PNAS

Supporting Information for

A peptide-binding domain shared with an Antarctic bacterium facilitates *Vibrio cholerae* human cell binding and intestinal colonization

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Supporting Information Text

Plasmid construction:

Plasmid pKEK2100 was created by PCR amplification of the R6K ori and oriT from pCVD442 (1) with primers R6K oriT F and R6K oriT R, and cloning via In Vivo Assembly (IVA) (2) into pKEK2094 PCR amplified with pUC118 ori F and pUC118 ori R. ΔPBD, ΔRIII_4, ΔIGL, ΔSBD, ΔUKD, and Δ5D deletion constructs were synthesized as ds DNA with approximately 600 bp flanking the deleted domain coding sequence (IDT), which were as indicated in Fig. S1. The Δ5D construct was PCR amplified with the Universal Δ Up and Universal Δ Dwn primers listed in Table S1, then digested with *NotI* and *ApaI* and ligated into pKEK229 digested similarly to form pKEK2054. The constructs ΔPBD, ΔRIII_4, ΔIGL, and ΔSBD were PCR amplified with the corresponding primers listed in Table S1 and cloned via IVA (2) into pKEK2100 PCR amplified with primers pUC118 MCS F and pUC118 MCS R. These fragments were then digested with NotI and ApaI and ligated into pKEK229 digested similarly to form pKEK2119 (ΔSBD), pKEK2120 (ΔIGL), pKEK2121 (ΔPBD), and pKEK2124 (ΔRIII_4). The ΔUKD construct was PCR amplified with ΔUKD F and ΔUKD R, and cloned via IVA (2) into pKEK2200 PCR amplified with primers pUC118 MCS F and pUC118 MCS R to form pKEK2157 (ΔUKD).

The deletions in *frhB* and *frhD* were constructed by Splicing by Overlap Extension (3) with the primers listed in Table S1, and the resulting deletion fragments were inserted into pKEK2100 PCR-amplified with primers pUC118 MCS F and pUC118 MCS R by IVA (2), resulting in pKEK2110 and pKEK2114. These plasmids were then digested with NotI and ApaI, and ligated into pKEK229, resulting in plasmids pKEK2122 (Δ*frhB*) and pKEK2123 (Δ*frhD*). The deletions in *lapD* and *lapG* in strains FY_VC_12120 and Fy_VC_11863 were PCR-amplified with the indicated primers and cloned by IVA (2) into pKEK2200 PCR-amplified with primers pUC118 MCS F and pUC118 MCS R to create pKEK2265 and 2266 respectively.

The Mp PBD was synthesized as a 620 bp ds DNA fragment (IDT), then PCR-amplified with primers MpIBP RIII-3 F and MpIBP RIII-3 R. This fragment was inserted via IVA (2) into pKEK2121 PCR-amplified with primers RIII-3VC>MP F and RIII-3VC>MP R, resulting in pKEK2335, which has the Vc PBD replaced with Mp PBD ($frhA^{MpPBD}$). pKEK2121 was also PCR-amplified with RIII-3VC>AbR F and RIII-3VC>AbR F, and a KanR fragment PCR-

amplified from SAD034 with primers ABD123 and ABD124 (4); these were assembled by IVA (2) resulting in pKEK2447, which replaces PBD with KanR.

***V. cholerae* strain construction:**

The *V. cholerae* mutants were generated through allelic exchange using the plasmids pKEK229 (5) or pKEK2200 (6) via *sacB* counterselection. The Vc strain expressing $FrhA^{MpPBD}$ was created by first introducing *frhA*::KanR onto the Vc chromosome from pKEK2447, resulting in KKV3451. The $FrhA^{MpPBD}$ in pKEK2335 was then introduced into the KKV3451 chromosome by replacing KanR, resulting in KKV3540.

Purification and Labelling of FrhA-PBD:

FrhA-PBD and its attendant split domain, which correspond to the red and orange domains in Figure 1B, respectively, spanning amino acids 1,155-1,460, were produced together as one recombinant protein. The codon-optimized DNA (GeneArt) for this 35-kDa construct was cloned between the *Nde*I and *Xho*I sites of the pET28a plasmid vector and transfected into *E. coli* BL-21 (DE3). After IPTG-induction of the *E. coli* culture, cells were harvested and lysed by sonication as previously described (7). FrhA-PBD was purified by affinity chromatography on nickel-NTA and size-exclusion chromatography on a HiLoad 16/60 Superdex-75 size-exclusion column (GE Healthcare) with a yield of 5 mg/L of culture.

FrhA-PBD in HEPES buffer (pH 8.5) was incubated in covered tubes for 2 h with fluorescein-5-isothiocyanate (FITC) or tetramethylrhodamine (TRITC) at a 20:1 molar ratio. The mixtures were then passed through a size-exclusion column equilibrated with 20 mM Tris-HCl (pH 9.0), 200 mM NaCl, 5 mM $CaCl_2$ followed by elution with the same buffer.

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aacctagcatctataattcagattaaagaagactag
N L A S I I Q I K E D –

Fig. S1A. Amino acid sequence of FrhA *V. cholerae* O395 *frhA* gene (VC395_1738) and predicted amino acid sequence. The LapG cleavage site PAAG and the "RTX repeat" (COG2931) are in bold, as well as aa 884-1460, which were used to generate polyclonal αFrhA. The red highlighted nucleotides corresponding to aa 1155-1350 were deleted in ΔPBD, the grey highlighted nucleotides corresponding to aa 1351-1460 were deleted in ΔRIII_4, the yellow highlighted nucleotides corresponding to aa 1461-1659 were deleted in ΔIGL, the green highlighted nucleotides corresponding to aa 1677-1853 were deleted in ΔSBD, and the blue highlighted nucleotides corresponding to aa 1854-2016 were deleted in ΔUKD. The Δ5D mutant has a deletion corresponding to aa 1155-2016.

```
CLUSTAL O(1.2.4) multiple sequence alignment

MPPBD      eefevseiaaswvsythgesvttfdgts--dlggvdndsakdqirwgnpaeskqsgygfi     58
VCPBD      DSFTVSGVVANWTSWSNGTNVTTFDGTNAPNGGGLDNDSGKDQIRWGQPASSYSSGYGFI     60
           :.* ** :.*.*.*:::* .*******.  : **:****.*******:**.* .******

MPPBD      dndsnlegrfdlnqdisvgtfthynypvysggaitsaemsvefsvldhlgvstpvtltvn     118
VCPBD      DNDSALNGEFALNQDIILGTFTHYNYPVYSGGAITSASMDVAFSVTDAHGVLTPVTLKLN     120
           **** *:*.* ***** :*******************.*.* *** *  ** *****.:*

MPPBD      fdhnetpntndvnasrdivtvqnthvtferdgdiytvqivgfrevgnpdgevvtsiytne     178
VCPBD      FDHNETPNTNNPEASKDIIKVGNTNVTFENAGALYTLQVIGFRIPGT--NQIVTEIRTGE     178
           **********: :**:**:.* **:****. * :**:*::***  *.  .::**.* *.*

MPPBD      naatsyelvvrvvegdgy  196
VCPBD      NATNSYELVVRVGPGEGY  196
           **:.********  *:**
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Fig. S1B. Amino acid alignment of FrhA-PBD and *Mp*lBP-PBD. These are the exact amino acids replaced in FrhA^{MpPBD}. Conserved residues are noted by asterisk, conserved residues in the ligand-binding site highlighted in red.

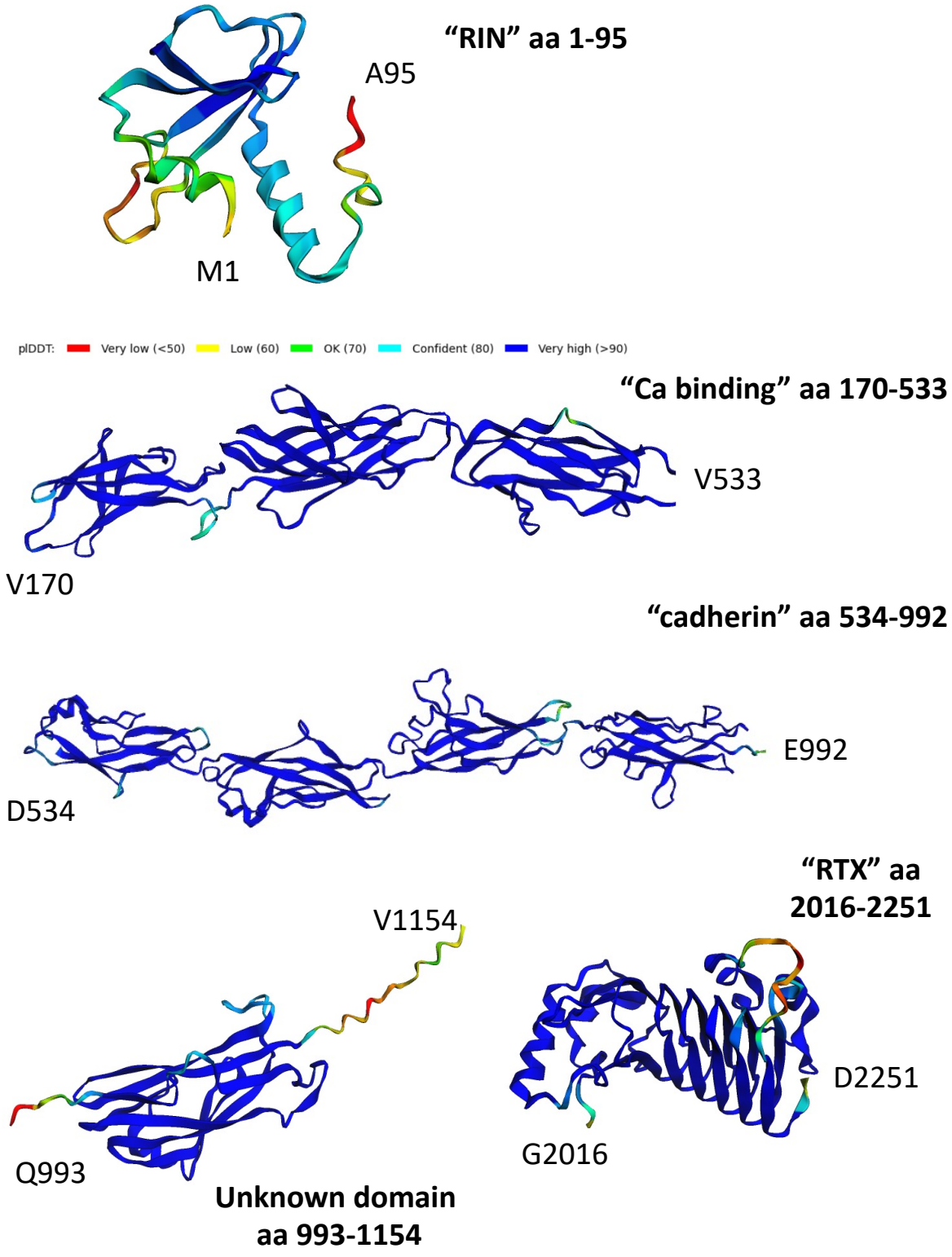
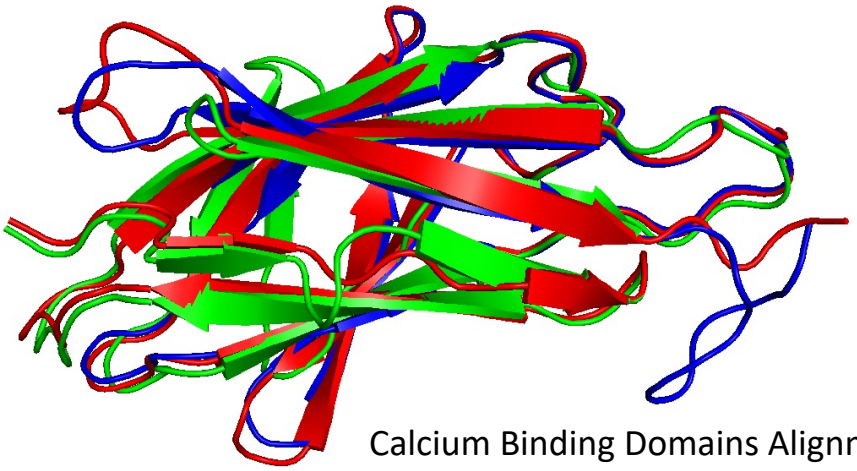
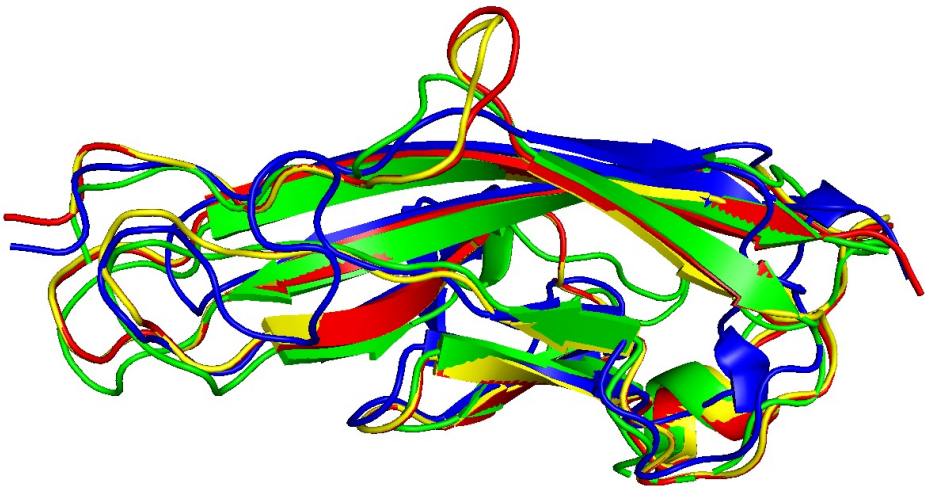


Fig. S2.A. AlphaFold predictions of various domains of FrhA



Calcium Binding Domains Alignment
R->G->B = N -> C Terminal appearance



Cadherin Domains Alignment
Y->R->G->B = N -> C Terminal appearance

Fig. S2.B. Structural alignment of predicted calcium binding domains and cadherin domains.

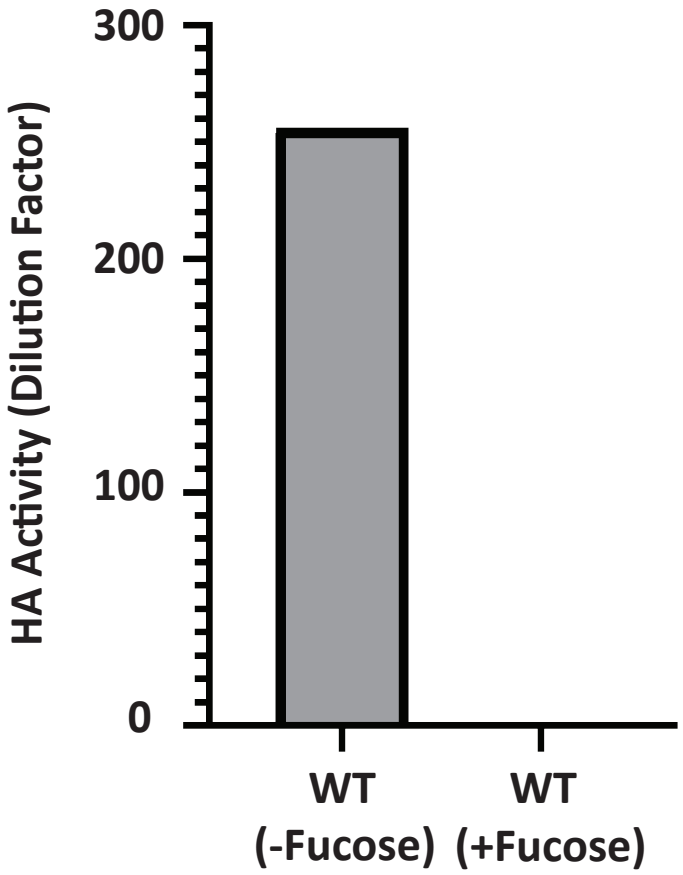


Fig. S3: Fucose inhibits *V. cholerae* HA activity. Hemagglutination of human O erythrocytes by *V. cholerae* strain KKV598 (WT) either without or with the addition of 100 mM fucose.

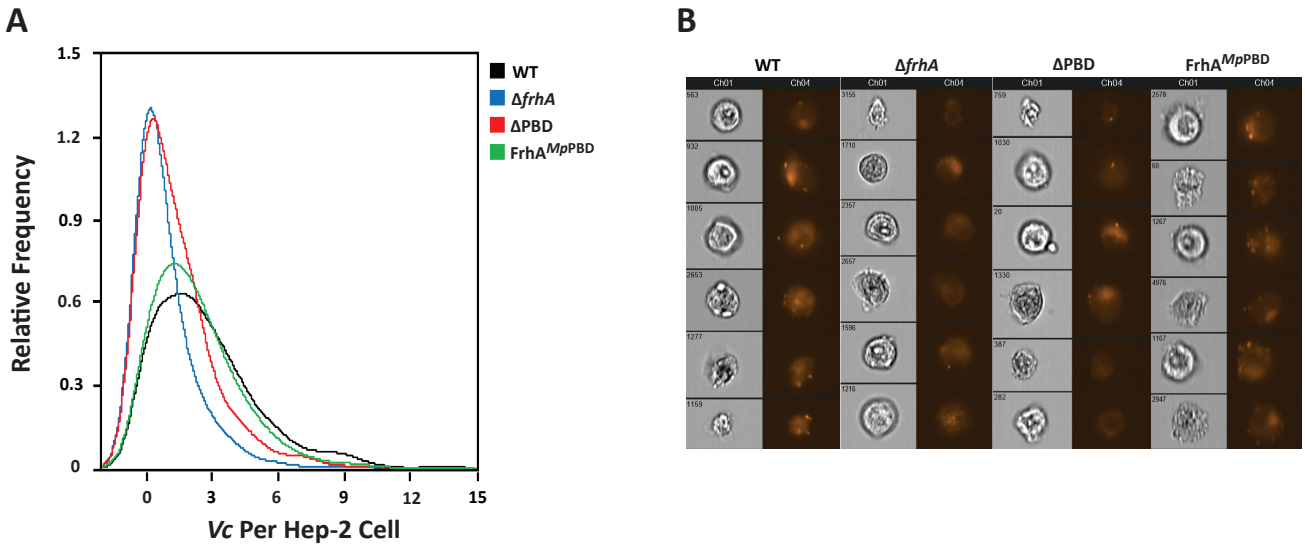


Fig. S4: *V. cholerae* binding to Hep-2 cells. **A.** Histogram of V. cholerae cells/Hep-2 cell as measured by Image Flow Cytometry; corresponds to Fig. 2A. **B.** Representative Hep-2 cells; corresponds to Fig. 2A and Fig. S4A.

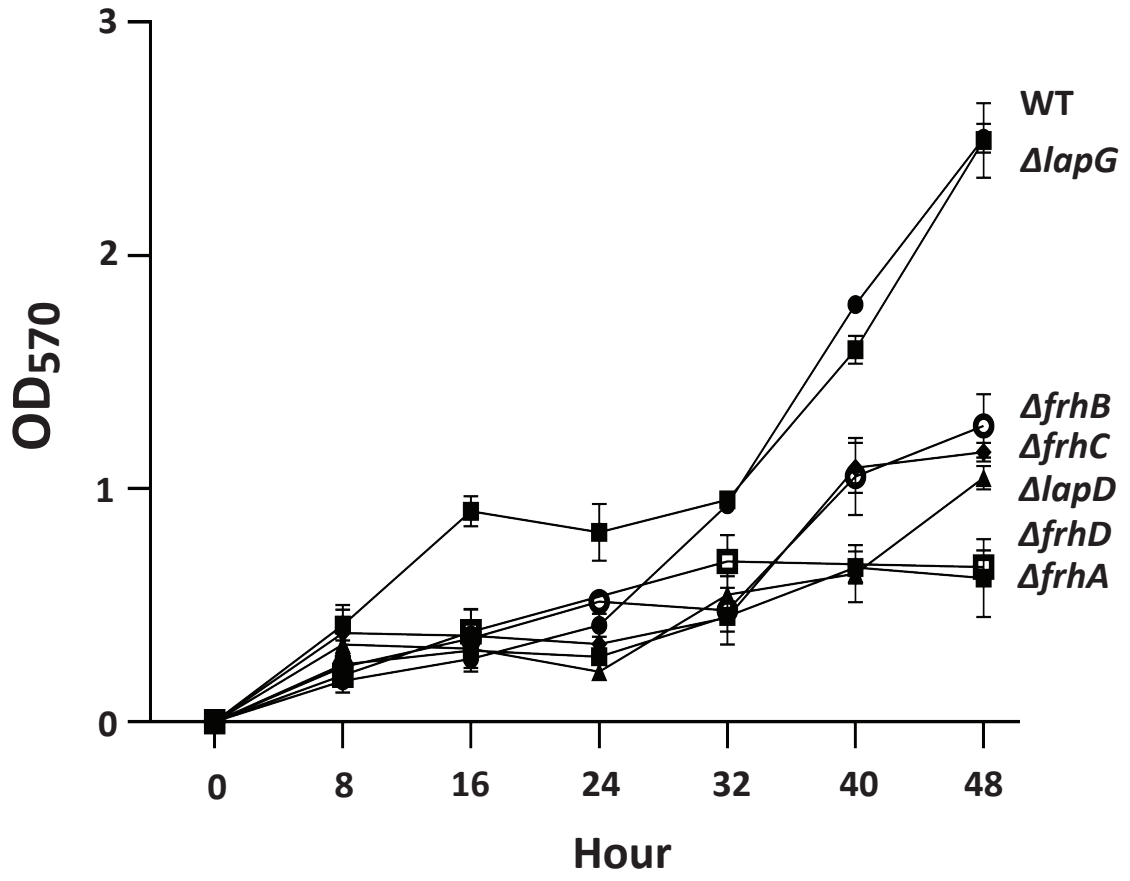


Fig. S5: Biofilm formation by *V. cholerae frhBCD* strains. *V. cholerae* strains KKV598 (WT), KKV3540 KKV3002 (Δ*frhA*), KKV3032 (Δ*frhB*), KKV2075 (Δ*frhC*), KKV3033 (Δ*frhD),* KKV2956 (Δ*lapD)* and KKV2957 (Δ*lapG*) were monitored for biofilm formation over 48 h, as described in Methods**.**

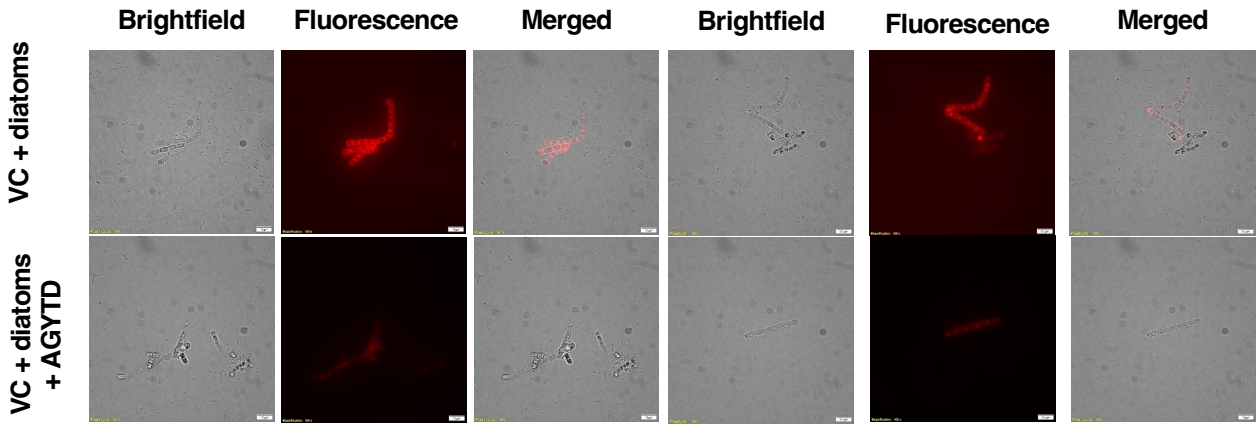


Fig. S6A: *V. cholerae* binds to diatoms via PBD: RFP-expressing *V. cholerae* were incubated with the diatom *E. spinifer* for 24 h, either without or with 500 mM AGYTD, and imaged by brightfield and fluorescence microscopy.

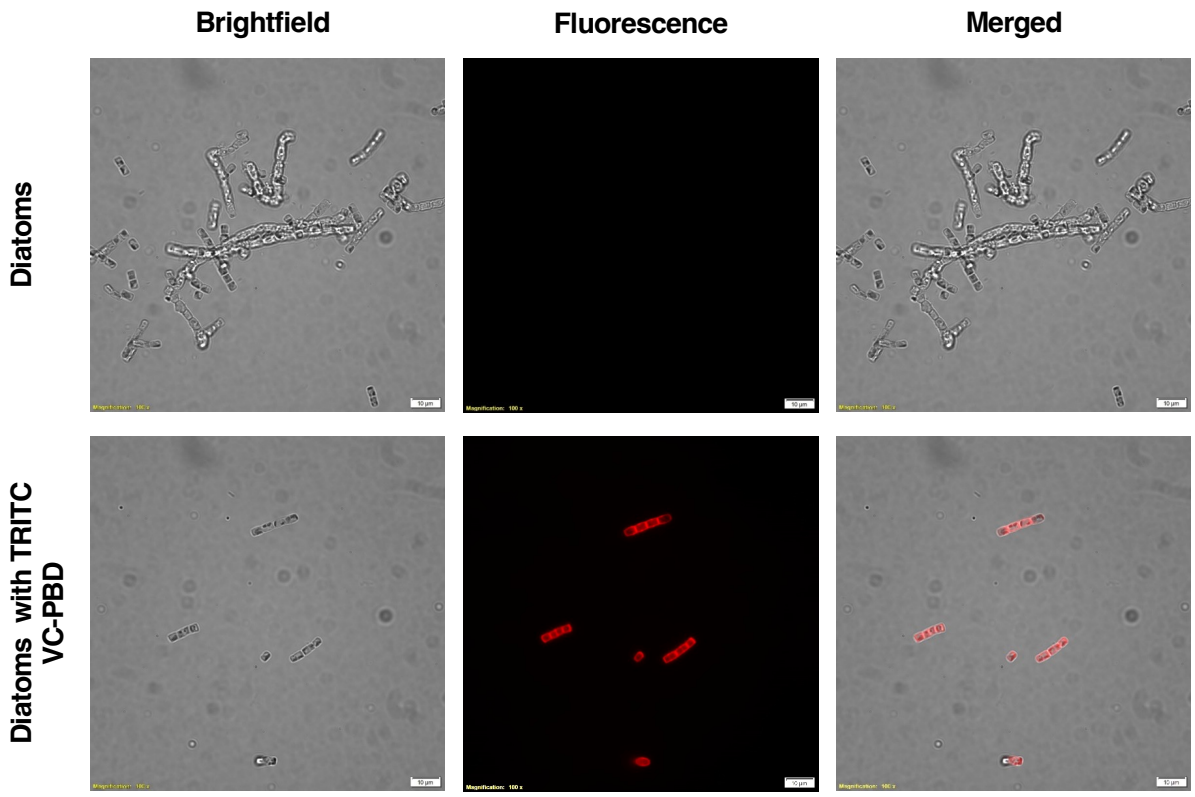


Fig S6B. *V. cholerae* PBD binds to diatoms: *E. spinifer* were incubated with TRITC-FrhA-PBD for 24 h and imaged by brightfield and fluorescence microscopy.

Table S1: *V. cholerae* binding to Hep-2 cells

Population	Count	Mean Bacteria per cell	Std. Dev.	Percent of Mean (WT)	Significance	Percent of Mean (Mp)	Significance
WT	491	2.668	2.343	1	NA	1.1789	***
ΔfrhA	1556	0.8573	1.38	0.3213	*****	0.3788	-
ΔPBD	499	1.359	1.681	0.5093	*****	0.6005	-
WT +AGYTD	918	1.291	1.756	0.4838	*****	0.5704	-
WT +YTAGD	1319	2.146	2.14	0.8043	**	0.9482	-
$FrhA^{MpPBD}$	1881	2.263	1.917	0.8482	**	1	NA
$FrhA^{MpPBD}$ +AGYTD	1915	1.193	1.371	0.4471	-	0.5271	*****
$FrhA^{MpPBD}$ +YTAGD	1655	2.056	2.049	0.7706	-	0.9085	**

Table S2: Oligonucleotides used in this study

primer	**sequence**
Universal Δ Up	GCGCACTAGTGCGGCCGC
Universal Δ Dwn	CGCGTCTAGATCAGGGCCC
R6K_oriT R	AAAAGGCCAGGAACCG TTTTTGTCCGGTGTTGGGTTGAAG
R6K_oriT F	cactgagcgtcagacc CCATGTCAGCCGTTAAGTGTTCC
pUC118 ori R	ggtctgacgctcagtggaacg
pUC118 ori F	CGGTTCCTGGCCTTTTGCTG
ΔPBD F	CGAGCTCGGTACCCGG ACTGCGAAAGGTGCAGAGGC
ΔPBD R	CTTGCATGCCTGCAGG CCGTCGCATCATCAACAGGG
ΔSBD F	CGAGCTCGGTACCCGG CCGTGGTGGCCGGTAATGT
ΔSBD R	CTTGCATGCCTGCAGG CACACCATCATGATCAGTAATCACGTCAT
ΔRIII_4 F	CGAGCTCGGTACCCGG CTTGATGACCGTGGTGAAGTGGA
ΔRIII_4 R	CTTGCATGCCTGCAGG TAGCCATTGATAGTCAGGTTCGGCA
ΔIGL F	CGAGCTCGGTACCCGG GGATGTTGCGTTCTCAGTGACAGA
ΔIGL R	CTTGCATGCCTGCAGG GGTATCGGTATCATTCAGTGAAGTGGAAA
ΔUKD F	CGAGCTCGGTACCCGG TACCGGAGCAACTGGAACTCTGA
ΔUKD R	CTTGCATGCCTGCAGG AACCATCATTTTTCTCTACTTTGAATGACGATGA
ΔlapD FC F	CGAGCTCGGTACCCGG_TCCCGCATCAGAGCAAGAGC
ΔlapD FC R	CTTGCATGCCTGCAGG_TAATCAGCAGTGACGCTATCAACACATC
ΔlapG FC F	CGAGCTCGGTACCCGG_ACTGAATCGGCGGTCGGC

ΔlapG FC R	CTTGCATGCCTGCAGG_ACGGCAAACATACCAATCACTAAGATGC
frhB FC F	CGAGCTCGGTACCCGG_ATTGAACGTGTGATTCAACAGCTTGAAAAAG
frhB FC R	CTTGCATGCCTGCAGG AAACACAAAGAACTCTATAAGCAAGCTCAAGACA
frhB SOE F	ATGTAGTTTGGTGTCTTTAAATAGACGAAC
frhB SOE R	TTAAAGACACCAAACTACATGCCTCTGACC
frhD FC F	CGAGCTCGGTACCCGG_TTTTCCGCCACACCTGTGATGT
frhD FC R	CTTGCATGCCTGCAGG_CTTTACGTTTCGACGCAGGTTTAATTTGT
frhD SOE F	ACAATTATTGGCGCTGAGGGAATAGCCCGC
frhD SOE R	CCCTCAGCGCCAATAATTGTTAAGGATATGCTATCTGCGGCG
RIII-3VC>MP F:	GAATTGCCATCCACATCAGGC
RIII-3VC>MP R:	AACAACAATGTCCACTTCACCAC
MpIBP RIII-3 F:	AGTGGACATTGTTGTT gaagagtttgaagtgtctgagattgc
MpIBP RIII-3 R:	ATGTGGATGGCAATTC ataaccatccccctcaaccac
RIII-3VC>AbR R:	GTCGACGGATCCCCGGAAT AACAACAATGTCCACTTCACCAC
RIII-3VC>AbR F:	GAAGCAGCTCCAGCCTACA GAATTGCCATCCACATCAGGC
229 dPBD atbtc F	GAAGCAGCTCCAGCCTACAAACAACAATGTCCACTTCA
229 dPBD atbtc R	GTCGACGGATCCCCGGAATGAATTGCCATCCACATCAG
pUC118 MCS F	CCTGCAGGCATGCAAGCTT
pUC118 MCS R	CCGGGTACCGAGCTCGAATTC
ABD123	ATTCCGGGGATCCGTCGAC

ABD124	TGTAGGCTGGAGCTGCTTC

Table S3: Plasmids used in this study

Plasmid	**Description**	**reference**
pFY_116	pGP704::Tn7-gfp	(8)
pFY_117	pGP704::Tn7-rpf	(8)
pFY_118	pUX-BF13	(8)
pKEK 229	Sucrose-counterselectable suicide vector: AmpR	(5)
pKEK 2054	Δ5D in pKEK229	This study
pKEK2094	Cloning vector; pUC origin; AmpR	(6)
pKEK2100	pKEK2094 with R6K ori and oriT; AmpR	This Study
pKEK2106	ΔSBD in pKEK2100	This Study
pKEK 2107	ΔIGL in pKEK2100	This study
pKEK 2108	ΔPBD in pKEK2100	This study
pKEK 2109	ΔRIII_4 in pKEK2100	This study
pKEK2110	Δ*frhB* in pKEK2100	This study

pKEK2114	Δ*frhD* in pKEK2100	This study
pKEK2119	ΔSBD in pKEK229	This Study
pKEK2120	ΔIGL in pKEK229	This Study
pKEK2121	ΔPBD in pKEK229	This Study
pKEK2122	Δ*frhB* in pKEK229	This study
pKEK2123	Δ*frhD* in pKEK229	This study
pKEK2124	ΔRIII_4 in pKEK229	This Study
pKEK2157	ΔUKD in pKEK2200	This Study
pKEK 2200	Sucrose-counterselectable suicide vector; CmR	(6)
pKEK 2265	Δ*lapD* in pKEK2200	This study
pKEK 2266	Δ*lapG* in pKEK2200	This study
pKEK 2270	*hapR* expression vector; Tc^R	(9)

pKEK 2285	pBAD-*tfoXqstR* expression vector; AmpR	(9)
pKEK 2335	$frhA^{MPPBD}$ in pKEK 229	This Study
pKEK2447	frhA::KanR in pKEK229	This Study

Table S4: *V. cholerae* strains used in this study

Strain	Description	Reference/Source
O395	*V. cholerae* wildtype	(10)
FY_Vc_12114	Δ*lacZ;* Δ*frhA*	(11)
FY_VC_12120	Δ*lacZ;* Δ*lapD*	(6)
Fy_VC_11863	Δ*lacZ;* Δ*lapG*	(6)
SAD034	ΔVC1807::KanR	(4)
KKV598	Δ*lacZ*	(5)
KKV2075	Δ*lacZ;* Δ*frhC*	(12)
KKV2759	Δ*lacZ;* frhA$^{\Delta 5D}$	This study
KKV2942	Δ*lacZ;* *frh*A$^{\Delta PBD}$	this study
KKV2956	Δ*lacZ,* Δ*lapD*	this study
KKV2957	Δ*lacZ,* Δ*lapG*	this study
KKV2985	Δ*lacZ;* *frh*A$^{\Delta SBD}$	this study
KKV2986	Δ*lacZ;* *frh*A$^{\Delta RIII_4}$	this study
KKV2987	Δ*lacZ;* *frh*A$^{\Delta IGL}$	this study
KKV2988	Δ*lacZ;* Tn7-rfp	This study
KKV3009	Δ*lacZ;* *frh*A$^{\Delta UKD}$	this study
KKV3032	Δ*lacZ,* Δ*frhB*	this study
KKV3033	Δ*lacZ,* Δ*frhD*	this study
KKV3451	Δ*lacZ,* Δ*frhA*::KanR	This study
KKV3540	Δ*lacZ;* *frhA*^{MpPBD}	this study

SI References

1. M. S. Donnenberg, J. B. Kaper, Construction of an *eae* deletion mutant of enteropathogenic *Escherichia coli* by using a positive-selection suicide vector. *Infect. Immun.* **59**, 4310-4317 (1991).
2. J. Garcia-Nafria, J. F. Watson, I. H. Greger, IVA cloning: A single-tube universal cloning system exploiting bacterial In Vivo Assembly. *Sci Rep* **6**, 27459 (2016).
3. R. M. Horton, H. D. Hunt, S. N. Ho, J. K. Pullen, L. R. Pease, Engineering hybrid genes without the use of restriction enzymes: gene splicing by overlap extension. *Gene* **77**, 61-68 (1989).
4. A. B. Dalia, E. McDonough, A. Camilli, Multiplex genome editing by natural transformation. *Proc Natl Acad Sci U S A* **111**, 8937-8942 (2014).
5. N. E. Correa, C. M. Lauriano, R. McGee, K. E. Klose, Phosphorylation of the flagellar regulatory protein FlrC is necessary for *Vibrio cholerae* motility and enhanced colonization. *Mol. Microbiol.* **35**, 743-755 (2000).
6. A. Mejia-Santana, C. J. Lloyd, K. E. Klose, New cloning vectors to facilitate quick allelic exchange in gram-negative bacteria. *Biotechniques* **70**, 116-119 (2021).
7. S. Guo *et al.*, Molecular basis for inhibition of adhesin-mediated bacterial-host interactions through a peptide-binding domain. *Cell Rep* **37**, 110002 (2021).
8. J. C. Fong, K. Karplus, G. K. Schoolnik, F. H. Yildiz, Identification and characterization of RbmA, a novel protein required for the development of rugose colony morphology and biofilm structure in Vibrio cholerae. *J Bacteriol* **188**, 1049-1059 (2006).
9. C. J. Lloyd, A. Mejia-Santana, T. N. Dalia, A. B. Dalia, K. E. Klose, Natural Transformation in a Classical-Biotype Vibrio cholerae Strain. *Appl Environ Microbiol* **87** (2021).
10. J. J. Mekalanos, R. J. Collier, W. R. Romig, Enzymic activity of cholera toxin. II. Relationships to proteolytic processing, disulfide bond reduction, and subunit composition. *J. Biol. Chem.* **254**, 5855-5861 (1979).
11. G. Kitts *et al.*, A Conserved Regulatory Circuit Controls Large Adhesins in Vibrio cholerae. *mBio* **10** (2019).
12. K. A. Syed *et al.*, The Vibrio cholerae flagellar regulatory hierarchy controls expression of virulence factors. *J Bacteriol* **191**, 6555-6570 (2009).