

Supplementary Materials: AAU-Specific RNA Cleavage Mediated by MazF Toxin Endoribonuclease Conserved in *Nitrosomonas europaea*

Tatsuki Miyamoto ^{1,2}, Akiko Yokota ², Satoshi Tsuneda ^{1,*} and Naohiro Noda ^{1,2,*}

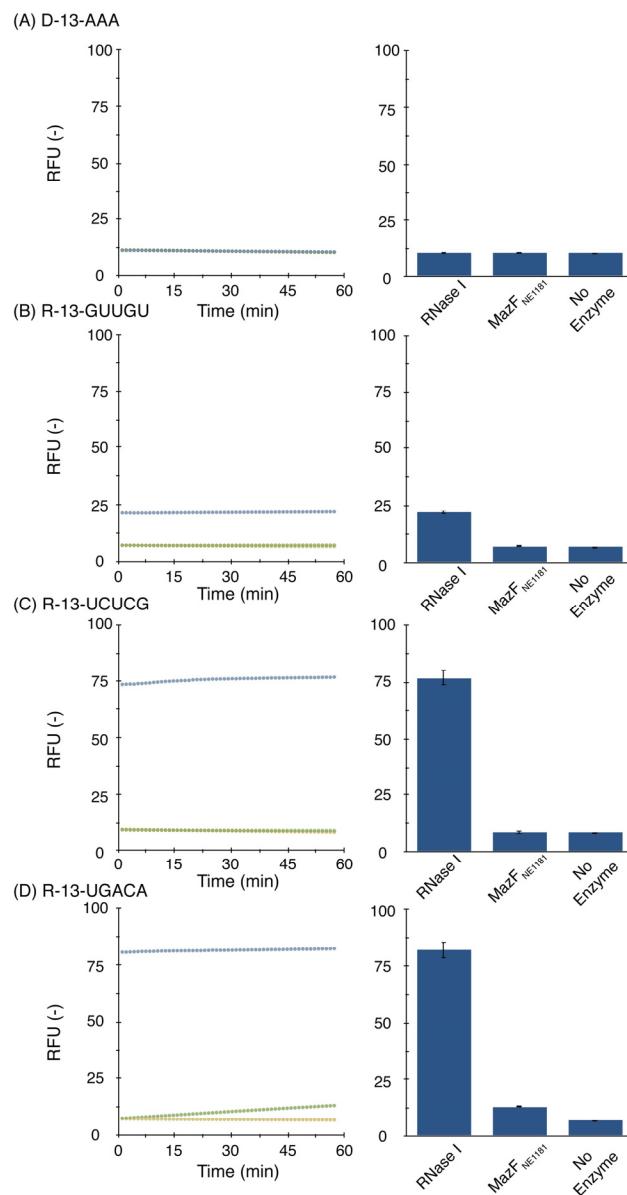


Figure S1. $\text{MazF}_{\text{NE1181}}$ -mediated sequence-specific RNA cleavage. Two hundred nanograms of $\text{MazF}_{\text{NE1181}}$ (green) was incubated with 20 pmol of fluorescent-modified oligonucleotides; (A) D-13-AAA; (B) R-13-GUUGU; (C) R-13-UCUCG; and (D) R-13-UGACA. In the control reactions, fluorescent intensities in the presence of 1 U of RNase I (blue) and in the absence of enzymes (yellow) at each time point (left) and end point (right) were measured.

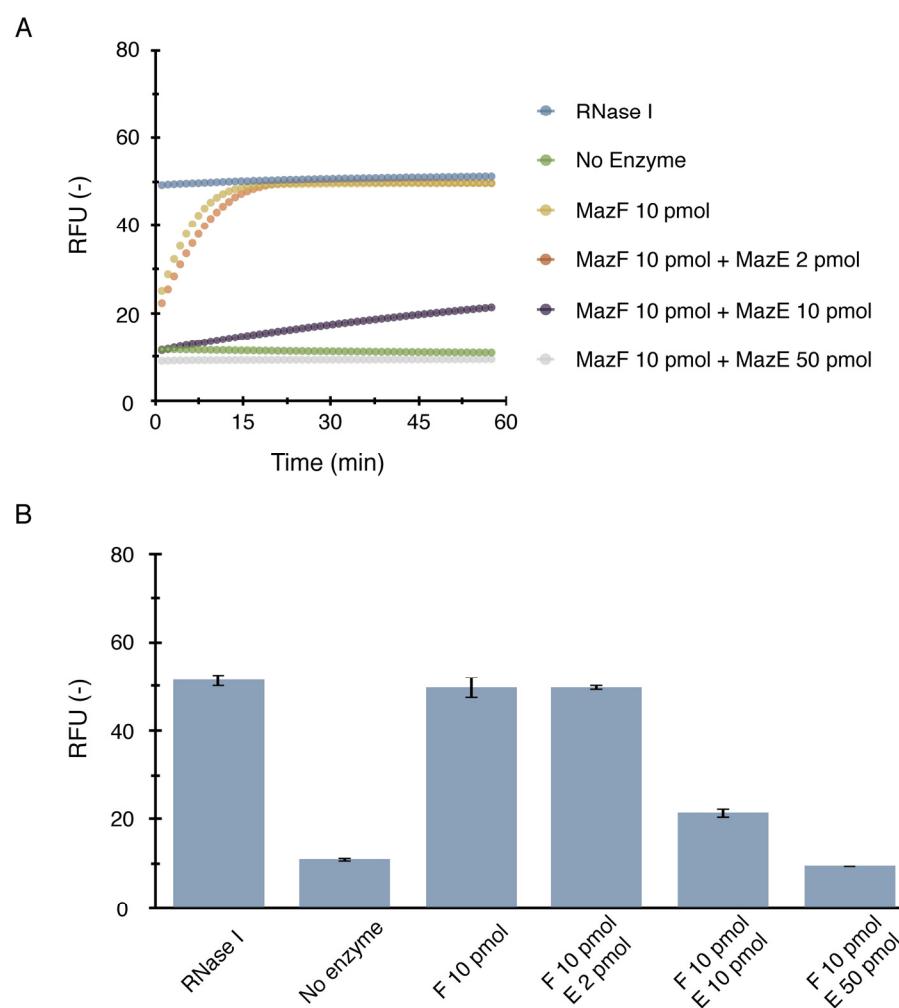


Figure S2. Neutralization of $\text{MazF}_{\text{NE1181}}$ -mediated RNA cleavage. Ten picomoles of $\text{MazF}_{\text{NE1181}}$ were pre-incubated with 2 (vermillion), 10 (purple), or 50 (gray) pmol of $\text{MazE}_{\text{NE1182}}$, and mixed with 20 pmol of DR-13-AAU. In the control reactions, fluorescent intensities in the presence of 1 U of RNase I (blue) and $\text{MazF}_{\text{NE1181}}$ (yellow) and in the absence of enzymes (green) were measured. (A) Fluorescent intensities at each time point; (B) Fluorescent intensities at end point.

Table S1. Twenty-five sequences with $\text{MazF}_{\text{NE1181}}$ cleavage

RNA Type	Rank	Position	Relative Coverage Increase	Coverage	Sequence (5' to 3') ^a
1000-1	1	725	4.93	25,940	CCCAA <u>A</u> TAGAC
	2	332	2.53	14,909	CAGAA <u>A</u> TCACT
	3	188	2.11	5407	CCTAA <u>A</u> ATGGAC
	4	392	1.78	22,871	CGCCA <u>A</u> ATCTCT
	5	703	1.68	5667	CGAGA <u>A</u> TCATG
1000-2	1	71	5.12	1151	ACCGA <u>A</u> TCCT
	2	192	2.49	3703	ATTTA <u>A</u> GTTC
	3	461	1.79	6739	CTTCA <u>A</u> TTTG
	4	277	1.75	5066	TCGTA <u>A</u> TGGTT
	5	628	1.68	16,116	GCGCAA <u>A</u> AGGAC
1000-3	1	533	2.19	1472	AATGA <u>A</u> ATATCG
	2	808	1.89	1061	TCCCA <u>A</u> TTCAA

	3	218	1.39	1067	TTGAA <u>A</u> TCCACC
	4	849	1.39	1294	TTCGA <u>A</u> TTTCG
	5	726	1.36	1015	ATTCA <u>A</u> TCTAC
	1	98	52.27	9200	TCCC <u>A</u> ATAGTT
	2	480	2.88	10,727	CCTGA <u>A</u> TACAC
1000-4	3	663	2.21	7650	CCTTA <u>A</u> TAAGC
	4	335	2.06	4102	GCCCA <u>A</u> TACGT
	5	798	1.81	3956	AGCTA <u>A</u> TCGGA
	1	36	27.44	1427	TCGGA <u>A</u> TCTTT
	2	715	9.34	8969	CATGAA <u>A</u> ATGAA
1000-5	3	432	1.81	18,470	CGCGAA <u>A</u> AGGAT
	4	716	1.61	14,443	ATGAA <u>A</u> ATGAAC
	5	362	1.58	9653	TACGA <u>A</u> ATGGC

a: Underlined letters represent the base with significant coverage increase.

Table S2. Genes consisting of *mer* operon.

Locus	Gene Symbol	Product Name	Length (bp)	Number of AAU Triplets
NE0842	<i>merT</i>	mercuric transport protein	351	0
NE0841	<i>merP</i>	mercury scavenger protein	276	0
NE0840	<i>merC</i>	mercury transport protein	423	2
NE0839	<i>merA</i>	mercuric reductase	1686	9
NE0838	<i>merD</i>	transcriptional regulator, MerR family	366	2
NE2575	<i>merE</i>	mercury resistance protein	237	0
NE0843	<i>merR</i>	transcriptional regulator, MerR family	426	4