGTACATCCGGAGACGCGTTTTTTTTTTTTTTTTATGAAGAACCTAGCCACCACCC AATTCCCAGCATTGCGATtCTCCCTATAGTGAGTCGTATTA	99

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3. Peng, N., Deng, L., Mei, Y., Jiang, D., Hu, Y., Awayez, M., Liang, Y. and **She, Q.** (2012) A synthetic arabinose-inducible promoter confers high levels of recombinant protein expression in hyperthermophilic archaeon Sulfolobus islandicus. *Appl Environ Microbiol*, **78**, 5630-5637.

4. Peng, W., Feng, M., Feng, X., Liang, Y.X. and **She, Q.** (2015) An archaeal CRISPR type III-B system exhibiting distinctive RNA targeting features and mediating dual RNA and DNA interference. *Nucleic Acids Res*, **43**, 406-417.