Supplementary Materials: Pore-Forming Cardiotoxin VVA2 (Volvatoxin A2) Variant I82E/L86K is an Atypical Duplex-Specific Nuclease

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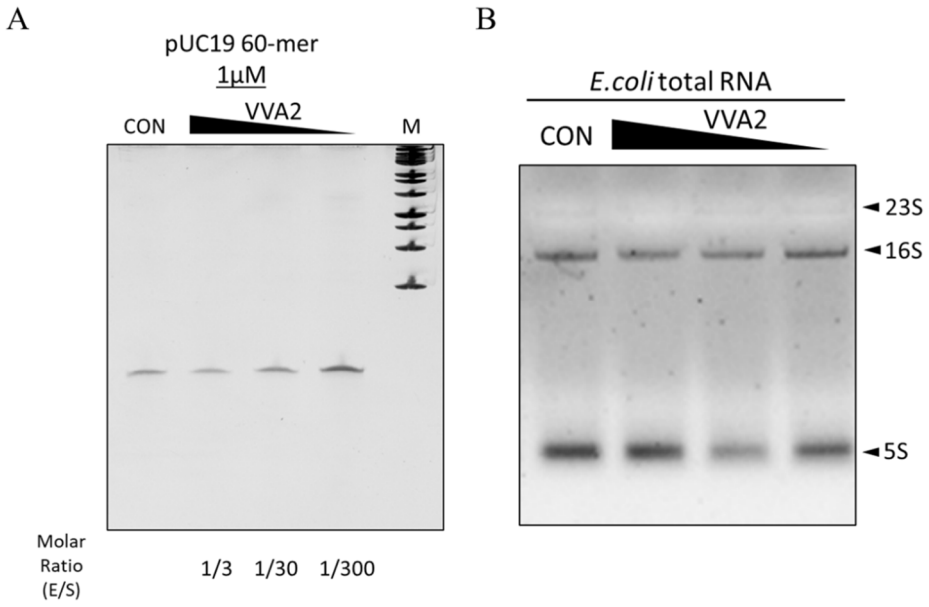
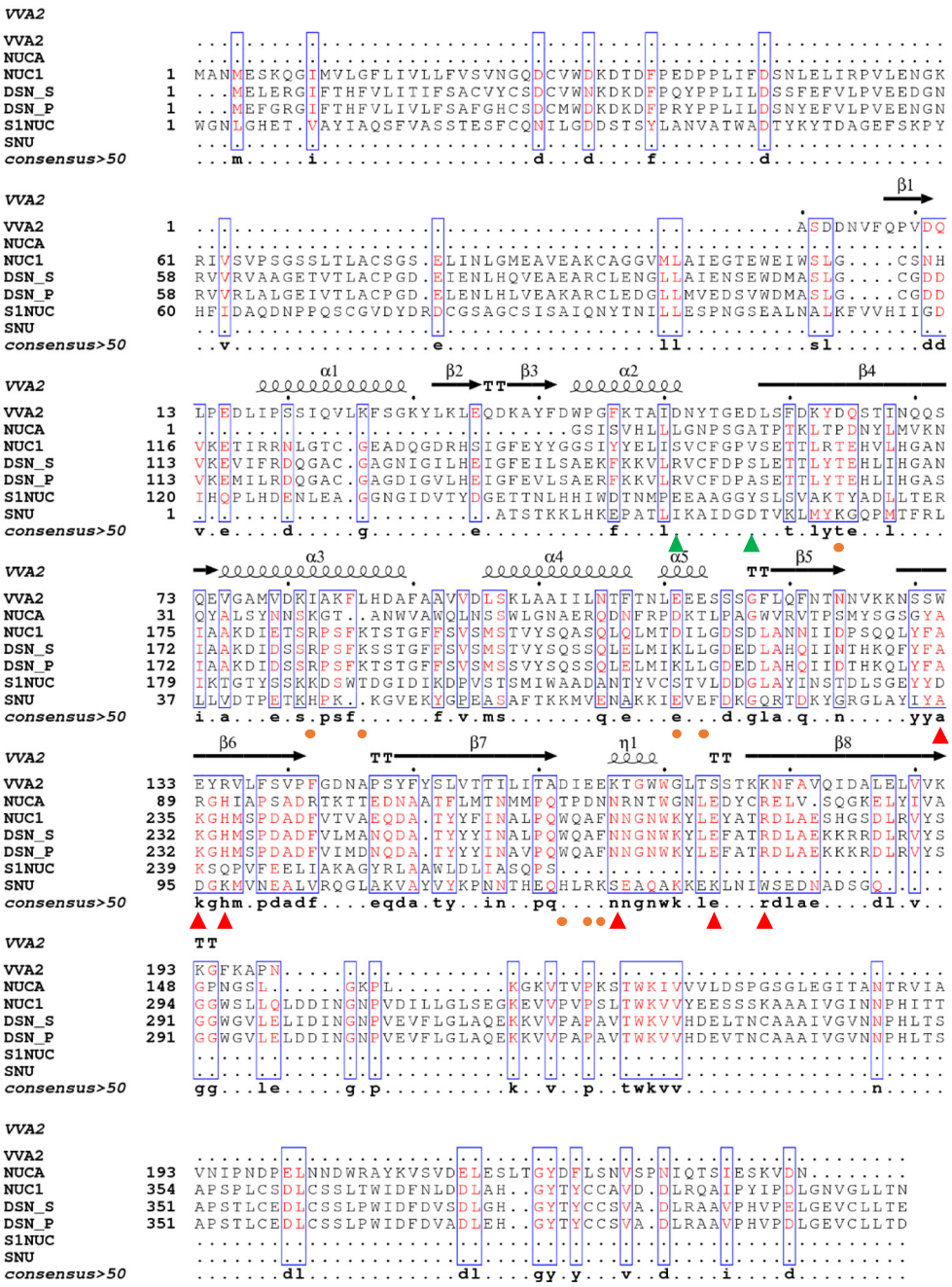


Figure S1. The nuclease activity of VVA2 variant on ssDNA and *E. coli* total RNA under optimal conditions. (**A**) Nuclease activity of Re-VVA2 I82E/L86K on 60-mer ssDNA mimics a fragment from pUC19. 1 μM ssDNA and 3.3 nM/33 nM/330 nM Re-VVA2-I82E/L86K under 25 mM Mg^{2+} were used to assay its nuclease activity at 55℃ for 2 h in reaction buffer (50 mM Tris pH 7.5, 200 mM NaCl, nuclease-free) as stated in Material and Methods. 8 M Urea/ 15% acrylamide gel stained with EtBr was used to analyze the product. CON: control group, ssDNA 60-mer incubated with other reagents except VVA2. M: DNA marker (100 bp DNA Ladder Dye Plus, Takara). (**B**) Nuclease activity of Re-VVA2 I82E/L86K on E. coli total RNA and yeast rRNA. Series diluted Re-VVA2-I82E/L86K (833 nM, 83.3 nM, and 8.3 nM) in reaction buffer (50 mM Tris pH 7.5, 200 mM NaCl, nuclease-free) was used under 25 mM Mg^{2+} at 55℃ for 1.5 h. 1% agarose gel was used to analyze the products after 75% ethanol precipitation. CON: The control group.

A


VVA2
NUCA
NUC1
DSN_S
DSN_P
S1NUC
SNU
consensus>50
β1
α1
β2
β3
α2
β4
α3
α4
α5
β5
β6
β7
η1
β8

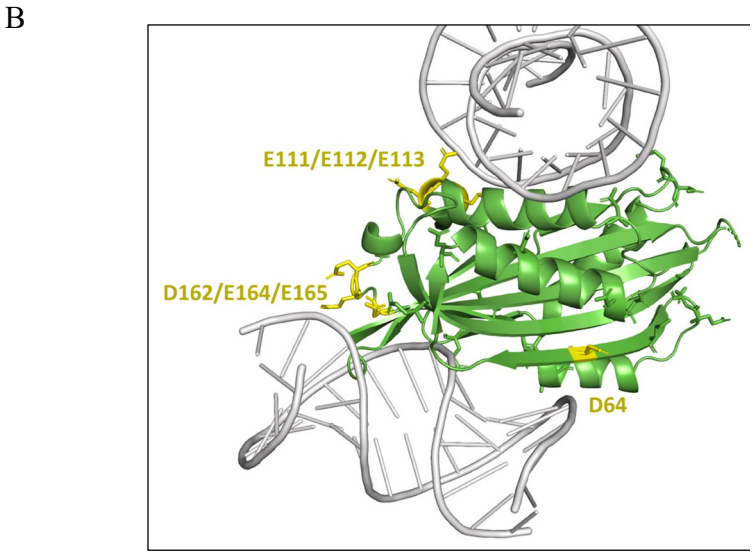


Figure S2. The multiple sequence alignment and structural comparison of VVA2 and other members of sugar non-specific nuclease. (**A**) The multiple sequence alignment and structural comparison of VVA2 and other members of sugar non-specific nuclease. The alignment was conducted as stated in Material and Methods. Related active sites were highlighted with red (for duplex-specific nucleases) and green (for non-specific nucleases). Amino acids of VVA2 involved in the mutagenesis study in this work were marked with orange dots. SNU: nuclease from *Staphylococcus aureus* (PDB code: 1STN_1); NUCA: nuclease A, nuclease from *Anabaena* sp. (PDB code: 2O3B_1); VVA2: Volvatoxin A2 (PDB code: 1VGF_A); S1NUC: S1 nuclease, nuclease from *Aspergillus oryzae* RIB40 (PDB code: 5FBF_1); NUC1: duplex-specific nuclease from *Paralithodes camtschaticus* (GenBank: AF520591.1). DSN1: duplex-specific nuclease from *Scylla paramamosain* (GenBank: AFP19103.1); DSN2: duplex-specific nuclease from *Portunus pelagicus* (GenBank: ADZ58187.1); (**B**) Molecular docking of 16-mer DNA (PDB code: 1CDW) on VVA2 (PDB code: 1PP0_A), as stated in Material and Methods. VVA2 is shown in the green cartoon; 16-mer DNA is shown in grey90. All the Glu and Arg are shown as sticks. Amino acids around the two clefts are shown in yellow. The codes of selected residues for further mutagenesis studies are marked in yellow and in bold.

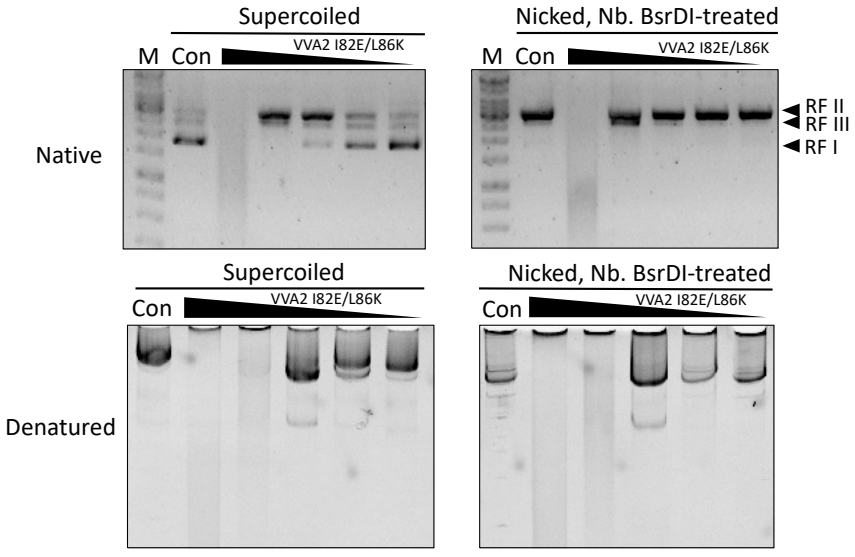


Figure S3. The comparison of nuclease activity of Re-VVA2 I82E/L86K on supercoiled and nicked dsDNA. Re-VVA2 I82E-L86K (16790 nM - 134.32 nM, 5-fold dilution) was used to cleave both supercoiled and nicked (by Nb. BsrDI) pUC19 (22.26 nM) at 37℃ for 1.5 h. 1% agarose gel (upper) and 8 M Urea/ 6% acrylamide gel (lower) was used to analyze the sample after ethanol precipitation. M: GeneRuler 1 kb DNA Ladder. CON: control group, supercoiled/nicked pUC19 incubated with other reagents except for VVA2.

Table S1. The cleavage sites of Re-VVA2-I82E/L86K on pUC19 and pET28a.

No.	Substrate	Order	Sequence information	Location on plasmid
1	pUC19	Top: 5′-3'	TGGAT GGAGG//CGGAT AAAGT	1915
		Bottom: 3′-5'	ACCTA CCTCC//GCCTA TTTCA	
2	pUC19	Top: 5′-3'	GTTTG CCGGA//TCAAG AGCTA	2395
		Bottom: 3′-5'	CAAAC GGCCT//AGTTG TCGAT	
3	pUC19	Top: 5′-3'	GGTGG GTTAC//ATCGA ACTGG	1415
		Bottom: 3′-5'	CCACC CAATG//TAGCT TGACC	
4	pUC19	Top: 5′-3'	TACGG ATGGC//ATGAC AGTAA	1625
		Bottom: 3′-5'	ATGCC TACCG//TACTG TCATT	
5	pUC19	Top: 5′-3'	AAAGT TGCAG//GACCA CTTCT	2135
		Bottom: 3′-5'	TTTCA ACGTC//ATGGT GAAGA	
6	pUC19	Top: 5′-3'	ACTGA TTAAG//CATTG GTAAC	1930
		Bottom: 3′-5'	TGACT AATTC//GTAAC CATTG	
7	pUC19	Top: 5′-3'	GATGC CGCAT//AGTTA AGCCA	937
		Bottom: 3′-5'	CTACG GCGTA//TCAAT TCGGT	
8	pUC19	Top: 5′-3'	CCAGT GGCGA//TAAGT CGTGT	2566
		Bottom: 3′-5'	GGTCA CCGCT//ATTCA GCACA	
9	pUC19	Top: 5′-3'	GGCAT GACAG//TAAGA GAATT	2120
		Bottom: 3′-5'	CCGTA CTGTC//ATTCT CTTAA	
10	pUC19	Top: 5′-3'	TGAGA TAGGT//GCCTC ACTGA	1633
		Bottom: 3′-5'	ACTCT ATCCA//CGGAG TGACT	
11	pUC19	Top: 5′-3'	CATAC CTCGC//TCTGC TAATC	2527
		Bottom: 3′-5'	GTATG GAGCG//AGACG ATTAG	
12	pUC19	Top: 5′-3'	TACAC TTTAT//GCTTC CGGCT	553
		Bottom: 3′-5'	ATGAG AAATA//CGAAG GCCGA	
13	pUC19	Top: 5′-3'	GTTCT TCTAG//TGTAG CCGTA	2469
		Bottom: 3′-5'	CAAGA AGATC//ACATC GGCAT	
14	pUC19	Top: 5′-3'	CCGTA TTGAC//GCCGG GCAAG	1533
		Bottom: 3′-5'	GGCAT AACTG//CGGCC CGTTC	
15	pUC19	Top: 5′-3'	CGGAT GGCAT//GACAG TAAGA	1627
		Bottom: 3′-5'	GCCTA CCGTA//CTGTA ATTCT	
16	pUC19	Top: 5′-3'	AAAGC GCCAC//GCTTC CCGAA	37
		Bottom: 3′-5'	TTTCG CGGTG//CGAAG GGCTT	
17	pUC19	Top: 5′-3'	TCCGG CTCGT//ATGTT GTGTG	565
		Bottom: 3′-5'	AGGCC GAGCA//TACAA CACAC	
18	pET28a	Top: 5′-3'	TTTGA CGAGG//GGAAA TTAAT	4620
		Bottom: 3′-5'	AAACT GCTCC// CCTTT AATTA	
19	pET28a	Top: 5′-3'	GGACT GTTGG//GCGCC ATCTC	581
		Bottom: 3′-5'	CCTGA CAACC//CGCGG TAGAG	
20	pET28a	Top: 5′-3'	GGCAT CAGTG//ACCAA ACAGG	2667
		Bottom: 3′-5'	CCGTA GTCAC// TGGTT TGTCC	

21	pET28a	Top: 5′-3' CGCTG CGCTC//GGTCG TTCGG Bottom: 3′-5' GCGAC GCGAG//CCAGC AAGCC	3137
22	pET28a	Top: 5′-3' GCGCT GGCAG//TGTTC CTGCG Bottom: 3′-5' CGCGA CCGTC//ACAAG GACGC	4371
23	pET28a	Top: 5′-3' GAGTT TGGAA//CAAGA GTCCA Bottom: 3′-5' CTCAA ACCTT//GTTCT CAGGT	5050
24	pET28a	Top: 5′-3' GCACC GGGAT//CTCGA CCGAT Bottom: 3′-5' CGTGG CCCTA//GAGCT GGCTA	1902

Table S2. Primers used for cloning and site-direct mutagenesis[1] studies of VVA2.

Variants	Restriction enzyme	Forward primer (5′-3′)	Reverse primer (5′-3′)
VVA2-I82E/L86K	BamH I & Hind III	AATTCCGGATCCATGGCAA GTGATGAT	GGAATTAAGCTTTTAAAGCTT ATTCGG
VVA2- D64A/I82E/L86K	-	GATAAATATGCTCAGAGCA CC	GGTGCTCTGAGCATATTTATC
VVA2- E111A/E112A/E113A/ I82E/L86K	-	ACCAACCTGGCAGCAGCAAG CAGCAGC	GCTGCTGCTTGCTGCTGCCAG GTTGGT
VVA2- D162A/E164A/E165A/ I82E/L86K	-	ATTACCGCAGCTATTGCAGC AAAAACCGGT	ACCGGTTTTTGCTGCAATAGC TGCGGTAAT
VVA2- E111A/I82E/L86K	-	ACCAA CTGGCAGAAGAAAGCAGCA GC	GCTGCTGCTTTCTTCTGCCAGG TTGGT
VVA2- E112A/I82E/L86K	-	ACCAACCTGGAAGCAGAAA GCAGCAGC	GCTGCTGCTTTCTGCTTCCAGG TTGGT
VVA2- E111A/E112A/I82E/L86 K	-	ACCAACCTGGCAGCAGAAA GCAGCAGC	GCTGCTGCTTTCTGCTGCCAG GTTGGT

[1] Overlapping PCR method was used for site-direct mutagenesis