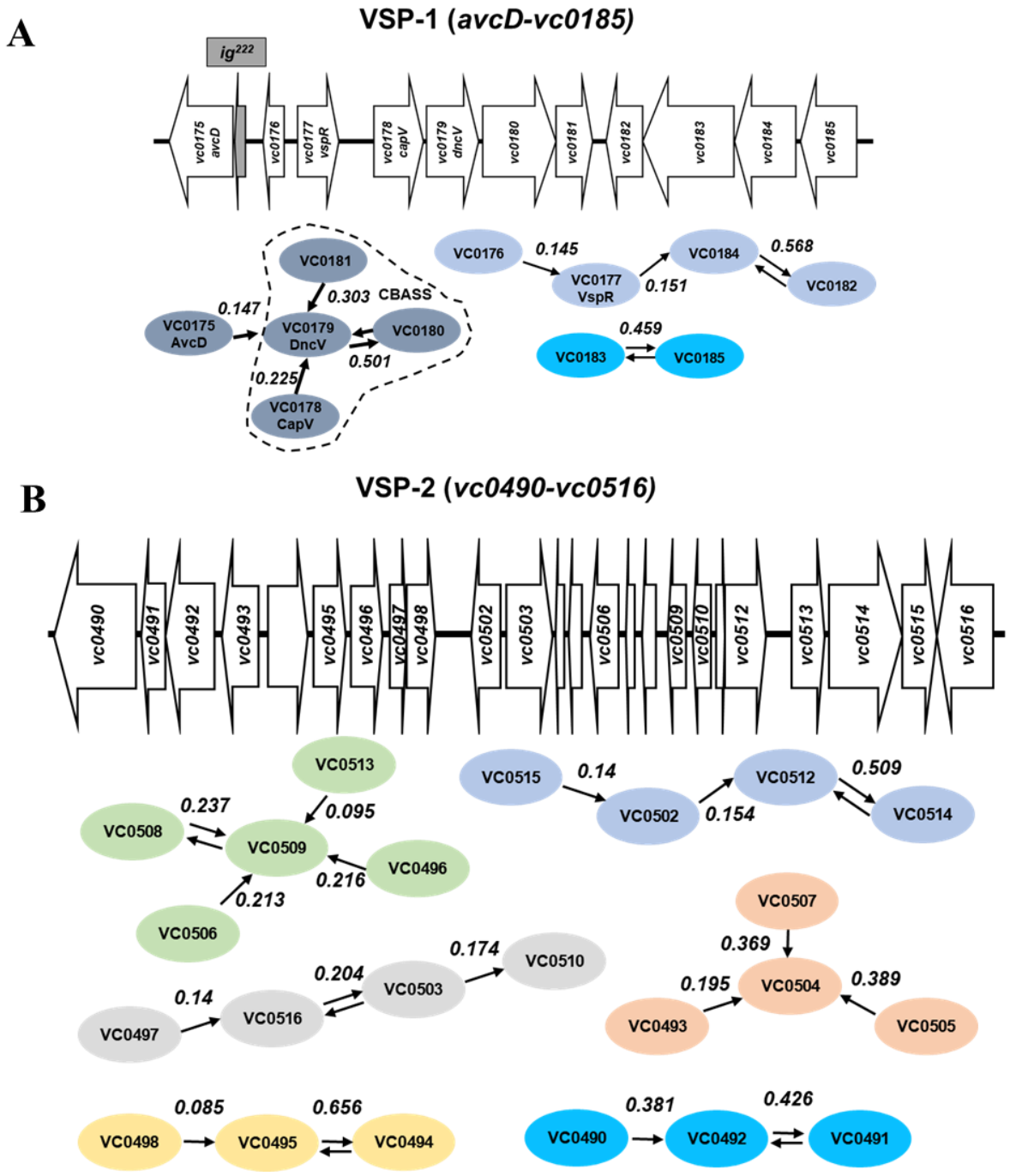
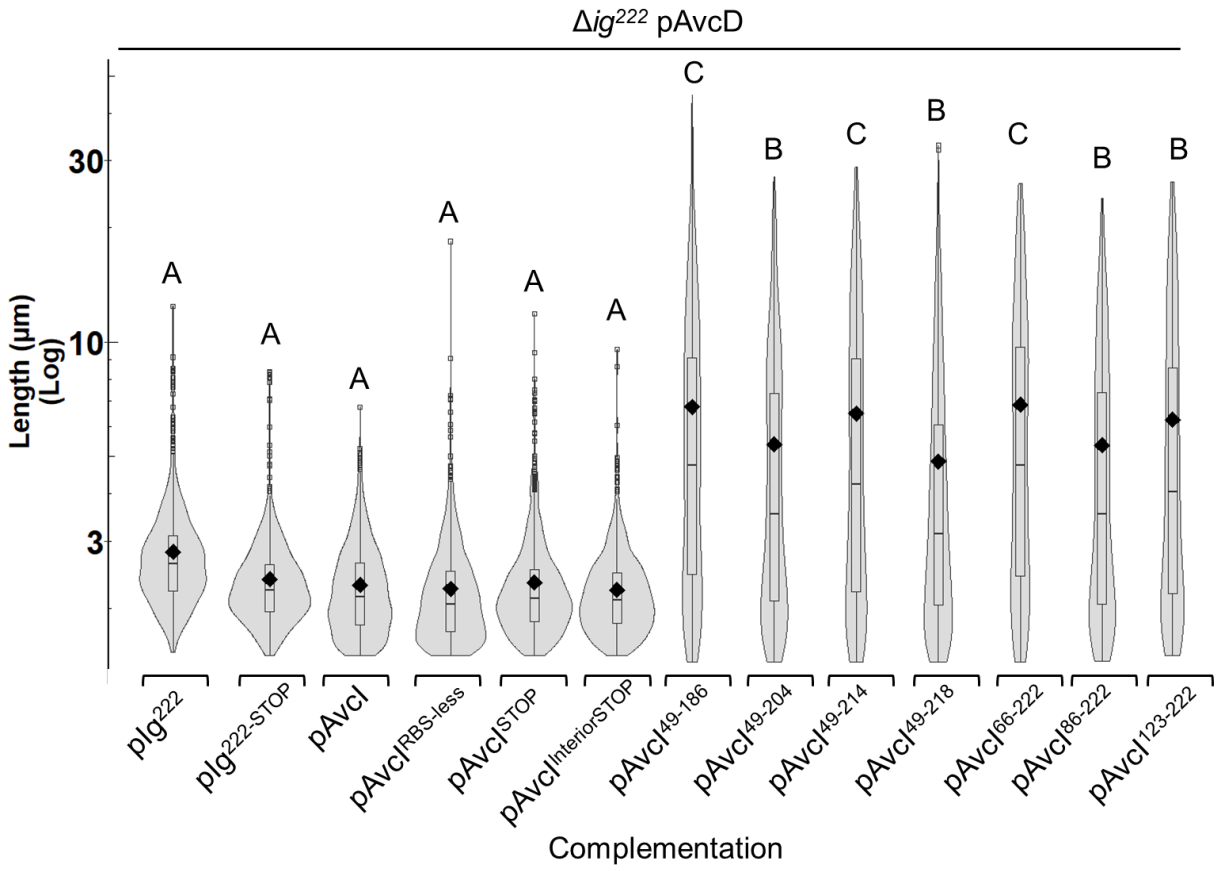
**Supplemental**

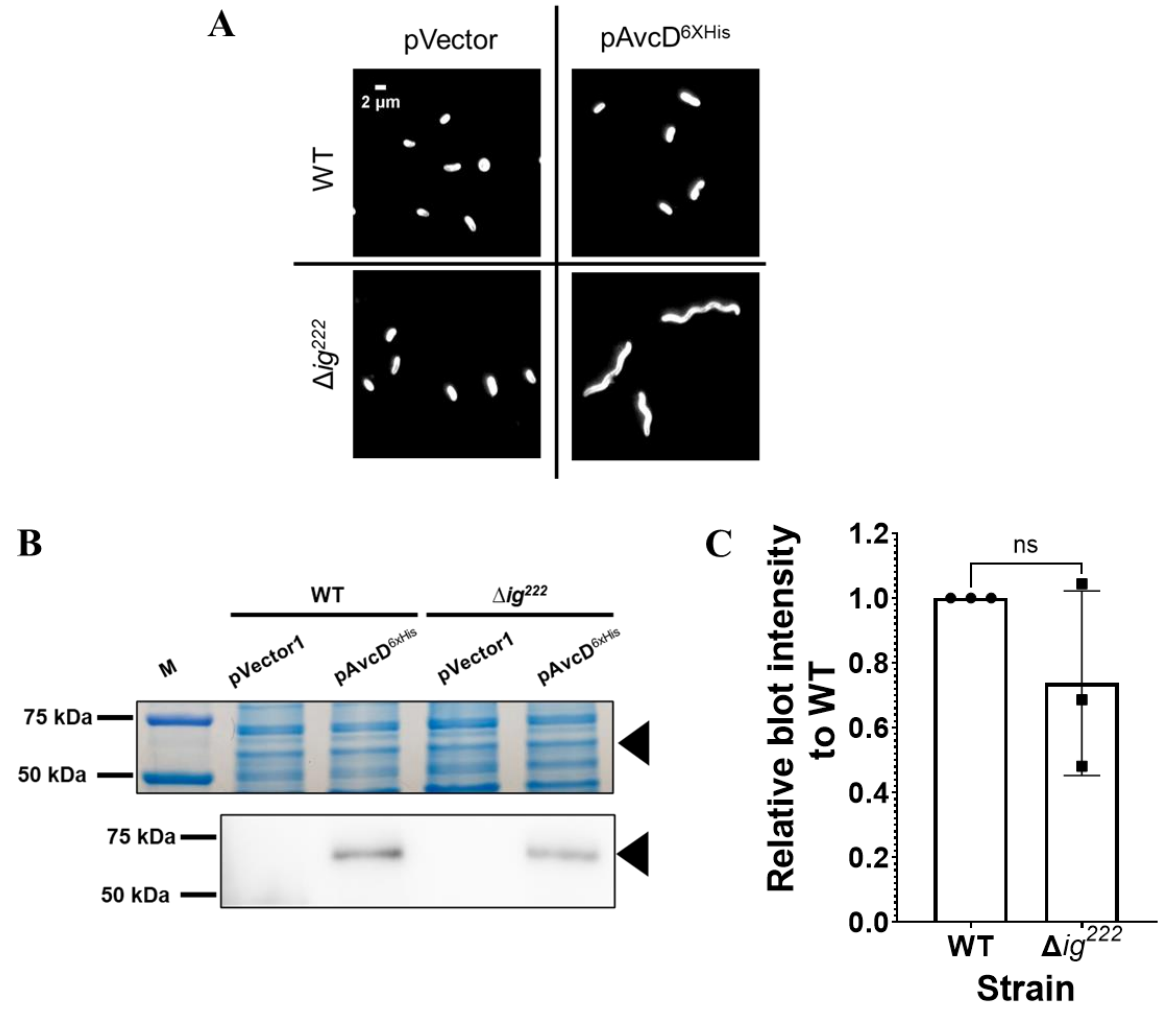



**Supplemental Figure 1. VSP-1 and VSP-2 schematic and predicted gene networks (MRS).** Cartoon of VSP-1 (**A**) and VSP-2 (**B**) from El Tor *V. cholerae* N16961 and gene network predictions from Correlogy. Arrows indicate the highest partial correlation $\boldsymbol{W_{ij}}$ each gene has to another (ovals). Two arrows are presented pointing in opposing directions where the highest correlation $\boldsymbol{W_{ij}}$ is reciprocal between two genes. MRS = maximum relatedness subnetwork

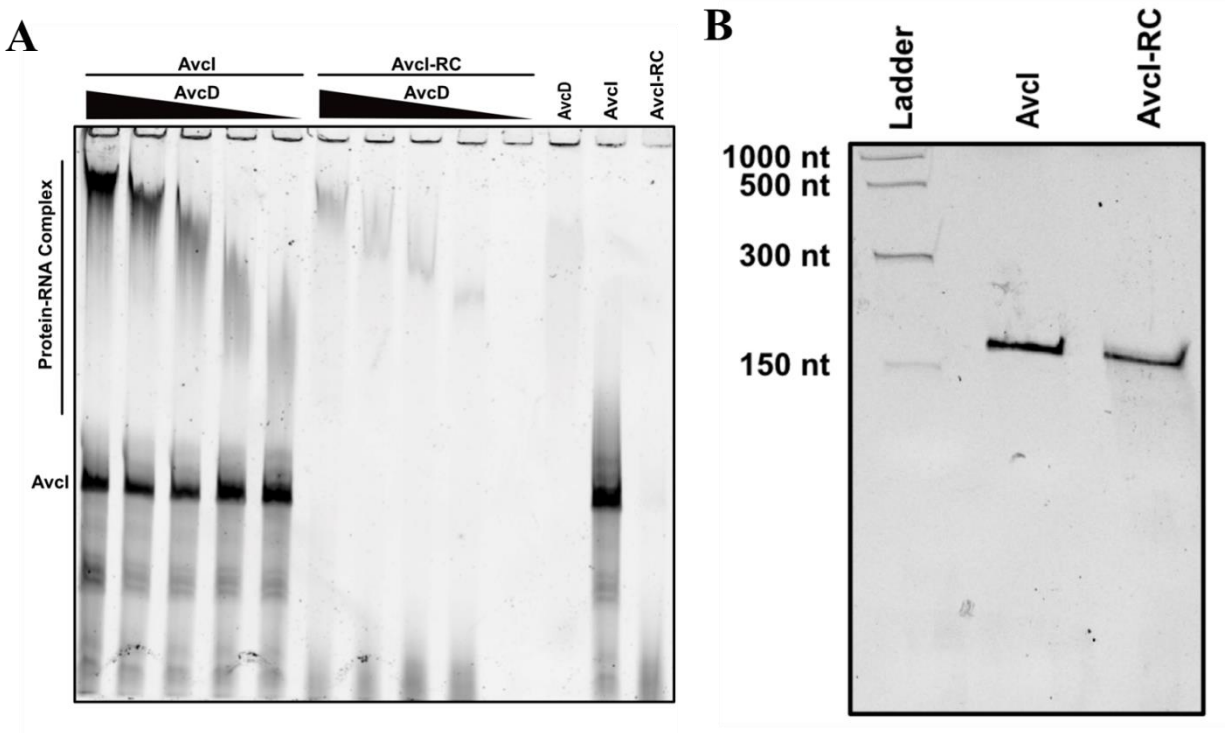



**Supplemental Figure 2. Complementation of various *ig*$^{222}$ constructs to prevent AvcD induced cell filamentation.**

Cell length distributions of Δ*ig*$^{222}$ *V. cholerae* expressing pAvcD. All cell length distributions represent ~750-1000 cells measured per strain with summary statistics: mean (diamonds), median (horizontal black line), interquartile range (box), and data below and above the interquartile range (vertical lines). Different letters indicate significant differences at $p < 0.05$, according to Tukey's post-hoc test.

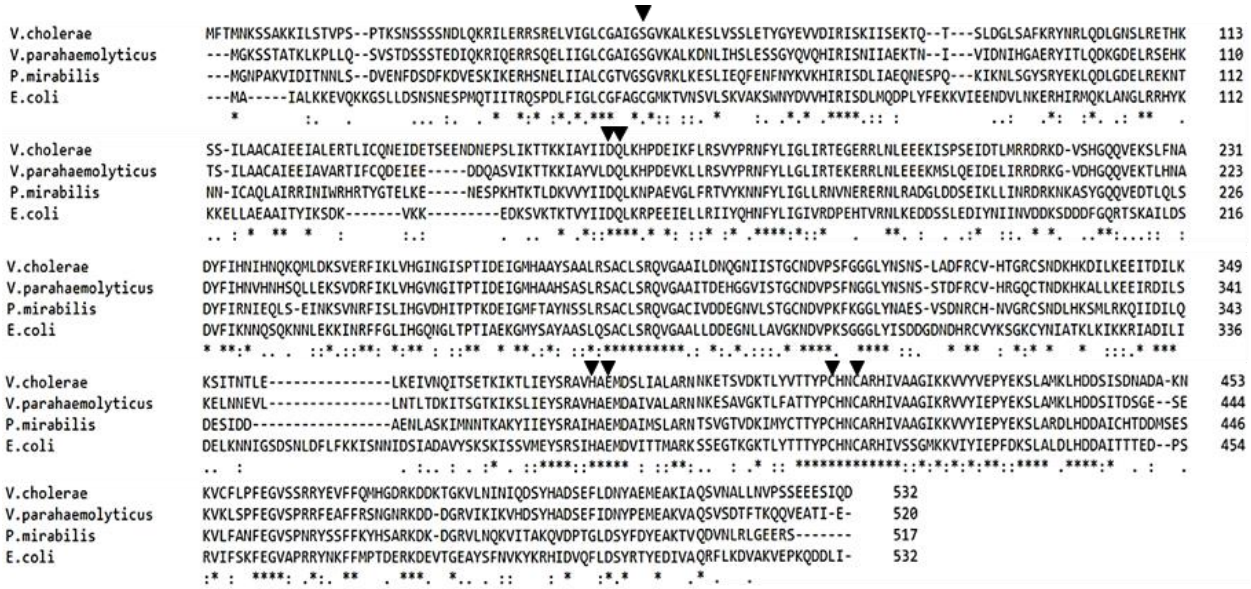



**Supplemental Figure 3. AvcD C-terminal 6x Histidine fusion maintains the same activity as the WT AvcD enzyme and the presence of *avcI* does not reduce the abundance of AvcD.** (**A)** Representative images of WT *V. cholerae* and Δ*ig*$^{222}$ cultures maintaining an empty vector plasmid (pVector1) or $P_{tac}$-inducible *avcD-6xHIS* plasmid (pAvcD$^{6xHis}$) grown in the presence of 100 µM IPTG for 2 h. Cells were stained with FM4-64 prior to imaging and performed in biological triplicate. (**B)** Representative coomassie stained PAGE gel (top) and matched anti-6x His antibody Western blot (bottom) of whole cell lysates normalized to total protein from *V. cholerae* WT and Δ*ig*$^{222}$ cultures maintaining pVector1 or pAvcD$^{6xHis}$. Black triangles correspond to AvcD$^{6xHis}$ (60.6 kDa). Analysis was performed in biological triplicate and the relative signal intensity (**C.**) was the determined by comparing the intensities of AvcD$^{6xHIS}$ from paired WT and Δ*ig*$^{222}$ lysates probed on the same blots.



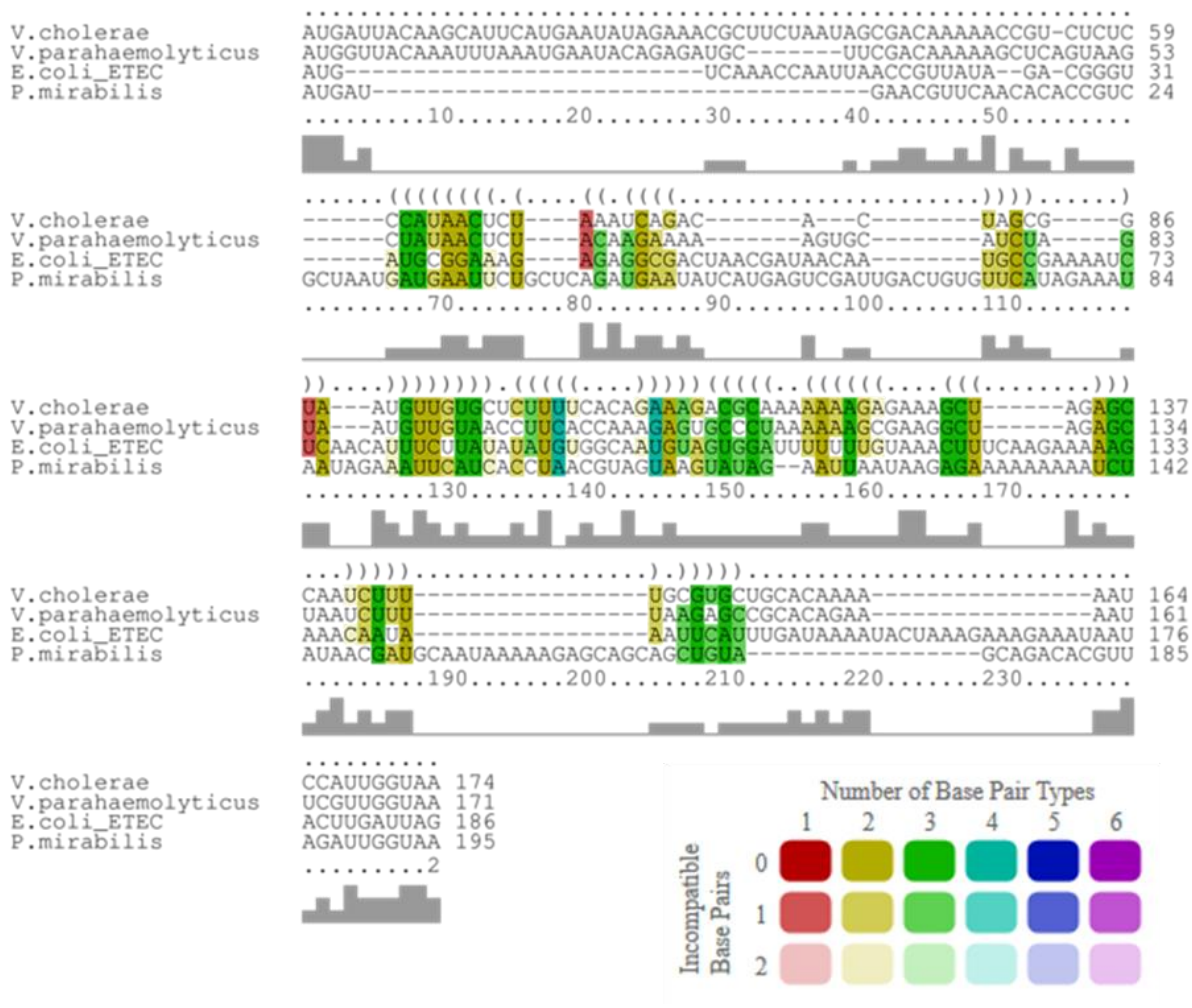

**Supplemental Figure 4. AvcD-AvcI complex formation in solution and Denaturing urea PAGE analysis of AvcI and AvcI-RC.**

(**A)** AvcD forms a complex with AvcI in an AvcD concentration-dependent manner as determined by EMSA. Trace quantities of AvcI reverse complement (AvcI-RC) binding to AvcD in a non-specific manner is observed. **(B)** AvcI and AvcI-RC run at essentially equivalent molecular weights on a 7 M urea denaturing PAGE. Low range ssRNA ladder (NEB).

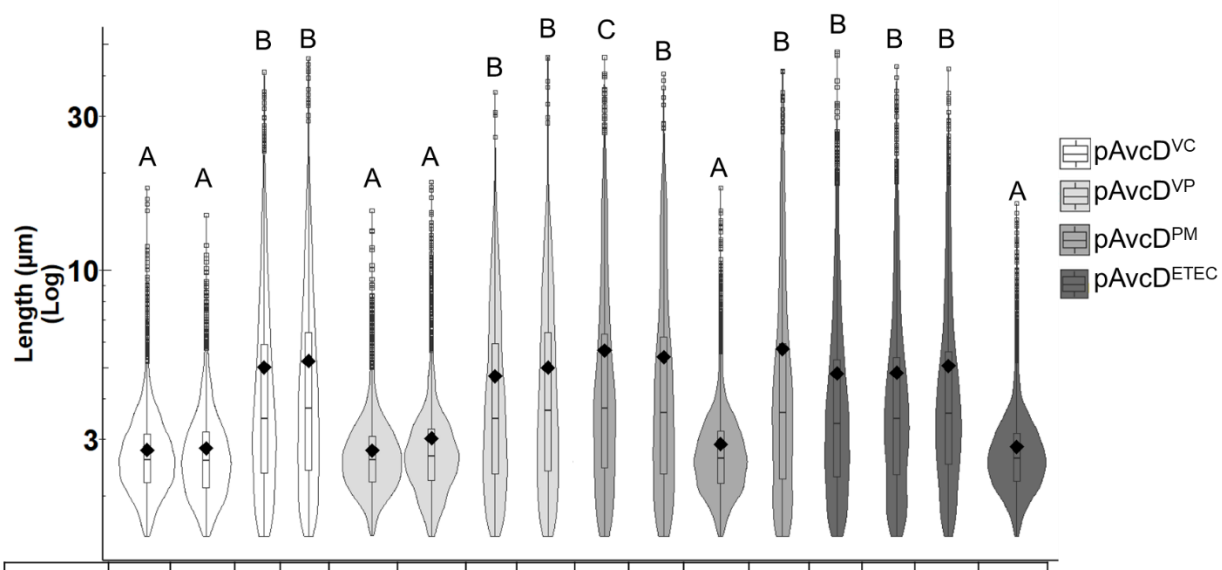



**Supplemental Figure 5. ClustalW multiple sequence alignment of AvcD homologs show conservation of likely active site residues explored in this study.**

Amino acid alignment of the *V. cholerae* AvcD and three homologs using EMBL-EBI ClustalW[42]. "*****" indicates 100% identity, "**:**" indicates >75%, and "**.**" Indicates >50% similarity. Black triangles indicate conserved residues in *V. cholerae* AvcD targeted for site-directed mutagenesis.

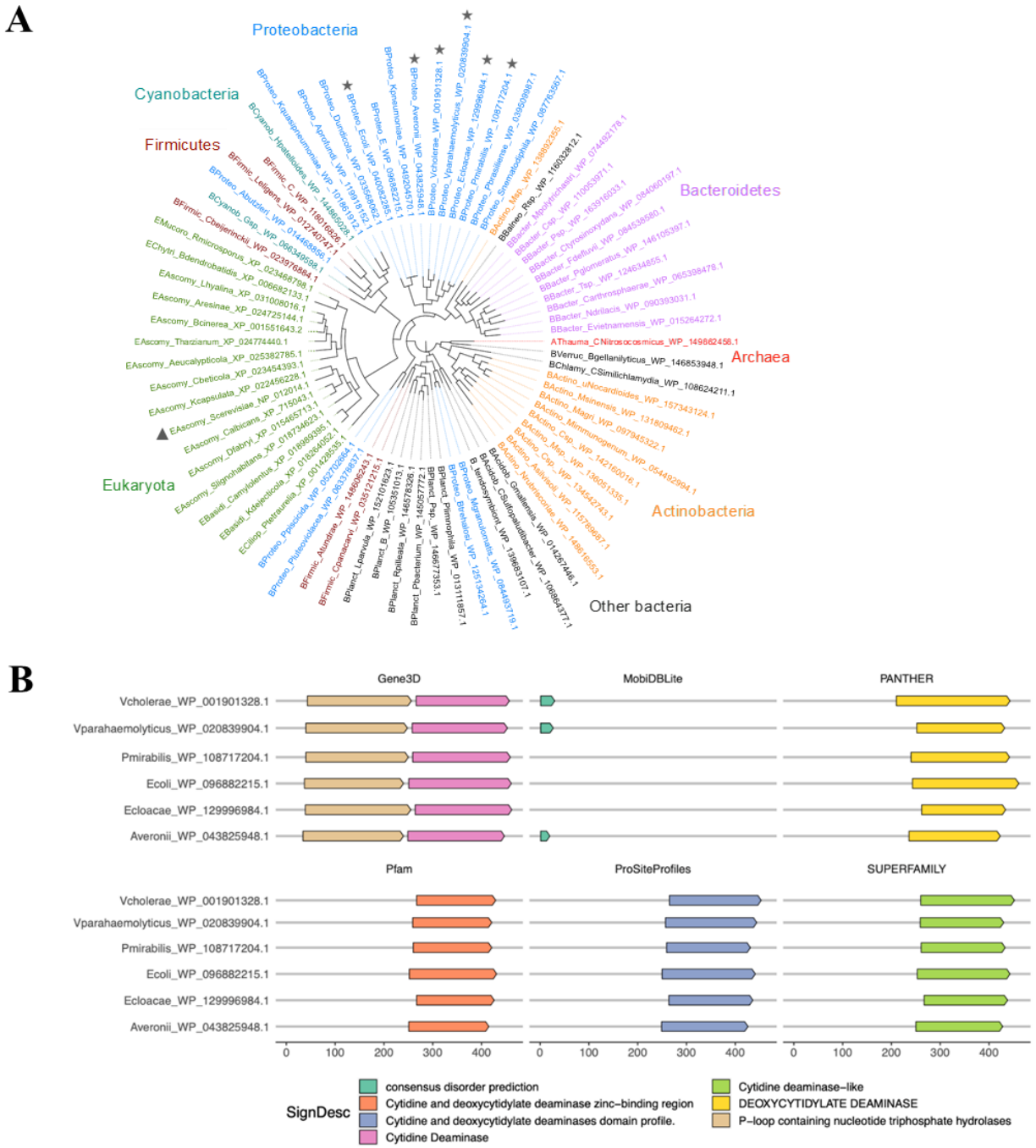



**Supplemental Figure 6. Nucleotide multiple sequence alignment of AvcI and homologs.** Nucleotide alignment of *V. cholerae* AvcI and three homologs using LocARNA[43]. The average secondary structure is indicated in dot-bracket notation (top). Consensus identities are correlated with the height of the bars below the corresponding nucleotide. Compatible base pairs are colored according to the number of different types C-G (1), G-C (2), A-U (3), U-A (4), G-U (5) or U-G (6) of compatible base pairs in the corresponding columns. The color saturation decreases with the number of incompatible base pairs.




| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pAvcI$^{VC}$ | + | – | – | – | + | – | – | – | + | – | – | – | + | – | – | – |
| pAvcI$^{VP}$ | – | + | – | – | – | + | – | – | – | + | – | – | – | + | – | – |
| pAvcI$^{PM}$ | – | – | + | – | – | – | + | – | – | – | + | – | – | – | + | – |
| pAvcI$^{ETEC}$ | – | – | – | + | – | – | – | + | – | – | – | + | – | – | – | + |

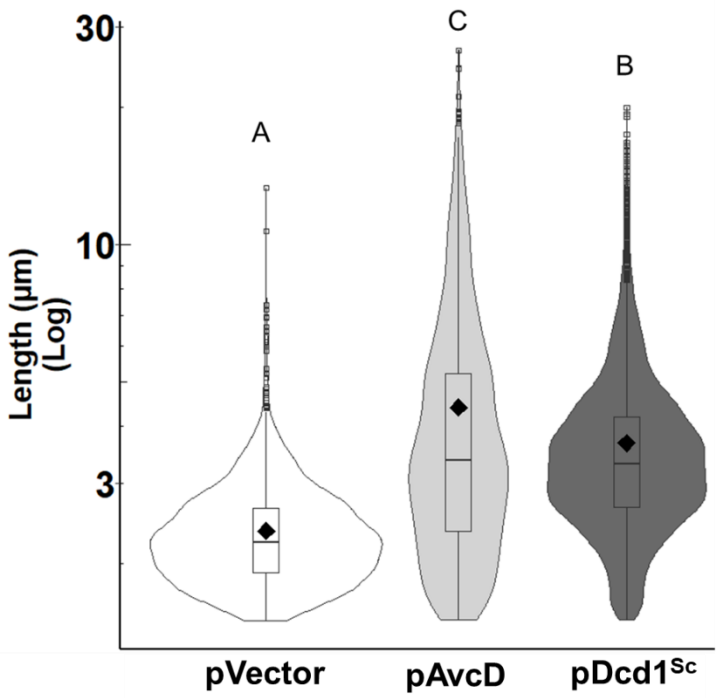
**Supplemental Figure 7. Cross-species inhibition of *avcD* and *avcI* homologs.**
Cell length distributions of *E. coli* co-expressing various combinations of $P_{tac}$-inducible plasmids encoding homologs of *avcD* and *avcI*. All cell length distributions represent ~1000-3000 cells measured per strain with summary statistics: mean (diamonds), median (horizontal black line), interquartile range (box), and data below and above the interquartile range (vertical lines). Different letters indicate significant differences at $p < 0.05$, according to Tukey's post-hoc test. VC = *Vibrio cholerae*, VP = *Vibrio parahaemolyticus*, PM = *Proteus mirabilis*, ETEC = *E. coli* ETEC .



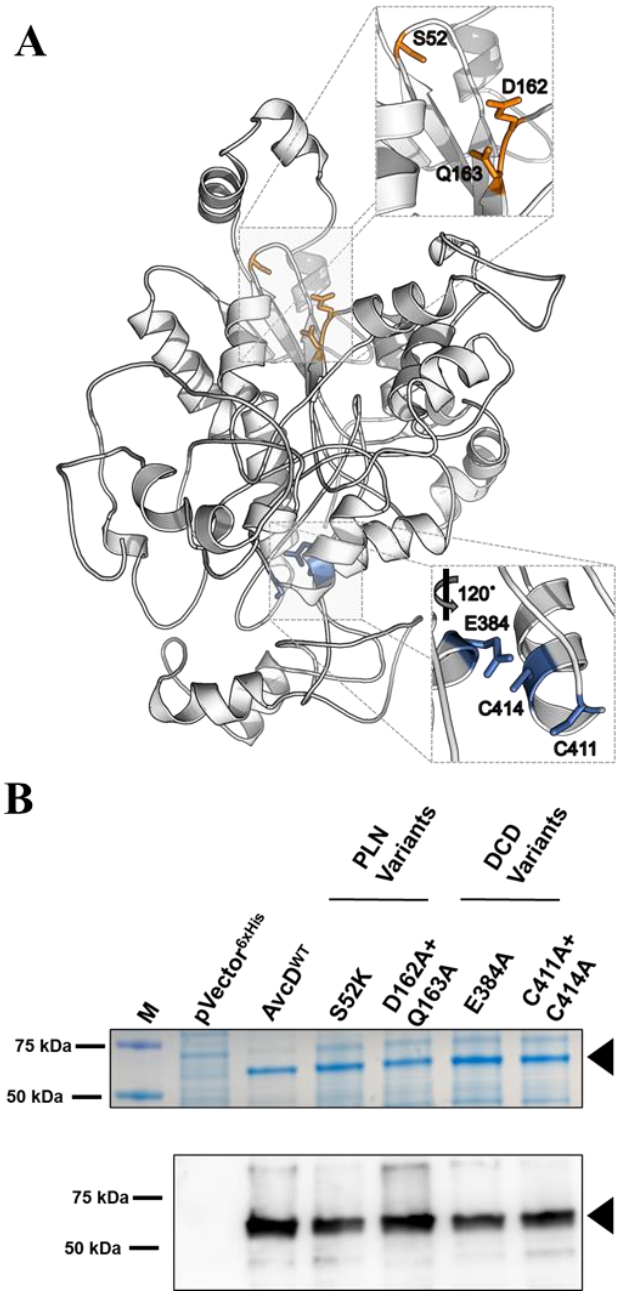

**Supplemental Figure 8. Phylogenetic analysis and domain architectures of the six AvcD query proteins.**

(**A**) Phylogenetic tree of AvcD homologs from representative phyla across the tree of life. Stars indicate the six proteobacterial starting points for the homology search, as well as the eukaryotic *Saccharomyces cerevisiae dcd1* (triangle), explored in Fig. S9. (**B**) Domain architecture and secondary structure predictions for the six proteobacterial starting points (query proteins) were predicted using InterProScan[49] (Methods). Results from six main analyses are shown here for the query proteins: Gene3D (including CATH structure database), Pfam, ProSiteProfiles, PANTHER,

and SUPERFAMILY protein domain profile databases, and MobiDBLite for disorder prediction. No transmembrane regions (using TMHMM) or membrane/extracellular localization were predicted for any of the proteins (using Phobius); hence not shown. Numbers (bottom) indicate the amino acid position of predicted domains and features.

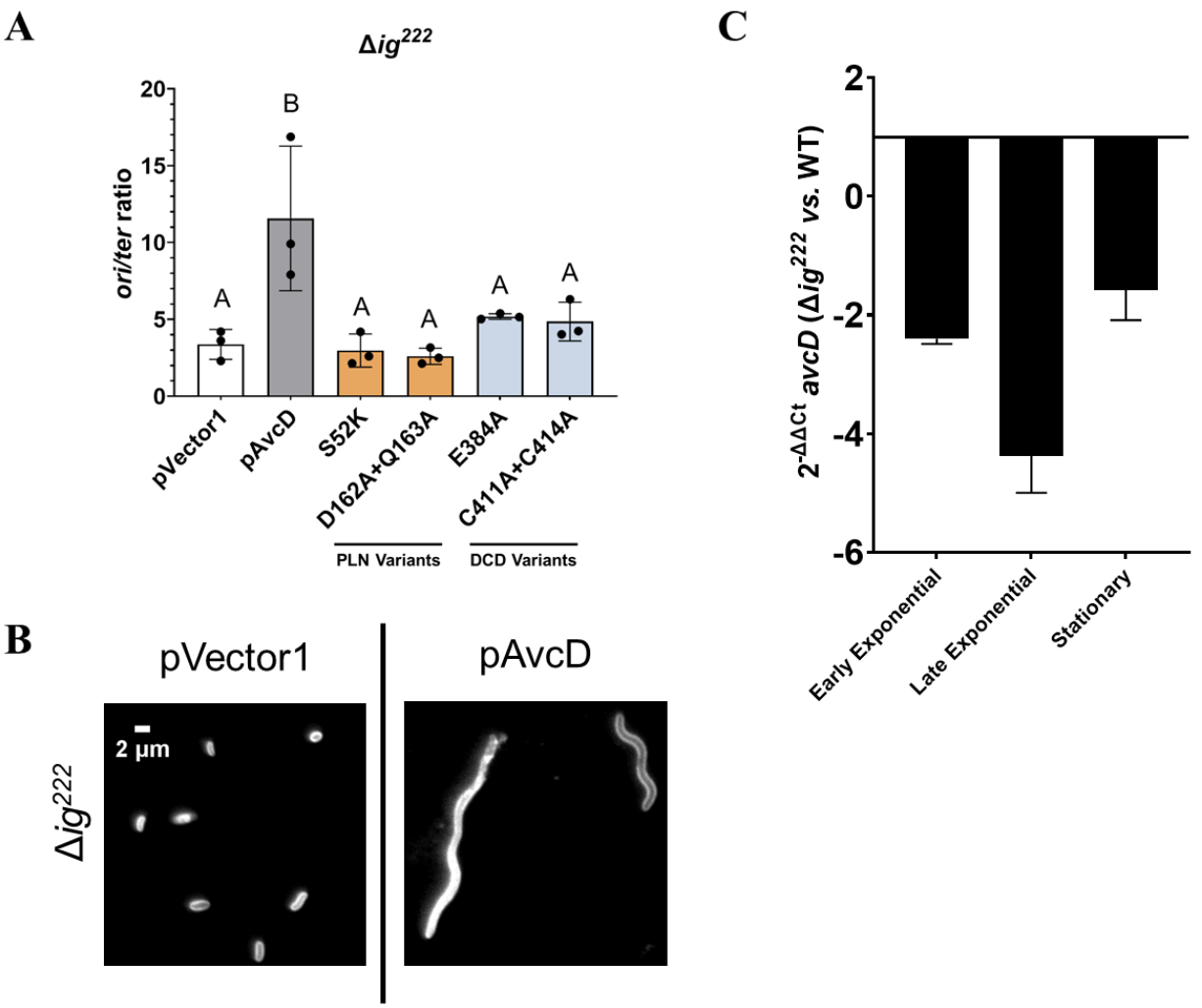



**Supplemental Figure 9. AvcD homolog from *Saccharomyces cerevisiae*, Dcd1, also induces filamentation in *E. coli*.**
Cell length distributions of *E. coli* expressing pAvcD, a $P_{tac}$-inducible plasmid encoding *dcd1* from *S. cerevisiae* (pDcd1$^{Sc}$), or pVector1. All cell length distributions represent ~1000-3000 cells measured per strain with summary statistics: mean (diamonds), median (horizontal black line), interquartile range (box), and data below and above the interquartile range (vertical lines). Different letters indicate significant differences at $p < 0.05$, according to Tukey's post-hoc test.

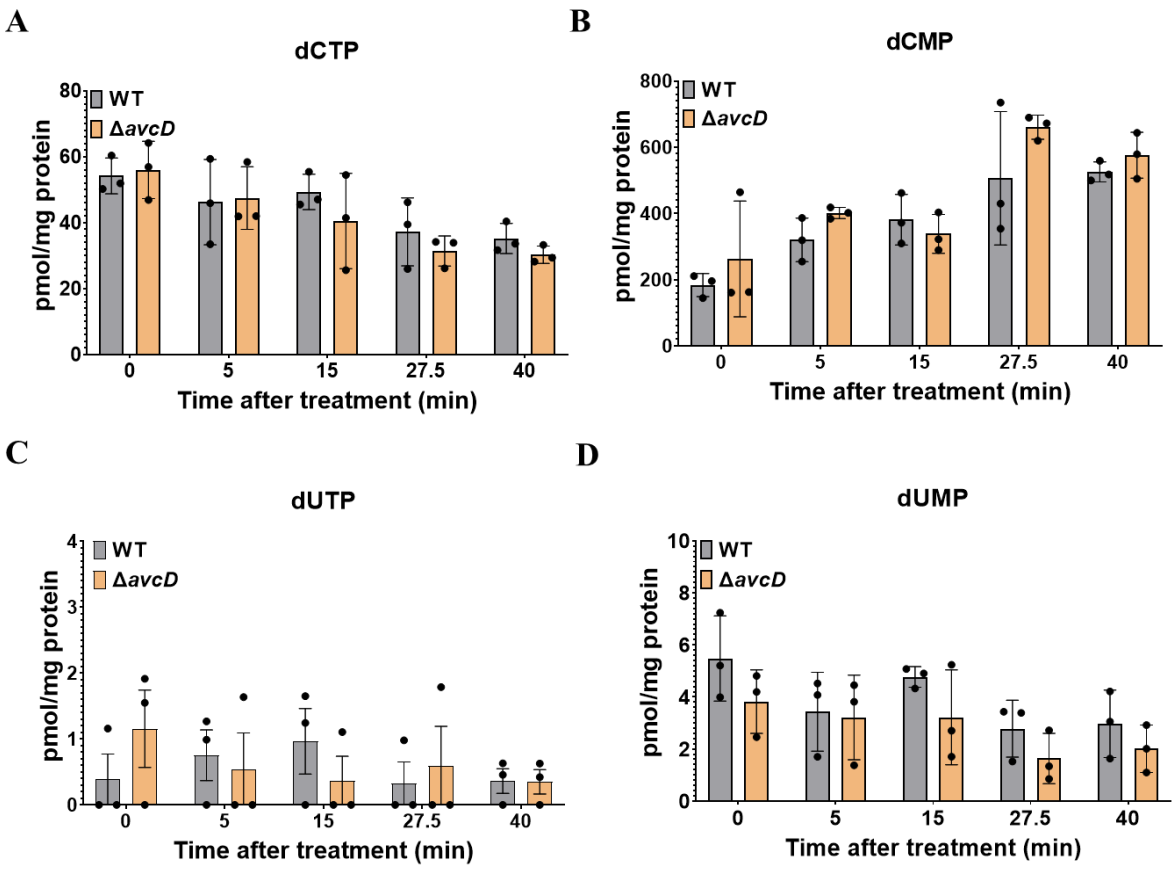



**Supplemental Figure 10. Mutations in conserved residues of AvcD do not affect the stability of the protein.**

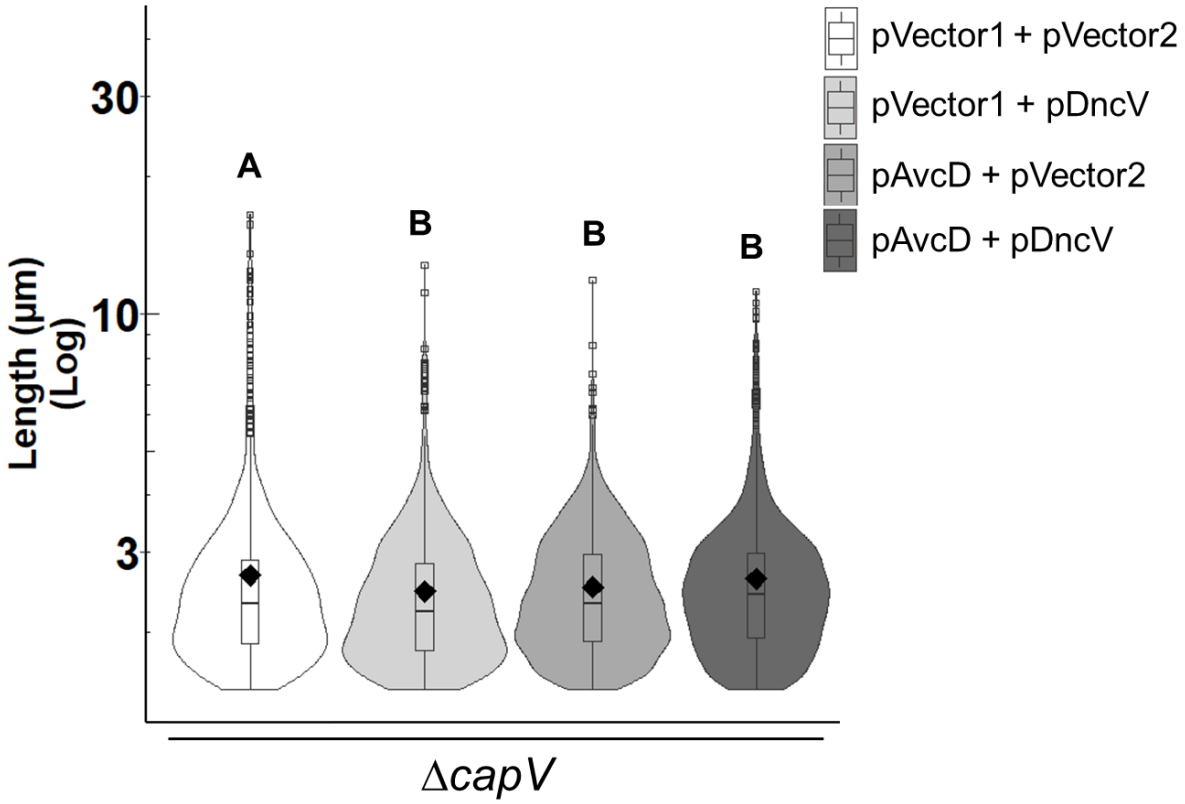
(**A**) Phyre2[12] predicted structure of AvcD from *V. cholerae* El Tor. Insets highlight conserved residues of the PLN (top) and DCD (bottom) domains selected for mutagenesis. (**B**) Representative Coomassie stained gel (top) and anti-6x His antibody Western blot (bottom) of whole cell lysates from *E. coli* BL21(DE3) cells maintaining an empty vector ($pVector^{6xHis}$), inducible C-terminal 6x histidine tagged *avcD* (WT) or *avcD* variants (S52K, D162A + Q163A, E384A, and C411A + C414A) grown in the presence of 1 mM IPTG for 3 h. Sample inputs were normalized by culture $OD_{600}$ and resolved by SDS-PAGE. Three biological replicates of each strain were analyzed with similar results. Black triangles correspond to the predicted molecular weight of the AvcD tagged fusions (60.6 kDa). M = molecular weight marker.




**Supplemental Figure 11. AvcD activity induces TLD-phenotype.**
(**A)** *V. cholerae* mutant expressing the indicated AvcD variants. *ori/ter* ratios of Chromosome 1 in Δ*ig*$^{222}$ *V. cholerae* strains expressing the indicated pAvcD construct and quantified using qRT-PCR. Each bar represents the mean ± SEM, *n*=3. Different letters indicate significant differences (*n*=3) at $p < 0.05$, according to Tukey's post-hoc test. (**B**) Representative images of Δ*ig*$^{222}$ cultures maintaining an empty vector plasmid pVector 1 or pAvcD grown in the presence of 100 µM IPTG for 8 h. Cells were stained with FM4-64 prior to imaging and performed in biological triplicate. (**C**) Relative difference in *avcD* expression between Δ*ig*$^{222}$ and WT *V. cholerae* at three different growth phases using qRT-PCR and an endogenous *gyrA* control. Data represent the mean ± SEM of three biological replicates.

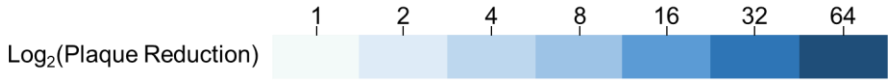



**Supplemental Figure 12. Cessation of global translation, by treatment with spectinomycin, does not liberate AvcD enzymatic activity.**
Intracellular concentration of dCTP (**A**), dCMP (**B**), dUTP (**C**), and dUMP (**D**) of WT and Δ*avcD* *V. cholerae* during spectinomycin treatment (200 µg/mL) measured by UPLC-MS/MS. Data represent the mean ± SEM of three biological replicate cultures. No statistically significant differences in nucleotide concentrations were observed between strains at any time point as determined by Two-way ANOVA with Šídák's multiple-comparison test.




**Supplemental Figure 13. Ectopic expression of DncV and AvcD does not lead to filamentation in the Δ*capV* mutant of *V. cholerae.***
Cell length distributions measured from three biological replicates of *ΔcapV V. cholerae* cultures co-expressing either two empty vectors, pDncV and an empty vector, pAvcD and an empty vector, or pDncV and pAvcD grown in the presence of 100 µM IPTG for 8 h. Distributions represent ~1200-1700 cells measured per strain. Different letters indicate significant differences at $p < 0.05$, according to Tukey's post-hoc test.

| | Coliphages | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Myoviridae* | | | *Podoviridae* | | *Siphoviridae* | | | | *Microviridae* |
| AvcID systems | T2 | T4 | T6 | T3 | T7 | λ-vir | T5 | SECΦ18 | SECΦ27 | SECΦ17 |
| VC | 2 | 2 | 2 | 8 | 1 | 1 | 2 | 1 | 1 | 1 |
| VP | 1 | 1 | 4 | 64 | 1 | 1 | 64 | 8 | 1 | 1 |
| PM | 1 | 8 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| ETEC | 1 | 1 | 1 | 64 | 1 | 1 | 1 | 4 | 4 | 4 |




**Supplemental Figure 14. Summary of Figure 4A.** Data are the mean of the three biological replicates rounded to the nearest fold of plaque reduction relative to empty vector control. VC = *Vibrio cholerae*, VP = *Vibrio parahaemolyticus*, PM = *Proteus mirabilis*, ETEC = *E. coli* ETEC.

**Supplementary Table 1.** Bacterial strains and phages used in this study.

| Strains | Name in this Study | Relevant Characteristics | Source or reference |
|---|---|---|---|
| ***E. coli*** | | | |
| DH10b | | *F-mcrA Δ(mrr-hsdRMS-mcrBC) Φ80lacZΔM15 ΔlacX74 recA1 endA1 araD139Δ(ara, leu)7697 galU galK λrpsL nupG* | ThermoFisher Scientific |
| BW29427 | | *RP4-2(TetSkan1360::FRT)*, *thrB1004*, *lacZ58*(M15), *ΔdapA1341::[erm pir$^+$]*, *rpsL*(strR), *thi-*, *hsdS-*, *pro-* | Lab Stock |
| BL21(DE3) | | *F- ompT hsdSB(rB -mB +) gal dcm (DE3)* | Lab Stock |
| MG1655 | | *F- lambda- ilvG- rfb-50 rph-1* | Lab Stock |
| 078:H11 H10407 (ETEC) | ETEC | Wild type | [41] |
| ***V. cholerae*** | | | |
| C6706str2 | WT and VC | Wild type O1 El Tor; Sm$^R$ | [30] |
| CR01 | ΔVSP-1 | O1 El Tor ΔVSP-1 | This study |
| CR02 | ΔVSP-2 | O1 El Tor ΔVSP-2 | This study |
| CR03 | ΔVSP-1/2 | O1 El Tor ΔVSP-1/2 | This study |
| BYH206 | Δ*ig$^{222}$* | O1 El Tor Δ*ig$^{222}$ between vc0175-vc0176 position in N16961 chromosome I [177,230-177,008]* | This study |
| BYH207 | Δ*vc0176* | O1 El Tor Δ*vc0176* | This study |
| GS05 | Δ*avcD* | O1 El Tor Δ*avcD* | This study |
| WLN5105 | Δ*capV* | O1 El Tor Δ*capV* | [8] |
| ***V. parahaemolyticus*** | | | |
| O1:Kuk str. FDA_R31 | VP | Wild type | [39] |
| ***P. mirabilis*** | | | |
| AR379 | PM | Wild type | [40] |
| ***S. cerevisiae*** | | | |
| yMK839 | Sc | *MATa leu2-3 trp1 ura3-52* | [11] |
| **Phages** | | | |
| T2 | T2 | Wild type | ATCC |
| T3 | T3 | Wild type | ATCC |
| T4 | T4 | Wild type | ATCC |
| T5 | T5 | Wild type | ATCC |
| T6 | T6 | Wild type | ATCC |
| T7 | T7 | Wild type | ATCC |
| $\lambda_{virulent}$ | $\lambda_{vir}$ | Wild type | Gift from M. Laub |
| SECϕ17 | SECϕ17 | Wild type | Gift from M. Laub |
| SECϕ18 | SECϕ18 | Wild type | Gift from M. Laub |
| SECϕ27 | SECϕ27 | Wild type | Gift from M. Laub |

**Supplementary Table 2:** Plasmids Descriptions

| Plasmids | Name in this Manuscript | Relevant characteristics | Source or Reference |
|---|---|---|---|
| pEVS141 | pVector1 | pEVS143 without $P_{tac}$; $Km^r$ | [34] |
| pEVS143 | | Broad-host range $P_{tac}$ overexpression vector; $Km^r$ | [32] |
| pMMB67EH | pVector2 | Broad-host range $P_{tac}$ overexpression vector; $Amp^r$ | [33] |
| pKAS32 | | Suicide vector for mutant construction, $Amp^r$ | [31] |
| pET28b | $pVector^{6xHis}$ | T7 promoter; $Km^r$ | Novagen |
| pBRP353 | pDncV | pMMB67EH::*dncV*; $Amp^r$ | [8] |
| pCMW204 | pAvcD | pEVS143::*avcD;* $Km^r$ | This study |
| pGBS87 | pAvcD/$pAvcD^{VC}$ | pMMB67EH::*avcD*; $Amp^r$ | This study |
| pGBS65 | $pAvcD^{6xHis}$ | pET28b::*avcD-6xHis C-term*; $Km^r$ (*only* in *E. coli* BL21(DE3)) | This study |
| pGBS98 | $pAvcD^{6xHis}$ | pEVS143::*avcD-6xHis C-term;* $Km^r$ (*only* in *V. cholerae*) | This study |
| $pAvcD^{4-532}$ | | pET28b::$avcD^{4-532}$*-6xHis N-term;* $Km^r$ | This study |
| pGBS71 | $pAvcD^{E384A}$ | pEVS143::*avcD-E384A;* $Km^r$ | This study |
| pGBS82 | $pAvcD^{E384A}$ | pET28b::*avcD-E384A-6xHis C-term*; $Km^r$ (*only* for in vitro and western blot) | This study |
| pGBS81 | $pAvcD^{C411A+C414A}$ | pEVS143::*avcD-C411A+C414A*; $Km^r$ | This study |
| pGBS75 | $pAvcD^{C411A+C414A}$ | pET28b::*avcD-C411A+C414A-6xHis C-term*; $Km^r$ | This study |
| pGBS103 | $pAvcD^{S52K}$ | pEVS143::*avcD-S52K;* $Km^r$ | This study |
| pGBS114 | $pAvcD^{S52K}$ | pET28b::*avcD-S52K-6xHis C-term*; $Km^r$ | This study |
| pGBS106 | $pAvcD^{D162A+Q163A}$ | pEVS143::*avcD-D162A+Q163A;* $Km^r$ | This study |
| pGBS116 | $pAvcD^{D162A+Q163A}$ | pET28b::*avcD-D162A+Q163A-6xHis C-term*; $Km^r$ | This study |
| pGBS80 | $pIg^{222}$ | pEVS143::$Ig^{222}$*, (position in N16961 chromosome I [177,230-177,008]);* $Km^r$ | This study |
| pGBS108 | $pIg^{222\text{-}STOP}$ | pEVS143::$ig^{222}$-1C>T, 2T>A; $Km^r$ | This study |
| pGBS110 | pAvcI | pEVS143::*avcI* (*position in N16961 chromosome I [177,181-177,008]);* $Km^r$ | This study |
| pAW01 | $pAvcI^{RBS\text{-}less}$ | pEVS143:*avcI* without RBS; $Km^r$ | This study |
| pGBS111 | $pAvcI^{STOP}$ | pEVS143::*avcI*-1A>T, 2T>A, 3G>A; $Km^r$ | This study |
| pGBS118 | $pAvcI^{InteriorSTOP}$ | pEVS143::*avcI*-17A>T, 18T>A, 19G>A; $Km^r$ | This study |
| pBYH49 | $pAvcI^{49\text{-}186}$ | pEVS143::$Ig^{222}$ truncation (49-186 NT); $Km^r$ | This study |
| pBYH52 | $pAvcI^{49\text{-}204}$ | pEVS143::$Ig^{222}$ truncation (49-204 NT); $Km^r$ | This study |
| pBYH53 | $pAvcI^{49\text{-}214}$ | pEVS143::$Ig^{222}$ truncation (49-214 NT); $Km^r$ | This study |

| | | | |
|---|---|---|---|
| pBYH54 | pAvcI$^{49\text{-}218}$ | pEVS143::*Ig$^{222}$* truncation (49-218 NT); Km$^r$ | This study |
| pBYH55 | pAvcI$^{66\text{-}222}$ | pEVS143::*Ig$^{222}$* truncation (66-222 NT); Km$^r$ | This study |
| pBYH56 | pAvcI$^{86\text{-}222}$ | pEVS143::*Ig$^{222}$* truncation (86-222 NT); Km$^r$ | This study |
| pBYH57 | pAvcI$^{123\text{-}222}$ | pEVS143::*Ig$^{222}$* truncation (123-222 NT); Km$^r$ | This study |
| pGBS124 | pAvcD$^{ETEC}$ | pEVS143::*avcD* from *Escherichia coli* O78:H11 H10407 (ETEC); Km$^r$ (*only* for mass spec experiment) | This study |
| pGBS125 | pAvcI$^{ETEC}$ | pEVS143::*avcI* from *E. coli* O78:H11 H10407 (ETEC); Km$^r$ | This study |
| pGBS126 | pAvcD$^{ETEC}$ | pMMB67EH::*avcD* from ETEC; Amp$^r$ | This study |
| pAW07 | pAvcI$^{VP}$ | pEVS143::*avcI* from *V. parahaemolyticus* O1:Kuk str. FDA_R31; Km$^r$ | This study |
| pAW06 | pAvcD$^{VP}$ | pMMB67EH::*avcD* from *V. parahaemolyticus* O1:Kuk str. FDA_R31; Amp$^r$ | This study |
| pAW02 | pAvcI$^{PM}$ | pEVS143::*avcI* from *P. mirabilis* AR379; Km$^r$ | This study |
| pAW04 | pAvcD$^{PM}$ | pMMB67EH::*avcD* from *P. mirabilis* AR379; Amp$^r$ | This study |
| pBRP15 | | pMMB67EH without $P_{tac}$; Amp$^r$ | This study |
| pBYH64 | | pBRP15::*avcI-avcD* operon with its upstream intergenic region position in *V. cholerae* N16961 [177,759-176,932]; Amp$^r$ | This study |
| pBYH67 | | pBRP15:: *avcI-avcD* operon with its upstream intergenic region position in *V. parahaemolyticus* O1:Kuk str. FDA_R31 (CP006004) [468,152-466,174]; Amp$^r$ | This study |
| pBYH65 | | pBRP15:: *avcI-avcD* operon with its upstream intergenic region position in *P. mirabilis* AR379 (NZ_CP029133), [3,698,504-3,700,828]; Amp$^r$ | This study |
| pBYH63 | | pBRP15:: *avcI-avcD* operon with its upstream intergenic region position in ETEC (NC_017723.1), [2,280-4,414]; Amp$^r$ | This study |
| pBYH69 | | pEVS143::*dcd1* from *Saccharomyces cerevisiae*; Km$^r$ | This study |
| pBYH81 | pAvcID$^{VP\text{-}avcD\text{S47K}}$ | pBYH67::*avcD*-S47K; Amp$^r$ | This study |
| pBYH82 | pAvcID$^{VP\text{-}avcD\text{E376K}}$ | pBYH6::*avcD*-E376K; Amp$^r$ | This study |
| pBYH83 | pAvcID$^{VP\text{-}avcD\text{S47K+E376K}}$ | pBYH67::*avcD*-S47K+E376K; Amp$^r$ | This study |
| pCRR01 | | Deletion construct for ΔVSP-1, Amp$^r$ | This study |
| pCRR02 | | Deletion construct for ΔVSP-2, Amp$^r$ | This study |
| pBYH36 | | Deletion construct for *Δig$^{222}$*, Amp$^r$ | This study |
| pBYH37 | | Deletion construct for *Δvc0176,* Amp$^r$ | This study |
| pGBS88 | | Deletion construct for Δ*avcD,* Amp$^r$ | This study |

**Supplementary Table 3:** Oligonucleotides used in this study

| Name | Primer use | Sequence | Reference |
|---|---|---|---|
| ***Vector Construction*** | | | |
| CMW3009 | *avcD* F[1] EcoRI + RBS[3] (pEVS143-AvcD) | GGAAACAGCCTCGACAGGCCTAGGAGGAAGCTAAATTGTTTACAATGAATAAGTCCTCCG | This study |
| CMW3010 | *avcD* R[2] BamHI (pEVS143-AvcD) | CATAAAGCTTGCTCAATCAATCACCGGATCCTAGTCTTGGATGCTCTCTTC | This study |
| CMW3025 | *avcD* F EcoRI + RBS (pMMB67EH-AvcD) | ATTTCACACAGGAAACAGAGGAGCTAAGGAAGCTAAATTGTTTACAATGAATAAGTCCTC | This study |
| CMW3026 | *avcD* R BamHI (pMMB67EH-AvcD) | CCTGCAGGTCGACTCTAGAGCTAGTCTTGGATGCTCTC | This study |
| CMW3066 | *avcD*+6His R BamHI (pEVS143-AvcD-$His_6$) | GCTTGCTCAATCAATCACCGTTAGTGGTGGTGGTGGTGGTGCTCGATGTCTTGG | This study |
| CMW3079 | *Ig*$^{222}$ F EcoRI + RBS (pEVS143-Ig$^{222}$) | CAGCCTCGACAGGCCTAGGAGGAGCTAAGGAAGCTAAACTGTTCGCAAATCATACTTTAG | This study |
| CMW3080 | *Ig*$^{222}$ R BamHI (pEVS143-Ig$^{222}$, pEVS143-AvcI & pEVS143-AvcI 3' end truncations and interior stop codon) | GCTTGCTCAATCAATCACCGTTACCAATGGATTTTTTGTG | This study |
| CMW3081 | *Ig*$^{222\text{-}STOP}$ F EcoRI + RBS (pEVS143- Ig$^{222\text{-}STOP}$) | CAGCCTCGACAGGCCTAGGAGGAGCTAAGGAAGCTAAATAGTTCGCAAATCATAGTTTAG | This study |
| CMW3093 | *avcD* F NcoI (pET28b-AvcD-$His_6$) | AACTTTAAGAAGGAGATATACATGTTTACAATGAATAAGTCCTCCGC | This study |
| CMW3094 | *avcD* R XhoI (pET28b-AvcD-$His_6$) | CTCAGTGGTGGTGGTGGTGGTGCTCGATGTCTTGGATGCTCTCTTCTTCACTCGATGG | This study |
| CMW3102 | *avcI* F EcoRI + RBS (pEVS143-AvcI & pEVS143-AvcI 5' end truncations) | CTCGACAGGCCTAGGAGGAGCTAAGGAAGCTAAAATGATTACAAGCATTCATGAATATAG | This study |
| CMW3103 | *avcI* F EcoRI + RBS (pEVS143-AvcI$^{STOP}$) | CTCGACAGGCCTAGGAGGAGCTAAGGAAGCTAAATAAATTACAAGCATTCATGAATATAG | This study |
| CMW3128 | *avcI*$^{49\text{-}186}$ F EcoRI + RBS (pEVS143-AvcI$^{49\text{-}186}$) | ACAGCCTCGACAGGCCTAGGAGGAGCTAAGGAAGCTAAAATGATTACAAG | This study |
| CMW2129 | *avcI*$^{49\text{-}186}$ R BamHI (pEVS143-AvcI$^{49\text{-}186}$) | GCTTGCTCAATCAATCACCGGGCTCTAGCTTTCTCTTTTTTTGCGTCTTTC | This study |
| CMW3162 | *avcD*$^{ETEC}$ F EcoRI + RBS (pEVS143-AvcD$^{ETEC}$) | ACAGCCTCGACAGGCCTAGGAGGAGCTAAGGAAGCTAAAATGGCTATAGCTTTGAAAAAG | This study |
| CMW3163 | *avcD*$^{ETEC}$ R BamHI (pEVS143-AvcD$^{ETEC}$) | GCTTGCTCAATCAATCACCGTTAAATCAAGTCATCTTGTTTTG | This study |
| CMW3164 | *avcD*$^{ETEC}$ F EcoRI + RBS (pMMB67EH-AvcD$^{ETEC}$) | AATTTCACACAGGAAACAGAGGAGCTAAGGAAGCTAAAATGGCTATAGCTTTGAAAAAGG | This study |

| | | | |
|---|---|---|---|
| CMW3165 | *avcD*$^{ETEC}$ F BamHI (pMMB67EH-AvcD$^{ETEC}$) | CCTGCAGGTCGACTCTAGAGTTAAATCAAGTCATCTTGTTTTGG | This study |
| CMW3166 | *avcI*$^{ETEC}$ F EcoRI + RBS (pEVS143-AvcI$^{ETEC}$) | ACAGCCTCGACAGGCCTAGGAGGAGCTAAGGAAGCTAAAATGTCAAACCAATTAACCG | This study |
| CMW3167 | *avcI*$^{ETEC}$ F BamHI (pEVS143-AvcI$^{ETEC}$) | GCTTGCTCAATCAATCACCGCTAATCAAGTATTATTTCTTTCTTTAGTATTTTATC | This study |
| CMW3180 | *avcI*$^{VP}$ F EcoRI + RBS (pEVS143-AvcI$^{VP}$) | ACAGCCTCGACAGGCCTAGGAGGAGCTAAGGAAGCTAAAATGGTTACAAATTTAAATG | This study |
| CMW3181 | *avcI*$^{VP}$ R BamHI (pEVS143-AvcI$^{VP}$) | GCTTGCTCAATCAATCACCGTTACCAACGAATTTTCTGTGCGGCTCTTAAAAG | This study |
| CMW3184 | *avcD*$^{VP}$ F EcoRI + RBS (pMMB67EH-AvcD$^{VP}$) | CAATTTCACACAGGAAACAGAGGAGCTAAGGAAGCTAAAATGGGAAAATCCTCTA | This study |
| CMW3185 | *avcD*$^{VP}$ R BamHI (pMMB67EH-AvcD$^{VP}$) | CCTGCAGGTCGACTCTAGAGTTATTCAATAGTGGCTTCTACTTGTTGCTTTGTGAATG | This study |
| CMW3189 | *avcI* F EcoRI (pEVS143-AvcI) | ACAGCCTCGACAGGCCTAGGATGATTACAAGCATTCATGAATATAGAAACGCTTC | This study |
| CMW3192 | *avcI*$^{PM}$ F EcoRI + RBS (pEVS143-AvcO$^{PM}$) | ACAGCCTCGACAGGCCTAGGAGGAGCTAAGGAAGCTAAAATGAACGTTCAAC | This study |
| CMW3193 | *avcI*$^{PM}$ R BamHI (pEVS143-AvcI$^{PM}$) | GCTTGCTCAATCAATCACCGTTACCAATCTAACGTGTCTGCTACAGCTGC | This study |
| CMW3196 | *avcD*$^{VP}$ F EcoRI + RBS (pMMB67EH-AvcD$^{PM}$) | CAATTTCACACAGGAAACAGAGGAGCTAAGGAAGCTAAAATGGGTAATCC | This study |
| CMW3197 | *avcD*$^{VP}$ R BamHI (pMMB67EH-AvcD$^{PM}$) | CCTGCAGGTCGACTCTAGAGTTAACTTCTCTCTTCACCTAAACGAAGATTTAC | This study |
| CMW3200 | *avcI*$^{49-204}$ R BamHI (pEVS143-AvcI$^{49-204}$) | GCTTGCTCAATCAATCACCGTGCAGCACGCAAAAGATTG | This study |
| CMW3201 | *avcI*$^{49-214}$ R BamHI (pEVS143-AvcI$^{49-214}$) | GCTTGCTCAATCAATCACCGGGATTTTTTGTGCAGCAC | This study |
| CMW3202 | *avcI*$^{49-218}$ R BamHI (pEVS143-AvcI$^{49-218}$) | GCTTGCTCAATCAATCACCGCAATGGATTTTTTGTGCAGCACGCAAAAGA | This study |
| CMW3203 | *avcI*$^{66-222}$ F EcoRI + RBS (pEVS143-AvcI$^{66-222}$) | ACAGCCTCGACAGGCCTAGGAGGAGCTAAGGAAGCTAAAGAATATAGAAACG | This study |
| CMW3204 | *avcI*$^{86-222}$F EcoRI + RBS (pEVS143-AvcI$^{86-222}$) | ACAGCCTCGACAGGCCTAGGAGGAGCTAAGGAAGCTAAAATAGCGACAAAAAC | This study |
| CMW3205 | *avcI*$^{123-222}$ F EcoRI + RBS (pEVS143-AvcI$^{123-222}$) | ACAGCCTCGACAGGCCTAGGAGGAGCTAAGGAAGCTAAAAGACACTAGCG | This study |
| CMW3306 | *avcI-avcD* w/ upstream intergenic regions from *V. cholerae* F EcoRI (pAvcI-AvcD$^{VC}$) | CGGGAAACCTGTCGTGCCAGCTAGTCTTGGATGCTCTC | This study |
| CMW3307 | *avcI-avcD* w/ upstream intergenic | CCTGCAGGTCGACTCTAGAGATAGAGACACTATATTTAGTGTTTAATTAAC | This study |

| | | | |
|---|---|---|---|
| | regions from *V. cholerae* R BamHI (pAvcI-AvcD$^{VC}$) | | |
| CMW3308 | *avcI-avcD* w/ upstream intergenic regions from ETEC F EcoRI (pAvcI-AvcD$^{ETEC}$) | CGGGAAACCTGTCGTGCCAGTTAAATCAAGTCATCTTGTTTTGGTTC | This study |
| CMW3309 | *avcI-avcD* w/ upstream intergenic regions from ETEC R BamHI (pAvcI-AvcD$^{ETEC}$) | CCTGCAGGTCGACTCTAGAGAGGCTCCGCTGAGAAAAAATTC | This study |
| CMW3310 | *avcI-avcD* w/ upstream intergenic regions from *P. mirabilis* F EcoRI (pAvcI-AvcD$^{PM}$) | CGGGAAACCTGTCGTGCCAGTTAACTTCTCTCTTCACCTAAAC | This study |
| CMW3311 | *avcI-avcD* w/ upstream intergenic regions from *P. mirabilis* R BamHI (pAvcI-AvcD$^{PM}$) | CCTGCAGGTCGACTCTAGAGTGCTTTAACTCCTAAAGG | This study |
| CMW3312 | *avcI-avcD* w/ upstream intergenic regions from *V. parahaemolyticus* F EcoRI (pAvcI-AvcD$^{VP}$) | CGGGAAACCTGTCGTGCCAGTTATTCAATAGTGGCTTCTAC | This study |
| CMW3313 | *avcI-avcD* w/ upstream intergenic regions from *V. parahaemolyticus* R BamHI (pAvcI-AvcD$^{VP}$) | TGCCTGCAGGTCGACTCTAGAGTCACTTTGCTGATTTAAGCAGAT | This study |
| CMW3335 | *dcd1$^{Sc}$* F EcoRI (pEVS143-Dcd1) | ACAGCCTCGACAGGCCTAGGAGGAGCTAAGGAAGCTAAAATGTTAATTGGTGTAAG | This study |
| CMW3336 | *dcd1$^{Sc}$* R BamHI (pEVS143-Dcd1) | GCTTGCTCAATCAATCACCGTTAAATCATCACAATTCTTGGTTC | This study |
| EWAvcDFwd | *avcD$^{4-532}$* F NdeI (pAvcD$^{4-532}$) For protein purification | GTGCCGCGCGGCAGCCATATGAATAAGTCCTCCGCAAA | This study |
| EWAvcDrev | *avcD$^{4-532}$* R XhoI (pAvcD$^{4-532}$) For protein purification | TGGTGGTGGTGGTGGTGCTTAGTCTTGGATGCTCTCTTCTT | This study |
| ***Site-directed Mutagenesis*** | | | |
| CMW3011 | *avcD* (E384A) F (pEVS143-AvcD$^{E384A}$ & pET28b-AvcD$^{E384A}$) | CAAGAGCGGTTCATGCTGCAATGGATTCTCTTATAGC | This study |
| CMW3012 | *avcD* (E384A) R (pEVS143-AvcD$^{E384A}$ & pET28b-AvcD$^{E384A}$) | GCTATAAGAGAATCCATTGCAGCATGAACCGCTCTTG | This study |

| | | | |
|---|---|---|---|
| CMW3013 | *avcD* (C411A + C414A) F (pEVS143-AvcD$^{C411A+C414A}$) | TATATGTTACGACATATCCGGCTCACAACGCTG CGCGACACATCGTTGCTG | This study |
| CMW3014 | *avcD* (C411A + C414A) R (pEVS143-AvcD$^{C411A+C414A}$) | CAGCAACGATGTGTCGCGCAGCGTTGTGAGCC GGATATGTCGTAACATATA | This study |
| CMW3021 | *avcD* (K55A) F (pEVS143-AvcD$^{K55A}$) | GCTATTGGCTCTGGTGTAGCGGCATTAAAAGAG AGTTTAGTTAGTTCTCTTGAGACATAT | This study |
| CMW3022 | *avcD* (K55A) R (pEVS143-AvcD$^{K55A}$) | ATATGTCTCAAGAGAACTAACTAAACTCTCTTTT AATGCCGCTACACCAGAGCCAATAGC | This study |
| CMW3104 | *avcD* (D162A + Q163A) F (pEVS143-AvcD$^{D162A+Q163A}$) | CGCATACATCATCGCGGCGTTAAAGCACCCTGA TGAAATCAAATTCC | This study |
| CMW3105 | *avcD* (D162A + Q163A) R (pEVS143-AvcD$^{Q162A+Q163A}$) | GGAATTTGATTTCATCAGGGTGCTTTAACGCCG CGATGATGTATGCG | This study |
| CMW3110 | *avcD* (S52K) F (pEVS143-AvcD$^{S52K}$) | CCTCTGTGGGGCTATTGGCAAAGGTGTAAAGG CATTAAAAGAGAG | This study |
| CMW3111 | *avcD* (S52K) R (pEVS143-AvcD$^{S52K}$) | CTCTCTTTTAATGCCTTTACACCTTTGCCAATAG CCCCACAGAGG | This study |
| CMW3112 | *avcD* (S52P) F (pEVS143-AvcD$^{S52P}$) | CCTCTGTGGGGCTATTGGCCCGGGTGTAAAGG CATTAAAAGAGAG | This study |
| CMW3113 | *avcD* (S52P) R (pEVS143-AvcD$^{S52P}$) | CTCTCTTTTAATGCCTTTACACCCGGGCCAATA GCCCCACAGAGG | This study |
| CMW3114 | *avcD* (S52W) F (pEVS143-AvcD$^{S52W}$) | CCTCTGTGGGGCTATTGGCTGGGGTGTAAAGG CATTAAAAGAGAG | This study |
| CMW3115 | *avcD* (S52K) R (pEVS143-AvcD$^{S52W}$) | CTCTCTTTTAATGCCTTTACACCCCAGCCAATAG CCCCACAGAGG | This study |
| CMW3118 | *avcI* (interior alternative frame stop) F (pEVS143-AvcI17A>T, 18T>A, 19G>A) | AAGGAAGCTAAAATGATTACAAGCATTCTAAAAT ATAGAAACGCTTCTAATAGCG | This study |
| CMW3119 | *avcI* (interior alternative frame stop) R (pEVS143-AvcI17A>T, 18T>A, 19G>A) | CGCTATTAGAAGCGTTTCTATATTTTAGAATGCT TGTAATCATTTTAGCTTCCTT | This study |
| CMW3448 | *avcD$^{VP}$* (S47K) F | ATTGGTCTTTGTGGAGCTATAGGCAAGGGTGTG AAAGCACTAAAAGATAAC | This study |
| CMW3449 | *avcD$^{VP}$* (S47K) R | GTTATCTTTTAGTGCTTTCACACCCTTGCCTATA GCTCCACAAAGACCAAT | This study |
| CMW3450 | *avcD$^{VP}$* (E376A) F | GAGAGCTGTACACGCAGCAATGGATGCCATTGT TG | This study |
| CMW3451 | *avcD$^{VP}$* (E376A) R | CAACAATGGCATCCATTGCTGCGTGTACAGCTC TC | This study |
| ***Gene Deletion*** | | | |
| CMW2794 | ΔVSP-2 up[4] F; CR02 & CR03 | GTGGAATTCCCGGGAGAGCTCGGCTTGTTCACT ATCGTAATAATGC | This study |
| CMW2795 | ΔVSP-2 up R; CR02 & CR03 | GGAGGGGCCACCACTGGGAGGGCACCAGATTC | This study |
| CMW2796 | ΔVSP-2 down[5] F; CR02 & CR03 | GCCCTCCCAGTGGTGGCCCCTCCCAGGT | This study |

| | | | |
|---|---|---|---|
| CMW2797 | ΔVSP-2 down R; CR02 & CR03 | AGCTATAGTTCTAGAGGTACGGGCATTAAGGTG GTGGAAACCG | This study |
| CMW2814 | ΔVSP-1 up F; CR01 & CR03 | GTGGAATTCCCGGGAGAGCTGGCTTTACTGTTA TTCGC | This study |
| CMW2815 | ΔVSP-1 up R; CR01 & CR03 | TACCATGTAGTAGCGGTATCGAGATTCC | This study |
| CMW2816 | ΔVSP-1 down F; CR01 & CR03 | GATACCGCTACTACATGGTAACGAACTCTTC | This study |
| CMW2817 | ΔVSP-1 down R; CR01 & CR03 | AGCTATAGTTCTAGAGGTACCGCTAAGTTTGTG GATGC | This study |
| CMW2970 | Δ*vc0176* up F; BYH207 | ATAACAATTTGTGGAATTCCCGGGAGAGCTGGG AATCGAATATTGAGAG | This study |
| CMW2971 | Δ*vc0176* up R; BYH207 | ATATAGTGTCTCTATTTATGGCTCATAATCTTGA AG | This study |
| CMW2972 | Δ*vc0176* down F; BYH207 | GATTATGAGCCATAAATAGAGACACTATATTTAG TGTTTAATTAAC | This study |
| CMW2973 | Δ*vc0176* down R; BYH207 | TGCGCATGCTAGCTATAGTTCTAGAGGTACTAT GAAACTTATTTCTATACTCTCAG | This study |
| CMW3067 | Δ*avcD* up F; GS05 | GTGGAATTCCCGGGAGAGCTACTATATTTAGTG TTTAATTAACAAAAAAC | This study |
| CMW3068 | Δ*avcD* up R; GS05 | CAGACTAAAGCCTGAAATTATGAAACTTATTTCT ATAC | This study |
| CMW3069 | Δ*avcD* down F; GS05 | TAATTTCAGGCTTTAGTCTGGAAAATTCACTTTT C | This study |
| CMW3070 | Δ*avcD* down R; GS05 | AGCTATAGTTCTAGAGGTACACATGGAGCATGA TCAGG | This study |
| CMW3071 | Δ*Ig*$^{222}$ up F; BYH206 | ATAACAATTTGTGGAATTCCCGGGAGAGCTTCT CAAAGAAGCACGTAAAAAAG | This study |
| CMW3072 | Δ*Ig*$^{222}$ up R; BYH206 | CAAGAATTAACGTGGTAAAGTGCGCACATTCTA C | This study |
| CMW3073 | Δ*Ig*$^{222}$ down F; BYH206 | AATGTGCGCACTTTACCACGTTAATTCTTGATTA GC | This study |
| CMW3074 | Δ*Ig*$^{222}$ down R; BYH206 | TGCGCATGCTAGCTATAGTTCTAGAGGTACTCA TTTTCTTCTGAGGTTTC | This study |
| ***qPCR*** | | | |
| CMW2926 | *gyrA* F | TGGCCAGCCAGAGATCAAG | This study |
| CMW2927 | *gyrA* R | ACCCGCAGCGGTACGA | This study |
| CMW3206 | *avcD* F | TCGACCAGTTAAAGCACCCT | This study |
| CMW3207 | *avcD* R | CCTTCTGTACGGATCAAGCCA | This study |
| CMW3208 | *avcI* F | GTGAATGGATATTTCGGTGGA | This study |
| CMW3209 | *avcI* R | TTGTCGCTATTAGAAGCGTT | This study |
| CMW3288 | *oriI* F | CAGGTGAACCAGCAAAATCGA | [71] |
| CMW3289 | *oriI* R | TGGTATTGAAGCTCAATGCGG | [71] |
| CMW3290 | *terI* F | TTCAAGCTGAGGCGGATTTG | [71] |
| CMW3291 | *terI* R | GCTCATTGGCTTCTTGTGCTT | [71] |
| ***In vitro Transcription Synthesis*** | | | |
| EJW002 | *avcI* RNA F | GACCATGATTACGCCATAATACGACTCACT ATAGGGATGATTACAAGCATTCATG | This study |
| EJW003 | *avcI* RNA R | [mU][mU]ACCAATGGATTTTTTGTGC | This study |
| EJW016 | *avcI*-RC RNA F | GACCATGATTACGCCATAATACGACTCACT ATAGGGTTACCAATGGATTTTTTG | This study |

| | | | |
|---|---|---|---|
| EJW017 | *avcI*-RC RNA R | [mA][mU]GATTACAAGCATTCATG | This study |

[1]F = Forward
[2]R= Reverse
[3]RBS= Ribosomal Binding Site
[4]Up= Amplifies Upstream Fragment
[5]Down= Amplifies Downstream Fragment

**Supplementary Table 4:** Maximum conservation of homologs from different phylogenetic lineages.

**AvcD homologs summary table**

Lineages and percentage similarities of AvcD homologs containing both DCD and PLN domains

| DomArch.Gene3D | Lineage | Max%Positive |
|---|---|---|
| PLN+DCD | Bacteria>Proteobacteria | 100.00 |
| PLN+DCD | Bacteria>Bacteroidetes | 58.80 |
| PLN+DCD | Bacteria>Balneolaeota | 56.02 |
| PLN+DCD | Bacteria>Actinobacteria | 55.63 |
| PLN+DCD | Archaea>Thaumarchaeota | 53.65 |
| PLN+DCD | Bacteria>Firmicutes | 52.27 |
| PLN+DCD | Bacteria>Planctomycetes | 52.27 |
| PLN+DCD | Bacteria | 51.88 |
| NABP+PLN+DCD | Bacteria>Proteobacteria | 51.09 |
| PLN+DCD | Bacteria>Acidobacteria | 49.70 |
| PLN+DCD | Bacteria>Verrucomicrobia | 48.69 |
| PLN+DCD | Bacteria>Chlamydiae | 45.25 |
| PLN+DCD+NABP | Bacteria>Proteobacteria | 42.48 |
| PLN+DCD+NABP+NABP | Bacteria>Proteobacteria | 39.85 |
| PLN+PLN+DCD | Bacteria>Proteobacteria | 36.75 |
| PLN+DCD | Bacteria>Cyanobacteria | 34.63 |
| PLN+DCD | Eukaryota>Ascomycota | 27.63 |
| PLN+DCD | Eukaryota>Ciliophora | 27.57 |
| PLN+DCD | Eukaryota>Basidiomycota | 25.10 |
| PLN+DCD | Eukaryota>Chytridiomycota | 23.77 |
| PLN+DCD | Eukaryota>Mucoromycota | 22.63 |
| PLN+DCD | Eukaryota>Apicomplexa | 19.96 |
| PLN+DCD | Eukaryota>Streptophyta | 19.39 |
| PLN+Znf_CCHC+DCD | Eukaryota>Ascomycota | 16.92 |


**Abbreviations**. PLN, P-loop containing nucleotide triphosphate hydrolases; DCD, Cytidine Deaminase domain 2; NABP, Nucleic acid-binding proteins; Znf_CCHC, Zinc finger CCHC-type

**Supplementary Table 5:** Absolute intracellular concentration of dNTPs from Figure 2E

| | Absolute Intracellular dNTP Concentration in pmol/mg of Total Protein | | | | |
|---|---|---|---|---|---|
| Nucleotides | pVector | pAvcD$^{WT}$ | pAvcD$^{S52K}$ | pAvcD$^{E384A}$ | pAvcD$^{ETEC}$ |
| dATP | 37.8 ± 2.6 | 60.9 ± 9.1 | 32.3 ± 1.8 | 35.0 ± 2.9 | 29.5 ± 2.9 |
| dCTP | 33.2 ± 3.6 | 8.3 ± 1.5 | 28.9 ± 1.6 | 34.7 ± 3.7 | 11.8 ± 0.9 |
| dGTP | 213.4 ± 22.7 | 229.5 ± 69.6 | 178.5 ± 8.9 | 211.9 ± 18.4 | 163.9 ± 9.7 |
| dTTP | 57.1 ± 3.7 | 82.47 ± 23.3 | 44.9 ± 1.2 | 53.9 ± 2.1 | 51.9 ± 11.1 |
| dUTP | 5.4 ± 0.5 | n.d. | 4.7 ± 0.4 | 5.6 ± 0.5 | n.d. |
| dCMP | 3579.1 ± 1242.7 | 592.8 ± 113.9 | 3429.7 ± 1113.2 | 3513.9 ± 975.2 | 972.6 ± 318.1 |
| dUMP | 46.6 ± 14.5 | 35.4 ± 1.4 | 45.7 ± 16.5 | 43.5 ± 5.6 | 49.8 ± 16.3 |