1 Supplemental Information

	<- fur promoter
VC2105	TGTTGCTTTGC <mark>ATTATA</mark> GGTGAGTCACCGCGAACAATAAACCACG
VSAK1 10623	TAAT-CTATTC <mark>ATTATA</mark> GGGTACTACAGCGTAACAATAAACCTCG
VS 2257	TATT-AATACC <mark>ATTATA</mark> GTTTAGTCAGGCATAACAATAAACCACG
VV1 0174	TATT-CTGAAC <mark>ATTATA</mark> GGTTAACCAGAGATAACAATAAACCACG
VIBHAR 01340	TATT-CCTATC <mark>ATTATA</mark> GGCGAAGCGTACTGAACATTAAACCACG
VP0834	
VSAL 10834	
VF 0811	
VI_0011	
	Candidate promotor boyog for VC2105
VC2105	
VC210J	
VSARI_10025	
VS_2257	
VVI_01/4	
VIBHAR_01340	
VP0834	AACTTCAAAGGGTTAATAGAATCTTT <u>TTGTAAA</u> ATTGGAAA
VSAL_I0834	<u>TTGAAA</u> CCTTATCTGCAAGATCTCT <u>TACAAA</u> AAAATAGATG <u>TTGATC</u> TTTTGTTAATA
VF_0811	<u>TTGAAA</u> CCTTATATGCAAGATCTCT <u>TACAAA</u> AAAATAGATG <u>TTGATC</u> TTTTGTTAACAAAGAA
	*** * * * **
	FadR site
VC2105	CAAATCAC <u>TGAACA<mark>AAGTGCTCTTACCAGTT</mark>ACAAT</u> AATTTAACAACGATAGCATCATTA 4.03
VSAK1_10623	AATGTCACTTTACA <mark>AACACGTCTGACCAGTT</mark> ACAATTCGTTTACAT 4.71
VS_2257	TATCTCACT <u>TATAG<mark>TGGAGGTACGACCAGCC</mark>AGGTGACGGTG??</u>
VV1 0174	TAGGTCGCTTATAT <mark>TGCTCATCTGACCAGTT</mark> ACAATTTAATAACAA 3.53 (weak)
VIBHAR 01340	CAGGTCGATTATAT <mark>TACAGATCAGACCAGTT</mark> ACATATTGATAACAT 4.79
VP0834	TGGGTCGATTATAT <mark>TACAGATCTGACCAGTT</mark> ACAATTTTGTAACAT 4.48
VSAL I0834	ACTATT <mark>ATGAGGTCTGACCAGAA</mark> TT4.72
VF 0811	ACTATT <mark>ATGAGGTCTGACCAGAA</mark> TT
	* ****
	AACIUL CACACITI FadR consensus in Vibrio spp.
VC2105	0 Latte חיים בככם בכם היים היים היים היים היים היים היים הי
VC210J	
VSARI_10025	
VB_2237	
VVI_UI/4	
VIBHAR_UI340	TGGAACGCACTACGGAC
VPU834	GAGCTTAGAGAGGGCATGGACTGCA <mark>ATG</mark>
VSAL_10834	AAAGGGAAATAAAT <mark>ATG</mark>
AF. 0811	AAAGAG-ATTAAAT <mark>ATG</mark>

- 3 Fig.S1 Sequence alignment analyses for the promoters of the vc2105 (and/or
- 4 its equivalent) gene

- 5 The VC2105 gene is labeled in red. The translation initiation site "ATG" is in
- 6 red with green background. The predicted FadR-specific palindrome is
- 7 highlighted in yellow background. The promoter of *fur* gene on the opposite
- 8 strand and adjacent to the vc2105 (and/or its equivalent) is indicated in blue
- 9 background.



- 10
- 11 Fig.S2 Sequence alignment and structural analyses for Vibrio FadR
- 12 regulatory protein
- 13 **A.** Sequence alignment of eight *Vibrio* FadR orthologues with the prototype
- 14 version of *E. coli and Salmonella*

15	The final output of the multiple alignments of FadR protein sequences was
16	given with the program ESPript 2.2 (<u>http://espript.ibcp.fr/ESPript/cgi-</u>
17	bin/ESPript.cgi). Identical residues are white letters with red background,
18	similar residues are red letters in white background, the varied residues are in
19	grey letters, and gaps are denoted with dots. The protein secondary structure
20	was shown in cartoon (on top) (1), α : alpha-helix; β : beta-sheet; T: Turn; η :
21	coil.
22	In relative to that of <i>E. coli</i> FadR, an extra-40aa insert seen in Vibrio
23	homologues is highlighted with a blue line.
24	B. Ribbon structure of the E. coli FadR in monomer
25	C. Ribbon structure of the monomeric Vibrio FadR
26	D. Superposition of FadR structures of <i>E. coli</i> and <i>Vibrio</i>
27	
28	The crystal structure of <i>E. coli</i> FadR is generated using PDB: 1E2X, whereas
29	the counterpart of Vibrio is illustrated with V. alginolyticus FadR (PDB: 5DV1).
30	The structure (enriched in α -helix) formed by the extra-40aa insert is indicated
31	in blue. N: N-terminus; C: C-terminus.





35 putative thioesterase

- A. Multiple sequence alignment of vc2105 and its orthologues
- **B.** Ribbon structure of Pa5062, a putative thioesterase from *Pseudomonas*
- *aeruginosa* (PDB: 1SH8)
- **C.** Modeled structure of vc2105 using Pa5062 as the structural template
- **D.** Superposition of structures of vc2105 and Pa5062
- **E.** Phylogenetic tree of vc2105 and its orthologues



45 **Fig.S4** Binding of VcFadR to *plsB*

46 **A.** Gel shift assay for the interaction of the VcFadR with *plsB* promoter

47 **B.** Long chain acyl-CoA species release VcFadR from the cognate *plsB*

48 promoter region

44

49 7% native PAGE was applied into the gel shift assays, and a representative

50 result was given. In each assay of Panel A (20 μL in total), levels of VcFadR

51 were denoted with a triangle on right hand (0.1, 0.5, 2, and 5 pmol), whereas

- 52 the DIG-labeled *plsB* probe is around 0.2 pmol. In **Panel B**, the VcFadR
- 53 protein (~5 pmol) was incubated with the DIG-labeled *plsB* probe (0.2 pmol).
- 54 When required, acyl-CoA species of varied acyl length (~50 pmol) were

- 55 supplemented. The super-shifted DNA probe band is indicated with a triangle.
- 56 Minus sign denotes no addition of either FadR protein or acyl-CoA.



59 **Fig.S5** Oleate does not significantly induce expression of *V. cholerae fur*

60 The strain used here refers to FYJ379 with a *fur_*vc-lacZ transcriptional fusion

61 integrated on chromosome. Overnight cultures were collected for

62 measurement of LacZ activity. The data was expressed in average ± standard

63 deviation (SD).

65 Supplemental references

- 1. Feng Y, Li M, Zhang H, Zheng B, Han H, Wang C, Yan J, Tang J, Gao GF. 2008.
- Functional definition and global regulation of Zur, a zinc uptake regulator in a *Streptococcus suis* serotype 2 strain causing streptococcal toxic shock syndrome. Journal of bacteriology
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