SUPPLEMENTARY FILE

Identification and Characterization of a HEPN-MNT Family Type II Toxin-Antitoxin in *Shewanella oneidensis*

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Running head: a HEPN-MNT Family Type II TA in *S. oneidensis*

Table S1. Oligonucleotides used for plasmid construction gene knockout, site-directed mutagenesis (target mutated nucleotides are in red font) and DNA sequencing. Restriction enzyme sites are underlined. f indicates forward primer and r indicates reverse primer.

Purpose/Name	**Sequence (5'-3')**
Plasmid construction	
pHEG-*SO_3165*-f	GGAATTCATGCAACAACTAAATGAAAATA
pHEG-*SO_3165*-r	CCCAAGCTTTCAAAGTGACTCCCCCCTATGAG
pHEG-*SO_3166*-f	GGAATTCATGACATTATTATCAATAAAATA
pHEG-*SO_3166*-r	CCCAAGCTTTCACTCTGCTTTTATCACATCAA
pCA24N-*SO_3165*-f	GCCCAACAACTAAATGAAAATAA
pCA24N-*SO_3165*-r	CCAAGTGACTCCCCCCTATGAGC
pCA24N-*SO_3166*-f	GCCAATGACATTATTATCAATAA
pCA24N-*SO_3166*-r	CCCTCTGCTTTTATCACATCAAT
pET28b-*SO_3165*-f	CTAGCCATGGGCATGCAACAACTAAATGAAAATAA
pET28b-*SO_3165*-r	CCCAAGCTTCTAGTGGTGGTGGTGGTGGTGAAGTGACTCCCCCCTATGAGCTTTGGC
pET28b-*SO_3166*-f	CTAGCCATGGGCATGAATGACATTATTATCAATAA
pET28b-*SO_3166*-r	CCCAAGCTTCTAGTGGTGGTGGTGGTGGTGCTCTGCTTTTATCACATCAATAAATTG
pBS(Kan)-*SO_3165*-500 up-f	GTCTAGGGTACCAGTACCGCGCGTATATTGAGCG
pBS(Kan)-*SO_3166*-r	GAGCACGAGCTCTCACTCTGCTTTTATCACATCA
FAM-*SO_3166*-r	CGAGAGTGGCTGAGTAATAA
pHGR03-$P_{so\_3165\text{-}3166}$-f	CCGGAATTC TGCCTACGAGGCCAGAATTTAAA
pHGR03-$P_{so\_3165\text{-}3166}$-r	CGCGGATCCGTAATCATGGTCATAGGTCCATTGCACGATTTGA
PCR and DNA sequencing	
pCA24N-f	GATAACAATTTCACACAGAATT
pCA24N-r	GTCAGAGGTTTTCACCGTCATCA

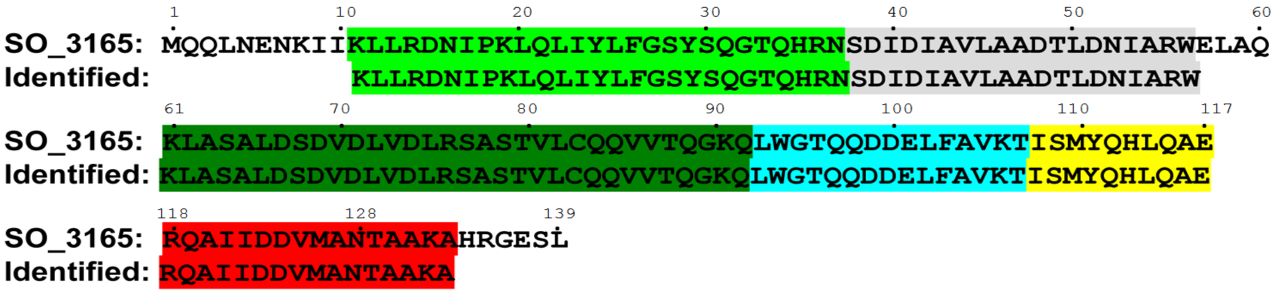
PHEG-f	CACCTCGCTAACGGATTCACC
PHEG-r	CCAATACGCAAACCGCCTC
pET28b-f	TAATACGACTCACTATAGGG
pET28b-r	TATGCTAGTTATTGCTCAG
pBS(kan)-f	GCCTTTGAGTGAGCTGATACCGCTC
pBS(kan)-r	GTGATGGTTCACGTAGTGGGCCATCG
CRSID-F	GATGTTGAAGTGGCGAGC
CRSID-V	ATGGATGCAAGCAAATGC
P*SO_3165*-f	TACGAGGCCAGAATTTAAAA
P*SO_3165*-r	AGGTCCATTGCACGATTTGA
Gene knockout	
delta *SO_3166*-5-O	GGGGACAAGTTTGTACAAAAAAGCAGGCTCGAACGCTCCGCTTACAGATA
delta *SO_3166*-5-I	GGTCCGGGTTCGCTATCTATTTCAAAGTGACTCCCCCTATGAG
delta *SO_3166*-3-O	GGGACCACTTTGTACAAGAAAGCTGGGTTATGCGAGCCATCCTGAATC
delta *SO_3166*-3-I	ATAGATAGCGAACCCGGACCAGATGTTGACAGAACAAGAAACG
delta *SO_3165-3166*-5-O	GGGGACAAGTTTGTACAAAAAAGCAGGCTCGAACGCTCCGCTTACAGATA
delta *SO_3165-3166*-5-I	GGTCCGGGTTCGCTATCTATTAGGTCCATTGCACGATTTGATGAATA
delta *SO_3165-3166*-3-O	GGGACCACTTTGTACAAGAAAGCTGGGTTATGCGAGCCATCCTGAATC
delta *SO_3165-3166*-3-I	ATAGATAGCGAACCCGGACCAGATGTTGACAGAACAAGAAACG
delta *SO_3166*-l-f	GACTGGGATAGTAACGGCTCAAA
delta *SO_3166*-s-f	ATCCGCTATCAAGAATGCGAATC
delta *SO_3166*-l-r	GCTTTGGTCCGTATGTTGATTGA
delta *SO_3166*-s-r	ACTTTAGGGCAAGCACAACAGCG
Single amino acid substitution	
SO_3166 R97G-f	GAAAATGGTCGGCCTG**G**GAAACATCGCAGTACA

SO_3166 R97G-r	TGTACTGCGATGTTTC**C**CAGGCCGACCATTTTC
SO_3166 H102A-f	TTGAGTTCTTGGTAGTCA**GC**TACTGCGATGTTTCGCAGGC
SO_3166 H102A-r	GCCTGCGAAACATCGCAGTA**GC**TGACTACCAAGAACTCAA
SO_3166 Y104A-f	TATCGAGGTTGAGTTCTTGG**GC**GTCATGTACTGCGATGTTTC
SO_3166 Y104A-r	GAAACATCGCAGTACATGAC**GC**CCAAGAACTCAACCTCGATA

Table S2. Mass spectrometry results of the co-purified protein with SO_3166-CHis. Peptide fragments observed were highlighted in different colors, and their loci in SO_3165 protein were also shown.

Reference				P (pro)	Score	Coverage	MW	Accession	Peptide (Hits)	
Scan(s)	Peptide	MH+	z	P (pep)	XC	DeltaCn	Sp	RSp	Ions	Count
5542	K.TISM*YQHLQAER.Q	1492.72129	2	2.56E-04	3.72	0.46	654.5	1	17/22	2
5745	R.QAIIDDVM*ANTAAK.A	1476.73627	2	2.15E-03	3.19	0.54	507.0	1	20/26	2
8253	K.QLWGTQQDDELFAVK.T	1777.87554	2	4.56E-06	4.73	0.66	1445.2	1	21/28	2
8447	R.NSDIDIAVLAADTLDNIAR.W	2000.02947	2	2.29E-04	2.81	0.54	451.9	1	16/36	2
9743	K.LASALDSDVDLVDLRSASTVLCQQVVTQGK.Q	3188.64677	3	1.55E-10	6.77	0.73	1913.4	1	40/116	2
9932	K.LLRDNIPKLQLIYLFGSYSQGTQHR.N	2960.61049	3	2.22E-15	6.72	0.68	2843.9	1	43/96	2

toxin-antitoxin system antidote Mnt family (SO_3165) [*Shewanella oneidensis*]



SO_3165: MQQLNENKIIKLLRDNIPKLQLIYLFGSYSQGTQHRNSDIDIAVLAADTLDNIARWELAQ
Identified: KLLRDNIPKLQLIYLFGSYSQGTQHRNSDIDIAVLAADTLDNIARW

SO_3165: KLASALDSDVDLVDLRSASTVLCQQVVTQGKQLWGTQQDDELFAVKTISMYQHLQAE
Identified: KLASALDSDVDLVDLRSASTVLCQQVVTQGKQLWGTQQDDELFAVKTISMYQHLQAE

SO_3165: RQAIIDDVMANTAAKAHRGESL
Identified: RQAIIDDVMANTAAKA

Figure S1. Gene organization of *SO_3165* and *SO_3166* in *S. oneidensis*. Transcriptional start site C (-30 bp upstream of the start codon of *SO_3165*) for the operon is indicated with an arrow. The sequence encoding *SO_3165* is shown in orange letters and the sequence encoding *SO_3166* is shown in blue letters. The start and stop codons for *SO_3166* and *SO_3165* are highlighted in green and square frames, respectively. The primer used for primer extension experiment is underlined.

CATCCCGTATTCATCAAATCGTGCAATGGACCT

ATGCAACAACTAAATGAAAATAAAATCATAAAGTTACTGCGAG
ACAACATCCCAAAGTTGCAATTAATCTACCTGTTTGGCTCTTAT
TCGCAGGGAACACAACACCGCAACAGCGATATCGATATCGCGG
TACTCGCCGCTGACACTTTAGACAATATTGCACGCTGGGAACT
TGCCCAAAAACTCGCTTCGGCGTTGGACTCAGATGTTGATCTG
GTTGATTTACGCTCAGCATCCACCGTGTTATGCCAACAGGTAGT
CACTCAAGGGAAGCAACTTTGGGGAACACAACAAGATGATGA
ACTTTTTGCCGTAAAGACCATCTCAATGTACCAACATCTACAAG
CCGAAAGACAGGCAATTATTGATGATGTAATGGCCAACACCGC
AGCCAAAGCTCATAGGGGGGAGTCACTTTGAATGACATTATTA
TCAATAAAATAGCCACCATCAAACGCTGTATAAAGCGCATCCA
ACAAGTCTATGGTGATGGCAGCCAGTTCAAACAAGACTTCACC
TTGCAAGATAGCGTTATCCTCAATTTACAACGCTGCTGCGAAGC
CTGTATCGACATAGCCAATCATATTAACCGCCAACAACAACTAG
GCATCCCCCAAAGTAGCCGTGATAGCTTTACATTACTCGCACAA
AATAACCTTATTACTCAGCCACTCTCGGATAACTTAAAGAAAAT
GGTCGGCCTGCGAAACATCGCAGTACATGACTACCAAGAACTC
AACCTCGATATCGTTGTGCATGTTGTACAGCATCATCTTGAGGA
TTTTGAGCAATTTATTGATGTGATAAAAGCAGAGTGA

SO_3165

SO_3166

Figure S2. Cell morphology examined by phase-contrast microscopy. Morphology of cells overproducing SO_3166 (MR-1/pHGE-*SO_3166*) vs. cells carrying empty plasmid (MR-1/pHGE) at 0 h and 10 h. Red arrows point to the "swollen" cells. Cells were grown in LB until the OD reached ~0.1. A concentration of 1 mM IPTG was added to overexpress *SO_3166* in *S. oneidensis*. Two independent cultures were used for each strain; only representative images are shown here. Scale bars indicate 10 μm.

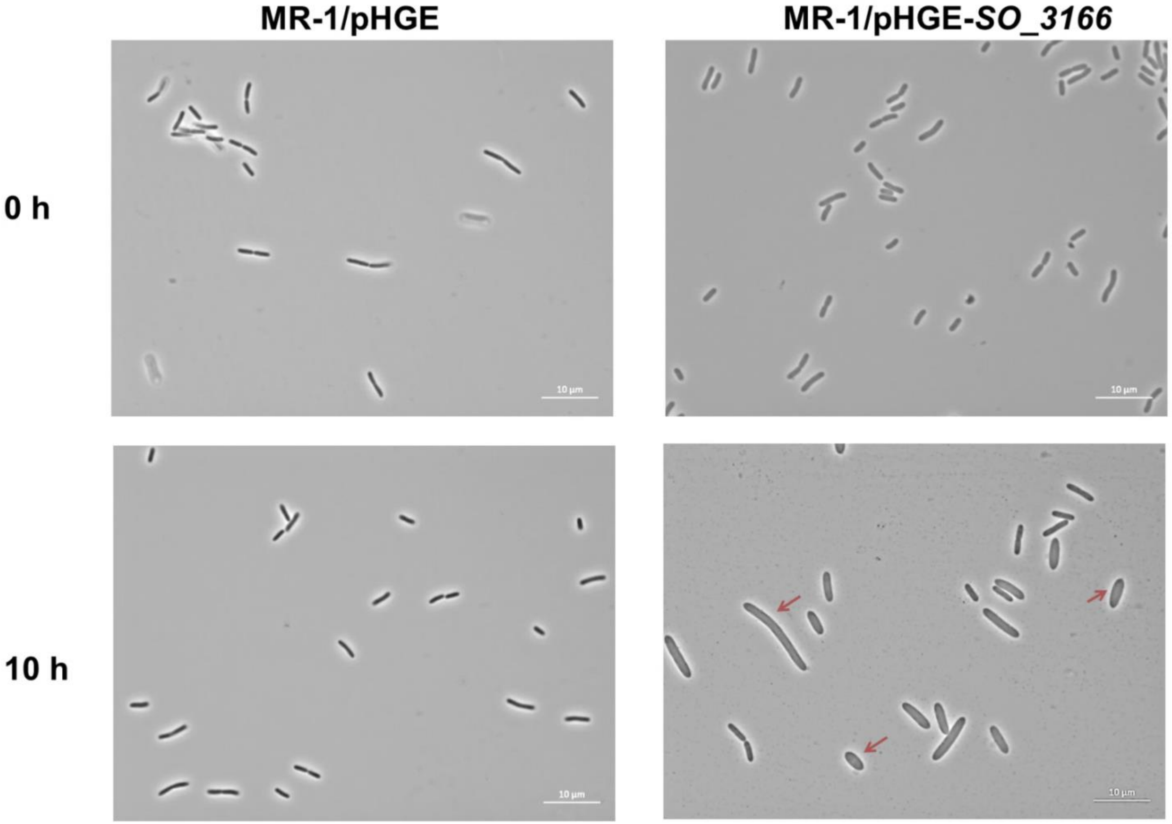


Figure S3. SO_3165 in *S. oneidensis* belongs to the MNT substrate-binding superfamily. The secondary structure of SO_3165 was predicted using the online PHYRE2 server (Kelley and Sternberg, 2009). The α-helices and β-sheets are shown. SO_3165 was predicted to have a similar secondary structure with the known secondary structure of nucleotidyltransferase 2 (np_343093.1) from *Sulfolobus solfataricus*. The MNT residues in the two proteins are boxed.

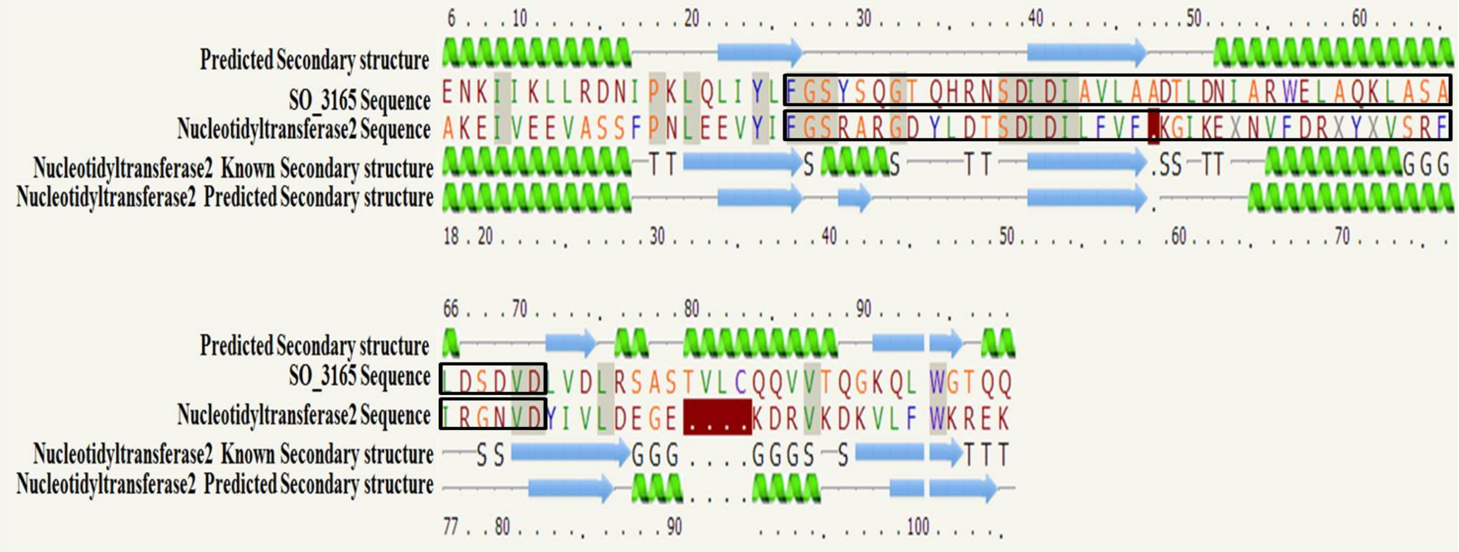


Figure S4. SO_3166 in *S. oneidensis* belongs to the HEPN superfamily. The secondary structure of SO_3166 was predicted using the online PHYRE2 server (Kelley and Sternberg, 2009). The α-helices and β-sheets are shown. The toxin SO_3166 has a similar secondary structure with the known secondary structure of the functional unknown HEPN family protein Yute 2 from *Bacillus Subtilis*. The HEPN residues in the two proteins are boxed, and conserved Rx4-6H motifs in the two proteins are also shown.

Reference:

Kelley, L.A., and Sternberg, M.J. (2009) Protein structure prediction on the Web: a case study using the Phyre server. *Nat Protoc* **4**: 363-371.