SUPPORTING INFORMATION

Materials and Methods

Plasmids and Strains. The pDawn and pPL-PCB(S) plasmids were provided by Andreas Möglich and J. Clark Lagarias, respectively. The plasmids pJT122 and pJT106b were purchased from Addgene (Plasmids 31396 and 31392, respectively). The *E. coli* strain JT2 was obtained from Jeff Tabor. BL21(DE3) strains transformed with pDawn-mCherry or pDawn-ClyA were maintained in 50 μg/mL kanamycin. JT2 strains transformed with pPL-PCB(S) and either pMars-mCherry or pMars-ClyA were maintained in 50 μg/mL kanamycin, 17 μg/mL chloramphenicol, and 100 ug/mL spectinomycin. Cultures were grown at 37°C with shaking (250 rpm) unless otherwise specified. SKBR3 cells were obtained from Matthew Lazzara and the cells were routinely cultured at 37°C in a humidified atmosphere with 5% CO_2.

Cloning. The Cytolysin A (*clyA)* gene from *E. coli* K12 (locus b1182) was codon optimized and synthesized (Integrated DNA Technologies). Standard restriction enzyme and ligase cloning was used to construct pET26b-ClyA (NdeI and XhoI restriction sites), pDawn-mCherry and pDawn-ClyA (NdeI and BamHI restriction sites). To construct pMars-mCherry and pMars-ClyA, the following DNA fragments from pJT122 (Addgene), pJT106b (Addgene), and pDawn were amplified by PCR. From pJT122, a 4.2kb fragment was amplified using Primers 1 and 2 (Supplementary Table 1). This backbone fragment included the cph8 gene, the kanamycin resistance gene, and the ColEI origin of replication and had XmaI and BglII restriction sites at each end. The pOmpC promoter and the cI repressor gene from pJT106b were amplified using Primers 3 and 4. The λ promoter and mCherry/ClyA from the pDawn plasmid were amplified using Primers 5 and 6. Using overlap assembly PCR with a 20 bp overhang, the two fragments

were combined to create an insert that was then ligated into the backbone amplified from pJT122 with *XmaI* and *BglII* to create either pMars-mCherry (with the mCherry reporter gene) or pMars-ClyA (with the ClyA gene).

Protein Purification and Cytotoxicity Assay. Overnight cultures of BL21(DE3) transformed with pDawn-ClyA or JT2 cultures transformed with pMars-ClyA and pPL-PCB(S) were diluted and allowed to grow to an OD_{600} of ~0.8 and then induced with 1 mM IPTG, blue light (~480 nm and 37.7 $\mu W/cm^2$), or red light (~650 nm and 17.7 $\mu W/cm^2$) (similar red light power magnitudes have been previously used to induce cph8[1]) at 25°C. Cultures were pelleted by centrifugation and sonicated. His-tagged proteins were column-purified using low-density nickel agarose beads (Gold Biotechnology). Samples were run on 1 mm 4-12% Bis-Tris SDS-PAGE gels and protein loading was standardized by the Bradford Assay. Cytotoxicity assays using the Cytotox-Fluor kit (Promega) were conducted according to the manufacturer's instructions with purified ClyA diluted in water. Emission values at 520 nm were normalized to calculate fold change over the negative control vehicle (water).

Light-Induction Time Courses. BL21(DE3) transformed with pDawn-mCherry or JT2 transformed with pMars-mCherry and pPL-PCB(S) were grown overnight in dark conditions and diluted into 50 mL cultures at an OD_{600} of ~0.1. Cultures were transferred to a shaker-incubator where they were illuminated continuously by blue or red light. For time course experiments, 5 mL of these cultures were transferred to dark conditions at the given illumination times. All cultures were assayed on fluorescent plate reader at the end of the 24 hour period.

Blood Agar Assays. Overnight cultures of BL21(DE3) transformed with pDawn-ClyA or JT2 transformed with pMars-ClyA and pPL-PCB(S) were diluted $1{:}10^7$ in LB and plated on Columbia agar plates seeded with 5% sheep erythrocytes (BD Diagnostics). Following 12 hours

of growth in the dark at 37°C for colony formation, plates were switched to either light (blue: ~480 nm, 37.1 μW/cm^2 or red: ~650 nm, 17.7 μW/cm^2) or dark conditions at 25°C for 24 hours prior to imaging. Negative controls of pDawn-mCherry and pMars-mCherry were prepared in the same fashion. All blood agar experiments were repeated in at least 3 independent trials.

Statistics. Cytotoxicity results and pDawn-mCherry time points were compared using one-way ANOVA with Bonferroni post-hoc comparisons. Red light-activated mCherry expression in pMars-mCherry was compared using two-way ANOVA with Bonferroni post-hoc comparisons. Data are presented as mean ± standard deviation (SD).

Reference:

1. Tabor, J. J., Levskaya, A., and Voigt, C. A. (2011) Multichromatic control of gene expression in *Escherichia coli*. *J. Mol. Biol. 405*, 315–24.

Supplementary Table 1: Primers used to construct pMars plasmids.

Number	Name	Sequence
1	XmaI-cph8 Fwd	TTGAAACCCGGGATGGCATTGGGTATCGTCTG
2	pJT122-BglII Rev	ATGGAGATCTGACGTTGATCGGCACGTAAG
3	XmaI-pOmpC Fwd	ACTACCCGGGACTAGAGTCCCTTGCATTTAC
4	cI Overlap	GCGAGGAAGCCTGCATAACG
5	pLambda Fwd Overlap	CGTTATGCAGGCTTCCTCGCTAACACCGTGCGTGTTGAC
6	pLambda-BglII Rev	GATCAGATCTCTTAATGCGCCGCTACAGGG