**Cmr3 regulates the suppression on cyclic oligoadenylate synthesis by tag complementarity in a Type III-B CRISPR-Cas system**

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Supplementary Table 1. Primers used in this work

|  |  |
| --- | --- |
| **Name** | **Sequence (5’~3’)** |
| Cmr3-up-NdeI | GCGATATCCATATGTATTTGCTAATAAAACCTTTAG |
| Cmr3-dw-SalI | GTACGTCGACAATTTCGACTACAGAACCCCAAC |
| Cmr3-M1-R-UP | GACTAAGGAAGGCTATGCTGCTATGGAGAAAATGCTCGATTTG |
| Cmr3-M1-L-DW | CTCCATAGCAGCATAGCCTTCCTTAGTCATCTTAG |
| Cmr3-M2-R-UP | GGATATAACTCCGGAGGAGCTATAAATAGTGG |
| Cmr3-M2-L-DW | TATAGCTCCTCCGGAGTTATATCCTCCCCAC |
| Cmr3-M3-R-UP | CCATATTATTGTTTGGAGCTATAAATAGTGGCTATTTTG |
| Cmr3-M3-L-DW | CTATTTATAGCTCCAAACAATAATATGGAGTTATATCC |
| Cmr3-M4-R-UP | CCATATTATTGGGATTTGCTATAAATAGTGGCTATTTTG |
| Cmr3-M4-L-DW | CTATTTATAGCAAATCCCAATAATATGGAGTTATATC |
| Cmr3-M5-R-UP | GTGGGGAGGAATATTATTGGGAGGAGCTATAAATAG |
| Cmr3-M5-L-DW | CCCAATAATATTCCTCCCCACTTGAATACTAC |
| Cmr3-M6-R-UP | GTGGGGAGGAGGAGGAGCTATAAATAGTGGC |
| Cmr3-M6-L-DW | ATAGCTCCTCCTCCTCCCCACTTGAATACTAC |
| Cmr3-M7-R-UP | GTAGTAGCAGCTGCAGGAGGATATAACTCCATATTATTG |
| Cmr3-M7-L-DW | GGAGTTATATCCTCCTGCAGCTGCTACTACCGAACCTAAAGGTT |
| MRS-up | ATGCCCCGGGATGTTAAACAAGTTAGG |
| MRS-dw | GGCACTCGAGAAAAAAAAGATTTTGCTTAATGGTG |

Supplementary Table 2. Plasmids used in this work

|  |  |  |
| --- | --- | --- |
| **Plasmids** | **Genotype and features** | **Reference** |
| pAC-MS1 | Derived from pSeSD1, carrying an artificial CRISPR locus with 10 copies of 43 nt SS1 spacer. | (1) |
| pcmr3α | Derived from pSeSD1, expressing His-tagged Cmr3α | This work |
| pAC-cmr3α | Derived from pAC-MS1, expressing both SS1 crRNA and a His-tagged Cmr3α | This work |
| pAC-cmr3α-M1 | Expressing both SS1 crRNA and a His-tagged Cmr3α mutant carrying alanine substitutions of I123 and Y124. | This work |
| pAC-cmr3α-M2 | Expressing both SS1 crRNA and a His-tagged Cmr3α mutant carrying deletion of I21, L22 and L23. | This work |
| pAC-cmr3α-M3 | Expressing both SS1 crRNA and a His-tagged Cmr3α mutant carrying substitution of G24 with phenylalanine. | This work |
| pAC-cmr3α-M4 | Expressing both SS1 crRNA and a His-tagged Cmr3α mutant carrying substitution of G25 with phenylalanine. | This work |
| pAC-cmr3α-M5 | Expressing both SS1 crRNA and a His-tagged Cmr3α mutant carrying deletion of Y18, N19 and S20. | This work |
| pAC-cmr3α-M6 | Expressing both SS1 crRNA and a His-tagged Cmr3α mutant carrying deletion of Y18, N19, S20, I21, L22 and L23. | This work |
| pAC-cmr3α-M7 | Expressing both SS1 crRNA and a His-tagged Cmr3α mutant carrying substitution of F13, K14and W15 with alanine. | This work |

Supplementary Table 3. DNA oligos to generate DNA template for the in vitro transcription assay

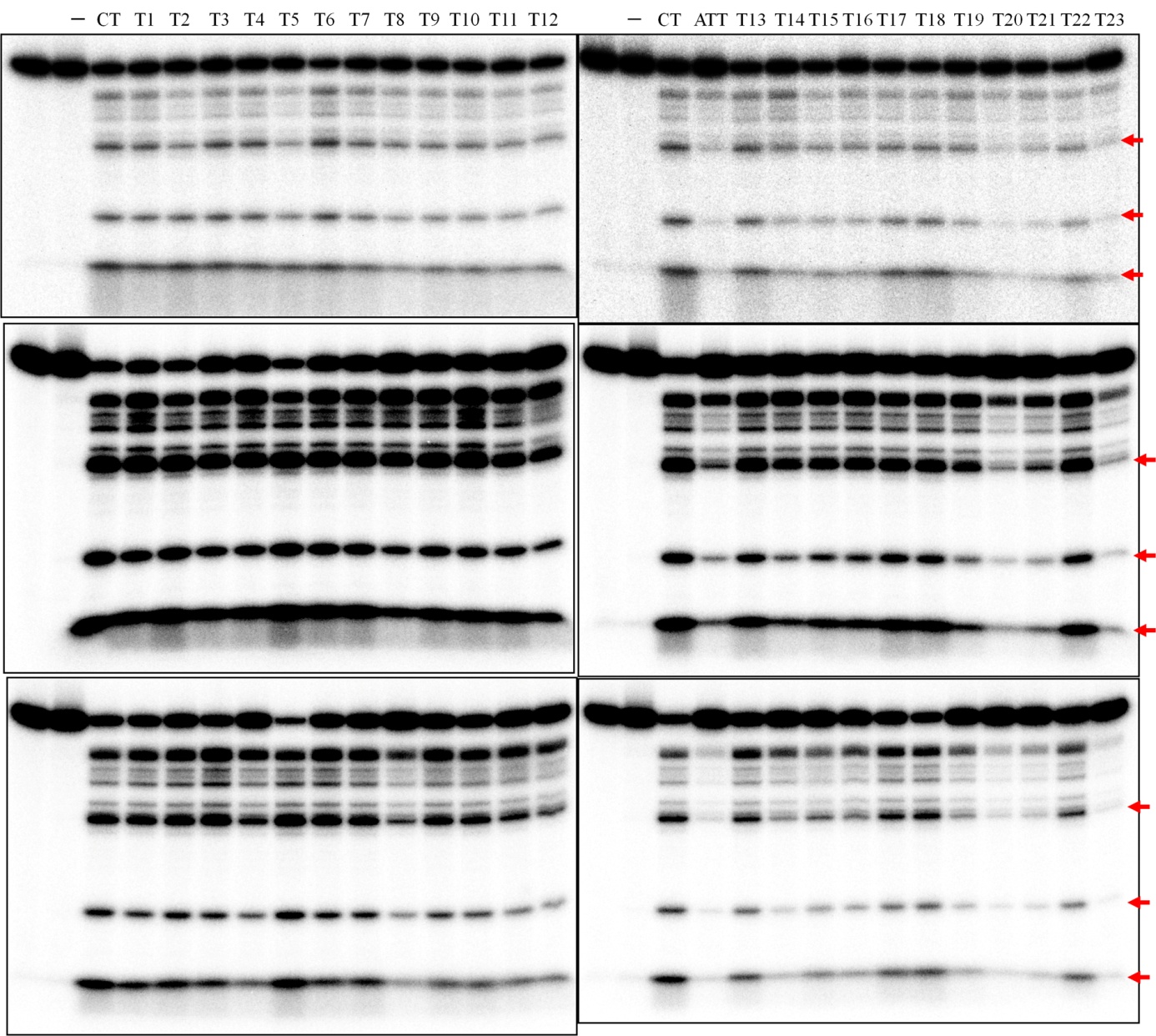
|  |  |
| --- | --- |
| Name | Sequences(5’ ̴ 3’) |
| SS1-46-Fwd | TAATACGACTCACTATAGGGTGTTAAGTCTGGTTTCCCTCCAGGGTATCTAAGCTT |
| T1-Rev | TGTTTTTTCAAAGCTTAGATACCCTGGAGG |
| T2-Rev | TTATTTTTCAAAGCTTAGATACCCTGGAGG |
| T3-Rev | TTTATTTTCAAAGCTTAGATACCCTGGAGG |
| T4-Rev | TTTTATTTCAAAGCTTAGATACCCTGGAGG |
| T5-Rev | TTTTTGTTCAAAGCTTAGATACCCTGGAGG |
| T6-Rev | TGATTTTTCAAAGCTTAGATACCCTGGAGG |
| T7-Rev | TTAATTTTCAAAGCTTAGATACCCTGGAGG |
| T8-Rev | TTTAATTTCAAAGCTTAGATACCCTGGAGG |
| T9-Rev | TTTTAGTTCAAAGCTTAGATACCCTGGAGG |
| T10-Rev | TGTATTTTCAAAGCTTAGATACCCTGGAGG |
| T11-Rev | TGTTATTTCAAAGCTTAGATACCCTGGAGG |
| T12-Rev | TTATATTTCAAAGCTTAGATACCCTGGAGG |
| T13-Rev | TGAATTTTCAAAGCTTAGATACCCTGGAGG |
| T14-Rev | TTAAATTTCAAAGCTTAGATACCCTGGAGG |
| T15-Rev | TTTAAGTTCAAAGCTTAGATACCCTGGAGG |
| T16-Rev | TGATATTTCAAAGCTTAGATACCCTGGAGG |
| T17-Rev | TGATTGTTCAAAGCTTAGATACCCTGGAGG |
| T18-Rev | TTAATGTTCAAAGCTTAGATACCCTGGAGG |
| T19-Rev | TGTAATTTCAAAGCTTAGATACCCTGGAGG |
| T20-Rev | TGAAATTTCAAAGCTTAGATACCCTGGAGG |
| T21-Rev | TTAAAGTTCAAAGCTTAGATACCCTGGAGG |
| T22-Rev | TGAATGTTCAAAGCTTAGATACCCTGGAGG |
| T23-Rev | TGAAAGTTCAAAGCTTAGATACCCTGGAGG |
| CT-Rev | TTTTTTTTCAAAGCTTAGATACCCTGGAGG |
| ATT-Rev | TTATTGAAAGTTCAAAGCTTAGATACCCTGGAGG |

Supplementary Table 4. RNA and DNA substrates used in this study.

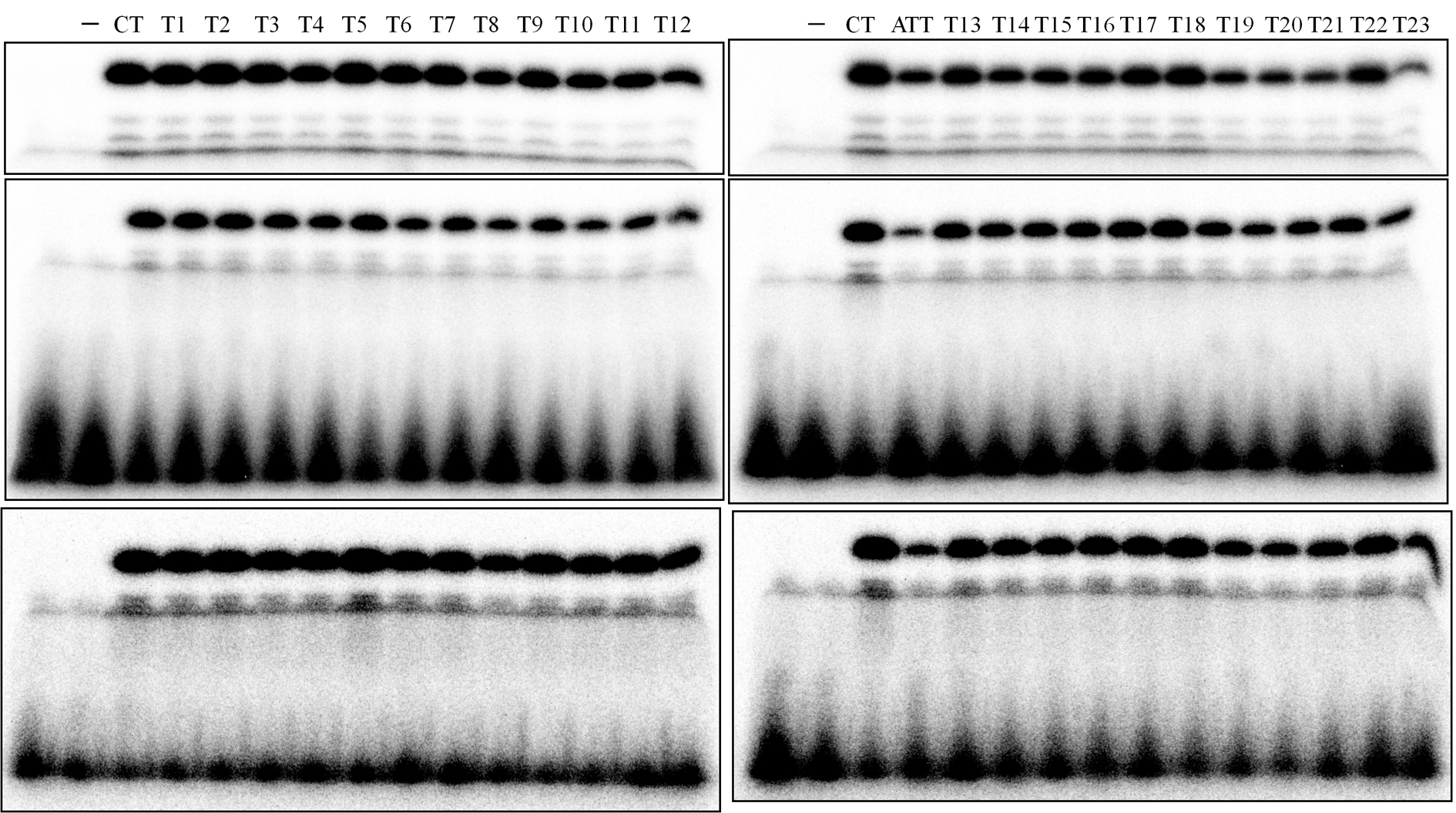
|  |  |  |
| --- | --- | --- |
| **Name** | **Sequence (5’~3’) and comments** | **Size (mer)** |
| RNA |  |  |
| SS1-46 | UGUUAAGUCUGGUUUCCCUCCAGGGUAUCUAAGCUUUGAAAAAAAA | 46 |
| DNA |  |  |
| S10 | ACTATAGGGAGaATAGAATGCCCCCATTATACAATATCTACGTTTTAGATGAcccccccc | 60 |
|  |

Supplementary Table 5. T-test of the difference of the relative cOA synthesis activity between wild type and each mutated Cmr-α-RNP. The p values derived from each mutated complex and each activator RNA are listed and those < 0.05 are shown in bold. The p values are further highlighted in red if Cmr3α mutation resulted in decreased activities or green at elevated activities.

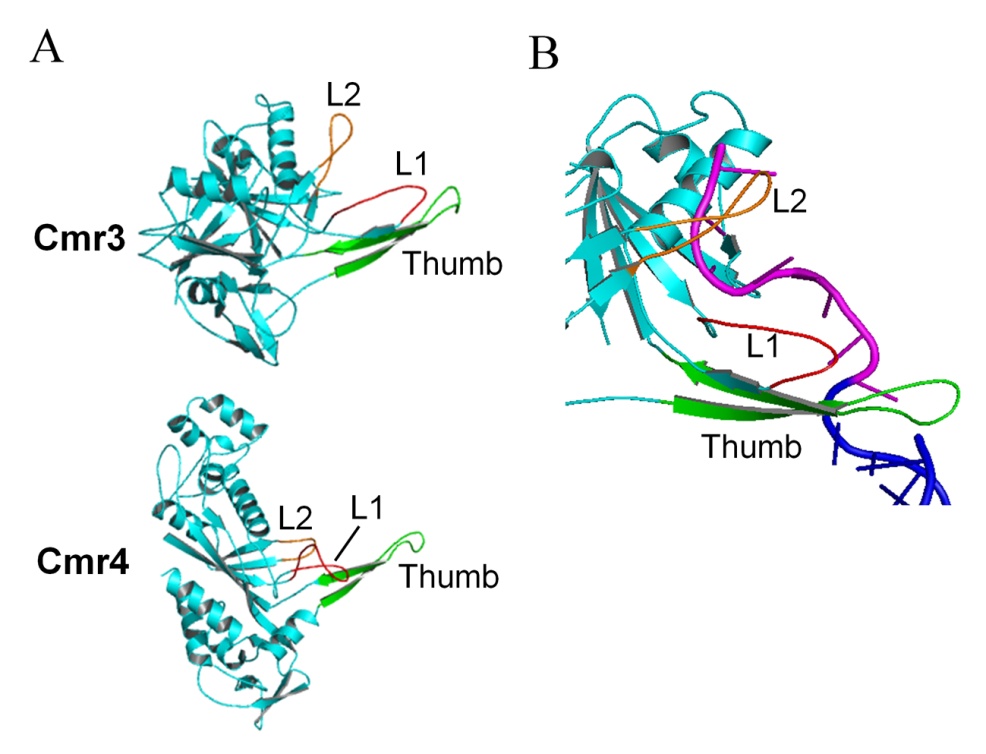
|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | M1 | M2 |  | M3 |  | M5 |
| T1 | 0.224692 | 0.315743 |  | 0.361633 |  | 0.570926 |
| T2 | 0.181143 | **0.03761** |  | 0.72335 |  | 0.648135 |
| T3 | 0.085569 | **0.027309** |  | 0.062331 |  | 0.671732 |
| T4 | 0.769568 | 0.249633 |  | 0.369268 |  | **0.023871** |
| T5 | 0.210257 | 0.195068 |  | 0.442248 |  | 0.145594 |
| T6 | 0.292525 | **0.039325** |  | 0.165012 |  | 0.88646 |
| T7 | 0.196536 | **0.027036** |  | 0.154184 |  | 0.980367 |
| T8 | 0.067998 | 0.340957 |  | 0.113082 |  | **0.008942** |
| T9 | 0.659345 | **0.016944** |  | 0.519521 |  | **0.036846** |
| T10 | 0.376532 | **0.039387** |  | 0.281622 |  | 0.218794 |
| T11 | 0.127611 | 0.068646 |  | 0.559029 |  | 0.378406 |
| T12 | 0.710317 | 0.050394 |  | 0.54289 |  | 0.488581 |
| T13 | 0.147748 | 0.227208 |  | 0.418707 |  | **0.049983** |
| T14 | **0.039676** | **0.006215** |  | 0.319659 |  | 0.157741 |
| T15 | 0.139704 | 0.164088 |  | 0.341246 |  | **0.016065** |
| T16 | 0.216748 | **0.004152** |  | 0.825972 |  | **0.042466** |
| T17 | 0.180856 | 0.916396 |  | 0.452718 |  | 0.443897 |
| T18 | 0.451832 | 0.334371 |  | 0.261926 |  | 0.310802 |
| T19 | 0.534571 | **0.032591** |  | 0.591317 |  | 0.147792 |
| T20 | **0.013141** | **0.00237** |  | 0.217918 |  | 0.95273 |
| T21 | **0.046943** | **0.007265** |  | 0.894416 |  | **0.004957** |
| T22 | 0.824426 | 0.145876 |  | 0.077042 |  | **0.01682** |
| T23 | **0.064275** | **0.003381** |  | 0.46702 |  | 0.571251 |
| ATT | **0.009152** | **0.000217** |  | 0.46312 |  | 0.592051 |



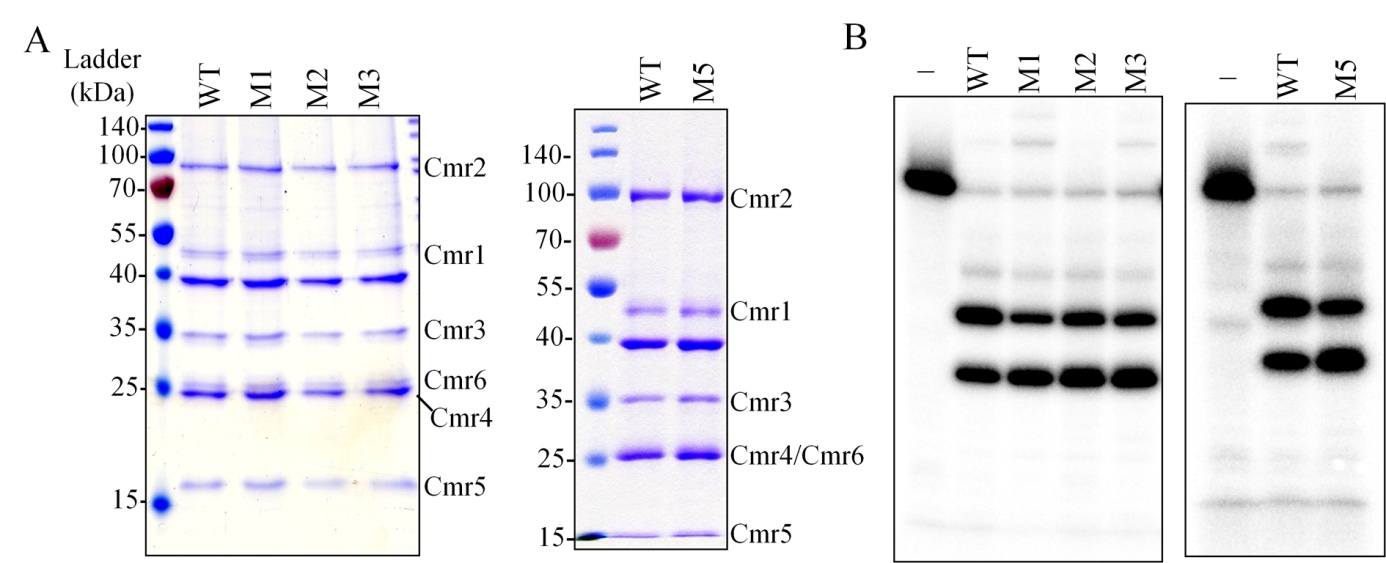
Supplementary figure S1. DNA cleavage gels for Figure 1. The three bands indicated by arrows were chosen for quantification.



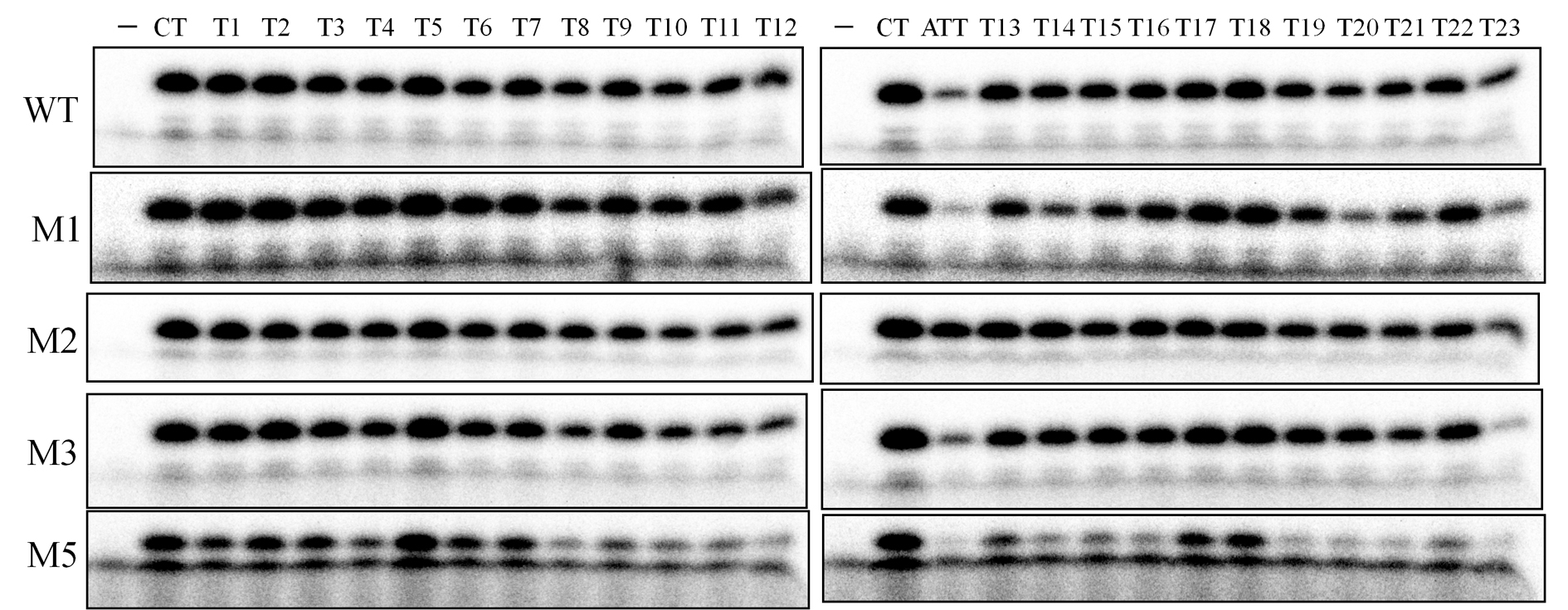
Supplementary figure S2. cOA synthesis gels for Figure 1.



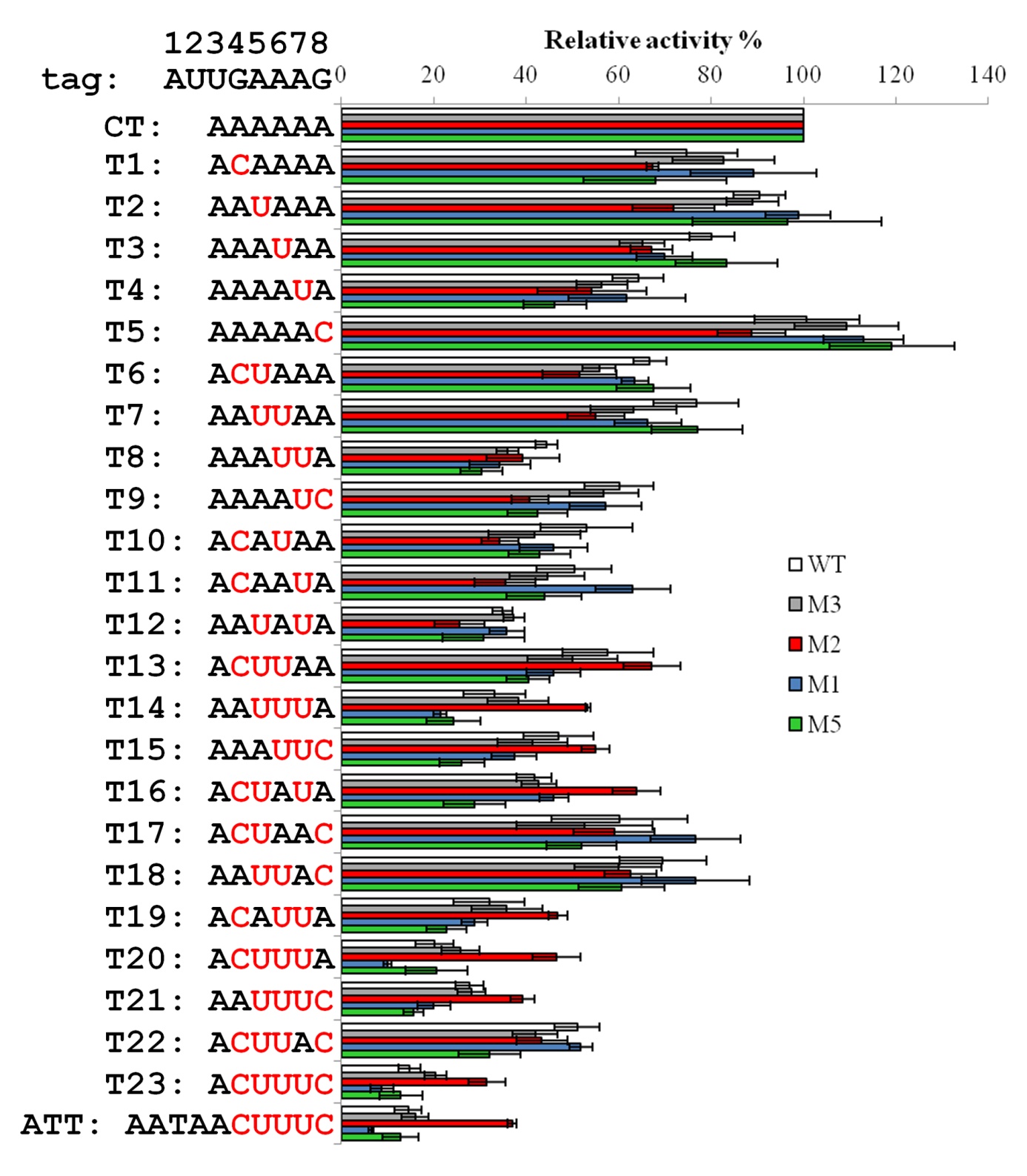
Supplementary figure S3. (A) Comparison of the structures of Cmr3 (upper panel) and Cmr4 (lower panel). Loop 1 (L1), loop 2 (L2) and thumb are shown in red, orange and green, respectively. (B) Interaction between Cmr3 and crRNA. The crRNA tag is shown in pink, while the spacer region of crRNA and target RNA are shown in blue. The structures are modified from *P. furiosus* Cmr3 and *Archaeoglobus fulgidus* Cmr4 (PDB: 3X1L, chain B and C respectively) using PyMOL (http://www.pymol.org)



Supplementary figure S4. SDS-PAGE analysis (A) and RNA cleavage activity (B) of wild type and Cmr3α mutant complexes.



Supplementary figure S5. Representing gels for Figure 3 and Supplementary figure S6.

Supplementary figure S6. The relative cOA synthesis activity of wild type and Cmr3α mutants in the presence of different activator RNA oligos. White: WT; gray: M3 (G24F); red: M2 (deletion of I21, L22 and L23); blue: M1 (I123A-Y124A); green: M5 (deletion of Y18, N19 and S20). Error bar represents SD of three independent experiments.