Cmr1 enables efficient RNA and DNA interference of a III-B CRISPR-Cas system by binding to target RNA and crRNA

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Supplementary data:

The file contains 4 tables and 6 figures.

Table S1. *Sulfolobus* strains used in this work

Strains	Genotype and features	Reference
S. islandicus E233	Δ*pyrEF*	(1)
S. islandicus Δcmr-β(Δβ)	Derived from E233, carrying deletion of IIIB Cmr-β locus including 7 *cmr*-β genes	(2)
S. islandicus ΔCmr1α(ΔβΔ1α)	Derived from ΔβE233, carrying deletion of *cmr1α* gene	This work
S. islandicus Cmr1α-M1(Δβ1α-M1)	Derived from ΔβE233, carrying a double mutation (W58A, F59A) donor DNA of *cmr1α*	This work
S. islandicus Cmr1α-M2(Δβ1α-M2)	Derived from ΔβE233, carrying a quadruple mutation (I52A, G54A, R57A, R61A) donor DNA of *cmr1α*	This work

Table S2. 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 *Sulfolobus* artificial mini-CRISPR cloning vector	(4)
pAC-SS1	An artifcial mini-CRISPR locus plasmid derived from pSe-Rp, carrying one spacer matching the protospacer 1 (SS1) of the *S. islandicus lacS* gene	(4)
pAC10-SS1	An artifcial mini-CRISPR locus plasmid derived from pSe-Rp, carrying an artifcial CRISPR locus with 10 S1 spacer of the *S. islandicus lacS* gene	(4)
pS10i	An invader plasmid carrying a target sequence of spacer 10 in CRISPR locus 2 in *S. islandicus*	(5)
pAC-cmr6α-10His	Derived from pAC, carrying His-tagged Cmr6 and mini-CRISPR locus with 10 S1 spacer	This work
pGE-ΔCmr1α	A genome editing plasmid for deletion of the *cmr1α* gene in *S.islandicus*	This work
pGE-1α-M1	A genome editing plasmid for double mutation(W58A, F59A) of the *cmr1α* gene in *S.islandicus*	This work
pGE-1α-M2	A genome editing plasmid for quadruple mutation(I52A, G54A, R57A, R61A) of the *cmr1α* gene in *S.islandicus*	This work

Table S3. Oligonucleotides used in this work

Oligonucleotide	Sequence (5'-3')
10His-replace-F	GGCCGCACATCATCATCACCACCATCATCATCACCATTAAGCAAATCTTTTTTTTCCC
10His-replace-R	GGGAAAAAAAAGATTTGCTTAATGGTGATGATGATGGTGGTGATGATGATGTGC
MCS-fwd	ATGCCCCGGGATGTTAAACAAGTTAGG
MCS-rev	GGCACTCGAGAAAAAAAAGATTTTGCTTAATGGTG
△1α-SpF	AAAGGTAGATTAAGATGGTTCTTAAGGACTGTATATAATAGATT
△1α-SpR	TAGCAATCTATTATATACAGTCCTTAAGAACCATCTTAATCTAC
△1α-SOEF	TTGCACTGTTTACTGATAGAAGTAAATCCGTTATAATCCC
△1α-SOER	GATTATAACGGATTTACTTCTATCAGTAAACAGTGCAAAG
△1α-SalIF	ACGCGTCGACCTCAAGCAAGCCTGGATTAG
△1α-NotIR	AAGGAAAAAAGCGGCCGCCTTTTCCTACTCCATTGG
△1α-PCR-F	AACTGCAAATTGCCAAAT
△1α-PCR-R	GAGGGTCGTGGAAATAGGCT
Q_{Tar}-F	GTACTCGACAATGAACGAAC
Q_{Tar}-R	GAAACGCTCTTTATTCCATC
Q_{Ref}-F	AGCAGGATTACCAACAAGTG
Q_{Ref}-R	CATCCGCAATACCGTTTTC
lacS-RT-R	AGGTCTTTGATAATCTGCATC
1α-M1-SpF	AAAGGTAGATTAAGATGGTTCTTAAGGACTGTATATAATAGATT
1α-M1-SpR	TAGCAATCTATTATATACAGTCCTTAAGAACCATCTTAATCTAC
1α-M1-SOEF	AAGACGCTCTTAATCTACCCACTATTTCTTCTTCGTCAAC
1α-M1-SOER	AGTGGGTAGATTAAGAGCGGCTTTAAGGACTGTATATAAT
1α-M2-SpF	AAAGGTAGATTAAGATGGTTCTTAAGGACTGTATATAATAGATT
1α-M2-SpR	TAGCAATCTATTATATACAGTCCTTAAGAACCATCTTAATCTAC
1α-M2-SOEF	AGCTAAGAACCATGCTAATCTAGCCACTGCTTCTTCTTCGTCAAC
1α-M2-SOER	GCAGTGGCTAGATTAGCATGGTTCTTAGCTACTGTATATAAT
1α-M-SalIF	ACGCGTCGACCTCAAGCAAGCCTGGATTAG
1α-M-NotIR	AAGGAAAAAAGCGGCCGCTATAGAGTCTTCAGAAGTTT

Table S4. Nucleic acid substrates used in this work

Name	Sequence (5'-3')	Size (mer)
RNA		
SS1-46	UGUUAAGUCUGGUUUCCCUCCAGGGUAUCUAAGCUUUGAAAAAAAA	46
DNA		
S10	ACTATAGGGAGAATAGAATGCCCCCATTATACAATATCTACGTTTTAGATGACCCCCCCC	60

Supplementary figures

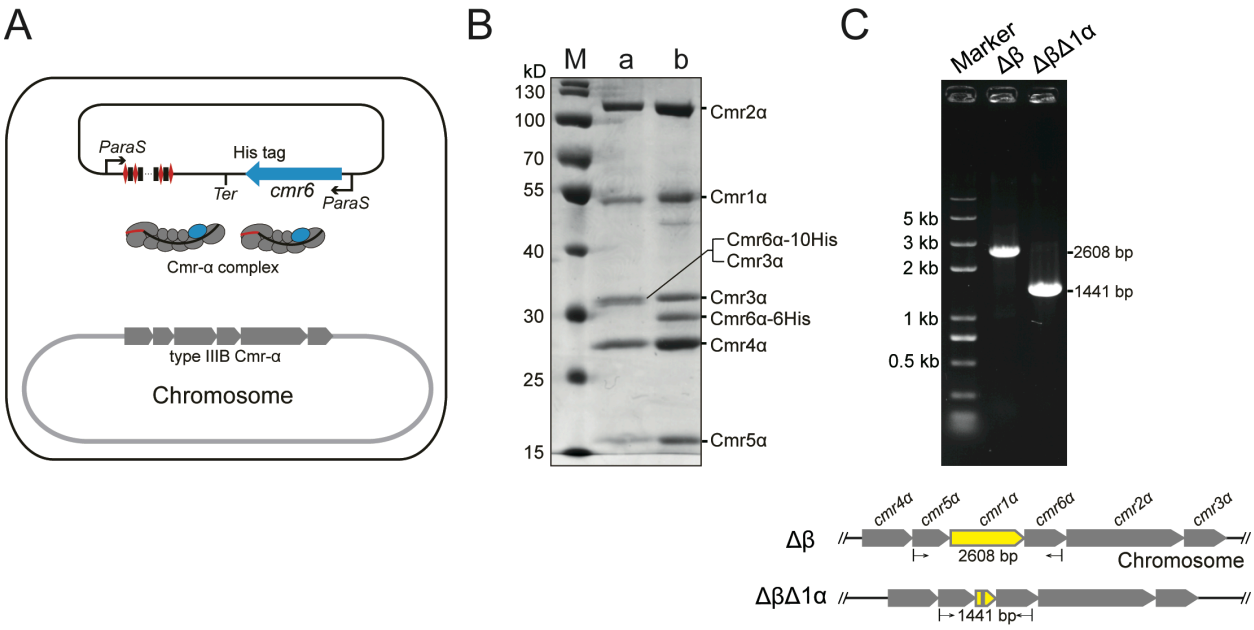
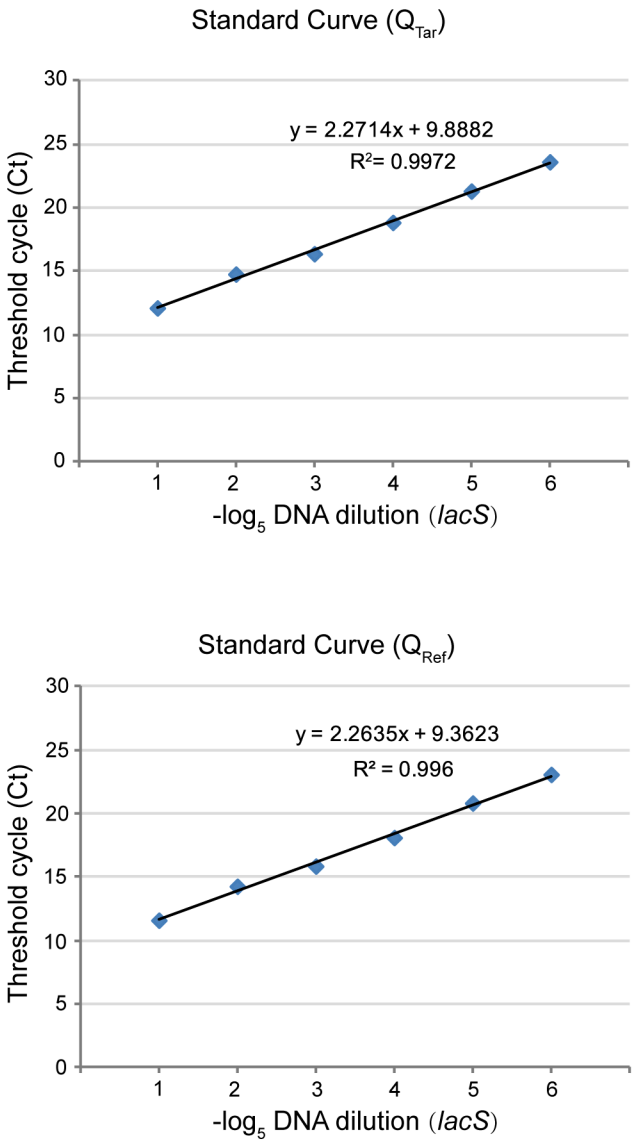


Figure S1

(A) Strategy for copurification of native Cmr-α complex using pAC-cmr6α-10His plasmid, a 10×His tag has substituted the 6×His tag that used in our previous study(6).

(B) SDS-PAGE of purified Cmr-α complex with 10×His-cmr6α(a) and 6×His-cmr6α(b).

(C) PCR verification of Cmr1α deletion mutant.



$slope = 1/\log_5 E$

$E = 5^{1/slope}$

$E(Q_{Tar}) = 5^{1/2.2714} = 2.03$

$E(Q_{Ref}) = 5^{1/2.2635} = 2.04$

Figure S2 . qPCR standardization – determination of amplification efficiency for Q_{Tar} and Q_{Ref} primer sets.

Standard curves were generated with five-fold series diluted template (plasmid DNA containing *lacS*) . Slope was determined as linear regression of Ct (y-axis) versus -$\log_5$ DNA dilution (x-axis). Efficiency of amplification for priner sets were calculated using the following equation: efficiency $E = 5^{1/slope}$, being $E\ (Q_{Tar}) = 2.03$ and $E\ (Q_{Ref}) = 2.04$.

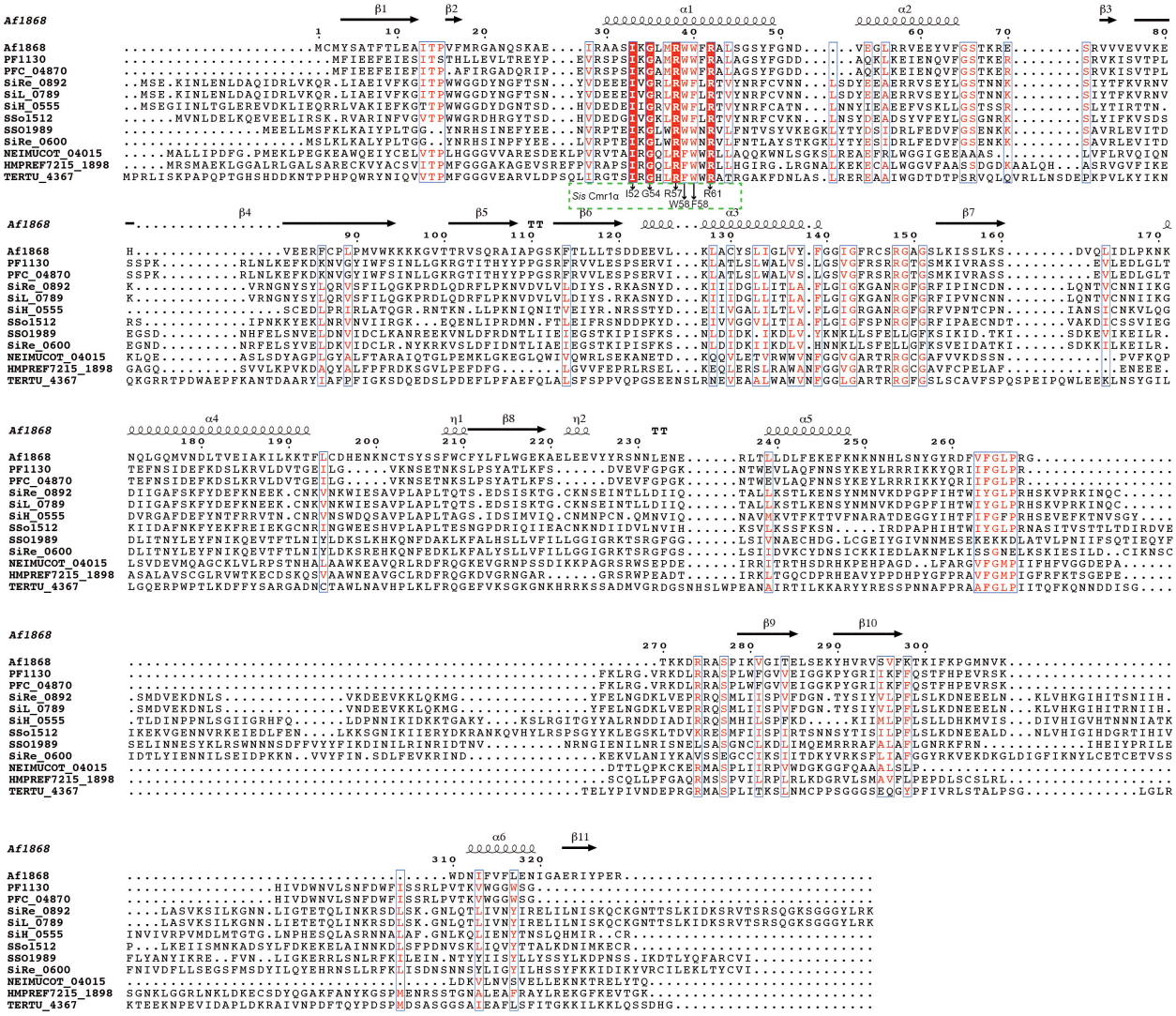


Figure S3. Sequence alignment of Cmr1 homologs.

Twelve homologues(Af1868, PF1130, PFC_04870, SiRe_0892, SiL_0789, SiH_0555, SSo1512, SSo1989, SiRe_0600, NEIMUCOT_04015, HMPREF7215_1898 and TERTU_4367) were selected and aligned using ESPript 3.x(http://espript.ibcp.fr) (7). Six highly conserved residues at N-termini were chosen to produce two Cmr1α mutants which were defined as follows: Cmr1α-M1(W58A, F59A), Cmr1α-M2(I52A, G54A, R57A, R61A).

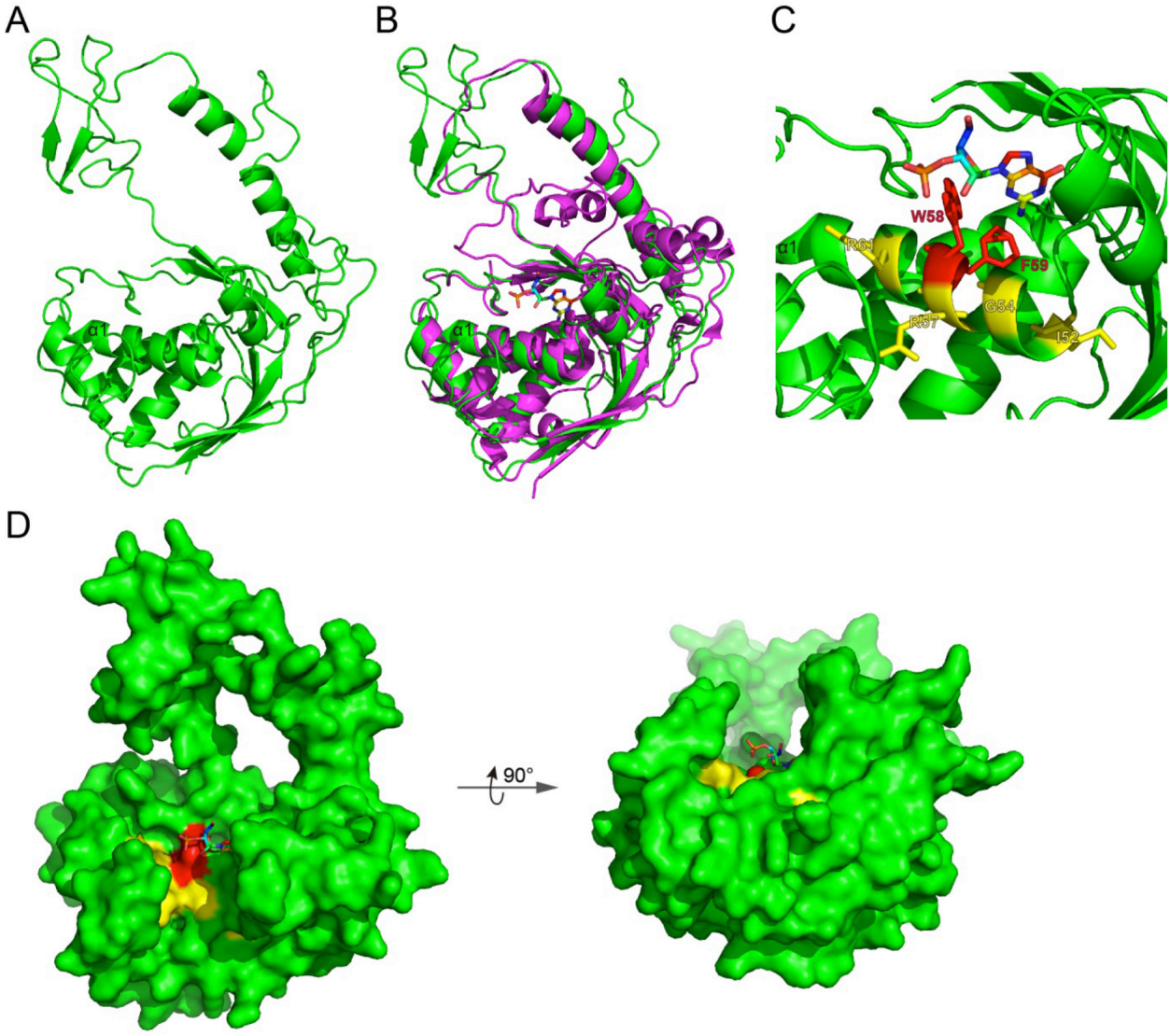


Figure S4. Structure model of *S. islandicus* Cmr1α

(A) Structural model of *S.islandicus* Cmr1α generated by SWISS-MODEL(8-11).

(B) The structures of *Sis* Cmr1α and *Pf* Cmr1(4W8X) are superimposed. A adenine ribonucleotide binds in the conserved groove.

(C) A zoom shows the ribonucleotide binding groove. Conserved residues mutated in this study are indicated in red(W58, F59) and yellow(I52, G54, R57, R61).

(D) Surface representation of Cmr1α and stereoview of the interaction between Cmr1α and bound ribonucleotide.

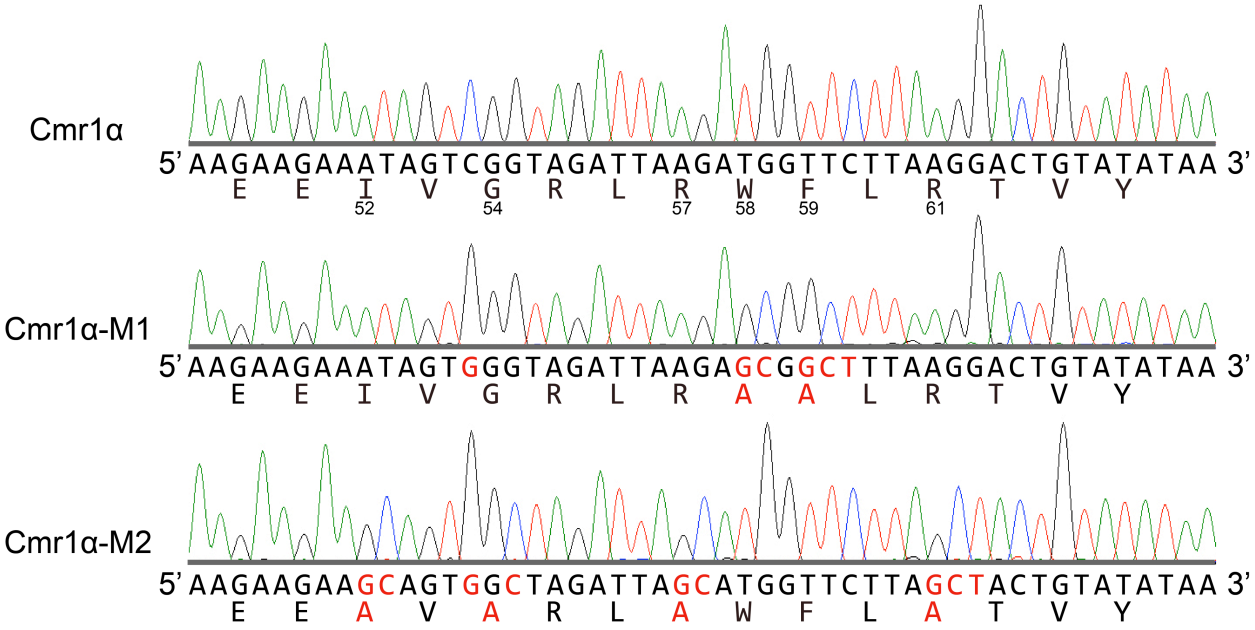


Figure S5. Sequencing results of *S. islandicus* cmr1α mutant genes

Chromatographs of the sequencing data generated from Sanger DNA sequencing. Mutated bases as well as the mutated amino acids are highlighted in red.

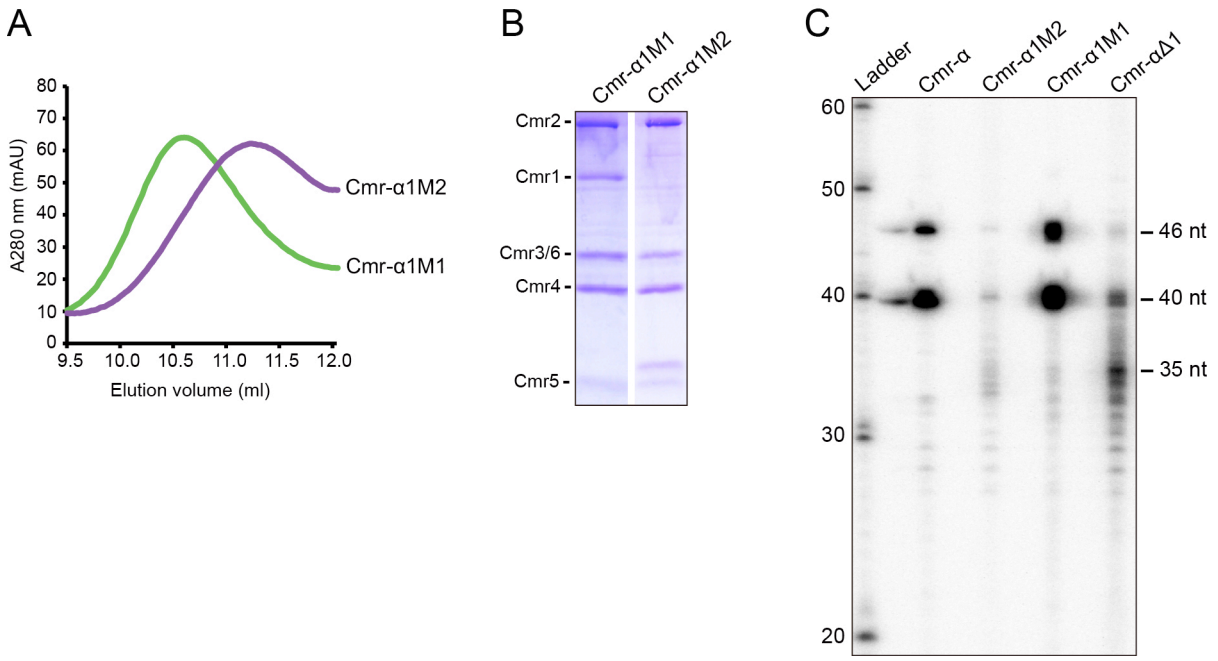


Figure S6

(A) Gel filtration profiles of Cmr-α1M1 (green) and Cmr-α1M2 (purple). A_{280}: UV absorbance at 280 nm.

(B) SDS-PAGE of purified Cmr-α1M1 and Cmr-α1M2 complexes.

(C) Denaturing gel electrophoresis analysis of crRNAs present in Cmr-α, Cmr-αΔ1, Cmr-α1M1 and Cmr-α1M2 complexes.

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