METHODS

Chemicals. All chemicals were purchased from Sigma Aldrich unless otherwise noted.

Molecular cloning. To construct the plasmid for *E. coli* BL21(A1) expression of ARGs, the gene cluster encoding *B. megaterium* gas vesicle proteins GvpBRNFGLSKJTU was amplified from pNL29 (ref. 19) (gift from M. Cannon) and cloned into pET28a using Gibson assembly. The amplicon included an additional 46 base pairs (bp) upstream of the *gvpB* start codon and 180 bp downstream of the *gvpU* stop codon. To generate hybrid gene clusters, the genes encoding GvpA and GvpC were amplified from *A. flos-aquae* and cloned into pET28-RNFGLSKJTU using Gibson assembly. A control gene encoding the green fluorescent protein (GFP) mNeonGreen[33] was similarly constructed in the pET28 vector. For expression of ARGs in *E. coli* Nissle 1917, the pET28 T7 promoter was replaced by the T5 promoter. For *S. typhimurium* expression, the ARG gene cluster was cloned into pTD103 (gift from J. Hasty). A plasmid encoding the luxCDABE gene cluster from *Photorhabdus luminescens* on the pTD103 backbone was also a gift from J. Hasty.

Bacterial expression. Plasmids encoding ARGs or GFP were transformed into chemically competent *E. coli* BL21(A1) cells (Thermo Fisher Scientific) and grown in 5 ml starter cultures in LB medium with 50 μg ml^{-1} kanamycin, 1% glucose for 16 h at 37 °C. Large-scale cultures in LB medium containing 50 μg ml^{-1} kanamycin and 0.2% glucose were inoculated at a ratio of 1:100 with the starter culture. Cells were grown at 37 °C to $OD_{600nm} = 0.5$, then induced with 0.5% L-arabinose and 0.4 mM IPTG for 22 h at 30 °C. For *E. coli* Nissle 1917 (Ardeypharm GmbH) the same protocol was followed, except constructs were electroporated into the cells and induction was performed at $OD_{600nm} = 0.3$ with 3 μM IPTG (*arg1*) and 3 nM *N*-(β-ketocaproyl)-L-homoserine lactone (AHL) (*lux*). Strain identity of *E. coli* Nissle 1917 cells was confirmed by PCR[34]. For *S. typhimurium* expression, the same protocol was followed, except constructs were electroporated into *S. typhimurium* ELH1301 (gift from J. Hasty) and expression was induced with 3 nM AHL.

Gas vesicle purification and quantification. Collected cells were centrifuged at 350*g* in 50 ml conical tubes for 4 h with a liquid height <10 cm to prevent collapse of gas vesicles by hydrostatic pressure. For ARG variants that produce a buoyant band of cells, the middle layer between the buoyant cells and the sedimented cells was removed and discarded. For ARG variants that do not produce a buoyant band, the supernatant was discarded. The remaining cells were resuspended in 8 ml SoluLyse-Tris (L200500 Genlantis) per 100 ml culture and 250 μl ml^{-1} lysozyme, and incubated for 1 h at 4 °C with rotation. Subsequently, 10 μl ml^{-1} DNaseI was added to the lysate and incubated for 10 min at 25 °C. The lysate was transferred to 2 ml tubes and centrifuged for 2 h at 400*g* at 8 °C. The subnatant was removed with a 21.5-gauge needle, and the supernatant containing the gas vesicles was transferred to a clean tube. PBS was added to the gas vesicles in a threefold volume excess and centrifugation, removal of subnatant and PBS dilution was repeated three times. Purified gas vesicles were quantified using the Micro BCA Protein Assay Kit (Thermo Fisher Scientific). Gas vesicles were collapsed with hydrostatic pressure before quantification. Bovine serum albumin was used to generate the standard curve. Absorbance measurements were taken on a Spectramax M5 spectrophotometer (Molecular Devices).

TEM sample preparation and imaging. Cells expressing ARGs, or purified gas vesicles, were exchanged into water or 10 mM HEPES pH 8.0 with 150 mM NaCl, respectively, via three rounds of buoyancy purification and buffer exchange as described above. Samples were deposited on Formvar/carbon 200 mesh grids (Ted Pella) that were rendered hydrophilic by glow discharging (Emitek K100X). For purified gas vesicles, 2% uranyl acetate was added for staining. The samples were then imaged on a FEI Tecnai T12 transmission electron microscope equipped with a Gatan Ultrascan CCD. Images were processed with Fiji[35].

Hydrostatic collapse pressure measurements. Cells expressing ARGs, or purified gas vesicles, were diluted to $OD_{600nm} = 1.0$ in PBS and 0.4 ml was loaded into an absorption cell (176.700-QS, Hellma GmbH). A single valve pressure controller (PC series, Alicat Scientific), supplied by a 1.5-MPa nitrogen gas source, applied hydrostatic pressure in the cell, while a microspectrometer (STS-VIS, Ocean Optics) measured the optical density of the sample at 500 nm. OD_{500nm} was measured from 0 to 1.2 MPa gauge pressure with a 10-kPa step size and a 7-s equilibration period at each pressure.

***In vitro* ultrasound imaging.** Phantoms for imaging were prepared by melting 1% (w/v) agarose in PBS and casting wells using a custom 3D-printed template. Cells at 2× the final concentration were mixed in a 1:1 ratio with molten agarose (at 50 °C) and immediately loaded into the phantom. The concentration of cells was determined before loading by measuring their OD_{600nm} after exposure to 1.2 MPa hydrostatic pressure to eliminate any contribution to light scattering from gas vesicles. The optical density was then converted into cells per ml using the relationship 1 OD = 8×10^8 cells ml^{-1} (https://www.genomics.agilent.com/biocalculators/calcODBacterial.jsp). Cell samples collected at early time points following induction, which had an optical density insufficient for loading, were first concentrated using centrifugation at 350*g*. Ultrasound imaging was performed using a Verasonics Vantage programmable ultrasound scanning system and L22-14v 128-element linear array transducer (Verasonics). 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 19 MHz, 67% intra-pulse duty cycle, and a one-cycle pulse. Samples were positioned 6 mm from the transducer face, which is the elevation focus of the L22-14v transducer, coupled through a layer of PBS. The transmit beam was also digitally focused at 6 mm. For imaging, the transmit voltage was 2 V and the *f*-number was 3, resulting in a peak positive pressure of 0.4 MPa. Backscattered ultrasound signals were filtered with a 7-MHz bandpass filter centred at 19 Mhz. Signals backscattered from four transmit events were summed before image processing. Pixel gain was set to 3 and persistence to 90.

For gas vesicle collapse using the L22-14 array, we set the *f*-number to 0.2 (thereby ensuring that all transducer elements were active) and scanned the transmit focus from 3 mm to 9 mm. During the 10-s collapse scan, single-cycle pulses were applied using a ray-lines protocol at 19 MHz with a frame rate of 12 frames per second. To measure gas vesicle collapse in ARG-expressing cells as a function of acoustic pressure, images were acquired as described above at a peak positive pressure of 0.4 MPa after sequentially exposing the samples to collapse pulses of increasing amplitude, with pressures that varied from 0.55 MPa to 4.7 MPa. To achieve complete collapse, we applied the maximal pressure of 4.7 MPa. Collapse data were fitted with a Boltzmann sigmoid function to facilitate visualization of collapse curves. This function is of the form $f(p) = \left(1 + e^{\frac{p-p_c}{s}}\right)^{-1}$ where p is the pressure, and p_c and s are fitted parameters representing the collapse midpoint and slope, respectively. For spectral unmixing, the two collapse pressures applied were 2.7 MPa and 4.7 MPa. Transducer output pressures were measured in a degassed water tank using a fibre-optic hydrophone (Precision Acoustics).

Plate-based induction and optical imaging. ARG and GFP constructs were transformed as described above, and the transformation mix after recovery was plated on two-layer LB-Agar plates. The underlayer contained 50 μg ml^{-1} kanamycin, 1.0% L-arabinose, and 0.8 mM IPTG. The overlayer contained 50 μg ml^{-1} kanamycin and 0.4% glucose. The overlayer was poured 30 min before plating, and each layer was 4 mm thick. Plates with transformants were incubated at 30 °C for 20 h and then imaged for white light scattering and green fluorescence using a Chemidoc MP instrument (Bio-Rad).

Cell growth, viability and microcin production assays. *E. coli* Nissle 1917 cells were transformed by electroporation with pET28 plasmids containing either the *arg1* or *lux* gene cluster under the T5 promoter. Transformed cells were grown in 5 ml starter cultures in LB medium containing 50 μg ml^{-1} kanamycin, 1% glucose for 16 h at 37 °C. The overnight cultures were diluted 1:100 in 50 ml of LB medium containing 50 μg ml^{-1} kanamycin and 0.2% glucose. Cultures were grown at 30 °C to $OD_{600nm} \approx 0.2–0.3$ and induced with 3 μM IPTG (+IPTG), or left uninduced (−IPTG). Both induced and uninduced cultures were allowed to grow for 22 h at 30 °C. For time point optical density measurements, 1 ml of the culture was taken out and measured. For plating after 22 h of growth, the cultures were diluted to a uniform OD_{600nm} of 0.2, before further serial dilution by a factor of 2×10^4 in LB supplemented with 50 μg ml^{-1} kanamycin and 0.2% glucose. 100 μl of the final dilutions was plated on two-layer LB agar plates using a cell spreader. The underlayer of the plates contained 50 μg ml^{-1} kanamycin and 9 μM IPTG. The overlayer contained 50 μg ml^{-1} kanamycin and 0.4% glucose. The overlayer was poured 30 min before plating, and each layer was 3 mm thick. Cells uniformly spread on the two-layer plates were allowed to grow at 30 °C for 21 h. Colonies were then imaged for light scattering using the Chemidoc MP instrument under white light transillumination and 605 ± 50 nm receive filter, and both opaque (gas vesicle-producing) and clear colonies were counted to determine total colony forming units per millilitre and the gas vesicle-expressing fraction. Plates had a minimum of 82 and a maximum of 475 total colonies, enabling manual counting.

To assay microcin production, *E. coli* Nissle 1917 cells containing *arg1* or *lux* were cultured as described above and spotted on microcin assay plates containing *E. coli* K-12 H5316 cells (gift from K. Hantke). Wild-type H5316 were grown in 5 ml LB medium, and H5316 cells transformed with pET plasmid containing mWasabi and KanR under a T5 promoter (H5316* cells) were grown in 5 ml LB medium containing 50 μg ml^{-1} kanamycin and 1% glucose for 16 h at 37 °C. Two-layer LB plates were used to assay the growth inhibition of H5316 cells by microcin peptides produced by Nissle 1917 cells. Plates used to assay with wild-type H5316 cells contained 20 ml of 1% LB agar at the bottom, and the top layer contained 2×10^7 H5316 cells in 20 ml of 0.3% LB agar. Plates using H5316* cells contained 20 ml of 1% LB agar with 50 μg ml^{-1} kanamycin, 50 μM desferal, and 3 μM IPTG, and the top layer contained 2×10^7 H5316* cells in 20 ml of 0.3% LB agar with 50 μg ml^{-1} kanamycin, 50 μM desferal, and 3 μM IPTG. Nissle cells containing