Supplementary Information

Synthetic Quorum Sensing Circuit to Control Consortial Biofilm Formation and Dispersal in a Microfluidic Device

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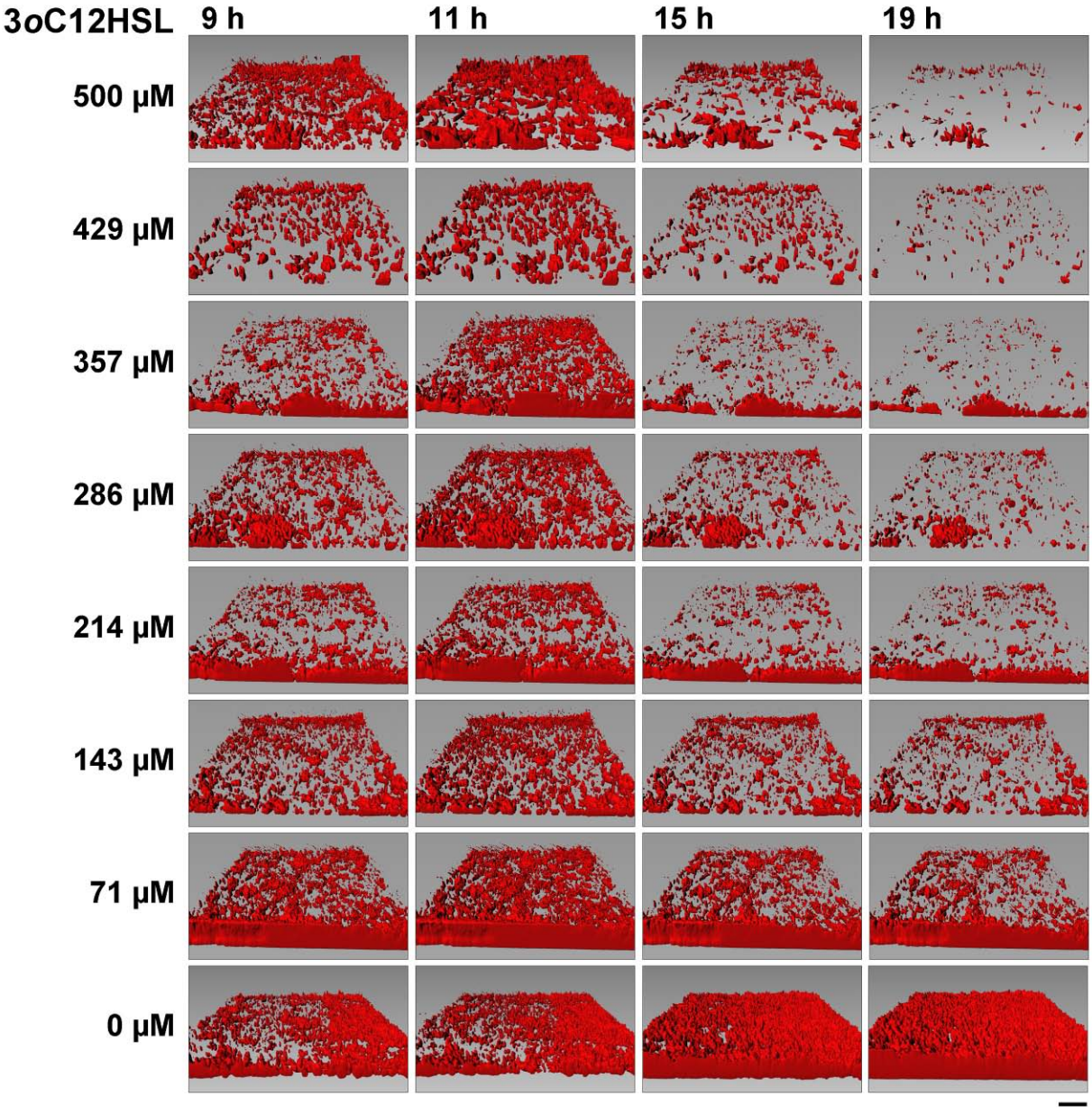
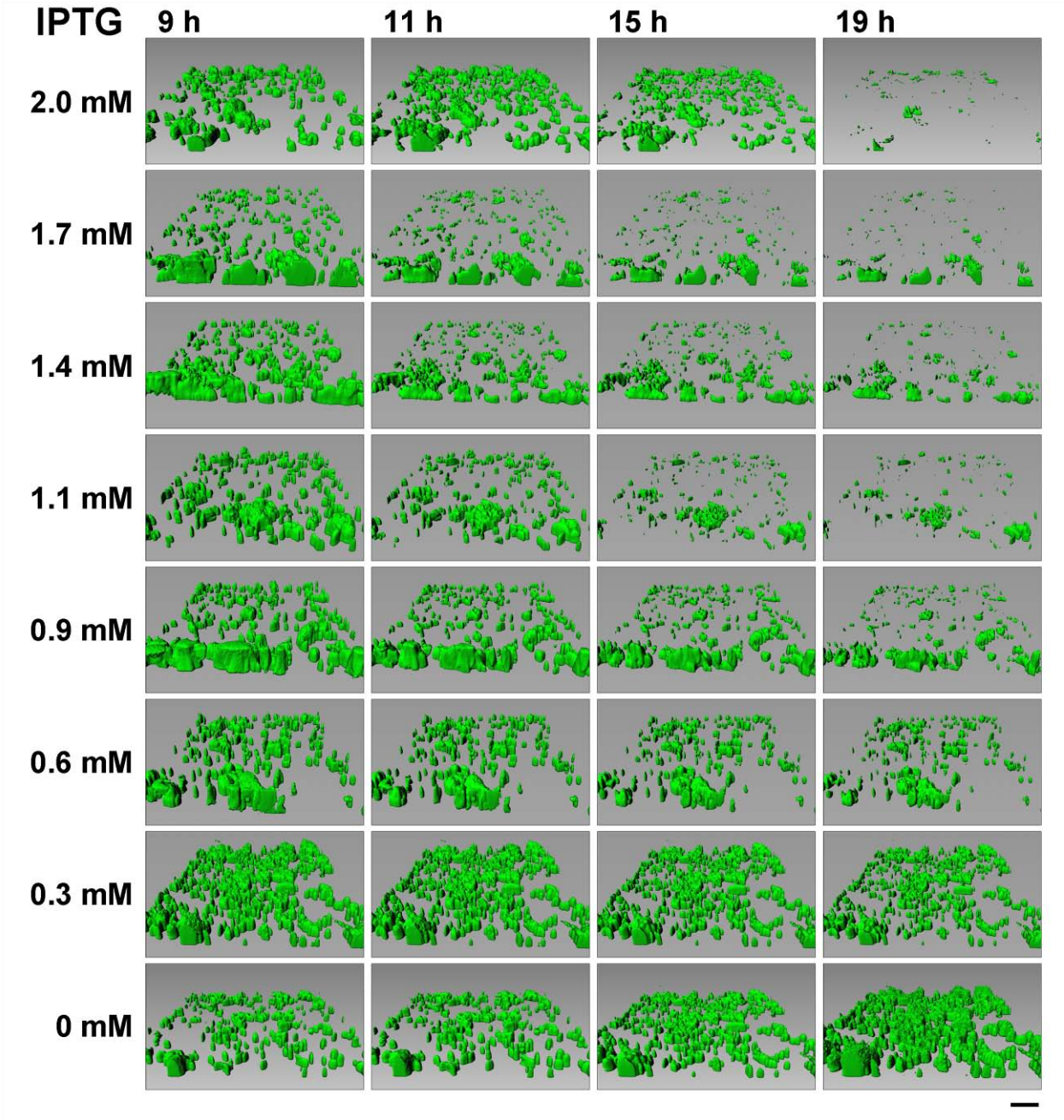
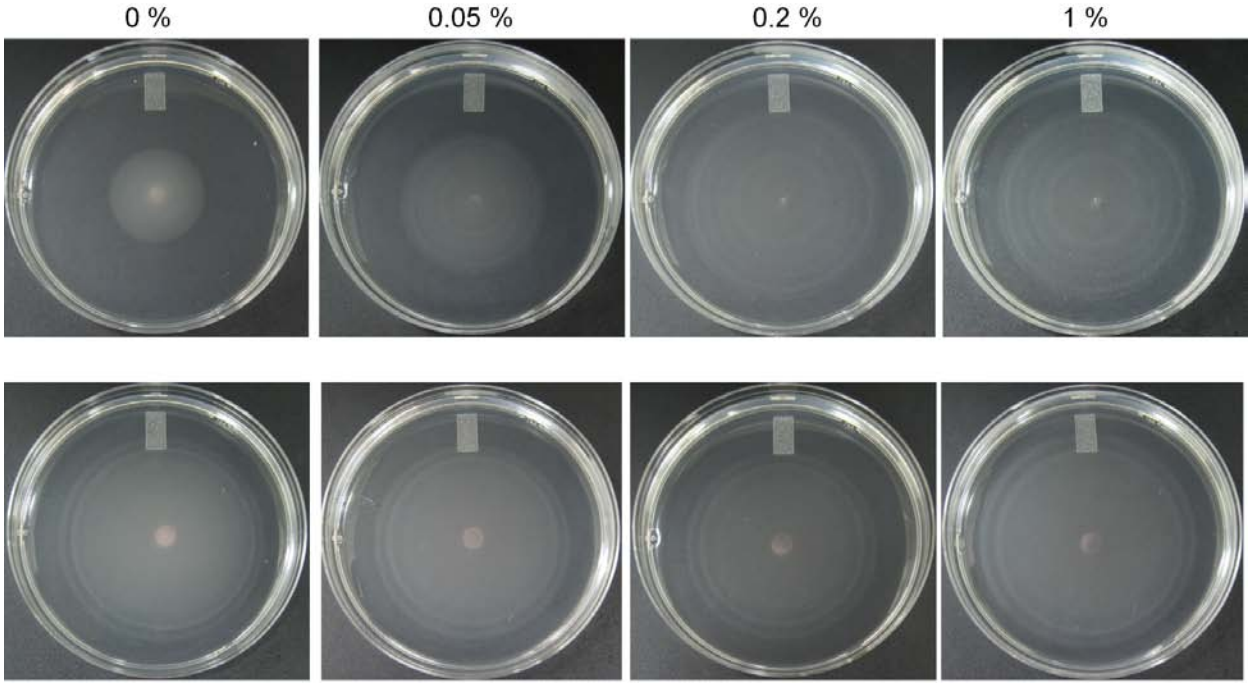


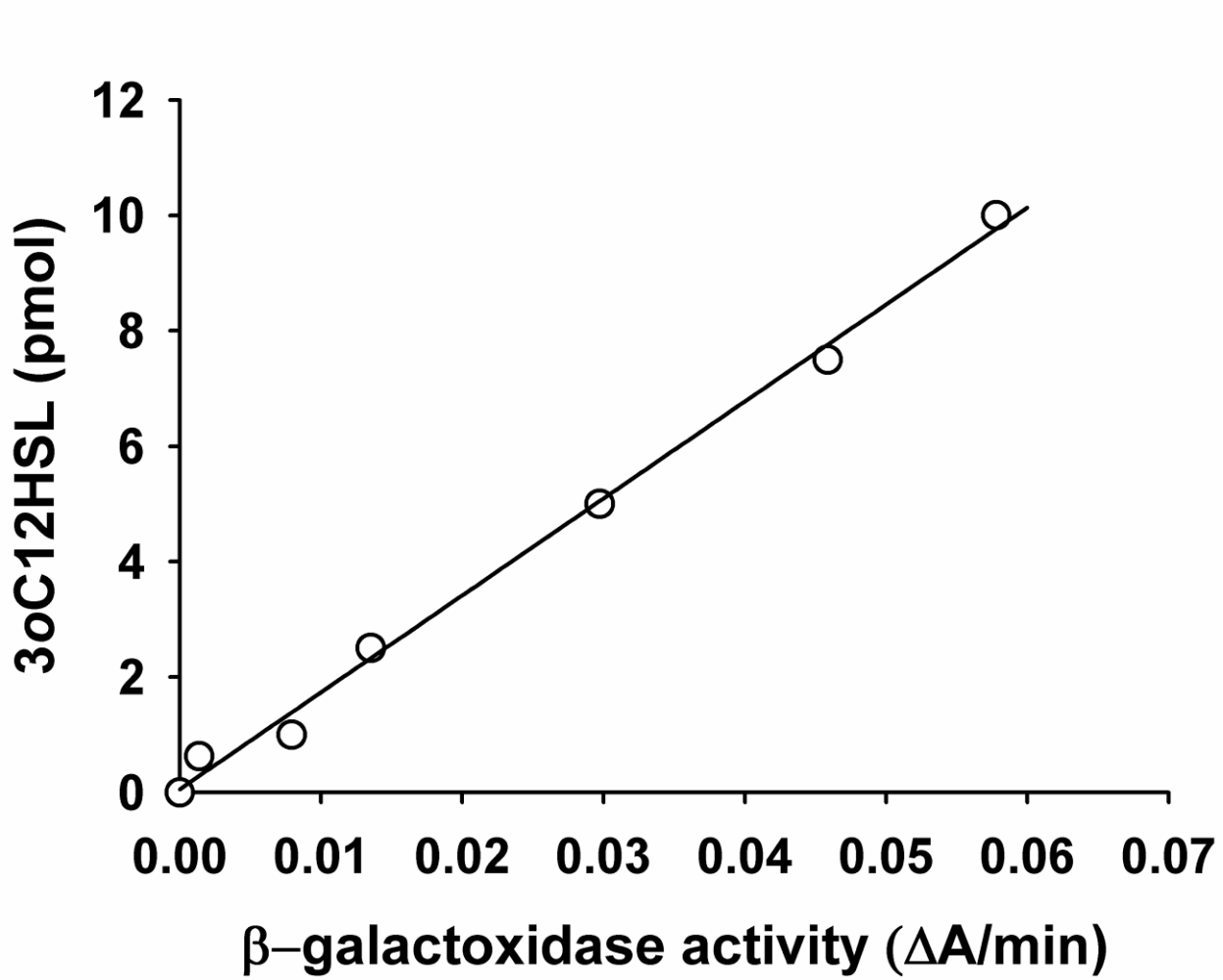
Supplementary Figure S1. Dispersal of the initial colonizer biofilm with different concentrations of 3*o*C12HSL. Robust biofilms of initial colonizer (*lasR*$^{+}$, *bdcAE50Q*$^{+}$, *rfp*$^{+}$ via *E. coli hha/*pBdcAE50Q-*rfp-lasR*) cells were developed for 9 h in each microchamber. Different concentrations of 3*o*C2HSL (0, 71, 143, 214, 286, 357, 429, and 500 µM) were added for 10 h to induce biofilm dispersal. Scale bar indicates 20 µm. Three independent cultures were tested, and representative images are shown.



Supplementary Figure S2. Dispersal of the disperser biofilm with different concentrations of IPTG. Robust biofilms of disperser cells (*lasI*$^{+}$, *hha13D6*$^{+}$, *gfp*$^{+}$ via *E. coli hha/*pHha13D6-*gfp-lasI*) were developed for 9 h in each microchamber. Different concentrations of IPTG (0, 0.3, 0.6, 0.9, 1.1, 1.4, 1.7, and 2.0 mM) were added for 10 h to induce biofilm dispersal. Scale bar indicates 20 μm. Three independent cultures were tested, and representative images are shown.



Supplementary Figure S3. Swimming motility for the two initial colonizer strains with arabinose. *E. coli hha*/pPBAD-*bdcAE50Q-rfp-lasR* (upper panel) and *E. coli hha*/pBdcAE50Q-*rfp-lasR* (lower panel, negative control) were inoculated and incubated for 16 h with different concentrations of arabinose (0, 0.05, 0.2, and 1%) on motility agar plates at 37°C. Three independent cultures were tested, and representative images are shown.



Supplementary Figure S4. Standard curve for determining 3*o*C12HSL concentrations. β-galactosidase activity was measured using reporter *E. coli* MG4/pKDT17 upon adding different amounts of 3*o*C12HSL.

Supplementary Table S1. Strains and plasmids used in this study. Km^R, Cm^R, Em^R, and Ap^R are kanamycin, chloramphenicol, erythromycin, and ampicillin resistance, respectively.

Strains and plasmids	Genotype/relevant characteristics	Source
Strains		
E. coli hha	BW25113 Δ*hha* Ω Km^R	[51]
E. coli MG4/pKDT17	Ap^R; P_{lasB}::*lasB*$^+$-*lacZ*$^+$ translational fusion, P_{lac}::*lasR*$^+$	[52]
P. aeruginosa PAO1	Wild-type	T. McDermott
Plasmids		
pCA24N-*hha13D6*	Cm^R; *lacI*^{q}, pCA24N $P_{T5\text{-}lac}$::*hha13D6*$^+$	[53]
pHha13D6-*gfp*	Cm^R; *lacI*^{q}, pCA24N $P_{T5\text{-}lac}$::*hha13D6*$^+$ P_{CP25}::*gfp*$^+$	This study
pHha13D6-*gfp-lasI*	Cm^R; *lacI*^{q}, pCA24N $P_{T5\text{-}lac}$::*hha13D6*$^+$ P_{CP25}::*gfp*$^+$-*lasI*$^+$	This study
pCA24N-*bdcAE50Q*	Cm^R; *lacI*^{q}, pCA24N $P_{T5\text{-}lac}$::*bdcAE50Q*$^+$	[54]
pBdcAE50Q	Cm^R; *lacI*^{q}, pCA24N P_{lasI}::*bdcAE50Q*$^+$	This study
pBdcAE50Q-*rfp-lasR*	Cm^R; *lacI*^{q}, pCA24N P_{lasI}::*bdcAE50Q*$^+$ P_{CP25}::*rfp*$^+$-*lasR*$^+$	This study
pCM18	Em^R; P_{CP25}::*gfp*$^+$	[55]
pGFP-*lasI*	Em^R; P_{CP25}::*gfp*$^+$-*lasI*$^+$	This study
pCM18-X	Em^R; *gfp*-disrupted	This study
pRFP-*lasR*	Em^R; P_{CP25}::*rfp*$^+$-*lasR*$^+$	This study
pDsRed-Express	Ap^R; P_{lac}::*rfp*$^+$	Clontech
pDsRed-*lasR*	Ap^R; P_{lac}::*rfp*$^+$-*lasR*$^+$	This study
pDsRed-BlpIX-*lasR*	Ap^R; P_{lac}::*rfp*$^+$-*lasR*$^+$ (BlpI site disrupted)	This study
pBAD/*Myc*-HisC	Ap^R; *araC*	Invitrogen
pPBAD-*bdcAE50Q*	Cm^R; *lacI*^{q}, pCA24N P_{BAD}::*bdcAE50Q*$^+$	This study
pPBAD-*bdcAE50Q-rfp-lasR*	Cm^R; *lacI*^{q}, pCA24N P_{BAD}::*bdcAE50Q*$^+$ P_{CP25}::*rfp*$^+$-*lasR*$^+$	This study
pPBAD-*hha13D6-gfp-lasI*	Cm^R; *lacI*^{q}, pCA24N P_{BAD}::*hha13D6*$^+$ P_{CP25}::*gfp*$^+$-*lasI*$^+$	This study

Supplementary Table S2. Primers used for constructing plasmids for the microfluidic biofilm engineering circuit. Underlined italic text indicates the restriction enzyme sites: AvaI in plasI-F and pBAD-AvaI-F, BlpI in lasI-F, lasI-R, rfp-lasR-F1, rfp-lasR-F3, and rfp-lasR-R, BseRI in plasI-R and pBAD-BseRI-R, and NotI in gfp-F1, lasR-F, and lasR-R. Italicized bold text indicates the site-directed mutation for disruption of the BlpI restriction site (5'-GCTGAGC to 5'-TCTGAGC) in BlpI-X-F and BlpI-X-R. Underlined bold text indicates the site-directed mutation site for the codon corresponding to truncation at GFP Y66 (5'-TAT to 5'-TA**A** for Y66X) in gfpX-F and gfpX-R.

Primer Name	**Primer Sequence (listed 5' to 3')**
Construction of the plasmid for the disperser cell (pHha13D6-*gfp-lasI*)	
gfp-F1	GGACTC*GCGGCCGC*TAAGGGCTTTGGCAGTTTATTCTTGACATGTAGTGAGGGGGCTGGT
gfp-F2	ACATGTAGTGAGGGGGCTGGTATAATAAAATAGTACTGTTCGGGTGAGCGGATAACAATT
gfp-F3	TTCGGGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGCGTAAAGGAGAAGAACTT
gfp-R	ATCAACGGTGGTATATCCAGTG
lasI-F	CCTGCA*GCTGAGC*TTCTTCAGCTTCCTATTTGGAGGAAGTG
lasI-R	GCTCGACG*GCTCAGC*AGGTCCCCGTCATGAAACCGCCAGTCGC
Construction of the plasmid for the initial colonizer cell (pBdcAE50Q-*rfp-lasR*)	
plasI-F	CGACGCCG*CTCGAG*GGGCTGTGTTCTCTCGTGTG
plasI-R	GCCGTGC*ATAGTTAATTTCTCCTC*TTTAATGGAAGCTGAAGAATTTATGC
lasR-F	GCACCT*GCGGCCGC*TAGTTTAAGAAGAACGTAGCGCTATGGCCTTGGTTGACGGTTTTC
lasR-R	GCCTGACG*GCGGCCGC*TCAGAGAGTAATAAGACCCAAATTAAC
BlpI-X-F	GTGCTCGCGGCGAACTCGGCGC***TCTGAGC***CTCAGCGTGGAAGCGG
BlpI-X-R	CCGCTTCCACGCTGAG***GCTCAGA***GCGCCGAGTTCGCCGCGAGCAC
BlpI-X-seq-F	GAACGCCTTCATCGTCGGCAACTACC
rfp-lasR-F1	GGACTC*GCTGAGC*GCTTTGGCAGTTTATTCTTGACATGTAGTGAGGGGGCTGGTATAATA
rfp-lasR-F2	GTGAGGGGGCTGGTATAATAAAATAGTACTGTTCGGGTGAGCGGATAACAATTTCACAC
rfp-lasR-F3	CCGGACTC*GCTGAGC*ATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGC
rfp-lasR-R	GCTCGACG*GCTCAGC*AGGTCCCGCTCAGAGAGTAATAAGACCC
gfpX-F	CAACACTTGTCACTACTTTCGGT**TAA**GGTGTTCAATGCTTTGCGAGATAC
gfpX-R	GTATCTCGCAAAGCATTGAACACC**TTA**ACCGAAAGTAGTGACAAGTGTTG
Replacing the *lasI* and *T5-lac* promoters with the *araBAD* promoter	
pBAD-AvaI-F	CGACGCCG*CTCGAG*AAACCAATTGTCCATATTGC
pBAD-BseRI-R	GCCGTGC*ATAGTTAATTTCTCCTC*TTTAATCAGTAGAGAGTTGCGATAAAAAGCG

SUPPLEMENTARY METHODS

Confocal microscopy. Images were taken every 1 to 2 h using a 40X/0.85 NA dry objective with a TCS SP5 scanning confocal laser microscope (Leica Microsystems, Wetzlar, Germany)[56]. Z-stack images were taken at a zoom level of 2 such that the image covered 90% of the width of the microchamber. Two individual positions per microchamber covering a total of 70% of the channel length were chosen for imaging. Using the confocal z-stack images, 3-D reconstruction of the biofilm architecture was performed using IMARIS 3D and 4D Real-Time Interactive Data Visualization software (Bitplane Inc., CT, USA). Biomass and average biofilm height were obtained using COMSTAT image-processing software[57].

Flow-cell biofilm experiments and biofilm volume analysis. Overnight cultures were diluted to a turbidity at 600 nm of 0.05 in LB-glucose and pumped through the flow-cell (BST model FC81, Biosurface Technologies, MT, USA) at 10 mL/h for 2 h, then LB-glucose was pumped for 48 h to form biofilms. The biofilms on the glass slides were visualized after robust biofilms were formed (48 h) using a confocal microscope. COMSTAT was used to analyze the biofilms formed at 13 positions. Biofilm volume was calculated by multiplying biofilm biomass and the surface area (1400 mm^2) of the flow-cell; biofilm volume of *E. coli hha*/pHha13D6-*gfp-lasI* in the flow-cell was less than 0.01 mm^3.

Replacing the *lasI* and *T5-lac* promoters with the *araBAD* promoter. To construct a plasmid with *bdcAE50Q* controlled by arabinose, the *lasI* promoter of pBdcAE50Q was replaced with the *araBAD* promoter by amplifying this promoter from pBAD/*Myc*-HisC (Invitrogen, CA, USA) using the pBAD-AvaI-F and pBAD-BseRI-R primers. The PCR fragment was cloned into the AvaI and BseRI restriction sites to construct pPBAD-*bdcAE40Q*. The P_{CP25}-*rfp-lasR* DNA sequence was amplified from pBdcAE50Q-*rfp-lasR* using the rfp-lasR-F1 and rfp-lasR-R primers, and the PCR fragment was cloned downstream of *bdcAE50Q* of pPBAD-*bdcAE50Q* using the BlpI restriction site. The final construct, pPBAD-*bdcAE50Q-rfp-lasR*, is identical to pBdcAE50Q-*rfp-lasR* (used for the initial colonizer strain) except that the *araBAD* promoter replaces the *lasI* promoter. The promoter replacement and the P_{CP25}-*rfp-lasR* insertion were confirmed by PCR and DNA sequencing.

To replace the somewhat leaky *T5-lac* promoter in pHha13D6-*gfp-lasI* with the *araBAD* promoter,

the *araBAD* promoter PCR fragment obtained using the pBAD-AvaI-F and pBAD-BseRI-R primers was cloned into the AvaI and BseRI restriction sites of pHha13D6-*gfp-lasI* to form the final construct pPBAD-*hha13D6-gfp-lasI*. The promoter replacement was confirmed by DNA sequencing.

3*o*C12HSL assay in biofilms. The flow-cell was disassembled, and biofilms samples were collected by wiping the coverslip, glass slides, and four sides of the flow-cell with paper towels (Kimwipes, 1.5 cm × 1.5 cm). This was repeated three times to ensure all of the biofilm cells were collected. The biofilm cells were resuspended in 5 mL of dH_2O, mixed, and centrifuged. The biofilm cells were resuspended in 3 mL of dH_2O and sonicated twice using a 60 Sonic Dismembrator (Fisher Scientific, PA, USA) at level 10 for 15 sec. 3*o*C12HSL was extracted[58] three times with a half volume of dichloromethane. The aqueous residue was removed after freezing the samples for 3 h at -20ºC. The solvent was evaporated via rotary evaporation, and the residue was resuspended in 200 µL of ethyl acetate. *E. coli* MG4/pKDT17 was used to assay the 3*o*C12HSL levels[52]. This reporter strain contains a copy of the *lasR* gene as well as a *lasB*::*lacZ* fusion. β-galactosidase activity was measured[59] at 25ºC based on the conversion of *o*-nitrophenyl-β-D-galactopyranoside to *o*-nitrophenol. Since the maximum 3*o*C12HSL detection limit by *E. coli* MG4/pKDT17 was measured as approximately 10 pmol using synthetic 3*o*C12HSL (Sigma-Aldrich, MO, USA) as the standard (**Supplementary Fig. S4**), 3*o*C12HSL samples were diluted to the picomolar range. Planktonic cultures of *E. coli hha*/pHha13D6-*gfp*, which does not produce 3*o*C12HSL, was used as a negative control. As additional controls, effluent from the flow-cell and planktonic cultures were used to compare with 3*o*C12HSL concentrations from the biofilm. Three independent cultures were used to assay 3*o*C12HSL concentrations in biofilms using the flow-cell device.

Swimming motility assay. To confirm that the plasmid was functional, swimming motility of *E. coli hha*/pPBAD-*bdcAE50Q-rfp-lasR* was examined on motility agar plates (1% tryptone, 0.25% NaCl, and 0.3% agar) with different concentrations of arabinose (0, 0.05, 0.1, and 0.2%). Chloramphenicol (30 µg/mL) was added to maintain the plasmid. Overnight cultures (8 µL) were used to inoculate the motility plates, and the swimming halo was measured after 16 h at 37°C.

SUPPLEMENTARY REFERENCES

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