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Supplementary Information for

Direct activation of a phospholipase by cyclic GMP-AMP in El Tor *Vibrio cholerae*

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Supplementary Information Text

Materials and Methods

Strain Construction. The strains and plasmids used in this study are listed in Supplementary Table S1. Plasmid inserts were generated by PCR using primers as listed in Supplementary Table S2, then inserted into vectors by restriction digest and ligation. Plasmids were transformed into *Escherichia coli* S17-1λ*pir* or *E. coli* BW29427. Transformants were checked for the presence of the correct plasmids and the inserts were confirmed by sequencing. Plasmids were transferred into *Vibrio cholerae* by conjugation with *E. coli*.

Overexpression of *dncV* was achieved from plasmid constructs derived from either pEVS143 or pMMB67EH by induction of the P_{tac} promoter following introduction of IPTG. Induction of either plasmid with 100 µM IPTG causes cell death (shown in Fig. 1 using pEVS143-dncV and in Fig. S5 using pMMB67EH-dncV). The pMMB67EH-derived constructs were used for the cosmid screen, colony morphology images, quantification of intracellular cyclic dinucleotides by UPLC-MS/MS, and lipid extraction experiments. The pEVS143-derived constructs were used in all growth curves, the transposon screen, and the membrane integrity microscopy images.

To delete genes from the *V. cholerae* genome, the 500-1000bp regions of genomic DNA immediately upstream and downstream of the gene intended for deletion were amplified using primers listed in Supplementary Table 2. Construct pGBS40 was constructed using three-piece Gibson Assembly of the upstream and downstream products and the suicide vector pKAS32 (1). All other gene deletion constructs were constructed by using SOE PCR to splice upstream and downstream products, followed by digestion and ligation into pKAS32. The plasmid was transformed into *E. coli*, then transferred to *V. cholerae* by conjugation. Allelic exchange was allowed to occur, then *V. cholerae* colonies were screened for successful deletion of the gene.

All mutations generated in this study were confirmed by Sanger sequencing. Classical *V. cholerae* strains were derived from the O395 isolate and El Tor *V. cholerae* strains were derived from C6706 str2, a streptomycin resistant isolate of C6706. Our laboratory strain of C6706 str2 is quorum sensing proficient and has not acquired the LuxO G333S quorum sensing mutation (2).

Culture Conditions. Unless otherwise noted, *V. cholerae* and *E. coli* cultures were grown in LB broth with aeration or on LB agar plates at 30 °C and 37 °C, respectively. Where appropriate, antibiotics were used at the following concentrations, unless otherwise noted: 100 µg/ml ampicillin (Amp), 100 µg/ml kanamycin (Kan), 100 µg/ml spectinomycin (Sp), 100 µg/ml streptomycin (Sm), and 5 µg/ml tetracycline (Tet). Addition of 100 µM or 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) to either agar plates or broth was used to induce transcription from the P_{tac} promoter. *E. coli* BW29427, a diaminopimelic acid (DAP) auxotroph, was additionally supplemented with 300 µg/mL DAP.

Transposon Mutagenesis Screen. El Tor *V. cholerae* carrying pEVS143-DncV was mutagenized using the mTn*10* delivery vector pDL1098 as previously described (3). The transposon library was plated on LB agar plates supplemented with Kan and Sp, and either 100 µM or no IPTG. Colonies on agar plates containing IPTG which were the same size as control colonies on plates without IPTG were grown overnight in LB supplemented with Kan and Sp, and their ability to suppress growth arrest caused by DncV overproduction was confirmed in a planktonic growth assay (as described below). The region of transposon insertion in these mutants was determined by Sanger sequencing and mapping to the *V. cholerae* N16961 genome (GenBank Accession No. NC_002505 and NC_002506).

Cosmid Library Screen. Construction of the cosmid library in pLAFR was initiated by digestion of C6706 str2 genomic DNA using Sau3AI. The digested DNA fragments were separated using gel electrophoresis and random fragments of ~23 kb were isolated and cloned into the pLAFR BamHI site. The cosmid library was maintained in *E. coli* DH5α and mobilized into *V. cholerae* O395 carrying pMMB67EH-DncV with the assistance of *E. coli* harboring the pRK2013 helper plasmid. Transconjugates were selected on LB agar plates supplemented with Amp, Tet, and 1000 µg/mL Sm. Additionally, isolates were confirmed to be *V. cholerae* using thiosulfate citrate bile salts sucrose (BD Difco^{TM} TCBS) agar plates. ~200 isolated *V. cholerae* transconjugate colonies were grown overnight at 35 °C in LB supplemented with antibiotics. Overnight cultures were subcultured into 96-well microplates, one containing 100 µM IPTG and one without, and grown at 35 °C. Hourly OD_{600} measurements were performed using an EnVision multimode plate reader (PerkinElmer). The criteria for cGAMP sensitivity was a >40% reduction in planktonic growth after 6 hours in the presence of IPTG compared to the complementary culture's growth in its absence. Two cosmids resulting in O395 cGAMP sensitivity were isolated, pCCD7 and pCCD13, and the El Tor *V. cholerae* C6706 genomic fragments harbored within the cosmids were identified using Sanger sequencing. The genomic fragment in pCCD7 begins 470 bp into the *vc0172* ORF and ends 644 bp into the *vc0187* ORF, while the pCCD13 fragment begins 104 bp into the *vc0172* ORF and ends 806 bp into the *vc0188* ORF.

Planktonic Growth Assays. *V. cholerae* and *E. coli* strains carrying pEVS143-derived plasmid constructs were streaked on LB agar plates supplemented with Kan, then colonies were grown overnight in LB with Kan. Overnight cultures were subcultured (1:1000 dilution) into 200 µL LB supplemented with Kan and either 100 µM or no IPTG in a 96-well microplate. 50 µl Light Mineral Oil (Millipore) was added to the top of each well. The cultures were grown for 10 hr in a BioTek Synergy HTX Plate Reader, with OD_{600} measurements every 30 min.

Colony Morphology Imaging. *V. cholerae* strains carrying pMMB67EH-derived plasmid constructs were streaked on LB agar plates supplemented with Amp and either 100 µM or no IPTG. Inoculated plates were inverted and grown at 35 °C. El Tor *V. cholerae* strains were grown for 14 hr and classical *V. cholerae* O395 strains were grown for 20 hr prior to imaging. Colonies were visualized using a Leica MZ6 modular stereomicroscope with transillumination and images were captured using an iPhone 6s (iOS 11.1.2).

Protein Purification. For use of CapV in the *in vitro* serine hydrolase and phospholipase assays, *E. coli* BL21(DE3) carrying pET28b-CapV-His_6 was first grown at 37 °C to $OD_{600} \approx 0.5$ in LB supplemented with Kan, and then expression was induced with 1 mM IPTG for 4 hr at 37 °C. The cells were collected by centrifugation, resuspended in binding buffer [50 mM sodium phosphate (pH 7.4), 300 mM NaCl, 20 mM imidazole (pH 7.4), 5% (vol/vol) glycerol] supplemented with DNase I, and lysed using a fluidizer. Lysate supernatant was filter-clarified through a 0.45 µm sterile syringe filter (Millipore) then applied to a HisTrap HP column (GE Healthcare) equilibrated with binding buffer. The column was then washed in binding buffer and His_6-tagged CapV was eluted with increasing concentrations of imidazole in binding buffer. Fractions containing CapV were pooled.

For use of CapV in the microscale thermophoresis binding assay, CapV was amplified from WN5144 and cloned into pET28b using Gibson Assembly (New England Biolabs) to remove extra amino acids between CapV and its C-terminal His_6-tag. *E. coli* BL21(DE3) carrying this modified pET28b-CapV-His_6 was first grown at 37 °C to $OD_{600} \approx 0.5$ in LB supplemented with

Kan, and then expression was induced with 1 mM IPTG for 16 hr at 18 °C. The cells were collected by centrifugation and lysed in buffer A [20 mM sodium phosphate (pH 7.3), 300 mM NaCl, 20 mM imidazole, 10% (v/v) glycerol] supplemented with 20 μg/mL DNase and 1 mM phenylmethylsulfonyl fluoride. Lysate supernatant was applied to His-60 Ni resin (Takara Bio USA) equilibrated in buffer A. The resin was then washed in buffer A and His_6-tagged CapV was eluted with increasing concentration of imidazole in buffer A. Fractions containing CapV were pooled and dialyzed against buffer B [20 mM sodium phosphate (pH 7.3), 300 mM NaCl].

Serine Hydrolase Assay. Assay buffer [50 mM sodium phosphate (pH 7.4), 300 mM NaCl, 10% (v/v) glycerol] containing 1.6μM His_6-tagged CapV, was mixed with varying concentrations of cGAMP (Invivogene) or other nucleotides and incubated at room temperature for 30 seconds. Then 1μM of fluorophosphonate-rhodamine probe (FP-Rh; a generous gift from Dr. Aimee Shen, Tufts University) was added to the reaction and incubated for 1 hr. The reaction was quenched by adding an equal volume of SDS loading dye and applying the mixture to a 12% SDS/PAGE gel. Gels were scanned using a Fujifilm Starion FLA-9000 image scanner with 532 nm excitation and BPG1 (570DF20) emission filter, then stained with Coomassie blue and imaged with a Syngene G:BOX Chemi XT4.

Phospholipase Assay. *In vitro* lipase assays were assembled in parallel using dioleoyl-PE (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine; Avanti Polar Lipids) as the lipase substrate. For each reaction, 100 µg dioleoyl-PE dissolved in chloroform was dried under N_2 gas, resuspended in 140 µL of reaction buffer [50 mM sodium phosphate (pH 7.5), 300 mM NaCl, 10% (v/v) glycerol, 5.4 mM decyl β-D-maltopyranoside], and dispersed by sonication for 4 x 5 s with a Misonix Sonicator 3000 (microprobe; power setting 1.5). Either additional buffer (control) or 500 nM His_6-tagged CapV was added to each reaction along with 1 µM cyclic dinucleotide ligand (cGAMP, c-di-GMP, or c-di-AMP; Axxora) in a total reaction volume of 150 µL. The reaction mixtures were sonicated a second time for 2 x 5 s as before. Reactions were incubated at ~ 23 °C for 22 hr and terminated by the addition of 800 µL organic lipid extraction buffer [methanol, chloroform, formic acid (20:10:1, v/v/v)]. Extraction mixtures were vortexed for 5 min, followed by the addition of 400 µL inorganic aqueous lipid extraction buffer [0.2 M H_3PO_4, 1 M KCl], and further vortexed for 10 s. Samples were centrifuged at 12k x g for 2 min, then the upper aqueous phase was discarded, while the lower organic phase was transferred to a new tube and dried under N_2 gas. The resulting desiccated lipids were dissolved in 18 µL chloroform and resolved by thin-layer chromatography (TLC). Neutral lipids were resolved on a silica gel TLC plate (EMD Chemical) while polar lipids were resolved on a silica gel TLC plate impregnated with ammonium sulfate. TLC mobile phases for neutral and polar lipids were composed of petroleum ether, ether, and acetic acid (80:20:1, v/v/v) and chloroform, methanol, glacial acetic acid, water (65:35:8:5, v/v/v/v), respectively. Lipids were visualized following brief exposure to iodine vapor. The co-migrated neutral lipid standards used for the identification of FFA and diacylglycerol (DAG) were linoleic acid and 1-2-dioleoyl-sn-glycerol, respectively (Avanti Polar Lipids).

Microscale Thermophoresis. His_6-tagged CapV was diluted to 200 nM in buffer B [20 mM sodium phosphate (pH 7.3), 300 mM NaCl]. 100 nM His_6-tagged CapV was labeled overnight using the RED-tris-NTA His-Tag Labeling kit (NanoTemper Technologies). Threefold titrations of cGAMP in milliQ water were incubated with 50 nM working stock solutions of labeled protein in the dark for 30 min at room temperature. After incubation, the samples were transferred into Standard Treated Capillaries (NanoTemper Technologies) and read in a Monolith NT.115 Blue/Red instrument at room temperature using 60% LED and medium MST power. Binding affinities were calculated from four experiments.

Lipid Extraction and Analysis. The extraction and analysis of *V. cholerae* lipids was based on a previously published procedure (4). Strains carrying pMMB67eh derived plasmids were grown overnight in LB supplemented with Amp. Overnight cultures were subcultured into 400 mL of LB with Amp and grown to $OD_{600} \approx 1.0$. Immediately following withdrawal of culture for the initial lipid extraction (T0), the bulk culture was induced by adding 1 mM IPTG. The second and third extractions were performed 30 (T30) and 60 (T60) minutes post-induction, respectively. All lipid extractions were performed on sample volumes normalized to the equivalent of 27 mL of culture at an $OD_{600} = 1.0$. The cells were collected by centrifugation at 4 °C. 5 mL organic extraction buffer [methanol, chloroform, 0.1 formic acid (20:10:1, v/v/v)] was added to the cell pellets, followed by 10 min of vortexing. 2.5 mL inorganic aqueous buffer [0.2 M H_3PO_4, 1 M KCl] was then added, followed by another 10 min of vortexing. The mixture was centrifuged at 7000 x g for 3 min, then the lower liquid phase was collected and dried under N_2 gas. Dried lipid samples were resuspended in chloroform and loaded onto TLC plates. Polar lipids were separated on ammonium sulfate-impregnated silica gel TLC plates (EMD Chemical) with a solvent composed of acetone, toluene, and water (91:30:7.5, v/v/v). Neutral lipids were resolved on DC-Fertigplatten SIL G-25 TLC plates (Macherey-Nagel) using a solvent composed of petroleum ether, ether, and acetic acid (80:20:1, v/v/v). PE, PG, and FA lipid standards used are dioleoyl-PE (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine), dioleoyl-PG (1,2-dioleoyl-sn-glycero-3-phospho-(1'-rac-glycerol)), and linoleic acid (Avanti Polar Lipids). All lipids were visualized by brief exposure to iodine vapor and lipids of interest were mechanically isolated from TLC plates. Pentadecanoic acid (15:0) was added as an internal standard and a transesterification reaction was performed to convert the lipid acyl groups into fatty acid methyl esters by adding 1 mL 1 N methanolic HCL solution and incubating at 80 °C for 30 min. After the solution cooled to room temperature, 1 mL 0.9% NaCl and 1 mL hexane were added. The mixture was centrifuged at 3000 x g for 3 min, after which the upper liquid phase was collected, dried with N_2 gas, and resuspended in hexane. Isolated fatty acid methyl esters were loaded on gas chromatography (Agilent Technology 7890A GC system) for identification and quantification using a previously described GC method (4, 5).

UPLC-MS/MS Quantification of Intracellular Cyclic Dinucleotides. El Tor *Vibrio cholerae* Δ*capV* carrying pMMB67EH-DncV was grown overnight in LB supplemented with Amp at 35 °C. Overnight cultures were subcultured and grown to $OD_{600} \approx 1.0$. An initial sample (0 min) of 1.4 mL culture was removed and the remaining bulk cultures were each divided into two flasks. Expression of *dncV* was induced by the addition of 1mM IPTG to one of the paired flasks, while the other remained as an uninduced control. Subsequent 1.4 mL samples were collected from paired flasks at 15, 30, and 60 minutes after division of the bulk culture. Cells were immediately harvested by centrifugation (1 min, 21k x g), the supernatant was removed by aspiration, and cell pellets were resuspended in 200 µL ice cold extraction buffer [acetonitrile, methanol, ultra-pure water, formic acid (2:2:1:0.02, v/v/v/v)]. Extraction suspensions were incubated on ice for 1 hr, then cellular debris was pelleted by centrifugation (2 min, 21k x g), and the supernatant was transferred to a new tube and dried overnight in a speed vac. Dried extracts were dissolved in 100 µL ultra-pure water. A standard curve was generated by adding pooled cyclic dinucleotides (cGAMP, c-di-GMP, c-di-AMP; Axxora) of known concentrations to rehydrated extracts collected from uninduced cultures of El Tor *V. cholerae* Δ*capV* containing pMMB67EH-DncV. Experimental samples and cyclic dinucleotide standards (1.95 – 120 nM) were analyzed by UPLC-MS/MS using an Acquity Ultra Performance LC system (Waters) coupled with a Quattro Premier XE mass spectrometer (Waters). Using negative-ion mode electrospray ionization with multiple-reaction monitoring, the parent>daughter ions, cone voltages, and collision energies used to monitor c-di-GMP, c-di-AMP, and cGAMP were (689.16>344.31, 50.0 V, and 34.0 V), (657.00>134.00, 56.0 V, and 50.0 V), and (673.24>343.93, 50.0 V, and 34.0 V), respectively. General buffer preparations, UPLC gradients, and MS/MS parameters were performed using a previously published method (6). The intracellular concentrations of cyclic dinucleotides were calculated using a previously published method (7).

FM 4-64 Microscopy. El Tor *V. cholerae* carrying pEVS143-DncV was grown overnight in LB supplemented with Kan. Overnight cultures were subcultured into 10 mL of LB with Kan and grown to $OD_{600} \approx 0.3$. Each culture was then split into two and 100 µM IPTG was added to one of the new cultures. Both induced and uninduced cultures were grown for 2 hr, then FM 4-64FX (Molecular Probes) dye was added to both to a final concentration of 2 µg/mL. Cells were fixed in 4% paraformaldehyde then immediately examined with an Olympus BX60 microscope. FM4-64 signal was visualized using an X-cite exacte fluorescence lightsource (FM 4-64FX: Ex565 Em744). Images were captured using a Hamamatsu ORCA-ER camera and Volocity 6.1 software (PerkinElmer) and processed using ImageJ software.

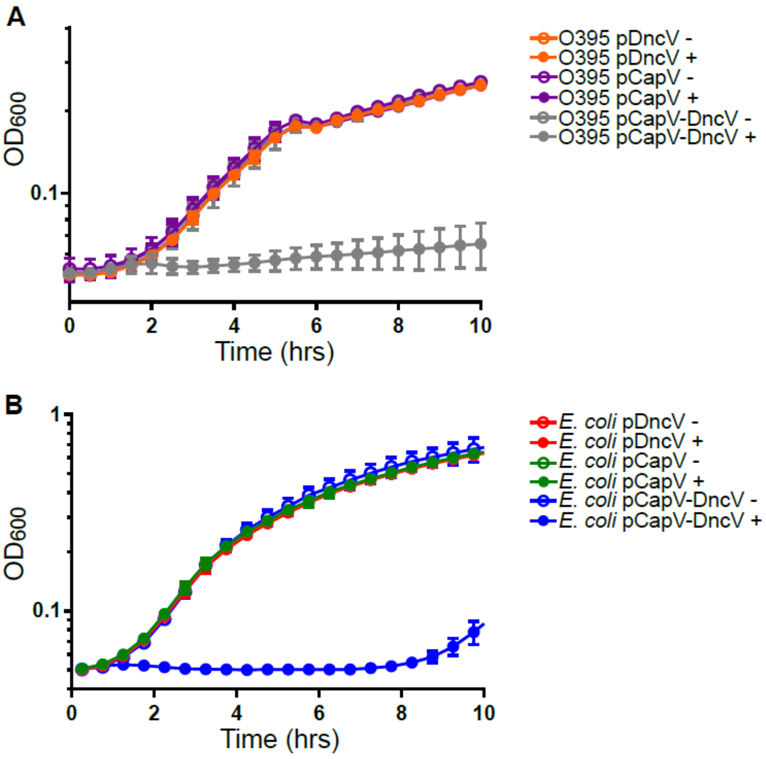

Fig. S1. Co-expression of *capV* and *dncV*, but not independent expression of either gene alone, induces planktonic growth arrest in classical *V. cholerae* and *E. coli*. *(A)* Growth curves of the classical *V. cholerae* strain O395 carrying a P_{tac}-inducible plasmid encoding either *dncV* (pDncV), *capV* (pCapV), or both genes (pCapV-DncV), grown in the absence (-) or presence (+) of 100µM IPTG. *(B)* Growth curves of *E. coli* S17-1λ*pir* carrying pDncV, pCapV, or pCapV-DncV grown in the absence (-) or presence (+) of 100 µM IPTG. Each data point represents the mean ± SD of six biological replicates and growth curves are representative of at least three independent experiments.

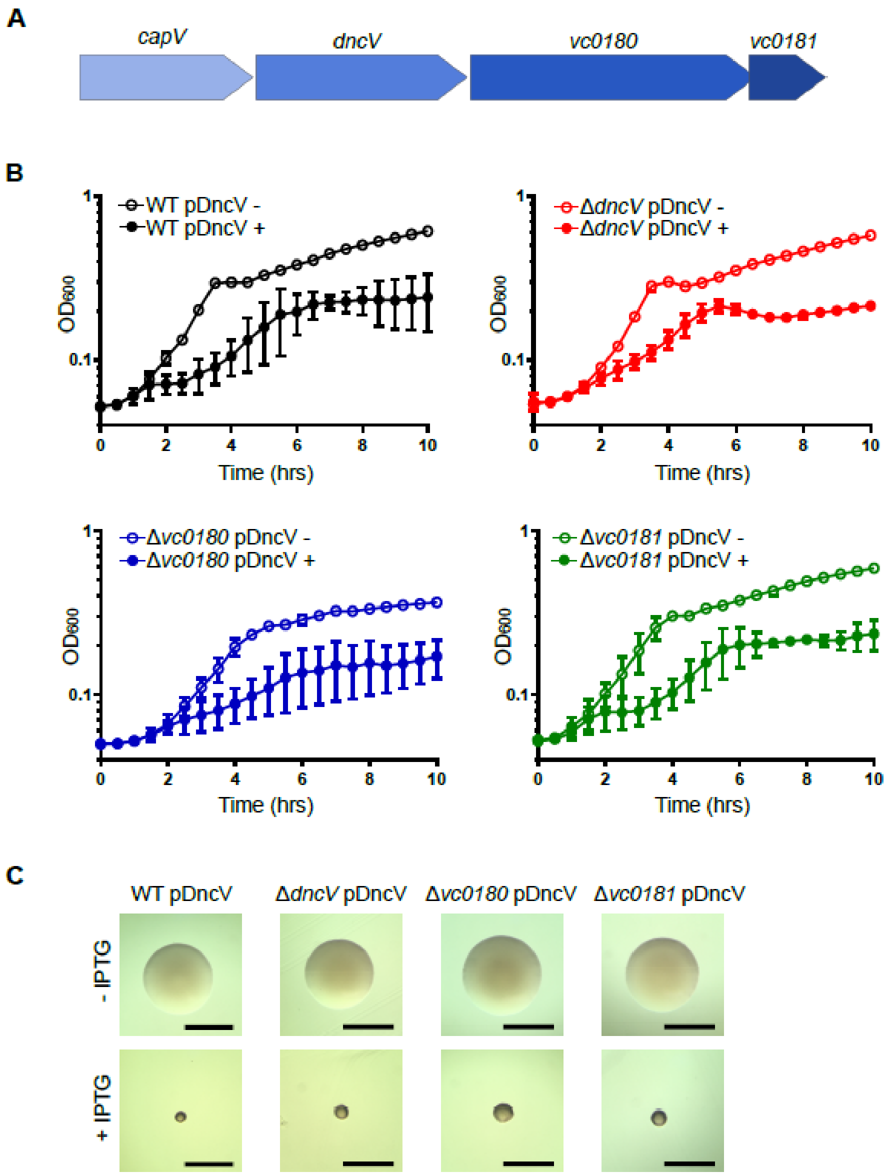

Fig. S2. Deletion of genes downstream of *capV* does not suppress cGAMP-induced growth arrest. *(A)* Cartoon representation of the putative four gene operon that includes *capV*, *dncV* as well as two additional hypothetical genes: *vc0180* and *vc0181*. *(B)* Growth curves of El Tor *V. cholerae* WT and Δ*dncV*, Δ*vc0180*, and Δ*vc0181* mutants, each carrying pDncV, grown in the absence (-) or presence (+) of 100 µM IPTG. Each data point represents the mean ± SD of six biological replicates. *(C)* Colony morphologies of El Tor *V. cholerae* WT, Δ*dncV*, Δ*vc0180*, and Δ*vc0181*, each carrying pDncV, grown on solid agar plates in the absence (top) or presence (bottom) of 100 µM IPTG. Scale bars: 1 mm. Growth curves and colony images are representative of at least three independent experiments.

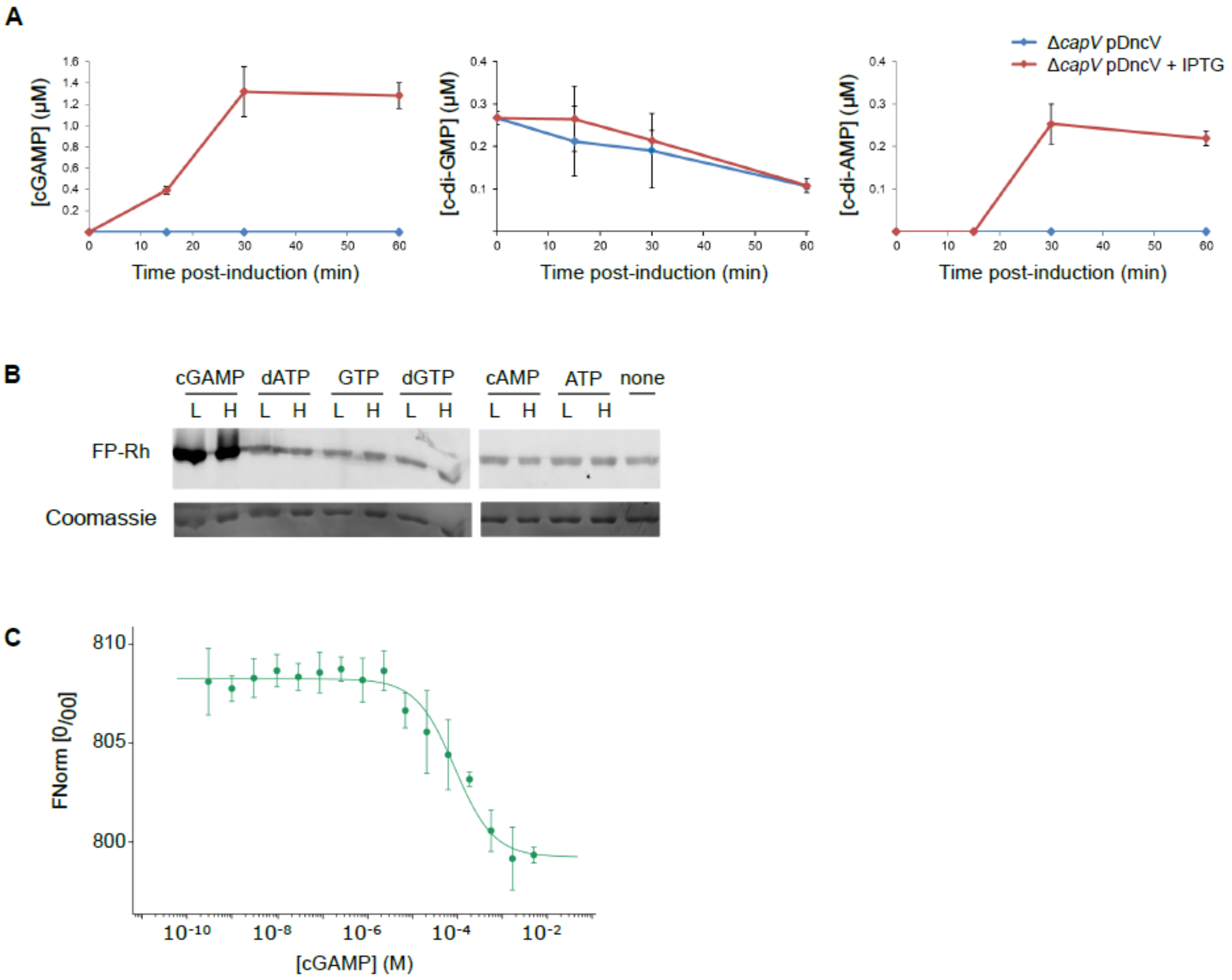

Fig. S3. Overexpression of *dncV* leads to an increase in intracellular cGAMP, and CapV activation is specific to cGAMP. *(A)* Quantitation by UPLC-MS/MS of intracellular concentrations of cyclic dinucleotides extracted from El Tor *V. cholerae* Δ*capV* carrying pDncV, grown in the absence (blue) and presence (red) of 1 mM IPTG. Each data point represents the mean ± SD of four biological replicates. *(B)* Covalent labeling (top) of the CapV active-site serine by a reactive rhodamine-labeled fluorophosphonate probe (FP-Rh). 1.6 µM His$_6$-tagged CapV was mixed with both FP-Rh and either 1.75 µM (L) or 1 mM (H) cGAMP, dATP, GTP, dGTP, cAMP, or ATP. Bottom panel shows Coomassie blue staining of CapV. *(C)* MicroScale Thermophoresis (MST) quantification (FNorm; normalized fluorescence) for cGAMP binding to CapV. cGAMP was titrated between 0.5 M and 0.03 nM with 50 nM His$_6$-tagged CapV. cGAMP binds to CapV with a K$_d$ of 8.6 ± 1.9 µM. Binding affinity was calculated from four independent experiments.

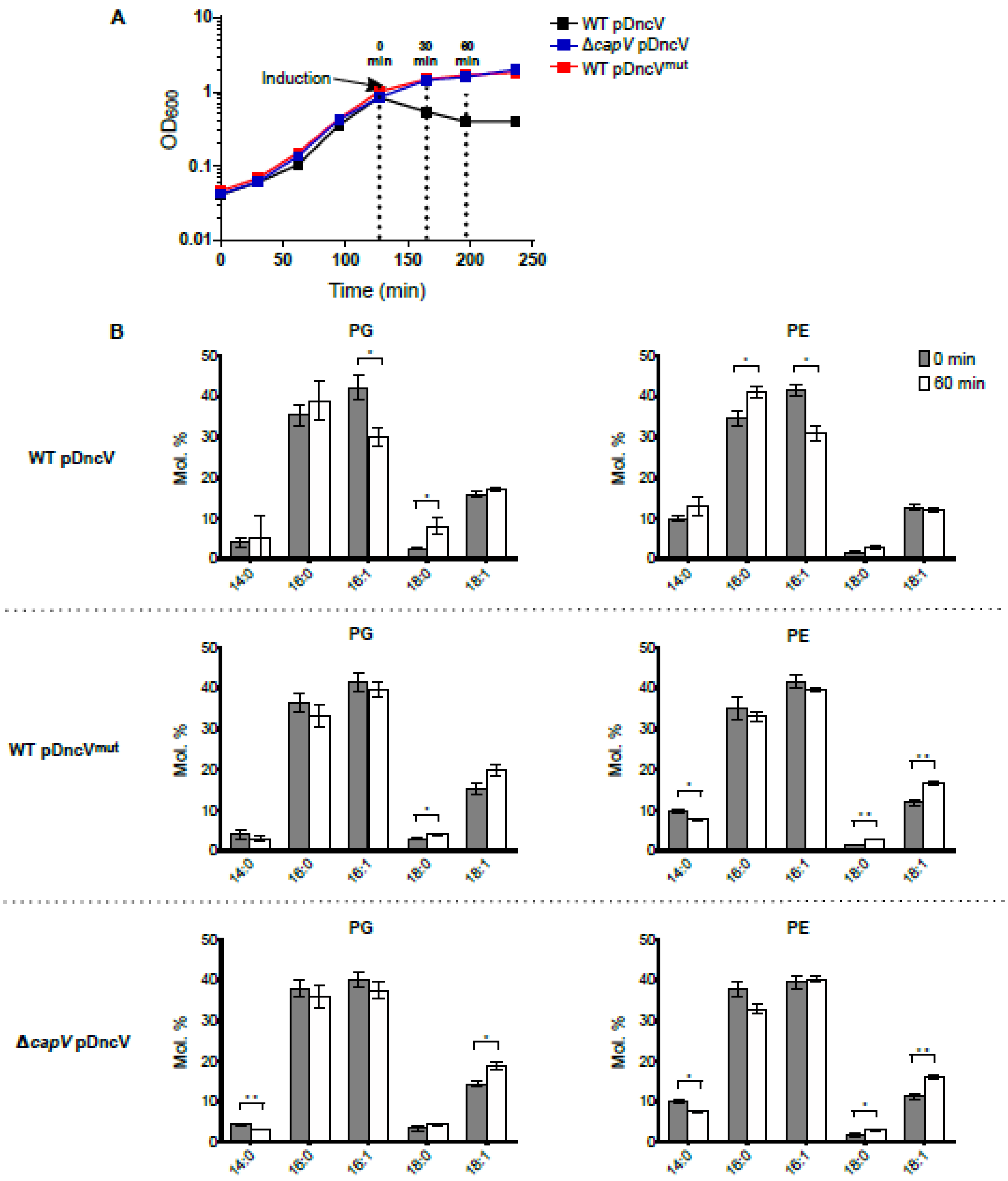

Fig. S4. cGAMP-activated CapV degrades phospholipids in the cell membrane. *(A)* Growth curves of strains during lipid extraction experiments. El Tor *V. cholerae* WT carrying a P_{tac}-inducible plasmid encoding either *dncV* (pDncV) or a catalytically inactive mutant (pDncV^{mut}) and Δ*capV* carrying pDncV were grown to $OD_{600} \approx 1.0$ then induced with 1 mM IPTG. Arrow denotes time of induction, and time points above graph (0, 30, 60 min) denote where samples were removed for lipid extraction. Growth curves are representative of three independent experiments. *(B)* The proportion (molar percentage) of fatty acid species present in the phosphatidylglycerol (PG) and phosphatidylethanolamine (PE) fractions collected from El Tor *V. cholerae* WT carrying pDncV (top) or pDncV^{mut} (middle), and from El Tor *V. cholerae* Δ*capV* carrying pDncV (bottom). Each data point represents the mean ± SD of three independent experiments (*p<0.01, **p<0.001).

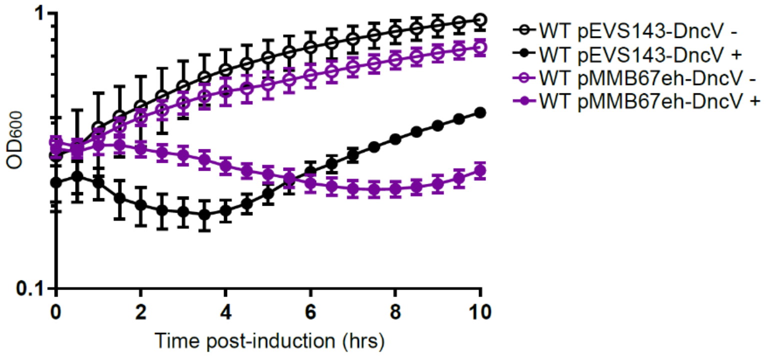

Fig. S5. El Tor *V. cholerae* growth arrest due to overexpression of *dncV* can be achieved using either of two P_{tac}-inducible *dncV* constructs. Growth curves of El Tor *V. cholerae* WT carrying either of the two P_{tac}-inducible *dncV* plasmids used in this manuscript (pEVS143-DncV and pMMB67EH-DncV) following induction of exponential phase cultures in the absence (-) or presence (+) of 100 µM IPTG. Each data point represents the mean ± SD of six biological replicates, and the growth curves are representative of at least three independent experiments. pMMB67EH-DncV was used for the cosmid screen, colony morphology images, quantification of intracellular cyclic dinucleotides by UPLC-MS/MS, and lipid extraction experiments. pEVS143-DncV was used in all growth curves, the transposon screen, and the membrane integrity microscopy images.

Table S1. Bacterial strains used in this study.

Name	**Relevant genotype**	**Marker**	**Source**
E. coli strains			
WN006	S17-1λ*pir* pKAS32	Amp^r	(1)
WN0092	S17-1λ*pir* pEVS143	Kan^r	(8)
WN4563	S17-1λ*pir* pEVS143-DncV	Kan^r	This study
WN4565	S17-1λ*pir* pKAS32-Δ*dncV*	Amp^r	This study
WN5022	S17-1λ*pir* pKAS32- Δ*vc0180*	Amp^r	This study
WN5023	S17-1λ*pir* pKAS32- Δ*vc0181*	Amp^r	This study
WN5055	S17-1λ*pir* pEVS143-CapV	Kan^r	This study
WN5080	S17-1λ*pir* pKAS32- Δ*capV*	Amp^r	This study
WN5133	S17-1λ*pir* pEVS143-CapV-DncV	Kan^r	This study
WN5144	BL21(DE3) pET28b-CapV-His$_6$	Kan^r	This study
WN5346	XL10-Gold (Agilent) pEVS143-CapV(S62A)-DncV	Kan^r	This study
WN5547	XL10-Gold (Agilent) pEVS143-DncV(D131A/D133A)	Kan^r	This study
BW1000	BW29427 pMMB67EH-DncV	Amp^r	This study
BW1001	BW29427 pMMB67EH-DncV(D131A/D133A)	Amp^r	This study
BW1002	BW29427 pMMB67EH	Amp^r	This study
BW1003	BW29427 pGBS40 (pKAS32- Δ*dncV*)	Amp^r	This study
BW1004	BW29427 pLAFR	Tet^r	This study
BW1005	BW29427 pCCD7 (VSP-I cosmid #1)	Tet^r	This study
BW1006	BW29427 pCCD13 (VSP-I cosmid #2)	Tet^r	This study
V. cholerae strains			
WN001	WT C6706 str2 O1 El Tor	Sm^r	(9)
WN4952	WT pEVS143-DncV	Kan^r	This study
WN5060	Δ*dncV* pEVS143-DncV	Kan^r	This study
WN5120	Δ*capV* pEVS143-DncV	Kan^r	This study
WN5137	Δ*capV* pEVS143-CapV-DncV	Kan^r	This study
WN5390	Δ*vc0180* pEVS143-DncV	Kan^r	This study
WN5392	Δ*vc0181* pEVS143-DncV	Kan^r	This study
WN5533	Δ*capV* pEVS143-CapV(S62A)-DncV	Kan^r	This study
WN5549	WT pEVS143-DncV(D131A/D133A)	Kan^r	This study
ET1000	WT pMMB67EH-DncV	Amp^r	This study
ET1001	Δ*capV* pMMB67EH-DncV	Amp^r	This study
ET1002	WT pMMB67EH-DncV(D131A/D133A)	Amp^r	This study
GS01	Δ*dncV* pMMB67EH-DncV	Amp^r	This study
WN5710	O395 WT pEVS143-CapV	Kan^r	This study
WN5712	O395 pEVS143-DncV	Kan^r	This study
WN5714	O395 pEVS143-CapV-DncV	Kan^r	This study
CL1000	O395 pLAFR pMMB67EH-DncV	Tet^r Amp^r	This study
CL1001	O395 pCCD7 pMMB67EH-DncV	Tet^r Amp^r	This study
CL1002	O395 pCCD13 pMMB67EH-DncV	Tet^r Amp^r	This study

Table S2. Primers used for plasmid and strain construction.

Name	**Primer use**	**Sequence**
Primers for plasmid construction		
WNTP0454	*dncV* F AvrII + RBS (pEVS143-DncV)	GGCCTAGGAATTCAGGAGCTAAGGAAGCTAAAATGACTTGGAACTTTCACCAGTA
WNTP0455	*dncV* R BamHI (pEVS143-DncV & pEVS143-CapV-DncV)	CCGGATCCTCAGCCACTTACCATTGTGCTGCT
CMW2373	*dncV* F EcoRI + RBS (pMMB67EH-DncV)	GAATTCAGGAGCTAAGGAAGCTAAAGTGAGAATGACTTGGAACTTTC
CMW2374	*dncV* R BamHI (pMMB67EH-DncV)	GGATCCTTCAGCCACTTACCATTGTGC
WNTP0596	*capV* F StuI + RBS (pEVS143-CapV)	GCGAGGCCTAATTCAGGAGCTAAGGAAGCTAAAATGCCAAATCCACCTGAATATG
WNTP0591	*capV* R BamHI (pEVS143-CapV)	CCGGATCCTTACTTAAATTTGCGGGCAGGTAC
WNTP0600	*capV* F SpeI + RBS (pEVS143-CapV-DncV)	GGACTAGTAATTCAGGAGCTAAGGAAGCTAAAATGCCAAATCCACCTGAATATG
WNTP0617	*capV* F KpnI (pET28b-CapV-His$_6$)	GACGGTACCCCAAATCCACCTGAATATGAACAC
WNTP0618	*capV* R BamHI (pET28b-CapV-His$_6$)	GCAGGATCCCGCTTAAATTTGCGGGCAGGTACTTT
Primers for site-directed mutagenesis		
WNTP0697/ CMW2381	*dncV*(D131A/D133A) F (pEVS143-DncV^{mut} & pMMB67EH-DncV^{mut})	AGCCTGGTCAAGAAATGGCTATTGCTGATGGAACCTATATGCC
WNTP0698/ CMW2755	*dncV*(D131A/D133A) R (pEVS143-DncV^{mut} & pMMB67EH-DncV^{mut})	GGCATATAGGTTCCATCAGCAATAGCCATTTCTTGACCAGGCT
WNTP0699	*capV*(S62A) F (pEVS143-CapV^{mut}-DncV)	CCAGAATTCCACCAATAGCAGTACCTGTAATCAGGTC
WNTP0700	*capV*(S62A) R (pEVS143-CapV^{mut}-DncV)	GACCTGATTACAGGTACTGCTATTGGTGGAATTCTGG
Primers for gene deletion		
CMW2462	Δ*dncV* up F; BW1003	GTGGAATTCCCGGGAGAGCTCCGCCCCACAATCCTGAGT
CMW2463	Δ*dncV* up R; BW1003	TGCTTCACTTTCTCCTCTAAGATTTACTTAAATTTGCG
CMW2464	Δ*dncV* down F; BW1003	TTAGAGGAGAAAGTGAAGCAGGAATTACATCATAC
CMW2465	Δ*dncV* down R; BW1003	AGCTATAGTTCTAGAGGTACCGCAGGGAGCTTTCATCGAAC
WNTP0456	Δ*dncV* up F; WN4565	GCGGGTACCTTGGGTTTGGCTTATGGAAAGAGC
WNTP0457	Δ*dncV* up R; WN4565	TGCTGATTTTTTCTGTGTAGTACTGGTGAAAGTTCC
WNTP0458	Δ*dncV* down F; WN4565	CACCAGTACTACACAGAAAAAATCAGCAGCACAATGGTAAGTGG
WNTP0459	Δ*dncV* down R; WN4565	AGGAATTCTTGGCACTCACAAACTTGCCACC
WNTP0592	Δ*capV* up F; WN5080	GCGGGTACCGCAGATACTAACAGGTGATGG
WNTP0593	Δ*capV* up R; WN5080	CTAACTGCCTTGCACCACCGCCATTCAAACTAAG
WNTP0594	Δ*capV* down F; WN5080	CGGTGGTGCAAGGCAGTTAGCTACTGAAGAGTTCACT
WNTP0597	Δ*capV* down R; WN5080	AAGGCGGCCGCCGATTCAGCGTGTCATACTGA
WNTP0551	Δ*vc0180* up F; WN5022	GCGGGTACCCGCGTCACTTAAGTCACTT
WNTP0578	Δ*vc0180* up R; WN5022	TTAGAGTTCTGCCTTTAGGCATTTGTTTAGCGGGCGT
WNTP0553	Δ*vc0180* down F; WN5022	GCCTAAAGGCAGAACTCTAAAGCGCTTCTC
WNTP0554	Δ*vc0180* down R; WN5022	AGGAATTCCCATAGACTCGTCGGAGACA
WNTP0555	Δ*vc0181* up F; WN5023	GCGGGTACCGGTGTGACCAGAGCTGATCG

WNTP0579	Δ*vc0181* up R; WN5023	AGTCCTTGCGCCGCGTTACTACATGTCCCATAATGACAAC
WNTP0557	Δ*vc0181* down F; WN5023	AGTAACGCGGCGCAAGGACTTTTGGCTTGG
WNTP0558	Δ*vc0181* down R; WN5023	AGGAATTCACCGCGTAGATCCTGCAAG

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