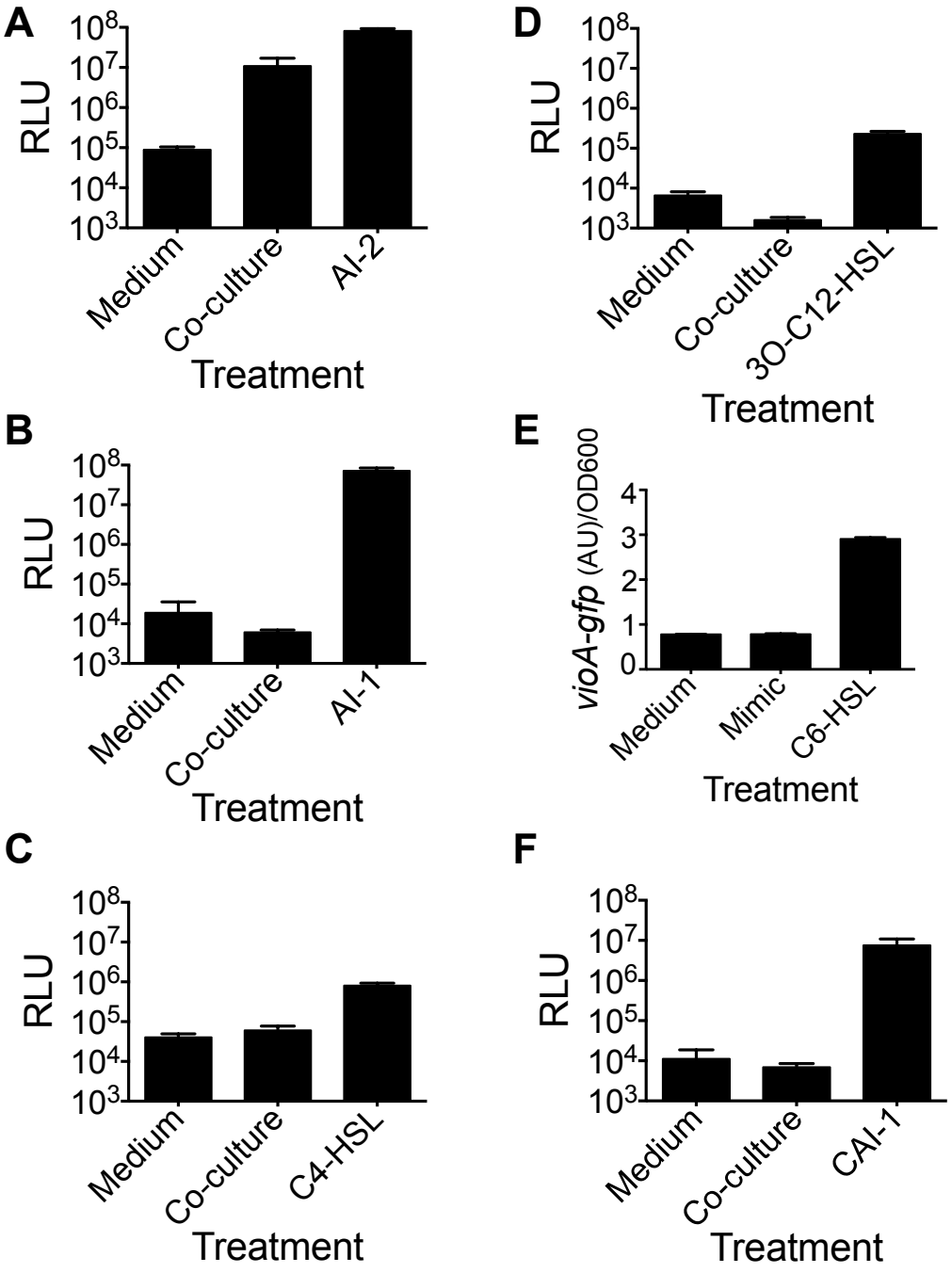

Figure S1 related to Figure 1: Caco-2 cells make an AI-2 mimic but not other autoinducer mimic activities. (A-D) Bioluminescence of bacterial quorum-sensing reporter strains during co-culture with Caco-2 cells. (A) *V. harveyi* TL26 (B) *V. harveyi* TL25 (C) *E. coli* p*rhlR*, p*rhlA-luxCDABE* (D) *E. coli* p*lasR*, p*lasB-luxCDABE* and (E) *gfp* expression from *E. coli* carrying p*cviR,* p*vioA-gfp* in response to conditioned medium from Caco-2 cells grown under PBS-treatment conditions. (F) Bioluminescence from *V. cholerae* WN1102 during co-culture with Caco-2 cells. The cognate autoinducers (A) 1 μM AI-2, (B) 100 nM AI-1, (C) 10 μM C4–homoserine lactone, (D) 2.5 nM 3O-C12-homoserine lactone, (E) 10 μM C6-homoserine lactone or (F) 1 μM CAI-1 ((*S*)-3-hydroxytridecan-4-one) were used as positive controls. Error bars represent SD for three replicates. In the figure, HSL denotes homoserine lactone.

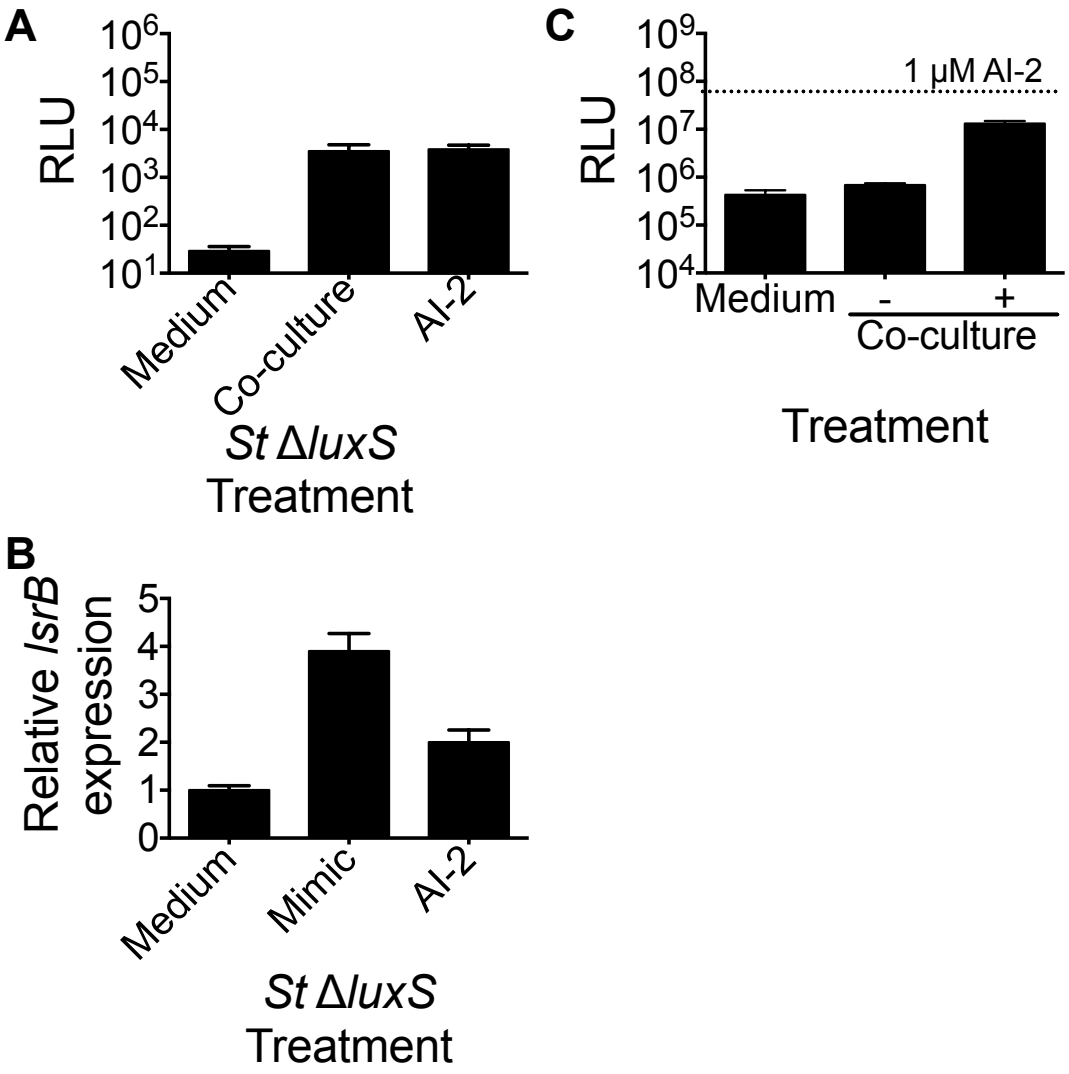

Figure S2 related to Figure 1: *S. typhimurium* responds to the AI-2 mimic and Caco-2 cells do not consitutively produce the AI-2 mimic. (A) Caco-2 cells were co-cultured with the Δ*luxS S. typhimurium* strain MET687 (denoted *St* Δ*luxS*) that carries an *lsr-luxCDABE* transcriptional reporter fusion. Light production from the *lsr-luxCDABE* fusion was measured. (B) *lsrB* mRNA was measured in strain MET687 following exposure to mammalian AI-2 mimic and compared to that in the no exposure (medium) control. Error bars represent SD for triplicate cultures. 50 μM AI-2 was included as a positive control. (c) Caco-2 cells were grown alone or in co-culture with *V. harveyi* TL26. Conditioned medium from each treatment was assayed for AI-2 mimic activity using *V. harveyi* TL26 as in Fig. 3 of the main text. Error bars represent SD for three replicates.

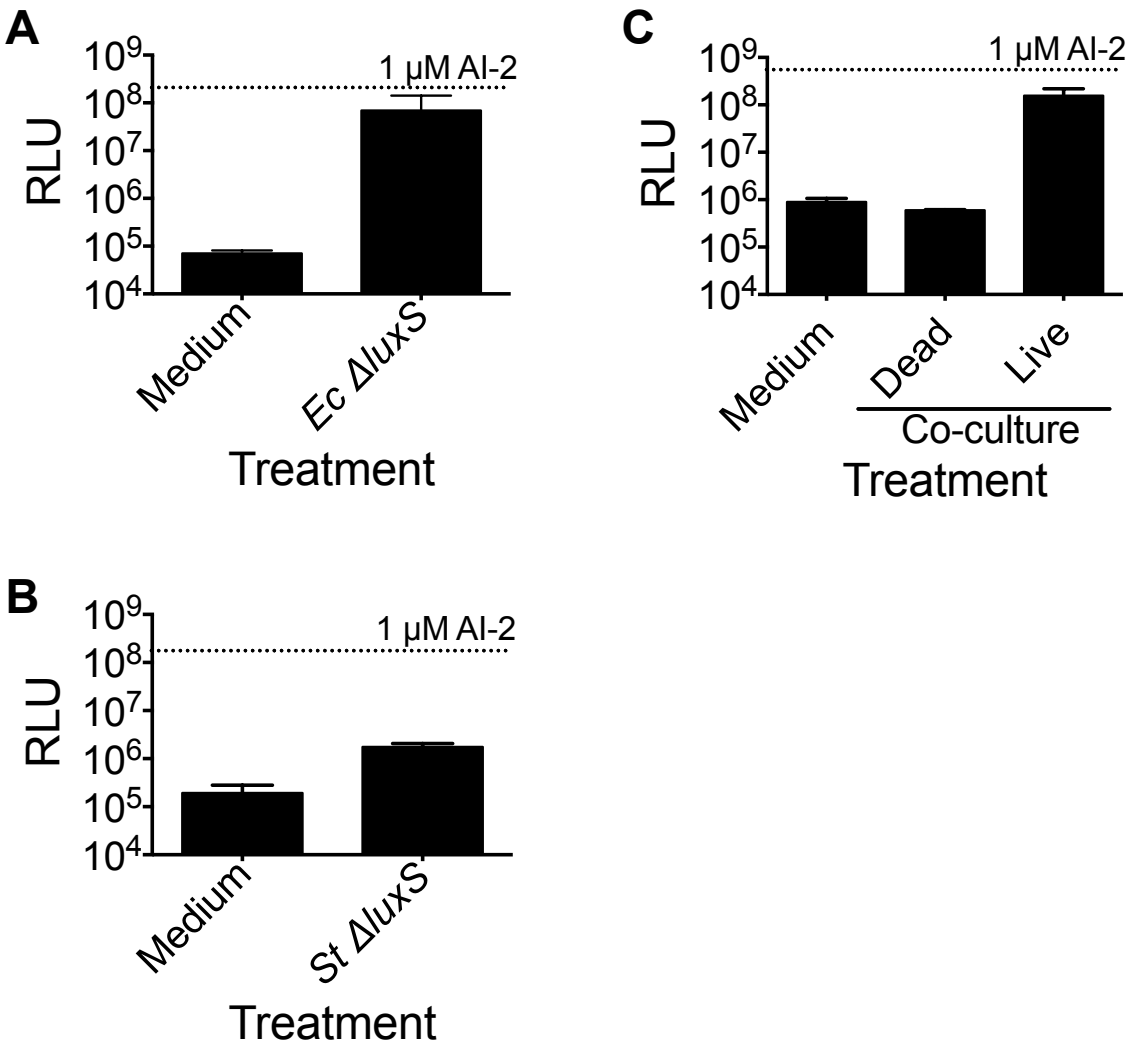

Figure S3 related to Figure 2: The AI-2 mimic is produced in response to *E. coli* and *S. typhimurium*. Caco-2 cells were co-cultured with Δ*luxS* strains of (A) *E. coli* (i.e. *Ec* Δ*luxS*) or (B) *S. typhimurium* (i.e. *St* Δ*luxS*). (C) Live or heat-killed *E. coli* cells were added to Caco-2 cells. Conditioned medium from each preparation was assayed for AI-2 mimic using *V. harveyi* TL26 as in Fig. 3 of the main text. Error bars represent SD for three replicates. 1 μM AI-2 was included as a positive control.

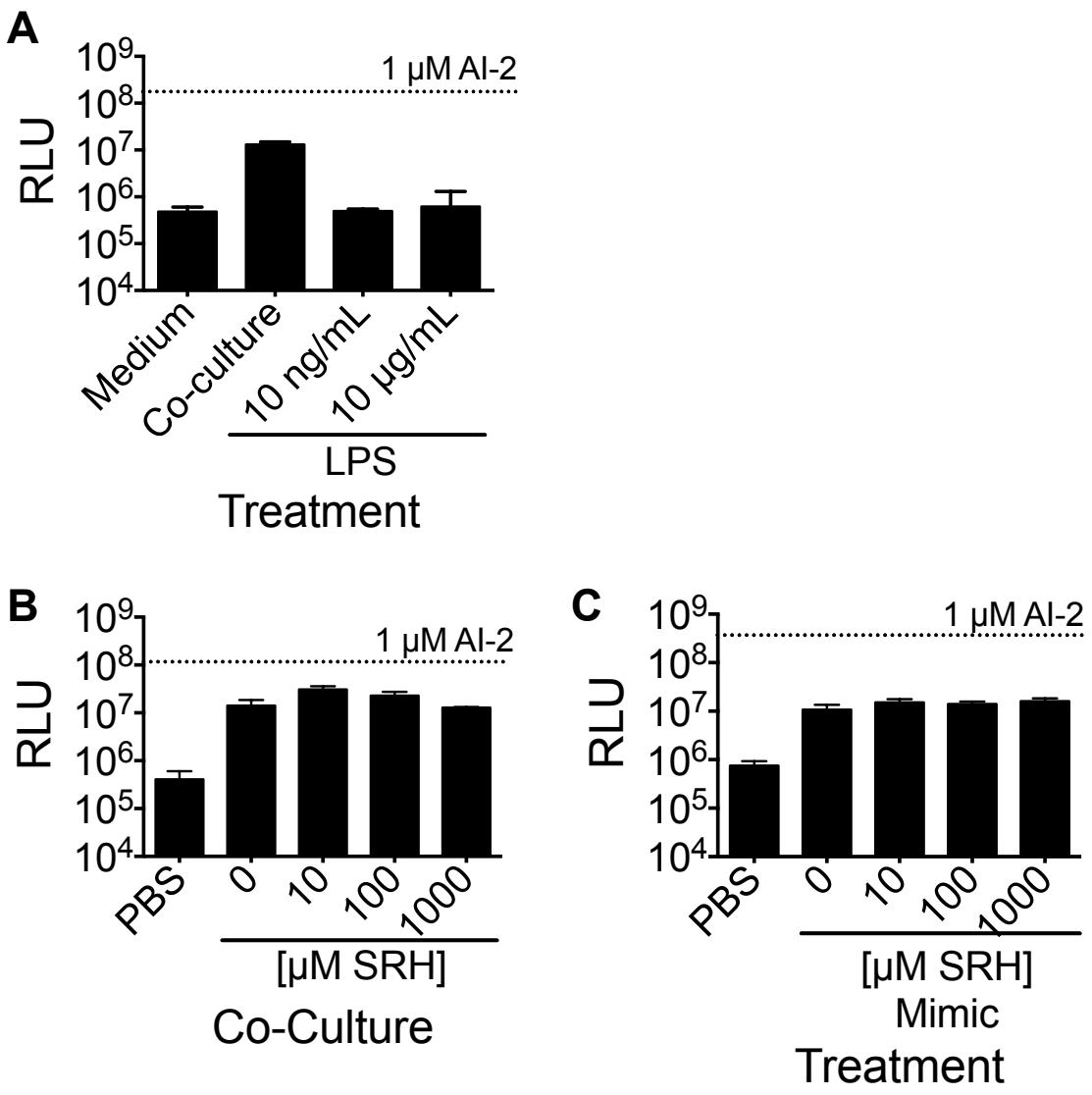

Figure S4 related to Figures 2 and 3: LPS and SRH do not stimulate AI-2 mimic production by Caco-2 cells. (A) LPS (10 ng/mL or 10 µg/mL, Sigma Aldrich) was added to Caco-2 cells under culture conditions. Conditioned medium was collected and assayed for AI-2 mimic activity using *V. harveyi* TL26 as in Fig. 3 of the main text. (B) Bioluminescence of *V. harveyi* TL26 during co-culture with Caco-2, or (C) with AI-2 mimic preparations from PBS-treated Caco-2 cells. In panels B and C, SRH was added at the specified concentrations prior to the incubations. 1 µM AI-2 was included as a positive control. Error bars represent SD for three replicates.

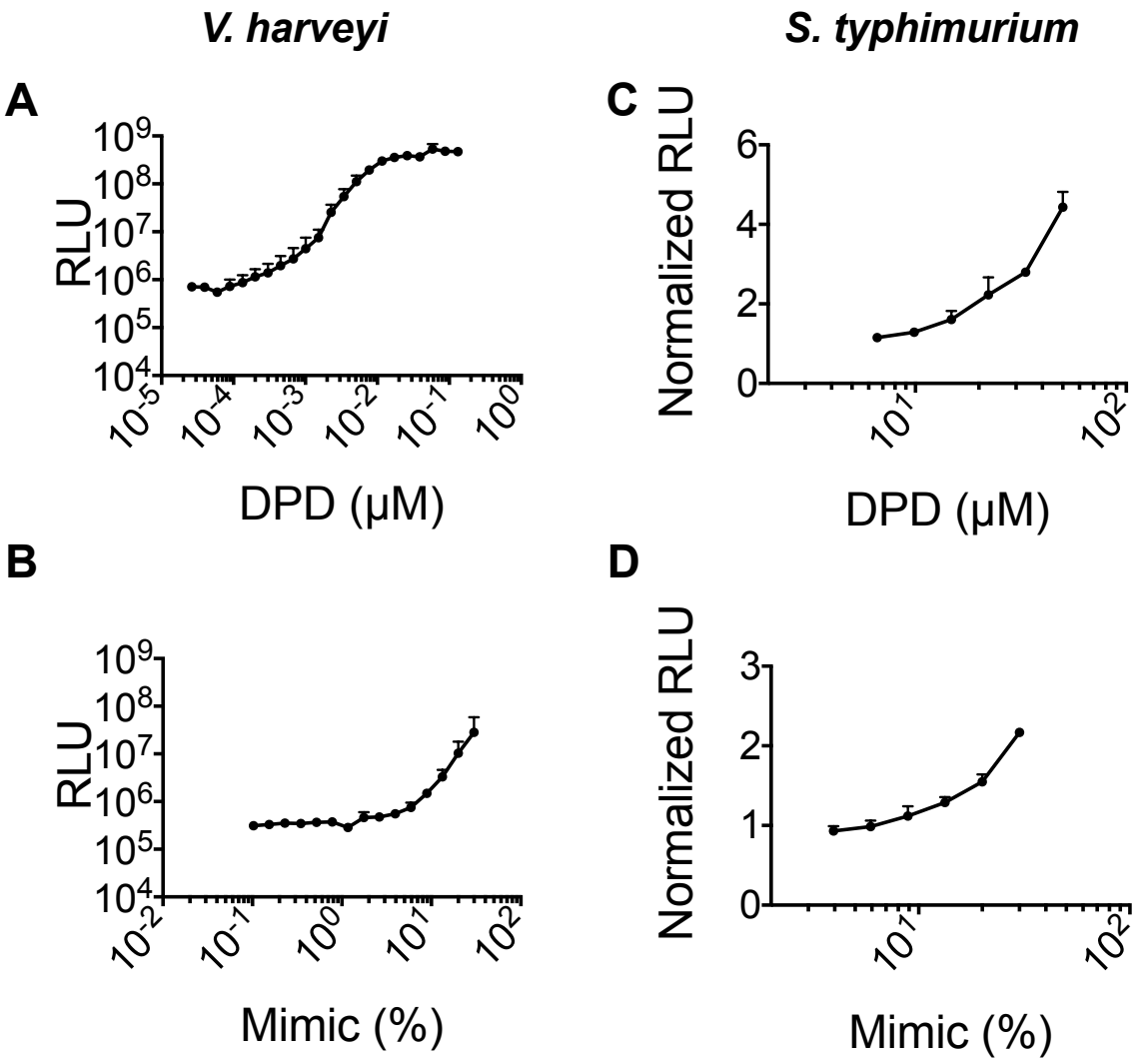

Figure S5 related to Figure 3: The AI-2 mimic and DPD elicit similar response patterns in *V. harveyi* and *S. typhimurium*. Light production from *V. harveyi* TL26 (A and B) and *S. typhimurium* MET687 (C and D) in response to (A and C) DPD or (B and D) AI-2 mimic prepared from PBS-treated Caco-2 cells. Error bars represent SD.

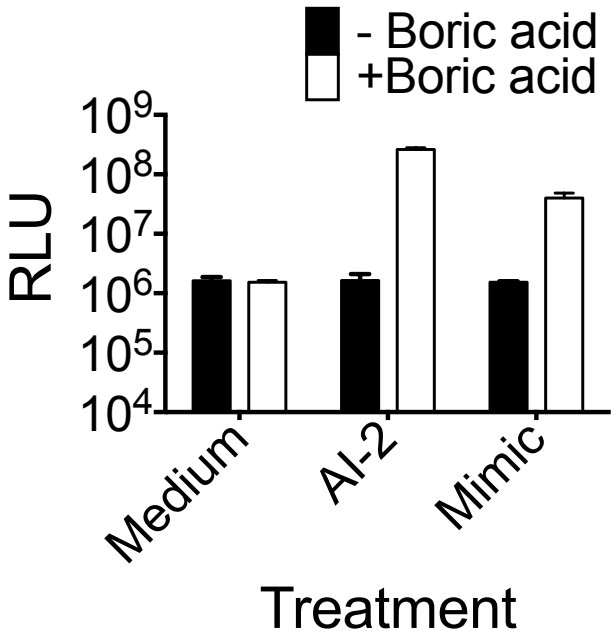

Figure S6 related to Figure 4: Maximal AI-2 mimic activity requires borate. Conditioned medium from PBS-treated Caco-2 cells was tested for AI-2 mimic activity using *V. harveyi* TL26 as in Fig. 3 of the main text. Absence (black) or presence (white) of 0.1 mM boric acid. Error bars represent SD for three replicates.

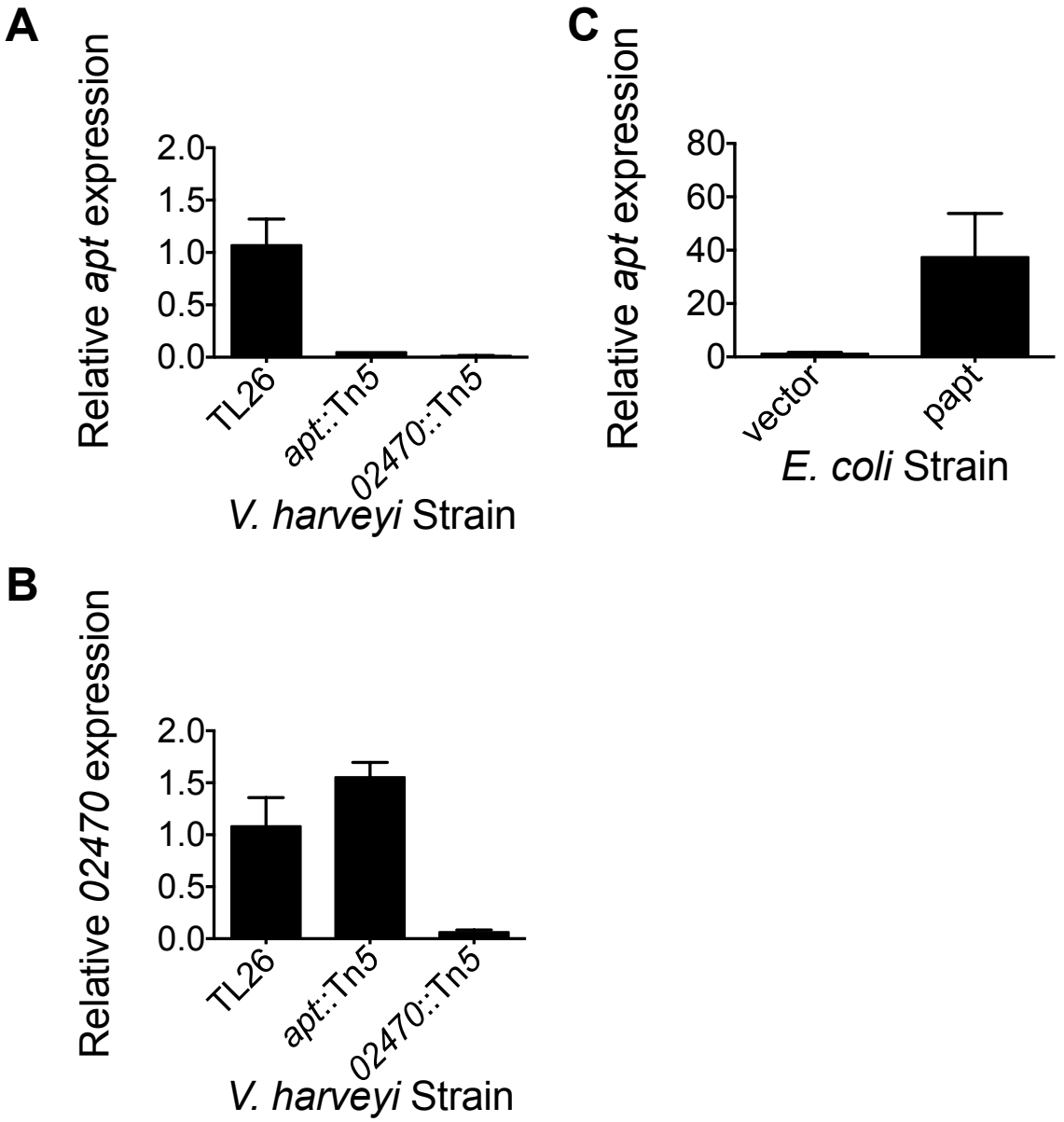

Figure S7 related to Figures 5 and 6: Regulation of *apt*. (A and B) Relative mRNA measured in *V. harveyi* in the absence of *apt* (A) or *VIBHAR_02470* (B) normalized to the levels in their presence. (C) Relative expression of *apt* in *E. coli* normalized to the background control plasmid. Error bars represent SD for three replicates.

Table S1, related to main experimental procedures:

Strain name	Genotype	Source
V. harveyi		
TL25	Δ*luxM*, Δ*luxPQ*, Δ*cqsS*	(Long et al., 2009)
TL26	Δ*luxN*, Δ*luxS*, Δ*cqsS*	(Long et al., 2009)
FED119/pFED368	Δ*luxN*, Δ*luxPQ*, Δ*luxS*/p*luxPQ*	(Neiditch et al., 2006)
FED119/pFED408	Δ*luxN*, Δ*luxPQ*, Δ*luxS*/p*luxP*(W167A) *luxQ*	(Neiditch et al., 2006)
ASI01	*tkt*::Tn*5*	This study
ASI02	*hldE*::Tn*5*	This study
ASI03	*apt*::Tn*5*	This study
ASI04	*VIBHAR02470*::Tn*5*	This study
ASI05	*tkt*::Tn*5*/p*tkt*	This study
ASI06	*tkt*::Tn*5*/p*ASI05*	This study
ASI07	*hldE*::Tn*5*/p*hldE*	This study
ASI08	*hldE*::Tn*5*/p*ASI05*	This study
E.coli		
S17-1λ*pir*	Wild-type	(de Lorenzo and Timmis, 1994)
BL21	Wild-type	Life Technologies
BL21Δ*luxS*	Δ*luxS*	This study
ASI09	BL21Δ*luxS*/p*apt*	This study
ASI11	BL21Δ*luxS*/p*ASI05*	This study
ELS1255	BL21/p*cviR*, p*vioA-gfp*	(Swem et al., 2009)
V. cholerae		
WN1102	Δ*cqsA*Δ*luxQ*/p*luxCDABE*	(Ng et al., 2011)
S. typhimurium		
MET687	Δ*luxS /pMET1047 (Plsr-luxCDABE)*	This study
MET715	*rpsL*, *putRA*::*kanR-lsr-lacZYA*, *luxS*::T-POP	(Taga et al., 2003)

Table S2, related to main experimental procedures:

Plasmid	Description	Source
pRL27	R6K origin, Tn*5* delivery plasmid	(Larsen et al., 2002)
pJV021	cmR removed from gfp-cmR expression cassette in pEVS143	(van Kessel et al., 2015)
pTL18	IPTG-inducible FLP recombinase, from pEVS143 and pCP20	(Long et al., 2009)
pFED368	Plasmid carrying WT *luxPQ*	(Neiditch et al., 2006)
pFED408	Plasmid carrying *luxP* W167A and WT *luxQ*	(Neiditch et al., 2006)
pASI05	kanR removed from pJV021, replaced with tetR.	This study
pASI06	pASI05 with *apt* (BamHI/NdeI)	This study
pASI08	pASI05 with *tkt* (BamHI/NdeI)	This study
pASI09	pASI05 with *hldE* (BamHI/NdeI)	This study

Table S3, related to main experimental procedures:

Primer	Sequence	Use
VC029	cagcaacaccttcttcacga	Sequencing Tn*5* insertion fwd
VC030	aacaaggccagggatgtaacg	Sequencing Tn*5* insertion rev
AI21	gctcaatcaatcaccggatcctcattagtctgctgcttgtatgct	Construction of pASI06
AI22	ggcgtaagcttaaggagatatacatatgcgaaacatcaacatcag	Construction of pASI06
AI23	gcttgctcaatcaatcaccggatcctcaatgaaaccaattctacctg	Construction of pASI09
AI24	ggaattcggcgtaagcttaaggagatatacatttagcctttaccgcctttgatgg	Construction of pASI09
AI25	aagcttgctcaatcaatcaccggatcctcattacgctagcagttctttgctgtg	Construction of pASI08
AI26	aggaattcggcgtaagcttaaggagatatacatatgcactcaggagcagacatgt	Construction of pASI08
AI27	ggagtatctggctgggccaacgttc	tetR cassette from pTL18 fwd
AI28	acatggctctgctgtagtgagtgg	tetR cassette from pTL18 rev
AI29	cgcaacccactcactacagcagagccatgtgccagaagtgagggagcca	Construction of pASI05
AI30	ggttggaacgttggcccagccagatactcctggatgatggggcgattcagg	Construction of pASI05
AI31	ccagcaaagacgtgacctta	qPCR for *apt* fwd
AI32	tgaatcggtaagggtaggaaatg	qPCR for *apt* rev
AI33	aacgctccaagagaggaaatag	qPCR for *VIBHAR02470* fwd
AI34	aacccagtgagacaacataaca	qPCR for *VIBHAR02470* rev
EC105	aacagcgttcagggctatgact	qPCR for *lsrB* fwd
EC106	atcacgcgctccggtaac	qPCR for *lsrB* rev
AI37	actcctacgggaggcagcagt	qPCR for 16S rRNA fwd
AI38	attaccgcggctgctggc	qPCR for 16S rRNA rev

Supplemental Experimental Procedures

Bacterial strains and growth conditions
V. harveyi strains TL25 (Δ*luxM,* Δ*luxPQ,* Δ*cqsS*), TL26 (Δ*luxN,* Δ*luxS*, Δ*cqsS*), FED-119 (Δ*luxN,* Δ*luxPQ,* Δ*luxS*) carrying pFED368 (wild-type LuxPQ) or pFED408 (wild-type LuxQ, LuxP W167A), and TL26 derivatives constructed in this study (Table S1) were grown in Luria-Murine (LM) medium with shaking at 30°C. *E. coli* strains S17-1λ*pir*, BL21(DE3) (Life Technologies), and derivatives (Table S1) were grown with aeration at 37°C in Luria-Bertani (LB) medium. *V. cholerae* strain WN1102 and *S. typhimurium* strains MET715 and MET687 were grown with aeration at 37°C in LB medium. Antibiotics, when appropriate, (Sigma) were used at the following concentrations: 10 μg/mL chloramphenicol, 100 μg/mL kanamycin, 50 μg/mL polymyxin B, 1000 μg/mL streptomycin, and 10 μg/mL tetracycline (except 5 μg/mL in liquid medium for *V. harveyi* and *V. cholerae*). Plasmid constructs (Table S2) were introduced into electrocompetent *E. coli* S17-1λ*pir* or *E. coli* BL21Δ*luxS* using 0.2 μm cuvettes (USA Scientific) and a Bio-Rad MicroPulser. Plasmids were transferred into *V. harveyi* by conjugation between *E. coli* S17-1λ*pir* and *V. harveyi* on LB plates, followed by isolation of exconjugants on LM plates containing polymyxin B and the appropriate antibiotic for plasmid maintenance. DNA manipulations were performed as previously described using Gibson techniques (Gibson et al., 2009). All reagents were purchased from New England Biolabs. PCR reactions used iProof DNA polymase (Bio-Rad). Plasmids were constructed as described in Table S2 using primers listed in Table S3 that were purchased from Integrated DNA Technologies.

Mammalian cell culture:
Caco-2, HeLa, A549, and J774A.1 cells were maintained in 1X DMEM, 20% FBS, 1X Penstrep (Invitrogen). Jurkat and U937 cells were maintained in 1X RPMI, 20% FBS, 1X Penstrep. All cell lines were maintained at 37°C, 5% CO_2. To isolate Caco-2, HeLa, A549, and J774.A1 cells that adhered to tissue culture plate surfaces, culture fluids were discarded, and attached cells were washed with sterile PBS. Cells were detached by addition of 4 mL 1X Trypsin-EDTA (Fisher) and incubation at 37°C, 5% CO_2 for 10 min. Cells were subsequently washed in the cell culture media described above. All cell lines were sub-cultured every 48 h.

Mammalian co-culture with bacteria:
Mammalian cells were plated in 24-well culture plates (Fisher scientific) at a seeding density of 200,000 cells per well (100,000 cells/mL) in co-culture medium. Co-culture medium is 1X DMEM without glucose, 20% FBS, 1X PenStrep (Invitrogen). Cultures were grown to confluence for 48 h at 37°C in the presence of 5% CO_2. Co-culture with bacteria was performed as follows: 1 mL (1:100 overnight dilution) *V. harveyi* TL26 or *V. harveyi* TL25 was back-diluted in Autoinducer Bioassay (AB) broth supplemented with 0.1 mM boric acid, and these cells were added to the mammalian cell culture. 1 mL (1:100 overnight dilution) of *V. harveyi* TL26 or *V. harveyi* TL25 in AB broth was also added to co-culture medium alone, in the absence of mammalian cells, as a negative control. Overnight cultures of *S. typhimurium* MET687 were back-diluted 1:100 in LB broth, and these cells were added to the mammalian cell culture as described above. The mammalian-bacterial co-cultures were incubated for 5 h at 30°C, with 5% CO_2. Following incubation, 100 μL of the co-culture was transferred, in triplicate, to 96-well black clear bottom plates and assessed for bioluminescence using an EnVision® Multilabel Reader.

Transwell co-culture experiments:
Mammalian cells were plated in 24-well culture plates (Fisher scientific) at a seeding density of 200,000 cells per well (100,000 cells/mL) in co-culture medium as described above. 1 mL co-culture medium was added to the upper chamber of the transwell. After mammalian cells were grown to confluence for 48 h, transwell co-culture with bacteria was performed as follows: 1 mL (1:100 overnight dilution) *V. harveyi* TL26, was back-diluted in AB broth supplemented with 0.1 mM boric acid, and added to the upper chamber of the transwell hanging above the Caco-2 cells. *V. harveyi* TL26, in the absence of Caco-2 cells, or Caco-2 cells, in the absence of *V. harveyi* TL26, were grown in this medium in transwells as negative controls. The co-cultures were incubated for 5 h at 30°C, with 5% CO_2. After incubation, *V. harveyi* TL26 cells from the transwell were transferred to 96-well black clear bottom plates and assessed for bioluminescence as described above. Overnight cultures of Δ*luxS S. typhimurium* or Δ*luxS E. coli* (live or heat-killed) were back-diluted 1:100 in LB broth, and were added to the upper chamber of a transwell as

described above. Following 5 h co-culture, 10% v/v conditioned medium from the upper chamber of the transwell was assayed, in triplicate, for AI-2 mimic activity using the *V. harveyi* TL26 detector strain.

AI-2 mimic production from PBS- or DSS-treated Caco-2 cells:
Caco-2 cells were detached from tissue culture plates using trypsin-EDTA. Isolated Caco-2 cells were washed in cell-culture medium to remove trypsin and serum components. Caco-2 cells were next incubated in either Dulbecco's PBS (DPBS; Invitrogen), or 2.5% dextran sulfate sodium prepared in DMEM medium (DSS; Sigma Aldrich) at a cell density of 200,000 cells/mL for 48 h at 37°C, in the presence of 5% CO_2. The cells were removed by centrifugation at 1,500 rpm for 5 min, followed by filtration with Stericup filter units (Millipore), and the conditioned medium preparations were stored at 4°C. 10% v/v conditioned media were tested for AI-2 mimic activity using the *V. harveyi* TL26 bioluminescence assay.

S-ribosylhomocysteine (SRH) conversion assay:
Caco-2 cells were co-cultured with *V. harveyi* TL26 as described above for 5 h at 30°C, with 5% CO_2. 0, 10, 100, or 1000 µM SRH was added during the co-culture period. Following incubation, 100 µL of the co-culture was transferred, in triplicate, to 96-well black clear bottom plates and TL26 was assessed for bioluminescence using an EnVision® Multilabel Reader. Alternatively, AI-2 mimic was produced from PBS-treated Caco-2 cells as described above. 0, 10, 100, or 1000 µM SRH was added to these preparations followed by overnight incubation at 37°C with 5% CO_2. 10% v/v of these preparations were tested for AI-2 mimic activity using the TL26 bioluminescence assay.

Caco-2 cell permeability assay:
Caco-2 cells were grown to confluence at a cell density of 200,000 cells/mL in the apical chamber of a 0.4 µm transwell (Becton, Dickinson) in 24-well plates for 48 h in DMEM, PBS or 2.5% dextran sulfate sodium prepared in DMEM medium. 200 µL of 50 µM Lucifer Yellow (LY) was added to the top chamber of a transwell plate containing a monolayer of Caco-2 cells. 600 uL fresh medium (DMEM, PBS, or 2.5% DSS) was placed in the bottom chamber. The plate was incubated at 37°C with orbital shaking (60 rpm) for 1 h. Aliquots of 200 µL were transferred from the basal chamber, in triplicate, to a solid 96-well black plate (Fisher), and transfer of LY was assessed by measuring fluorescence using an Envision Multilabel plate reader (Excitation/Emission wavelength 480/530nm). LY transfer through a transwell without Caco-2 cells was used as a positive control.

Lactate dehydrogenase assay:
The Pierce™ LDH Cytotoxicity Assay Kit (Thermo Fisher) was used to assay lactate dehydrogenase release. Caco-2 cells were grown to confluence (200,000 cells/mL) in 96-well plates for 48 h at 37°C, 5% CO_2 in DMEM, PBS, or 2.5% DSS prepared in DMEM medium. Caco-2 cells were also treated with 10% v/v water to assess spontaneous LDH activity. Caco-2 cells treated with the kit-provided lysis buffer for 45 minutes at 37°C, 5% CO_2 served as the maximum LDH release control. Controls containing medium but no cells were included to determine LDH background activity. Triplicate 50 µL aliquots of cell-free culture fluids from the incubations were transferred to 96-well plates, and 50 µL of the kit-provided reaction mixture was added to each sample well. Plates were incubated in the dark at room temperature for 30 min, after which 50 µL of stop solution from the kit was added to each sample. Absorbance was measured at 490 nm and 680 nm on a Synergy™ Mx plate reader (Biotek). To determine LDH activity, the 680 nm absorbance value was subtracted from the 490 nm absorbance reading. % cytotoxicity was determined by comparing the LDH activity in the samples to the maximum LDH release control activity.

LuxP-AI-2 mimic binding assay:
1 mL of AI-2 mimic was lyophilized to powder and resuspended with 7.7 nmol of LuxP or BSA in 100 µL total volume. For the AI-2 control, an approximately equal molar ratio of AI-2 to protein was used. Reactions were incubated overnight in phosphate buffer in the presence of 2 mM boric acid at room temperature. Following overnight incubation, samples were concentrated in Amicon Ultra-0.5 mL Centrifugal Filters; 10,000 molecular weight cutoff (EMD Millipore). The concentrated product was washed once with 100 µL PBS and filtered again to remove unbound AI-2 mimic or AI-2. The resulting concentrated product was removed from the filter and incubated at 70°C for 5 minutes to denature protein and to release AI-2 mimic or AI-2. Denatured protein was pelleted and the resulting supernatant was tested

for activity using the bioluminescence assay. AI-2 capture by LuxP was used as a positive control. AI-2 mimic capture by BSA was used as a negative control.

Screen for V. harveyi TL26 mutants defective in stimulating AI-2 mimic production or in AI-2 mimic detection:
V. harveyi TL26 was conjugated with *E. coli* S17 λ*pir* containing pRL27 overnight on LB plates at 30°C, and the mixture was subsequently plated onto LM agar plates containing kanamycin and polymyxin B to select for exconjugants harboring Tn*5* insertions. A total of ~5,000 colonies from each of six separate matings were pooled and resuspended in 5 mL of LM medium supplemented with kanamycin and polymyxin B. These approximately 30,000 Tn*5* insertion mutants were screened for decreased bioluminescence during co-culture with Caco-2 cells. Specifically, individual colonies were placed into 100 μL of AB broth supplemented with 0.1 mM boric acid in separate wells of 96-well plates previously seeded with 50,000 Caco-2 cells. These co-cultures were grown for 5 h at 30°C in the presence of 5% CO_2. To screen for defects in stimulation or detection of the mammalian AI-2 mimic, *V. harveyi* TL26 transposon mutants were assayed for those exhibiting low bioluminescence during co-culture but high bioluminescence in the presence of 100 nM synthetic AI-2. To map the locations of the Tn*5* insertion sites, total DNA was prepared from the strains, digested with KpnI, ligated, and transformed into *E. coli* S17λ*pir*. The transposon contains an R6K origin enabling replication in *pir*$^+$ *E. coli*. Plasmids were isolated from kanamycin resistant transformants and the DNA flanking the transposons was sequenced with primers VC029 and VC030 (Table S2). Sequences were analyzed by BLAST against the *V. harveyi* genome (ATCC BAA‐1116).

Quantitative real-time PCR analysis
Vibrio harveyi TL26, *apt*::Tn*5*, *02470*::Tn*5* strains were grown overnight at 30°C in LM medium and diluted 1:1000 in LM medium and shaken for 6 h, after which cell pellets were isolated and flash frozen with liquid nitrogen. Pellets were stored at −80°C prior to RNA isolation using the RNeasy Mini Kit (Qiagen). RNA was quantified and 2 μg of RNA was converted to cDNA with Superscript III reverse transcriptase (Invitrogen). Quantitative real-time PCR analysis was performed with primers for *apt* and *VIBHAR_02470*. *E. coli* p*apt* overexpressing strains or control *E. coli* carrying the empty vector were grown overnight at 37°C in LB medium with appropriate antibiotic and diluted 1:500 in LB medium and shaken for 6 h, after which cell pellets were isolated and quantitative real-time PCR analysis was performed for *apt*. *S. typhimurium* MET687 was grown overnight at 37°C in LB medium and diluted 1:1000 in fresh medium supplemented with 50 μM AI-2 or 20% v/v AI-2 mimic from PBS-treated Caco-2 cells. The cultures were shaken for 6 h at 30°C, after which, cell pellets were isolated and quantitative real-time PCR analysis was performed for *lsrB* as described above. In all cases, 16S rRNA served as the internal control.

S. typhimurium bioluminescence assays
S. typhimurium MET687 was grown overnight in LB medium with shaking at 37°C. The culture was diluted 1:1000 in the presence of a 1.5-fold dilution series of DPD (starting at 50 μM DPD) or PBS-derived AI-2 mimic (starting at 30% v/v AI-2 mimic) in a 96-well plate. Cells were grown for 24 h in Synergy™ Mx plate reader (Biotek). Bioluminescence and OD_{600} were measured every 20 min. The results from the 7-hour reading are shown. Normalized RLU are defined as counts per minute per mL per OD_{600} normalized to an untreated control.

Supplemental References

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