Synthesis of the photocaged erythromycin 3. The E-9-oxime of erythromycin (2)\(^1\) (150 mg, 0.200 mmol) was dissolved in dry acetone (8 ml) under a nitrogen atmosphere. 6-Nitropiperonyl chloride (432 mg, 2.00 mmol) and K\(_2\)CO\(_3\) (193 mg, 1.40 mmol) were added and the resulting mixture was heated under reflux for 17 h, cooled to room temperature, and concentrated under vacuum. The crude product was dissolved in 20 ml of ethyl acetate, washed with saturated sodium bicarbonate solution (2 x 3 ml) and brine (5 ml), and dried over anhydrous Na\(_2\)SO\(_4\). The filtrate was concentrated and purified by column chromatography, eluting with 100:1:1 DCM/MeOH/TEA, providing 3 (161 mg, 87% yield) exclusively as the E isomer as reported previously in the literature\(^1\), as a light yellow solid. \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 7.59 (s, 1H), 6.97 (s, 1H), 6.11 (s, 2H), 5.38 (s, 2H), 5.05 (dd, \(J = 11.1\) Hz and 1.5 Hz, 1H), 4.89 (d, \(J = 4.8\) Hz, 1H), 4.40 (d, \(J = 7.5\) Hz, 1H), 4.02 (s, 1H), 3.98 (d, \(J = 9.3\) Hz, 2H), 3.86-3.71 (m, 1H), 3.66 (s, 1H), 3.56 (d, \(J = 10.8\) Hz, 1H), 3.51-3.43 (m, 1H), 3.42 (s, 1H), 3.30 (s, 3H), 3.20 (dd, \(J = 7.5\) Hz and 10.5 Hz, 1H), 3.03-2.97 (m, 2H), 2.91-2.82 (m, 2H), 2.67 (q, \(J = 6.6\) Hz, 1H), 2.49-2.30 (m, 2H), 2.27 (s, 6H), 2.16 (d, \(J = 10.2\) Hz, 1H), 2.01-1.83 (m, 2H), 1.70-1.56 (m, 4H), 1.54-1.52 (m, 1H), 1.49-1.39 (m, 4H) 1.29-1.26 (m, 4H), 1.25-1.20 (m, 5H), 1.19-1.15 (m, 5H), 1.12-1.04 (m, 12H), 0.82 (t, \(J = 7.2\) Hz, 3H); MS [M+H] calcd C\(_{45}\)H\(_{74}\)N\(_3\)O\(_{17}\) 928.5, found 928.6.
**$^1$H NMR spectrum of caged erythromycin 3**

![NMR spectrum](image)

**Plasmid Maps**

![Plasmid maps](image)

**Fig. S1** Plasmid maps of pMLGFP and pJZ12.
Sequence of pMLGF3

tctagtctacagtctacagacacgccttagtggctactaactcgacgacggttgtgctgcctggatcggccacactcatgcgtgttggcctctctagccgacagtctcgctcagttgcacggtgtgctacatccacatctccatctcaacctccctctctatctctatcattctatattttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttt
Sequence of pJZ12

tcattccgctgttatggccgcgtttgtctcattccacgcctgacactcagttccgggtaggcagttcgct

Electronic Supplementary Material (ESI) for Molecular BioSystems
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Cloning of pMLEGFP. pMLGFP (3957 bp, 500 ng) was digested with SpeI and PmeI at 37 °C for 1 hour, and separated on a 1% agarose gel in TBE buffer at 80 V for 45 minutes. The digested plasmid backbone was excised from the gel and purified with the Qiagen Gel Extraction kit. EGFP was amplified from pEGFP-N1 by PCR with Pfu Ultra DNA polymerase (Stratagene) with forward (5’-GAACTAGTATGGTGAGCAAGGGCGAG-3’) and reverse (5’TACTTGTACAGCTCGTCCATGCC-3’, the 5’ terminus was phosphorylated) primers (IDT). The PCR product was verified by gel electrophoresis with a band apparent at ~800bp, and was purified, digested with SpeI and purified again on an ion-exchange column (Qiagen). Digested pMLGFP (50 ng) was then ligated with the PCR-amplified EGFP in 1:6, 1:3, and 1:0 ratios using T4 DNA ligase (NEB). Ligations were performed at 4 °C overnight, and then transformed into GC5 competent cells. Clones from the 1:6 ligation were sequenced to verify proper sequence and orientation of the EGFP gene. Positive clones were co-transformed into Top10 cells (genetically similar to DH10B) with pJZ12.

Evaluation of erythromycin analogues. E. coli cells (GeneHogs DH10B) containing pJZ12 and pMLGF were grown to saturation in LB broth containing ampicillin (50 μg/mL) and tetracycline (15 μg/mL). An aliquot (40 μL) was spread onto agar plates (10 cm) pre-treated with 40 μL of erythromycin or 9-oxime erythromycin (0.5 mg/mL in DMSO) and incubated at 37 °C overnight, followed by visualization on a transilluminator (365 nm, 25 W).
Fig. S2 GFP expression in bacteria induced by erythromycin 1 (left) and 9-oxime erythromycin 2 (right).

**Erythromycin decaging time course.** *E. coli* (GeneHogs DH10B) containing pMLGFP and pJZ12 were grown at 37 °C overnight in LB media containing ampicillin (50 µg/mL) and tetracycline (15 µg/mL). Cells were diluted 50-fold into a 25 mL culture and grown to OD<sub>600</sub> = 0.6 at 37 °C. Compound (either erythromycin 1, 9-oxime erythromycin 2, or caged erythromycin 3 in DMSO) was added to a final concentration of 1 µM. Cells (1 mL) treated with compounds were transferred to a 10 cm petri dish and irradiated at 365 nm for 5 min. The cells were then transferred to a 96 well plate and grown for 12 hours at 37 °C, then pelleted by centrifugation and re-suspended in lysis buffer (100 µL, 50 mM Tris Base, 1 mM EDTA, 100 mM NaCl, 0.2% Triton X) and fluorescence was read on a plate reader (395/509 ex/em, Biotek Synergy 4 Microplate Reader).

**Bacterial logic gate.** *E. coli* (Top10, Invitrogen) containing pMLEGFP and pJZ12 were at 37 °C overnight in LB media containing ampicillin (50 µg/mL) and tetracycline (15 µg/mL). Cells were diluted 50-fold into a 25 mL culture and grown to OD<sub>600</sub> = 0.6 at 37 °C. 3 was added to a final concentration of 1 µM. Cells that were to receive I₁ were irradiated for 5 minutes at 365 nm in a petri dish (5 cm), and then were transferred to a 96 well plate, and grown overnight, as well as cells that were not irradiated. Cells were pelleted by centrifugation and resuspended in lysis buffer and were imaged on a Typhoon scanner.

**Spatial control of EGFP expression.** *E. coli* cells (Top10, Invitrogen) containing pJZ12 and pMLEGFP were grown to saturation in LB broth containing ampicillin (50 µg/mL) and tetracycline (15 µg/mL). An aliquot (10 µL) was spread onto agar plates (5 cm) pre-treated with 3 or 2 (10 µL, 0.5 mg/mL in DMSO stock solution). Plates containing 3 were placed on a transilluminator (UVP High Performance Transilluminator, 365 nm, 25 W) and one half of the plate was covered with an aluminum foil mask. The other side of the plate was irradiated for 5 minutes at 365 nm. Plates were then incubated overnight at 37 °C, and then imaged for EGFP expression. (Typhoon FLA 7000, GE; FAM filter 473 nm/[Y520]).

**Bandpass filter experiment.** *E. coli* (Top10, Invitrogen) containing pMLEGFP and pJZ12 were at 37 °C overnight in LB media containing ampicillin (50 µg/mL) and tetracycline (15 µg/mL). Cells were diluted 50-fold into a 25 mL culture and grown to OD<sub>600</sub> = 0.6 at 37 °C. 3 was added to a final concentration of 1 µM to 15 mL of cells. 1 mL aliquots of cells were then transferred to separate petri dishes (5 cm) and irradiated for 0 sec, 1 sec, 10 sec, 100 sec, 500 sec, 1000 sec, 3000 sec and 4000 sec on a transilluminator (365 nm, 25W), transferred to a 96 well plate and allowed to grow overnight at 37°C. Cells were pelleted by centrifugation and resuspended in lysis buffer. Fluorescence was read on a plate reader.
**UV Toxicity Study.** Top10 cells were grown in LB media to $OD_{600} = 0.56$ at 37 °C, and then 5 mL aliquots were irradiated for 30, 45, or 60 minutes (365 nm, 25W), transferred to a 96 deep well block and allowed to grow at 37 °C. The optical density was recorded every 4 hours, for 12 hours, and after 22 hours incubation. Cells exhibited virtually no growth after being irradiated for 60 min.