Supporting Information for

Hybrid promiscuous (Hypr) GGDEF enzymes produce cyclic AMP-GMP (3', 3'-cGAMP)

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This file includes:

Extended Materials and Methods

General Reagents and Oligonucleotides

All oligonucleotides were purchased from Elim Biopharmaceuticals (Hayward, CA) or IDT (Coralville, IA). *Geobacter sulfurreducens* PCA was obtained from the laboratory of John Coates at UC Berkeley. Genomic DNA from *G. sulfurreducens* was isolated using the Purelink Genomic DNA mini kit (Invitrogen). Genomic DNA from *Myxococcus xanthus* was obtained from the laboratory of David Zusman at UC Berkeley. Additional GGDEF domain-containing synthase genes were purchased as gBlocks from IDT (Table S3). Cyclic dinucleotide standards were purchased from Axxorra (Farmingdale, NY) or enzymatically synthesized. DFHBI was chemically synthesized following literature protocols (1).

Molecular Cloning

For untagged constructs used for flow cytometry screening with fluorescent biosensors, gene sequences were amplified from genomic DNA and inserted into the MCS2 region of pCOLADuet-1. For C-terminal 6x-His-tagged constructs, gene sequences were inserted between NdeI and XhoI restriction sites of pET24a or pET31b. For N-terminal 6xHis-MBP-tagged constructs, gene sequences were inserted between BamHI and XhoI restriction sites of a custom pET16-derived vector from reference (2). pET28a containing *E. coli* BL21 (DE3)-derived *yhdH* between the NdeI and EcoRI cut sites was provided by the M. Chang lab at the University of California at Berkeley.

Significance Analysis for Fluorescence Biosensor Screen

Fluorescence turn-on was analyzed by the Student's t-test using 1 tail and 2 sample equal variance parameters, $p<0.01$ was the cut-off for significant turn-on. For the cdiG biosensor, the significance test was between candidate GGDEF signal and pCOLA signal. The cAG biosensor is ~100-fold selective for cAG over cdiG, but some fluorescence above background is still observed for cdiG synthases. Thus, for the cAG biosensor, the significance test was between candidate GGDEF signal and WspR signal.

Liquid Culture Growth of *E. coli* BL21 (DE3) Star for Nucleotide Extraction

Overnight starter cultures of BL21 (DE3) Star cells containing the pRARE2 plasmid (Invitrogen) and genes encoding dinucleotide cyclase enzymes in pET24a (or pET31b for GSU1656; pET-MBP for ACP_2467, Calni_1629, and DEFDS_0689) were inoculated into LB media and grown aerobically to an OD_{600} ~ 0.3. Cultures were then induced with 1 mM IPTG at 28 ºC for 4 h. Cells were harvested by centrifugation at 4,700 rpm for 15 min at 4 ºC, and pellets were stored at -80 ºC.

Cell Extraction of *E. coli*

Cyclic dinucleotides were extracted as described previously (3), with the following modifications. A frozen cell pellet from 100 mL of liquid culture was thawed and resuspended in 1.4 mL extraction buffer (40% methanol, 40% acetonitrile, 20% ddH_2O). The cell solution was incubated at ambient temperature with agitation for 20 min. After centrifugation for 5 min at 13,200 rpm, the supernatant was carefully removed and stored on ice. The remaining pellet was extracted twice more as described, with 700 µL extraction solvent each time. The combined supernatants were evaporated to dryness by rotary evaporation, and the dried material was resuspended in 300 µL ddH_2O. The extract was filtered through a 3 kDa MWCO Amicon Ultra-4 Protein Concentrator (Millipore) and used immediately or stored at -20 °C.

LC-MS Analysis of *E. coli* Cell Extracts

LC-MS analysis of *E. coli* cell extracts was performed using an Agilent 1260 Quadrupole LC-MS with an Agilent 1260 Infinity liquid chromatograph equipped with a diode array detector. Sample volumes of 20 µL were separated on a Poroshell 120 EC C18 column (50 mm length x 4.6 mm internal diameter, 2.7 µm particle size, Agilent) at a flow rate of 0.4 mL/min. For analysis of cell extracts and purified protein, a linear elution program of 0 to 10% B over 20 min. Solvent A was H_2O + 0.05 % TFA and solvent B was MeCN + 0.05 % TFA. Under the former conditions, the cyclic dinucleotides in extracts were found to always elute in the order of cdiG (7.3±0.3 min), cAG (7.6±0.3), and cdiA (7.9±0.4 min). Due to slight variability in retention times, the assignment of cyclic dinucleotide identity was made through analysis of the mass spectra. Shown in figures

are the MS spectra from integrating the retention time region containing all three cyclic dinucleotides (6 to 8 min).

Extract samples were analyzed by MS in the positive ion mode using the range of *m/z* = 600 to 800. When a broader range of 100 to 1000 *m/z* was used, the expected mass for the corresponding cyclic dinucleotide was present, but was not the most abundant ion peak observed, even with the standards. This observation suggests that the relative ionization of cyclic dinucleotides is low under these conditions, and furthermore the cyclic dinucleotides may not be fully resolved from other small molecules present in the extract. Thus the UV absorbance peaks detected at 254 nm may not be solely attributable to cyclic dinucleotides.

For high-resolution and tandem MS/MS, lysate was first fractionated on a Agilent 1260 Infinity liquid chromatograph equipped with a diode array detector and analytical-scale fraction collector as previously described (4). High-resolution mass spectrometry (HRMS) and tandem mass spectrometry (MS/MS) measurements of collected fractions were performed as previously described (4) using an Agilent 1200 liquid chromatograph (LC) that was connected in-line with an LTQ-Orbitrap-XL hybrid mass spectrometer equipped with an electrospray ionization (ESI) source (Thermo Fisher Scientific). This instrumentation is located in the QB3/Chemistry Mass Spectrometry Facility at UC Berkeley.

Overexpression and Purification of Dinucleotide Cyclase Enzymes

Full-length proteins with N-terminal His-6-MBP tags encoded in a pET16-derived plasmid were overexpressed in *E. coli* BL21 (DE3) star cells harboring a pRARE2 human tRNA plasmid and were grown in LB/carb/chlor for 10 h after induction at OD_{600} ~ 0.7 with 1 mM IPTG. Cells were lysed by sonication in a lysis buffer containing 25 mM Tris-HCl (pH 8.2), 500 mM NaCl, 20 mM imidazole, and 5 mM beta-mercaptoethanol. Clarified lysate was bound to Ni-NTA agarose (QIAGEN), and resin was washed with lysis buffer prior to elution with lysis buffer supplemented with 500 mM imidazole. Proteins were dialyzed overnight at 4 ºC against buffer containing 25 mM Tris-HCl (pH 7.5), 100 mM NaCl, 5% (v/v) glycerol, and 1 mM DTT. Protein purified in this way was

concentrated to ~5-10 mg/mL, flash frozen in liquid nitrogen, and stored at -80 ºC. Protein with C-terminal His-6x tags encoded in pET24a were overexpressed and purified similarly, with the cells grown in LB/kan/chlor.

Pulldown Assays to Detect Protein Self-Association

After inoculating fresh LB cultures from overnight starter cultures of *E. coli* BL21 (DE3) Star cells containing the pRARE2 plasmid (Invitrogen), C-terminal His-tagged enzymes in pET24a or pET28a, and C-terminal HA-tagged enzymes in pET22b, cells were grown aerobically to an OD_{600} ~ 0.3, then induced with 1 mM IPTG at 28 ºC for 4 h. Cells were harvested by centrifugation at 4,700 rpm for 15 min at 4 ºC, and pellets were stored at -80 ºC. The cells were resuspended in lysis buffer (25 mM Tris-HCl (pH 8.2), 500 mM NaCl, 20 mM imidazole, and 5 mM beta-mercaptoethanol), and lysed using a Biospec MiniBeadBeater-16. Lysates were cleared by centrifugation at 4 ºC at 13,000 rpm for 45 min. Clarified lysates were suspended in Ni-NTA resin (QIAGEN). The resin was washed five times with lysis buffer. The proteins were eluted with lysis buffer supplemented with 500 mM imidazole. The epitope-tagged HA protein was detected with HRP-conjugated BMG-3F10 anti-HA rat antibody (Roche, 1:1000). Validation information for the antibody is available on the manufacturer website.

Isothermal Titration Calorimetry

Samples of MBP-tagged GSU1658 R393A were first dialyzed into buffer containing 50 mM Tris-HCl [pH 7.5], 100 mM NaCl, and 1 mM TCEP, and then concentrated using a 5 mL Amicon Ultra MWCO 10 kDa concentrator (Millipore). Protein samples and buffer were then loaded onto a Microcal Auto-iTC200 isothermal titration calorimeter (Malvern, Worcestershire, UK). Details of the dissociation experiment and subsequent analysis have been described previously (5, 6). Briefly, 3.1 µL samples of MBP-GSU1658 R393A (at concentrations of 111 µM and 119 µM) were injected into the cell filled with buffer (400 µL volume) at 4 min intervals. The measurement was obtained at 28 ºC. Both measurements of each state were analyzed with Origen software (OrigenLab) to obtain ΔH and K_D values for the dissociation reaction. An additional fit parameter was also used upon data evaluation to eliminate constant background heat produced by technical

effects and dilution of titrant. However, in all cases, best fit curves corresponded to enthalpic changes in excess of 100 kcal/mol, likely a result of aggregate dissociation instead of dimer dissociation.

Size Exclusion Chromatography

GSU1658 was monitored by size-exclusion chromatography (SEC) by using a Superdex 200 HiLoad 26/60 column (GE Healthcare). Dialysis buffer (100 mM Tris-HCl, pH 7.5, 100 mM NaCl, 1 mM DTT, 5% glycerol) was used as running buffer for the protein samples. Runs were performed on an ÄKTA*purifier* (GE Healthcare) FPLC system at a flow rate of 0.5 ml/min.

In Vitro Activity Assay of Dinucleotide Cyclases using Radiolabeled NTPs

In vitro activity assays were performed as previously described by Kranzusch et al., with the following modifications (2). Enzyme (10 µM) was incubated in a solution of 50 mM Tris-HCl [pH 7.5], 100 mM NaCl, 10 mM $MgCl_2$, and 5 mM dithiothreitol with the indicated amounts of ATP and GTP and ~0.1 µCi radiolabeled [α-^{32}P]-ATP or [α-^{32}P]-GTP (Perkin Elmer) as indicated. Reactions were incubated at 28 ºC for 1 h. The total concentration of radiolabeled nucleotide did not exceed 66 nM, and so we expect that this does not significantly affect the results of any ratio-based experiments performed. Following incubation, the reaction was treated with 20 units of Calf Intestinal Alkaline Phosphatase (NEB) at 28 ºC for 30 min to digest the unincorporated NTPs. Reactions were terminated by heating to 95 ºC for 30 s. The reaction mixture (1 µL) was then spotted onto a PEI-cellulose F Thin-Layer Chromatography plate (Millipore), and allowed to dry for 15 min at room temperature. TLC plates were developed using 1 M KH_2PO_4, pH 3.6. Plates were dried overnight post-development, and radiolabeled products were detected using a Phosphor-image screen (GE Healthcare) and a Typhoon scanner (GE Healthcare).

In Vitro Activity Assay of Dinucleotide Cyclases using LC-MS

In vitro activity assays were performed as described above, with omission of both radiolabeled nucleotides and digestion with Calf Intestinal Alkaline Phosphatase. After termination of the reactions by heating to 95 ºC for 30 s, reactions were filtered using a

0.45 µm filter, and analyzed by LC-MS. For LC-MS analysis, an elution program of 0% B for 5 minutes, followed by a linear gradient from 0 to 5% B over 10 min, was used. Solvent A was H_2O + 0.05 % TFA and solvent B was MeCN + 0.05 % TFA. Under these conditions, the cyclic dinucleotides in extracts were found to always elute in the order of cdiG (8.7 ± 0.3 min), cAG (10.6 ± 0.3 min), and cdiA (11.0 ± 0.5 min). Due to slight variability in retention times, the assignment of cyclic dinucleotide identity was made through analysis of the mass spectra. Shown in figures are the MS spectra from integrating the retention time region containing all three cyclic dinucleotides (8 to 12 min).

Bioinformatic Analysis of GGDEF Variants

A Python-based program was developed to extract alignment data for a library of 42,747 putative GGDEF domain-containing proteins from the Pfam database (accession PF00990, http://pfam.xfam.org/, accessed 06/05/2014). In particular, positions critical for catalytic activity (i.e. the GG[D/E]EF sequence) and selectivity (i.e. positions 344 and 326 in PleD) were identified and analyzed for each sequence. Given previous results with some DGCs possessing altered signature motifs, we assigned any diguanylate cyclase with a [G/A/S]G[D/E][F/Y] motif to be active.

Growth of *Myxococcus xanthus*

Wild type (DZ2) *M. xanthus* was cultured at 32 ºC in liquid CYE medium (7) to an OD_{600} ~0.6. For liquid grown cultures, cells were pelleted and frozen at -80 ºC until use. For cultures grown on agar, liquid culture (25 mL at OD_{600} ~0.6) was poured onto a CYE plate (150 mm diameter, 1.5% (w/v) agar), and incubated for 24 h at 32 ºC to allow the cells to settle onto the agar surface. Excess liquid culture was then discarded. The cells attached to the agar surface were incubated at 32 ºC for another 24 h before being harvested using a cell scraper.

LC-MS Analysis of *Myxococcus xanthus* Cell Extracts

LC-MS analysis of *M. xanthus* cell extracts was performed using an Agilent 6530 Accurate-Mass Q-TOF LC-MS with an Agilent 1290 Infinity UHPLC. This instrumentation is located in the laboratory of Professor Michelle Chang at UC Berkeley.

Samples were separated on a Poroshell 120 SB-Aq column (50 mm length x 2.1 mm internal diameter, 2.7 µm particle size, Agilent) at a flow rate of 0.6 mL/min. For analysis of cell extracts, a linear elution program of 0 to 20% B over 4 min with an initial hold at 0% B for the first 0.2 min was used. Solvent A was H_2O + 0.1% formic acid and solvent B was MeCN. MS data were collected from 0.9 to 2.4 min.

Extract samples were analyzed by MS in the positive ion mode using the range of m/z = 50 to 1100 or 1700. MS/MS measurements were performed with a fragmentation voltage of 150 V and a collision energy of 20 V.

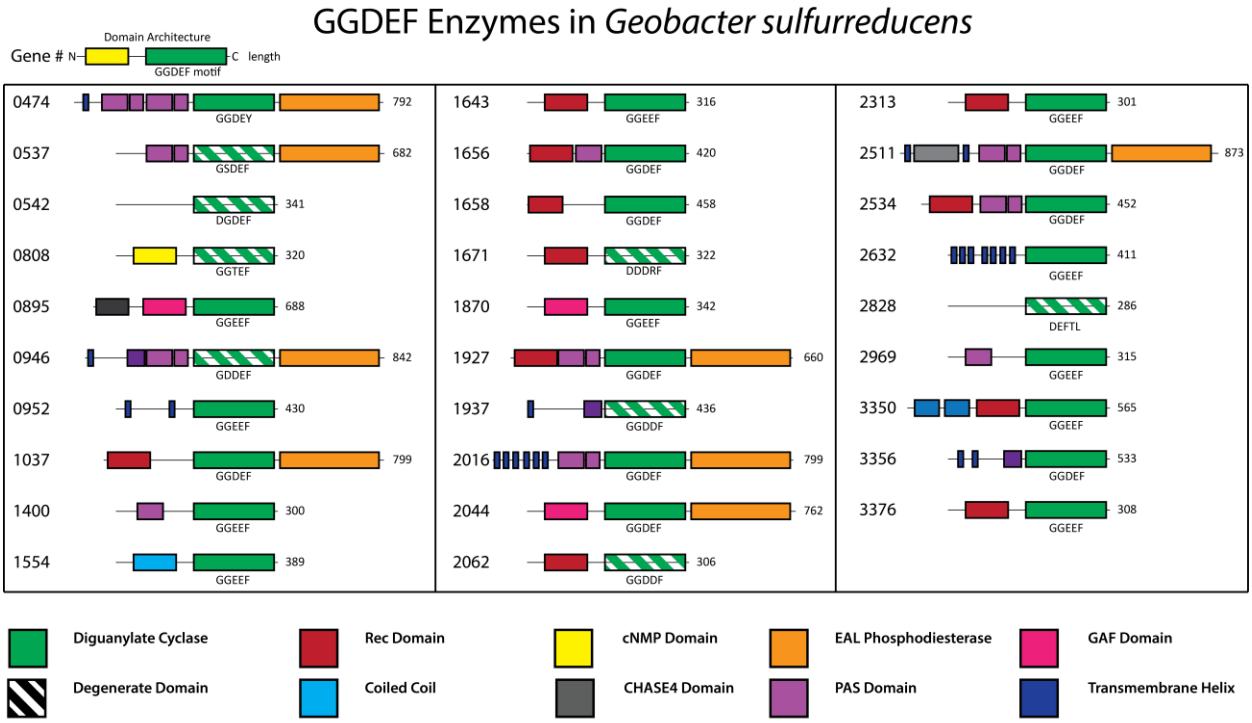

Figure S1 – Domain architecture of GGDEF-domain containing proteins in *Geobacter sulfurreducens* PCA. Proteins tested for cdiG- and cAG-synthase activity are shown. REC, response receiver regulator domain found in two-component regulatory systems; cNMP, cyclic nucleotide monophosphate binding domain; EAL, cdiG-specific phosphodiesterase domain; GAF, domain present in cGMP phosphodiesterases, adenylyl cyclases, and FhlA, sometimes associated with phytochromes; CHASE4, cyclase/histidine kinase associated extracellular sensor domain; PAS, PER/ARNT/SIM domain involved in oxygen, light, and redox state sensing. The residues corresponding to the “signature” “GGDEF” motif are shown below the GGDEF domain for each.

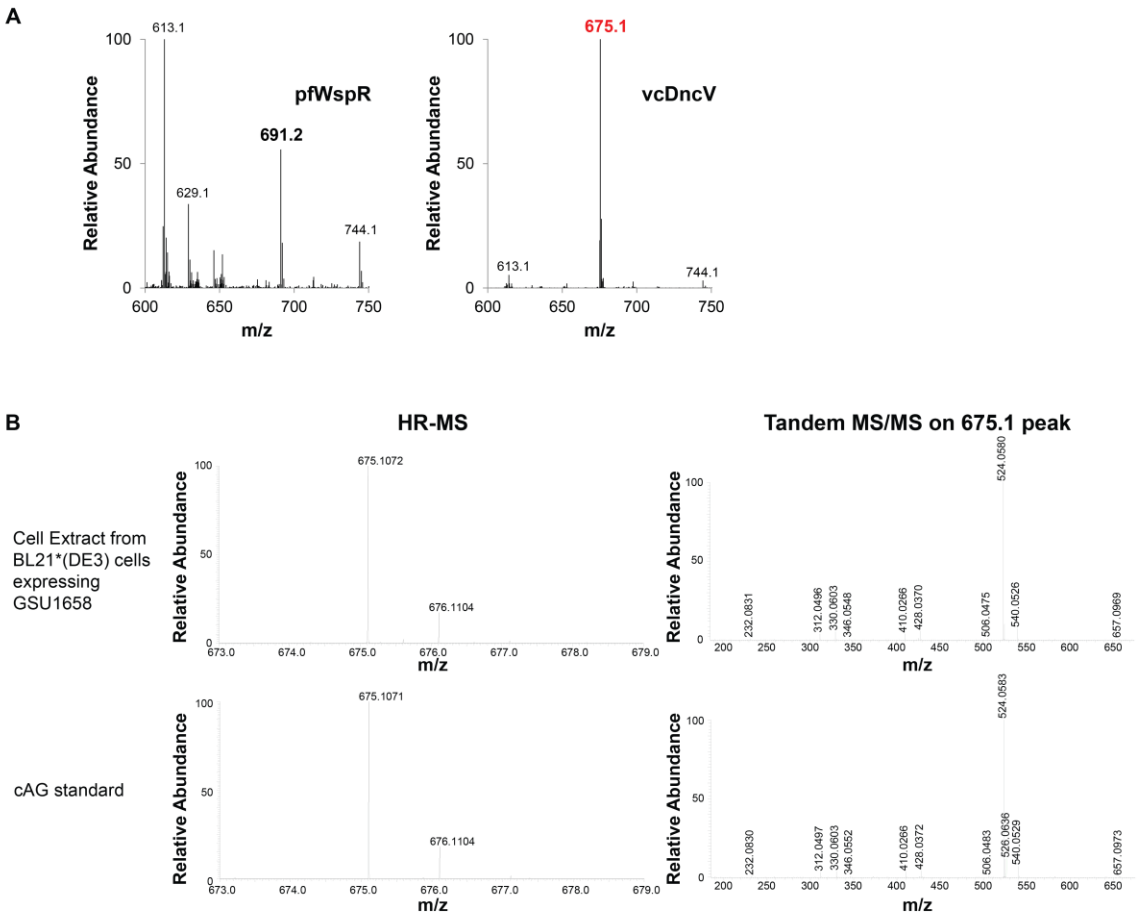

Figure S2 – Cell extraction of enzyme standards and GSU1658. (A) HPLC-MS of lysates from BL21 star (DE3) cells expressing control enzymes. Left: WspR, a cdiG (m/z = 691.1) synthase; right: DncV, a cAG (m/z = 675.2) synthase. **(B)** Mass spectrometry analysis of lysate from BL21 star (DE3) cells expressing GSU1658 or synthetic 3',3'-cAG standard. Left: High-resolution mass-spectrometry; Right: Tandem MS/MS of the 675.1 peak observed.

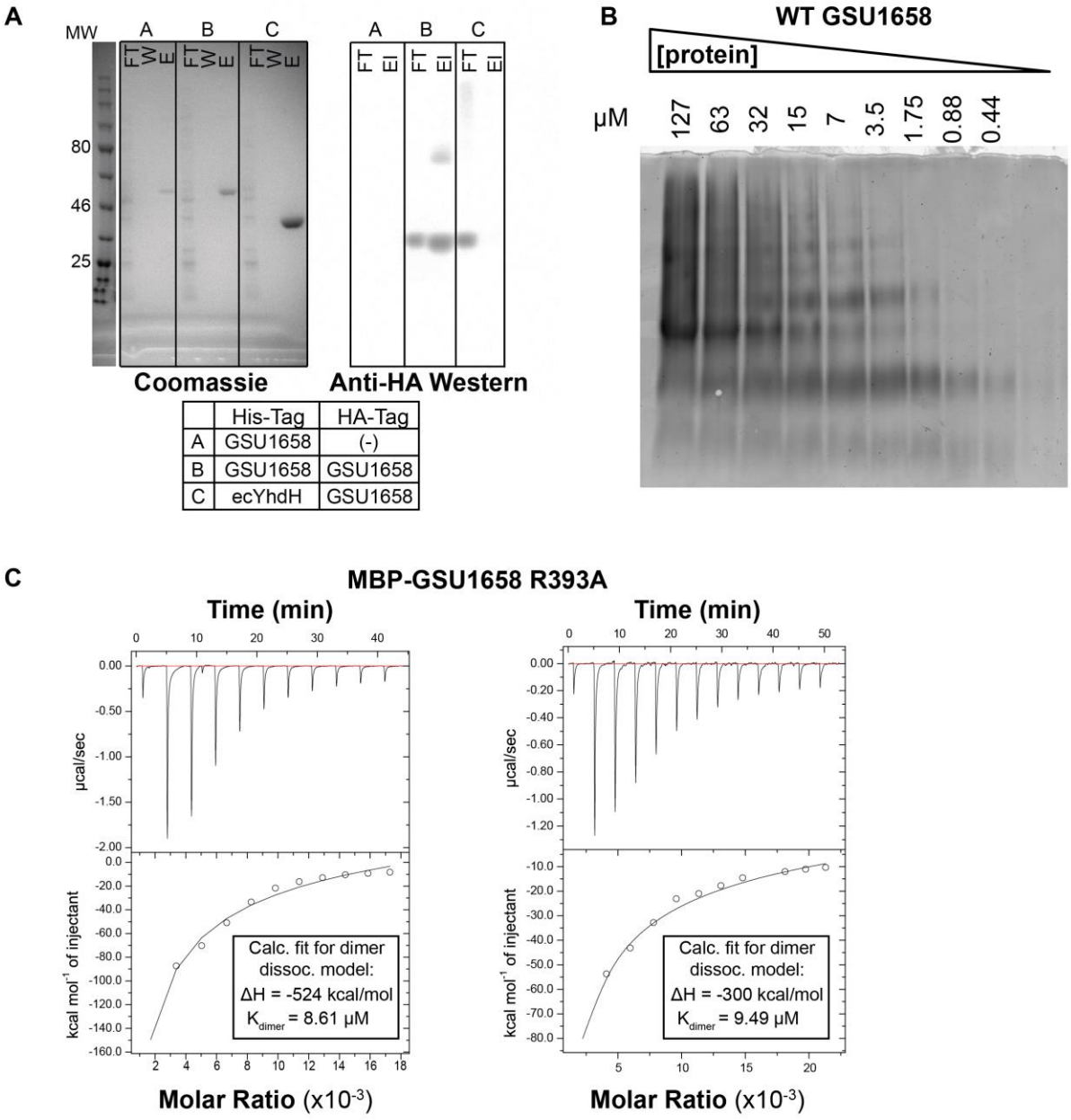

Figure S3 – Analysis of GSU1658 dimerization. (**A**) Pull-down of differentially tagged GSU1658 constructs. His-tagged WT GSU1658 or BL21-derived YhdH was co-expressed with a plasmid control containing no enzyme or HA-tagged WT GSU1658, and the cell lysate purified by Ni-NTA affinity chromatography. Samples were then immunoblotted against the HA epitope. (**B**) Native PAGE analysis of GSU1658-6xHis conformers. Samples were visualized with SYPRO®-Orange (Life Technologies) staining. (**C**) Dilution ITC of MBP-GSU1658 R393A mutants. GSU1658 at 111 and 119 μM were diluted into buffer and the heat change measured over multiple rounds of injection. Enthalpy change and K_{dimer} values were obtained by fitting heat changes to a dimer dissociation model; the high enthalpy values suggest this model is incorrect.

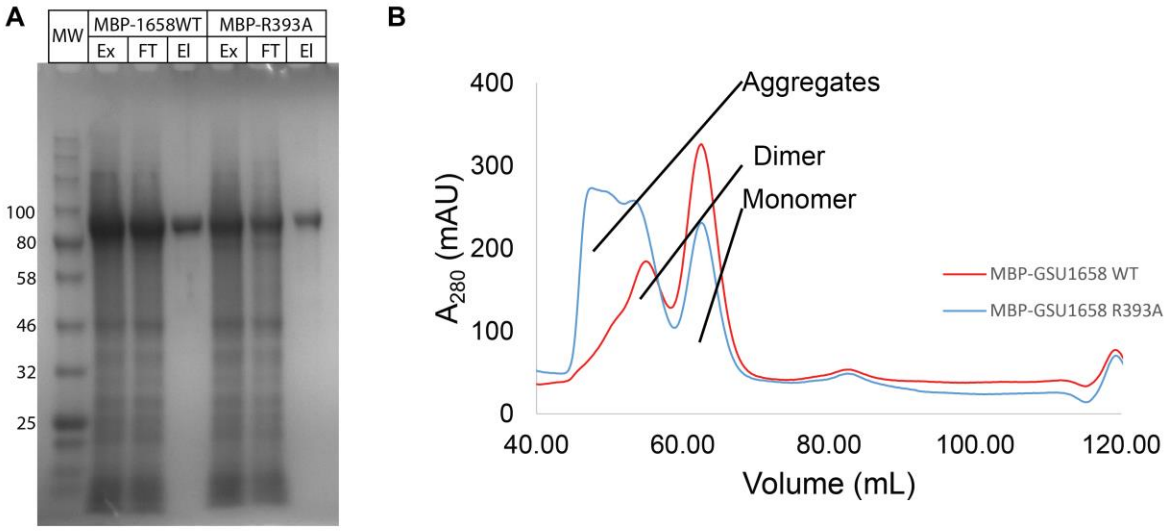

Figure S4 – Purification of MBP-tagged GSU1658. (**A**) SDS-PAGE gel analysis of fractions from the purification of MBP-tagged WT- and R393A-GSU1658. Gels were stained with GelCode Blue (Thermo Scientific). Ex, extract; FT, flow-through; El, elution. (B) Size-exclusion chromatography of MBP-tagged variants of GSU1658. MBP-GSU1658 (WT or R393A, 50 μM, 1 mL) was analyzed by SEC. Shown is the A_{280} trace starting at the void volume of ~40 mL.

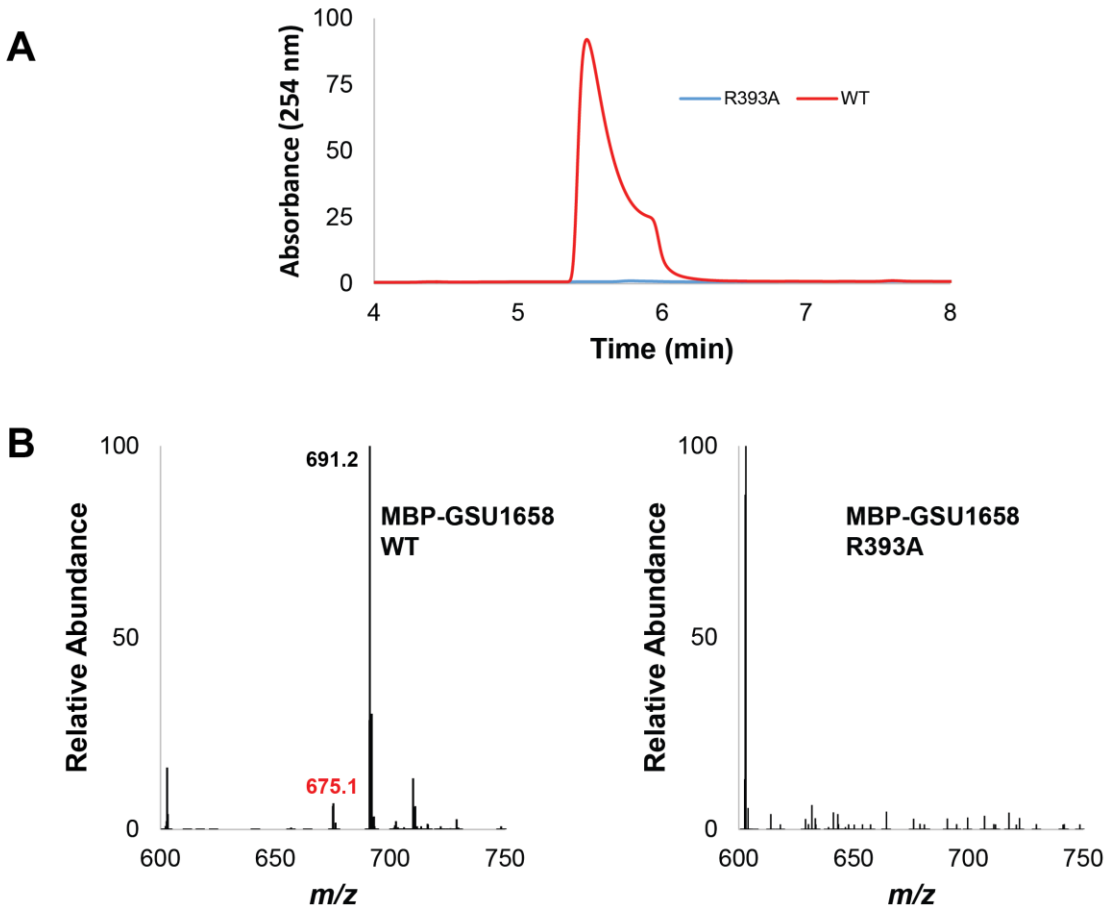

Figure S5 – R393A mutation of GSU1658 ablates CDN binding to I-site. (**A**) Overlay of UV-Vis spectra and (**B**) MS spectra from HPLC-MS analysis of nucleotides bound to MBP-tagged GSU1658 constructs. The MS spectra are from integrating the retention time region containing all three cyclic dinucleotides (6 to 8 min). Expected masses are for cdiG (m/z = 691) and cAG (m/z = 675).

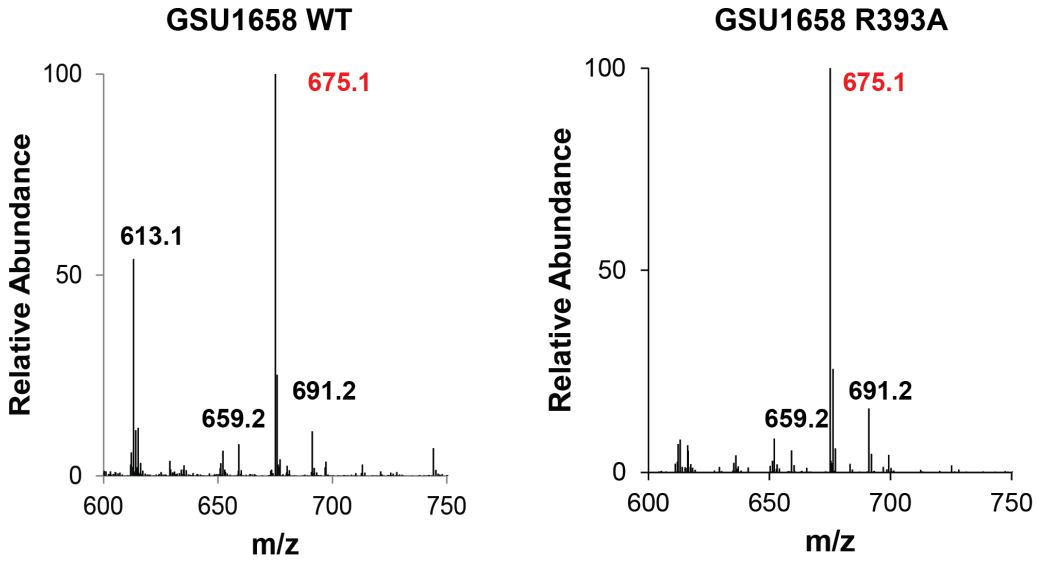

Figure S6 – HPLC-MS analysis of lysate from cells expressing the GSU1658 wild-type and R393A mutant. The MS spectra shown integrates the retention time region containing all three cyclic dinucleotides (6 to 8 min). Expected masses are for cdiG (m/z = 691), cAG (m/z = 675), and cdiA (m/z = 659).

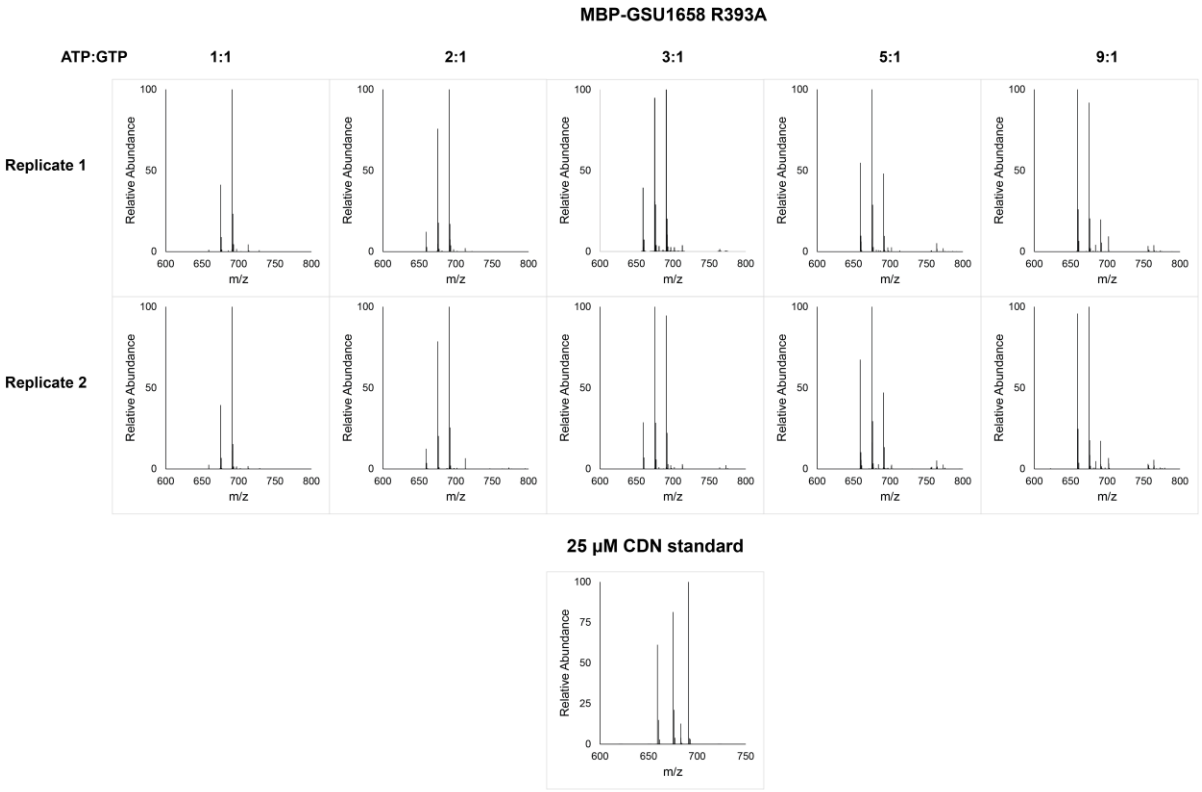

Figure S7 – LC-MS analysis of CDN product distribution for MBP-tagged GSU1658 R393A mutant. LC-MS analysis of *in vitro* enzyme reactions with varying ratios of ATP to GTP. Shown is the MS spectra from integrating the retention time region containing all three cyclic dinucleotides (6 to 8 min). Expected masses are for cdiG (m/z = 691), cAG (m/z = 675), and cdiA (m/z = 659).

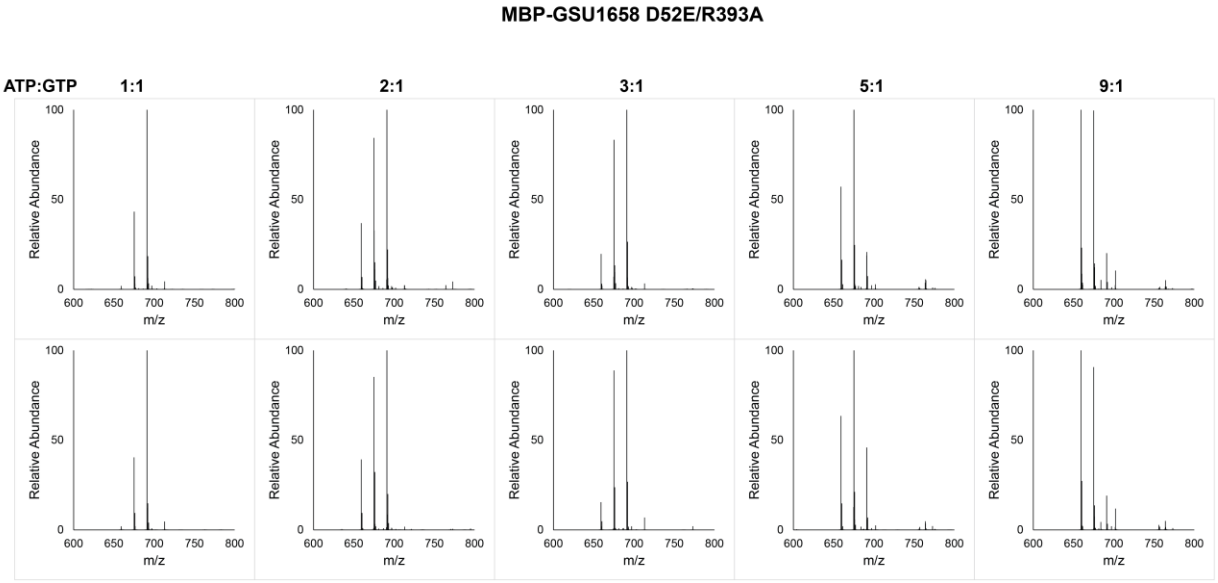

Figure S8 – LC-MS analysis of CDN product distribution for MBP-tagged GSU1658 D52E/R393A mutant. LC-MS analysis of *in vitro* enzyme reactions with varying ratios of ATP to GTP. Shown is the MS spectra from integrating the retention time region containing all three cyclic dinucleotides (6 to 8 min). Expected masses are for cdiG (m/z = 691), cAG (m/z = 675), and cdiA (m/z = 659).

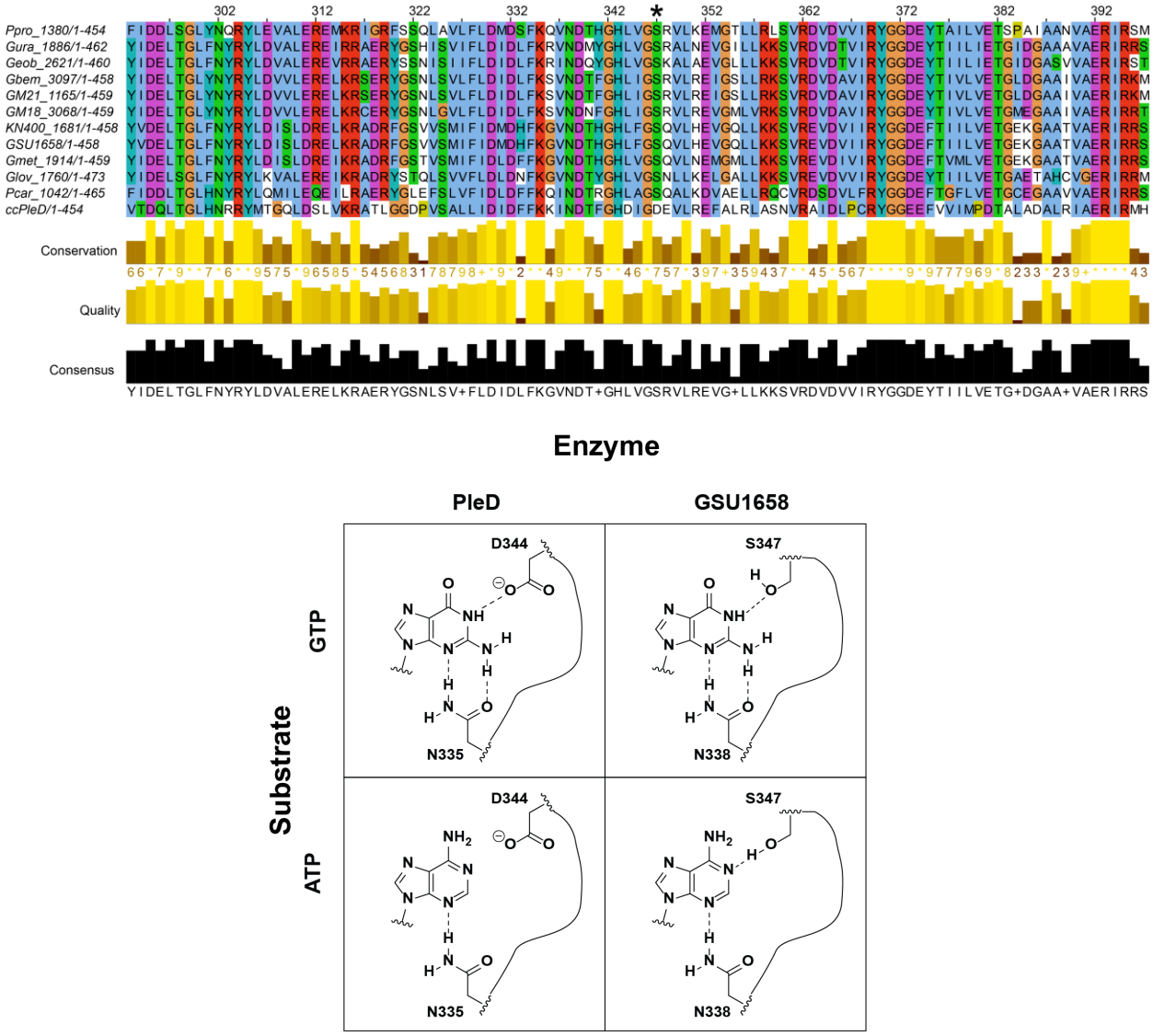

Figure S9. Alignment of representative *Geobacter* GGDEF domains and proposed model for nucleotide recognition. (**A**) Sequence alignment of the GGDEF domain of PleD, a canonical diguanylate cyclase, with all HyprA GGDEF domains in sequenced *Geobacter* and *Pelobacter* species. The encoding gene is conserved and found in the same genomic location, 5' to the histidyl tRNA synthetase gene *hisS*. The position of the substrate-binding aspartate, D344 in PleD, and its S347 counterpart in GSU1658 is marked with an asterisk. Ppro, *Pelobacter propionicus*; Gura, *Geobacter uraniireducens*; Geob, *Geobacter daltonii FRC-32*; GM21, *Geobacter sp. (Strain M21)*; GM18, *Geobacter sp. M18*; KN400, *Geobacter sulfurreducens KN400*; GSU, *Geobacter sulfurreducens PCA*; Gmet, *Geobacter metalireducens*; Glov, *Geobacter lovleyi*; Pcar, *Pelobacter carbinolicus*. Alignments were performed using the MUSCLE alignment program with the standard settings in JalView (8). (**B**) Proposed model for purine nucleotide recognition by PleD versus GSU1658.

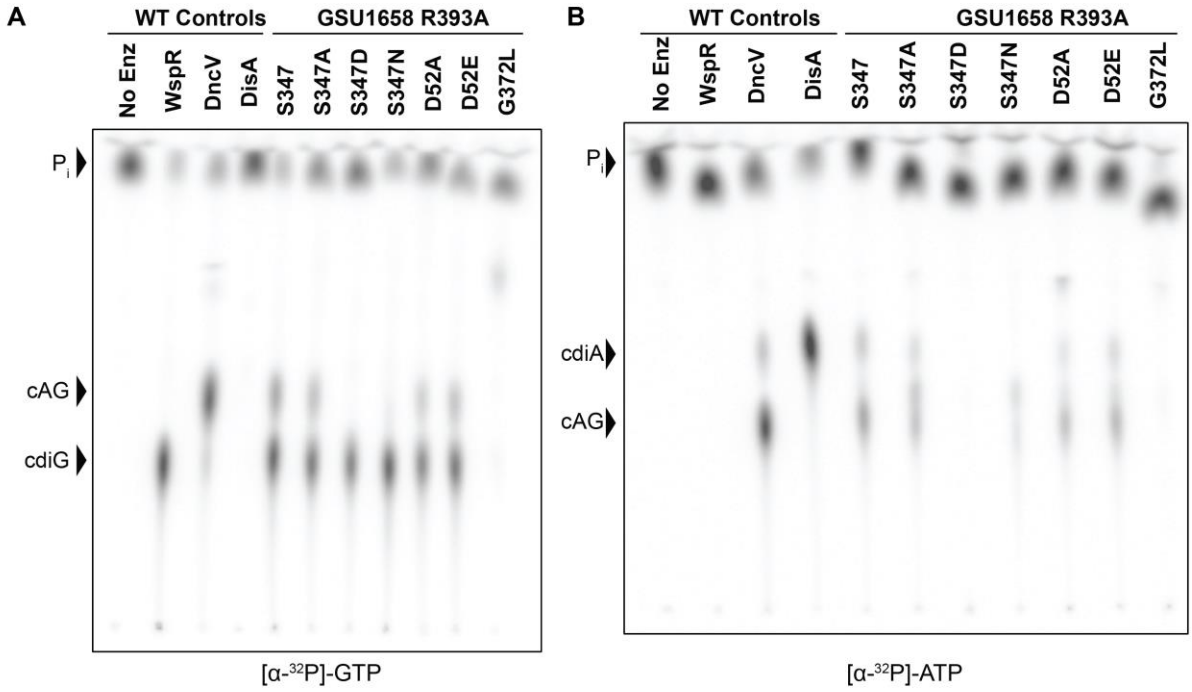

Figure S10. Activity assay of GSU1658 mutants with radiolabeled NTPs. Cellulose thin layer chromatography of radiolabeled products from enzymatic reactions with 1:1 ATP to GTP substrates in excess and doped with trace amounts of α-^{32}P-labeled (**A**) GTP or (**B**) ATP. (**A**) is the full TLC plate for the inset shown in Figure 3. The positions of the cyclic dinucleoide products (cAG, cdiG, cdiA) and inorganic phosphate (P_i) are marked.

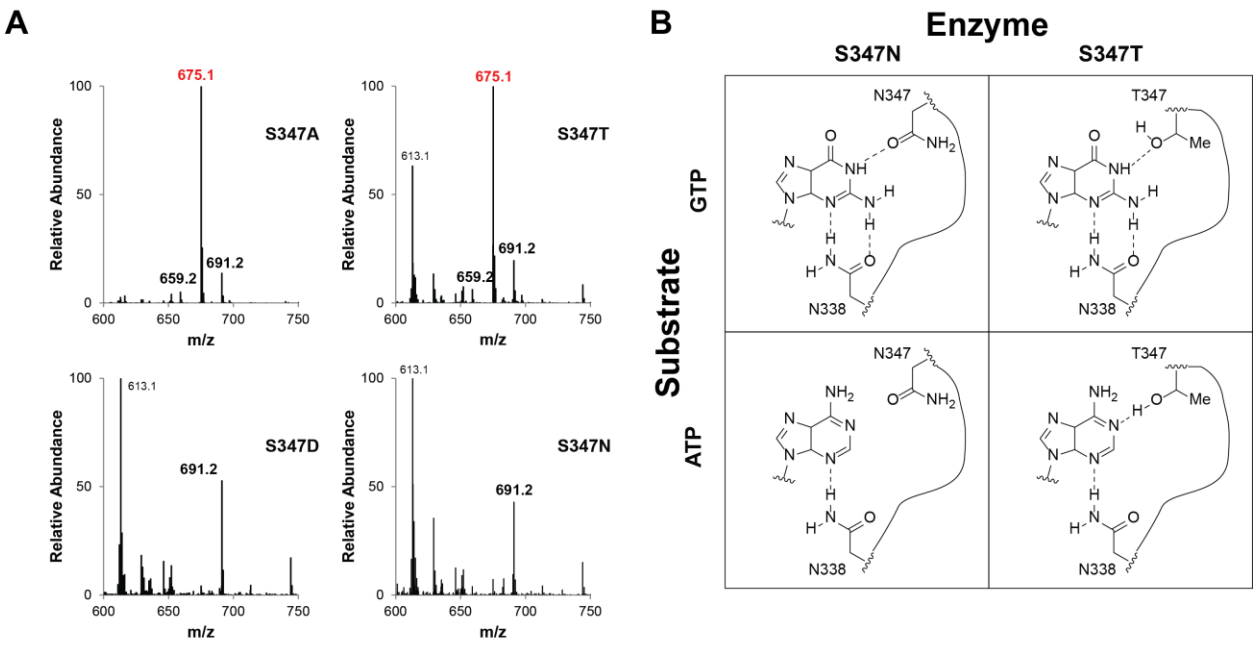

Figure S11. Analysis of specificity residue mutations in GSU1658. (**A**) LC-MS analysis of extracts from cells expressing GSU1658 single mutants in pET24a with C-terminal 6x-His tags. (**B**) Proposed model for purine nucleotide binding by S347N and S347T GSU1658 mutants.

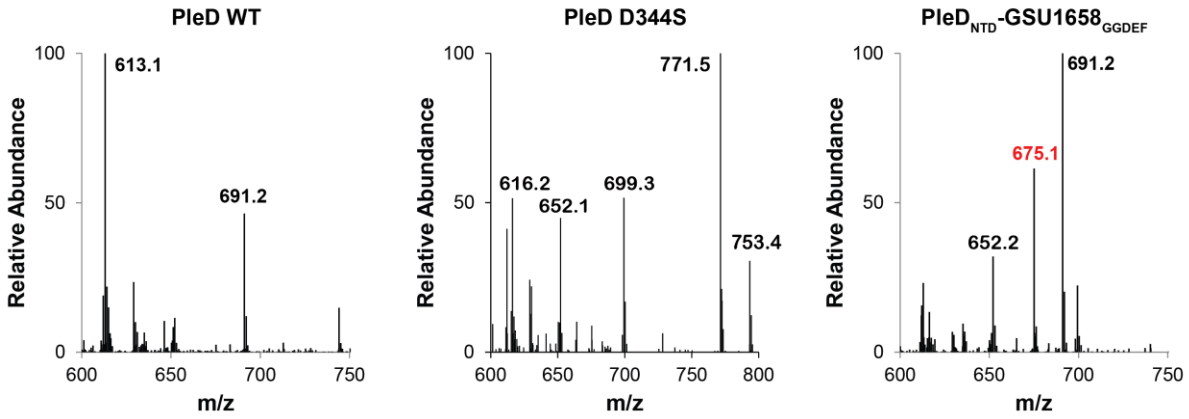

Figure S12 – HPLC-MS analysis of PleD and associated mutants. LC/MS analysis of *E. coli* cell extracts overexpressing PleD variants as shown. PleD$_{NTD}$-GSU1658$_{GGDEF}$ is a fusion between residues 1-293 of PleD, and residues 297-458 of GSU1658. Shown is the MS spectra from integrating the retention time region containing all three cyclic dinucleotides (6 to 8 min). Expected masses are for cdiG (m/z = 691) and cAG (m/z = 675).

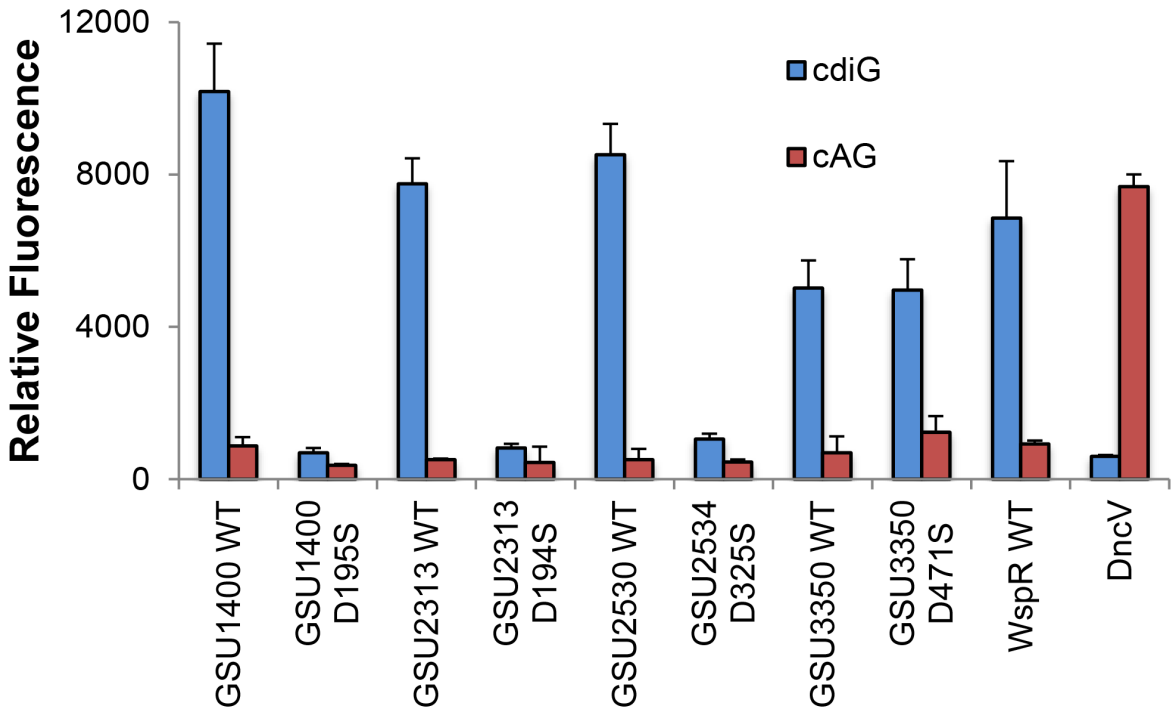

Figure S13. Analysis of D-to-S mutations of several diguanylate cyclases from *Geobacter sulfurreducens*. Average fluorescence measured by flow cytometry (n=3, 10,000 cells per run) of *E. coli* BL21 (DE3) Star cells co-expressing the cdiG-selective biosensor DP17-Spinach2 (blue) or cAG-selective biosensor Gm0970-p1-4delA-Spinach (red) along wild-type or selectivity site D-to-S mutants of validated diguanylate cyclases from *G. sulfurreducens* PCA.

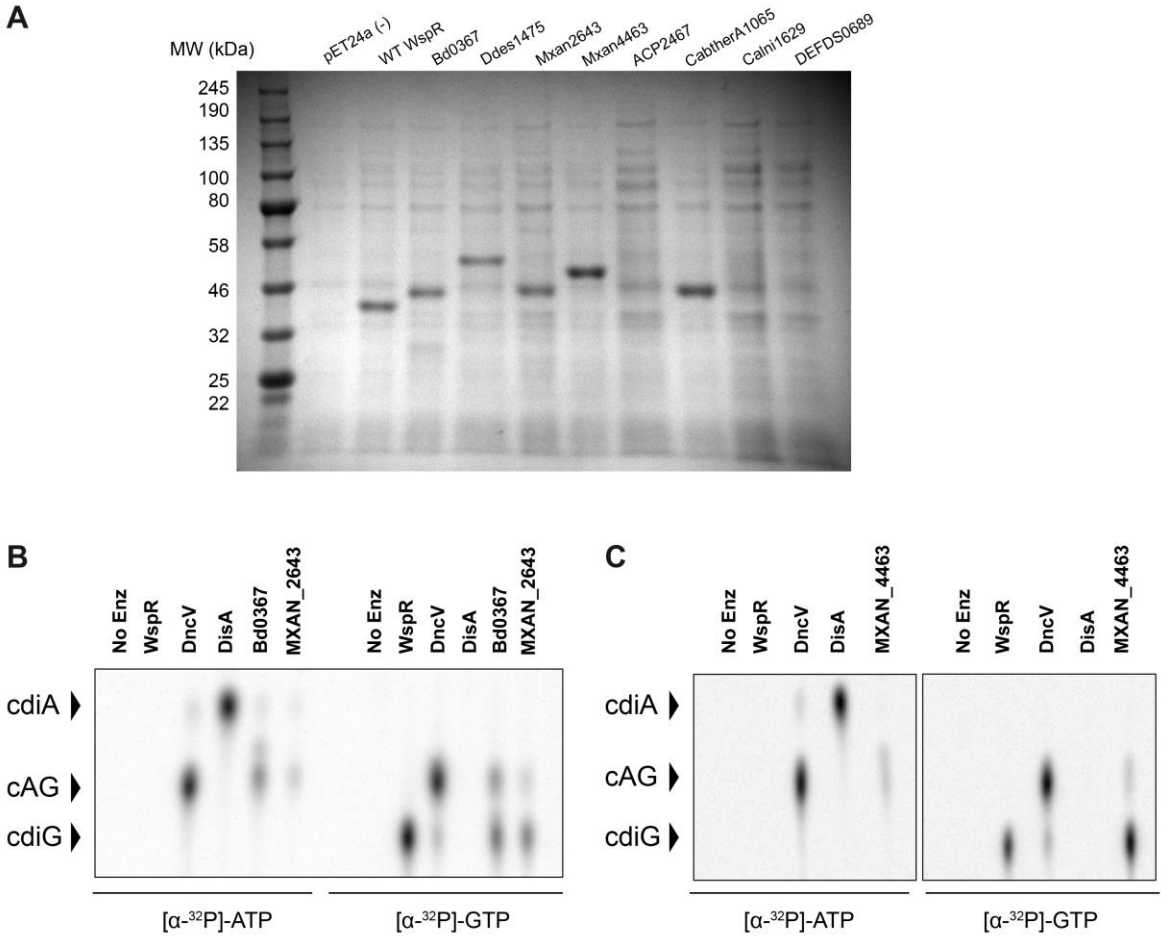

Figure S14. Activity assay for additional Hypr GGDEF domains. (A) SDS-PAGE gel analysis of lysates from cells expressing C-terminal 6x-His constructs in pET24a, or (for ACP2467, Calni1629, and DEFDS0689) N-terminal 6x-His-MBP constructs. Gel was stained with GelCode Blue (Thermo Scientific). (**B**) Cellulose thin layer chromatography showing cyclic dinucleotide region of radiolabeled products from enzymatic reactions of MBP-tagged I-site mutations of Bd0367 (R260A) or MXAN_2643 (R292A) with 1:1 ATP to GTP substrates in excess and doped with trace amounts of α-^{32}P-labeled GTP or ATP. (**C**) As in (B), with Wild-type C-terminal 6x-His tagged MXAN_4463.

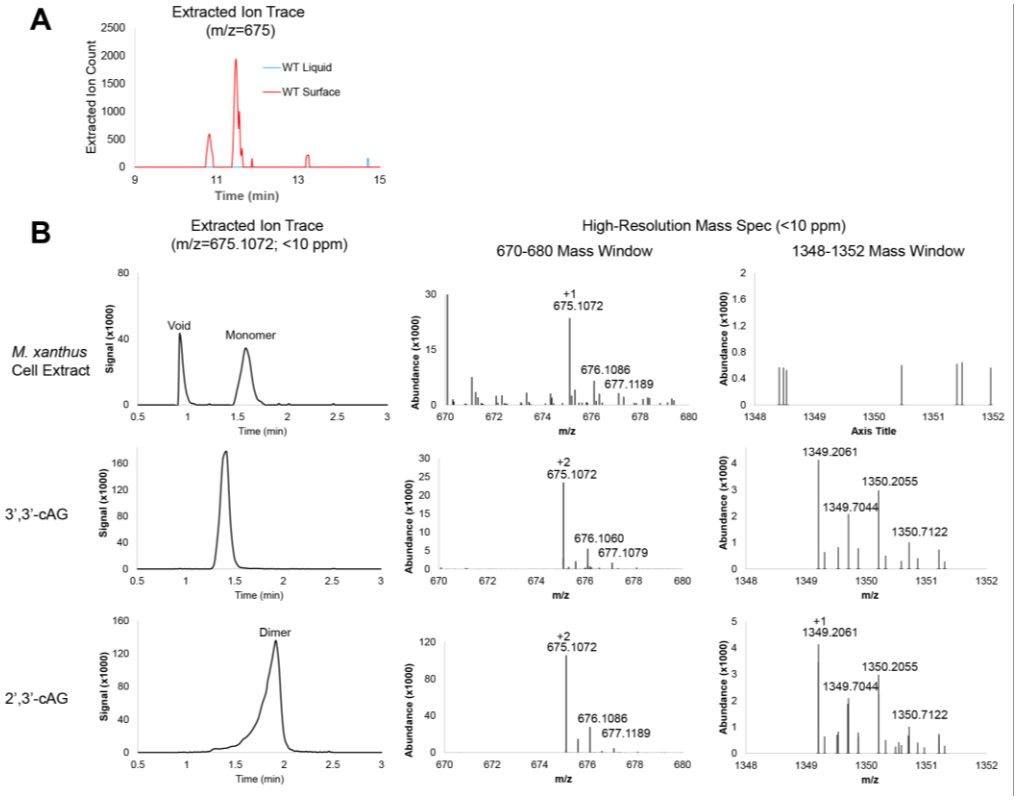

Figure S15. Mass spectrometry analysis of *Myxococcus xanthus* cell extracts. (**A**) Mass spectrometry analysis of second independent biological replicate of *M. xanthus* cell extracts from surface- or liquid-grown WT samples. Shown is the extracted ion trace for cAG (m/z=675) normalized to the weight of extracted cells. The instrumentation and LCMS protocol used in this case was the same as described for *E. coli* cell extract. (**B**) Mass spectrometry analysis of lysate from surface-grown WT *M. xanthus* strain DZ2 or synthetic 3',3'-cAG and 2',3'-cAG standards. Left: Extracted ion trace for cAG (m/z=675.1072). Right: High-resolution mass spectra of the monomer (m/z=675.1072) and dimer (m/z=1349.2064)

Using the same instrumentation as used for analysis of *E. coli* cell extracts, we detect cAG only in WT *M. xanthus* grown on agar surface, not for liquid culture samples.

In the extract from surface-grown *M. xanthus*, we observe two peaks, one at the void volume (1 min), and the other which is intermediate between the retention time of the 3',3' and 2',3' cAG standards. However, the high-resolution mass spectrum shows that it matches the chemical formula for cAG. The slight discrepancy in retention times compared to synthetic standards is because both synthetic standards appear to elute as dimers, as we observe the dimer mass (1349 mass, +1 charge) as well as the 675 mass as a +2-charged species. In contrast, the cAG present in cell extracts elutes as the monomer (675 mass, +1 charge). We further validated that the peak is 3',3'-cAG by tandem MS/MS, as seen in Figure S16.

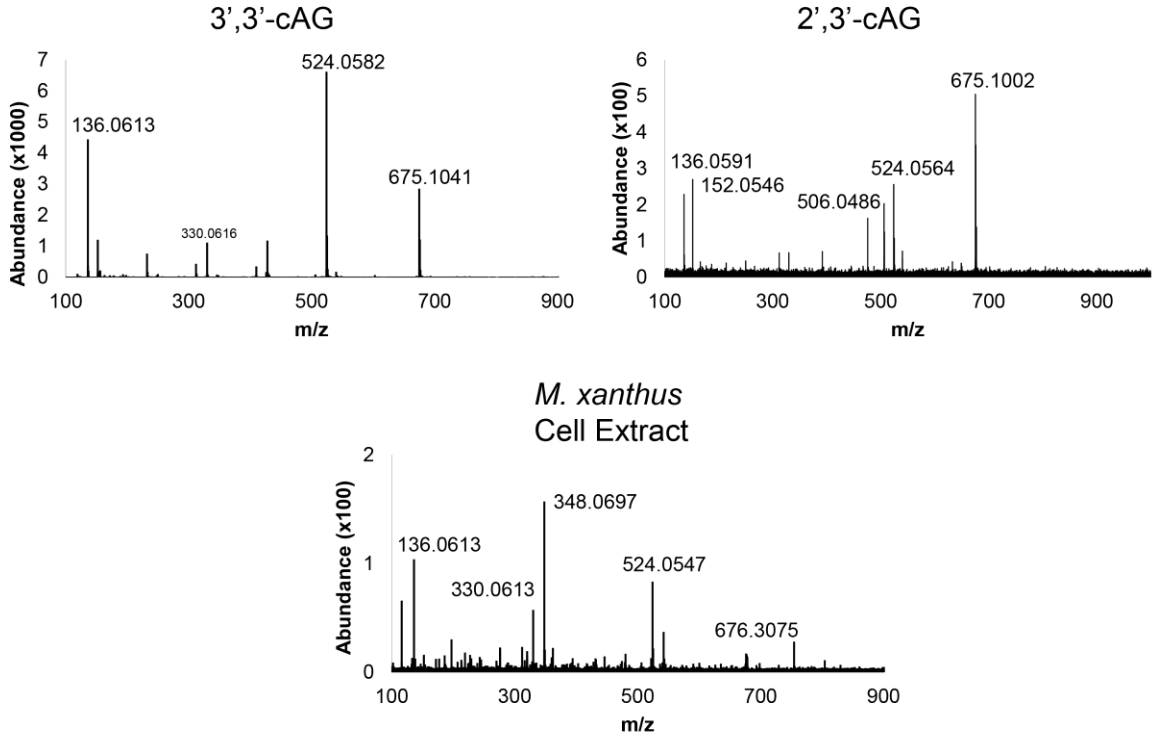

Figure S16. Tandem MS/MS of parent ion (m/z=675.1072) in cyclic dinucleotide standards and *Myxococcus xanthus* cell extracts.

Tandem MS/MS analysis of the parent ion shows the cAG present in cell extracts has key fragmentations of 136, 330, and 524, which correspond to the 3',3'-cAG synthetic standard . In contrast, the key fragmentation of m/z=506 corresponding to 2',3'-cAG is not observed in the cell extract sample. A related linear dinucleotide containing a 2',3'-cyclic phosphate has been known to have fragmentation masses of m/z=152 and 540 (9), which we do not observe in the cell extract sample. There is one additional peak in the extract tandem MS/MS spectrum corresponding to AMP, which may be attributed to possible fragmentation differences between the monomeric and dimeric forms of the cyclic dinucleotide.

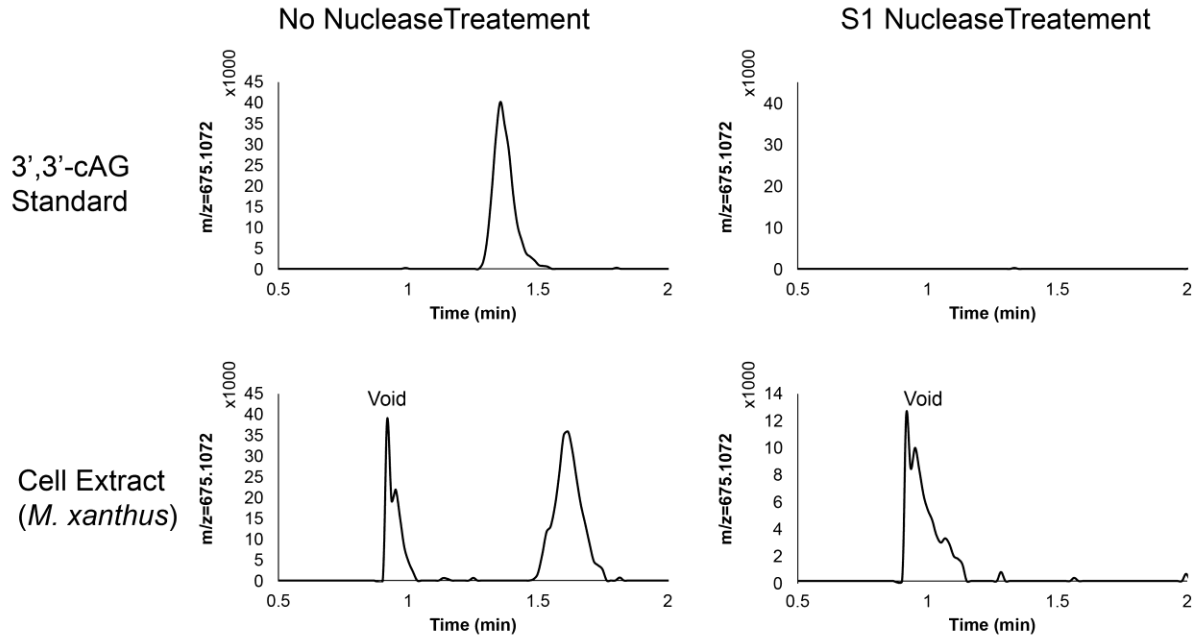

Figure S17. Effect of nuclease treatment on *Myxococcus xanthus* cell extracts. Extracted ion trace for m/z=675.1075 with a <10 ppm cutoff of 3',3'-cAG standard (Top) or *M. xanthus* strain DZ2 extracts (bottom) untreated (Left) or treated with S1 Nuclease (Right).

S1 nuclease cleaves 3'-5' phosphodiester bonds, and is shown to cleave 3',3'-cAG. As shown, S1 nuclease is able to cleave both m/z=675 peaks occurring at the void volume and at ~1.6 min in the *M. xanthus* cell extract, further supporting our assignment for the compound as 3',3'-cAG.

Table S1. GGDEFs containing variant residues at the PleD 344 alignment position. Genes listed are only those which possess a functional GGDEF motif, which we consider [G/A/S]G[D/E]EF.

UniProt KB Accession	Gene names	Organism	Selectivity Residue
A3IYP6	CY0110_05007	*Cyanothece sp. CCY0110*	Y
A1ALA3	Ppro_0492	*Pelobacter propionicus (strain DSM 2379)*	T
A5G6K7	Gura_3266	*Geobacter uraniireducens (strain Rf4) (Geobacter uraniumreducens)*	T
B5EC68	Gbem_3531	*Geobacter bemidjiensis (strain Bem / ATCC BAA-1014 / DSM 16622)*	T
C6E666	GM21_3597	*Geobacter sp. (strain M21)*	T
D3PC46	DEFDS_0689	*Deferribacter desulfuricans (strain DSM 14783 / JCM 11476 / NBRC 101012 / SSM1)*	T
E1WZI7	BMS_1301	*Halobacteriovorax marinus (strain ATCC BAA-682 / DSM 15412 / SJ) (Bacteriovorax marinus)*	T
E8WQD1	GM18_0558	*Geobacter sp. (strain M18)*	T
Q46S25	Reut_B4711	*Cupriavidus pinatubonensis (strain JMP 134 / LMG 1197) (Ralstonia eutropha (strain JMP 134))*	T
A1ANS6	Ppro_1380	*Pelobacter propionicus (strain DSM 2379)*	S
A5GF71	Gura_1886	*Geobacter uraniireducens (strain Rf4) (Geobacter uraniumreducens)*	S
A7HAD5	Anae109_1474	*Anaeromyxobacter sp. (strain Fw109-5)*	S
B3E1R0	Glov_1844	*Geobacter lovleyi (strain ATCC BAA-1151 / DSM 17278 / SZ)*	S
B3EB82	Glov_1760	*Geobacter lovleyi (strain ATCC BAA-1151 / DSM 17278 / SZ)*	S
B4UJZ3	AnaeK_1471	*Anaeromyxobacter sp. (strain K)*	S
B5E8T5	Gbem_3097	*Geobacter bemidjiensis (strain Bem / ATCC BAA-1014 / DSM 16622)*	S
B8EMQ6	Msil_3853	*Methylocella silvestris (strain BL2 / DSM 15510 / NCIMB 13906)*	S
B8J0V0	Ddes_1475	*Desulfovibrio desulfuricans (strain ATCC 27774 / DSM 6949)*	S
B8J555	A2cp1_1566	*Anaeromyxobacter dehalogenans (strain 2CP-1 / ATCC BAA-258)*	S
B9M0W8	Geob_2621	*Geobacter daltonii (strain DSM 22248 / JCM 15807 / FRC-32)*	S
C0QB51	HRM2_17460	*Desulfobacterium autotrophicum (strain ATCC 43914 / DSM 3382 / HRM2)*	S
C1F1G0	ACP_2467	*Acidobacterium capsulatum (strain ATCC 51196 / DSM 11244 / JCM 7670 / NBRC 15755 / NCIMB 13165 / 161)*	S
C6E353	GM21_1165	*Geobacter sp. (strain M21)*	S
C7QHC7	Caci_0111	*Catenulispora acidiphila (strain DSM 44928 / NRRL B-24433 / NBRC 102108 / JCM 14897)*	S
E3FYP4	STAUR_3377	*Stigmatella aurantiaca (strain DW4/3-1)*	S
E3T615		*uncultured bacterium 293*	S
E4TFG3	Calni_1629	*Calditerrivibrio nitroreducens (strain DSM 19672 / NBRC 101217 / Yu37-1)*	S
E6PWY9	CARN3_0369	*mine drainage metagenome*	S
E8RE90	Despr_2994	*Desulfobulbus propionicus (strain ATCC 33891 / DSM 2032 / 1pr3)*	S
E8V865	AciPR4_3292	*Terriglobus saanensis (strain ATCC BAA-1853 / DSM 23119 / SP1PR4)*	S
E8WHW1	GM18_3068	*Geobacter sp. (strain M18)*	S
E8WYM1	AciX9_0547	*Granulicella tundricola (strain ATCC BAA-1859 / DSM 23138 / MP5ACTX9)*	S
F2NID6	Desac_2520	*Desulfobacca acetoxidans (strain ATCC 700848 / DSM 11109 / ASRB2)*	S
F8CEK8	LILAB_20895	*Myxococcus fulvus (strain ATCC BAA-855 / HW-1)*	S

F8CQQ7	LILAB_30450	*Myxococcus fulvus (strain ATCC BAA-855 / HW-1)*	S
G2LH77	Cabther_A1065	*Chloracidobacterium thermophilum (strain B)*	S
H8MHV0	pleD2 COCOR_03316	*Corallococcus coralloides (strain ATCC 25202 / DSM 2259 / NBRC 100086 / M2) (Myxococcus coralloides)*	S
H8MXI3	cph2C COCOR_05401	*Corallococcus coralloides (strain ATCC 25202 / DSM 2259 / NBRC 100086 / M2) (Myxococcus coralloides)*	S
Q08TQ2	STIAU_4749	*Stigmatella aurantiaca (strain DW4/3-1)*	S
Q08YB4	STAUR_4818 STIAU_0908	*Stigmatella aurantiaca (strain DW4/3-1)*	S
Q1D3Y9	MXAN_4463	*Myxococcus xanthus (strain DK 1622)*	S
Q1D911	MXAN_2643	*Myxococcus xanthus (strain DK 1622)*	S
Q1IKE0	Acid345_3659	*Koribacter versatilis (strain Ellin345)*	S
Q1JVE0	Dace_0065	*Desulfuromonas acetoxidans DSM 684*	S
Q2IKI3	Adeh_2393	*Anaeromyxobacter dehalogenans (strain 2CP-C)*	S
Q39UD1	Gmet_1914	*Geobacter metallireducens (strain GS-15 / ATCC 53774 / DSM 7210)*	S
Q3A5R5	Pcar_1042	*Pelobacter carbinolicus (strain DSM 2380 / Gra Bd 1)*	S
Q5ZPC6		*Angiococcus disciformis*	S
Q6MQU2	pleD Bd0367	*Bdellovibrio bacteriovorus (strain ATCC 15356 / DSM 50701 / NCIB 9529 / HD100)*	S
Q74CL4	GSU1658	*Geobacter sulfurreducens (strain ATCC 51573 / DSM 12127 / PCA)*	S
A0AK16	lwe1930	*Listeria welshimeri serovar 6b (strain ATCC 35897 / DSM 20650 / SLCC5334)*	N
A0AK17	lwe1931	*Listeria welshimeri serovar 6b (strain ATCC 35897 / DSM 20650 / SLCC5334)*	N
A0Q1B8	NT01CX_2347	*Clostridium novyi (strain NT)*	N
A0YR26	L8106_11667	*Lyngbya sp. (strain PCC 8106) (Lyngbya aestuarii (strain CCY9616))*	N
A1S6Z8	Sama_1949	*Shewanella amazonensis (strain ATCC BAA-1098 / SB2B)*	N
A1SR97	Ping_0142	*Psychromonas ingrahamii (strain 37)*	N
A1SZB6	Ping_3143	*Psychromonas ingrahamii (strain 37)*	N
A1UF51	Mkms_2261	*Mycobacterium sp. (strain KMS)*	N
A3DC33	Cthe_0273	*Clostridium thermocellum (strain ATCC 27405 / DSM 1237 / NBRC 103400 / NCIMB 10682 / NRRL B-4536 / VPI 7372) (Ruminiclostridium thermocellum)*	N
A3IWY5	CY0110_23131	*Cyanothece sp. CCY0110*	N
A3YE50	MED121_21460	*Marinomonas sp. MED121*	N
A4B9U6	MED297_20957	*Reinekea blandensis MED297*	N
A4BH36	MED297_14975	*Reinekea blandensis MED297*	N
A4E847	COLAER_00587	*Collinsella aerofaciens ATCC 25986*	N
A4U2M2	MGR_1840	*Magnetospirillum gryphiswaldense*	N
A5VMQ1	Lreu_1888	*Lactobacillus reuteri (strain DSM 20016)*	N
A5ZSV1	RUMOBE_02079	*Blautia obeum ATCC 29174*	N
A6CN78	BSG1_01135	*Bacillus sp. SG-1*	N
A6TIC3	KPN_pKPN3p05967	*Klebsiella pneumoniae subsp. pneumoniae (strain ATCC 700721 / MGH 78578)*	N
A6VRU9	Mmwyl1_0236	*Marinomonas sp. (strain MWYL1)*	N
A8DJI0	YS_M60-F11.073	*Chloracidobacterium thermophilum*	N

A8DW58	v1g49651	*Nematostella vectensis (Starlet sea anemone)*	N
A8G1K2	Ssed_4373	*Shewanella sediminis (strain HAW-EB3)*	N
A8GYS2	Spea_0130	*Shewanella pealeana (strain ATCC 700345 / ANG-SQ1)*	N
A8GZL9	Spea_0428	*Shewanella pealeana (strain ATCC 700345 / ANG-SQ1)*	N
A8RMS1	CLOBOL_02006	*Clostridium bolteae (strain ATCC BAA-613 / WAL 16351)*	N
A8S3F1	CLOBOL_06594	*Clostridium bolteae (strain ATCC BAA-613 / WAL 16351)*	N
A8UBZ9	CAT7_10515	*Carnobacterium sp. AT7*	N
A8UU60	HG1285_16780	*Hydrogenivirga sp. 128-5-R1-1*	N
A9AXC8	Haur_4216	*Herpetosiphon aurantiacus (strain ATCC 23779 / DSM 785)*	N
A9D5G8	KT99_02136	*Shewanella benthica KT99*	N
B0C8G1	AM1_5154	*Acaryochloris marina (strain MBIC 11017)*	N
B0JT77	MAE_12990	*Microcystis aeruginosa (strain NIES-843)*	N
B0TJH3	Shal_2411	*Shewanella halifaxensis (strain HAW-EB4)*	N
B0TMZ0	Shal_4188	*Shewanella halifaxensis (strain HAW-EB4)*	N
B1BA96	CBC_A0861	*Clostridium botulinum C str. Eklund*	N
B1KNY3	Swoo_4765	*Shewanella woodyi (strain ATCC 51908 / MS32)*	N
B1LZ02	Mrad2831_2428	*Methylobacterium radiotolerans (strain ATCC 27329 / DSM 1819 / JCM 2831)*	N
B1MWP5	LCK_00134	*Leuconostoc citreum (strain KM20)*	N
B1WSQ7	cce_4288	*Cyanothece sp. (strain ATCC 51142)*	N
B1XL77	SYNPCC7002_A2587	*Synechococcus sp. (strain ATCC 27264 / PCC 7002 / PR-6) (Agmenellum quadruplicatum)*	N
B2A0U2	Nther_2407	*Natranaerobius thermophilus (strain ATCC BAA-1301 / DSM 18059 / JW/NM-WN-LF)*	N
B2A818	Nther_2224	*Natranaerobius thermophilus (strain ATCC BAA-1301 / DSM 18059 / JW/NM-WN-LF)*	N
B2J5G5	Npun_R3941	*Nostoc punctiforme (strain ATCC 29133 / PCC 73102)*	N
B3PET3	CJA_1657	*Cellvibrio japonicus (strain Ueda107) (Pseudomonas fluorescens subsp. cellulosa)*	N
B5JXV5	GP5015_1671	*gamma proteobacterium HTCC5015*	N
B5U200		*uncultured bacterium*	N
B6ARH3	CGL2_11390004	*Leptospirillum sp. Group II '5-way CG'*	N
B6BGA1	SMGD1_1002	*Sulfurimonas gotlandica (strain DSM 19862 / JCM 16533 / GD1)*	N
B6BIR1	SMGD1_1897	*Sulfurimonas gotlandica (strain DSM 19862 / JCM 16533 / GD1)*	N
B6WPU3	DESPIG_00058	*Desulfovibrio piger ATCC 29098*	N
B7KYX3	Mchl_0308	*Methylobacterium extorquens (strain CM4 / NCIMB 13688) (Methylobacterium chloromethanicum)*	N
B8CH18	swp_0168	*Shewanella piezotolerans (strain WP3 / JCM 13877)*	N
B8D025	Hore_21320	*Halothermothrix orenii (strain H 168 / OCM 544 / DSM 9562)*	N
C0BQ78	BBPC_0428 BIFPSEUDO_02518	*Bifidobacterium pseudocatenulatum DSM 20438 = JCM 1200 = LMG 10505*	N
C0BWF0	CLOHYLEM_04111	*[Clostridium] hylemonae DSM 15053*	N
C0D7P9	CLOSTASPAR_05296	*[Clostridium asparagiforme] DSM 15981*	N
C0QXE8	BHWA1_00306	*Brachyspira hyodysenteriae (strain ATCC 49526 / WA1)*	N
C0WEP4	ACDG_01935	*Acidaminococcus sp. D21*	N

C0YZG5	HMPREF0535_1180	*Lactobacillus reuteri MM2-3*	N
C2EFU2	HMPREF0545_0514	*Lactobacillus salivarius DSM 20555 = ATCC 11741*	N
C2EUE6	HMPREF0549_1082	*Lactobacillus vaginalis DSM 5837 = ATCC 49540*	N
C2JZI8	HMPREF0539_2323	*Lactobacillus rhamnosus LMS2-1*	N
C5EJH1	CBFG_00456	*Clostridiales bacterium 1_7_47FAA*	N
C6WGK8	Amir_0355	*Actinosynnema mirum (strain ATCC 29888 / DSM 43827 / NBRC 14064 / IMRU 3971)*	N
C6WVQ3	Mmol_1094	*Methylotenera mobilis (strain JLW8 / ATCC BAA-1282 / DSM 17540)*	N
C7XH20	HMPREF5045_00103	*Lactobacillus crispatus 125-2-CHN*	N
C9A618	ECBG_00198	*Enterococcus casseliflavus EC20*	N
C9AAA1	ECBG_01681	*Enterococcus casseliflavus EC20*	N
C9CHJ5	ECAG_00228	*Enterococcus casseliflavus EC10*	N
C9CIF0	ECAG_00128	*Enterococcus casseliflavus EC10*	N
C9CKC7	ECAG_01191	*Enterococcus casseliflavus EC10*	N
C9YL14	CDR20291_1266	*Peptoclostridium difficile (strain R20291) (Clostridium difficile)*	N
D0DEJ0	HMPREF0508_00079	*Lactobacillus crispatus MV-3A-US*	N
D1C7L1	Sthe_0405	*Sphaerobacter thermophilus (strain DSM 20745 / S 6022)*	N
D1CCW0	Tter_1719	*Thermobaculum terrenum (strain ATCC BAA-798 / YNP1)*	N
D2RKZ4	Acfer_1386	*Acidaminococcus fermentans (strain ATCC 25085 / DSM 20731 / VR4)*	N
D2RKZ5	Acfer_1387	*Acidaminococcus fermentans (strain ATCC 25085 / DSM 20731 / VR4)*	N
D4CHU0	CLOM621_09029	*Clostridium sp. M62/1*	N
D4IYS5	CIY_00120	*Butyrivibrio fibrisolvens 16/4*	N
D4LR02	CK5_18170	*Blautia obeum A2-162*	N
D4LW73	CK5_02480	*Blautia obeum A2-162*	N
D4MNW8	CL3_00230	*butyrate-producing bacterium SM4/1*	N
D5Q675	HMPREF0220_2407	*Peptoclostridium difficile NAP08*	N
D5S2D4	HMPREF0219_2715	*Peptoclostridium difficile NAP07*	N
D5U625	Bmur_0413	*Brachyspira murdochii (strain ATCC 51284 / DSM 12563 / 56-150) (Serpulina murdochii)*	N
D5XCW0	TherJR_2804	*Thermincola potens (strain JR)*	N
D6DM36	CLS_36110	*[Clostridium] cf. saccharolyticum K10*	N
D6XT12	Bsel_1436	*Bacillus selenitireducens (strain ATCC 700615 / DSM 15326 / MLS10)*	N
D6XZ65	Bsel_2859	*Bacillus selenitireducens (strain ATCC 700615 / DSM 15326 / MLS10)*	N
D7CN39	Slip_1361	*Syntrophothermus lipocalidus (strain DSM 12680 / TGB-C1)*	N
D8IA76	BP951000_2233	*Brachyspira pilosicoli (strain ATCC BAA-1826 / 95/1000)*	N
D9S411	FSU_2241	*Fibrobacter succinogenes (strain ATCC 19169 / S85)*	N
D9T0X8	Micau_1801	*Micromonospora aurantiaca (strain ATCC 27029 / DSM 43813 / JCM 10878 / NBRC 16125 / INA 9442)*	N
D9T2P9	Micau_6102	*Micromonospora aurantiaca (strain ATCC 27029 / DSM 43813 / JCM 10878 / NBRC 16125 / INA 9442)*	N
E0RAP7	PPE_01378	*Paenibacillus polymyxa (strain E681)*	N
E0S298	bpr_I1183	*Butyrivibrio proteoclasticus (strain ATCC 51982 / DSM 14932 / B316) (Clostridium proteoclasticum)*	N
E0SCN2	yddV Dda3937_02950	*Dickeya dadantii (strain 3937) (Erwinia chrysanthemi (strain 3937))*	N

E1IH26	OSCT_2627	*Oscillochloris trichoides DG-6*	N
E1QZ42	Olsu_0541	*Olsenella uli (strain ATCC 49627 / DSM 7084 / CIP 109912 / JCM 12494 / VPI D76D-27C) (Lactobacillus uli)*	N
E2SNJ1	HMPREF0983_02712	*Erysipelotrichaceae bacterium 3_1_53*	N
E3EFH1	PPSC2_07110	*Paenibacillus polymyxa (strain SC2) (Bacillus polymyxa)*	N
E3R434	LBKG_01059	*Lactobacillus crispatus CTV-05*	N
E3YRK3	NT05LM_2248	*Listeria marthii FSL S4-120*	N
E3YRK4	NT05LM_2249	*Listeria marthii FSL S4-120*	N
E3ZRW6	NT03LS_2247	*Listeria seeligeri FSL N1-067*	N
E3ZRW7	NT03LS_2248	*Listeria seeligeri FSL N1-067*	N
E4NFT5	KSE_45840	*Kitasatospora setae (strain ATCC 33774 / DSM 43861 / JCM 3304 / KCC A-0304 / NBRC 14216 / KM-6054) (Streptomyces setae)*	N
E5WJ43	HMPREF1013_02467	*Bacillus sp. 2_A_57_CT2*	N
E5Y1N9	HMPREF0179_00110	*Bilophila wadsworthia 3_1_6*	N
E6QNG4	CARN6_2294	*mine drainage metagenome*	N
E6TZP4	Bcell_3107	*Bacillus cellulosilyticus (strain ATCC 21833 / DSM 2522 / FERM P-1141 / JCM 9156 / N-4)*	N
E6U113	Bcell_2063	*Bacillus cellulosilyticus (strain ATCC 21833 / DSM 2522 / FERM P-1141 / JCM 9156 / N-4)*	N
E6VA14	Varpa_3212	*Variovorax paradoxus (strain EPS)*	N
E7GSI6	HMPREF9474_03881	*[Clostridium] symbiosum WAL-14163*	N
E8WZ00	AciX9_2892	*Granulicella tundricola (strain ATCC BAA-1859 / DSM 23138 / MP5ACTX9)*	N
E9USG8	NBCG_01687	*Nocardioidaceae bacterium Broad-1*	N
F0EFX9	HMPREF9087_0076	*Enterococcus casseliflavus ATCC 12755*	N
F0EJB5	HMPREF9087_1553	*Enterococcus casseliflavus ATCC 12755*	N
F0EPR5	HMPREF9087_3407	*Enterococcus casseliflavus ATCC 12755*	N
F0RXB1	SpiBuddy_0117	*Sphaerochaeta globosa (strain ATCC BAA-1886 / DSM 22777 / Buddy) (Spirochaeta sp. (strain Buddy))*	N
F0SZ73	Sgly_2922	*Syntrophobotulus glycolicus (strain DSM 8271 / FlGlyR)*	N
F2F2E2	SSIL_0971	*Solibacillus silvestris (strain StLB046) (Bacillus silvestris)*	N
F2M179	LAB52_07155	*Lactobacillus amylovorus (strain GRL 1118)*	N
F3LIZ8	IMCC1989_1671	*gamma proteobacterium IMCC1989*	N
F3MTT0	AAULR_17209	*Lactobacillus rhamnosus MTCC 5462*	N
F3S3A2	SXCC_00522	*Gluconacetobacter sp. SXCC-1*	N
F4AAH9	CbC4_1639	*Clostridium botulinum BKT015925*	N
F4BN82	yhcK CAR_c18940	*Carnobacterium sp. (strain 17-4)*	N
F4FH21	VAB18032_07575	*Verrucosispora maris (strain AB-18-032)*	N
F5JFM0	AGRO_3972	*Agrobacterium sp. ATCC 31749*	N
F5LAL3	CathTA2_2948	*Caldalkalibacillus thermarum TA2.A1*	N
F5LCV7	HMPREF9413_3556	*Paenibacillus sp. HGF7*	N
F6B3T5	Desca_2416	*Desulfotomaculum carboxydivorans (strain DSM 14880 / VKM B-2319 / CO-1-SRB)*	N
F6CN01	Desku_3271	*Desulfotomaculum kuznetsovii (strain DSM 6115 / VKM B-1805 / 17)*	N
F7QUH5	LSGJ_00968	*Lactobacillus salivarius GJ-24*	N

F7S1E1	A28LD_2356	*Idiomarina sp. A28L*	N
F7UXQ1	EGYY_28350	*Eggerthella sp. (strain YY7918)*	N
F8CDF6	LILAB_26855	*Myxococcus fulvus (strain ATCC BAA-855 / HW-1)*	N
F8FBD9	KNP414_03968	*Paenibacillus mucilaginosus (strain KNP414)*	N
F8I0J4	WKK_01730	*Weissella koreensis (strain KACC 15510)*	N
F8KDS1	LRATCC53608_0991	*Lactobacillus reuteri ATCC 53608*	N
F9DPS8	HMPREF9372_0808	*Sporosarcina newyorkensis 2681*	N
F9S3C5	VII00023_19474	*Vibrio ichthyoenteri ATCC 700023*	N
F9UBV0	ThimaDRAFT_2402	*Thiocapsa marina 5811*	N
G0EP50	Bint_1301	*Brachyspira intermedia (strain ATCC 51140 / PWS/A) (Serpulina intermedia)*	N
G0VQR3	MELS_1543	*Megasphaera elsdenii DSM 20460*	N
G1V667	HMPREF0178_03014	*Bilophila sp. 4_1_30*	N
G2DZG9	ThidrDRAFT_1432	*Thiorhodococcus drewsii AZ1*	N
G2LDT7	Cabther_A2208	*Chloracidobacterium thermophilum (strain B)*	N
G2LE99	Cabther_A0561	*Chloracidobacterium thermophilum (strain B)*	N
G2MXY0	Thewi_2388	*Thermoanaerobacter wiegelii Rt8.B1*	N
G2ZC65	LIV_1891	*Listeria ivanovii (strain ATCC BAA-678 / PAM 55)*	N
G2ZC66	LIV_1892	*Listeria ivanovii (strain ATCC BAA-678 / PAM 55)*	N
G3J2F6	Mettu_3032	*Methylobacter tundripaludum SV96*	N
G4L0E2	OBV_17720	*Oscillibacter valericigenes (strain DSM 18026 / NBRC 101213 / Sjm18-20)*	N
G4Q567	Acin_0817	*Acidaminococcus intestini (strain RyC-MR95)*	N
G5FI24	HMPREF1020_04120	*Clostridium sp. 7_3_54FAA*	N
G5HCX5	HMPREF9469_00437	*[Clostridium] citroniae WAL-17108*	N
G6B9S8	HMPREF1122_02608	*Peptoclostridium difficile 002-P50-2011*	N
G6BFS5	HMPREF1123_00856	*Peptoclostridium difficile 050-P50-2011*	N
G6FMV2	FJSC11DRAFT_0199	*Fischerella sp. JSC-11*	N
G6XN69	ATCR1_00425	*Agrobacterium tumefaciens CCNWGS0286*	N
G7M2F7	CDLVIII_2446	*Clostridium sp. DL-VIII*	N
G7RV34	PUUH_pUUH2392p0067	*Klebsiella pneumoniae*	N
G7VXU3	HPL003_15520	*Paenibacillus terrae (strain HPL-003)*	N
G8PE99	PECL_22	*Pediococcus claussenii (strain ATCC BAA-344 / DSM 14800 / JCM 18046 / KCTC 3811 / P06)*	N
G8QI44	Dsui_3113	*Azospira oryzae (strain ATCC BAA-33 / DSM 13638 / PS) (Dechlorosoma suillum)*	N
G8QIN9	Dsui_3167	*Azospira oryzae (strain ATCC BAA-33 / DSM 13638 / PS) (Dechlorosoma suillum)*	N
G8QR99	SpiGrapes_0926	*Sphaerochaeta pleomorpha (strain ATCC BAA-1885 / DSM 22778 / Grapes)*	N
G9X349	HMPREF9629_00806	*Peptostreptococcaceae bacterium ACC19a*	N
G9XC84	HMPREF9628_00292	*Peptostreptococcaceae bacterium CM5*	N
H1G8Q6	HMPREF0557_00376	*Listeria innocua ATCC 33091*	N
H1G8Q7	HMPREF0557_00377	*Listeria innocua ATCC 33091*	N

H1LGS0	HMPREF9104_01798	*Lactobacillus kisonensis F0435*	N
H1WNM7	LEUCOC10_01345	*Leuconostoc citreum LBAE C10*	N
H2J665	Marpi_0688	*Marinitoga piezophila (strain DSM 14283 / JCM 11233 / KA3)*	N
H5UVE1	MOPEL_132_00660	*Mobilicoccus pelagius NBRC 104925*	N
H6CGY3	WG8_1543	*Paenibacillus sp. Aloe-11*	N
H6NB78	PM3016_5343	*Paenibacillus mucilaginosus 3016*	N
H7F339	KKC_02784	*Listeria fleischmannii subsp. coloradonensis*	N
H7F340	KKC_02789	*Listeria fleischmannii subsp. coloradonensis*	N
H8FVX9	PHAMO_40078	*Phaeospirillum molischianum DSM 120*	N
H8N0H1	pleD3 COCOR_04267	*Corallococcus coralloides (strain ATCC 25202 / DSM 2259 / NBRC 100086 / M2) (Myxococcus coralloides)*	N
H9UHF0	Spiaf_0851	*Spirochaeta africana (strain ATCC 700263 / DSM 8902 / Z-7692)*	N
I0BPW9	B2K_27610	*Paenibacillus mucilaginosus K02*	N
I0IKW4	LFE_0186	*Leptospirillum ferrooxidans (strain C2-3)*	N
I0IQK6	LFE_1877	*Leptospirillum ferrooxidans (strain C2-3)*	N
I0JPE6	HBHAL_3671	*Halobacillus halophilus (strain ATCC 35676 / DSM 2266 / JCM 20832 / NBRC 102448/ NCIMB 2269) (Sporosarcina halophila)*	N
I0X6Y9	MSI_21000	*Treponema sp. JC4*	N
I0X855	MSI_16880	*Treponema sp. JC4*	N
I0XA21	MSI_9180	*Treponema sp. JC4*	N
I1B2E0	C357_01298	*Citreicella sp. 357*	N
Q03VX4	LEUM_1556	*Leuconostoc mesenteroides subsp. mesenteroides (strain ATCC 8293 / NCDO 523)*	N
Q04DU8	OEOE_1517	*Oenococcus oeni (strain ATCC BAA-331 / PSU-1)*	N
Q08T21	STAUR_4235 STIAU_6437	*Stigmatella aurantiaca (strain DW4/3-1)*	N
Q18BU0	CD630_14190	*Peptoclostridium difficile (strain 630) (Clostridium difficile)*	N
Q1D603	MXAN_3735	*Myxococcus xanthus (strain DK 1622)*	N
Q1JW04	Dace_0162	*Desulfuromonas acetoxidans DSM 684*	N
Q1WTG6	LSL_1024	*Lactobacillus salivarius (strain UCC118)*	N
Q221T2	Rfer_0469	*Rhodoferax ferrireducens (strain ATCC BAA-621 / DSM 15236 / T118) (Albidiferax ferrireducens)*	N
Q2B6K1	B14911_06261	*Bacillus sp. NRRL B-14911*	N
Q2RLY1	Moth_0223	*Moorella thermoacetica (strain ATCC 39073)*	N
Q2VZS3	amb4098	*Magnetospirillum magneticum (strain AMB-1 / ATCC 700264)*	N
Q3MFD3	Ava_0679	*Anabaena variabilis (strain ATCC 29413 / PCC 7937)*	N
Q5FJ86	LBA1413	*Lactobacillus acidophilus (strain ATCC 700396 / NCK56 / N2 / NCFM)*	N
Q6ALR6	DP1980	*Desulfotalea psychrophila (strain LSv54 / DSM 12343)*	N
Q7CZY1	Atu1119	*Agrobacterium fabrum (strain C58 / ATCC 33970) (Agrobacterium tumefaciens (strain C58))*	N
Q8Y5Z1	lmo1912	*Listeria monocytogenes serovar 1/2a (strain ATCC BAA-679 / EGD-e)*	N
Q8Y5Z2	lmo1911	*Listeria monocytogenes serovar 1/2a (strain ATCC BAA-679 / EGD-e)*	N
Q8YMN7	all4896	*Nostoc sp. (strain PCC 7120 / UTEX 2576)*	N
Q8YPG9	all4225	*Nostoc sp. (strain PCC 7120 / UTEX 2576)*	N

Q92A96	lin2026	*Listeria innocua serovar 6a (strain CLIP 11262)*	N
Q92A97	lin2025	*Listeria innocua serovar 6a (strain CLIP 11262)*	N
Q9K8H0	BH3036	*Bacillus halodurans (strain ATCC BAA-125 / DSM 18197 / FERM 7344 / JCM 9153 / C-125)*	N
A0YP32	L8106_11277	*Lyngbya sp. (strain PCC 8106) (Lyngbya aestuarii (strain CCY9616))*	E
A5CYJ5	PTH_2757	*Pelotomaculum thermopropionicum (strain DSM 13744 / JCM 10971 / SI)*	E
D8IYM5	Hsero_2714	*Herbaspirillum seropedicae (strain SmR1)*	E
Q2BMC3	MED92_12931	*Neptuniibacter caesariensis*	E

Table S2. List of genes tested for Hypr activity. Genes codon-optimized for *Escherichia coli* K12 strains have an asterisk next to the gene. All codon-optimized genes were ordered from IDT.

Gene	UniProt ID	Nucleotide Sequence(5'→3')
GSU1658	Q74CL4	ATGGAACGGATTCTCGTTGTCGAAGATGACCGTTTTTTTTCGTCAGATGTATGTTGATCTCCTGAAAGAGGAGGG ATACGAGGTCGATACCGTGGCATCGGGCACCGAGGGGTTGAAGCGGCTTGAGAAGCAAGAATACCACCTCGT CATTACCGACCTGGTCATGCCCGGAATGAGCGGTATCGAGGTGTTGTCCCGCGTCAAGCAGAAAGCTCCGAA CGTCGATGTCATCCTCGTCACCGGTCACGCCAACCTCGAATCGGCCGTCTATGCCCTCAAGAATGGTGCCCGC GATTATATTCTCAAACCGTTCAACCATGATGAATTCAAGCACACCGTGGCACTTTGCTTTGAGCAGCGGAGGCT TATCAACGAAAACTACGAGCTCAAGGAGCTGCTGAATCTTTTTCAAGTTGGGCAGAACATAGCCAACTGTATCG ACTTGGAACGGCTCTCTGCGGTTGTGGTCGATGCTTTCTGCAAGGAGGTCGGAGTTTCACGCGCTATCGGCCT CTTTCCCGAAAAGAGCGAACCCCACGCCCTCAAGGAGCTGAGGGGGCTTGAGCCTGAAGTTGCAGCCGCTCT TGCCGAAAAAGCTCTTACCCTTTGCAGTGACGCCGCGGAGACGGCAGGGGGCTTTCGACGGCTCGACGGTTC CCATTTTTCCGATGGTCTCCTGCGAACTGCGGGGATTAATGGCGCCCTTGTGGTTAGCATCCGCCAGCGTACG CTCCTGCAGGGAGTGCTTCTGCTGGTCAATGACCAGGGCAAGCCGTTCCCTGCCGTGTTCAAACATAAAAGCA TCCAGTTTTTGCTGGAGCAGGCATCGCTTGCCTTCGACAACGCCCTGCGTTACTCCAGCGCCCGCGACATGCT CTATGTTGACGAACTCACGGGACTCTTCAACTACCGTTACCTTGACATCTCGCTGGACCGGGAGTTGAAGCGG GCTGACCGATTCGGCTCGGTAGTTTCCATGATCTTCATCGACATGGACCACTTCAAGGGAGTCAACGACACCC ACGGCCATCTTTTTGGGAGCCAGGTCCTCCATGAAGTAGGTCAATTGCTCAAGAAGTCGGTCCGTGAGGTCGA TGTAATCATTCGCTACGGTGGCGACGAGTTCACCATAATTCTGGTGGAAACCGGTGAAAAGGGCGCTGCAACC GTGGCTGAAAGGATTCGTCGCTCCATCGAGGACCACCACTTTCTGGCCTCTGAAGGGCTCGATGTCCGGCTCA CCGCAAGTCTCGGCTACGCCTGTTATCCCCTTGACACCCAGTCCAAAATGGAACTTCTCGAACTGGCGGACAA AGCCATGTATAGGGGCAAGGAAGAGGGCAAAAACCGTGTATTCCGGGCAACGGCAATCCGTTGA
Mxan2643	Q1D911	ATGAATCCCGCGGACCTCCTGTCGGCCATGAAGCGGACAGTGGAGCAGTTGGCCGCCTTCAATGAGATGGCG AAGGCCCTGACGTCCACGCTCGAGCTCCGCGAGGTGCTGGCGCTGGTGATGCAGAAGGTCAGCAGCCTGCT GCTGCCTCGCAACTGGTCGCTCATCCTCCAGGACGAGCGCACCGGAAAGCTCTACTTCGAAATCGCGGTGGG TGACGGCGCGGACGTGCTCAAGGGCCTCCAGCTCAACCCGGGCGAGGGCATTGCCGGCGCCGTCTTCACGT CCGGCGCGGCGCGGCTCGTCCATGACGTGGGTGGGGACCCCAGCTTCTCGCCACGCTTCGATGAAGCCTCC GCCTTCCACACCCGCTCCATCCTCGCGGTGCCGCTGCTGGCCCGGGGCCGGGTCCTGGGCATCATCGAACT GGTGAACGGGCCCATGGACCCCCCCTTCACCAACGAGGACCTCACCATTCTCACCGCCATCGCGGACTACGC GGCCATCGCGATTGAGAACGCGCGCAACTTCCGGCGGGTGCAGGAGTTGACGATTACGGACGAGCACACCG GCTGCTACAACGCCCGGCACCTGCGCGCCTTGCTGGACCAGGAGGTGAAGCGCTCGGAGCGCTTCAGCCAC CCGCTGTCGCTCGTCTTCCTGGACCTGGACCACTTCAAGAGCATCAACGACACCCATGGGCACCTGGTGGGT AGCGCCACCTTGAAGGAAGTGGGGGACCTGCTGATGACCCTGGGCCGGCAGAACCTGGACGCCGTCTTCCG CTACGGCGGCGACGAGTTCGCCATGTTGCTGGTGGAGACGGACCCGGAGGGCGCGGCCGTCATCGGCCAGC GCGTCTGCGAGGCCTTTCGGGGGCGGGGCTTCCTCCTGGAGCAGGGCCTGGACGTGCGCCTCACCGCCAGC GTGGGCGTGGCCACCTACCCGGACCATGCCTCGTCCGCGCTGGACCTCATCCGCGCGGCGGACTTCGCCAT GTACGCGGCCAAGGCCCGGGGCCGGGACGCGCTCTGCATCGCCGAGCCCATTGCTCCGAACGGCGGCACA GGCTCCCACGAGTTCCCGGAGCGGTAG
Mxan4463	Q1D3Y9	ATGGCGCGAATCCTCCTCGTCGACGACGAAAAGATCGCCCGCACCCTGTACGGCGACTACCTCACCGCCGTG GGACACGCCGTCACGGCGGTGGGCACGCTACAAGAGGCAAAGGAAGCACTCGCAGGCGACCGTTTCGACGC GGTGGTGACGGACCTCATCCTCCCCGGTGGTGACGGCATGGAGGTCCTGCGGCACGTGCGGGAACATCACC CGGGCGTGGAGGTGGTGGTCATCACTGGCCTGGAGAAGGTGGACCCCGCCGTGCGCGCCATCAAGAGCGGC GCCGCGGAGTACCTCGTCAAGCCGGTGGCCCCGGAGGCCCTGCAGCACGCCGTGCGCCGAGCGCTCACCAC GCGCGACCTGATGCAGGAGAACGCGTCGCTGCGCCGCCATGTGGCCATGTTGGAGGCGGGGCAACGCATCG CCACCACCCTGGACCGCGAGAAGCTGGCCTCGGCCACCGCCAGCGCGCTGCAGAGCATGGCCTCCGCCAGC GCCGTGGTCCTGCTGGAGCGCGACTCTGCCTTCGCGCTGCGGCGCCACGGCACCAGCGGCCTGTCCACCGC GCTGGAAGAGCCGCTCATCGCCGAGCTCATCGAACGCCTGACGAACGAACGCGGTCCGCGCGAGCTGGACG GCATGGACGCGCCCTTTCCTCGCGCAATCTCCTTCCCCGCGCTGGAGGGTGACGCCGTGCTGGGACACGCG GTGCTCTTCTTCGGCGGCACGGGCGCGGAGTGGGCGGGCGAGACGGCCAGCTTCCTGGTTCGCAACTGGGC GCTCGCGCTGCGCAACCTCGGCCGCTTCGCCGCGGTGGAGGACCTGGCGTACGTCGACGACCTCACGCGCC TGTTCAACACCCGCTACCTGCACCTGGTGGTGGACCGCGAGGTCCAGGACGCGCTCCAGTCACAGCGCACCT TCAGCCTGCTGTTCCTGGACCTGGACCACTTCAAGTCCATCAACGATACCCATGGCCACCTCGTGGGCTCCAA GGTGCTGGTGGAGGCGGCGCGCGTGGTGAAGGGCTGCGTGAGAGACCACGACGTCGTCGCGCGCTACGGC GGAGACGAATACGTGGTGGTGCTGCGCAACACCGACTCCGGCGGCGCGCTCAAGGTGGCCGAGCGCATCCG ACGCACCATGGAGACGCACAACTTCCTGGCGCGCGAAGGCCTGTCGCTCAAGCTCACCACGTGTATCGGCGT GGCCAGCTTCCCCGAGCACGCCCAGGACAAGGCCACGCTGTTGGACCTGTCGGACCGGGCCATGTACCGCG GCAAGCGGGGCTCGCGGAACGTCGTCTACATGGCGGCGAAGGACCTGGAGGCCCCACCGGCCGAGCGCCG GCAGGCCCACTCCGCGTCCTGA

Ddes1475	B9J0V0	ATGCTGAACAAGTCAAGCATCATACCAGAACATATACAGCTCGAATCACACGACCCTGTCTGGGAGTGGCACA CGACATCCGACAGGCTTTTCATGAGCGTAGGCGCCCTTGCCCAGCTACGCATGGACGGCAAACCGCCGCGCA GCATGAAAGATTATCTGGAGCACTGCCCCCTCGAAAGCCTGGCTCCCCTTCTTGAATCTATGGAAAAAGCGCT CAACGGCTCCCACGGGCCGCACCTTGAAGTGTTTTATCCTTTTGACAGTTTTCTGGTACGGTCTCAGATACTGG TCTTGCGGCGCGACGTTTTCGGTCGCGGAACCCTGGTAACAGGCTGCAACGTGGCTATGGACAGACAAAGGC TTGCACCCACTGCTGCCGCCGCCCCCGTGCCGGCACCCCAGCCCCCGCCCCGAAGCCTGGCCGAAGCCGCC GTTCCTTCCACGGCCCGCAGCGACGCCAGCCGCCTCATGCTGGCCCTCAACGCCGCCAGCGATGGCCTGTG GGACTGGGACCCCAGCACCAATGCCATTTATTTCAGTCCCCGCTACCTCGACATGCTTGGCTACACCAGCGAA GAATTCCCCCCCCTGTCCACATCATGGACCAGCAAGGTACACCCCGACGATTACGACAACATCGTTCCCATGC AGATTGAATTCATCAACAACCCCAAAATGGGCGACAGCTTTGAATGCACCTACCGGATGCAGCGCGGCGACGG CACCTGGGCATGGATTCTCAGCCGGGGCTATGTGACTCACCGCGACGCAAGCGGCAAGGCCATCCGCGTTGT GGGCCTGCACACAGACGTCAGCGCGAGCCAGGGCGACAGGGCACGGCTTGAAGAGCTGGTGCGTAACGACG CCCTTACCGGGCTGCGCAGCCGCACCTATTATGGAATGACTGTCGACAAGCTGGAACAGCAGCAGATGCGGC CCGTCAGCATCATCATCGCCGACATGGACGGACTCAAAATGGTCAACGACCATGTAGGCCATACCGAAGGCAG CGAAATGCTCTGCCAGGCAGCCATCATACTGCGCGGCAGTCTCAATGCCACCGACTGTATCGCCCGCATGGG CGGCGATGAATTTGCCGCCATTGTGCCGGGTTGCGCCAAGGAAGACCTTGAGGCGCTCATCCAGCGGGTCAG AGACGCTTTTGATGCCTATAATGCCGACCCGGACCATGTGCCGACACACATGTCTGTGGGCGGAGCATGCGCT GACGACATGAACACCACCCTGGCCCAGGCCCTGTCGGAGGCGGATCGCAACATGCTGGCCGTCAAGCACGAA AGCAGCCCAAAGTGGCGCCTGCGCATAAAAAACTGGATAGAAAACCGGACCGGCAAAACAATTCAGCTTGAAG ACAGCCGCTACAGGATGTCCCCCACGCACGACGACTCTTGA
Bd0367 WT *	Q6MQU2	ATGTCGCGCGCCGAAGTGACGCTCGTATGTAAAATGAGCTTTGAAGTATCGCCGAAGCAACCAAAGAGCCGCC GTATCCTGGTTATCGACGACGATAAGGACTCATTAGAAATTTTATTGGAACCCCTGCGCTGGGAAGGTTATGAC GCGCGTGGCGTGACTACCGAAGCGGAGGCGCATAAATTAATCGAGTCATGGATTCCGCATATCGTGATCCTGG ATTGGATGGCCCCGTCAATGGCCGGCCTGCGCGTTCTGAAATCCGTACGCGAACGCCTGAGTCATGTCTCGT GTGTCTTTGTATCGGAAAATTCTTCCACAGAGGCTATTATTGAGGCTTTGGATTCGGGCGCCGACGATTATATT GTAAAGCCATTCGTGCCATTAGAGTTGTTAGCACGCATCCGCTCTCAACTGCGCATCCGCGATCTGCACGAGC AGCTGCTGTTTGCCAACGAAAAATTAAAGGAACTGGTTGATACCGACGATTTAACCGGTTTATATAATATGCGTA GCTTATACCAGCGTCTGGATTTTGAAATGGAACGTGGCCGCCGCTTCCACCGCGACGTGTGCGTGGTCATGAT GGACATGGACTATTTCAAAACCGTGAATGATGGACACGACCACTTATTCGGGAGTTATGTGCTGAGCGAAGTT GGTAAAATCATTCGCGCCAACACTCGTAACATCGATATCCCGGCACGTTATGGGGGGGATGAGTTTCTGATGG TCCTGACCGAAACTAATCATGCGGGCGCTATGTATTTTTGCGAGCGCCTGCGCGAAAATATTGAAAAAACAACC TTTCGTAACGGCGAGGACAGCATGAAATTGACAGCCTCACTGGGCTTTGCGATCACCATCCCCGGCGAAAACA TCAGCGCGCGTGAACTGGTTCGCCGCGCCGACCACGCTCTGTATCAGGCAAAACGCGCCGGGCGCAACCAG GTGGCGCATTACAAACCGGAGAGCGCGCCCGTAGTTGAGATCAAGTCGGCAGTGCACAAACGCCGTAAAGCC GCCGGTTAA
CabtherA_1065 WT *	G2LH77	ATGAACCTTAAACTGGGCGCCATCTTACGTCCGGTTAATAGCCTCAACCAAACACAGAAACTGCAAGCCAACCC ACTTGCCCGCCCGCGTCAAGCGCGTCCGGCACTGGTGCACATGCGTGGAGATTATCTGGGCTCAAGCTTTCG TATTGAACATGCCATTACGCGCATTGGACGCGGATCAGACGCAGAGTTACGTTTAGAAAATGATGACGAAGCA AGTCGCTTACACGCCCGTATTGAGCGCCTGGAAACACCTACAGGGCATTTCCAATATTGGTTGACCGATCTGC GCTCTACCAACGGGACCCAACTGAATGGTATTCCGCTGGTGCCGGGCGAGGCAGTGTTGCTGCATGATGGCG ATAAATTTAGTATCGGCCGTCATATCCTCAAGTTCACTTTTTTAGACGATATTGATGAGGAGTTTCATCGTCGTA TCACCGAACTCATCACTCATGATGACTTAACCGGTCTGCTGAACCGCAAATCGTTCATCCTGGAAATGCAGCGT GAGATGGCCCGTAGTAACCGCTACGGTCACCCATTTGGCCTGCTGATGATGGATATTGATCATTTTAAGCGTGT CAATGATACCTATGGTCACCTGGTTGGTTCTCAGGTATTACGCGAGGTGGCTACCGTTATCCGCGAAACACTG CGTGACTCTGACATTGCAGGTCGTTATGGTGGAGAAGAATATATTGCCCTCTTACCAGAAACCGATCGCCTGC GCGCACACGAAGCGGCCGAGCGCATTCGTCAAGCAATCGAACGCACCCCGTTCACAGCAAGCCTCAACGTGC CGCACCACAAGTTACGTCTGACCATTAGTATTGGGATCGCGAGTTATCCGGGGGACGCAGCCCAAATTAATGA TCTGATCCAGCGCGCGGATGAAGCGATGTATGAAGCAAAACGCCGCGGTCGTAATCTGGTGCAGACGACGGG CCAATCGGCGGCCAATCGCGCGACCCCCCCTTCACTTCCATTGCCGCCGCCGTCTGGAGATGACAGTCCCAC GGAGCATCTGACCGTGGAGCAGCCGCAGCCTGTCAAACCTTAG
DEFDS_0689 R248A *	D3PC46	ATGTATGAAAGCCTGAAACGCAACATCTTCGTCATTCTGACAAGCATTCTCCTTATTTATGAAACCTATAACAAA ACAAATGAGAATTTGCTGCTGCTGACTTCTTTACTGCTCACGTGCTATATTGCTGCAACGTTGATCAAAAAGGTT GAACTGGATGAAGTACTGTTTGCTCTGTTCGTGATTTTAATCGGTTATCTGAGCATCGCAAACCGTGAGTTCATT TATTTTCAAATTCTCGCGATTACATTTTTGGTATTTGATTCGAAGTTCTACGTGATTAAAGTGATCCTGGCGATTC TGTTGATTCTCTTCGATTTATTCTACTTGAACATTTCGATCCTTTCCACGTTTAGCCTGATGATTCTCTATTCCTT ATTCTTTTCTATCTTCATCAAATTGCTGATTGATCGTTTGGAAGAAGAAATCGACGAACTGTCCATTACGGACGA CCTCACGGGTCTGCTGAACCAAAAAGGATTCCTGAAAAAGTTTGAGGAAGAATATTATCGTAGCGTTCGCTACA AGAAAAATTTTACCGTTATCATGTTGGATAGCGATGATCTGAAGAAAGTTAATGACACTTATGGGCACAAATACG GGACCAAAGTTATTCTGTTCATCGCGGATGAAATTAAGAAGAACATTCGCCGTACCGACTTTGCTTGCCGCTAC GGCGGTGACGAGTTTATGATCTGTCTGGTTGAAACACCTATCAACAACGGCAAAATTTTCGCGGAAGCGCTGA AAAACAACATTGCAATGAAACCGGTATTTACCGATAAAGGCCGTGGTTTCAATGTGACAGTGTCGGTGGGGGTT GTTGGTTATCCGCACACAAGCGAAAAGTCGTTCGAGCTGCTTGATCTGGTTGACAAAGCGCTGTACGAAGCAA AAAACAAAGGCAAAAATCGCGTTGAGATCCTGACCAAAAATTCTTCCTTA
Calni_1629 R268A *	E4TFG3	ATGATTGATAACAAGATTAAACACTTTCAGTATAAGATTGCAGAAGTCTTATATCTCTTTGCGTTTACTATCATCA TCGCCTCCATTAAATTTCTTGATTCTACCAGTAACAAAGCAAACTACGCGATCTTAGTGTTTTTCCTGATCTTCAT CATCCTGAAATTTAGCATTGACGATAATCTGTTCAGCTCAAAAATCCTGTCTTATTATCTGCTGTTCCAGTCACA AAACATTTTTGCCGCGTTCATTAACGGGTCAACTCCAAATCTGTTAATCTTTATGTCGATGCTGGGCTTGCTGAT TTTTAGCATTGTGCTGTATGATAAAAAATATCTCGTCGTCCACTTTATTGTGACCGGTATCCTTGCGTTCGTTTTC TTCACAACATTTGATTCGAAAGAGAGTTTTGTGTTCTTCATCTCACTTCCTTTCATCTTTATTATTTCGTTAAACTT TAATAAGATCTACATTACTACCCGTAACCTGATCACCGAGTTATCTATTACTGATGAGATGACCGGCCTCCTCAA CCAGAGCGGGTTTATGAAGAAGATCGAAGAAGAATTTTATCGCAGCCAGCGTTACCAGAAAACGTTTTCAGTTC TGATGATCGATTCAGACAATTTAAAACTGATTAATGATACTTATGGCCATAAATATGGGAGCATCGTCATCAAAT CCATTGCGGAAGTCATTAAGACTAACATTCGCCGTACAGACTTCGCGGCGCGCTATGGCGGAGATGAATTCAT TCTGTGCCTGGTGGAAACTGATTTAGACGGGGCTCTGGAAGTGGCAGAGGCGATCCGTAAGCAGTTCGAGCT

		GAAAAGCTTCTTTACCAAAGATGAGAAGAAGTTCACAATCACGATTAGTATCGGAGTAAGCAACTATCCTAAAA GCGGCGATTCTCTGATGGATGTGATTGAACTTGCGGACAAGGCCATGTACCATAGCAAGAACAGCGGTAAGAA CAAGACGAGTTTCCTGCTGAAGAAC
ACP_2467 WT *	C1F1G0	ATGGACGCCCACACTATCGTCAGTCTGCCGCCCACTTGGAACCAAGGGATGTCTGCCGAAGCGCGCAATCAG AACTGGAAGGATTTGGTGGTCTTCCATAACTTAGCACGCGCTCTGACCTCCTCCCTGGAGCTTGATTCGGTGC TGCATGCAATCATGGAACAGATGCGTCAATTCTTCGAACCGGAGACCTGGTCGTTGCTTATCCTGGATGAAACA ACCCAGGAATTGTATTACGCGGTTGCAGTCGGACAGTCCGAAGCGGCTCTGCGTAATGTGCGTGTGCCGCTG GGAGAAGGCATGGCGGGTTGGGTGGCCCAACATGGCGAGTCCCTCATCGTGCCGGATCTGGAACAAGATCC GCGCTTCGCCGCGACCTCGGATGCCCGCACCCCAATGCGTAGCGCGATCTGCATGCCACTGCTCTCACGCCA ACGCACCCTGGGCGTGATTCAACTGTTTAACTGCCGCCTGGAAAGCATGACCGAATACACCATTAGCTTCCTG CATATCCTGTGCGACTATGCGGCGATTGCAATCGAAAATGCACGTGCAGTGGAGAAAATCCAGGCCCTGACGA TTACGGATGACTGTACCGGCTTATACAACCAACGTCATCTCCAGCAGAAGATCGAAGAAGAGGTCACCCGTGC TCGTCGTCACCACCATCCATTCTCAGTCATCTTTCTGGATCTTGACCATTTCAAACAAATCAATGACCAACACGG GCACTTAATCGGGAGCCGCCTTCTGGCGGGTATTGGCCAGTGCCTCCGCCTGCACATTCGCCCGGGAGACCA TGCCTTTCGCTATGGTGGCGATGAATTTATCTTACTGCTTCCAGAAACCACAAAAGCGGAAGCCGAGCAGATTG CGCGCAACCTGCGTCAAAAACTGCGTAGCCATGTCTTCGAGATGGGCAGCGATCTCCGTTTGCAGGTTTCGGC CTCATTCGGTGTCGCCAGTTTTCCGGAGGATGGCCGTACGGGCCATCAGATCATTCGTATGGCCGATGCAATG ATGTATTTGGTGAAAGGCTCTACGCGCGACGACGTGGCAGTTGCGGACCGTAATACCGAACTGCTCCGCAACT CA

Table S3. List of primer sequences. Restriction sites are denoted by an underline.

#	**Nucleotide Sequence (5'→3')**	**Purpose**
1	GAG AGA CAT ATG GAT TTC ACA AAA ATC TCC G	For Primer for GSU0474 into pCOLA
2	GAG AGA CTC GAG TTA CGC TGT AAC GCG GCA G	Rev Primer for GSU0474 into pCOLA
3	GAG AGA CAT ATG CCC TTG CGC AAG AA	For Primer for GSU0537 into pCOLA
4	GAG AGA CTC GAG TTA CGG TTG AAG TGA CCT GAG C	Rev Primer for GSU0537 into pCOLA
5	GAG AGA CAT ATG TCC GGC GAC ATT CTG	For Primer for GSU0542 into pCOLA
6	GAG AGA CTC GAG CTA TTT CAC GAC AAC CTT GTT CTT G	Rev Primer for GSU0542 into pCOLA
7	GAG AGA CAT ATG TCC AGG AAC CAC CTG C	For Primer for GSU0808 into pCOLA
8	GAG AGA AGA TCT CTA ACG GGA AAC GGT GTT GC	Rev Primer for GSU0808 into pCOLA
9	GAG AGA CAT ATG CCC CAT GTG AAC CTG	For Primer for GSU0895 into pCOLA
10	GAG AGA CTC GAG TCA TGG CAG GTT GAG CG	Rev Primer for GSU0895 into pCOLA
11	GAG AGA CAT ATG AAG ATT CGG AGC ACC CT	For Primer for GSU0946 into pCOLA
12	GAG AGA CTC GAG CTA CCC CTC TTC GGC CCT	Rev Primer for GSU0946 into pCOLA
13	GAG AGA CAT ATG TCG GCA GAA AAA GAA CAG AC	For Primer for GSU0952 into pCOLA
14	GAG AGA CTC GAG CTA ACC TTT GAC GGC CTC CAG	Rev Primer for GSU0952 into pCOLA
15	GAG AGA CAT ATG GGC AGG GAG GGC	For Primer for GSU1037 into pCOLA
16	GAG AGA CTC GAG TCA CCT TCC CCG CGC	Rev Primer for GSU1037 into pCOLA
17	GAG AGA CAT ATG AAG CCT GAC ACC ACC TTC	For Primer for GSU1400 into pCOLA
18	GAG AGA CTC GAG CTA TGC GCA GGT GAC GC	Rev Primer for GSU1400 into pCOLA
19	GAG AGA CAT ATG CCG CGA AAG AAG AAA AC	For Primer for GSU1554 into pCOLA
20	GAG AGA CTC GAG TCA GAC GTC GGC GCG	Rev Primer for GSU1554 into pCOLA
21	GAG AGA CAT ATG ACG GAT GAA CAG AGA CAA TG	For Primer for GSU1643 into pCOLA
22	GAG AGA AGA TCT TCA GAG TTG TTC GCT GCA CAC	Rev Primer for GSU1643 into pCOLA
23	GAG AGA CAT ATG CCC CCT CCG CTT C	For Primer for GSU1656 into pCOLA, pET16b
24	GAG AGA CTC GAG TTA TGC AGG TAA TAC GCA GCA TTT TTT A	Rev Primer for GSU1656 into pCOLA, pET16b, pET-MBP
25	GAG AGA CAT ATG GAA CGG ATT CTC GTT GTC	For Primer for GSU1658 into pCOLA, pET24a

26	GAG AGA CTC GAG TCA ACG GAT TGC CGT TGC	Rev Primer for GSU1658 into pCOLA
27	GAG AGA CAT ATG ACA GAT GCC ATT ACG GAT G	For Primer for GSU1671 into pCOLA
28	GAG AGA CTC GAG TCA ATG AAG CTG GAC TCC CTT G	Rev Primer for GSU1671 into pCOLA
29	GAG AGA CAT ATG GAA CTC AGC CCC GAG	For Primer for GSU1870 into pCOLA
30	GAG AGA CTC GAG TCA TGG CTC ATC CTC TCT TCT G	Rev Primer for GSU1870 into pCOLA
31	GAG AGA CAT ATG CGA AAA GAG GGC AAG G	For Primer for GSU1927 into pCOLA
32	GAG AGA AGA TCT CTA GCG CGA CCG AGC G	Rev Primer for GSU1927 into pCOLA
33	GAG AGA CAT ATGACCCTCGCCGAAG	For Primer for GSU1937 into pCOLA
34	GAG AGA CTC GAG TCAGGGGTGCATTGACAG	Rev Primer for GSU1937 into pCOLA
35	GAG AGA CAT ATG GCC CAG ACT TCA TTG AC	For Primer for GSU2016 into pCOLA
36	GAG AGA AGA TCT TTA CGG GGC TGA GTT CAG ACT G	Rev Primer for GSU2016 into pCOLA
37	GAG AGA CAT ATG GCG AAT CTC AAG CGA TAT AAT	For Primer for GSU2044 into pCOLA
38	GAG AGA CTC GAG TCA GCA CCA GGT TCC GAA AC	Rev Primer for GSU2044 into pCOLA
39	GAG AGA CAT ATGAGATCTGACCTGAGAATAGCC	For Primer for GSU2062 into pCOLA
40	GAG AGA CTC GAG TCAGTACTTACGTCGGTCGAC	Rev Primer for GSU2062 into pCOLA
41	GAG AGA CAT ATG CGA ATT CTC ATC GCC	For Primer for GSU2313 into pCOLA
42	GAG AGA CTC GAG TCA TGG TGA TCC CGC CTG	Rev Primer for GSU2313 into pCOLA
43	GAG AGA CAT ATG GTT GCG TTC TTC ACA CAG TA	For Primer for GSU2511 into pCOLA
44	GAG AGA CTC GAG TCA TTC CCT CGG CGC	Rev Primer for GSU2511 into pCOLA
45	GAG AGA CAT ATG GCC GAA TCA CGT CC	For Primer for GSU2534 into pCOLA
46	GAG AGA CTC GAG CTA GCA CGG GGA TCC GG	Rev Primer for GSU2534 into pCOLA
47	GAG AGA CAT ATG AAC ACC CTG ACG GCA	For Primer for GSU2632 into pCOLA
48	GAG AGA CTC GAG TCA GGT GCT CAC CTG GTT GC	Rev Primer for GSU2632 into pCOLA
49	GAG AGA CAT ATG ACT GAA TTG ACG GAG TTC GTA G	For Primer for GSU2828 into pCOLA
50	GAG AGA CTC GAG TCA TCC GTT CAC TGC GCC	Rev Primer for GSU2828 into pCOLA
51	GAG AGA CAT ATG CCC AAC AAC GAC AGC	For Primer for GSU2969 into pCOLA
52	GAG AGA CTC GAG TCA GGG TGA CGC GGA C	Rev Primer for GSU2969 into pCOLA
53	GAG AGA CAT ATG ACG CGC CGG C	For Primer for GSU3350 into pCOLA
54	GAG AGA CTC GAG TCA ATC GGT TCC GTC CG	Rev Primer for GSU3350 into pCOLA
55	GAG AGA CAT ATG AGA CGA GCA AGC CTG AAA	For Primer for GSU3356 into pCOLA
56	GAG AGA AGA TCT TCA GGA GGC CGA AAC GG	Rev Primer for GSU3356 into pCOLA
57	GAG AGA CAT ATG GCG ATG ACA GCC CTC	For Primer for GSU3376 into pCOLA
58	GAG AGA CTC GAG TTA TGT CGA GCC TGA CAT GAG CTC	Rev Primer for GSU3376 into pCOLA
59	GAG AGA CTC GAG ACG GAT TGC CGT TGC	Rev Primer for GSU1658 into pET24a
60	CAACCGTGGCTGAAGCCATTCGTCGCTCCATC	For Primer to Quickchange GSU1658 R393A
61	GATGGAGCGACGAATGGCTTCAGCCACGGTTG	Rev Primer to Quickchange GSU1658 R393A
62	CCATCTTTTTGGGGCCCAGGTCCTCCATG	For Primer to Quickchange GSU1658 S347A
63	CATGGAGGACCTGGGCCCCAAAAAGATGG	Rev Primer to Quickchange GSU1658 S347A
64	CGGCCATCTTTTTGGGGATCAGGTCCTCCATGAAG	For Primer to Quickchange GSU1658 S347D
65	CTTCATGGAGGACCTGATCCCCAAAAAGATGGCCG	Rev Primer to Quickchange GSU1658 S347D
66	GGCCATCTTTTTGGGAATCAGGTCCTCCATGAAG	For Primer to Quickchange GSU1658 S347N

67	CTTCATGGAGGACCTGATTCCCAAAAAGATGGCC	Rev Primer to Quickchange GSU1658 S347N
68	GTAATCATTCGCTACCTTGGCGACGAGTTCACC	For Primer to Quickchange GSU1658 G371L
69	GGTGAACTCGTCGCCAAGGTAGCGAATGATTAC	Rev Primer to Quickchange GSU1658 G371L
70	CCACCTCGTCATTACCGCACTGGTCATGCCCGGAATG	For Primer to Quickchange GSU1658 D52A
71	CATTCCGGGCATGACCAGTGCGGTAATGACGAGGTGG	Rev Primer to Quickchange GSU1658 D52A
72	CGTCATTACCGAGCTGGTCATGCCC	For Primer to Quickchange GSU1658 D52E
73	GGGCATGACCAGCTCGGTAATGACG	Rev Primer to Quickchange GSU1658 D52E
74	GAGAGAGGATCCATGGACGCCCACACTATC	For Primer for ACP_2467 into pET-MBP
75	GAGAGACTCGAGTCATGAGTTGCGGAGCAGTTC	Rev Primer for ACP_2467 into pET-MBP
76	AGAGACAT ATGTCGCGCGCCG	For Primer for Bd0367 into pET24a
77	AGAGACTCGAGACCGGCGGCTTTACG	Rev Primer for Bd0367 into pET24a
78	AGAGACATATGAACCTTAAACTGGGCGC	For Primer for Cabther_A1065 into pET24a
79	AGAGACTCGAGAGGTTTGACAGGCTGCG	Rev Primer for Cabther_A1065 into pET24a
80	GAGAGAGGATCCATGATTGATAACAAGATTAAACAC	For Primer for Calni_1629 into pET-MBP
81	AGAGAGCTCGAGTCAGTTCTTCAGCAGGAAACTC	Rev Primer for Calni_1629 into pET-MBP
82	GAGAGAGGATCCATGTATGAAAGCCTGAAACG	For Primer for DEFDS_0689 into pET-MBP
83	AGAGAGCTCGAGTCATAAGGAAGAATTTTTGGTCAGG	Rev Primer for DEFDS_0689 into pET-MBP
84	GAGAGACATATGCTGAACAAGTCAAGCATC	For Primer for Ddes_1475 into pET24a
85	GAGAGACTCGAGAGAGTCGTCGTGCGTGG	Rev Primer for Ddes_1475 into pET24a
86	AGAGACATATGAATCCCGCGGACCTC	For Primer for Mxan_2643 into pET24a
87	AGAGAGCGGCCGCTCCGGGAACTCGTGG	Rev Primer for Mxan_2643 into pET24a
88	AGAGACATATGGCGCGAATCCTCC	For Primer for Mxan_4463 into pET24a
89	AGAGACTCGAGGGACGCGGAGTGGGC	Rev Primer for Mxan_4463 into pET24a
90	GAGAGACATATGAGCGCCCGGATCCTCG	For Primer for ccPleD into pET24a
91	GAGAGACTCGAGTCAGGCGGCCTTGCCG	Rev Primer for ccPleD into pET24a
92	CTGGTCGGTGACGGC	LIC primers for GSU1658-PleD fusion (rev; primes to PleD 293)
93	GCCGTCACCGACCAGCTCACGGGACTCTTCAACTAC	Lic primers for GSU1658-PleD fusion (overhang with PleD NTD; primes to 1658 f 297)
94	CTTCGGTCACGATATCGGCAGTGAGGTGCTGCGCGAGTTC	For primer to Quickchange PleD D344S
95	GAACTCGCGCAGCACCTCACTGCCGATATCGTGACCGAAG	Rev primer to Quickchange PleD D344S
96	GGGCACCAGATGGGAAGCGACCTCCTCAAAATG	Forward primer for Round-the-horn of GSU1400 D195S
97	GAAACTGTCGTTGATCTCCTTGAAG	Reverse primer for Round-the-horn of GSU1400 D195S
98	CATCAGACCGGAAGCGAGGTGCTGTGC	Forward primer for Round-the-horn of GSU2313 D194S
99	ACCGTAACGGTCGTTTACCC	Reverse primer for Round-the-horn of GSU2313 D194S
100	CCACGACGCCGGCAGTGTGCTCCTGATGG	Forward primer for Round-the-horn of GSU2534 D325S
101	CCGAAGACATCGTTCACTCC	Reverse primer for Round-the-horn of GSU2534 D325S
102	CACCTCCGGGGCAGCGAGGTCCTCAG	Forward primer for Round-the-horn of GSU3350 D471S
103	GCCGTGGCAGTCGTTG	Reverse primer for Round-the-horn of GSU3350 D471S
104	GAGAGGATCCATGAATCCCGCGGACCTC	For primer for Mxan_2643 into pET-MBP
105	GAGAGAGTCGACTCATCCGGGAACTCGTGG	Rev primer for Mxan_2643 into pET-MBP
106	GAGAGGATCCATGTCGCGCGCCG	For primer for Bd0367 into pET-MBP

107	GAGAGACTCGAGTCAACCGGCGGCTTTACG	Rev primer for Bd0367 into pET-MBP
108	GCAGGGCGGGGCTTCCTC	For primer to Quickchange Mxan_2643 R292A
109	AAAGGCCTCGCAGACG	Rev primer to Quickchange Mxan_2643 R292A
110	GCGCTGCGCGAAAATATTGAAAAAAC	For primer to Quickchange Bd0367 R260A
111	CTCGCAAAAATACATAGCG	Rev Primer to Quickchange Bd0367 R260A

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