## nature research

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## **Reporting Summary**

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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For all st	tatistical and	alyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.				
n/a Co	nfirmed					
	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.					
	A description of all covariates tested					
$\boxtimes \square$	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 Bayesi	an 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					
$\boxtimes \square$	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.				
Softw	vare and	d code				
Policy in	nformation a	about availability of computer code				
Data c	collection	Microscopy data collected using Micro-Manager (versions 1.4beta and 2.0gamma).				
		Custom Micro-Manager plugin for autofocusing is available on the Holden lab Github page: https://github.com/HoldenLab/DeepAutoFocus.				
Data analysis  Videos analysed using Fiji (v1.53) with open-source plugins PureDenoise, StackReg, and TrackMate (v6.0.1); and custom code as Holden lab Github page: https://github.com/HoldenLab/VerciniAnalysisJ.		Videos analysed using Fiji (v1.53) with open-source plugins PureDenoise, StackReg, and TrackMate (v6.0.1); and custom code available on the Holden lab Github page: https://github.com/HoldenLab/VerciniAnalysisJ.				
		Further data analysis done using Matlab with custom code available on the Holden lab Github page: https://github.com/HoldenLab/ring-fitting2.				
For manus	scripts 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				

## Data

Policy information about availability of data

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

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.

- 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

All source data for figures and results in this paper can be found in the Figshare repository: https://doi.org/10.25405/data.ncl.c.5652010.v1

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Please select the or	ne below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.
∑ Life sciences	Behavioural & social sciences Ecological, evolutionary & environmental sciences
For a reference copy of t	he document with all sections, see <a href="mailto:nature.com/documents/nr-reporting-summary-flat.pdf">nature.com/documents/nr-reporting-summary-flat.pdf</a>
Life scien	ices study design
All studies must dis	close on these points even when the disclosure is negative.
Sample size	No a priori sample size calculations were performed. No specific sample size was chosen, as the number of pillars per wafer, and the number of microholes per FoV meant a moderate to large sample size for each measurement, and were sufficiently large for robust statistical analysis.
Data exclusions	No data was excluded.
Replication	The number of independent replicates for any new results, defined as the number of experiments done using independently-prepared samples (e.g. wafers), is described where the results appear.
Randomization	Allocating experimental groups was not relevant for this study, as all bacterial cells of a particular strain are genetic clones.
Blinding	Blinding was neither possible nor necessary for this study, as 1) all bacterial cells of a particular strain are genetic clones and 2) analyses were not sufficiently subjective to require researcher blinding.
Reportin	g for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed 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.

Materials & experimental systems	Methods		
n/a Involved in the study	n/a Involved in the study		
Antibodies	ChIP-seq		
Eukaryotic cell lines	Flow cytometry		
Palaeontology and archaeology	MRI-based neuroimaging		
Animals and other organisms	•		
Human research participants			
Clinical data			
Dual use research of concern			