

VapC Toxins Drive Cellular Dormancy under Uranium Stress for the Extreme Thermoacidophile *Metallosphaera prunae*

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Table S1. RNase activity of total RNA degrading VapC proteins on the generic RNA substrate (IDT DNA). Activity was obtained by calculating initial reaction velocity (increase in fluorescence reading over time). The 'no VapC added' control fluorescence was subtracted from the EDTA, VapBC and VapC data prior to determination of initial reaction velocity. Further, the EDTA, VapBC and VapC initial reaction velocities were divided by the VapC loading. The VapC4 and VapC8 loading was 0.01 μ g while VapC7 was 0.001 μ g. The VapC:VapB mass ratio was 1:2 for all VapCs. 25 mM EDTA + VapC (EDTA); VapB + VapC (VapBC); Biological replicate #1 VapC (VapC-1); Biological replicate #2 VapC (VapC-2).

VapC	EDTA (RFU/ μ g·min)	VapBC (RFU/ μ g·min)	VapC-1 (RFU/ μ g·min)	VapC-2 (RFU/ μ g·min)
VapC4	-2.17E+03 \pm 1.54E+2	-1.09E+02 \pm 1.50E+02	1.07E+04 \pm 2.21E+02	3.09E+04 \pm 2.74E+02
VapC7	-2.55E+04 \pm 1.62E+03	-3.37E+03 \pm 1.72E+03	2.60E+05 \pm 2.97E+03	1.68E+05 \pm 1.62E+03
VapC8*	-2.33E+03 \pm 2.13E+02	2.30E+01 \pm 2.38E+02	2.57E+04 \pm 2.62E+02	2.60E+04 \pm 2.58E+02
(*) The activity of VapC8 when treated with VapB4 was 2.25E+02 \pm 2.00E+02				

Table S3. Base-Pairing Probabilities for *M. sedula*/*M. prunae* 16S rRNA.
Sequence displayed 5' → 3'

1	10	20	30	40																																			
C	U	G	C	C	C	U	A	A	U	U	C	C	G	G	U	U	G	A	U	C	C	U	G	C	C	G	G	A	C	C	G	A	U	C	G	C	U	A	
41	50	60	70	80																																			
U	A	G	G	G	G	U	A	G	G	C	G	C	U	A	A	G	C	C	A	U	G	G	G	A	G	U	C	G	U	A	C	G	C	U	C	G	G	G	G
81	90	100	110	120																																			
A	A	G	A	G	G	G	C	G	U	G	G	C	G	A	C	C	G	C	U	G	A	G	U	A	A	C	A	C	G	U	G	G	C	U	A	A	C	C	
121	130	140	150	160																																			
U	G	C	C	C	U	U	G	G	C	A	U	C	U	G	G	A	U	A	A	C	C	C	C	G	G	G	A	A	A	C	U	G	G	G	G	C	U	A	A
161	170	180	190	200																																			
U	C	C	G	G	A	G	C	G	G	C	A	A	G	G	G	A	U	C	U	G	G	A	A	U	G	A	U	U	C	U	C	U	G	C	C	U	A		
201	210	220	230	240																																			
A	A	A	G	C	C	U	C	U	C	G	G	C	U	G	A	U	C	C	G	U	C	G	A	G	A	G	G	C	G	C	C	A	A	G	G	A	U		
241	250	260	270	280																																			
G	G	G	G	C	U	G	C	G	G	C	C	C	A	U	C	A	G	G	C	U	G	U	U	G	G	G	G	A	G	U	A	A	A	G	G	U	C		
281	290	300	310	320																																			
C	C	C	A	A	A	C	C	G	A	U	A	A	C	G	G	G	U	A	C	G	G	C	C	G	U	G	G	A	G	C	G	G	G	A	G	C	C		
321	330	340	350	360																																			
C	C	C	A	G	U	U	G	G	G	C	A	C	U	G	A	G	A	C	A	A	G	G	C	C	C	A	G	G	C	C	C	A	G	G	C	C	C	C	
361	370	380	390	400																																			
C	G	C	A	C	C	A	G	A	G	G	C	G	A	A	C	G	U	C	C	C	A	A	U	G	C	G	G	A	A	A	C	C	G	U	G	A			
401	410	420	430	440																																			
G	G	G	C	C	U	A	G	U	G	C	C	U	C	G	C	A	A	G	A	G	A	G	G	C	U	U	U	U	C	U	C	C							
441	450	460	470	480																																			
A	G	U	C	C	A	G	A	A	A	G	G	U	G	G	A	G	G	A	U	A	A	G	C	C	G	G	G	G	G	C	G	C	A	A	G	A	C	U	G
481	490	500	510	520																																			
U	G	U	C	A	G	C	C	G	C	C	G	U	A	A	U	A	C	C	A	G	C	C	C	C	C	C	C	C	C	G	A	G	U	G	A	U	C	G	
521	530	540	550	560																																			
C	A	C	G	U	U	A	U	G	G	C	U	A	A	A	A	G	C	G	C	C	C	G	U	A	G	C	C	G	C	C	U	G	U	A	A				
561	570	580	590	600																																			
A	G	U	C	A	C	C	G	U	U	A	A	A	G	A	C	C	C	G	G	C	U	C	A	A	C	U	C	G	G	G	A	A	C	G	G	C			
601	610	620	630	640																																			
C	G	U	G	A	U	A	C	A	G	G	C	U	A	G	C	G	G	G	C	G	G	A	G	A	G	G	U	C	G	G	A	G	G	U					
641	650	660	670	680																																			
A	C	U	C	C	C	G	G	A	G	U	A	G	G	G	G	C	G	A	A	U	C	C	U	C	A	G	A	U	C	C	C	G	G	A	G	G	A		
681	690	700	710	720																																			
C	C	A	C	C	A	G	U	G	G	C	G	A	A	A	G	C	G	U	C	C	G	C	U	A	G	A	A	C	G	C	C	C	G	A	C				

Base-Pairing probability color scale



Table S3. Continued

721	730	740	750	760
G UGAGGGGC G AAAGCCGGG G UAGCAAUA G GAUUA GAUA C				
761	770	780	790	800
C GUAGUAGU C CCGGCUGUA A ACGAUGCAG G CUAGGUCUC G				
801	810	820	830	840
C GUAGGCUU U GUGCCUGCC C GGUGCCGCA G GAA AACUGG U				
841	850	860	870	880
A AGCCCGCC G CCUGGGGAG U ACGGCCGCA A GGCUGAAAC U				
881	890	900	910	920
U AAAGGA AU U GGC GGGGA G CACCACAAG G GGUGGAACC U				
921	930	940	950	960
G CCGCUC AA U UGGAGUC AA C GGCUGGA AU C UCACCGGGG G				
961	970	980	990	1000
A GACCCG CAG G AUGACGGCC A GGCUAACGA C CUUGCCAGA C				
1001	1010	1020	1030	1040
U CCGCGAGA G GAGGUGCAU G CCGUCGCC A GCUCGUUU G				
1041	1050	1060	1070	1080
U GAAAU GUC C GGUUAAGUC C GGCAACGAG C GAGACCCCC A				
1081	1090	1100	1110	1120
C UUCUAGUU G GUAACCGUC U CUCCGGAGA C GGUCCACAC U				
1121	1130	1140	1150	1160
A GAAGGACU G CCGGUGUUA A ACCGGAGGA A GGAGGGGGC C				
1161	1170	1180	1190	1200
A CGGCAGGU C AGCAUGCCC C GAAACUJCC G GGCCGCACG C				
1201	1210	1220	1230	1240
G GGUUACAA U GGCAGGGAC A GCGGGAUCC G ACCCCGAGA G				
1241	1250	1260	1270	1280
C GGAAGGCA A UCCACACAAA C CCUGCCUCA G UUGGGAUUG A				
1281	1290	1300	1310	1320
G GGCUGAAA C UCGCCUCUG U GAACGAGGA A UCCCUAGUA A				
1321	1330	1340	1350	1360
C CCGCGGUC A ACAACCCGC G GUGAAUACG U CCCUGCUCU U				
1361	1370	1380	1390	1400
U GCACACAC C GCCCGUCGC U CCACCCGAG U GGAGGGGAA G				
1401	1410	1420	1430	1440
U GAGGCCUC U UGCCCCUCG G GGUGGGAGG U CGAGCUUCU C				
1441	1450	1460	1470	1480
C UCCCGGAG G GGGGAGAAG U CGUAACAAG G UAGCCGUAG G				
1481	1490	1500	1510	1520
G GAACCUUC G GCUUGGAUCA C CUCACA AU				

Base-Pairing probability color scale



Table S4. Base-Pairing Probabilities for Msed_1804.

Sequence displayed 5' → 3'

1	10	20	30	40
G G G A U G A A A U U C C C U A A G C U A G U C A A G G C U U A C U G C C C C A				
41	50	60	70	80
A G U G U A A G A C U C A C A C U G A C C A C U C C A U C U C C C U U U A C A A				
81	90	100	110	120
G G G U G G A A A G A G G A G G G A G A U C G C A G A G G G A C A G C G C A G G				
121	130	140	150	160
U A C A A C A G G A A G A A C C U A G G U U A C G G A A G C A C A A G G A A G C				
161	170	180	190	200
C A G U U C C C A A G A G G U U C G C C A A G G U C A C G A A G A A G C A G A C				
201	210	220	230	240
C C U G A U G U U U A A G U G C C A G A A G U G C G G U U A C A C C U A C U C C				
241	250	260	270	280
A A G C U A G G G A U G A G G G U A A A G A A G C U C G A A C U A G U U G A G G				
281	290			
U G A U C A A A U G A				

Base-Pairing probability color scale



Table S5. Base-Pairing Probabilities for Msd_1802.
Sequence displayed 5' → 3'

1	10	20	30	40
G GGAUGAUC U ACAACAGGU A CCCUCUCCC C CGUGAGGGG G				
41	50	60	70	80
A CAUUCUAA U UGCGACCGU G AAGCAGGUU U UUGAUUACG G				
81	90	100	110	120
U AGUUAACGU C ACGCUAGAC G AGUAUGGUG G GCUUCAGGC C				
121	130	140	150	160
U UCGUUCGC U GGAGUGAGA U AAGUACCAG G UGGGUUAAG A				
161	170	180	190	200
A CAUAAGGG A CGUGGUAAA G GAGGGAAAGG A AGAUCAUUC U				
201	210	220	230	240
C AAGGUGAU U UAGAGUUGAC C GCAAGAAAG G GUCAGUUGA C				
241	250	260	270	280
G UUUCCCUG A AGAAGGUCA A UGACGACGA U AGGAGGAAG A				
281	290	300	310	320
A GAACGCC C A GUGGAAGAG G AUACAGAA G A UCGACAAGA U				
321	330	340	350	360
C CUGGAGAU A GUGGCGCAG A AGCUCAGA A GAGCGAGAA G				
361	370	380	390	400
G AAGCAUGG G AAGCAGGUGG C CUGGAAGCU C GAGGAGAA G U				
401	410	420	430	440
A CCGCGACG U GUACGAGGC C CUCCAGAA G G CAUCUAAGG A				
441	450	460	470	480
A GGGGAGAA G GUGCUCUUG G AUGCGGGGG U GCCAGAGAU C				
481	490	500	510	520
U GGAUUAA G C CCAUCCUUG A GGABGGCCAG C AAACACGGG G				
521	530	540	550	560
A AAGAGAAGA A GGUUAAGGA G AGCAAAAGUG G UCCUGGUGA A				
561	570	580	590	600
G AGCCUGGA C CCUGAUGGU G UGGACAAGA U CAGGAAGGU G				
601	610	620	630	640
U UCGACCUC G AGGAUGAGG G GGACAJAAG G AUAAUUCACG A				
641	650	660	670	680
U UGGUGCCC C AAGGUUAUAG G GUUGAGGUU U CAGGGACC G A				
681	690	700	710	720
C CCGAAGGC U GUAGCACAG A GGCUCGAGG A GGUCGUACA G				
721	730	740	750	760
A GGAUACUA G AAGAGGGCAA A AGAGGAGGG C GUUUCAGCG G				
761	770	780	790	800
A GGUUGCCA A AGUGA				

Base-Pairing probability color scale



Table S6. Base-Pairing Probabilities for Msd_0355.
Sequence displayed 5' → 3'

1	10	20	30	40
G G G A U G A G U A U A A C C C A G U C U U A U U A C A U G G A U U U A G G A A				
41	50	60	70	80
C A C U G A C C C U A G A G A A G G A U C A A G U A A U U A G A A A G U G U A U				
81	90	100	110	120
A G C U A U C C U A G G A A U A A G A G G A U C A G G A A A A U C A A A U A C U				
121	130	140	150	160
G C G A A A G U G U U C G C U G A G G A A C U C A U C A A G G A G A A A G U U C				
161	170	180	190	200
C C C U A G U U A U A G U G G A U C C A G A C G G U G A A U A U A G G G G G C U				
201	210	220	230	240
U G A G G C U A U A A U U U U G A U A A A U U U A A C G U U G A U C C A G A G				
241	250	260	270	280
G A A G U G G U U A A C C U U C U U A U G G U U G G U A A A A G C G U G G U C C				
281	290	300	310	320
U U G A U G U U A A U G A C U G G A A C G A G G A A G U A U U C A A G U U C C U				
321	330	340	350	360
G A C C A C C U U C U U C A A C A U C C U U U G G G A G A C U U C C A A G A U U				
361	370	380	390	400
U A U A G G A G G G A C A U C U U C G U C C U C C U A G A G G A G G C A C A U G				
401	410	420	430	440
A G U U A U C C C C C A G G G A A C A A G A A C C C C G C U U A G C G A C G U				
441	450	460	470	480
C U U G G U G A G G A U U G C C C U U C G A G G G A G A A A A G A G G G G G A U U				
481	490	500	510	520
G G G A U G A U C C U G G U G A G U C A G A G A U C A G C C A A G G U A A A C A				
521	530	540	550	560
A G G A U G U C C U U A C C C A G A G C G A G A U C U A U U U C C U C C A C A A				
561	570	580	590	600
G G U G G U U C A C C C A G U C G A C G U A A G G G U U U A C A A G G A A A U A				
601	610	620	630	640
C U A C C U C U A A A A U C C A G G G A A A U U G A G G C U G A G A U A A A G G				
641	650	660	670	680
C C A U G G A A A C U G G A G A U G C G A U A U U U U A C A A G G A U G G G G A				
681	690	700	710	720
A C U A A G A A A G G U U A G A A U U A G A A A G U U U G A U G A G U U U C C G				
721	730	740	750	760
U C G G U C A U C C A G A G C G G U G A A A A G G C G G A A A U A U A A				

Base-Pairing probability color scale



Table S7. Primers and Ek/LIC vectors used for cloning VapC toxins and PIN domain proteins.

Displayed sequences 5' → 3'.

Gene ID	Annotation	Fwd. primer	Rev. primer	Vector
Msed_0302	PIN 1	GAC GAC GAC AAG ATG ATC TCC TTA CTG CAA G	GAG GAG AAG CCC GGT TAA TCA ACG AAC TCC AC	pRSF
Msed_0338	VapC1	GAC GAC GAC AAG ATG AGT TAC CTC TTCGAT TC	GAG GAG AAG CCC GGT TAC CTC TCA ACT TCA TCA AG	pRSF
Msed_0411	VapC2	GAC GAC GAC AAG ATG GCG GGA CAG GAA TTG	GAG GAG AAG CCC GGT CAT ATT ACC TTA TAA TC	pRSF
Msed_0739	PIN 2	GAC GAC GAC AAG ATG GAG AAA GTG ATA TTT G	GAG GAG AAG CCC GGT CAT TTC CCC CTT TG	pRSF
Msed_0864	VapC3	GAC GAC GAC AAG ATG AGG GTT CTT CTC GAC	GAG GAG AAG CCC GGC TAG GGC GGG AGA TC	pRSF
Msed_0899	VapC4	GAC GAC GAC AAG ATG GCA AGG TAC GTG ATTG	GAG GAG AAG CCC GGT CAT TTC CCA AGT G	pET46
Msed_0908	VapC5	GAC GAC GAC AAG ATG GAG AAG GAG AAG TGC CTA G	GAG GAG AAG CCC GGT TAG AGT AGG TCA TCT GAG	pRSF
Msed_1184	VapC6	GAC GAC GAC AAG ATG CAG AAG AAT AAA TAT TTC	GAG GAG AAG CCC GGT TAT ATC TTG TCA ATT TCA C	pRSF
Msed_1214	VapC7	GAC GAC GAC AAG ATG AGG TTG ATC GTT GAT AC	GAG GAG AAG CCC GGC TAG CCT GGG CAG	pRSF
Msed_1245	VapC8	GAC GAC GAC AAG ATG CAG AGG ACG CAT ATA G	GAG GAG AAG CCC GGC TAG ATC GTC TCA AC	pRSF
Msed_1307	VapC9	GAC GAC GAC AAG ATG ATA TTC TTG GAT GCA AAC	GAG GAG AAG CCC GGT CAC TTT ATC CAG ATC	pET46
Msed_1385	VapC10	GAC GAC GAC AAG ATG AAA TAT TTC GAC ACT AG	GAG GAG AAG CCC GGC TAT TCC AGT AGG TTT AC	pET46
Msed_1534	VapC11	GAC GAC GAC AAG ATG CAA AGG TAC ATT CTT GAC	GAG GAG AAG CCC GGC TAG GAA AGA GGT ACA AAG	pRSF
Msed_2242	VapC12	GAC GAC GAC AAG ATG GGG ACA GGT GGT TCT G	GAG GAG AAG CCC GGC TAA ATA GTG CGA AA	pRSF

Table S8. Primers used for cloning VapB anti-toxins into pET-46 vector. The vector was constructed by assembling the gene fragment and vector backbone using Gibson Assembly. Gene specific nucleotides (underlined) vector specific nucleotides (not underlined). Displayed sequences 5' → 3'.

Gene ID	Annotation	Fwd. primer	Rev. primer
Msed_0898	VapB4	CAT CAC GTG GAT GAC GAC GAC AAG <u>ATG AGC TGG GTC ACA GTG</u>	CGG TGG CAG CAG CCT AGG TTA ATT <u>AAT CAC GTA CCT TG CC ATC</u>
Msed_1215	VapB7	CAT CAC GTG GAT GAC GAC GAC AAG <u>ATG AGC GAC ACA ATC TCC</u>	CGG TGG CAG CAG CCT AGG TTA ATT <u>AAC GAT CAA CCT CAT CCC TAT C</u>
Msed_1246	VapB8	CAT CAC GTG GAT GAC GAC GAC AAG <u>ATG AGA AAG ACA TTG GTT CG</u>	CGG TGG CAG CAG CCT AGG TTA ATT <u>AAC TAC CGC TAT ATG CGT C</u>
pET46	Vector Backbone	TAA TTA ACC TAG GCT GCT G	CTT GTC GTC GTC ATC CAC

Table S9. MS2 bacteriophage primer name, sequence and location adapted from Zhu et. al. (2008)

Location on MS2	ID	Primer sequence (3' → 5')
2026-2007	M1	TCTCTATTTATCTGACCGCG
156-137	S1	CCCTATCAAGGGTACTAAA
3435-3416	E1	GAGCACACCCACCCCGTTTA
3097-3077	E3	GGTCCGTCCCACCGAAGAAC
3231-3212	E5	AGAACTTGCGTTCTCGAGCG
3326-3307	E6	TATAACGCGCACGCCGGCGG
3158-3139	E4	CGGAGTCTTGGTGTATACCG
2621-2602	E2	GCGGATACGATCGAGATATG
2271-2252	M9	GCGCACATTGGTCTCGGACC
2244-2225	M8	CCGCTCTCAGAGCGCGGGGG
1843-1824	M7	GCAATTGATTGGTAAATTC
1915-1896	M6	AATTCGTCCCTTAAGTAAGC
1806-1787	M5	GAAGATCAATACATAAAGAG
1663-1644	M4	TGCATTGCCTTAACAATAAG
1476-1457	M3	TACAGGTTACTTTGTAAGCC
1302-1283	M2	GAGCCGTTGCCTGATTAATG
1140-1121	S7	AGCGTCAACGCTTATGATGG
1078-1061	S6	AGCATCCCACGGGGGCCG
976-957	S5	GGTTCAAGATACCTAGAGAC
859-840	S4	GACGGCCATCTAACTTGATG
511-492	S3	CTTCGGTCGACGCCCGGTTC
86-67	S2	TAGCCATGGTAGCGTCTCGC

Table S10. Primers used in messenger RNA degradation assays. Upper case indicates gene specific portion while lower case represents the T7 promoter sequence.

Primer ID	Primer Sequence (5' → 3')
Msed_0355F T7	taatacgactcactatagggATGAGTATAACCCAGTCTTATTA
Msed_0355R	TTATATTTCCGCCTTTTCAC
Msed_1538F T7	taatacgactcactatagggATGGAGGGAGTTTATCTCGT
Msed_1538R	TCATGTGGAGATCACGATAAATC
Msed_1802F T7	taatacgactcactatagggATGATCTACAACAGGTACCCTCT
Msed_1802R	TCACTTGGCAACCTCCGC
Msed_1804F T7	taatacgactcactatagggATGAAATTCCTAAGCTAGTCAA
Msed_1804R	TCATTTGATCACCTCAACTAGTT

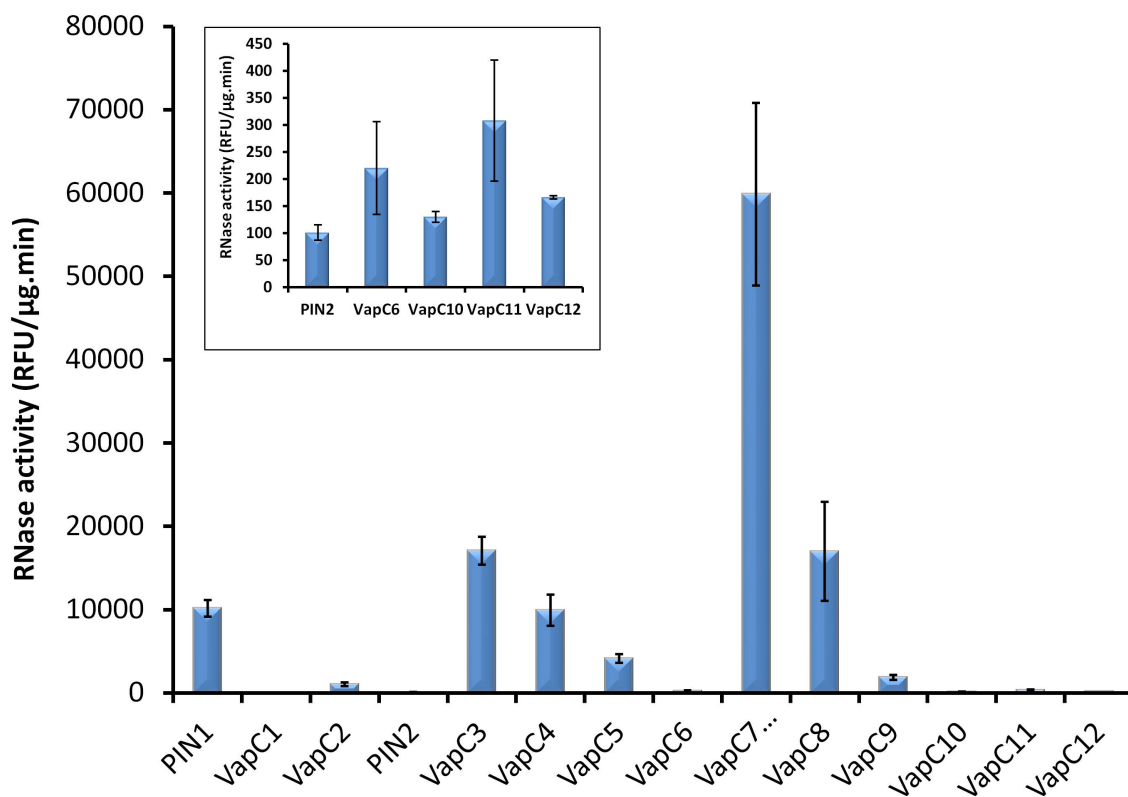


Figure S1. Initial RNase activity screen of VapC toxins and PIN domain proteins. RNase activity of VapC Toxins/PIN domain proteins on the generic RNA substrate (IDT DNA) was obtained by calculating initial reaction velocity (increase in fluorescence over time). PIN domain protein (PIN2) and VapC toxins, which had low activity, are shown in the inset. VapC7 had the highest RNase activity ($598,633 \pm 109,774$ RFU/ $\mu\text{g}\cdot\text{min}$); the VapC7 activity has been scaled down by a factor of 10 to be able to represent it along with the other VapCs/PIN domain proteins.

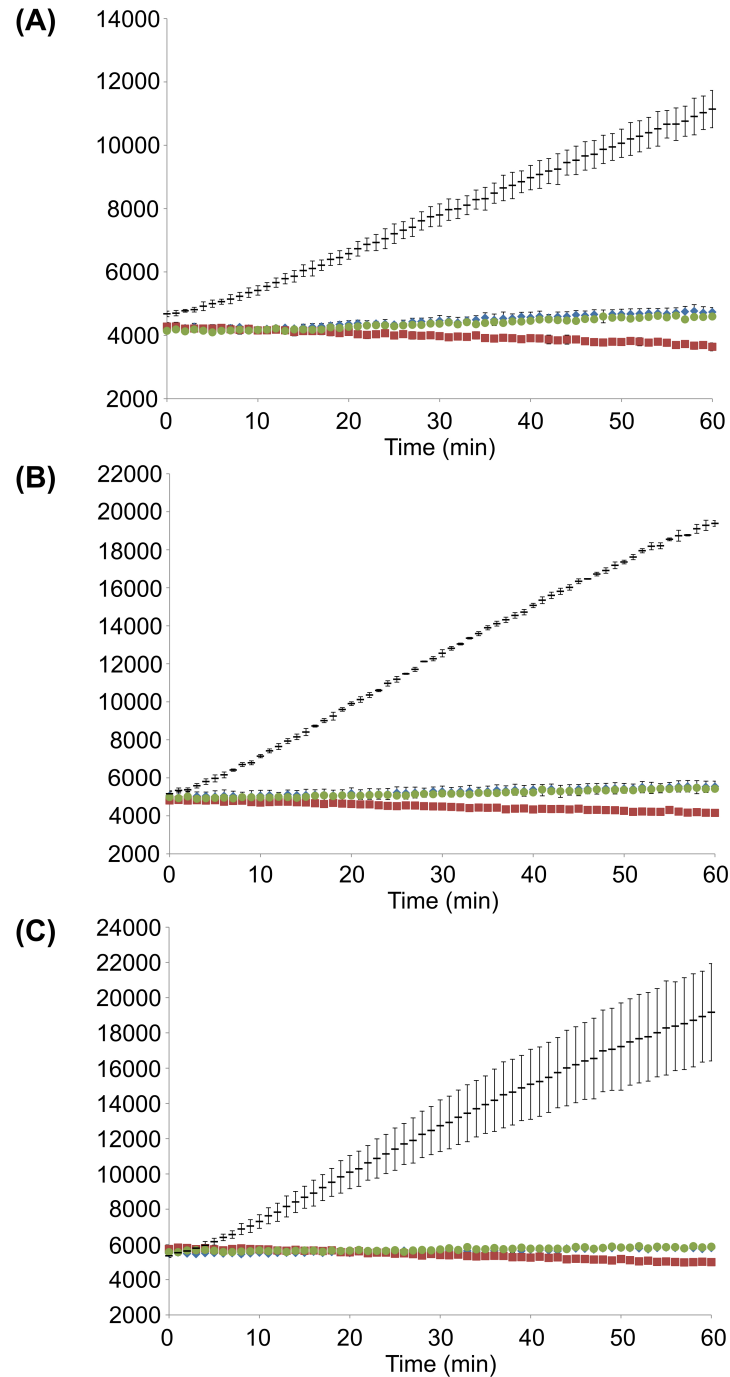


Figure S2. RNase activity of total RNA degrading VapC toxins. RNase activity of total RNA degrading VapC toxin proteins using the generic RNA substrate (IDT DNA). The RNA substrated was treated with (A) VapC4 (Msed_0899), (B) VapC7 (Msed_1214) and (C) VapC8 (Msed_1245). VapC4 and VapC8 reactions were loaded with 0.01 μg protein while VapC7 was loaded with 0.001 μg protein. The VapC:VapB mass ratio was 1:2 for all VapCs. No VapC added (\blacklozenge); 25 mM EDTA + VapC (\blacksquare); VapB + VapC (\bullet); VapC ($-$).

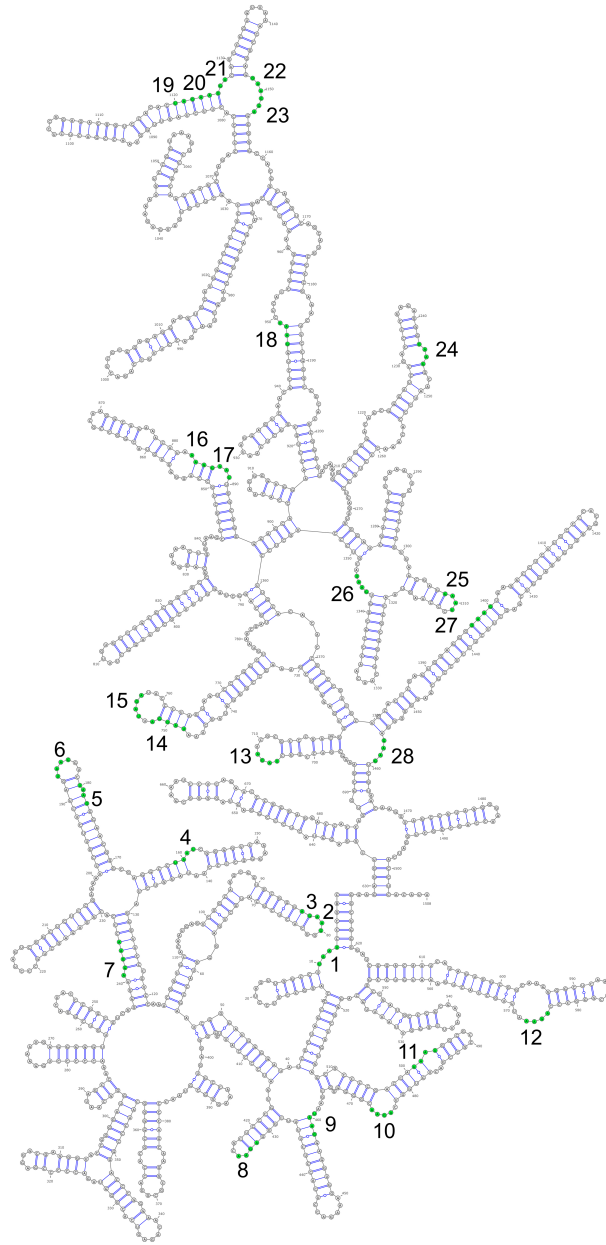


Figure S3. Predicted secondary structure of *M. sedula*/*M. prunae* 16S rRNA. The 16S rRNA contains a total of 28 consensus motifs (See **Table 1**), shaded green and indicated by numbers. The RNA structure and base pairing probabilities (see **Table S6**) were determined using RNAfold (<http://rna.tbi.univie.ac.at/>) with default settings followed by drawing in VARNA (<http://varna.lri.fr/>). The structure represents the minimum free energy structure.

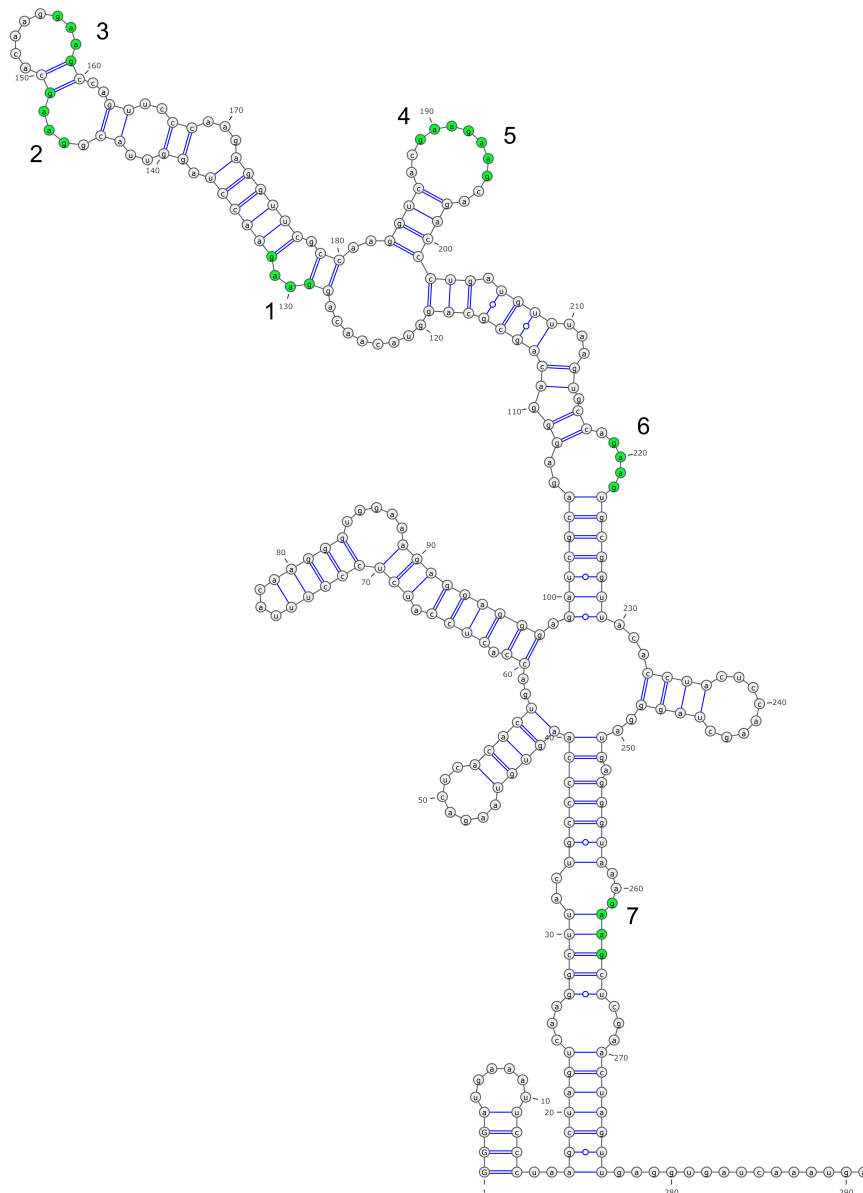


Figure S4. Predicted secondary structure of Mse_1804. Mse_1804 contains a total of 7 VapC4 (Msed_0899) consensus motifs (GAAG), shaded green and indicated by numbers. The RNA structure and base pairing probabilities (See **Table S7**) were determined using RNAfold (<http://rna.tbi.univie.ac.at/>) with default settings followed by drawing in VARNA (<http://varna.lri.fr/>). The structure represents the minimum free energy structure. The mRNA molecule includes a GGG at the beginning, runoff from T7 promoter.

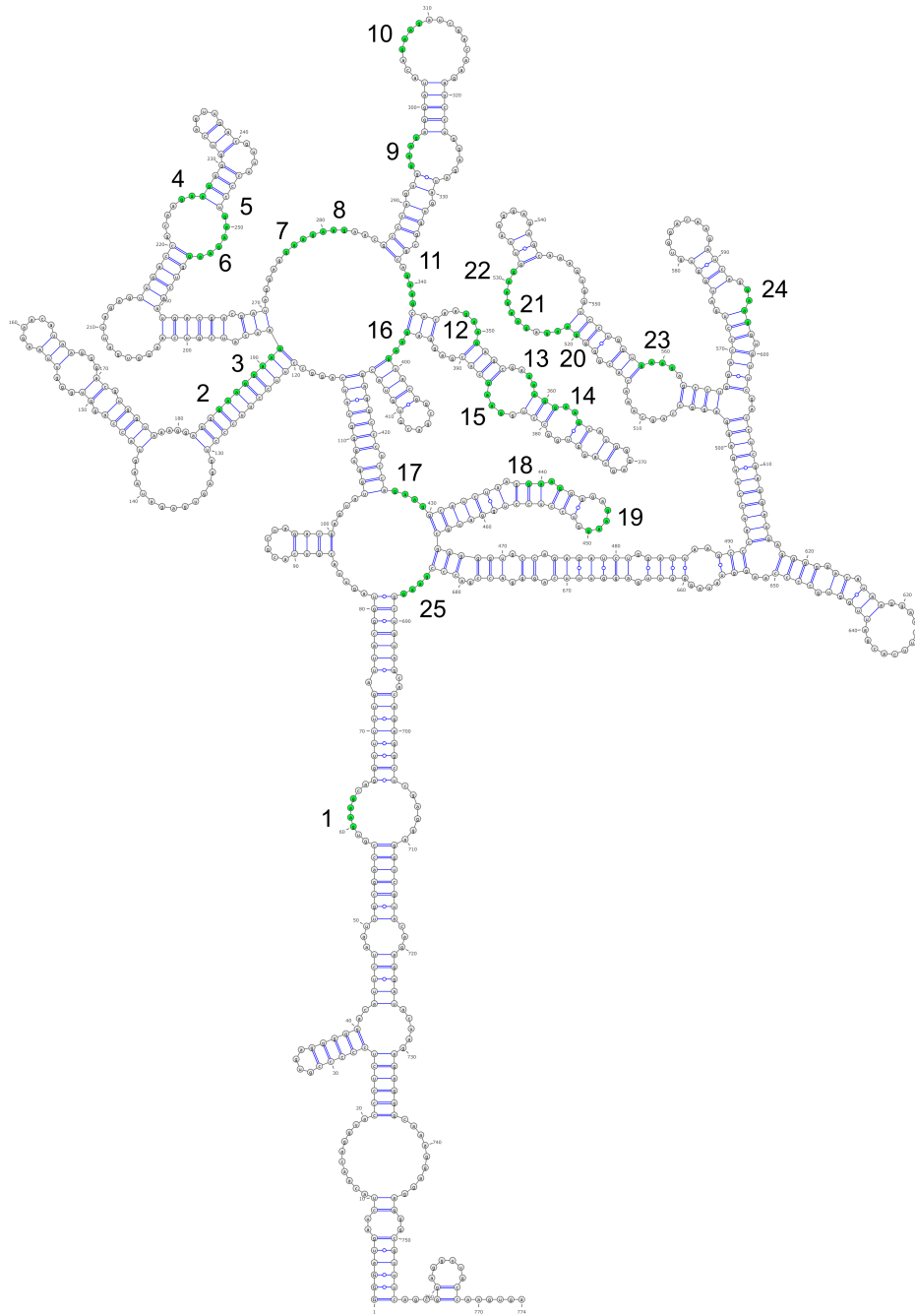


Figure S5. Predicted secondary structure of Msed_1802. Mse_1802 contains a total of 25 VapC4 (Msed_0899) consensus motifs (GAAG), shaded green and indicated by numbers. The RNA structure and base pairing probabilities (See **Table S8**) were determined using RNAfold (<http://rna.tbi.univie.ac.at/>) with default settings followed by drawing in VARNA (<http://varna.lri.fr/>). The structure represents the minimum free energy structure. The mRNA molecule includes a GGG at the beginning, runoff from T7 promoter.

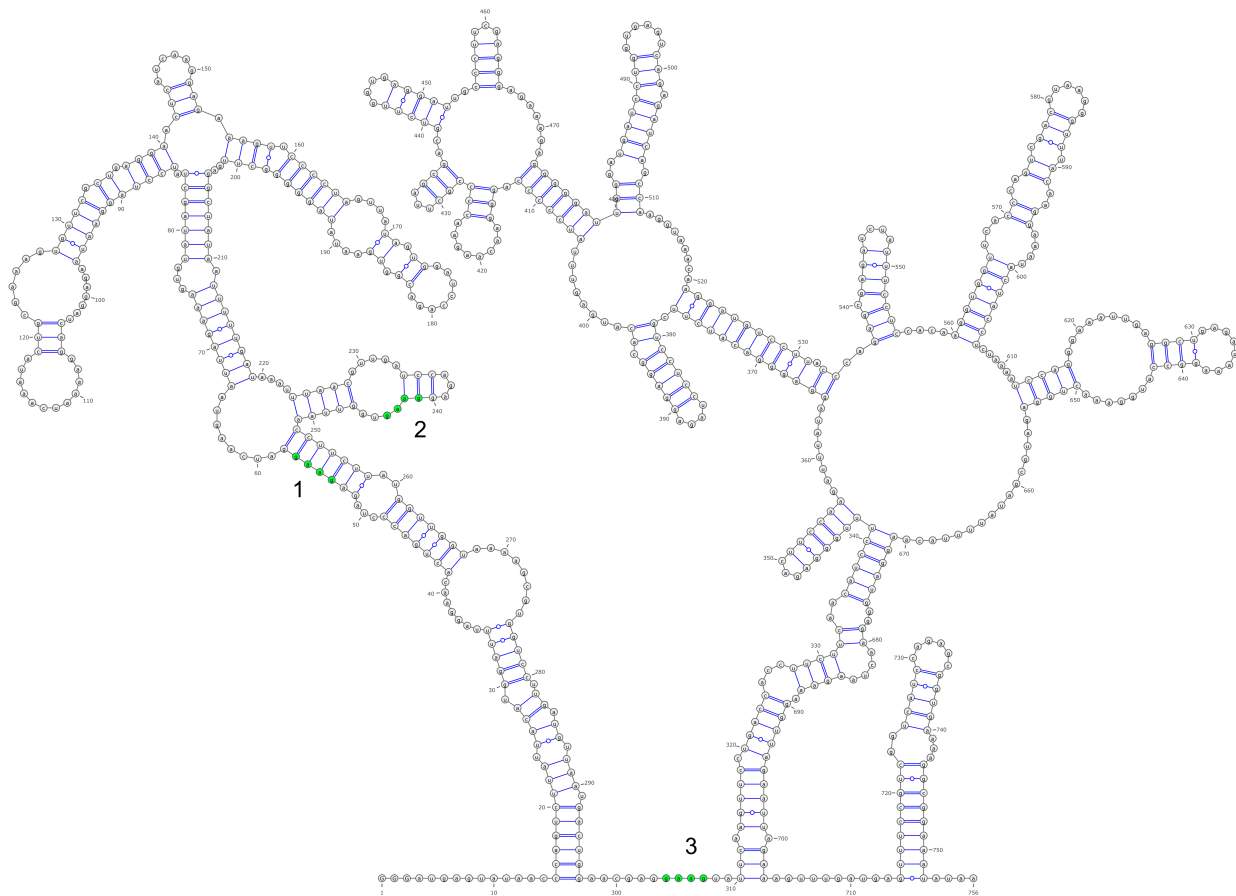


Figure S6. Predicted secondary structure of Msed_0355. Mse_0355 contains a total of 3 VapC4 (Msed_0899) consensus motifs (GAAG), shaded green and indicated by numbers. The RNA structure and base pairing probabilities (See **Table S9**) were determined using RNAfold (<http://rna.tbi.univie.ac.at/>) with default settings followed by drawing in VARNA (<http://varna.lri.fr/>). The structure represents the minimum free energy structure. The mRNA molecule includes a GGG at the beginning, runoff from T7 promoter.