Dynamic biofilm architecture confers individual and collective mechanisms of viral protection

Lucia Vidakovic1, Praveen K. Singh1, Raimo Hartmann1, Carey D. Nadell1,2* and Knut Drescher1,3*

In nature, bacteria primarily live in surface-attached, multicellular communities, termed biofilms1–6. In medical settings, biofilms cause devastating damage during chronic and acute infections; indeed, bacteria are often viewed as agents of human disease7. However, bacteria themselves suffer from infections; indeed, bacteria are often viewed as agents of biofilms. Biofilms cause devastating damage during chronic and acute infections, yet little is known about the interactions between biofilms and phages. A number of studies have determined the outcome of biofilms exposed to phages8–23, but have not provided a mechanistic understanding of the mechanisms that govern phage–biofilm interactions. To address this open question, we have developed a method for visualizing infection and the spread of lytic T7 phages in living Escherichia coli biofilms at single-cell resolution (Supplementary Fig. 1).

Biofilm size, matrix composition, internal architecture and cellular physiology can vary dramatically during biofilm growth24,25, so we hypothesized that phage susceptibility may vary as a function of biofilm developmental stage. To test this possibility, biofilms of varying ages—grown in microfluidic flow chambers at room temperature (24–26 °C)—were exposed to a continuous influx of phages and imaged by confocal microscopy every 30–60 min for 12 h. We discovered that biofilms that had grown for 48 h or less were rapidly eradicated as a result of phage exposure. By contrast, biofilms that had grown for 60 h or more were collectively protected from phage-mediated killing, with minimally detectable background infection (Fig. 1). For such biofilms, infected cells were rare, were always located on the outer biofilm edges, and did not result in propagation of infection further into the biofilm interior. We confirmed that the protection of biofilms from phage exposure was not due to a shift to stationary phase (Supplementary Fig. 2a), nor was it due to mutations that rendered cells physiologically resistant, as bacterial populations within biofilms grown for 72 h were still susceptible to phage killing after being dispersed and regrown in liquid culture (Supplementary Fig. 2b). The emergence of collective phage protection after 60 h of biofilm growth therefore seems to rely on properties that are specific to biofilms.

The extracellular matrix is an essential and unique feature of biofilm communities, and we hypothesized that the matrix structure might be implicated in phage protection. To understand the impact of the matrix on phage spread, we generated mutants, each lacking one of the Escherichia coli matrix components: proteinaceous curli fibres, flagellar filaments, cellulose, poly-β-1,6-N-acetyl-d-glucosamine (PGA), colanic acid and type 1 fimbriae8. Each of these single-gene knockout mutants was able to produce three-dimensional communities that formed an extracellular matrix and therefore, by definition, was able to form biofilms. Mature biofilms composed of these mutants deficient in any one of these matrix components retained protection from phages, except for mutants lacking curli amyloid fibres (Fig. 2a). Biofilms lacking curli were quickly eradicated by phages (Fig. 2a and Supplementary Fig. 3). A curli operon (csgBAC) transcriptional reporter, together with visualization of secreted CsgA via immunofluorescence, confirmed that curli are produced predominantly in the upper region of biofilms (Fig. 2b, c) and that curli fibres localize in the space between cells, but also cover most cells on the biofilm outer edge (Supplementary Fig. 4). This curli localization in the upper regions of our flow chamber biofilms is consistent with previous reports24,25. Furthermore, curli production initiates between 48 and 60 h after the start of biofilm growth (Fig. 2b, c), corresponding exactly to when biofilms gain protection against phage infection. Biofilms produced by a strain harbouring a csgD promoter mutation (termed csgD*), which causes curli expression at earlier time points during biofilm growth24, show a corresponding earlier shift from phage susceptibility to phage protection (Fig. 2d).

Transcriptional activity profiles of other matrix components were weak in comparison with that of curli, or did not correspond well in space and time with the emergence of phage protection (Fig. 2b and Supplementary Figs. 5 and 6). These results establish that bacteria residing within mature (≥60 h) biofilms are collectively protected from phage infection in a manner dependent on curli amyloid fibres within the extracellular matrix, while cells inside the biofilm remain physiologically susceptible to phages (Supplementary Fig. 2a,b). This is a novel strategy of phage infection avoidance, distinct from other mechanisms of phage immunity, such as CRISPR-Cas, restriction–modification, abortive infection, or alteration of phage...
Fig. 1 | Susceptibility of biofilms to phage exposure as a function of biofilm age. Lines denote the mean biofilm biomass at time $t$, normalized by the biomass at the time of phage exposure $t_0$, and shaded areas denote standard error of the mean ($t_{uni}, n = 8$; $t_{dco}, n = 10$; $t_{gfp}, n = 3$; $t_{csg}, n = 4$; $t_{csg}, n = 7$). In some cases, the standard error is narrower than the line denoting the mean value. Arrows at the start of each curve denote the introduction of phages to the system, and different coloured curves represent different biofilm ages at first phage exposure. Young biofilms were destroyed by propagating waves of phage infection and host cell lysis. Biofilms older than 60 h showed little phage infection and continued to increase in size despite the continuous flux of phases across the biofilms. In the bottom panels, red cells are uninfected and phage-infected cells are cyan due to a phage-encoded green fluorescent protein.

binding sites*. In analogous experiments using T5 phages instead of T7 phages, we observed an identical transition from phage susceptibility to protection as a function of biofilm age (Supplementary Fig. 7), suggesting that this mode of phage protection is generic to different kinds of bacterial viruses.

To understand how curli fibres confer phage protection, we investigated if the biofilm architecture changes upon curli production and how such changes impact phage mobility inside biofilms. To track individual phages, we conjugated purified T7 virions to a fluorescent dye (Alexa Fluor 488). Labelled phages were then incubated with mature biofilms (72 h) of wild-type E. coli, as well as each of the matrix mutants described above. For biofilms produced by wild-type cells, or by mutants lacking PGA ($\Delta$pgaC), colanic acid ($\Delta$wcaE), or cellulose ($\Delta$bcSA), phages accumulated only on the outer biofilm periphery (Fig. 3a–d). Biofilms lacking curli fibres ($\Delta$csgB), by contrast, permitted phages to diffuse freely through their entire volume (Fig. 3e). Using recently developed single-cell resolution biofilm imaging techniques†, we discovered that cells inside biofilms lacking curli fibres were less densely packed (Fig. 3h) and displayed a high cell–cell alignment (Fig. 3i). Such a biofilm architecture generated pores between cells that were wide enough for the phages to diffuse into the biofilm.

Surprisingly, biofilms produced by cells lacking flagella ($\Delta$fliC) also permitted phage diffusion (Fig. 3g), without allowing phage infection (Fig. 2a). This observation suggests that there is a second mechanism of phage protection that is active even when phages have penetrated into the biofilm interior. To understand why the $\Delta$fliC biofilm architecture permits phage diffusion, we investigated this architecture at high resolution, finding cell- and curli-free regions into which the phages were able to diffuse (Supplementary Fig. 8).

The presence of such unoccupied regions yields a biofilm architecture with a lower cell–cell alignment and a distribution of local cell densities that is broader and shifted to lower values, compared with the wild type (Fig. 3h,i). Together with the data for the curli mutant discussed above, these results indicate that cell–cell spacing, rather than cell–cell alignment, is the key biofilm architecture parameter that determines phage transport in biofilms.

To determine how the gaps in the biofilm architecture of $\Delta$fliC biofilms arise, we investigated the activity of the $fliC$ and csgBAC promoters in biofilms. Consistent with previous reports of the inverse regulation of flagella and curli, we found that in $\Delta$fliC biofilms, promoters of flagella and curli synthesis were both active, but never in the same cell (Supplementary Fig. 9a). A $\Delta$fliC biofilm therefore contains a fraction of cells that produce neither curli nor flagella (Supplementary Fig. 9a,b), resulting in cracks in the architecture that are exploited by phages (Supplementary Fig. 8). Interestingly, in biofilms of a strain lacking the flagellar master regulator FlhDC,
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protection, but instead have an indirect effect via the impact of flagella themselves are not directly required for mediating phage (Fig. 4a). Co-incubation of T7 phages and the preformed curli network formed a three-dimensional matrix when incubated on their own curli fibres, spontaneously assemble into filaments, which then

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by fluorescence and electron microscopy (Fig. 4b,c). However, the work resulted in binding of phages to the amyloid fibres, as verified

3, poly-β-1,6-N-acetyl-α-glucosamine (c, n = 3), colanic acid (d, n = 6) or the flagellar master regulator (f, n = 7), phages could be observed only on the outer periphery of 72 h biofilms. For mutants lacking curli fibres (e, n = 4) or flagellin (g, n = 7), however, phages could readily diffuse through the biofilms. h, Single-cell-resolution analysis of biofilm architecture, showing that ΔcsgB and ΔfliC mutants produce less densely packed biofilms than wild-type cells or ΔflhDC. i, Biofilms lacking curli fibres showed higher cell–cell alignment, measured in terms of the nematic order parameter, whereas ΔfliC showed lower cell–cell alignment, compared with wild-type biofilms. Cell–cell alignment within ΔflhDC biofilms resembled that observed for wild-type biofilms. Lines denote means and shaded areas denote standard error of the mean (nWT = 9; nΔcsgB = 9; nΔflhDC = 5; nΔfliC = 24).

nearly all cells in the biofilm produce curli (Supplementary Fig. 9c), which results in a dense cell packing inside the biofilm that phages cannot invade (Fig. 3f). The ΔfliC and ΔflhDC mutants show that flagella themselves are not directly required for mediating phage protection, but instead have an indirect effect via the impact of flagella regulation on curli production.

Our results thus far establish that curli fibres arrange the biofilm architecture so as to prevent phage diffusion. However, it remains unclear whether the fibres themselves also prevent phage diffusion through the pores between cells. To resolve this question, we constructed minimal synthetic biofilms composed of purified curli fibres and fluorescent microbeads with a size similar to bacterial cells (1.0 μm diameter). Purified CsgA proteins, the monomers of curli fibres, spontaneously assemble into filaments, which then formed a three-dimensional matrix when incubated on their own (Fig. 4a). Co-incubation of T7 phages and the preformed curli network resulted in binding of phages to the amyloid fibres, as verified by fluorescence and electron microscopy (Fig. 4b,c). However, the curli mesh by itself did not prevent the diffusion of phage virions (Fig. 4b). Clusters of beads incubated on their own also permitted diffusion of phages, due to the large pore size between close-packed beads (Fig. 4d). Remarkably, when beads were incubated with in vitro polymerized curli fibres, they spontaneously formed clusters embedded within the curli mesh and each curli-embedded bead cluster—like a wild-type biofilm—prevented the diffusion of phages into its inner volume (Fig. 4e). Electron microscopy revealed that curli fibres localize in the pore space between beads (Fig. 4f) and can directly capture phage virions, implying that even a sparse distribution of curli fibres in the pores is sufficient to prevent phage diffusion through biofilm pores.

Because ΔfliC biofilms permitted phage diffusion (Fig. 3g), but not overall phage infection (Fig. 2a), we hypothesized that curli fibres might additionally protect cells on an individual basis, a mechanism that might also take effect in planktonic cultures. Consistent with this idea, we found that when T7 phages were added to shaken liquid cultures of wild-type or ΔcsgB cells, the planktonic wild-type populations were indeed more protected from phage infection than ΔcsgB populations (Supplementary Fig. 10). Electron microscopy confirmed that self-produced curli fibres can cover individual cells in planktonic culture (Fig. 4g). Importantly, E. coli exhibits bistable expression of the csgBA operon in planktonic culture26,28 and we therefore tested the possibility that in a wild-type population, only those cells that produce and embed themselves in curli show higher survival, whereas non-producing cells become infected and lyse. We immunostained the curli fibres in a planktonic wild-type population exposed these cells to our engineered T7 phages. We found that cells completely covered by curli were protected from phage infection, as predicted, while neighbouring daughter cells that were not producing curli became infected and lysed (Fig. 4h). Binding of phages to the surrounding curli fibres (Fig. 4b,c) is likely to also prevent phages from attaching to the host cell exterior, yet it remains an open question to what extent phages with different host–cell adsorption mechanisms can be shielded by the curli mesh. Complete coverage of the cell exterior by curli fibres was necessary for T7 phage protection.
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Fig. 4 | Reconstruction of minimal synthetic biofilms recapitulates phage diffusion prevention and phage–cell attachment prevention in vivo. a, Curli fibre monomer CsgA was purified and shown by electron microscopy to polymerize in vitro (n = 2). b, Curli fibres alone, visualized by immunostaining (orange), permit free diffusion of phage virions (cyan) into the curli mesh (n = 3). c, Phages can bind to curli fibres that were polymerized in vitro before phage exposure, as indicated by arrows (n = 3). d, Fluorescent beads (magenta) were used as artificial replacements for bacterial cells. 1 μm diameter beads permit diffusion of phage virions throughout the interior of bead clusters. e, When beads are combined with curli polymers to produce minimal artificial biofilms, bead clusters are protected from phage diffusion (n = 4). f, Preformed curli fibres, when incubated with beads, localize between individual beads and wrap around bead clusters, as shown by electron microscopy (n = 3). g, Individual cells that are capable of curli production (but incapable of flagella and cellulose production) embed themselves in a dense mesh made out of self-produced curli fibres (n = 3). h, Individual bacteria (red) that are surrounded by curli (yellow) are not infected by phages and can divide normally, whereas cells that are not surrounded by curli become infected (grey) and then lyse (white dashed line) (n = 5). i, Bacteria that are not completely surrounded by curli are not protected from phage infection (n = 5).

of planktonic cells; cells that were incompletely covered remained unprotected and became infected and lysed over time (Fig. 4i).

Bacterial biofilms are ubiquitous, complex structures with broad ecological and medical impact, and they play a central role in microbial ecology and natural history. Here, we have demonstrated that amyloid fibres, which constitute only a single component of the E. coli extracellular matrix, reorganize the biofilm architecture such that susceptible bacteria achieve protection on the individual and collective cell level from exposure to phages. Investigating the general relationship between biofilm matrix production and biofilm–phage interactions, as well as modifying the diffusivity and infectivity of phages in biofilms, are therefore important directions for developing the next generation of therapeutic phages, for editing microorganisms and for a fundamental understanding of phage–bacteria co-evolution in nature13,29,30.

Methods

Bacterial strains. All strains used in this study are derivatives of E. coli strain AR31103 and are listed in Supplementary Table 1. Chromosomal modifications inside the E. coli genome were generated by lambda red recombination.

The scarless lambda red system was applied to construct chromosomal transcriptional reporter fusions, 6x-His tagged csgA, curli complementation and curli overexpression strains. Transcriptional reporter fusions were constructed by inserting mKate2 into the chromosome, either extending the native operon or creating an artificial operon. To complement the ΔcsgB mutant, csgB was inserted under control of its native promoter into the attB site of E. coli. All strains constitutively express a fluorescent protein under control of the tac promoter (with the operator deleted) inserted into the attB site of E. coli.

T7 phage modification. We engineered recombinant T7 phage using the T7select415-1 phage display system (Millipore) with standard molecular biology techniques. We cloned the E. coli codon-optimized sfgfp (superfolder green fluorescent protein) gene under control of the strong T7 phi10 promoter downstream of the T7select415-1 10B capsid gene31. Infected host cells produced SfGFP rapidly enough to clearly fluoresce in the green channel before subsequently lysing and releasing a cohort of viable progeny phages (Supplementary Fig. 1).

Growth and phage infection experiments in the plate reader. Bacterial growth under constant shaking was monitored using a shaking incubator plate reader (Tecan Spark 10M). Optical density and fluorescence intensity measurements were performed every 5 min. Bacteria were grown at 37 °C, where curli fibre expression does not occur (Supplementary Figs. 1 and 2 and refs. 13,29). For cases in which curli production was to be induced among cells in liquid culture, the growth temperature was set to 28 °C (Supplementary Figs. 2c and 10).

Biofilm growth in microfluidic channels. Bacteria were grown in lysogeny broth (LB) medium overnight at 37 °C and shaken at 250 T/min. The cells were washed and diluted 1:80 in 0.9% NaCl, inoculated into polydimethylsiloxane microfluidic devices and incubated in the channels for 1 h at room temperature (24–26 °C) to colonize the glass substrate. Syringes containing tryptone broth (10 g l−1) were connected with polyethylene tubing to the inlets of the microfluidic chambers.

Residual planktonic cells were then removed from the chamber by increasing the flow to 50 μl min−1 for 40 s. The flow rate was decreased to 0.1 μl min−1 for the remainder of the experiments, and the microfluidic channels and the inflowing medium were kept at room temperature (24–26 °C).

Bacteriophage amplification and purification. E. coli AR3110 was grown under shaking conditions at 37 °C until reaching an optical density at 600 nm (OD600) of 1.0. T7 phages were added, and the culture was incubated for an additional 3 h for phage amplification. The lysate was filter-sterilized (0.22 μm pore size) and stored at 4 °C. To generate fluorescent phage virions, further purification was necessary. After phage amplification, DNAseI was added to the culture lysate, which was then incubated for 30 min at 37 °C. Following DNA digestion, NaCl was added to a final concentration of 0.5 M and the lysate was incubated for 1 h at 4 °C. Phages were separated from cell debris by centrifugation for 10 min at 8,000 g (4 °C). The supernatant containing T7 phages was filtered (pore size 0.22 μm). PEG6000 (10% final concentration) was added and phages were allowed to precipitate overnight at 4 °C. After centrifugation for 15 min at 8,000 g (4 °C) the phages were suspended in phosphate-buffered saline (PBS). To obtain pure phage cultures, density gradient centrifugation (62.5–21% CsCl) was performed overnight at 100,000 g, followed by dialysis against PBS. The resulting highly purified T7 phages were stored at 4 °C.

Bacteriophage labelling with fluorescent dye. Purified phages (100 μl, 1012 p.f.u. ml−1) mixed with sodium carbonate (0.1 M final) were incubated with 0.1 mg Alexa Fluor 488 for 1 h at room temperature under continuous shaking. The reaction mixture was dialysed against PBS to separate phages from unbound dye. Fluorescently labelled phages were stored at 4 °C.

Phage–biofilm interaction experiments and analysis. E. coli biofilms were exposed to T7 phages after growth for a defined time at room temperature. Immediately after addition of T7 phages (109 p.f.u. ml−1) or T7 phages...
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was computed as the average nematic order parameter $S_{\text{neighbourhood}}$. Order and cell density were calculated per cell with respect to local spherical individualization by watershedding and/or $k$-means clustering. Local nematic planes. Clumped structures were segmented as described previously. Unlike in the previous study, segmentation was performed according to an established protocol. For protein purification, a prepacked Ni-NTA column was used instead of resin. Successful protein purification was verified by western blotting using an anti-6x-His antibody conjugated to Alexa Fluor 488 (Qiagen product no. 35310). Electron microscopy was used to confirm the polymerization of purified CsgA into curli fibres.

In vitro biofilm reconstruction. For in vitro biofilm reconstruction, fluorescent beads (with a 100 nm silver core, platelet to create bead aggregates. Fractions of the pellet were inserted into microfluidic channels with a poly-l-lysine-coated surface and incubated with fluorescently labelled phages (10 P.f.u. ml$^{-1}$) for 1 h under constant flow (1 µl min$^{-1}$). Curli fibres were allowed to polymerize from purified CsgA and were stained with Congo red (4 mg ml$^{-1}$) for 1 h at room temperature. These stained, pre-polymerized fibres were then incubated with beads for 1 h under constant shaking in the presence of 1% BSA. The curli–bead mixture was then inserted into a microfluidic channel containing a poly-l-lysine-coated surface. Finally, fluorescently labelled phages (10 P.f.u. ml$^{-1}$) were inserted under constant flow (1 µl min$^{-1}$) for 1 h. Phase diffusion through the in vitro reconstructed biofilms was visualized via confocal microscopy.

Phage interaction with single cells. To study phage infection of single cells that are protected by curli. The signal was interpreted to originate from Alexa Fluor 488 and indicate phage infection reporter activity and was displayed in cyan.

Biofilm architecture characterization at single-cell level. To investigate biofilm architectural properties at the single-cell level, biofilms were imaged and segmented as described previously. Unlike in the previous study, segmentation of cells in the biofilm was performed using a three-dimensional edge detection algorithm instead of thresholding single 2D planes. Clumped structures were individualized by watersheding and/or $k$-means clustering. Local nematic order and cell density were calculated per cell with respect to local spherical neighbourhoods of 3 µm radius. For the local cell density, the fraction of occupied cell volume in the local neighbourhood was calculated. The local nematic order was computed as the average nematic order parameter $S_{\text{neighbourhood}}$ (1/2) in the local neighbourhood, where $n$ is the orientation vector of the focal cell and $n_i$ are the orientation vectors of cells in the local neighbourhood. One imaging operation within a microfluidic channel was defined as one replicate. Three to five locations were imaged per channel.

Scanning electron microscopy of curli fibres. To visualize in vitro polymerized CsgA curli fibres via electron microscopy, curli fibres were deposited onto a poly-l-lysine-coated glass slide for 1 h. Afterwards, the fibres were fixed for 5 min with 4% paraformaldehyde, washed three times in dH$_2$O (each 5 min) and dehydrated in a graded ethanol series followed by critical point drying. Samples were sputter-coated with platinum using a sputter coater (Sputter Coater SCD050, Bal-Tec) and imaged using a scanning electron microscope (JOEL JSM 7500-F). To visualize T7 phages bound to in vitro polymerized curli fibres, both were incubated together for 1 h under constant shaking. Unbound phages were removed via centrifugation (15,000 r.p.m, 3 min) and washing of the curli fibre pellet with PBS was performed before deposition of the sample onto the poly-l-lysine-coated glass slide. Preparation of samples involving curli fibres and beads required incubation of beads with preformed curli in the presence of 1% BSA for 1 h under constant shaking before placing the bead–curli mixture on a poly-l-lysine-coated glass slide.

Life Sciences Reporting Summary. Further information on experimental design and reagents is available in the Life Sciences Reporting Summary.

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Author contributions
C.D.N. conceived the topic. C.D.N. and K.D. designed the project. L.V. and P.K.S. generated strains and acquired data. R.H. developed new analytical software. L.V., R.H., C.D.N. and K.D. analysed and interpreted the data. L.V., C.D.N. and K.D. wrote the paper with the help of all authors.

Competing interests
The authors declare no competing financial interests.

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Experimental design

1. Sample size
   Describe how sample size was determined.
   Each of the n replicates of each experiment is the average of thousands of bacterial cells as a sample size. Each experiment was replicated at least 3 times, but usually n>3. The n for each experiment is indicated in each figure.

2. Data exclusions
   Describe any data exclusions.
   No data were excluded.

3. Replication
   Describe whether the experimental findings were reliably reproduced.
   Each experiment was replicated n times (where n is given in each figure for each experiment). Although the exact quantification differs between replicates, the qualitative result is the same, so that it is reasonable to say that the "replication was successful".

4. Randomization
   Describe how samples/organisms/participants were allocated into experimental groups.
   There were thousands of bacterial cells sampled for each of the n replicates. Because of the large sample size for each replicate, a representative number of samples were collected for each replicate. There was no allocation of samples into experimental groups, beyond conducting separate biological replicates.

5. Blinding
   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
   Blinding of group allocation is irrelevant to our data analysis, because there was no allocation to experimental groups, beyond collecting n replicates, all of which were analyzed equally.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.
6. Statistical parameters
For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

| n/a | Confirmed |
|-----|-----------|
| ✗   | The exact sample size \((n)\) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.) |
| ✗   | A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ✗   | A statement indicating how many times each experiment was replicated |
| ✗   | The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section) |
| ✗   | A description of any assumptions or corrections, such as an adjustment for multiple comparisons |
| ✗   | The test results (e.g. \(P\) values) given as exact values whenever possible and with confidence intervals noted |
| ✗   | A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range) |
| ✗   | Clearly defined error bars |

See the web collection on statistics for biologists for further resources and guidance.

7. Software
Policy information about availability of computer code

Matlab (version R2016b) was used to analyze data. A detailed description of the analysis is provided in the Materials and Methods section.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

8. Materials availability
Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

All materials that were used in this study are available from standard commercial suppliers or from the corresponding author.

9. Antibodies
Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

All antibodies that were used are listed in the Materials and Methods section, including part numbers and supplier information.

10. Eukaryotic cell lines
a. State the source of each eukaryotic cell line used.

b. Describe the method of cell line authentication used.

c. Report whether the cell lines were tested for mycoplasma contamination.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

11. Description of research animals
Provide details on animals and/or animal-derived materials used in the study.

not applicable
Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

not applicable