**Supplemental Material For:**

**The ribonuclease activity of Csm6 of the Type III-A CRISPR-Cas System is needed for CRISPR-Cas immunity**

Kawanda Foster1, Joshua Kalter2, Walter Woodside1, Rebecca M. Terns3 and Michael P. Terns1,2,4\*

1Department of Microbiology,

2Department of Biochemistry and Molecular Biology,

3Office for Proposal Enhancement,

4Department of Genetics, University of Georgia, Athens, GA 30602, USA;

\*Corresponding author: Michael P. Terns, mterns@uga.edu

Table S1: RNA and DNA substrates used in this study

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| **Name** | **Associated Figure(s)** | **Sequence (5’-3’)** |
| RNA1 | 1,3,4 | CCCCCCCCCCAUGAUGAUG |
| RNA2 | 1 | UUGUAGUAUGCGGUCCUUGCGGCUGAGAGCACUUCAG |
| RNA3 | 1 | CUGAAGUGCUCUCAGCCGCAAGGACCGCAUACUACAA |
| DNA1 | 1 | CTGAAGTGCTCTCAGCCGCAAGGACCGCATACTACAA |
| DNA2 | 1 | TTGTAGTATGCGGTCCTTGCGGCTGAGAGCACTTCAG |
| RNA4 | 2 | AUUGAAAGUUGUAGUAUGCGGUCCUUGCGGCUGAGAGCACUUCAGUCGUUAUCUCUUACGAAGUCUU |
| RNA5 | 2-4 | GUUACAAUAAGACCAAAAUAGAAUUGAAAG |
| RNA6 | 3 | AUGAGUAUUCAACAUUUCCGUGUCGCCCUUAUUCCCU |
| RNA7 | 3 & 4 | AUUGAAAGUUGUAGUAUGCGGUCCUUGCGGCUGAGAGCA |

Table S2: Oligonucleotides used for mutagenesis

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| **Oligo** | **Alteration** | **Sequence (5’-3’)** |
| LLACsm6H360A-FP (2771) | Csm6 H360A | AGGACCGCAATAAAGTTGCAGCAAGTCTGCAGGCATTTGAC |
| LLACsm6H360A-RP (2772) | Csm6 H360A | GTCAAATGCCTGCAGACTTGCTGCAACTTTATTGCGGTCCT |
| SEPCsm6H369A-FP (2767) | Csm6 H369A | CCTGCGTAATAGCATTGCCGCAAACCTGGATACCCTGAAC |
| SEPCsm6H369A-RP (2768) | Csm6 H369A | GTTCAGGGTATCCAGGTTTGCGGCAATGCTATTACGCAGG |
| AIOCsm6\_Tag/Mut#1 (3672) | LLA H360A &/or tag | ACTGGAAGAACTGAAATAACCTAGGCGCTTCAACGGAACG |
| AIOCsm6\_Tag/Mut#2 (3673) | LLA H360A &/or tag | AACTGCGCTAATCAGGATTTTCATACCGCTGCTTCCATGGTGGTGATGATGATGCATTATTTACCTCCTTTATCCATG |
| AIOCsm6\_Tag/Mut#3 (3674) | LLA H360A &/or tag | ATGAAAATCCTGATTAGCGCAGTT |
| AIOCsm6\_Tag/Mut#4 (3675) | LLA H360A &/or tag | TGCAACTTTATTGCGGTCCT |
| AIOCsm6\_Tag/Mut#5 (3676) | LLA H360A &/or tag | ACCGCAATAAAGTTGCAGCAAGTCTGCAGGCATTTGAC |
| AIOCsm6\_Tag/Mut#6 (3677) | LLA H360A &/or tag | GTTTTCCATGAGCAAACTGAAACGTTTTCATCGCTCTGGAGTGA |
| Csm6\_Del\_InvPCR#1 (4419) | SEP & STH Csm6 del | TGCCCTACTTGGTCTCCGAGAC |
| Csm6\_Del\_InvPCR#2 (4420) | SEP & STH Csm6 del | CTCGAGAGGTTACAGCCTGCATAATG |
| STHCsm6H347A-FP (2769) | Csm6 H347A | CAGCCGTAATAAAGTTGCCGCAAGCCTGAGTCCGCTG |
| STHCsm6H347A-RP (2770) | Csm6 H347A | CAGCGGACTCAGGCTTGCGGCAACTTTATTACGGCTG |
| WW\_LLAAIO\_mut2R (3186) | LLA H13A/D14A | GTGCCACGATAGATAATTTTGCCAATAGCAGCCAGCAGGCTACCACAAACC |
| WW\_LLAAIO\_mut3F (3187) | LLA H13A/D14A | ATTGGCAAAATTATCTATCGTGGCAC |
| WW\_LLAAIO\_mut4R (3188) | LLA & D576A/D577A | CGCCACCGGCATAAATAAC |
| WW\_LLAAIO\_mut5F (3189) | LLA D576A/D577A | GTTATTTATGCCGGTGGCGCCGCCCTGTTTATGATTGGTGCATGGC |
| P1\_revision (3197) | LLA H13A/D14A & D576A/D577A | AAGCAACCTCGAGGCTGTGGTCTA |
| P6\_revision (3198) | LLA H13A/D14A | AAAAGCCATATGTTAGCGTTCACG |
| SthSOE\_P1 for (3892) | STH H15A/D16A & D575A/D576A | GGGAGAGGATCCATAAAGGAGG |
| SthSOE\_P2 rev (3893) | STH H15A/D16A | TGCACGCTGAATAACTTTACCAATGGCTGCCAGCAGTGCACCATAGAACAG |
| SthSOE\_P3 for (3894) | STH H15A/D16A | ATTGGTAAAGTTATTCAGCGTGCA |
| SthSOE\_P4 rev (3895) | STH D575A/D576A | ACCACCGGCATAGATAATGGA |
| SthSOE\_P5 for (3896) | STH D575A/D576A | TCCATTATCTATGCCGGTGGTGCAGCAGTTTTTGCAATTGGTAGCTGGC |
| SthSOE\_P6 rev (3897) | STH H15A/D16A & D575A/D576A | CGCAGCCATATGTTAATCTTTACG |
| Sep csm1F (5043) | SEP H14A/D15A & D586A/D587A | CCTCTAGTGGCTGGCTAAG |
| Sep csm1R (5044) | SEP H14A/D15A & D586A/D587A | CGCAGCCATATGTTAGTCG |
| Sep ggdd F (5045) | SEP D586A/D587A | GCAGCACTGTTTCTGATTGGTGCATGG |
| Sep ggdd R (5046) | SEP D586A/D587A | TCCGCCACTATAAATTGCGGTAA |
| Sep hd F (5047) | SEP H14A/D15A | GCAGCAATTGGCAAAATTATCTATCGTAGCG |
| Sep hd R (5048) | SEP H14A/D15A | CAGCAGGCTGCCATACAT |

Figure S1: Purification of tagged Csm proteins

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**2**

**1**



Purification of Csm proteins using co-expression plasmids than result in expression of a 6x-Histidine tag on either Csm3 (lane 1) or Csm6 (lanes 2 and 3) following separation by SDS-PAGE and Coomassie blue staining. In all sampes Csm1-6 proteins are co-expressed. The first lane shows that Csm1-5 proteins co-purify when His-tagged Csm3 is affinity purified. Lanes 2 and 3 show that no additional Csm proteins co-purify with His-tagged Csm6. The identities of the proteins in each major band were determined by mass spectrometry.