Supplementary Information
"Synchronized cycles of bacterial lysis for *in vivo* delivery"

Host Strains and Culturing

Strains with our lysis circuit were grown in LB media with 50 μg ml^{-1} and 34 μg ml^{-1} of the respective antibiotics (kanamycin and chloramphenicol) along with 0.2% glucose in a 37^{o}C shaking incubator. The glucose was added in order to decrease expression from the luxR promoter, as this promoter has a binding site for the CAP-cAMP activating complex[1]. When glucose levels in the cell are high, levels of cAMP are low, decreasing the transcriptional activation from CAP-cAMP.

For the microfluidics experiments we selected a non-motile *S. typhimurium* host for our circuit, SL1344 (M913: *fliGHI* mutant)[2]. For well-plate co-culture experiments and *in vivo* experiments, we utilized an attenuated *S. typhimurium* host, SL1344 (ELH1301: Δ*phoPQ* Δ*aroA*)[3], that was shown in previous work to have good efficiency in plasmid retention[4]. For *in vivo* experiments where the luminescence expression of the circuit was measured, we utilized another attenuated strain of *S. typhimurium* SL1344 as the circuit host (ELH430: Δ*phoPQ*) where luminescence expression was higher (see Extended Data Fig. 3a). For the constitutive luminescence case we used a well characterized bacterium with a constitutive luminescence cassette, *E. coli* Nissle 1917 integrated with p16Slux[5]. A list of strains and plasmids are shown in Extended Data Table 1.

For co-culture experiments, we used the HeLa cell line in DMEM (complete medium) supplemented with 10% fetal bovine serum and appropriate antibiotics for the circuit. HeLa cells were initially grown with penicillin/streptomycin (CellGro 30-002-CI) in the growth medium and placed inside a tissue culture incubator at 37^{o}C with 5% CO_2 before the experiment. For loading on a microfluidic chip or well-plate, HeLa cells were initially washed with dPBS and dis-adhered with 0.05% or 0.25% trypsin EDTA. Cells were then pelleted and resuspended in DMEM + FBS and the appropriate antibiotics.

Plasmids

Our circuit is composed of two plasmids, an activator plasmid and a lysis/therapeutic plasmid. The main activator plasmid is pTD103LuxI sfGFP which was used in previous work from our group[6]. This plasmid contains the ssrA-LAA degradation tag (amino-acid sequence of AANDENYALAA) on LuxI and sfGFP, a superfolding green fluorescent protein variant[7]. pTD103LuxI (-LAA) was constructed by removing the ssrA-LAA tag from LuxI. Most of the construction was done using the CPEC method of cloning[8].

The lysis plasmids were constructed using the modular pZ plasmid set-up with a p15a origin of replication and a chloramphenicol resistance marker[9]. The lysis gene, E from the bacteriophage ϕX174, was kindly provided by Lingchong You and was taken from the previously reported ePop plasmid via PCR[10]. The E gene was placed under the expression of the LuxR-AHL activatable

luxI promoter. In order to construct the therapeutic versions of the lysis plasmids, we extracted the *hlyE* gene from the genomic DNA of *E. coli* strain MG1655 via PCR and inserted it into the lysis plasmid. The promoter used to drive expression of HlyE was the *tac* promoter for *in vitro* characterization, and the *luxI* promoter for *in vivo* testing. The CDD-iRGD and mCCL21 genes were synthesized as fragments before inserting into the appropriate plasmids.

For the plasmids used in the circuit strains tested *in vivo*, we inserted two stabilizing elements, the hok/sok system and alp7 partitioning system, into the activator and lysis/therapeutic plasmids. Recent work has shown that addition of the hok/sok toxin-antitoxin system and the alp7AR cassette from the *B. subtilis* plasmid pLS20 enables plasmid retention *in vivo*[11,12]. See Extended Data Fig. 5 for maps of the the plasmids used in this study.

Microscopy and Microfluidics

The microscopy and microfluidics techniques described here are similar to those reported previously from our group[13]. Briefly, our microfluidic devices were constructed from PDMS (polydimethylsiloxane) which was molded and baked on a silicon wafer with micron-scale features formed by cross-linked photoresist. Individual devices (formed by a set of features transferred from the wafer to the PDMS) were then cut out of the baked PDMS and holes were punctured in the devices to allow for the connection of fluid lines. The devices were then bonded onto coverslips and placed on a microscope stage for cell loading and imaging. Fluid lines were connected to the devices from various syringes supplying media, cells, or acting as waste reservoirs. The

flow direction in the device was controlled by changing the relative heights between the relevant syringes resulting in hydrostatic pressure driven flow.

We cultured cells to an optical density (A_{600nm}) of approximately 0.1 (using 1.5 ml cuvettes from Plastibrand) before loading a device. Before loading, devices were 'wetted' with the media syringes to remove bubbles in the channels[14]. Devices were loaded from the cell port by lowering the designated waste port such that the relative changes in height of the cell loading syringe and waste syringe resulted in flow of cells from the cell port to a waste port. Once cells were loaded in the traps, the flow direction was reversed allowing media to flow into all ports, thus supplying the trapped cells with a continuous perfusion of nutrients. In these microfluidic experiments we added 0.075% Tween20 to the media and cell suspension fluid to prevent cells from adhering to channels and ports within the device. Experiments for characterizing circuit behavior in Fig. 1-2 were done in a side-trap array device, as described previously[13]. The device used with the GFP sink is also arranged as a side-trap array. See Extended Data Fig. 2a for a schematic of the trap and sink.

For the co-culture experiments on the chip, we utilized the side-trap array device and added fluidic resistance on all of the inlet/outlet ports with sinuous channels to increase the dynamic range of achievable flow rates. For mammalian cell loading, cells were trypsinized, pelleted, and resuspended in 0.5 - 1.0 mL DMEM+FBS+antibiotics (Kanamycin and Chloramphenicol) before loading. Cells were loaded under a light microscope such that un-adhered cells were localized in the media channels under near-stagnant flow conditions. The microfluidic chip and syringe appa-

ratus was then carefully placed in the CO_2 incubator while avoiding any changes in the relative heights of the syringes to maintain near-stagnant flow. The cells were allowed to adhere within 2 - 4 hours before slightly raising the media syringe to supply fresh media to the channels, and then left to proliferate overnight. On the next day, the chip and syringe apparatus was transferred to the microscope under a temperature and CO_2 environmental chamber as described previously[15]. The bacterial culture was then prepared and loaded as described above before imaging. If flow rate was too high, it was lowered when the bacterial population reached the quorum threshold (indicated by the appearance of sfGFP) to allow for better diffusion of the released therapeutic into the mammalian growth channel. For a schematic of the main steps involved, refer to Extended Data Fig. 2d.

For microscopy we used the same system as described in our previous work[6]. Briefly, we used a Nikon Eclipse TI epifluorescent microscope with phase-contrast based imaging. For the acquisition of images, we used a CoolSNAP HQ2 CCD camera from Photometrics. The microscope and acquisition was controlled by the Nikon Elements software. A plexiglass incubation chamber connected to a heating unit, which encompassed a wide area around the stage, was used in order to maintain the temperature of the microfluidic device. Phase-contrast images were taken at 60x magnification at 50-200ms exposure times. Fluorescent imaging at 60x was performed at 150ms for GFP, 30% setting on the Lumencor SOLA light source. Images were taken every 2 - 3 minutes for the course of a typical experiment. In order to estimate the flow rate in the device channel, we measured the length of traces of fluorescent beads (1.0 μm) upon 200ms exposure of fluorescent light to estimate the average fluid velocity. Further information on the analysis of these images is

presented in the Data Analysis section below.

Well-plate Experiments

For the viability experiments, we seeded a monolayer of HeLa cells on standard tissue culture 96-well flat bottom plates (Fisher Scientific) with penicillin and streptomycin antibiotics. We grew the four bacterial strains from Fig. 3d in 50 mL cultures to an optical density of 0.08 before pelleting and re-suspending in 1.2 mL media with the appropriate antibiotics. The bacterial cultures were then grown for one hour and then pelleted in a 1.5 mL micro-centrifuge tube. 100 μL of each resulting supernatant was then added to three HeLa culture wells. Thereafter we implemented the protocol for the Vybrant MTT Cell Proliferation Assay Kit (V-13154, Molecular Probes) to measure HeLa cell viability.

For the variable seeding experiments (Fig. 3e-f), we also utilized the same well-plates. We grew the SLC + HlyE strain to an optical density of $\sim$0.07 before pelleting the cells and re-suspending in 1 mL of fresh media and antibiotics. Variable volumes of this dense culture were then seeded to three wells for each respective case, and imaging was performed at 20X magnification.

Data Analysis

Fluorescence intensity profiles were obtained by analyzing frames from the fluorescent channel and plotting the mean pixel intensity over time. The period measured is the peak-to-peak period of

the fluorescence profile. The number of cells in the trap was found by analyzing the phase-contrast images in ImageJ. Since bacteria formed a monolayer in the growth chamber, we first estimated the average area of an individual bacterial cell and the average void fraction (open space between bacteria in the trap). Taking into account the μm/pixels of the image, we measured the area of the trap taken up by cells using ImageJ and divided by the average area of a bacterial cell. This value was then multiplied by (1 - void fraction) to yield the total estimated number of cells in the trap. Bacteria that were not close to the main group of cells were counted individually and added to the final number. The fraction of cells lysed per period was estimated by dividing the number of lysed bacteria by the maximum number of bacteria before lysis. Plots were generated by using MATLAB.

In order to estimate the flowrate in the media channel, we imaged fluorescent 1 μm beads at 20X and analyzed the images using MATLAB. The length of the fluorescent bead traces was measured in pixels and converted to microns. The length of the trace was then divided by the exposure time (200 ms) to yield the flow velocity. In this study we report the median flow velocity because it is less sensitive to outliers in the bead traces.

Modeling

To describe the dynamic behavior of the SLC we developed an ordinary differential equation model where the populations dynamics track the intracellular concentrations of the variables LuxI (I) and the lysis protein E (L). The population variables are cell population number (N) and extra-cellular AHL (H) (we assume LuxR-AHL binding is fast). This model can be thought of as a system where we follow a surviving lineage (via a single cell) throughout the experiment which responds to extracellular AHL, which in turn increases with cell number. Once the extracellular AHL threshold is reached, the intracellular production of the Lux driven genes, LuxI and E, are brought to the ON state (second stable state), due to the positive feedback provided by the activation term P_{lux}. Increase in the lysis protein leads to a rapid reduction of cell number via the killing term, γ_N, modeled as a hill function to switch killing ON/OFF based on the concentration of lysis protein. Once AHL and lysis protein levels decay, P_{lux} and γ_N turn back to their OFF stable states, allowing cell number to rise and repeat the process. Since firing is dependent on a threshold value of AHL, the SLC can be thought of as a circuit displaying integrate-and-fire behavior.

Maximum cell population (N_0) is defined by the maximum number of cells that could fit inside a single cell trap. Cells leave the trap as the consequence of cell growth (μ_G). The rate of cell degradation through lysis is described by hill function γ_N. We assumed AHL difusion through the cell membrane to be fast, allowing for dynamic description of total AHL (H) in the trap. Production of AHL (H) is proportional to the product of cell population (N) and per-cell concentration of LuxI (I). AHL dilution is inversely proportional to N due to the increased blockage of AHL

clearance from the trap as the result of cell accumulation. Internal production of LuxI and lysis proteins is described by P_{lux}. Degradation of both proteins is due to cell growth (μ_G) as well as some basal degradation (γ_I and γ_L). In addition, LuxI is further degraded by ClpXP machinery (γ_C). ClpXP-mediated degradation is used as a proxy for temperature since it is affected by changes in temperature[16].

$$\frac{dN}{dt} = \mu_G N(N_0 - N) - \gamma_N N \quad (1)$$

$$\frac{dH}{dt} = bNI - \frac{\mu H}{1 + N/N_0} \quad (2)$$

$$\frac{dL}{dt} = C_L P_{lux} - \gamma_L L - \mu_G L \quad (3)$$

$$\frac{dI}{dt} = C_I P_{lux} - \gamma_I I - \mu_G I - \gamma_C I \quad (4)$$

$$P_{lux} = \alpha_0 + \frac{\alpha_H (H/H_0)^4}{1+(H/H_0)^4}$$

$$\gamma_N = \frac{kL^n}{L_0^n + L^n}$$

Parameters:

We chose model parameters to qualitatively fit the experimental population and GFP (proxy for LuxI) trajectories (Fig 2a). Increased protein production and degradation in *S. typhimurium* as compared to *E. coli* was reported in previous work[17]. We find that the production and degradation terms in our model (E, LuxI production and basal degradation rates: C_L, C_I, γ_L, γ_I) account for the qualitative differences in behavior between *E. coli* and *S. typhimurium* (Fig. 2b).

Model parameter values:

μ_G (Dilution due to cell growth) 0.2; N_0 (Maximum cell population size) 10; k (Maximum rate of cell lysis) 10; L_0 (Conc. of lysis gene resulting in half maximum lysis) 2; n (Hill coefficient of lysis function) 2; b (AHL production rate) 25; μ (Maximum AHL clearance rate due to flow) 12; C_L (Lysis gene copy number) 0.5; C_I (LuxI copy number) 1 ; α_0 (Lux promoter basal production) 0.5; α_H (Lux promoter AHL induced production) 35; H_0 (AHL binding affinity to Lux promoter) 5; γ_L (Basal degradation of lysis protein) 2; γ_I (Basal degradation of LuxI) 2; γ_C (ClpXP degradation of LuxI) 12.

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Supplementary Videos

Supplementary Video 1. This video shows timelapse fluorescence microscopy of the Synchronized Lysis Circuit (SLC) in Strain 1 (*S. typhimurium*, no ssrA tag on LuxI) at 60X magnification. Images were taken every 2 min at the bottom portion of a 100x100μm chamber.

Supplementary Video 2. This video shows timelapse fluorescence microscopy of the SLC in Strain 2 (*S. typhimurium*, ssrA tag on LuxI) at 60X magnification. We observe a longer lysis period with a higher degradation efficiency on LuxI. The chamber size is 100x100μm and images were taken every 2 min.

Supplementary Video 3. This video shows timelapse fluorescence microscopy of the SLC in Strain 13 (*E. coli*) at 60X magnification. The chamber size is 100x100μm and images were taken every 2 min.

Supplementary Video 4. Video of bacteria and cancer cell co-culture on a microfluidic device at 60X magnification. Strain 3 (non-motile *S. typhimurium*, SLC with HlyE) was loaded in the growth chambers while HeLa cells grow in the main channel of the device. Timelapse fluorescence microscopy images were taken every 3 min.

Supplementary Video 5. Second video of bacteria and cancer cell co-culture on a microfluidic device at 60X magnification. Strain 3 (non-motile *S. typhimurium*, SLC with HlyE) was loaded in the growth chambers while HeLa cells grow in the main channel of the device. Timelapse fluorescence microscopy images were taken every 3 min.

Supplementary Video 6. Video of bacteria and cancer cell co-culture in a tissue culture well-plate at 20X magnification. Strain 4 (motile *S. typhimurium*, SLC with HlyE) was loaded in the well with a monolayer HeLa cells. Timelapse fluorescence microscopy images were taken every 1 min.