

SUPPLEMENTARY INFORMATION

***Vibrio cholerae* senses human enteric α -defensin 5 through a CarSR two-component system to promote bacterial pathogenicity**

Yutao Liu^{1, #}, Tingting Xu^{2, #}, Qian Wang¹, Junxi Huang², Yangfei Zhu³, Xingmei Liu¹,
Ruiying Liu¹, Bin Yang^{1, *}, Kai Zhou^{2, *}

¹ TEDA Institute of Biological Sciences and Biotechnology, Nankai University,
TEDA, Tianjin 300457, P. R. China;

² Shenzhen Institute of Respiratory Diseases, Shenzhen People's Hospital (The Second
Clinical Medical College, Jinan University; The First Affiliated Hospital, Southern
University of Science and Technology), Shenzhen 518020, Guangdong, P. R. China;

³ Laboratory Department of Shenzhen People's Hospital (The Second Clinical Medical
College, Jinan University; The First Affiliated Hospital, Southern University of Science
and Technology), Shenzhen 518020, Guangdong, P. R. China

[#] Yutao Liu, Tingting Xu contributed equally to this work.

^{*} To whom correspondence may be addressed. E-mail: yangbin@nankai.edu.cn or

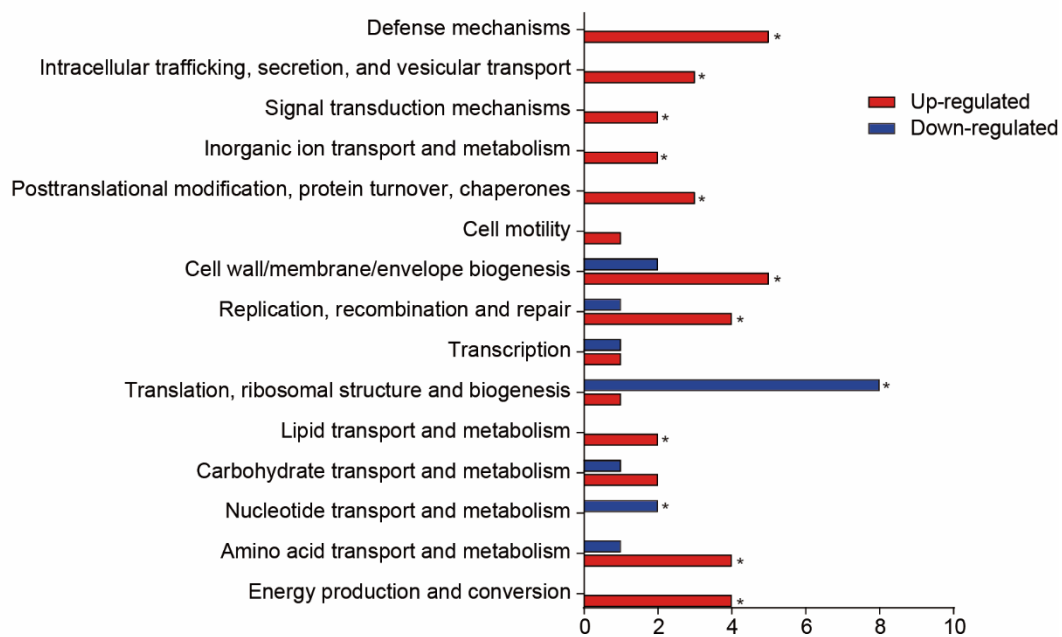
Kai_Zhou@zju.edu.cn

This Supplementary Information includes:

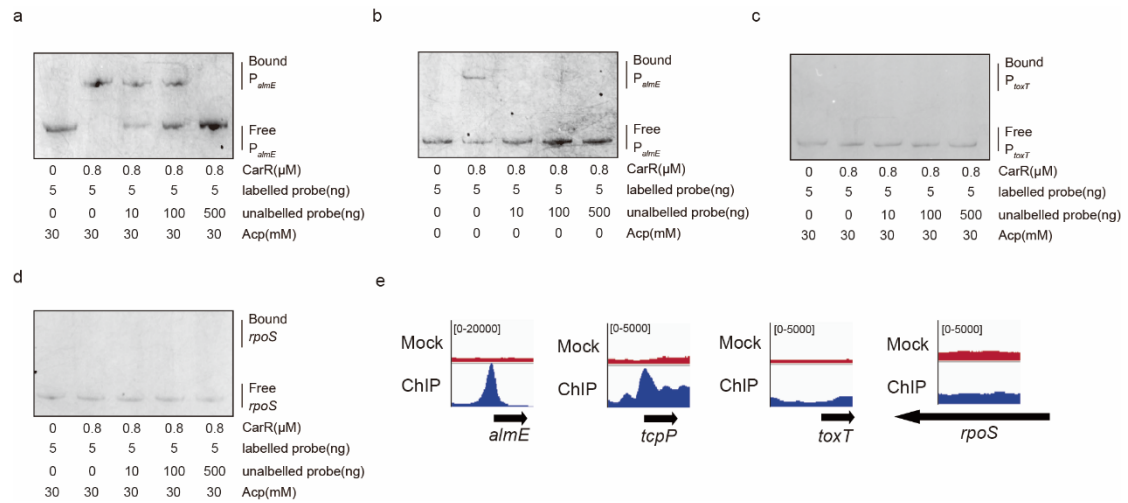
Supplementary Figures 1 to 4

Supplementary Tables 1 to 2

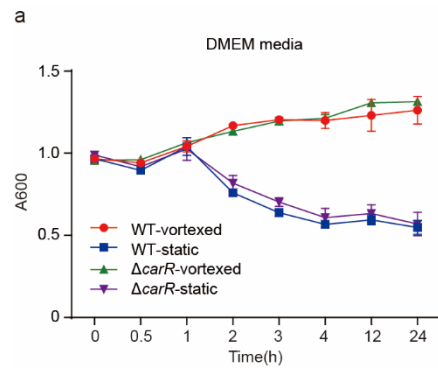
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Supplementary Figure 1. (a) COG analysis of HD-5-induced genes in *V. cholerae*. Bars represent the number of upregulated (red) or downregulated (blue) genes. The significant enrichment of a given COG in the sets of up- or downregulated genes was determined using Fisher's exact test. COG categories that are significantly enriched are indicated by asterisks ($P < 0.05$).



Supplementary Figure 2. (a, b) CarR bound to P_{almE} with (a) or without (b) 30mM acetyl phosphate. (c, d) CarR bound to P_{toxT} and rpoS with 30mM acetyl phosphate (negative control). (e) Original sequence peaks of the CarR binding regions in the P_{almE}, P_{tcpP}, P_{toxT}, rpoS according to ChIP-seq analyses.



Supplementary Figure 3. (a) Autoaggregation assays of CarR. Data are presented as mean $\pm$ SD (n = 3).

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CTGAGAACTAAGGCTAGATATAGATTTTGTGGTCTGATAGTTTCGAGTGATAGAAAAAGGC
AGTAAAAGCCAACGTAATGATGCACGCAAGTAATAAAAAAACCGAAATTATTTTTTTTATCATT
AGTTTAACTCTAAGTTTAAATGGTTATCACGGAGTACTTCGTGATAATTAGTTAAAAATGAAATT
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ACCAAGTTAATAGATATGGAATAGGCACTATAGGGGGGAGTGCTAAACCGAATGAATTATAATG
AGAATTACTTTCTGATATCGTGACTGGTTATTTTTTTAATGCTGTTTTATTTTTTTGAATGGCTT
CarR binding site      CRP binding site
GGTTATTATGCAATTAAGTTCTCATTATCAACTGCAGAATTAGATTGCAAATAATTATATAAAAAA
AphA binding site      AphB binding site      HapR binding site
AATAGACATAAAAAAAATGGGTAGTTATTATTAAGAAAATGTAAAGTAATGGGGTATGTCCGCGT
└─┬─┘ tcpP translation start

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Supplementary Figure 4. (a) Promoter organization of *tcpP*. Sequences were derived from *V. cholerae* EL2382. The translation start site of *tcpP* is indicated by bent arrows. The binding sites of different regulators in the promoter region of *tcpP* are underlined with solid lines. Small RNA TarA is outlined in blue.

Supplementary Tables

Supplementary Table 1. Strains and plasmids used in this study.

Strains	Genotype or description	Source
E12382	<i>Vibrio Cholerae</i> O1 El Tor strain E12382	Shanghai CDC*
S-17	<i>E. coli</i> S17-1/ λ pir strain	Lab collection
DH5 α	<i>E. coli</i> DH5 α / λ pir strain	Lab collection
$\Delta carR$	<i>carR</i> deletion mutant in E12382	This work
$\Delta tcpP$	<i>tcpP</i> deletion mutant in E12382	This work
$\Delta carR\Delta tcpP$	<i>tcpP</i> deletion mutant in $\Delta carR$	This work
$\Delta carR\Delta lacZ$	<i>carR</i> deletion mutant in $\Delta lacZ$	This work
$\Delta ctxAB$	<i>ctxAB</i> deletion mutant in E12382	This work
WT (pBAD33)	E12382 containing pBAD33	This work
$\Delta lacZ$ (pBAD33)	$\Delta lacZ$ containing pBAD33	This work
$\Delta carR^+$	$\Delta carR$ containing pBAD33- <i>carR</i>	This work
RstA-ChIP	$\Delta carR$ containing pBAD33- <i>carR</i> -3 $\times$ FLAG	This work
GV26	BL21(DE3) containing pET28a- <i>carR</i>	This work
Plasmids		
pBAD33	Bacteria expression vector, CmR	Lab collection
pRE112	Suicide vector for mutation, CmR	Lab collection
pET28a	T7 expression vector, KmR	Lab collection
pBAD33- <i>carR</i>	pBAD33 carrying <i>rstA</i> from E12382, CmR	This work
pBAD33- <i>carR</i> -FLAG	pBAD33 carrying <i>rstA</i> -3x FLAG from E12382, CmR	This work

*, Shanghai CDC, Shanghai Municipal Center for Disease Control & Prevention, China.

Supplementary Table 2. Primers used in this study (5'-3').

Primers for gene mutation		
<i>carR</i>	P1	GCTCTAGACATCCAAATCGAGCGCTTTACCGA
<i>carR</i>	P2	GGTTTCCTTTAAATCAACTTAGGTGGGGACCTCGTATTTACGGTTTAT
<i>carR</i>	P3	ATAAACCGTAAATACGAGGTCCCCACCTAAGTTGATTAAAGGAAAC
<i>carR</i>	P4	CGGAGCTCTTACGGATGAGGTTAACCAATGCC
<i>tcpP</i>	P1	GGGGTACCAAAAGCCAACGTAATGATGCACGC
<i>tcpP</i>	P2	GACCTGTAGCGCCCCCCCCAAGCTTTATTTACATTTTCTTAATAATAA
<i>tcpP</i>	P3	TTATTATTAAGAAAATGTAAAGTAAAGCTTGGGGGGCGCTACAGGTC
<i>tcpP</i>	P4	TCCCCCGGGCAACCGCGAACATTAGGGTAAAGA
<i>lacZ</i>	P1	CGGGATCCAGACCGGGAGCTAACGTA
<i>lacZ</i>	P2	GGCTTATTGTGGATCGGAGAAGTTGCGCATAAG
<i>lacZ</i>	P3	CCACAATAAGCCAGAGAGCCT
<i>lacZ</i>	P4	GTCTCGAGGATCGCTGTATTTCACTCG
<i>ctxAB</i>	P1	GGGGTACCCTACCACAGCGGACAGGCTCTA
<i>ctxAB</i>	P2	TTAAACAAAGGGAGCATTATGATATAAAAAAGCCCACCTC
<i>ctxAB</i>	P3	GAGGTGGGCTTTTTTATATCATAATGCTCCCTTTGTTTAA
<i>ctxAB</i>	P4	TCCCCCGGGCAGTCAAAGGCTACTGTTGGGAA
Primers for pBAD33 identifying		
pBAD33	F	ATGCCATAGCATTTTTATCC
pBAD33	R	GATTTAATCTGTATCAGG
Primers for complement construction		
<i>carR</i>	F	CGAGCTCATGTCTAACCAACCCAGTCTCT
<i>carR</i>	R	CTCTAGATTACCAAGTGTGTCAGGCACAAATA
Primers for ChIP strain construction		
<i>carR</i>	F	CGGAGCTCATGTCTAACCAACCCAGTCTCT
<i>carR</i>	R	CTCTAGATTACTATTTATCGTCGTCATCTTTGTAGTCGATATCATGAT CTTTATAATCACCGTCATGGTCTTTGTAGTCCCAAGTGTGTCAGGCACAA
Primers for protein purification		
<i>carR</i>	F	CATGCCATGGCCATGTCTAACCAACCCAGTCTCT
<i>carR</i>	R	GGCTCGAGCCAAGTGTGTCAGGCACAA
Primers for DNase I foot-printing		
<i>P_{tcpP}</i>	F	TGGAATAGGCACTATAGGGG
<i>P_{tcpP}</i>	R	GGGAAATTGATAAATCACGC
Primers for EMSA		
<i>P_{almE}</i>	F	GATGTTGCGTCTATTGGC
<i>P_{almE}</i>	R	CTGTTTAACCCATAATGCAG
<i>P_{tcpP}</i>	F	TGGAATAGGCACTATAGGGG
<i>P_{tcpP}</i>	R	GGGAAATTGATAAATCACGC
<i>P_{toxT}</i>	F	AAATTCTAATTATAAAAACGCAAAT
<i>P_{toxT}</i>	R	TGCGTTCTACTCTGAAGATATATA

<i>rpoS</i>	F	CGTGCGGTTGAGAAATTCGA
<i>rpoS</i>	R	TGCCTTATCTCCATCCCCAC
Primers for qRT-PCR		
<i>rrsA</i>	F	ACCTTACCTACTCTTGACATCCA
<i>rrsA</i>	R	CCCAACATTTACACAACACGAG
<i>carR</i>	F	AACCAAAGCCGACGCCACTG
<i>carR</i>	R	CCATCATACTCAATCCCCCGAAG
<i>carS</i>	F	GCGAGCATTATTGAGGACACGGA
<i>carS</i>	R	AAGGGTTGCAGAGAAGGGAGGT
<i>tcpP</i>	F	ATGGGGTATGTCCGCGTGAT
<i>tcpP</i>	R	TTTGGACAGGGGGCAGGATG
<i>toxT</i>	F	CGTTGGGCAGATATTTGTGGTG
<i>toxT</i>	R	CACTTGGTGCTACATTCATGGTTG
<i>tcpA</i>	F	CATTCGCAATTACAGTCGGTGG
<i>tcpA</i>	R	CAAAGCTTCTCAACATGCGTGA
<i>ctxA</i>	F	CTCAGACGGGATTTGTTAGGC
<i>ctxA</i>	R	CTATCTCTGTAGCCCCTATTACG
<i>almE</i>	F	AACAGATCCAAGAGACGCTGA
<i>almE</i>	R	GACGTAACGGTTCCACATCC
Primers for ChIP-qRT		
<i>P_{vc1318}</i>	F	TGCCAGTAAACAAGCAGTGA
<i>P_{vc1318}</i>	R	CGAATGTAAGTTTGGGCAGC
<i>P_{vca1078}</i>	F	CGCGAGCTGTAATGTTGACT
<i>P_{vca1078}</i>	R	TGCGGCGATATGATACCAAG
<i>P_{vc0973}</i>	F	AGTTGGTTAGATTTGCCCTGG
<i>P_{vc0973}</i>	R	GCACCCAGAAGAGTACGTTT
<i>P_{vc0633}</i>	F	ATTAGATTGCGTGCAATTTG
<i>P_{vc0633}</i>	R	AGCAATCAGAGTCTTGTTTCATCT
<i>P_{vca0227}</i>	F	GGCTCAATGGGACTGGAAAC
<i>P_{vca0227}</i>	R	GCAGCCCAATGAATACGAGA
<i>P_{almE}</i>	F	TGAACAGTTAGGGTTAGCGGT
<i>P_{almE}</i>	R	ATGGCATTGTCAGCTACCGA
<i>P_{tcpP}</i>	F	GGGGCAAATGTCACAGGAA
<i>P_{tcpP}</i>	R	TGATAAATCACGCGGACATACC
<i>P_{toxT}</i>	F	CAAGTGGTCAAATACTATGTTCTCA
<i>P_{toxT}</i>	R	TCCCAATCATTGCGTTCTACTC
<i>rpoS</i>	F	CGTGCGGTTGAGAAATTCGA
<i>rpoS</i>	R	TGCCTTATCTCCATCCCCAC