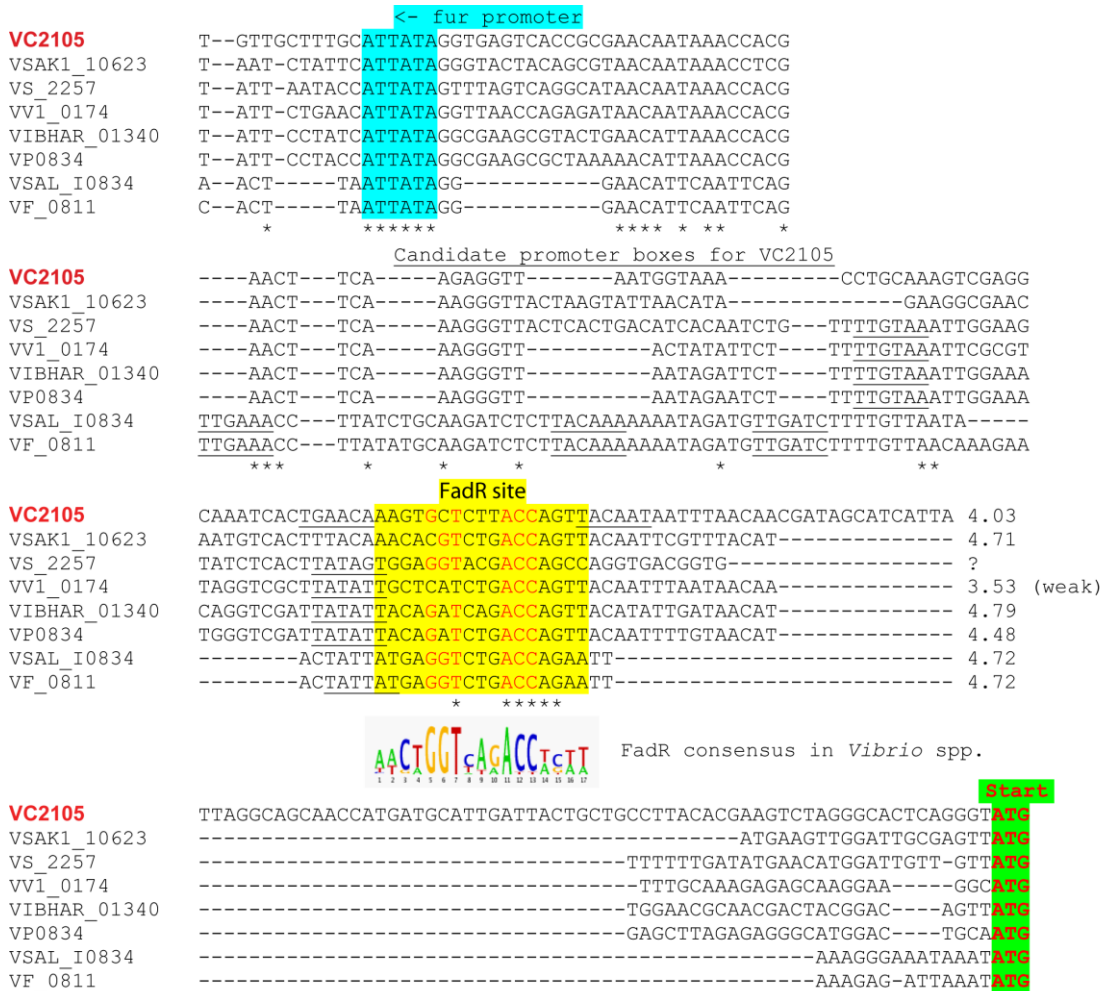


1 **Supplemental Information**



2

3 **Fig.S1** Sequence alignment analyses for the promoters of the *vc2105* (and/or

4 its equivalent) gene

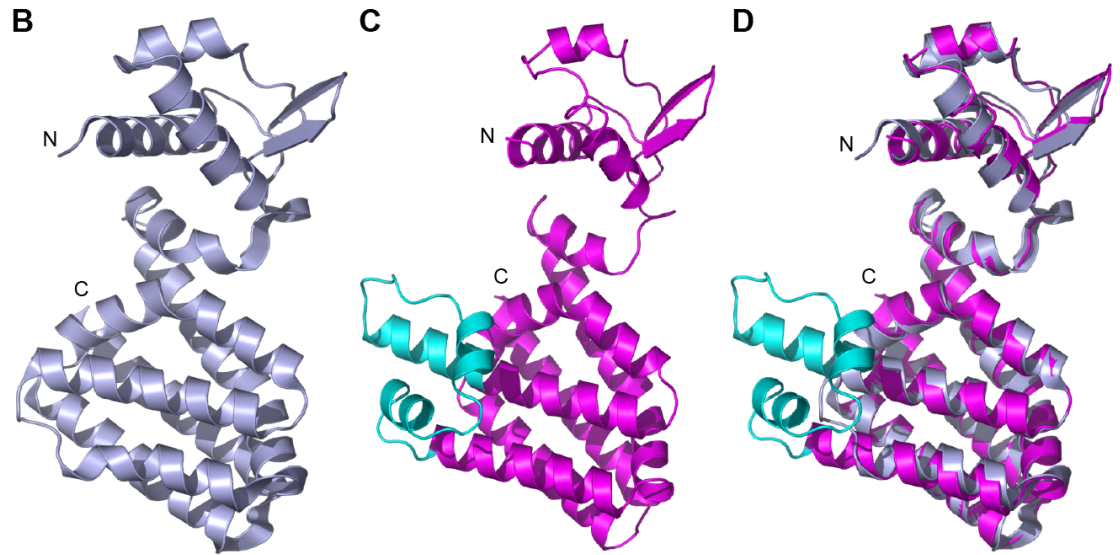
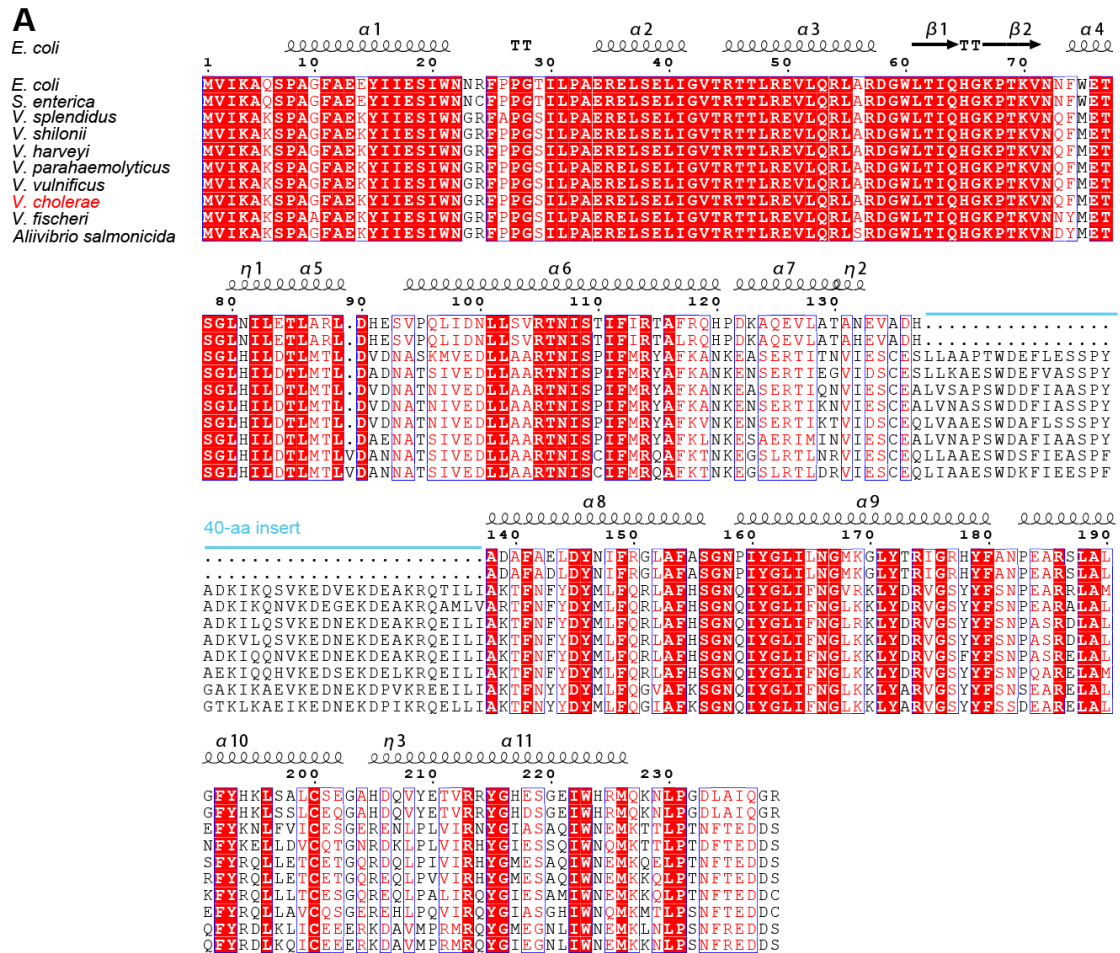
5 The *VC2105* gene is labeled in red. The translation initiation site “ATG” is in

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 ([http://espript.ibcp.fr/ESPript/cgi-](http://espript.ibcp.fr/ESPript/cgi-bin/ESPript.cgi)
17 [bin/ESPript.cgi](http://espript.ibcp.fr/ESPript/cgi-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 *E. coli* 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

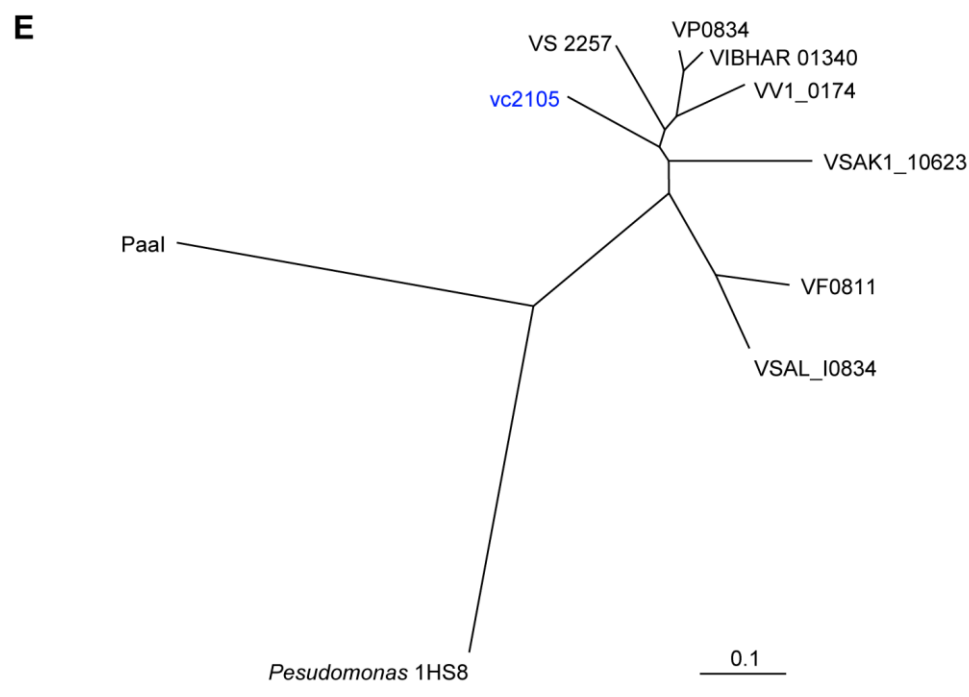
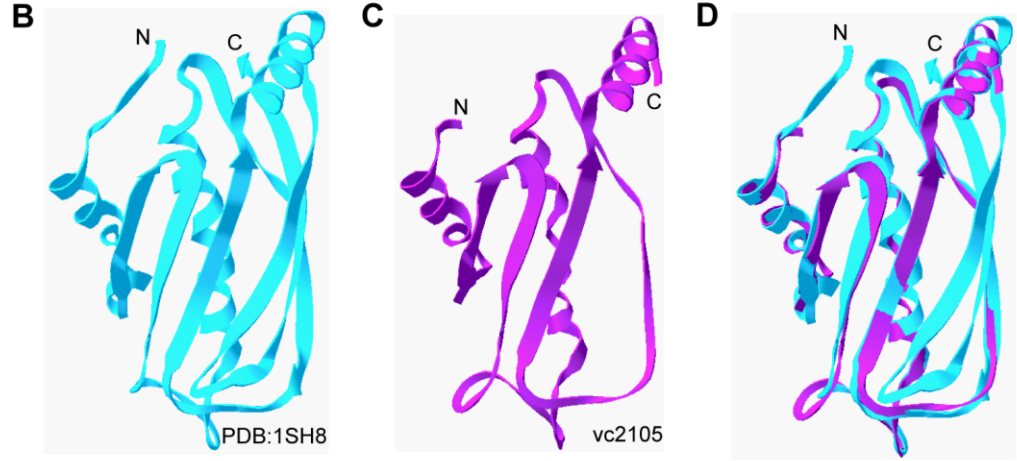
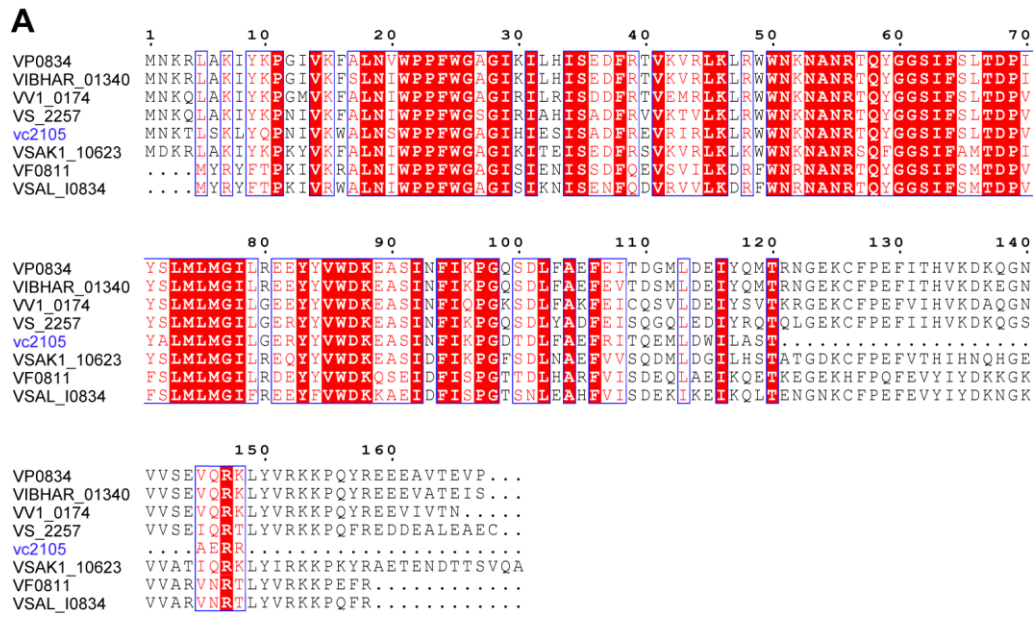
26 **D.** Superposition of FadR structures of *E. coli* and *Vibrio*

27

28 The crystal structure of *E. coli* 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.

32



33

34 **Fig.S3** Bioinformatics analyses of vc2105 (and/or its equivalents), encoding a

35 putative thioesterase

36

37 **A.** Multiple sequence alignment of vc2105 and its orthologues

38 **B.** Ribbon structure of Pa5062, a putative thioesterase from *Pseudomonas*

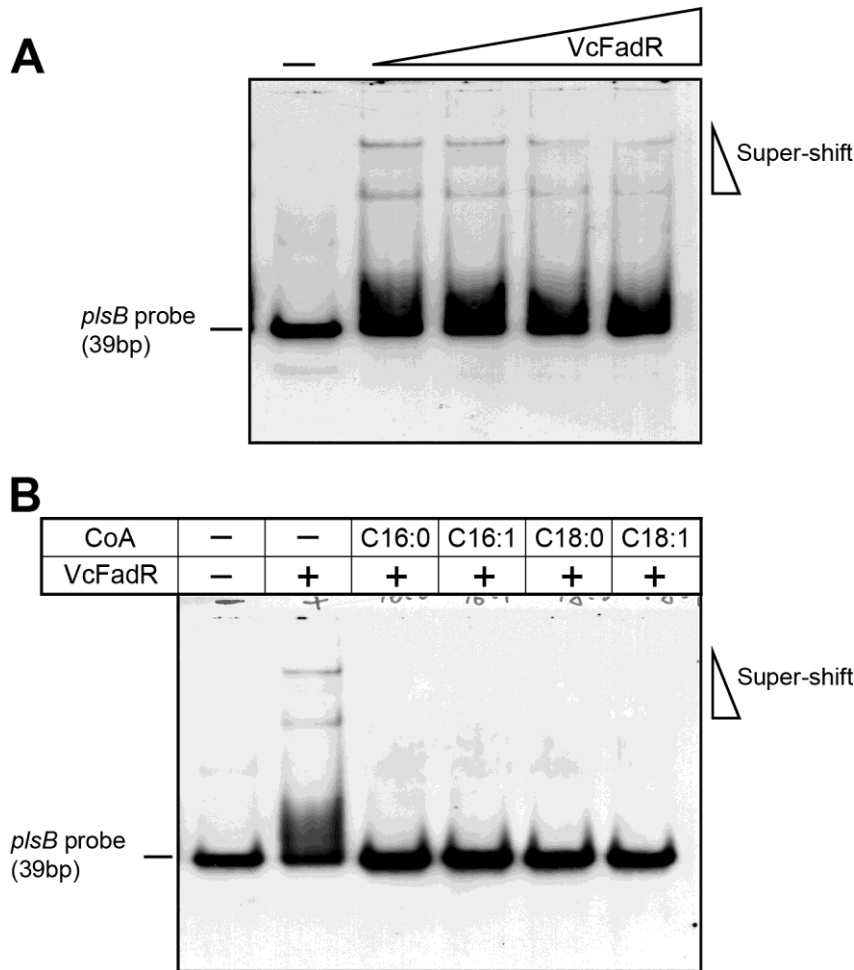
39 *aeruginosa* (PDB: 1SH8)

40 **C.** Modeled structure of vc2105 using Pa5062 as the structural template

41 **D.** Superposition of structures of vc2105 and Pa5062

42 **E.** Phylogenetic tree of vc2105 and its orthologues

43



44

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

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

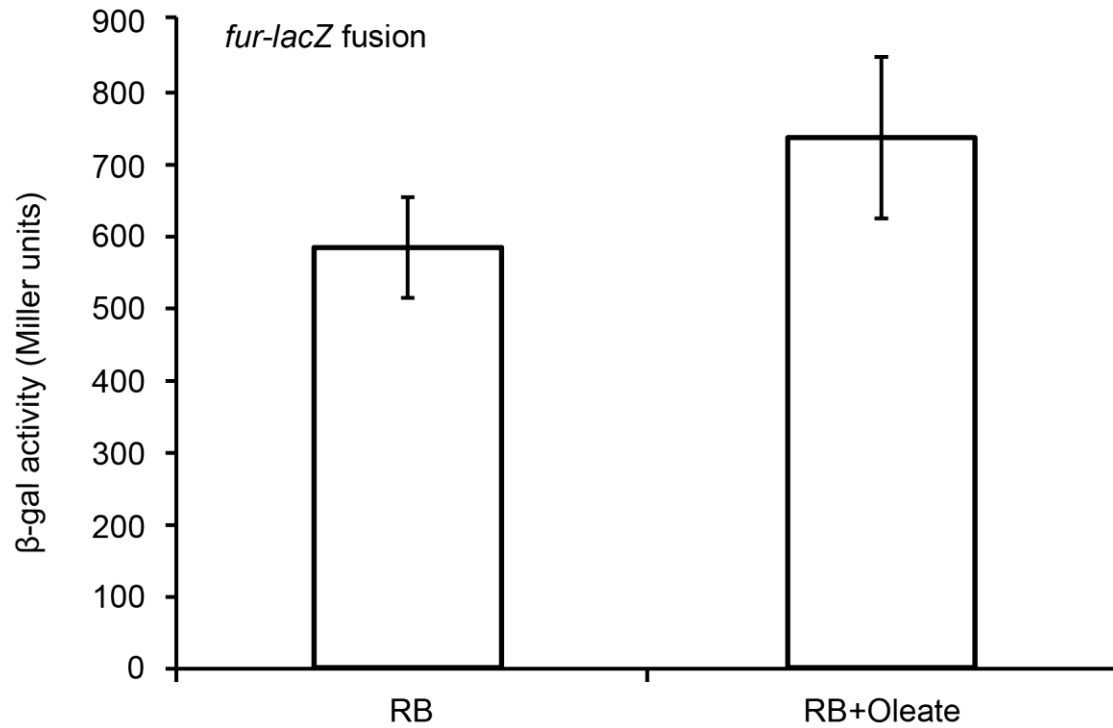
47 **B.** Long chain acyl-CoA species release VcFadR from the cognate *pIsB*
 48 promoter region

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 μ L 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 *pIsB* probe is around 0.2 pmol. In **Panel B**, the VcFadR
 53 protein (\sim 5 pmol) was incubated with the DIG-labeled *pIsB* probe (0.2 pmol).
 54 When required, acyl-CoA species of varied acyl length (\sim 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.

57



58

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 \pm standard

63 deviation (SD).

64

65 **Supplemental references**

- 66 1. **Feng Y, Li M, Zhang H, Zheng B, Han H, Wang C, Yan J, Tang J, Gao GF.** 2008.
67 Functional definition and global regulation of Zur, a zinc uptake regulator in a *Streptococcus*
68 *suis* serotype 2 strain causing streptococcal toxic shock syndrome. *Journal of bacteriology*
69 **190:7567-7578.**

70