

## Supplementary information

### MARTX toxin of *Vibrio vulnificus* induces RBC phosphatidylserine exposure that can contribute to thrombosis

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## Supplementary figure legends:

**Supplementary Fig. 1 Shape changes of RBCs infected with *V. vulnificus*.** RBCs were infected with *V. vulnificus* at various MOIs and incubated for 1 h. *V. vulnificus* only (left) and the shape changes of the human RBCs infected with *V. vulnificus* (red arrowheads) at various MOIs and incubated for 1 h (right) were analyzed by TEM. The representative images by TEM among 5 independent experiments were presented. Red arrowhead indicates *V. vulnificus*; white arrow, echinocyte; and white arrowhead, spherocyte. Scale bar is presented. MOI, multiplicity of infection; and TEM, transmission electron microscopy.

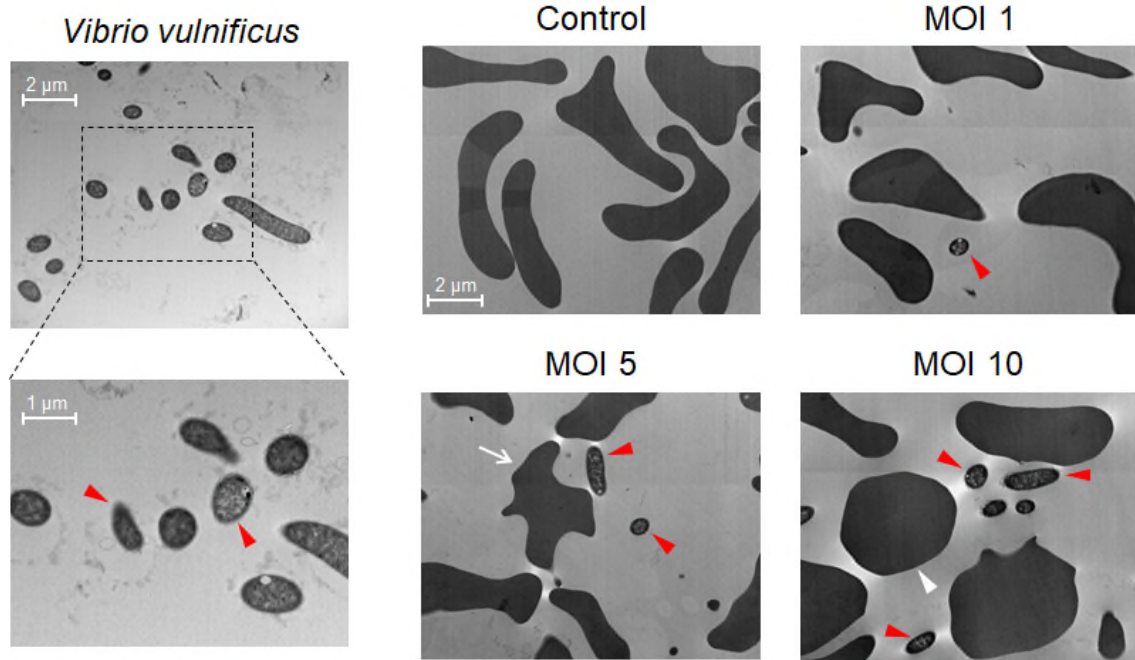
**Supplementary Fig. 2 Effects of *V. vulnificus* on the MV generation, and PS exposure of human RBCs.** a, b RBCs were infected with *V. vulnificus* at various MOIs and different incubation times. MV generation (a) and PS exposure (b) were determined (n=6, two-way analysis of variance (ANOVA) followed by Duncan's multiple range test). The means  $\pm$  SE were calculated from at least five independent experiments. Control, uninfected; MOI, multiplicity of infection; PS, phosphatidylserine; and MV, microvesicle.

**Supplementary Fig. 3 Effect of purified Annexin V on thrombin generation in *V. vulnificus*-infected RBCs.** Human RBCs were incubated in the presence or absence of purified Annexin V for 10 min. And then, the pre-incubated RBCs were infected with *V. vulnificus* at 5, and 10 MOIs for 30 min, and thrombin generation were determined (n=5, two-tailed Student's *t*-test). The means  $\pm$  SE. were calculated from at least five independent experiments. Control, uninfected; and MOI, multiplicity of infection.

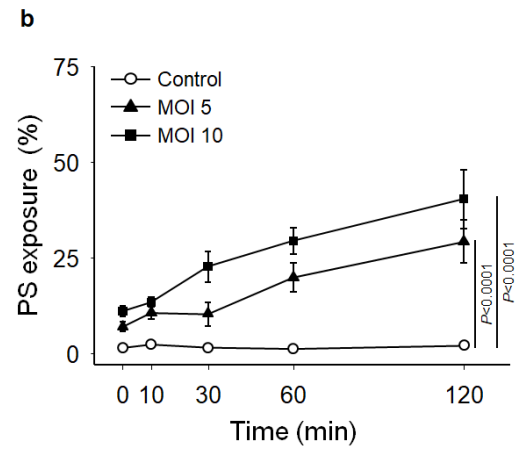
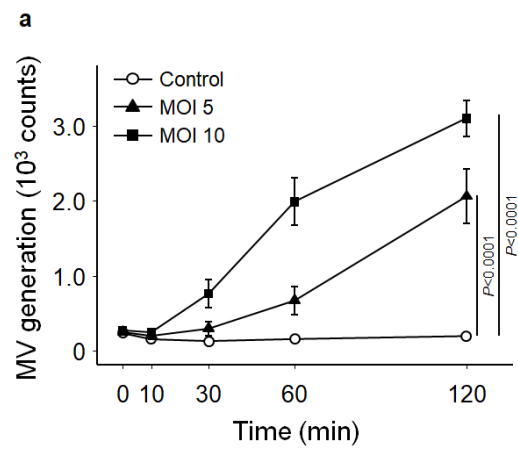
**Supplementary Fig. 4 Characteristics of the *V. vulnificus* mutant strains used in the study.** a Schematic diagrams of the *rtxA* structural gene of the *V. vulnificus* WT, *rtxA::nptI*

mutant, and revertant strains. The *rtxA::nptI* mutant carries the *nptI* gene cassette inserted at the 5'-end of *rtxA* and produces no MARTX toxin. The revertant carries the WT *rtxA* gene restored from the *rtxA::nptI* by removing the *nptI* insertion as indicated with dimmed symbol, and thus produces the WT-MARTX toxin. Arrows indicate transcriptional directions of each gene. **b, c** Human RBCs were infected with different strains of *V. vulnificus* at an MOI of 10 and incubated for 1 h, and then PS exposure (**b**) (n=5, two-tailed Student's *t*-test) and thrombin generation (**c**) (n=6, two-tailed Student's *t*-test) were determined. **d** Human epithelial cells (HeLa cells, 1 x 10<sup>5</sup> cells) originally purchased from ATCC (CCL-2) were infected with different strains of *V. vulnificus* at an MOI of 10 and incubated for 150 min, and then bleb formation (red arrowheads) was observed. Scale bar is presented. The means ± SE. were calculated from at least five independent experiments. WT, wild type;  $\Delta$ *rtxA*, a mutant producing no MARTX toxin; *rtxA::nptI*, a mutant producing no MARTX toxin; Rev, a revertant producing the WT-MARTX toxin; *nptI*, aminoglycoside 3'-phosphotransferase gene; *EF-rtxA*, a mutant producing the EF-MARTX toxin; PS, phosphatidylserine; control, uninfected; and MOI, multiplicity of infection.

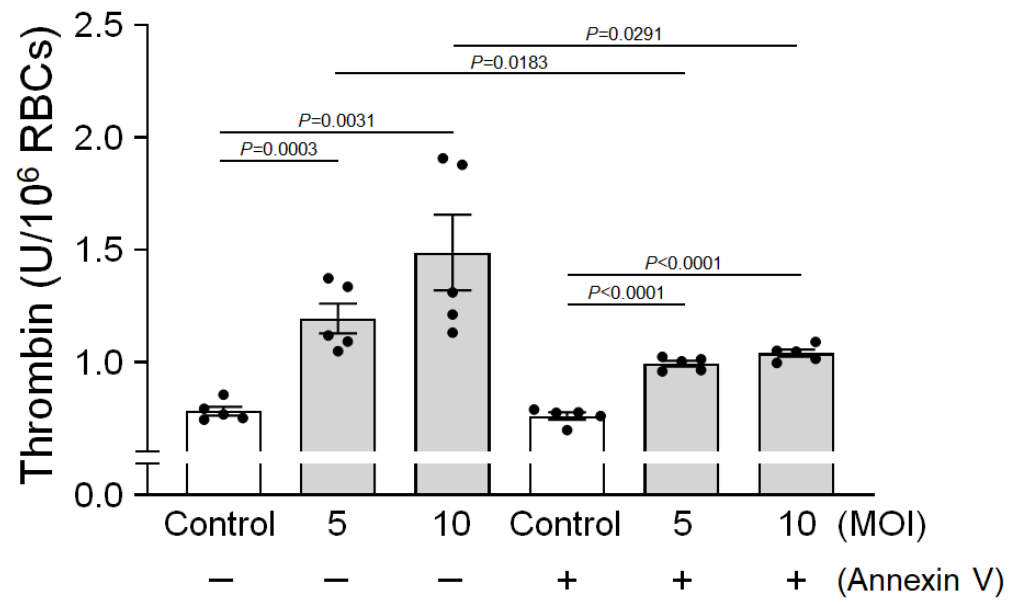
Supplementary Fig. 1



Supplementary Fig. 2

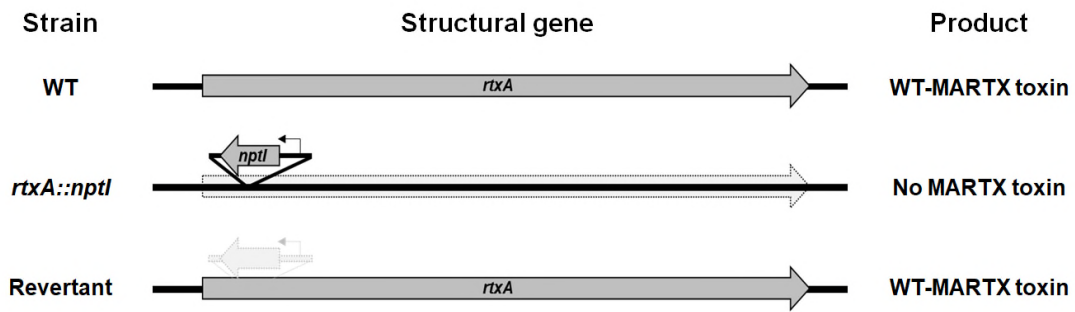


Supplementary Fig. 3

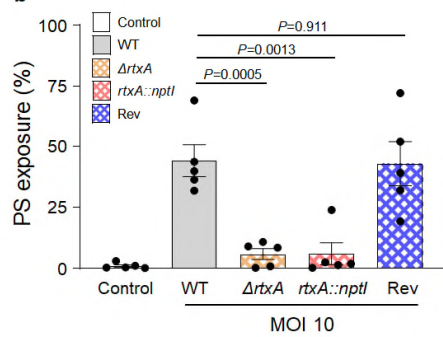


## Supplementary Fig. 4

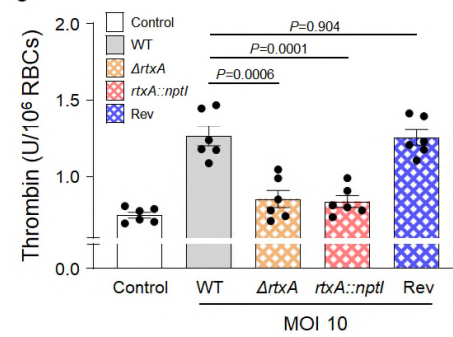
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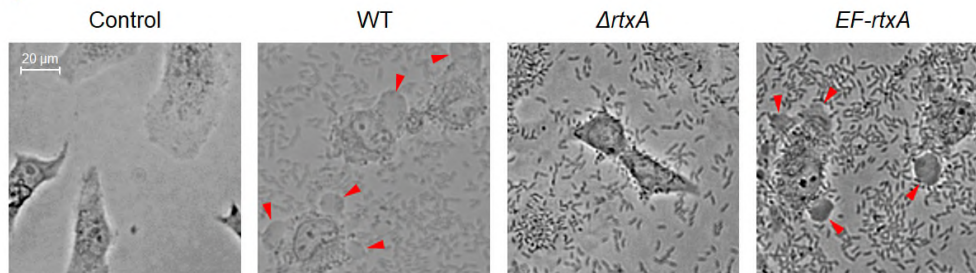
b



c



d



**Supplementary Table 1 Bacterial strains and plasmids used in this study**

Strain or plasmid	Relevant characteristics <sup>a</sup>	Reference or source
<b>Bacterial strains</b>		
<i>Vibrio vulnificus</i>		
WT	Clinical isolate MO6-24/O, wild type, WT-MARTX toxin	(Wright et al., 1990) <sup>1</sup>
<i>ΔrtxA</i>	MO6-24/O with <i>ΔrtxA::nptI</i> , Km <sup>r</sup> , no MARTX toxin	(Lee et al., 2007) <sup>2</sup>
<i>EF-rtxA</i>	MO6-24/O with <i>EF-rtxA</i> , EF-MARTX toxin	This study
<i>rtxA::nptI</i>	MO6-24/O with <i>rtxA::nptI</i> , Km <sup>r</sup> , no MARTX toxin	(Lee et al., 2018) <sup>3</sup>
Revertant	MO6-24/O with the restored <i>rtxA</i> , Km <sup>s</sup> , WT-MARTX toxin	(Lee et al., 2018) <sup>3</sup>
<i>Escherichia coli</i>		
S17-1 λ pir	λ <i>pir</i> lysogen, <i>thi pro hsdR hsdM<sup>+</sup> recA</i> RP4-2 Tc::Mu-Km::Tn7, Tp <sup>R</sup> , Sm <sup>R</sup> , host for π-requiring plasmids, conjugal donor	(Simon et al., 1983) <sup>4</sup>
<b>Plasmid</b>		
pDS132	<i>oriR6K sacB oriT</i> RP4, Cm <sup>R</sup>	(Philippe et al., 2004) <sup>5</sup>
pDS_EF-MARTX	pDS132:: <i>EF-rtxA</i> , Cm <sup>R</sup>	(Lee et al., 2019) <sup>6</sup>

WT, wild type; <sup>a</sup> Km<sup>r</sup>, kanamycin-resistant; EF, effector-free; Km<sup>s</sup>, kanamycin-sensitive; Tp<sup>R</sup>, Trimethoprim-resistant; Sm<sup>R</sup>, streptomycin-resistant; Cm<sup>R</sup>, chloramphenicol-resistant.



**Supplementary Table 2 List of differentially expressed genes in *V. vulnificus* exposed to human RBCs**

Locus tag <sup>a</sup>	Annotation	RLE (DESeq2)		Log <sub>2</sub> fold change	P value
		Control	Exposed to RBCs		
<b>Up-regulation<sup>b</sup></b>					
<b>Amino acid transport and metabolism<sup>c</sup></b>					
VVMO6_01726	Homoserine O-succinyltransferase	136.78	369.50	1.43	2.56E-28
VVMO6_03431	2-aminoethylphosphonate	243.40	521.45	1.10	2.02E-22
VVMO6_03434	ATP-binding protein	311.33	781.19	1.33	6.42E-40
VVMO6_04200	2-keto-3-deoxy-D-arabino-Heptulosonate-7-phosphate synthase I alpha	113.39	288.97	1.35	1.63E-20
VVMO6_04207	Isochorismate pyruvate-lyase of siderophore biosynthesis	32.15	94.69	1.56	2.88E-11
<b>Carbohydrate transport and metabolism<sup>c</sup></b>					
VVMO6_02646	Glycosyltransferase	222.26	447.00	1.01	2.52E-16
VVMO6_03242	Metabolite transporter	935.99	2072.28	1.15	1.83E-41
<b>Coenzyme transport and metabolism<sup>c</sup></b>					
VVMO6_01252	Nicotinate phosphoribosyltransferase	536.63	1319.58	1.30	3.94E-60
VVMO6_03382	Radical SAM family protein HutW	127.16	295.72	1.22	8.23E-18
<b>Transcription<sup>c</sup></b>					
VVMO6_01093	Transcriptional activator MetR	70.83	252.16	1.83	4.33E-30
<b>Replication, recombination and repair<sup>c</sup></b>					
VVMO6_03779	RstA phage-related replication protein	16.74	35.71	1.09	2.80E-03

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<b>Cell wall/membrane/envelope biogenesis<sup>c</sup></b>					
VVMO6_03380	Ferric siderophore transport system periplasmic binding protein TonB	7.31	17.36	1.25	1.00E-02
VVMO6_04039	Putative outer membrane protein	1853.30	4221.61	1.19	6.68E-38
<b>Inorganic ion transport and metabolism<sup>c</sup></b>					
VVMO6_00202	Manganese superoxide dismutase	109.24	315.19	1.53	5.10E-25
VVMO6_04408	Aerobactin siderophore receptor IutA	99.93	209.56	1.07	3.22E-13
<b>Secondary metabolites biosynthesis, transport and catabolism<sup>c</sup></b>					
VVMO6_04208	2,3-dihydroxybenzoate-AMP ligase	64.23	214.36	1.74	2.16E-23
VVMO6_04209	Aryl carrier domain	5.54	15.06	1.44	1.74E-02
<b>General function prediction only<sup>c</sup></b>					
VVMO6_01352	Death on curing protein, Doc toxin	2.16	7.04	1.71	4.35E-02
<b>Intracellular trafficking, secretion, and vesicular transport<sup>c</sup></b>					
VVMO6_01206	<i>flp</i> pilus assembly protein CpaB	13.60	40.05	1.56	6.75E-06
<b>Function unknown<sup>c</sup></b>					
VVMO6_01693	Tricarboxylate transport protein TctC	320.99	1479.75	2.20	4.36E-147
VVMO6_01696	Tricarboxylate transport membrane protein TctA	116.38	404.24	1.80	8.37E-46
<b>Unclassified<sup>c</sup></b>					
VVMO6_01205	Hypothetical protein	15.46	31.96	1.05	3.10E-03
VVMO6_01356	Hypothetical protein	31.98	80.23	1.33	3.34E-07

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<b>VVMO6_01694</b>	Hypothetical protein	3.98	40.23	3.34	6.40E-12
<b>VVMO6_01695</b>	Tricarboxylate transport protein TctB	32.15	142.11	2.14	2.95E-25
<b>VVMO6_01834</b>	Predicted manganese transporter	53.99	167.38	1.63	8.27E-19
<b>VVMO6_02535</b>	Hypothetical protein	23.50	48.17	1.04	3.65E-04
<b>VVMO6_03008</b>	Hypothetical protein	21.29	44.66	1.07	4.20E-04
<b>VVMO6_03538</b>	Type II/IV secretion system ATPase TadZ/CpaE	37.02	77.53	1.07	4.77E-06
<b>VVMO6_03894</b>	Transposase	4.80	13.46	1.49	2.18E-02
<b>VVMO6_04161</b>	Hypothetical protein	81.05	240.29	1.57	4.25E-22
<b>Iron uptake<sup>d</sup></b>					
<b>VVMO6_04199</b>	Siderophore biosynthesis	120.07	278.71	1.21	2.39E-18
<b>VVMO6_04201</b>	Catechol siderophore ABC transporter	31.81	88.13	1.47	2.87E-09
<b>VVMO6_04206</b>	Siderophore biosynthesis	48.56	163.93	1.76	2.51E-21
<b>VVMO6_04210</b>	Catechol siderophore ABC transporter	98.78	216.15	1.13	1.56E-13
<b>Toxin<sup>d</sup></b>					
<b>VVMO6_03947</b>	MARTX toxin	1121.50	2578.14	1.20	2.72E-43
<b>Down-regulation<sup>b</sup></b>					
<b>Energy production and conversion<sup>c</sup></b>					
<b>VVMO6_01670</b>	Acylphosphate phosphohydrolase putative	4.49	0.35	-3.69	2.18E-02
<b>VVMO6_02002</b>	Glutamate symport protein	962.04	461.17	-1.06	3.18E-36
<b>VVMO6_02391</b>	Glutamate symport protein	3187.71	967.56	-1.72	2.21E-123
<b>VVMO6_03181</b>	Oligopeptide transport ATP-binding protein OppF	157.11	60.11	-1.39	4.93E-13
<b>VVMO6_03847</b>	Nitrite reductase	109.18	46.40	-1.23	8.32E-09
<b>VVMO6_03866</b>	Nitrite reductase	9012.01	4048.87	-1.15	3.44E-05

<b>VVMO6_04094</b>	Ferredoxin-type protein NapF	310.68	119.27	-1.38	1.11E-23
<b>VVMO6_04096</b>	Periplasmic nitrate reductase precursor	4850.36	1241.94	-1.97	3.59E-161
<b>VVMO6_04097</b>	Nitrate reductase cytochrome c550-type subunit	263.20	60.19	-2.13	7.57E-36
<b>VVMO6_04098</b>	Cytochrome c-type protein NapC	378.90	83.75	-2.18	9.81E-50
<b>VVMO6_04166</b>	L-lactate permease	3883.93	1851.31	-1.07	1.15E-57
<b>Amino acid transport and metabolism<sup>c</sup></b>					
<b>VVMO6_00054</b>	Ketol-acid reductoisomerase	9904.53	4244.73	-1.22	6.09E-75
<b>VVMO6_00199</b>	Aspartate ammonia-lyase	490.20	199.48	-1.30	3.59E-31
<b>VVMO6_01167</b>	Tyrosine-specific transport protein	913.90	413.44	-1.14	1.44E-36
<b>VVMO6_01655</b>	Sodium/glycine symporter GlyP	951.96	346.61	-1.46	1.18E-53
<b>VVMO6_02415</b>	Sodium/alanine symporter	955.08	349.24	-1.45	8.05E-53
<b>VVMO6_02533</b>	Sodium/alanine symporter	1281.58	456.23	-1.49	2.88E-65
<b>VVMO6_02972</b>	Amino acid ABC transporter	7313.88	2351.55	-1.64	2.70E-85
<b>VVMO6_03045</b>	Sodium/dicarboxylate symporter	1255.89	548.12	-1.20	1.37E-48
<b>VVMO6_03441</b>	Di-/tripeptide transporter	80.95	26.87	-1.59	1.89E-09
<b>VVMO6_03488</b>	Serine transporter	384.21	177.75	-1.11	1.59E-18
<b>Carbohydrate transport and metabolism<sup>c</sup></b>					
<b>VVMO6_00328</b>	Ribulose-phosphate 3-epimerase	359.94	173.56	-1.05	1.76E-17
<b>Coenzyme transport and metabolism<sup>c</sup></b>					
<b>VVMO6_00730</b>	Multidrug efflux pump component MtrF	160.67	65.49	-1.29	8.42E-13
<b>VVMO6_02721</b>	Uroporphyrinogen-III methyltransferase	6674.09	3319.38	-1.01	2.96E-33

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**Lipid transport and metabolism<sup>c</sup>**

<b>VVMO6_00780</b>	Butyryl-CoA dehydrogenase	2445.81	1085.24	-1.17	9.99E-38
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**Translation, ribosomal structure and biogenesis<sup>c</sup>**

<b>VVMO6_02205</b>	Endoribonuclease L-PSP	39.83	17.42	-1.19	4.79E-4
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**Transcription<sup>c</sup>**

<b>VVMO6_03062</b>	Transcriptional regulator	121.32	57.39	-1.08	9.48E-08
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<b>VVMO6_04439</b>	Transcriptional regulator	490.02	235.65	-1.06	3.40E-21
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**Cell wall/membrane/envelope biogenesis<sup>c</sup>**

<b>VVMO6_03345</b>	Capsular polysaccharide synthesis enzyme CpsI	104.12	51.71	-1.01	2.34E-06
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<b>VVMO6_04183</b>	Lipoprotein	436.29	216.01	-1.01	8.75E-19
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**Posttranslational modification, protein turnover, chaperones<sup>c</sup>**

<b>VVMO6_00209</b>	Heat shock protein 60 family co-chaperone Gro	396.09	101.56	-1.96	7.93E-44
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<b>VVMO6_00210</b>	Heat shock protein 60 family chaperone GroEL	9753.06	3465.84	-1.49	1.49E-74
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<b>VVMO6_00297</b>	ATP-dependent hsl protease ATP-binding subunit HslU	2433.27	1057.85	-1.20	2.10E-53
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<b>VVMO6_00298</b>	ATP-dependent protease HslV	553.43	150.15	-1.88	9.16E-48
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<b>VVMO6_02385</b>	Chaperone protein DnaK	10047.69	4211.13	-1.25	4.91E-65
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<b>VVMO6_02477</b>	ClpB protein	6924.19	3211.64	-1.11	3.10E-59
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<b>VVMO6_02966</b>	16 kDa heat shock protein A	2949.54	632.75	-2.22	8.41E-33
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<b>VVMO6_04438</b>	Glutaredoxin 3	9.24	2.54	-1.86	2.41E-02
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<b>VVMO6_04522</b>	Heat shock protein 60 family chaperone GroEL	7113.21	2770.01	-1.36	1.23E-51
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<b>VVMO6_04523</b>	Heat shock protein 60 family co-chaperone GroES	344.60	90.14	-1.93	1.29E-32
<b>Inorganic ion transport and metabolism<sup>c</sup></b>					
<b>VVMO6_00653</b>	Chromate transport protein ChrA	102.39	50.56	-1.02	1.47E-06
<b>VVMO6_00937</b>	Lead, cadmium, zinc and mercury transporting ATPase	1652.00	799.21	-1.05	2.12E-28
<b>VVMO6_02186</b>	Ferrous iron transport protein A	10.23	2.29	-2.16	8.89E-03
<b>VVMO6_02280</b>	Lead, cadmium, zinc and mercury transporting ATPase	839.91	407.86	-1.04	4.27E-22
<b>VVMO6_02509</b>	Sodium-dependent phosphate transporter	6498.65	2385.24	-1.45	2.25E-86
<b>VVMO6_03867</b>	Nitrate ABC transporter ATP-binding protein	2859.99	995.43	-1.52	8.29E-09
<b>VVMO6_03868</b>	Nitrate ABC transporter permease protein	2815.34	668.68	-2.07	6.61E-38
<b>VVMO6_03869</b>	Nitrate ABC transporter nitrate-binding protein	4960.46	1164.12	-2.09	2.22E-27
<b>VVMO6_04095</b>	Periplasmic nitrate reductase component NapD	442.22	123.19	-1.84	6.69E-32
<b>Secondary metabolites biosynthesis, transport and catabolism<sup>c</sup></b>					
<b>VVMO6_01992</b>	Hypothetical protein	411.85	174.84	-1.24	5.60E-25
<b>General function prediction only<sup>c</sup></b>					
<b>VVMO6_00722</b>	Sodium-dependent transporter	878.74	335.98	-1.39	5.36E-48
<b>VVMO6_02367</b>	Xanthine/uracil/thiamine/ascorbate permease family protein	620.68	288.94	-1.10	1.17E-26
<b>VVMO6_04325</b>	Phospholipid-binding protein	282.59	39.67	-2.83	1.73E-48
<b>VVMO6_04520</b>	Multimeric flavodoxin WrbA	15.05	6.39	-1.24	2.32E-02
<b>VVMO6_04524</b>	Acetyltransferase	43.92	20.73	-1.08	1.02E-03
<b>Signal transduction mechanisms<sup>c</sup></b>					

<b>VVMO6_03870</b>	Putative two-component response regulatory protein	579.57	129.13	-2.17	2.77E-73
<b>Defense mechanisms<sup>c</sup></b>					
<b>VVMO6_00539</b>	ABC-type multidrug transport system	227.40	78.06	-1.54	1.19E-21
<b>VVMO6_03667</b>	Multidrug resistance protein A	178.81	72.97	-1.29	1.47E-12
<b>VVMO6_04387</b>	Membrane fusion component of tripartite multidrug resistance system	15.39	7.15	-1.11	3.76E-02
<b>Function unknown<sup>c</sup></b>					
<b>VVMO6_00408</b>	Predicted transmembrane protein	40.21	18.84	-1.09	8.44E-04
<b>VVMO6_00453</b>	Hypothetical protein	559.71	247.17	-1.18	5.74E-27
<b>VVMO6_00887</b>	Hypothetical protein	108.91	50.59	-1.11	1.3E-07
<b>VVMO6_01713</b>	Hypothetical protein	9.35	2.78	-1.75	3.05E-02
<b>VVMO6_02105</b>	Membrane protein	125.60	60.84	-1.05	2.50E-07
<b>VVMO6_03810</b>	Hypothetical protein	39.17	18.91	-1.05	1.85E-03
<b>VVMO6_04285</b>	Membrane protein	14.34	5.21	-1.46	1.24E-02
<b>VVMO6_04437</b>	Membrane protein	21.42	10.31	-1.05	1.64E-02
<b>Unclassified<sup>c</sup></b>					
<b>VVMO6_00939</b>	Hypothetical protein	824.70	317.42	-1.38	2.46E-45
<b>VVMO6_01196</b>	Hypothetical protein	167.49	58.27	-1.52	3.90E-14
<b>VVMO6_01874</b>	Hypothetical protein	236.18	99.02	-1.25	2.31E-12
<b>VVMO6_01899</b>	Hypothetical protein	18.43	8.85	-1.06	4.03E-02
<b>VVMO6_02992</b>	Permease of the major facilitator superfamily	19.07	9.16	-1.06	3.28E-02
<b>VVMO6_03146</b>	Hypothetical protein in aerobactin uptake cluster	25.32	11.93	-1.09	9.13E-03
<b>VVMO6_03548</b>	Pyruvate/2-oxoglutarate dehydrogenase	15.93	7.18	-1.15	3.15E-02

complex dihydrolipoamide dehydrogenase component					
<b>VVMO6_03986</b>	Hypothetical protein	16.61	7.29	-1.19	3.83E-02
<b>VVMO6_04154</b>	Permease of the major facilitator superfamily	40.51	19.02	-1.09	1.48E-03
<b>VVMO6_04217</b>	Hypothetical protein	10.42	3.36	-1.63	2.70E-02
<b>VVMO6_04375</b>	Acyl-CoA synthetase	175.34	82.71	-1.08	1.09E-10
<b>VVMO6_04432</b>	Hypothetical protein	20.18	9.30	-1.12	2.04E-02
<b>VVMO6_04506</b>	Hypothetical protein	9.45	2.88	-1.71	2.55E-02
<b>VVMO6_04521</b>	Hypothetical protein	28.86	9.86	-1.55	5.05E-04
<b>Antiphagocytosis<sup>d</sup></b>					
<b>VVMO6_03346</b>	Capsular polysaccharide	44.61	16.59	-1.43	2.11E-05
<b>VVMO6_03350</b>	Capsular polysaccharide	149.86	52.07	-1.53	1.36E-14
<b>VVMO6_03351</b>	Capsular polysaccharide	595.28	230.69	-1.37	1.17E-26
<b>Chemotaxis and motility<sup>d</sup></b>					
<b>VVMO6_02252</b>	Flagella	3175.42	1519.97	-1.06	4.24E-54

<sup>a</sup> Locus tags are based on the database for the *V. vulnificus* MO6-24/O genome (GenBank accession numbers: CP002469 and CP002470, <https://www.ncbi.nlm.nih.gov/>).

<sup>b</sup>  $|\text{Log}_2 \text{fold change}| \geq 1.0$ , and  $P \text{ value} \leq 0.05$ .

<sup>c</sup> Clusters of orthologous genes (COG, <http://weizhong-lab.ucsd.edu/webMGA/>) category<sup>7</sup>.

<sup>d</sup> Virulence Factor Database (VFDB, <http://www.mgc.ac.cn/VFs/>) category<sup>8</sup>.



## Supplementary References

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