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Supplementary Information for

Identification of signaling pathways, matrix-digestion enzymes, and motility components controlling *Vibrio cholerae* biofilm dispersal

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- Movie S1

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Supplementary Information Text

DbfS is not equivalent to PhoQ

In *Escherichia coli*, low Mg^{2+} and cationic peptides activate PhoQ kinase activity (1). Sequence alignment of the DbfS sensory domain with that from PhoQ of *E. coli*, *Salmonella enterica*, and *Pseudomonas aeruginosa* revealed that DbfS lacks all of the key residues involved in Mg^{2+} binding (Fig. S2A) (2). To test if Mg^{2+} alters DfbS activity, we measured the *V. cholerae* biofilm lifecycle in response to low Mg^{2+} conditions in WT *V. cholerae* and in the Δ*dbfR* mutant. If, analogous to PhoQ, DfbS kinase activity is activated by low Mg^{2+}, when Mg^{2+} is limiting, WT *V. cholerae* should exhibit an altered biofilm dispersal phenotype while the Δ*dbfR* mutant would be impervious to Mg^{2+} changes (1). Fig. S2B shows that Mg^{2+} limitation does indeed inhibit *V. cholerae* biofilm dispersal, however, inhibition occurs in *both* the WT and the Δ*dbfR* strains. Mg^{2+} limitation did not alter *vpsL-lux* expression in either strain (Fig. S2C). Thus, Mg^{2+} does not control DfbS activity. We obtained the same results following exogenous addition of the cationic peptide C18G (Fig. S2D). Together, these results demonstrate that DfbS does not respond to the ligands that control PhoQ activity.

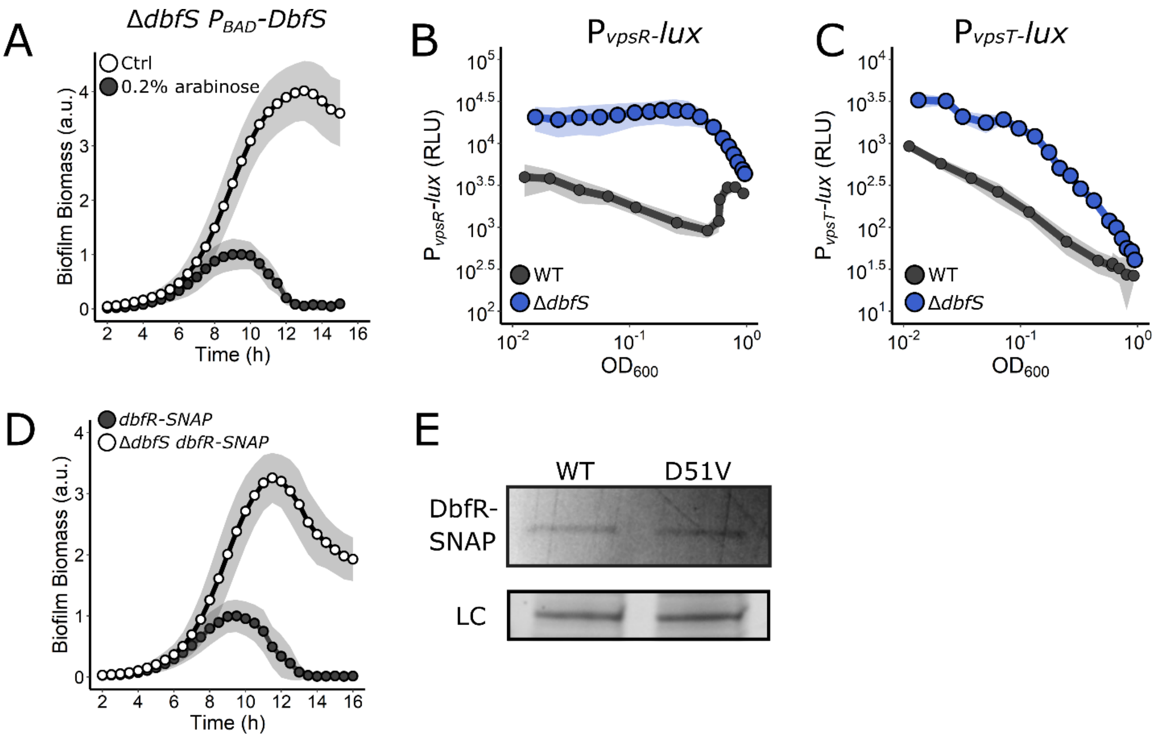

Fig. S1. Complementation, functional tagging, and mutagenesis of the DbfS-DbfR two-component system. (A) Quantitation of biofilm biomass over time measured by time-lapse microscopy for the Δ*dbfS* P_{BAD}-*dbfS* strain following addition of water (Ctrl) or 0.2% arabinose. (B) P_{vpsR}-*lux* and (C) P_{vpsT}-*lux* output for WT and the Δ*dbfS* strain over the growth curve. (D) As in A for SNAP-tagged DbfR in the WT and Δ*dbfS* strains. (E) Top panel: representative in-gel SDS-PAGE fluorescence following electrophoresis of *V. cholerae* cell lysates containing WT DbfS-SNAP or DbfS^{D51V}-SNAP that had been incubated with SNAP-Cell TMR Star. Bottom panel: Coomassie stained loading control (LC). For all biofilm measurements, N = 3 biological and N = 3 technical replicates, ± SD (shaded). a.u., arbitrary unit. For *lux* measurements, N = 3 biological replicates, ± SD (shaded). RLU, relative light units.

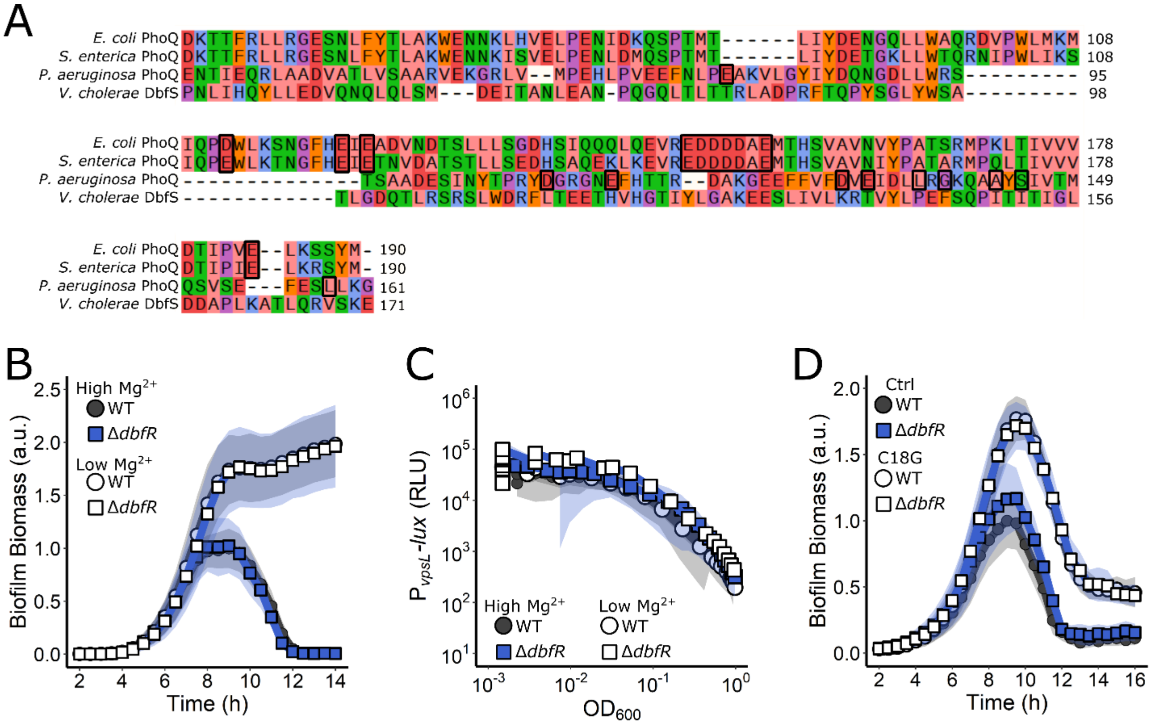

Fig. S2. DbfS is not functionally equivalent to PhoQ. (A) Alignment of the sensory domains of PhoQ from *E. coli*, *S. enterica*, and *P. aeruginosa* against that of *V. cholerae* DbfS. Black boxes indicate residues involved in Mg^{2+} binding in PhoQ. (B) Quantitation of biofilm biomass over time measured by time-lapse microscopy in high magnesium (10 mM) and limiting magnesium (10 µM) conditions for WT *V. cholerae* and the Δ*dbfR* strain. (C) The corresponding P_{vpsL}-*lux* outputs for strains and growth conditions in B over the growth curve. (D) As in B except following the addition of water or 5 µg/mL C18G. In all cases, *N* = 3 biological and *N* = 3 technical replicates, ± SD (shaded). a.u., arbitrary unit. For *vpsL-lux* measurements, *N* = 3 biological replicates, ± SD (shaded). RLU, relative light units.

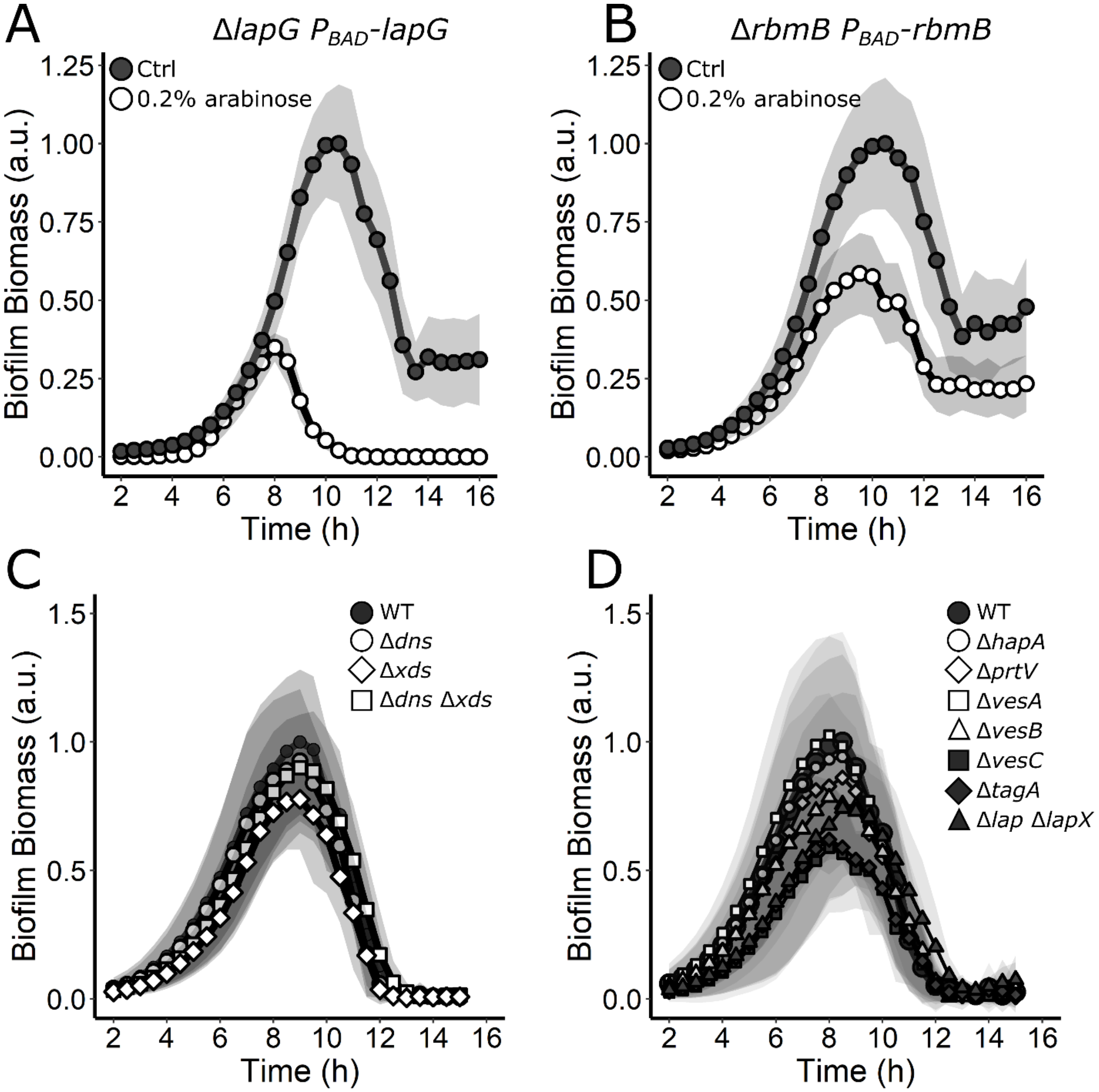

Fig. S3. Introduction of *lapG* and *rbmB* complements the Δ*lapG* and Δ*rbmB* biofilm defects, respectively, and assessment of the roles of extracellular DNAses and secreted proteases in *V. cholerae* biofilm dispersal. (A) Quantitation of biofilm biomass over time measured by time-lapse microscopy for the Δ*lapG* P_{BAD}-*lapG* strain following addition of water (Ctrl) or 0.2% arabinose. (B) As in A, but for the Δ*rbmB* P_{BAD}-*rbmB* strain. (C) Quantitation of biofilm biomass over time measured by time-lapse microscopy for WT *V. cholerae* and mutants lacking the designated DNAses. (D) Quantitation of biofilm biomass over time measured by time-lapse microscopy for WT *V. cholerae* and mutants lacking the designated proteases. In all cases, *N* = 3 biological and *N* = 3 technical replicates, ± SD (shaded). a.u., arbitrary unit.

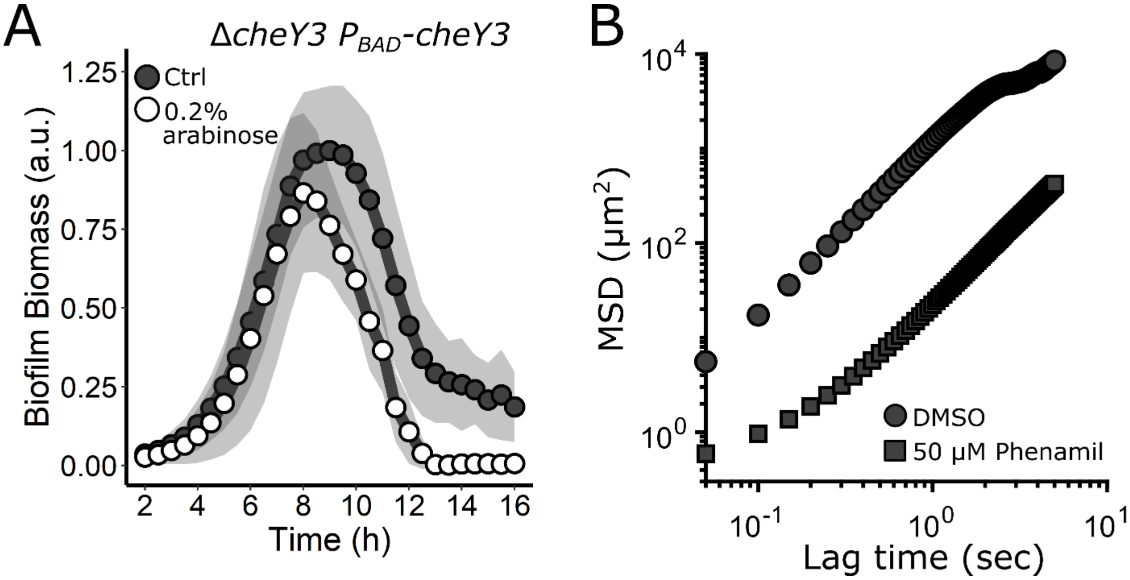

Fig. S4. Complementation of the Δ*cheY3* mutant and phenamil inhibition of *V. cholerae* motility. (A) Quantitation of biofilm biomass over time measured by time-lapse microscopy for the Δ*cheY3* P_{BAD}-*cheY3* strain following addition of water (Ctrl) or 0.2% arabinose. In all cases, *N* = 3 biological and *N* = 3 technical replicates, ± SD (shaded). a.u., arbitrary unit. (B) Mean squared displacement (MSD) of cell trajectories versus lag time for WT *V. cholerae* treated with DMSO solvent or 50 μM phenamil.

Table S1. Strains used in this study.

Strain Number	Genotype	Plasmid	Antibiotic Resistance	Parent
BB_Vc_0090	WT O1 El Tor biotype C6706str2	-	Sm	-
AB_Vc_761	Δ*vc1807*::Cm^{R} (Referred to as WT)	-	Sm, Cm	BB_Vc_0090
AB_Vc_705	Δ*cheY* Δ*vc1807*::Cm^{R}	-	Sm, Cm	BB_Vc_0090
AB_Vc_708	Δ*bipA* Δ*vc1807*::Cm^{R}	-	Sm, Cm	BB_Vc_0090
AB_Vc_839	Δ*mbaA* Δ*vc1807*::Cm^{R}	-	Sm, Cm	BB_Vc_0090
AB_Vc_711	Δ*potD1* Δ*vc1807*::Cm^{R}	-	Sm, Cm	BB_Vc_0090
AB_Vc_757	Δ*lapG* Δ*vc1807*::Cm^{R}	-	Sm, Cm	BB_Vc_0090
AB_Vc_758	Δ*rocS* Δ*vc1807*::Cm^{R}	-	Sm, Cm	BB_Vc_0090
AB_Vc_775	Δ*dbfS* Δ*vc1807*::Cm^{R}	-	Sm, Cm	BB_Vc_0090
AB_Vc_777	Δ*cdgI* Δ*vc1807*::Cm^{R}	-	Sm, Cm	BB_Vc_0090
AB_Vc_778	Δ*cdgG* Δ*vc1807*::Cm^{R}	-	Sm, Cm	BB_Vc_0090
AB_Vc_485	Δ*rbmB* Δ*vc1807*::Kan^{R}	-	Sm, Kan	BB_Vc_0090
AB_Vc_801	Δ*vc1807*::Kan^{R}	pEVS143-P_{vpsL}-*lux*::Cm^{R}	Sm, Cm, Kan	AB_Vc_479
AB_Vc_825	Δ*cheY* Δ*vc1807*::Kan^{R}	pEVS143-P_{vpsL}-*lux*::Cm^{R}	Sm, Cm, Kan	AB_Vc_705
AB_Vc_829	Δ*lapG* Δ*vc1807*::Kan^{R}	pEVS143-P_{vpsL}-*lux*::Cm^{R}	Sm, Cm, Kan	AB_Vc_757
AB_Vc_802	Δ*rbmB* Δ*vc1807*::Kan^{R}	pEVS143-P_{vpsL}-*lux*::Cm^{R}	Sm, Cm, Kan	AB_Vc_485
AB_Vc_815	Δ*dbfS* Δ*vc1807*::Kan^{R}	pEVS143-P_{vpsL}-*lux*::Cm^{R}	Sm, Cm, Kan	AB_Vc_775
AB_Vc_936	WT	pBBR1-P_{vpsR}-*lux*::Cm^{R}	Sm, Cm	BB_Vc_0090
AB_Vc_938	WT	pBBR1-P_{vpsT}-*lux*::Cm^{R}	Sm, Cm	BB_Vc_0090
AB_Vc_942	Δ*dbfS* Δ*vc1807*::Kan^{R}	pBBR1-P_{vpsR}-*lux*::Cm^{R}	Sm, Cm, Kan	AB_Vc_815
AB_Vc_944	Δ*dbfS* Δ*vc1807*::Kan^{R}	pBBR1-P_{vpsT}-*lux*::Cm^{R}	Sm, Cm, Kan	AB_Vc_815
AB_Vc_773	Δ*dbfR* Δ*vc1807*::Cm^{R}	-	Sm, Cm	BB_Vc_0090
AB_Vc_701	Δ*dbfRS* Δ*vc1807*::Cm^{R}	-	Sm, Cm	BB_Vc_0090
AB_Vc_788	*dbfR*^{D51V} Δ*vc1807*::Cm^{R}	-	Sm, Cm	BB_Vc_0090
AB_Vc_891	*dbfR*^{D51V} Δ*dbfS* Δ*vc1807*::Kan^{R}	-	Sm, Kan	BB_Vc_0090
AB_Vc_863	*dbfR-SNAP* Δ*dbfS* Δ*vc1807*::P_{BAD}-*dbfS*::Spec^{R}	-	Sm, Spec	BB_Vc_0090
AB_Vc_865	Δ*dbfS* Δ*vc1807*::P_{BAD}-*dbfS*::Spec^{R}	-	Sm, Spec	BB_Vc_0090
AB_Vc_879	*dbfR-SNAP* Δ*vc1807*::Kan^{R}	-	Sm, Kan	BB_Vc_0090
AB_Vc_881	*dbfR*^{D51V}*-SNAP* Δ*vc1807*::Kan^{R}	-	Sm, Kan	BB_Vc_0090
AB_Vc_859	Δ*lapG* Δ*vc1807*::P_{BAD}-*lapG*::Spec^{R}	-	Sm, Spec	AB_Vc_757
AB_Vc_898	Δ*lapD* Δ*vc1807*::Kan^{R}	-	Sm, Kan	BB_Vc_0090
AB_Vc_900	Δ*lapD* Δ*lapG* Δ*vc1807*::Kan^{R}	-	Sm, Kan	BB_Vc_0090
AB_Vc_948	Δ*lapG* Δ*rbmB* Δ*vc1807*::Kan^{R}	-	Sm, Kan	AB_Vc_182
AB_Vc_862	Δ*rbmB* Δ*vc1807*::P_{BAD}-*rbmB*::Spec^{R}	-	Sm, Spec	AB_Vc_485
BB_Vc_0252	Δ*dns*	-	Sm	BB_Vc_0090
BB_Vc_0253	Δ*xds*	-	Sm	BB_Vc_0090
BB_Vc_0254	Δ*dns* Δ*xds*	-	Sm	BB_Vc_0090
MJ_552	Δ*hapA* Δ*vc1807*::Kan^{R}	-	Sm, Kan	BB_Vc_0090

MJ_553	Δ*prtV* Δ*vc1807*::Kan^{R}	-	Sm, Kan	BB_Vc_0090
MJ_554	Δ*vesA* Δ*vc1807*::Kan^{R}	-	Sm, Kan	BB_Vc_0090
MJ_555	Δ*vesB* Δ*vc1807*::Kan^{R}	-	Sm, Kan	BB_Vc_0090
MJ_562	Δ*vesC* Δ*vc1807*::Kan^{R}	-	Sm, Kan	BB_Vc_0090
MJ_561	Δ*lap* Δ*lapX lacZ*::*Ptac-mKO* Δ*vc1807*::Kan^{R}	-	Sm, Kan	BB_Vc_0090
AB_Vc_792	Δ*tagA* Δ*vc1807*::Cm^{R}	-	Sm, Cm	BB_Vc_0090
AB_Vc_857	Δ*cheY* Δ*vc1807*::P_{BAD}-*cheY3*::Spec^{R}	-	Sm, Spec	AB_Vc_705
AB_Vc_715	*cheY*$^{D16K,\ Y109W}$ Δ*vc1807*::Kan^{R}	-	Sm, Kan	BB_Vc_0090
AB_Vc_732	Δ*vpsL* Δ*vc1807*::*Ptac-mScarletI::*Spec^{R}	-	Sm, Spec	BB_Vc_0090
AB_Vc_735	Δ*cheY* Δ*vpsL* Δ*vc1807*::*Ptac-mScarletI*::Spec^{R}	-	Sm, Spec	AB_Vc_705
AB_Vc_745	*cheY*$^{D16K,\ Y109W}$ Δ*vpsL* Δ*vc1807::Ptac-mScarletI*::Spec^{R}	-	Sm, Spec	AB_Vc_715

Table S2. DNA oligonucleotides and gene fragments used in this study.

Oligo #	Name	Purpose	Direction	5' to 3' Sequence
551	*cheY*_3000up	Cloning at *cheY3* locus	F	CAAGCGTTACAACTCGCAGCCTAG
552	*cheY*_3000down	Cloning at *cheY3* locus	R	CACAACCAGACCTACGCGCTGAC
553	*cheY*_100up	Cloning at *cheY3* locus	F	GGTGAGGTACTTGGAGTTAGTGAATCTC
554	*cheY*_100down	Cloning at *cheY3* locus	R	CACTGAAGCGCTCATCAATCTGAAAG
555	*cheY*_B	*cheY3* deletion	R	GAGCACCTTTTGCCGCAGCAAAAGCCTGAGTTTGAGATCAGTGATATTTAGTCATTCC
556	*cheY*_C	*cheY3* deletion	F	GGAATGACTAAATATCACTGATCTCAAACTCAGGCTTTTGCTGCGGCAAAAGGTGCTC
561	*cheY*_2700up	Cloning at *cheY3* locus	F	GATGACCGTGTCAGTTTGCAATCGAG
562	*cheY*_2700down	Cloning at *cheY3* locus	R	CTTCGGTGCTAACCAGTTTTGTAAG*TAGA*AC
563	*cheY*_up_R	Cloning at *cheY3* locus	R	GAGTTTGAGATCAGTGATATTTAGTCATTCCGAGTCC
564	*cheY*_down_R	Cloning at *cheY3* locus	R	GGCTTTTGCTGCGGCAAAAGGTGCTCTATTC
566	*cheY*_D16K_Y109W	Gblock for introduction of *cheY3* point mutation	F	GTTAAGTTCTTGGACTCGGAATGACTAAATATCACTGATCTCAAACTCAGTGGAGGCAATTTTGAATAAAAACATGAAGATCCTTATTGTTGATAAGTTTTCAACAATGCGCCGAATCGTTAAAAACCTACTTCGAGATCTGGGGTTCAATAACACGCAGGAAGCGGACGATGGCCTAACGGCATTGCCTATGCTCAAGAAAGGTGATTTTGACTTTGTAGTCACAGACTGGAATATGCCCGGTATGCAAGGTATTGACTTGCTTAAAAATATCCGTGCCGACGAAGAACTGAAGCACCTGCCTGTACTAATGATCACAGCAGAAGCCAAACGTGAGCAAATCATCGAAGCCGCTCAAGCAGGCGTGAATGGTTGGATCGTAAAACCATTTACCGCTGCTACGCTTAAAGAAAAAT*TAGA*CAAAATTTTTGAGCGTTTATAAGGCTTTTGCTGCGGCAAAAGGTGCTCTATTCACACGCGCAAAAG
545	*bipA*_3000up	Cloning at *bipA* locus	F	GCTGCGTGAGCAGTTGTAAATCGAG
546	*bipA*_3000down	Cloning at *bipA* locus	R	CAACGCTTTGTAGTTCGGGATTAGCATATA
547	*bipA*_100up	Cloning at *bipA* locus	F	GTCGACGATTTCAGCGCGACAGATC
548	*bipA*_100down	Cloning at *bipA* locus	R	GAGGTATTTCTGGATAGGTGGCATAGC
549	*bipA*_B	*bipA* deletion	R	GATGACTTATCTTACCAAACGAAAGTCAGTGACGGGGTTTGCTTCACTTTTTCATTGAGGCTG
550	*bipA*_C	*bipA* deletion	F	CAGCCTCAATGAAAAAGTGAAGCAAACCCCGTCACTGACTTTCGTTTGGTAAGATAAGTCATC
567	*bipA*_2700up	Cloning at *bipA* locus	R	CAGTGACTCGTCCAAAATGAGCACTG
568	*bipA*_2700down	Cloning at *bipA* locus	R	GATCTAAATCGCCACTGATCCCATCAAG
571	*mbaA*_3000up	Cloning at *mbaA* locus	F	GCGCGCTAATCTGAACTCAACCCATAAG
572	*mbaA*_2700up	Cloning at *mbaA* locus	F	CGTTAGCATTCCACGCGGTCAGTTAG
711	*mbaA*_KO2_B	*mbaA* deletion	R	GGAGGCATGAAGCCATGGGGAGATCTCGCTATGGTTTAGCTTCATATTGGTAAGTCACACTG
712	*mbaA*_KO2_C	*mbaA* deletion	F	CAGTGTGACTTACCAATATGAAGCTAAACCATAGCGAGATCTCCCCATGGCTTCATGCCTCC
575	*mbaA*_2700down	Cloning at *mbaA* locus	R	GATCTCATGACGCGCCTGACGGTATTTAAG
576	*mbaA*_3000down	Cloning at *mbaA* locus	R	CATCGTTCGCGATAGTGGGAAATTCAATAAAATG
577	*mbaA*_100up	Cloning at *mbaA* locus	F	GAAACCTGACATTGCCGCAATCAATGC

578	*mbaA*_100down	Cloning at *mbaA* locus	R	CCTGCTTCCAATCCGACATAATACTCTGC
539	*potD1*_3000up	Cloning at *potD1* locus	F	CTGGAATCCGGTATGTGTGTGATGGTTAG
540	*potD1*_3000down	Cloning at *potD1* locus	R	AGAGCGACTAGGTGTTATTGAACTTGGG
541	*potD1*_100up	Cloning at *potD1* locus	F	CTAAGAAAAGCATCAAATAGGCAGCCATTG
542	*potD1*_100down	Cloning at *potD1* locus	R	GATCTGGAAGAGATTAAGGCGCTCTC
543	*potD1*_B	*potD1* deletion	R	GGTGGCTTTTTAATGGGAGATAAAAGGCTACGTTCCCATAGTGTA*TAGA*AAGAACC
544	*potD1*_C	*potD1* deletion	F	GGTTCTTTCTATACACTATGGGAACGTAGCCTTTTATCTCCCATTAAAAAGCCACC
569	*potD1*_2700up	Cloning at *potD1* locus	F	CTGATGATTATTGGTACGAGTTTTCTGACTCGTG
570	*potD1*_2700down	Cloning at *potD1* locus	R	CGATAATCCAAATCAAATCGAGGTGCAGG
602	*lapG*_3000up	Cloning at *lapG* locus	F	CAAACAATTACCCGGTTATTGGGGATG
603	*lapG*_2700up	Cloning at *lapG* locus	F	GCATTCCGTCAAAGTGCTCGATATTCATC
604	*lapG*_100up	Cloning at *lapG* locus	F	GATCATTCCGGGAATGACCGCTTC
605	*lapG*_B	Cloning at *lapG* locus	R	CGACTAGTTGTTTGTATAGCGTCATAGTGCAGGGCGGGCTATTCCCTCAGCGCATTGCTTTG
606	*lapG*_C	*lapG* deletion	F	CAAAGCAATGCGCTGAGGGAATAGCCCGCCCTGCACTATGACGCTATACAAACAACTAGTCG
607	*lapG*_100down	*lapG* deletion	R	GTGTTGTTGACTTCAGAGCGTTGTTG
608	*lapG*_2700down	Cloning at *lapG* locus	R	GTCCAGCCATTAACCAGATCAACAC
609	*lapG*_3000down	Cloning at *lapG* locus	R	CAGCGGTACTGGAATTGTCCTTGC
774	*lapD*_3000up	Cloning at *lapD* locus	F	CGCGAATACAAGAAGCGATCATGCAG
775	*lapD*_2700up	Cloning at *lapD* locus	F	GCAAACTTCTGCTTAAGCTCAAGATACTTGC
776	*lapD*_100up	Cloning at *lapD* locus	F	CAATTGGCTGGGGACTCTTCGAGAC
777	*lapD*_B	*lapD* deletion	R	GTATCTTGCATGCCTCTGACCTTGGAGTGCCTACTCATCATAGCTAAC
778	*lapD*_C	*lapD* deletion	F	GTTAGCTATGATGAGTAGGCACTCCAAGGTCAGAGGCATGCAAGATAC
779	*lapD*_100down	Cloning at *lapD* locus	R	GTAAGCCGTTGATCAGTGCTTCAGGAG
780	*lapD*_2700down	Cloning at *lapD* locus	R	CTAACTACGCGCAGTATGTTGAGTTACAAGCG
781	*lapD*_3000down	Cloning at *lapD* locus	R	CGTTCAAGCACAAGGCGATA*TAGA*CG
784	*lapDG*_B	*lapDG* deletion	R	GTATCTTGCATGCCTCTGACCTTGGAGGGCGGGCTATTCCCTCAGCGCATTG
785	*lapDG*_C	*lapDG* deletion	F	CAATGCGCTGAGGGAATAGCCCGCCCTCCAAGGTCAGAGGCATGCAAGATAC
610	*rocS*_3000up	Cloning at *rocS* locus	F	CAACTCGAGCTTTTCTACCAACCTCAG
611	*rocS*_2700up	Cloning at *rocS* locus	F	GCATTTTACCGCCCCATTTTCGC
612	*rocS*_100up	Cloning at *rocS* locus	F	CTTCAGGCCAAGATCCTTTTCTACTGTG
613	*rocS*_B	*rocS* deletion	R	GGTTTCCACCAATCAGAGTAAAATTAACCCCTTAAAATACTACCAACTGTCCGTGCGCGACGACG
614	*rocS*_C	*rocS* deletion	F	CGTCGTCGCGCACGGACAGTTGGTAGTATTTTAAGGGGTTAATTTTACTCTGATTGGTGGAAACC
615	*rocS*_100down	Cloning at *rocS* locus	R	GAAACCGATATAAACCGCATCGGCA

616	*rocS*_2700down	Cloning at *rocS* locus	R	GTCACGTTATTAGGCTTGGCGTATTTC
617	*rocS*_3000down	Cloning at *rocS* locus	R	GCTGTTTGTTCACCTTAGGCTCG
533	*vc1639*_3000up	Cloning at *dbfS* locus	F	GCTTAGTGATCGCAGAGCTTGC
534	*vc1639*_3000down	Cloning at *dbfS* locus	R	GTGCACTGCATTATTGACTCGCTTAGC
535	*vc1639*_100up	Cloning at *dbfS* locus	F	CAAGATTTTGACCGCGATTCCAATAC
536	*vc1639*_100down	Cloning at *dbfS* locus	R	G*TAGA*GTTTCCAAACCTATAGGAG
626	*vc1639*_Real_B	*dbfS* deletion	R	CAACTGAAAATCCGTTTTTGCACCGCATTTAATTGGCATGCAACTGATACCCAAG
627	*vc1639*_Real_C	*dbfS* deletion	F	CTTGGGTATCAGTTGCATGCCAATTAAATGCGGTGCAAAAACGGATTTTCAGTTG
559	*vc1639*_2700up	Cloning at *dbfS* locus	F	CAATCGGTGGTGCGCAACTTATCTGAG
560	*vc1639*_2700down	Cloning at *dbfS* locus	R	GTTAATGACTTGGAGCAGAATTAAGTTAGCCGC
527	*vc1638*_3000up	Cloning at *dbfR* locus	F	GTAGGTCTTCTCGCACTTGTGTTTTG
528	*vc1638*_3000down	Cloning at *dbfR* locus	R	GTCCATAACCTTAGCGGAACTCATG
529	*vc1638*_100up	Cloning at *dbfR* locus	F	GACAATCAAGTCTTTCGTGTCGAATACAAC
530	*vc1638*_100down	Cloning at *dbfR* locus	R	CTTCCAGCAAATATTGATGGATGAGATTTGGG
628	*vc1638*_Real_B	*dbfR* deletion	R	GAGATTTAATTGGCATGCAACTGATACCCAAGGTCTGCTCGATTATTTTTTGATGGCACG
629	*vc1638*_Real_C	*dbfR* deletion	F	CGTGCCATCAAAAAATAATCGAGCAGACCTTGGGTATCAGTTGCATGCCAATTAAATCTC
557	*vc1638*_2700up	Cloning at *dbfR* locus	F	CACCATCCGGTTTGTGCATCATGATG
558	*vc1638*_2700down	Cloning at *dbfR* locus	R	GTGGCGTCAGATCCCAAAACTTGTTC
650	*dbfR*_D51V_B	Generating *dbfR*^{D51V}	R	CAATTTCGGTAGGCCGAGTACGAGTACGATGACGTCC
651	*dbfR*_D51V_C	Generating *dbfR*^{D51V}	F	GGACGTCATCGTACTCGTACTCGGCCTACCGAAATTG
736	*dbfR*_SNAP_deltaS_Gblock	Gblock for generating *dbfR-SNAP* and simultaneously deleting *dbfS*	F	CGCGGTCTTGGGTATCAGTTGCATGCCAATTCAGGAAGCGGCTCAGGCAGCGGATCAGGAATGGATAAGGATTGTGAAATGAAGAGAACAACTT*TAGA*TTCCCCACTAGGTAAAT*TAGA*ATTATCCGGTTGCGAACAAGGATTACATCGTATTATATTTTTAGGAAAAGGAACCAGTGCAGCAGACGCCG*TAGA*AGTACCAGCCCCCGCCGCAGTTTTAGGAGGACCAGAACCACTAATGCAAGCCACCGCTTGGTTAAACGCATATTTTCATCAACCAGAAGCCA*TAGA*AGAATTCCCAGTACCAGCCCTACACCACCCAGTATTTCAACAAGAATCATTTACGAGACAAGTATTATGGAAATTATTAAAAGTCGTCAAATTCGGAGAAGTTATCAGCTATAGTCACCTAGCCGCTCTTGCCGGTAATCCAGCAGCCACTGCCGCAGTTAAAACCGCATTATCAGGTAACCCAGTTCCCATATTAATTCCATGCCA*TAGA*GTAGTACAAGGAGATT*TAGA*CGTCGGCGGATATGAAGGAGGTTTAGCAGTTAAAGAATGGTTACTAGCACATGAAGGACA*TAGA*TTAGGTAAACCAGGATTAGGTTAAATGCGGTGCAAAAACGGATTTTCAGTTGC
734	*dbfR*_R	Generating *dbfR*-SNAP and deleting *dbfS*	R	TTGGCATGCAACTGATACCCAAGACCGCG
735	*dbfS*_down_F	Generating *dbfR*-SNAP and deleting *dbfS*	F	ATGCGGTGCAAAAACGGATTTTCAGTTGC
672	SNAP_UnivR	Generating *dbfR*-SNAP	R	TTAACCTAATCCTGGTTTACCTAATCTATGTCCTTCATGTGCTAGTAACC
718	*dbfR*_SNAP_E	Generating *dbfR*-SNAP	F	GACA*TAGA*TTAGGTAAACCAGGATTAGGTTAAGATGTGATCAAAACTGTGCGCGGTC

634	*cdgI*_3000up	Cloning at *cdgI* locus	F	CGATGCAAGTAGCTGAACAAGCAC
635	*cdgI*_2700up	Cloning at *cdgI* locus	F	GAATACATTGACGCCGAGCGCTTTG
636	*cdgI*_100up	Cloning at *cdgI* locus	F	GGGAGCAACTTCACTGTATTCAATGAGTG
637	*cdgI*_B	*cdgI* deletion	R	GATGCGATCATCATGAGCTACCTATTTTTGTAAAGGCCCGACTTCATTTTTTTCTACTCTC
638	*cdgI*_C	*cdgI* deletion	F	GAGAG*TAGA*AAAAAATGAAGTCGGGCCTTTACAAAAATAGGTAGCTCATGATGATCGCATC
639	*cdgI*_100down	Cloning at *cdgI* locus	R	GGTCAGCAGCTTTTGCAGCACTTTATTG
640	*cdgI*_2700down	Cloning at *cdgI* locus	R	GAGGTGCAACCTGCGTGTAACTGGATTTTC
641	*cdgI*_3000down	Cloning at *cdgI* locus	R	CCAGTGAGGCTATCAATATGCGCATC
642	*cdgG*_3000up	Cloning at *cdgG* locus	F	GTGTCGATTCCAGCGACAAGTGCCAATTTG
643	*cdgG*_2700up	Cloning at *cdgG* locus	F	GAATACACCGCAGAGCCGATAGTGAC
644	*cdgG*_100up	Cloning at *cdgG* locus	F	GATAAATGCTGCCCAGTCGGCATAAACACTGAG
645	*cdgG*_B	*cdgG* deletion	R	GCACAAATTAATAGTTAATTAGCTTAAATATTAATCAGACTGGATAGTTGAGGATCAATCCTGATCC
646	*cdgG*_C	*cdgG* deletion	F	GGATCAGGATTGATCCTCAACTATCCAGTCTGATTAATATTTAAGCTAATTAACTATTAATTTGTGC
647	*cdgG*_100down	Cloning at *cdgG* locus	R	TTGAGGCCATGC*TAGA*GCATGATGTTGAGC
648	*cdgG*_2700down	Cloning at *cdgG* locus	R	CCAGTAAATTCGGGTTATGAGGTAAAGGATG
649	*cdgG*_3000down	Cloning at *cdgG* locus	R	GATCGCCACTTTCCGCGATTGGATG
105	BBC1881	Cloning at *vc1807* locus	F	TTTAAAGGGGATCAGTGACCG
106	BBC1882	Cloning at *vc1807* locus	R	CAATTTTGCTTTTGGACCATCCC
270	*1807*_2700up	Cloning at *vc1807* locus	F	GGCCGGCACTTTGATTACAATC
271	*1807*_2700down	Cloning at *vc1807* locus	R	GTCTATATCAGAGCGCTTAAAGAGCG
721	P_{BAD}*_1807*_Univ_B	Generating P_{BAD}-*dbfS*	R	CATTTCACACCTCCTGCAGGTAC
722	P_{BAD}-*dbfS-1807*_C	Generating P_{BAD}-*dbfS*	F	GTACCTGCAGGAGGTGTGAAATGGGTATCAGTTGCATGCCAATTAAATCTCG
723	P_{BAD}-*dbfS-1807*_D	Generating P_{BAD}-*dbfS*	R	GTCGACGGATCCCCGGAATTTAATGGGATTTGACGGCTTTGGCTG
232	ABD123	Generating P_{BAD}-*dbfS*	F	ATTCCGGGGATCCGTCGAC
729	P_{BAD}-*lapG-1807*_C	Generating P_{BAD}-*lapG*	R	GTACCTGCAGGAGGTGTGAAATGAAACGTTGGATTGTGCTGTCTCTGG
730	P_{BAD}-*lapG-1807*_D	Generating P_{BAD}-*lapG*	F	GTCGACGGATCCCCGGAATCTACTCATCATAGCTAAC*TAGA*GG
731	P_{BAD}-*rbmB-1807*_C	Generating P_{BAD}-*rbmB*	R	GTACCTGCAGGAGGTGTGAAATGCTGTTATACTTAAATCAATTCAATAAAGAGGGTGG
732	P_{BAD}-*rbmB-1807*_D	Generating P_{BAD}-*rbmB*	F	GTCGACGGATCCCCGGAATTCAATCTTTAATAAAGTGCTGTATATAATAATGGTCGC
724	P_{BAD} *-cheY3-1807_Gblock*	Generating P_{BAD}-*cheY3*	F	GTACCTGCAGGAGGTGTGAAATGGAGGCAATTTTGAATAAAAACATGAAGATCCTTATTGTTGATGACTTTTCAACAATGCGCCGAATCGTTAAAAACCTACTTCGAGATCTGGGGTTCAATAACACGCAGGAAGCGGACGATGGCCTAACGGCATTGCCTATGCTCAAGAAAGGTGATTTTGACTTTGTAGTCACAGACTGGAATATGCCCGGTATGCAAGGTATTGACTTGCTTAAAAATATCCGTGCCGACGAAGAACTGAAGCACCTGCCTGTACTAATGATCACAGCAGAAGCCAAACGTGAGCAAATCATCGAAGCCGCTCAAGCAGGCGTGAATGGTTACATCGTAAAACCATTTACCGCTGCTACGCT

				TAAAGAAAAATTAGACAAAATTTTTGAGCGTTTATAAATTCCGGGGATCCGTCGAC
587	*tagA*_3000up	Cloning at *tagA* locus	F	GGGCTGCAAGAACTGGATCTGCTAC
588	*tagA*_2700up	Cloning at *tagA* locus	F	GAGCAAAATTACAAGCTCGATCTTCAGCTAAG
662	*tagA*_103bpD_B	Removes first 103 codons of *tagA* including start	R	GTCAAATACTGGTCGTTACTGGATGTTGCATTCTTTAACAAAAAAATAAAGACAAGGGAAACGTATTG
663	*tagA*_103bpD_C	Removes first 103 codons of *tagA* including start	F	CAATACGTTTCCCTTGTCTTTATTTTTTTGTTAAAGAATGCAACATCCAGTAACGACCAGTATTTGAC
591	*tagA*_2700down	Cloning at *tagA* locus	R	CCACCGAGGATACCATCCATCTTGATAATATG
592	*tagA*_3000down	Cloning at *tagA* locus	R	CTCTTGCCATCCATATGACATGATGTCTTTTG
593	*tagA*_100up	Cloning at *tagA* locus	F	GTGTGGCTTCATCCATTGACCTCCAATG
594	*tagA*_100down	Cloning at *tagA* locus	R	CCACTGCGAAATTAATTTTAGGATCAGCTTTAGC
664	*tagA*_150down	Cloning at *tagA* locus	R	GCAACCATACATCTTCCATTACTACCATAAGAG
519	Arbitrary Primer	Transposon localization	F	GGCCACGCGTCGACTAGTACNNNNNNNNNNAGAG
520	*Tn*5 specific Primer	Transposon localization	R	GAAGCCCTTAGAGCCTCTC
521	Arbitrary PCR cleanup	Transposon localization	F	AGGAACACTTAACGGCTGAC
522	Arbitrary PCR cleanup	Transposon localization	R	GGCCACGCGTCGACTAGTAC

Movie SI (separate file). Representative time-lapse images of WT *V. cholerae* undergoing biofilm dispersal. Also shown are the phenotypes of mutants identified in a screen for strains defective in dispersal. The mutant categories are designated by color: signal transduction, blue; matrix degradation, green; motility, red.

SI References

1. E. A. Groisman, The pleiotropic two-component regulatory system PhoP-PhoQ. *Journal of Bacteriology* **183**, 1835–1842 (2001).
2. L. R. Prost, M. E. Daley, M. W. Bader, R. E. Klevit, S. I. Miller, The PhoQ histidine kinases of *Salmonella* and *Pseudomonas* spp. are structurally and functionally different: evidence that pH and antimicrobial peptide sensing contribute to mammalian pathogenesis. *Mol Microbiol* **69**, 503–519 (2008).