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Last updated by author(s):	Mar 4, 2021

Reporting Summary

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For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section

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n/a	Confirmed
	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
	The statistical test(s) used AND whether they are one- or two-sided Only common tests should be described solely by name; describe more complex techniques in the Methods section.
x	A description of all covariates tested
x	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
	For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
×	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
×	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
×	Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated

Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

All software used for data collection are described in detail in the Methods section. Briefly, Zen Blue Lite (Zeiss), NIS-Elements (v4.60.00, Nikon), and AxioVision (v4.8, Zeiss) were used for microscopic image acquisition and ImageJ (v2.0, NIH) was used for processing and analysis. Manufacturer software from the CellASIC ONIX platform (MilliporeSigma) was used for microfluidic experiments, and the Nanowizard IV (JPK Instrument) Quantitative Imaging mode was used for atomic force microscopy. CytoFLEX software from Beckman Coulter (CytExpert, v2.4) was used for flow cytometry, and manufacturer software from Molecular Devices for the SpectraMax M3 was used for plate reader measurements.

Data analysis

ImageJ (v2.0, NIH) and Adobe Photoshop (Adobe) were used for image processing and analysis. FlowJo (v10, Becton Dickinson) was used for flow cytometry data processing and analysis. MATLAB (v2019b, Mathworks) was used for data processing of single-cell traces, and to augment the mathematical modeling performed in our work.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Source data are provided with this paper.

Field-spe	ecific reporting			
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	sclose on these points even when the disclosure is negative.			
Sample size Sample sizes were typically chosen based on the availability of experimental data. For single-cell measurements, all cells for w				
	perform measurements were used, with the exception of Fig. 2, for which we analyzed only 30 cells, and Fig. 3, for which we analyzed 10 to 20 cells. We found that these limited sample sizes provided sufficient data for the statistical tests described. For bulk culture experiments, all data from all experiments were used.			
Data exclusions	When performing the single-cell measurements of Figs. 2 and 3 described above, we chose the first 20 to 30 timelapses to perform cell length measurements on, because the smaller sample sizes provided sufficient data for the statistical tests described. All other timelapses were excluded in a pre-established manner in these measurements, because they were subsequent timelapses taken from the same treatment group of cells. For all other measurements and experiments, no data were excluded.			
Replication	All data were representative of at least two biological replicates, and the numbers of replications of each experiment are indicated as relevant. All attempts at replication were successful.			
Randomization	There were no preallocation considerations. We chose cell cultures to be subjected to treatment or control randomly.			
Blinding	As we were not aware of any potential sources of bias in our experiments, we were not blinded to allocation when performing experiments or performing measurements.			
We require informat	ng for specific materials, systems and methods ion from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, sted is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.			
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	es are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).			
All plots are contour plots with outliers or pseudocolor plots. **A numerical value for number of cells or percentage (with statistics) is provided.				
A numerical	value for number of cells of percentage (with statistics) is provided.			

Bacterial cells were grown as described in Methods and pipetted directly into 96-well plates.

We used a CytoFLEX S flow cytometer from Beckman Coulter.

Methodology

Instrument

Sample preparation

Software We used the manufacturer software from Beckman Coulter (CytExpert, v2.4) for data acquisition. FlowJo (v10, Becton Dickinson) was used for downstream data processing and analysis.

Cell population abundance

As bacterial cells were grown in the absence of any matrix or impurities in LB broth, we found that most scattering events corresponded to bacterial cells. We verified this by calibrating forward and side-scattering events with respect to the standard determined by a fluorescent cytoplasmic mCherry strain, as noted in the Methods section.

Gating strategy

Gating was based on FSC-A and SSC-A (area) measurements, according to the strategy shown in Supplementary Fig. 16.

Fluorescence was then measured with respect to the fluorophores FITC (green), Pb450 (blue), and APC-A (red).

x Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.