arg1 or *lux* genes were cultured at 30 °C for 22 h with or without 3 μM IPTG. Nissle cells with *arg1* were exposed to 1 MPa of hydrostatic pressure to facilitate the removal of kanamycin by centrifugation before spotting on H5316 plates. Nissle cells containing *arg1* and *lux* induced and uninduced with IPTG, as well as H5316* cells, were washed 3× in PBS by pelleting and adjusted to $OD_{600nm} = 1$ in LB. All cells were spotted in 2-μl volume on 5-mm sterile filter paper (Bel-Art Products), placed on the microcin assay plates. Unsupplemented LB and 100 mg ml^{-1} ampicillin (2 μl each) were similarly spotted as controls. After 17 h at 37 °C, the plates were imaged with the Chemidoc MP instrument with blue transillumination, and unfiltered light was collected to form an image. Images shown are representative of four experiments each.

Colony ultrasound. ARG and GFP constructs were transformed into BL21(A1) one-shot competent cells (Thermo Fisher Scientific) and plated onto LB agar two-layer inducer plates as described above. Plates were grown at 37 °C for 14h. The colonies were immobilized by depositing a 4 mm layer of 0.5% agarose–PBS gently onto the plate surface. Ultrasound imaging was performed using a L11-4v128-element linear array transducer (Verasonics) to obtain a larger field of view. The transducer was mounted on a computer-controlled 3D translatable stage (Velmex). Image acquisition was performed using conventional B-mode imaging using a 128-ray-lines protocol with a synthetic aperture to form a focused excitation beam. The transmit waveform was set to a frequency of 6.25 MHz, 67% intra-pulse duty cycle, and a four-cycle pulse. Colonies were positioned 20 mm from the transducer face, which is the elevation focus of the L11-4v transducer, coupled through a layer of PBS. The transmit beam was also digitally focused at 20 mm. For imaging, the transmit power was 2 V and the *f*-number was 3, resulting in a peak positive pressure of 0.61 MPa. To measure gas vesicle collapse in bacterial colonies as a function of acoustic pressure, images were acquired as described above at a peak positive pressure of 0.61 MPa after sequentially exposing the samples to collapse pulses at 6.25 MHz, with increasing amplitude from 0.61 MPa to 5.95 MPa. Pixel gain in the images was set to 0.1 and persistence to 20. Cross-sectional images of the plate (perpendicular to the plate surface) were acquired at spatial intervals of 250 μm using computer-controlled steps. The cross-sectional images were processed in MATLAB to form 2D images of the plate surface. First, the cross-sectional images were stacked to produce a 3D-volumetric reconstruction of the plate. We then summed the signals in a 2-mm slice of the volume parallel to and centred on the bacterial growth surface after thresholding to eliminate background, forming a 2D projection image of the plate. After ultrasound imaging, image processing, and acoustic phenotype prediction, the colonies were picked using 10-μl sterile pipette tips. Each colony was used to inoculate a 5-ml LB culture containing 50 μg ml^{-1} kanamycin culture. DNA was extracted from the cultures by mini-prep (PureYield, Promega) and sequenced to determine whether the plasmid contained GFP, *arg1* or *arg2*.

***In vivo* ultrasound and bioluminescence imaging.** All *in vivo* experiments were performed on BALB/c or SCID nude female mice, aged 14–15 weeks, under a protocol approved by the Institutional Animal Care and Use Committee of the California Institute of Technology. No randomization or blinding were necessary in this study. Ultrasound imaging was performed as follows. Mice were anaesthetized with 1–2% isoflurane, maintained at 37 °C on a heating pad, depilated over the imaged region, and imaged using an L22-14v transducer with the pulse sequence described above. For imaging of *E. coli* in the gastrointestinal tract, BALB/c mice were placed in a supine position, with the ultrasound transducer positioned on the lower abdomen, transverse to the colon. Anatomical landmarks including the bladder were used to identify the position of the colon. Prior to imaging, buoyancy-enriched *E. coli* Nissle 1917 expressing *arg1* or *lux* were mixed in a 1:1 ratio with 42 °C 4% agarose–PBS for a final bacterial concentration of 10^9 cells ml^{-1}. An 8-gauge needle was filled with the mixture of agarose and bacteria expressing either *arg1* or *lux*. Before it solidified, a 14-gauge needle was placed inside the 8-gauge needle to form a hollow lumen within the gel. After the agarose–bacteria mixture solidified at room temperature for 10 min, the 14-gauge needle was removed. The hollow lumen was then filled with the agarose–bacteria mixture expressing the other imaging reporter (*arg1* or *lux*). After it solidified, the complete cylindrical agarose gel was injected into the colon of the mouse with a PBS back-filled syringe. The same procedure was used with *E. coli* BL21 cells, except with the entire gel homogeneously composed of either *arg2*- or GFP-expressing cells. Introduction of gel into the colon is a common preparatory protocol for gastrointestinal ultrasound[36,37].

For imaging of *S. typhimurium* in tumours, we formed hind-flank ovarian tumour xenografts in SCID nude mice via subcutaneous injection of 5×10^7 OVCAR8 cells (provided by the National Cancer Institute tumour repository with certificate of authentication) with Matrigel. After tumours grew to dimensions larger than approximately 6 mm (14 weeks), they were injected with *arg1*-expressing *S. typhimurium*, (50 μl, 3.2×10^9 cells ml^{-1}). The tumours were then imaged with ultrasound, with anaesthetized mice in a prone position (homeostasis and imaging parameters as described above). Our animal protocol specified that animals with total tumour volume exceeding 2 cm^3, or showing signs of distress as assessed by the veterinary team, be euthanized.

For luminescence imaging, mice were anaesthetized with 100 mg kg^{-1} ketamine and 10 mg kg^{-1} xylazine and imaged using a Bio-Rad ChemiDoc MP imager without illumination, no emission filter, and an integration time of 5 min. The image was thresholded and rendered in ImageJ, and superimposed on a bright-field image of the mouse using GIMP.

Image processing. MATLAB was used to process ultrasound images. Regions of interest (ROIs) were defined to capture the ultrasound signal from the phantom well, colon, or tumour region. All *in vitro* phantom experiments had the same ROI dimensions. For *in vivo* experiments ROIs were selected consistently to exclude edge effects from the colon wall or skin. Mean pixel intensity was calculated from each ROI, and pressure-sensitive ultrasound intensity was calculated by subtracting the mean pixel intensity of the collapsed image from the mean pixel intensity of the intact image. Images were pseudo-coloured, with maximum and minimum levels adjusted for maximal contrast as indicated in accompanying colour bars.

For the multiplexed imaging of *arg1* and *arg2*, acoustic spectral unmixing was performed as previously described[20]. In brief, a spatial averaging filter (kernel size 30 × 30 pixels or 750 × 750 μm) was applied to the three acquired images (before collapse, after collapse with 2.7 MPa and after collapse with 4.7 MPa) to reduce noise. Then, pixel-wise differences between the first and second image, and between the second and third image were calculated, and multiplied by the inverse of the collapse matrix, α, representing the expected fractional collapse of each ARG type at each pressure ($\alpha = (0.7921, 0.5718; 0.2079, 0.4282)$), to produce the unmixed pixel intensities corresponding to the contributions from *arg2* and *arg1*.

Statistical analysis. For statistical significance testing, we used two-sided heteroscedastic *t*-tests with a significance level of type I error set at 0.05 for rejecting the null hypothesis. Sample sizes for all experiments, including animal experiments, were chosen on the basis of preliminary experiments to be adequate for statistical analysis.

Code availability. MATLAB code is available from the corresponding author upon reasonable request.

Data and code availability. *arg1* and *arg2* plasmid sequences are included in Supplementary Information, and plasmids will be available from Addgene. All other materials are available from the corresponding author upon reasonable request.

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