Amino acid divergence in the ligand binding pocket of *Vibrio* LuxR/HapR proteins determines the efficacy of thiophenesulfonamide inhibitors

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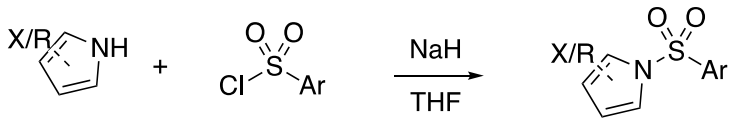
Supplementary Information

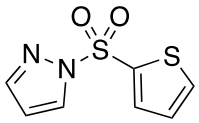
Synthesis of LuxR Inhibitors:

General Methods: ^{1}H NMR spectra were recorded at room temperature on a Varian I400 (400 MHz) or Varian VXR400 (400 MHz) spectrometer. Chemical shifts are reported in ppm from tetramethylsilane with the residual solvent resonance as the internal standard (CHCl3: δ 7.26 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constants (Hz), and integration. ^{13}C NMR spectra were recorded on a Varian I400 (100 MHz) or Varian VXR400 (100 MHz) spectrometer with complete proton decoupling. Chemical shifts are reported in ppm from tetramethylsilane with the solvent resonance as the internal standard (CDCl3: δ 77.16 ppm). High Resolution Mass Spectrometry (HRMS) analysis was obtained using Electron Impact Ionization (EI), Chemical Ionization (CI), Atmospheric Pressure Chemical Ionization (APCI) or Electrospray Ionization (ESI) and reported as m/z (relative intensity). ESI was acquired using a Waters/Micromass LCT Classic (ESI-TOF). Dichloromethane (DCM) and tetrahydrofuran (THF) were purified under a positive pressure of dry argon by passage through two columns of activated alumina. Triethylamine (Et_3N) and diisopropylethylamine (DIPEA) were distilled over CaH_2. All other reagents and solvents were used without purification. All work-up and purification procedures were carried out with reagent grade solvents (purchased from Sigma-Aldrich) in air. Thin-layer chromatography (TLC) was performed on Merck Silica Gel 60 F254 glass plates and visualized with UV and/or standard potassium permanganate, phosphomolybdic acid staining techniques. Standard column chromatography techniques using ZEOprep 60/40-63 µm silica gel was used for purification.

General procedure for synthesis of inhibitors 1, 3, 8, 10: (Nishida, 2009)

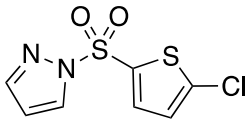


To a solution of amine (6 mmol) in tetrahydrofuran (15 mL) was added sodium hydride (60% in oil, 320 mg, 8 mmol) at room temperature, and the mixture was allowed to stir for 10 min. A solution of sulfonyl chloride (4 mmol) in tetrahydrofuran (5 mL) was added, and the mixture was allowed to stir for an additional 30 min. The reaction mixture was diluted with 20 mL water, and extracted with ethyl acetate (3 x 20 mL). The extract was washed with 20 mL saturated NaCl (brine), dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The resulting crude product mixture was purified via SiO_2 column chromatography in 10:1 Hexanes:EtOAc to give the desired product.



1B

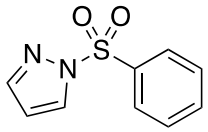
1-(thiophen-2-ylsulfonyl)-1*H*-pyrazole (**1B**): **^{1}H NMR (400 MHz, $CDCl_3$)**: δ 8.08 (d, *J* = 2.7 Hz, 1H), 7.82 (dd, *J* = 3.9, 1.4 Hz, 1H), 7.74 (d, *J* = 1.6 Hz, 1H), 7.71 (dd, *J* = 5.0, 1.4 Hz, 1H), 7.09 (dd, *J* = 5.0, 3.9 Hz, 1H), 6.39 (dd, *J* = 2.8, 1.6 Hz, 1H). **^{13}C NMR (101 MHz, $CDCl_3$):** δ 145.44, 136.73, 135.51, 135.49, 131.08, 127.87, 109.07. **HRMS (ESI):** Calculated for $C_7H_6O_2N_2NaS_2$ [M+Na^+]: 236.9763. Found: 236.9763.



1C

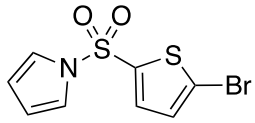
1-((5-chlorothiophen-2-yl)sulfonyl)-1*H*-pyrazole (**1C**): **^{1}H NMR (400 MHz, $CDCl_3$)**: δ 8.06 (d, *J* = 2.8 Hz, 1H), 7.77 (d, *J* = 1.6 Hz, 1H), 7.64 (d, *J* = 4.2 Hz, 1H), 6.94 (d, *J* = 4.2 Hz, 1H), 6.42 (dd,

J = 2.8, 1.6 Hz, 1H). **^{13}C NMR (101 MHz, $CDCl_3$):** (101 MHz, cdcl$_3$) δ 145.69, 141.32, 134.99, 134.33, 131.10, 127.22, 109.27. **HRMS (APCI):** Calculated for $C_7H_6ClN_2O_2S_2$ [M+H$^+$]: 248.9554. Found: 248.9554.



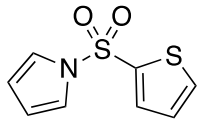
1E

1-(phenylsulfonyl)-1*H*-pyrazole (**1E**): **^{1}H NMR (400 MHz, $CDCl_3$)**: δ 8.10 (d, *J* = 2.8 Hz, 1H), 8.03 – 7.95 (m, 2H), 7.71 (d, *J* = 1.6 Hz, 1H), 7.66 – 7.57 (m, 1H), 7.56 – 7.46 (m, 2H), 6.38 (dd, *J* = 2.8, 1.6 Hz, 1H). **^{13}C NMR (101 MHz, $CDCl_3$):** δ 145.37, 137.05, 134.55, 131.24, 129.39, 128.03, 108.91. **HRMS (ESI):** Calculated for $C_9H_8O_2N_2NaS$ [M+Na$^+$]: 231.0199. Found: 231.0199.



3A

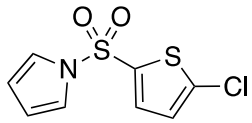
1-((5-bromothiophen-2-yl)sulfonyl)-1*H*-pyrrole (**3A**): **^{1}H NMR (400 MHz, $CDCl_3$):** δ 7.40 (d, *J* = 4.1 Hz, 1H), 7.13 (t, *J* = 2.3 Hz, 2H), 7.02 (d, *J* = 4.1 Hz, 1H), 6.32 (t, *J* = 2.3 Hz, 2H). **^{13}C NMR (101 MHz, $CDCl_3$):** δ 139.87, 133.46, 130.52, 122.26, 120.72, 114.31. **HRMS (APCI):** Calculated for $C_8H_7O_2NBrS_2$ [M+H$^+$]: 291.9096. Found: 291.9098



3B

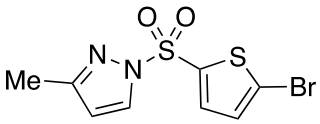
1-(thiophen-2-ylsulfonyl)-1*H*-pyrrole (**3B**): **^{1}H NMR (400 MHz, $CDCl_3$):** δ 7.63 (ddd, *J* = 13.5, 4.4, 1.4 Hz, 2H), 7.17 (t, *J* = 2.3 Hz, 2H), 7.04 (dd, *J* = 5.1, 3.8 Hz, 1H), 6.30 (t, *J* = 2.3 Hz, 2H). **^{13}C**

NMR (101 MHz, $CDCl_3$): δ 139.39, 133.80, 133.37, 127.54, 120.74, 113.90. **HRMS (EI):** Calculated for $C_8H_7NO_2S_2$ [M^+]: 212.9913. Found: 212.9916.



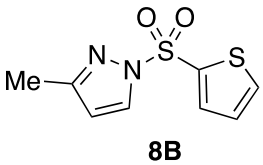
3C

1-((5-chlorothiophen-2-yl)sulfonyl)-1*H*-pyrrole (**3C**): **^{1}H NMR (400 MHz, $CDCl_3$):** δ 7.44 (d, *J* = 4.1 Hz, 1H), 7.14 (t, *J* = 2.3 Hz, 2H), 6.87 (d, *J* = 4.1 Hz, 1H), 6.32 (t, *J* = 2.3 Hz, 2H). **^{13}C NMR (101 MHz, $CDCl_3$):** δ 139.51, 136.99, 132.88, 126.99, 120.73, 114.34. **HRMS (EI):** Calculated for $C_8H_6O_2NClS_2$ [M^+]: 246.9528. Found: 246.9533.



8A

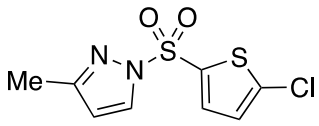
1-((5-bromothiophen-2-yl)sulfonyl)-3-methyl-1*H*-pyrazole (**8A**): **^{1}H NMR (400 MHz, $CDCl_3$):** δ 7.92 (d, *J* = 2.7 Hz, 1H), 7.55 (d, *J* = 4.1 Hz, 1H), 7.05 (d, *J* = 4.1 Hz, 1H), 6.21 (d, *J* = 2.7 Hz, 1H), 2.28 (s, 3H). **^{13}C NMR (101 MHz, $CDCl_3$):** δ 156.08, 137.68, 135.16, 132.02, 130.69, 123.51, 110.25, 14.01. **HRMS (ESI):** Calculated for $C_8H_7O_2N_2BrNaS_2$ [M+Na^+]: 330.9003. Found: 330.9004.



8B

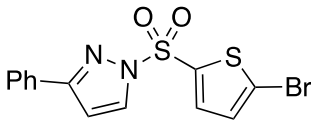
3-methyl-1-(thiophen-2-ylsulfonyl)-1*H*-pyrazole (**8B**): **^{1}H NMR (400 MHz, $CDCl_3$):** δ 7.96 (d, *J* = 2.7 Hz, 1H), 7.80 (dd, *J* = 3.9, 1.4 Hz, 1H), 7.68 (dd, *J* = 5.0, 1.4 Hz, 1H), 7.08 (dd, *J* = 5.0, 3.8 Hz, 1H), 6.20 (d, *J* = 2.7 Hz, 1H), 2.27 (s, 3H). **^{13}C NMR (101 MHz, $CDCl_3$):** δ 155.70, 137.22,

135.06, 134.93, 131.99, 127.71, 109.96, 13.98. **HRMS (ESI):** Calculated for $C_8H_8N_2O_2S_2Na$ [M+Na$^+$]: 250.9919. Found: 250.9920.



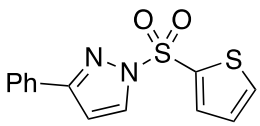
8C

1-((5-chlorothiophen-2-yl)sulfonyl)-3-methyl-1*H*-pyrazole (**8C**): **^{1}H NMR (400 MHz, $CDCl_3$):** δ 7.92 (d, *J* = 2.7 Hz, 1H), 7.59 (d, *J* = 4.2 Hz, 1H), 6.91 (d, *J* = 4.1 Hz, 1H), 6.21 (d, *J* = 2.8 Hz, 1H), 2.28 (s, 3H). **^{13}C NMR (101 MHz, $CDCl_3$):** δ 156.08, 140.71, 134.79, 134.54, 132.02, 127.10, 110.25, 14.00. **HRMS (ESI):** Calculated for $C_8H_7O_2N_2ClNaS_2$ [M+Na$^+$]: 283.9530. Found: 284.9531.



10A

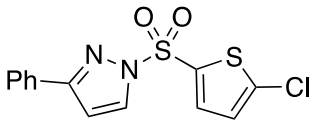
1-((5-bromothiophen-2-yl)sulfonyl)-3-phenyl-1*H*-pyrazole (**10A**): **^{1}H NMR (400 MHz, $CDCl_3$):** δ 8.08 (d, *J* = 2.8 Hz, 1H), 7.87 – 7.79 (m, 2H), 7.61 (d, *J* = 4.1 Hz, 1H), 7.45 – 7.37 (m, 3H), 7.05 (d, *J* = 4.1 Hz, 1H), 6.73 (d, *J* = 2.8 Hz, 1H). **^{13}C NMR (101 MHz, $CDCl_3$):** δ 157.47, 137.37, 135.42, 132.53, 131.12, 130.73, 129.48, 128.74, 126.49, 123.94, 107.10. **HRMS (ESI):** Calculated for $C_{13}H_9BrN_2O_2S_2Na$ [M+Na$^+$]: 390.9181. Found: 390.9182



10B

3-phenyl-1-(thiophen-2-ylsulfonyl)-1*H*-pyrazole (**10B**): **^{1}H NMR (400 MHz, $CDCl_3$):** δ 8.11 (d, *J* = 2.8 Hz, 1H), 7.88 – 7.79 (m, 3H), 7.68 (dd, *J* = 5.0, 1.4 Hz, 1H), 7.44 – 7.31 (m, 3H), 7.07 (dd, *J*

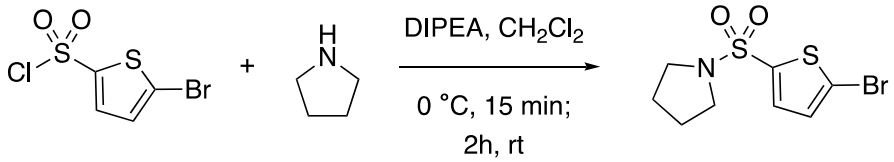
= 5.0, 3.9 Hz, 1H), 6.71 (d, *J* = 2.8 Hz, 1H). **^{13}C NMR (101 MHz, $CDCl_3$):** δ 157.17, 136.84, 135.40, 135.36, 132.55, 131.28, 129.36, 128.72, 127.79, 126.47, 106.90. **HRMS (ESI):** Calculated for $C_{13}H_{10}O_2N_2NaS_2$ [M+Na^+]: 313.0076. Found: 313.0078.



10C

1-((5-chlorothiophen-2-yl)sulfonyl)-3-phenyl-1*H*-pyrazole (**10C**): **^{1}H NMR (400 MHz, $CDCl_3$):** δ 8.08 (d, *J* = 2.8 Hz, 1H), 7.87 – 7.79 (m, 2H), 7.65 (d, *J* = 4.2 Hz, 1H), 7.41 (s, 1H), 7.43 – 7.33 (m, 2H), 6.92 (d, *J* = 4.1 Hz, 1H), 6.73 (d, *J* = 2.8 Hz, 1H). **^{13}C NMR (101 MHz, $CDCl_3$):** δ 157.47, 141.13, 134.79, 134.50, 132.51, 131.12, 129.47, 128.74, 127.12, 126.48, 107.08. **HRMS (ESI):** Calculated for $C_{13}H_9O_2N_2ClNaS_2$ [M+Na^+]: 346.9686. Found: 346.9688.

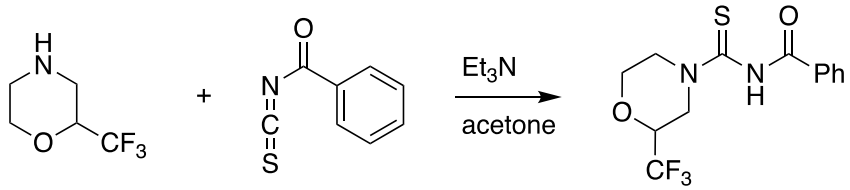
Synthesis of P007H4 (Keil, 2010)



The sulfonyl chloride (1.05 g, 4 mmol) was dissolved in dichloromethane (1.0 mL) and the resulting solution was added dropwise to a round-bottom flask containing a stirred solution of pyrrolidine (657 µL, 8 mmol) and diisopropylethyllamine (1.5 mL, 8 mmol) in dichloromethane (10 mL) at 0 °C. The resulting reaction mixture was allowed to stir at 0 °C for 15 min and then allowed to warm to room temperature. After 2 h, the resulting solution was washed with 10 mL each of saturated sodium bicarbonate, water, 1 N HCl and brine. The organic layer was dried with magnesium sulfate, and solvent was removed under reduced pressure to give 1-((5-

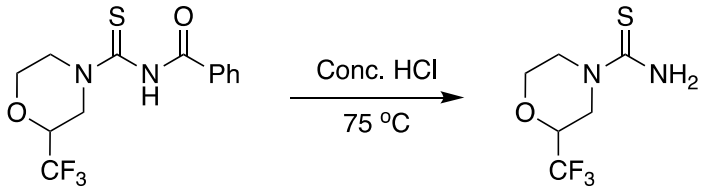
bromothiophen-2-yl)sulfonyl)pyrrolidine (**P007H4**). **^{1}H NMR (400 MHz, $CDCl_3$):** δ 7.30 (d, *J* = 4.0 Hz, 1H), 7.08 (d, *J* = 4.0 Hz, 1H), 3.29 – 3.22 (m, 4H), 1.85 – 1.74 (m, 4H). **^{13}C NMR (101 MHz, $CDCl_3$):** δ 137.98, 132.05, 130.47, 119.30, 48.20, 25.34. **HRMS (ESI):** Calculated for $C_9H_{10}BrNO_2S_2Na$ [M+Na^+]: 317.9229. Found: 317.9229.

Synthesis of P2065E16



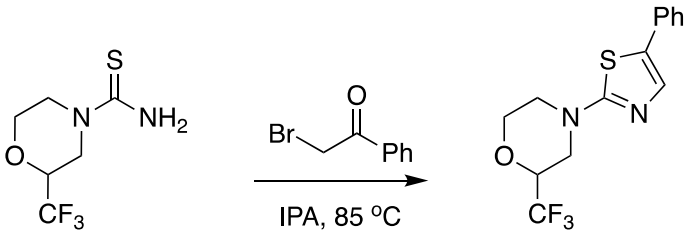
SI-1

To a flask purged and backfilled under nitrogen was added a solution of 2-trifluoromethyl-morpholine (100 mg, 0.522 mmol) in 0.5 mL dry acetone. Triethylamine (0.109 mL, 0.783 mmol) was added via syringe and the resulting solution allowed to stir at room temperature for 30 minutes. The solution was cooled to 0°C, and benzoyl isothiocyanate (0.702 mL, 0.522 mmol) was added dropwise. The resulting solution was allowed to stir for 30 min at 0°C and was then quenched with 1 mL water. The crude product was extracted with ethyl acetate (2 x 30 mL). The organic layers were combined, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The resulting crude product mixture was purified via SiO_2 column chromatography in 5:1 Hexanes:EtOAc to give N-(2-(trifluoromethyl)morpholine-4-carbonothioyl)benzamide (**SI-1**) in 62% yield (Buckley, 2018). **^{1}H NMR (400 MHz, $CDCl_3$):** δ 7.93 – 7.75 (m, 2H), 7.69 – 7.55 (m, 1H), 7.50 (t, *J* = 7.7 Hz, 2H), 5.10 (d, *J* = 94.4 Hz, 2H), 4.11 (s, 3H), 3.86 (s, 1H), 3.58 – 3.42 (m, 1H), 3.37 (dd, *J* = 13.4, 10.6 Hz, 1H).



SI-1 **SI-2**

Concentrated HCl (2 mL) was added to **SI-1** (102.6 mg, 0.322 mmol). The resulting solution was allowed to stir for 1.5 hours at 75°C, then cooled to 0°C. Water (10 mL) was added, followed by 50% sodium hydroxide (5 mL). The aqueous phase was extracted with 1:1 ethyl acetate: petroleum ether (3 x 30mL). The organic layers were combined, washed with water (10 mL), dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The resulting crude product mixture was purified via SiO_2 column chromatography in 1:1 Hexanes:EtOAc to give 2-(trifluoromethyl)morpholine-4-carbothioamide (**SI-2**) in 51% yield (Shreykar & Sekar, 2016). **^{1}H NMR (400 MHz, $CDCl_3$):** δ 6.33 (s, 2H), 4.75 (d, *J* = 13.3 Hz, 1H), 4.30 (d, *J* = 13.4 Hz, 1H), 3.92 (dqd, *J* = 12.1, 6.0, 3.0 Hz, 1H), 3.64 (td, *J* = 11.6, 3.0 Hz, 1H), 3.25 (ddd, *J* = 13.5, 11.3, 3.6 Hz, 1H), 3.18 (dd, *J* = 13.4, 10.6 Hz, 1H).

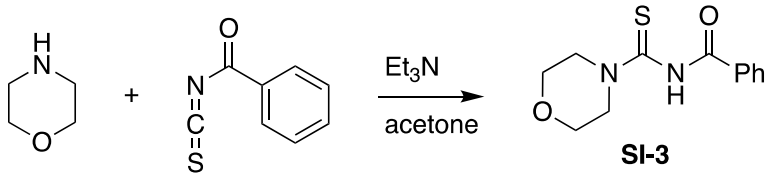


SI-2 **P2065E16**

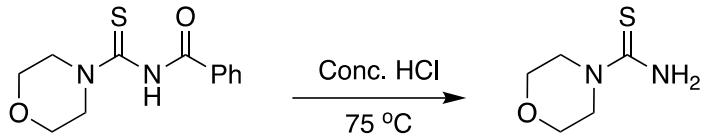
SI-2 (35 mg, 0.164 mmol) and 2-bromoacetophenone (33 mg, 0.164 mmol) were dissolved in isopropanol (5 mL). The resulting solution was heated with stirring for four hours at 85°C. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The resulting crude reaction mixture was purified via SiO_2 column chromatography (hexanes/EtOAc) to give 4-(5-phenylthiazol-2-yl)-2-(trifluoromethyl)morpholine (**P2065E16**) in 24% yield (Li *et al.*, 2014). **^{1}H NMR (400 MHz, $CDCl_3$):** δ 7.76 (d, *J* = 7.4 Hz, 2H), 7.32 (t, *J* = 7.7 Hz, 2H), 7.19 (d, *J* = 2.2 Hz, 1H), 6.79 (s, 1H), 4.10 – 3.91 (m, 3H), 3.75 (t, *J* = 15.1 Hz, 2H),

3.18 (dt, *J* = 38.2, 11.9 Hz, 2H). **HRMS (APCI):** Calculated for $C_{14}H_{13}F_3N_2OS$ [M^+]: 314.0701. Found: 314.0702.

Synthesis of P2065E16-CF_3



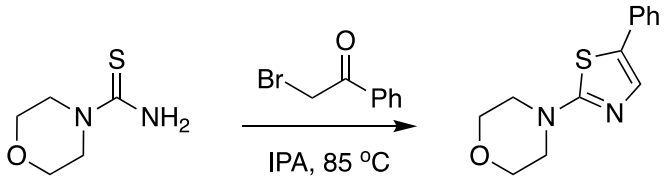
Morpholine (1.0 g, 0.99 mL, 11.5 mmol) was added to dry acetone (10 mL) under nitrogen. Triethylamine (1.74 g, 2.40 mL, 17.2 mmol) was added via syringe and the resulting solution was allowed to stir at room temperature for 30 minutes. The resulting solution was cooled to 0°C, and benzoyl isothiocyanate (1.87 g, 1.54 mL, 11.5 mmol) was added dropwise. The resulting solution was allowed to stir for 30 min at 0°C and was then quenched with water (20 mL). The aqueous layer was extracted with ethyl acetate (2 x 30 mL). The organic layers were combined, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The resulting crude product mixture was purified via SiO_2 column chromatography in 3:1 Hexanes:EtOAc to give *N*-(morpholine-4-carbonothioyl)benzamide (**SI-3**) in 57% yield. **^{1}H NMR (400 MHz, $CDCl_3$):** δ 8.55-8.50 (m, 1H), 7.81 (d, J = 7.6 Hz, 2H), 7.57 (t, J = 7.6 Hz, 1H), 7.46 (t, J = 7.6 Hz, 2H), 4.20 (s, 2H), 3.85 – 3.77 (m, 4H), 3.63 (s, 2H).



SI-3 **SI-4**

Concentrated HCl (4 mL) was added to **SI-3** (169 mg, 0.675 mmol). The resulting solution was heated with stirring for 1.5 hours at 75°C, then cooled to 0°C. Water (15 mL) was added, followed by 50% sodium hydroxide (10 mL). The aqueous phase was extracted with 1:1 ethyl

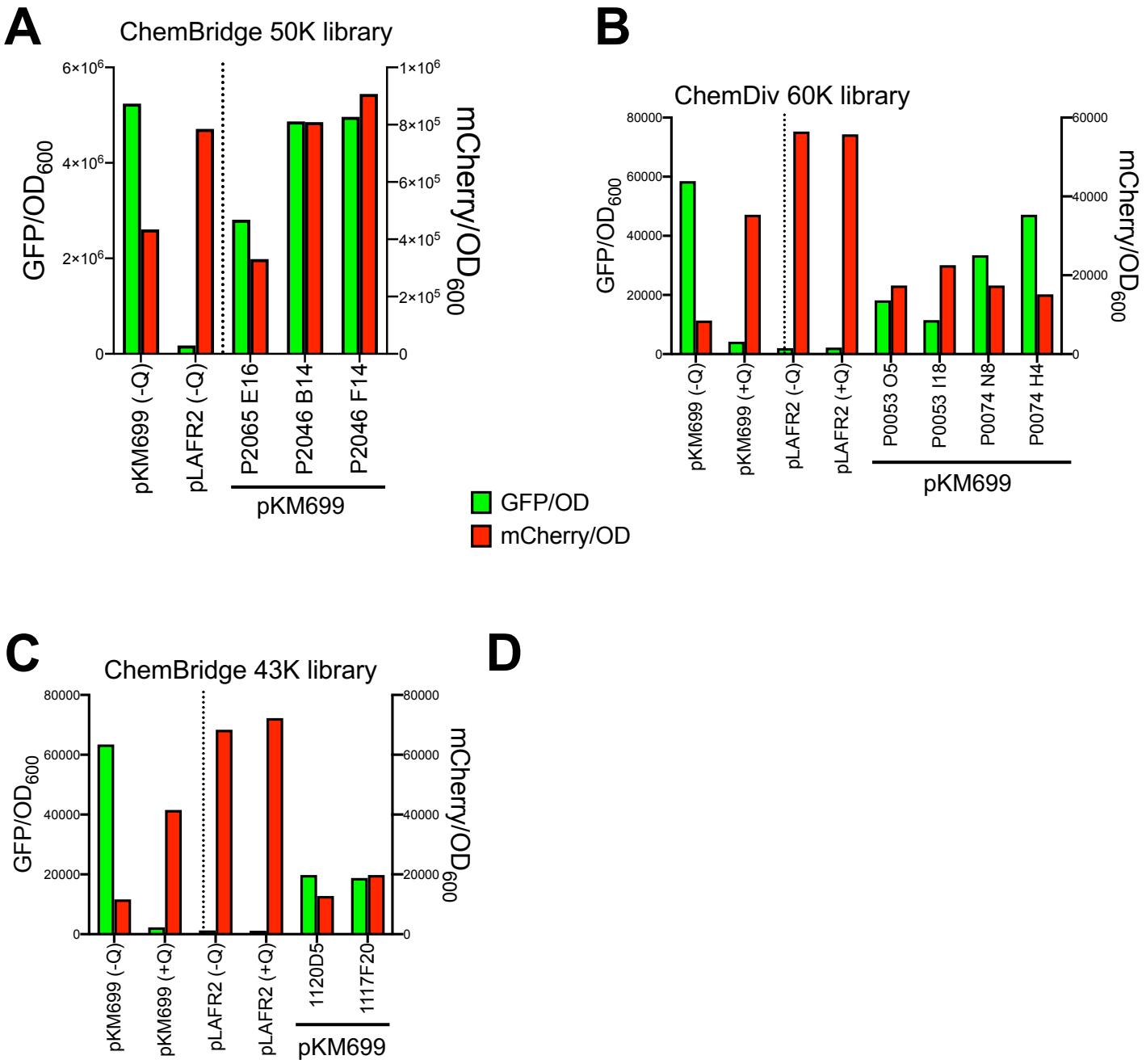
acetate: petroleum ether (3 x 30 mL). The combined organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure to provide morpholine-4-carbothioamide (**SI-4**) in 33% yield (no purification was necessary). **^{1}H NMR (400 MHz, $CDCl_3$):** δ 7.42 (s, 2H), 3.67 (t, *J* = 4.8 Hz, 4H), 3.52 (t, *J* = 4.8 Hz, 4H).



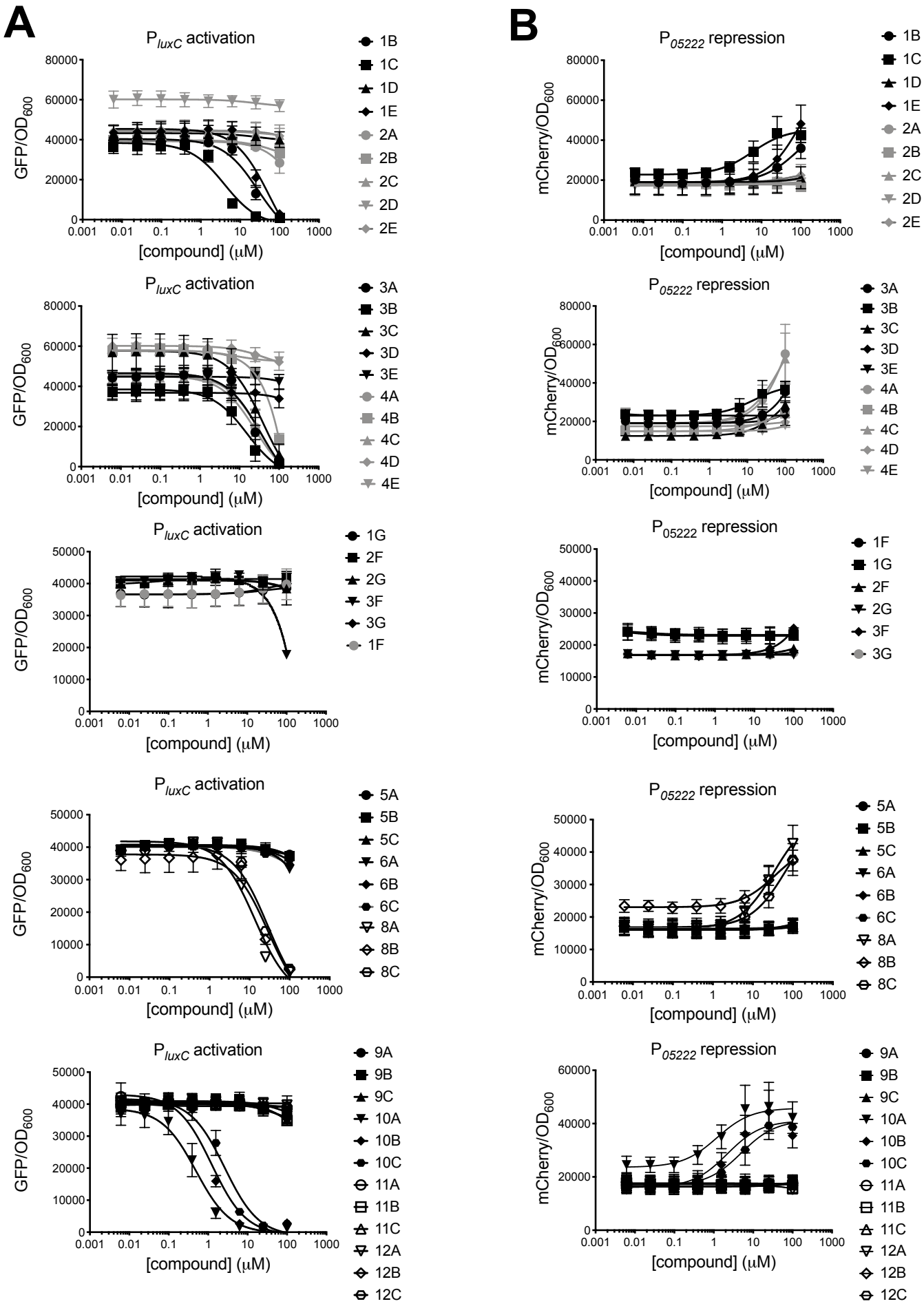
SI-4 **P2065E16-CF_3**

SI-4 (123 mg, 0.84 mmol) and 2-bromoacetophenone (167 mg, 0.84 mmol) were dissolved in isopropanol (5 mL). The resulting solution was heated with stirring for four hours at 85°C. The reaction was cooled to room temperature and concentrated under reduced pressure to give 4-(5-phenylthiazol-2-yl)morpholine (**P2065E16-CF_3**) in quantitative yield. δ 7.82 – 7.76 (m, 2H), 7.37 (t, *J* = 7.6 Hz, 2H), 7.31-7.25 (m, 2H), 3.70 (t, *J* = 4.9 Hz, 4H), 3.46 (t, *J* = 4.8 Hz, 4H). **^{13}C NMR (101 MHz, DMSO-d_6)**: δ 170.99, 148.82, 133.74, 129.02, 128.49, 126.42, 103.43, 65.71, 48.87. **HRMS (EI):** Calculated for $C_{13}H_{14}N_2OS$ [M^+]: 246.0827. Found: 246.0839.

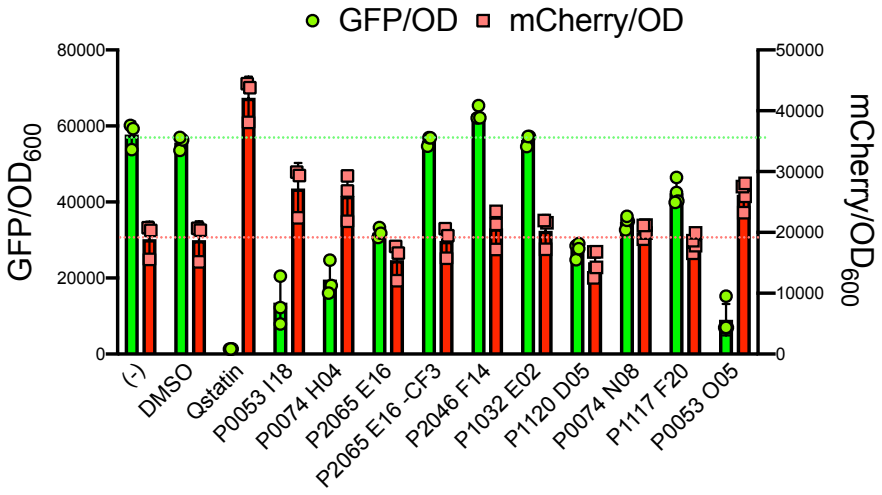
Tables

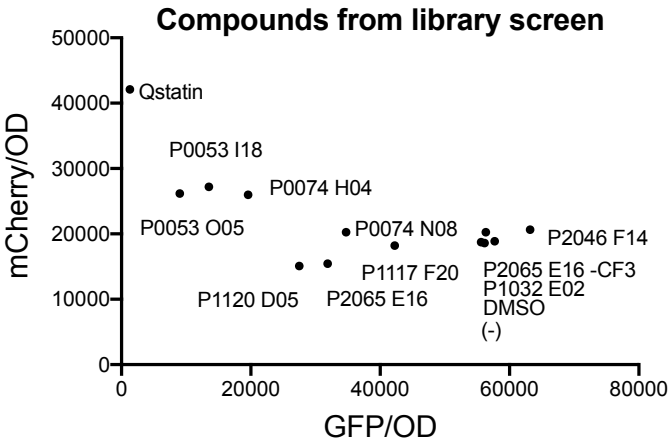


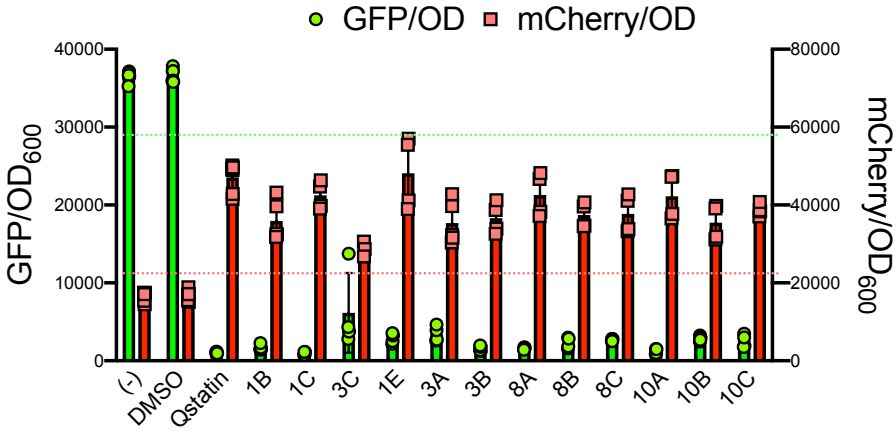
Supplemental Figure 1. (A-C) Data from the chemical library screens. Production of GFP (GFP/OD_{600}) or mCherry ($mCherry/OD_{600}$) in the presence of compounds in chemical libraries at a final concentration is 12.5 μg/ml. These assays were performed in the *E. coli* bioassay strain containing two plasmids: 1) pJV064, which contains divergent promoters for *V. campbellii luxCDABE* and *05222* driving expression of *gfp* and *mCherry*, respectively, and 2) either a plasmid expressing LuxR from its native promoter (pKM699) or empty vector (pLAFR2). Qstatin (Q+) was added at a final concentration of 5 μM (1.47 μg/ml) compared to the solvent DMSO (Q-) as positive and negative controls, respectively.

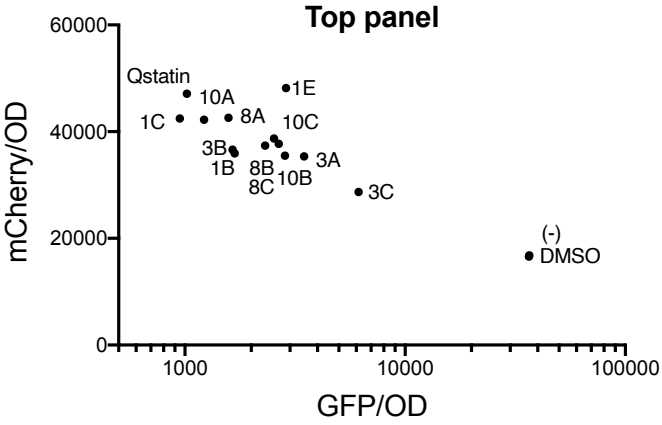


Supplemental Figure 2. Production of GFP (panel A; GFP/OD_{600}) or mCherry (panel B; mCherry/OD_{600}) in the presence of sulfonamide compounds titrated into the *E. coli* bioassay strain (pKM699, pJV064). Data shown represent the mean and standard deviation of at least three biological replicates.

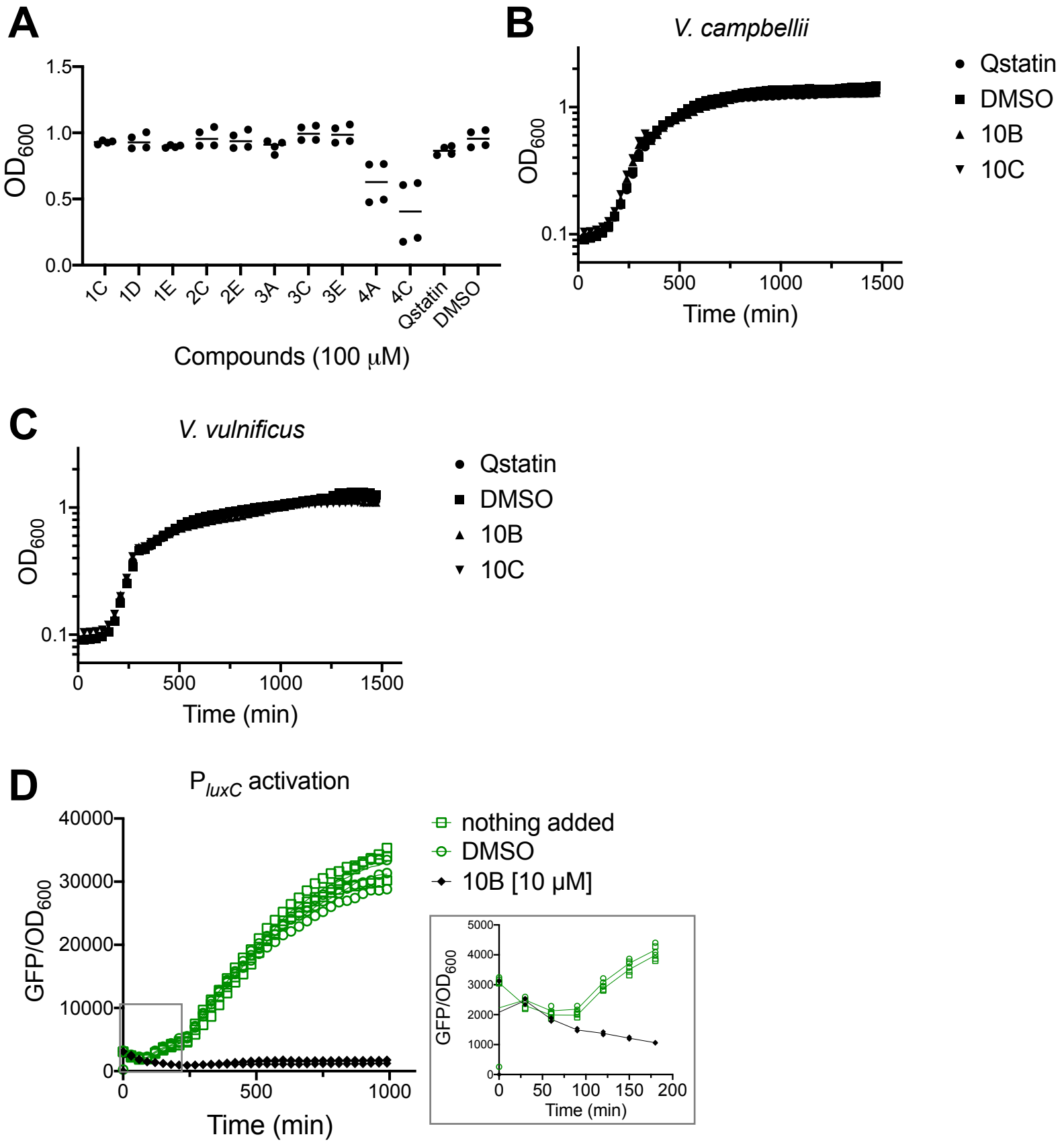




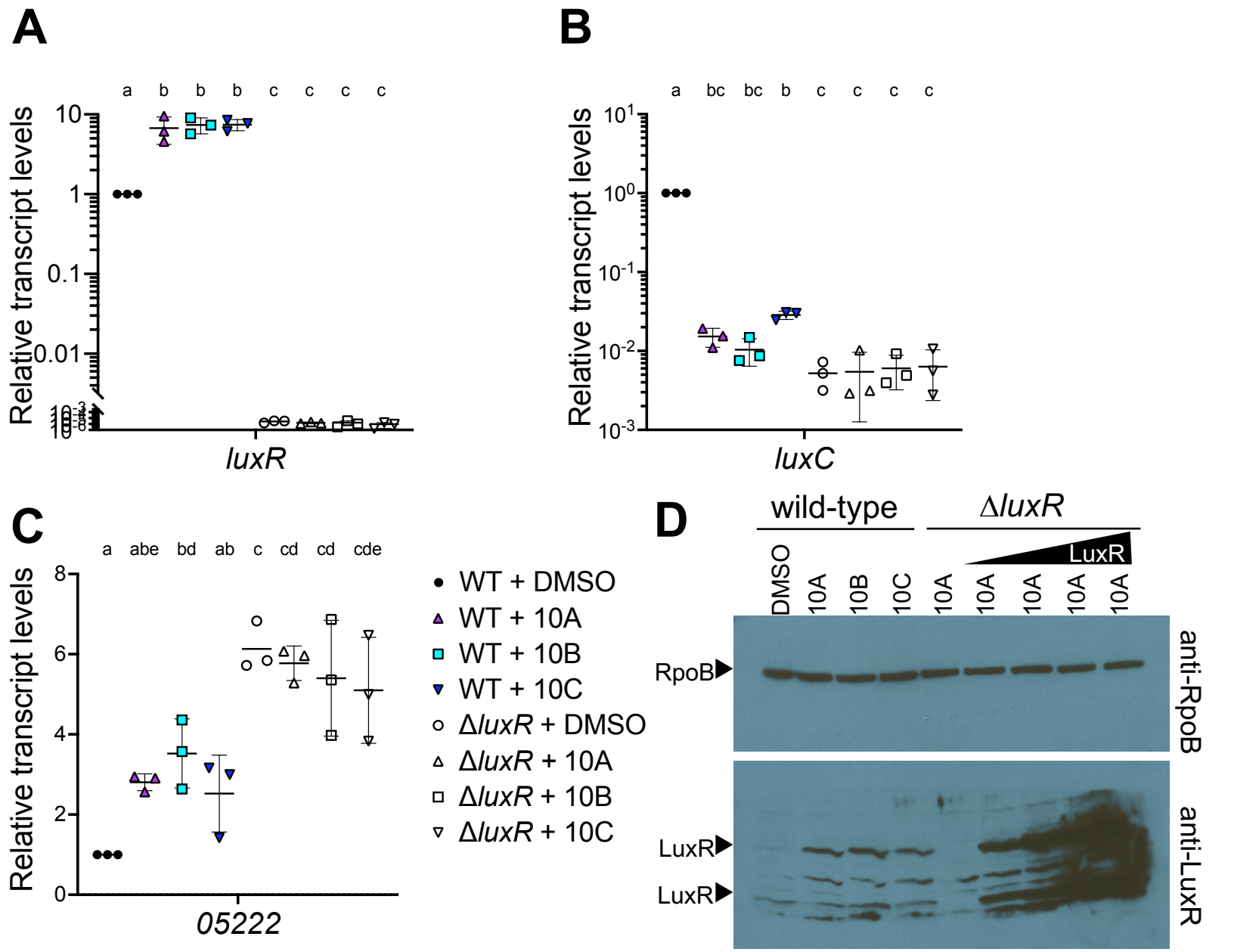




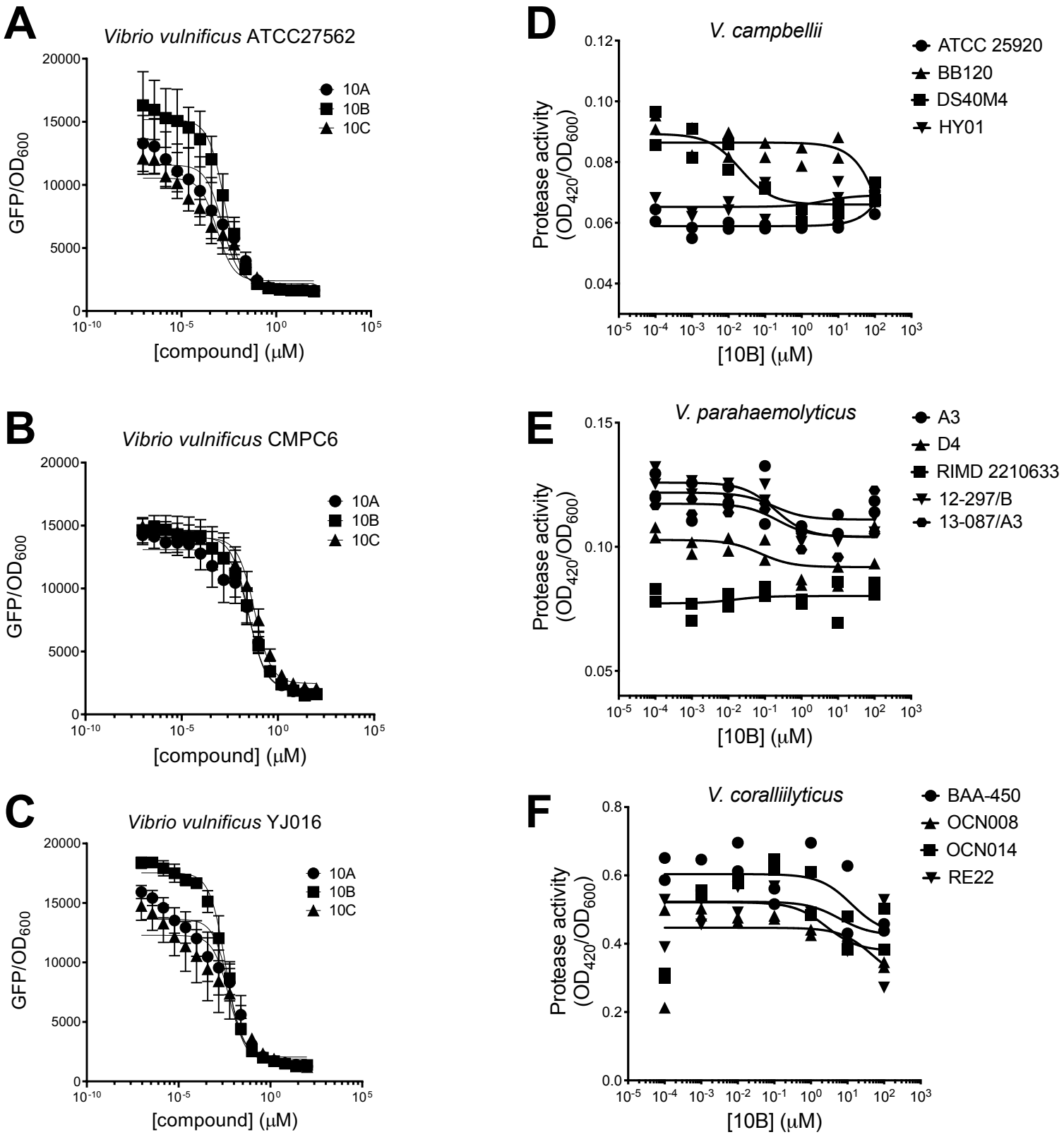
Supplemental Figure 3. Production of GFP (GFP/OD_{600}) or mCherry ($mCherry/OD_{600}$) in the presence of compounds at 100 μM in the *E. coli* bioassay strain containing plasmid pKM699 expressing LuxR (pJV064). These data are the same as those plotted in Figures 2D, 2E, 3B, and 3C.



Supplemental Figure 4. (A) Growth yield of *E. coli* bioassay strain (pKM699, pJV064) in the presence of compounds at 100 μM or an equal volume of DMSO. (B, C) Growth curves of *V. campbellii* BB120 (B) and *V. vulnificus* ATCC 27562 (C) in the presence of 25 μM 10B, 10C, Qstatin, or an equal volume of DMSO. (D) Production of GFP (GFP/OD_{600}) over a timecourse in the presence of 10 μM compound 10B added at time 0 to the *E. coli* bioassay strain (pKM699, pJV064). An equal volume of solvent DMSO was added as a negative control compared to nothing added. Inset is the first 180 minutes of the timecourse.

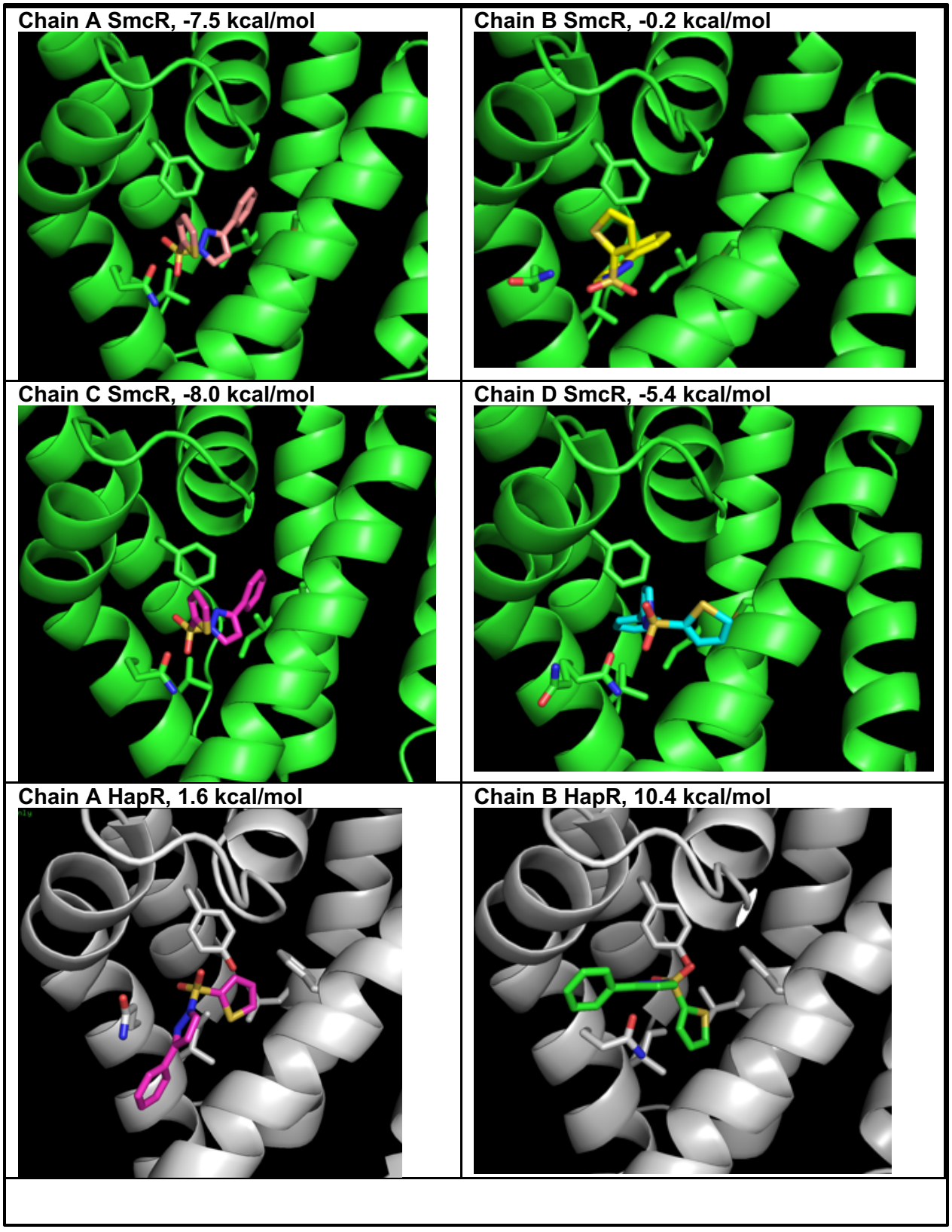


Supplemental Figure 5. (A, B, C) Transcript levels of *luxR* (A)*, luxC* (B), and *VIBHAR_05222* (*05222*; C) were determined by qRT-PCR from wild-type (BB1210) or Δ*luxR* (KM669) cultures treated with 10 μM of 10A, 10B, 10C, or an equivalent volume of DMSO. Relative transcript levels were calculated compared to *hfq* transcript levels. Error bars represent standard deviation of three biological replicates. Different letters indicate significant differences (one-way ANOVA followed by Tukey's multiple comparisons test, $n = 3$, $p = 0.05$; data were log-transformed for panels A and B prior to analysis). (D) Western blot analysis of lysates from wild-type (BB120) or Δ*luxR* (KM669) cultures treated with 10 μM of 10A, 10B, 10C, or an equivalent volume of DMSO. Prior to loading the gel, purified LuxR protein (0.9 μg, 1.8 μg, 4.5 μg, 9 μg) were added to four samples of KM669 lysate as a standard. Anti-LuxR or anti-RpoB antibodies were used to probe the same gel. LuxR runs as both a dimer and monomer on SDS-PAGE gels.

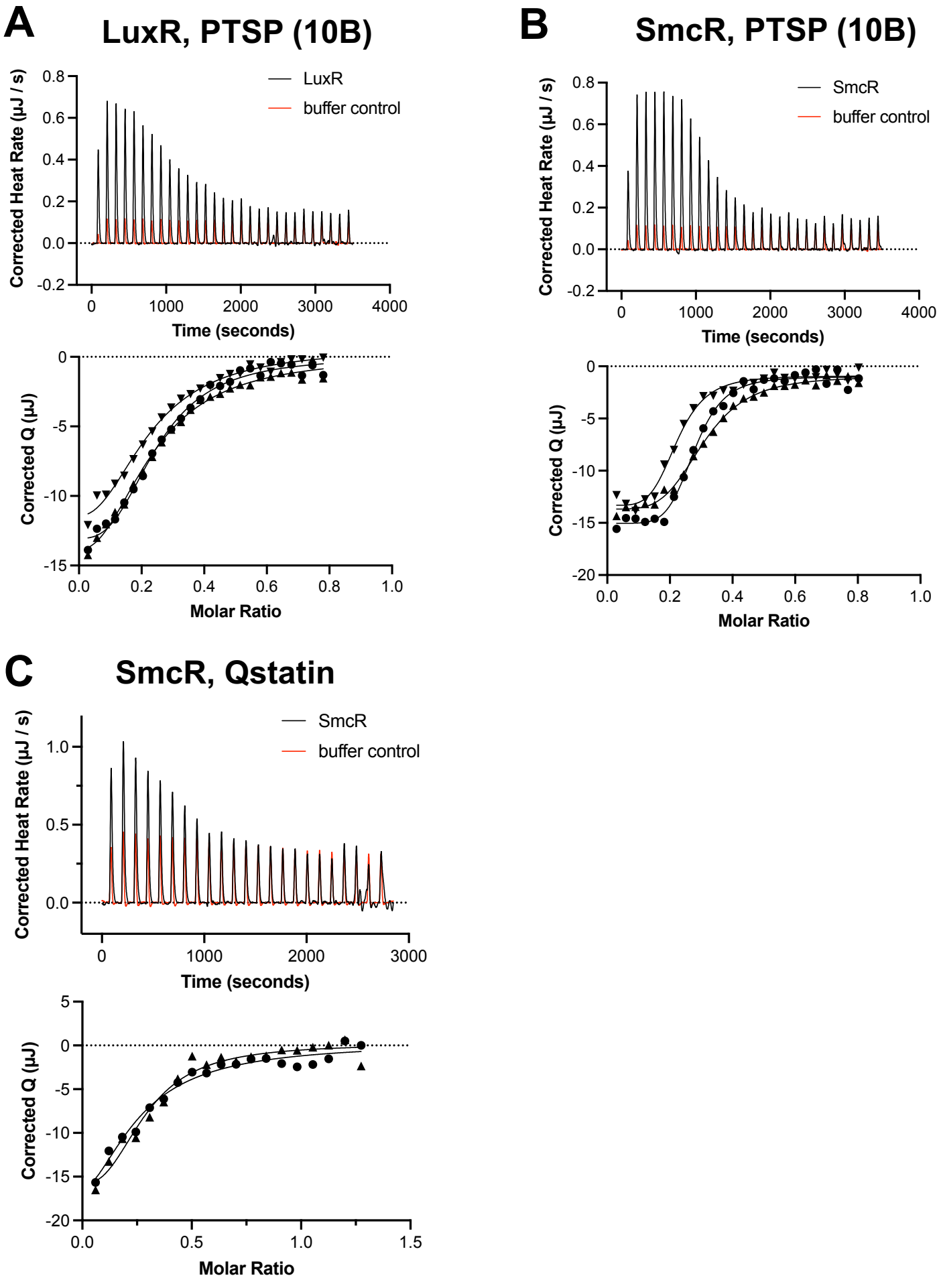


Supplemental Figure 6. (A-C) Titration of molecules from the top panel of thiophenesulfonamides in *V. vulnificus* strains. Data shown represent the mean and standard error of measurement of three biological replicates. (D-F) Protease activity (final assay OD_{420}/initial culture OD_{600}) for *Vibrio* strains in the presence of 10B titrated into the cultures. Two biological replicates are shown for each culture series.

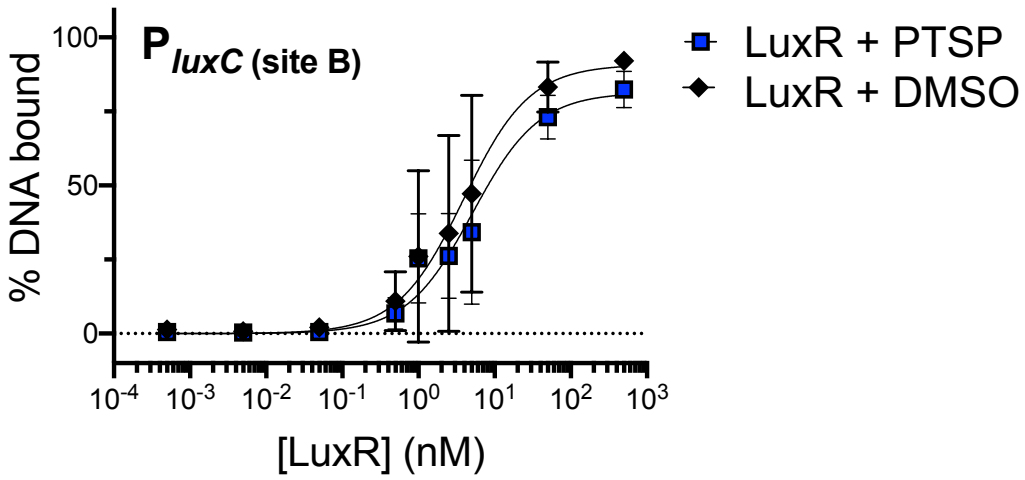
PTSP modelling in SmcR and HapR



Supplemental Figure 7. Autodock Vina modelling of PTSP into the four chains of SmcR (3KZ9) and two chains of HapR (2PBX). The predicted binding energies are shown.



Supplemental Figure 8. (A-C) ITC analysis of LuxR or SmcR proteins titrated against molecules. Top panels shows heats of exchange for one example replicate experiment; bottom panel shows the corrected Q (heat) and curve fit for three experiments for panels A and B, or two experiments for panel C.



Supplemental Figure 9. EMSAs with purified LuxR protein in varying concentrations incubated with radiolabeled dsDNA corresponding to the *V. campbellii luxC* promoter site B. DNA shifts were quantified using ImageJ, and the graphs show the mean and standard deviation for three biological replicates.

Table S1. Predicted binding energies for small molecules into SmcR (3KZ9) calculated using Webina (Kochnev *et al.*, 2020).

Molecule	Binding energy (kcal/mol)[a]
1A (Qstatin)	-5.6
1B	-5.7
1C	-6.0
1D	-5.6
1E	-6.4
1F	-6.5
1G	-6.6
2A	-5.4
2B	-5.4
2C	-5.9
2D	-5.5
2E	-6.1
2F	-6.3
2G	-6.5
3A	-5.5
3B	-5.6
3C	-6.1
3D	-5.4
3E	-6.4
3F	-6.7
3G	-6.8
4A	-5.4
4B	-5.6
4C	-5.1
4D	-5.8
4E	-6.4
5A	-4.9
5B	-4.6
5C	-4.9
6A	-5.2
6B	-5.7
6C	-5.9
8A	-6.3
8B	-6.5
8C	-6.2
9A	-5.8

9B	-5.6
9C	-5.8
10A	-7.9
10B (PTSP)	-7.2
10C	-7.8
11A	-5.3
11B	-6.8
11C	-5.8
12A	-5.5
12B	-5.4
12C	-5.6
P0053 I18	-4.4
P2046 B14	-3.1
P2046 F14	-1.5
P2065 E16	-5.8
P0053 O05	-7.2
P0074 H04	-5.5
P1117 F20	-6.8
P1120 D05	-5.5
P0074 N08	-7.5

a: Parameters: center_x=18, center_y=40, center_z=56, size_x=12, size_y=12, size_z=12

Table S2. Bacterial strains used in this study.

Name	Genotype/Notes	Reference
***E. coli* strains**		
S17-1λ*pir*	Conjugation strain, λ-*pir*, *recA thi pro hsdR⁻ M⁺* RP4: 2-Tc, Mu, Tp^R, Sm^R	(de Lorenzo & Timmis, 1994)
DH10B	str. K-12 F^- Δ(*ara-leu*)*7697*[Δ(*rapA'-cra'*)] Δ(*lac*)*X74*[Δ('*yahH-mhpE*)] duplication (514341-627601) [*nmpC-gltI*] *galK16 galE15* e14⁻(*icd*^{WT} *mcrA*) φ80d*lacZΔM15 recA1 relA1 endA1* Tn*10.10 nupG rpsL150* (Str^R) *rph⁺ spoT1* Δ*(mrr-hsdRMS- mcrBC)* λ⁻ Missense (*dnaA glmS glyQ lpxK mreC murA*) Nonsense (*chiA gatZ fhuA*? *yigA ygcG*) Frameshift(*flhC mglA fruB*)	Life Technologies
BL21(DE3)	*E. coli* str. B F^- *ompT gal dcm lon hsdS$_B$*($r_B^- m_B^-$) λ(DE3 [*lacI lacUV5-T7p07 ind1 sam7 nin5*]) [*malB*$^+$]$_{K-12}$(λ^S)	NEB
β3914	Conjugation strain; Δ*dapA*::(*erm-pir*); Km^r, Em^r, Tc^r	(Le Roux *et al.*, 2007)
***V. campbellii* strains**		
BB120	Wild-type	(Bassler *et al.*, 1997)
KM669	BB120 Δ*luxR*	(Pompeani *et al.*, 2008)

HY01	Wild-type	(Rattanama *et al.*, 2009)
ATCC 25920	(NBRC 15631/ CAIM 519)	ATCC
***V. coralliilyticus* strains**		
OCN008	Wild-type	(Lydick *et al.*, 2020, Ushijima *et al.*, 2013)
Δ*vcpR*	OCN008 Δ*vcpR*	(Guillemette *et al.*, 2020)
OCN014	Wild-type	(Ushijima *et al.*, 2014)
***V. cholerae* strains**		
E7946	wild-type 01 El Tor	(Miller *et al.*, 1989)
SAD793	E7946 Sm^R Δ*hapR::Spec*^{R}, pMMB67EH-tfox-kanR	(Simpson *et al.*, 2019)
***V. parahaemolyticus* strains**		
RIMD 2210633	Wild-type	ATCC
CAS-V03	RIMD2210633 Δ*opaR::Spec*^{R}, pMMB67EH-tfoX-kanR	(Simpson *et al.*, 2019)
D4	AHPND isolate	(Li *et al.*, 2017)
12-197/B	AHPND isolate	(Li *et al.*, 2017)
***V. vulnificus* strains**		
ATCC 27562	Wild-type	ATCC
CAS-vv015	ATCC 27562 Δ*smcR*	(Simpson *et al.*, 2019)
YJ016	Clinical isolate	(Chen *et al.*, 2003)
CMPC6	Clinical isolate	(Kim *et al.*, 2003)

Table S3. Oligonucleotides used in this study.

Name	Description	Reference
JCV1295	GATAACAATTTCACACAGGAAACAGAATTCATGGACTCAATTGCAAAGAGAC	luxR in pMMB, F
JCV1296	GCATGCCTGCAGGTCGACTCTAGAGGATCCTTAGTGATGTTCACGGTTGTAGA	luxR in pMMB, R
JCV1297	GATAACAATTTCACACAGGAAACAGAATTCATGGACGCATCAATCGAAAAAC	hapR n pMMB, F
JCV1298	GCATGCCTGCAGGTCGACTCTAGAGGATCCCTAGTTCTTATAGATACACAGCATATTGA	hapR in pMMB, R
JCV1299	GATAACAATTTCACACAGGAAACAGAATTCATGGACTCAATTGCAAAGAGAC	opaR in pMMB, F
JCV1300	GCATGCCTGCAGGTCGACTCTAGAGGATCCTTAGTGTTCGCGATTGTAGATGC	opaR in pMMB, R
JCV1301	GATAACAATTTCACACAGGAAACAGAATTCATGGATTCTATAGCTAAGAGACCG	vcpR in pMMB, F
JCV1302	GCATGCCTGCAGGTCGACTCTAGAGGATCCCTACTTGTAGATGCAAAGCATATCTAG	vcpR in pMMB, R
JCV1305	AAGTGCTTAATCATGTCGTTCGTCAGTACTCTAATTTCCTATCTGACAACATCGAT	SmcR F75Y
JCV1306	ATCGATGTTGTCAGATAGGAAATTAGAGTACTGACGAACGACATGATTAAGCACTT	SmcR F75Y
JCV1307	TTCACGCTAAAGAAAACATCGCCAACCTGACCAACGCAATGATTGAGCTTGTGGTG	SmcR I96L
JCV1308	CACCACAAGCTCAATCATTGCGTTGGTCAGGTTGGCGATGTTTTCTTTAGCGTGAA	SmcR I96L
JCV1309	CCACCAACCGTACTAATCAGTTGCTGATCCAAAACATGTTCATCAAAGCCATTGAA	SmcR V140I
JCV1310	TTCAATGGCTTTGATGAACATGTTTTGGATCAGCAACTGATTAGTACGGTTGGTGG	SmcR V140I
JCV1311	ATTTGGCAAACCTGTTCCACGGCATTTTCTACTCGCTGTTTGTTCAAGCAAACCGC	SmcR C170F
JCV1312	GCGGTTTGCTTGAACAAACAGCGAGTAGAAAATGCCGTGGAACAGGTTTGCCAAAT	SmcR C170F
JCV1313	GTGCTGAATTTTGTGGTTCGTCAGTTTTCCAACTTCTTGACCGATCACATCG	HapR Y76F
JCV1314	CGATGTGATCGGTCAAGAAGTTGGAAAACTGACGAACCACAAAATTCAGCAC	HapR Y76F
JCV1315	TGGATGTGAAAACCAACCTACAAACTATCTGCAAAGAGATGGTGAAATTGGC	HapR L97I
JCV1316	GCCAATTTCACCATCTCTTTGCAGATAGTTTGTAGGTTGGTTTTCACATCCA	HapR L97I
JCV1317	CACCAACCGAACTAACCAACTGCTGGTAAGAAACATGTTTATGAAAGCGATG	HapR I141V
JCV1318	CATCGCTTTCATAAACATGTTTCTTACCAGCAGTTGGTTAGTTCGGTTGGTG	HapR I141V
JCV1319	ATGGCCAGCCTGTTCCACGGCATCTGTTACTCCATCTTCTTACAAGTGAACC	HapR F171C
JCV1320	GGTTCACTTGTAAGAAGATGGAGTAACAGATGCCGTGGAACAGGCTGGCCAT	HapR F171C
AB324	ATAAATCAAATTTGTAACTTTATTTCTGAGATGACTGTCCCATTTATCTTATTGATAAATCTGCGTAAAAAAGAAAAAGCACAATTTTATTACTAGTGTTT	P_{vvpE} smcR, CAP, IHF BSs +25 bp left +12 bp right, F
AB325	AAACACTAGTAATAAAATTGTGCTTTTTCTTTTTTACGCAGATTTATCAATAAGATAAATGGGACAGTCATCTCAGAAATAAAGTTACAAATTTGATTTAT	P_{vvpE} smcR, CAP, IHF BSs +25 bp left +12 bp right, R
JDN54	TAATGTTATTTGTAACACTATAAATAAGTAA	P_{luxC} Site B, R
JDN55	TTACTTATTTATAGTGTTACAAATAACATTA	P_{luxC} Site B, F
JDN101	GGATCCTCTAGAGTCGACCT	pMMB amplify backbone, F

JDN102	GAATTCTGTTTCCTGTGTGAAATTG	pMMB amplify backbone, R
JDN103	GATAACAATTTCACACAGGAAACAGAATTCATGGACTCAATCGCAAAGAGA	smcR in pMMB, F
JDN104	GCATGCCTGCAGGTCGACTCTAGAGGATCCCTATTCGTGTTCGCGTTTATAGAT	smcR in pMMB, R
luxC right	TGTTCATTTAACTCAGATGGTGACT	luxC qRT right
luxC left	TTCTTCTTGAATACTCTTCGCTCTT	luxC qRT left
JCV737	TGGTTAACAGTGTTCTTCAATAGGATC	hfq qRT reverse
AB156	CGTGAGCGTATCCCGGTATCTAT	hfq qRT forward
AB154	CGCTGCTTGGAGTGGTAAGTA	05222 qRT forward
AB155	CGTCCGGTGTAGATGACCTTA	05222 qRT reverse
JCV745	GCAAAGAGACCTCGTACTAGG	luxR qRT
JCV746	GCGACGAGCAAACACTTC	luxR qRT

Table S4. Plasmids used in this study.

Name	Description	Reference
pAMS001	*hapR* F171C Y76F in pJV387	This study
pET28b	pET28b, 6X-his tag, Kan^R	EMD
pJN08	6x-his-*smcR* in pET28b	(Newman *et al.*, 2020)
pJN22	P_{tac}-*smcR* (*V. vulnificus* ATCC 27562), in pMMB67EH-kanR	(Newman *et al.*, 2020)
pJV064	P_{05222}-mCherry, P_{luxC}-*gfp*; p15a origin, CM^R	(van Kessel *et al.*, 2013b)
pJV387	P_{tac}-*hapR* (*V. cholerae* E7946), in pMMB67EH-kanR	This study
pJV388	P_{tac}-*luxR* (*V. campbellii* BB120), in pMMB67EH-kanR	This study
pJV389	P_{tac}-*opaR* (*V. parahaemolyticus* RIMD 2210633), in pMMB67EH-kanR	This study
pJV390	P_{tac}-*vcpR* (*V. coralliilyticus* OCN008), in pMMB67EH-kanR	This study
pJV391	*hapR* Y76F in pJV387	This study
pJV392	*hapR* L97I in pJV387	This study
pJV393	*hapR* I131V in pJV387	This study
pJV394	*hapR* F171C in pJV387	This study
pJV395	*smcR* F75Y in pMMB67EH-kanR	This study
pJV396	*smcR* I96L in pMMB67EH-kanR	This study
pJV397	*smcR* V140I in pMMB67EH-kanR	This study
pJV398	*smcR* C170F in pMMB67EH-kanR	This study
pJV79	*luxR* in pET28b	(van Kessel *et al.*, 2013a)
pKM699	*V. campbellii* P_{luxR}-*luxR* region (2.3 kbp fragment) in pLAFR2, Tet^R	(Hustmyer *et al.*, 2018)
pLAFR2	Vector cosmid, Tet^R	(Hustmyer *et al.*, 2018)
pMMB67EH-kanR	Vector control; kan^R	(Simpson *et al.*, 2019)

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