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SUMMARY

The conventional view is that high temperatures cause microbes to replicate slowly or die. In this view, microbes autonomously combat heat-induced damages. Yet, microbes co-exist with each other, raising the underexplored and timely question of whether microbes can cooperatively combat heat-induced damages at high temperatures. Here we use the budding yeast, *Saccharomyces cerevisiae*, to show that cells can help each other and their future generations survive and replicate at high temperatures. As a surprising consequence, even for the same temperature, a yeast population can either exponentially grow, never grow, or grow after unpredictable durations (hours-to-days) of stasis, depending on its population density. Through the same mechanism, yeasts collectively delay and can eventually stop their approach to extinction, with higher population-densities stopping faster. These features arise from yeasts secreting and extracellularly accumulating glutathione - a ubiquitous heat-damage-preventing antioxidant. We show that secreting glutathione, which eliminates harmful extracellular chemicals, is both necessary and sufficient for yeasts to collectively survive high temperatures. A mathematical model, generally applicable to any cells that cooperatively replicate by secreting molecules, recapitulates all these features. Our study demonstrates how organisms can cooperatively define and extend the boundaries of life-permitting temperatures.

Microbes live in a range of “habitable temperatures” (*1-3*). The conventional view is that increasing the temperature above some optimal value causes microbes to take more time to self-replicate and that once the temperature goes beyond the habitable range - into an "unlivable temperature" regime - microbes cannot replicate and they die (*1-4*) (Figs. 1a-b). In this textbook view, a microbe's ability to replicate at high temperatures hinges on whether the cell can combat heat-induced damages by itself such as misfolded proteins (*5-8*) which it cannot at sufficiently high temperatures, leading to its death (Fig. 1c). Yet a microbe often lives with other cells instead of alone, enabling them to work together for their collective survival (*9-13*). Given the timeliness of understanding how the rising global temperatures affect organisms, we used the budding yeast, *Saccharomyces cerevisiae*, to re-examine the conventional picture - one in which yeasts autonomously combat heat shocks - to investigate whether microbes can also collectively combat high temperatures to avoid becoming extinct (Fig. 1d).

As our starting point, we reproduced the well-known, textbook picture of how temperature affects microbial growths by measuring the population-level growth rates for a laboratory-standard ("wild-type") strain of haploid budding yeast in liquid cultures (*4,14-16*) (Fig. 1b and Supplementary Fig. 1). In this picture, the population growth-rate is zero for temperatures of 40 ^{O}C and higher (Fig. 1b and Supplementary Fig. 1). Despite being evidently true - as we reproduced it here - we discovered that this textbook picture (Fig. 1c) is misleading and requires a revision. In this paper, we revise this picture with experiments and a mathematical model, which reveal that, at sufficiently high temperatures, yeasts secrete and extracellularly accumulate glutathione - a major antioxidant for many species - that cleanses the extracellular environment of harmful reactive oxygen species whose high reactivity is damaging for cells. Thus, we discovered that yeasts help each other and their future generations replicate, survive, and avoid becoming extinct at high temperatures (Fig. 1d). In short, our work demonstrates the habitability of a temperature for a single-celled organism emerging as a community-level property, determined by interactions among the members of the microbial community.

RESULTS

Population-density determines replicability of cells and habitability of temperature

We re-examined the conventional, cell-autonomous picture by incubating populations of wild-type yeasts in liquid media at a conventionally-defined habitable temperature (~38 ^{O}C), unlivable temperature (~40 ^{O}C), and a transition temperature in between the two (~39 ^{O}C). This time, and

in contrast with the conventional picture (Supplementary Fig. 1), we precisely set the initial population-density (# of cells/mL) and studied its effect on population growth. With a flow cytometer, we counted the integer numbers of cells per volume to determine the population-density over time. These experiments revealed surprising behaviors. Specifically, at the supposedly-habitable temperature of ~38 ^{O}C, none of the replicate populations that started with a relatively low population-density (200 cells/mL) grew at all during ~12 days of incubation except for a small, transient growth that occurred for a few hours right after the transfer from 30 ^{O}C (Fig. 2a - red curves). At the same temperature (~38 ^{O}C), setting the initial population-density to be just five times larger (1,000 cells/mL) yielded a population whose behavior was completely unpredictable: it could either grow until it reached the carrying capacity (i.e., ~10^7 cells/mL) or not grow at all after the initial transient-growth (Fig. 2a - green curves). When the population did grow, it could wait four days or eight days or some other, unpredictable time before starting to grow (Fig. 2a - multiple green curves). Still, at the same temperature (~38 ^{O}C), setting the initial population-density to be again just five times larger (5,000 cells/mL) yielded populations that always grew exponentially and identically over time up to the carrying capacity (Fig. 2a - blue curves). Thus, at the supposedly "habitable" temperature of 38 ^{O}C, only the largest of the three initial population-densities led to the deterministic growth that the conventional picture states should be exhibited by every population (*1,14-16*). The same three, population-density-dependent growth behaviors also occur near the upper limit of the habitable temperatures (~39 ^{O}C) (Fig. 2b). Moreover, we found that populations with sufficiently many cells can grow at ~40 ^{O}C - a supposedly "unlivable" temperature (Fig. 2c - non-red curves). These results show that in order to determine whether a yeast-population grows or not, one must know *both* the temperature and the initial population-density.

Phase diagram summarizes population-level behaviors across temperatures

By incubating liquid cultures of populations with differing initial densities at multiple temperatures, we constructed a "phase diagram" (Fig. 2d and Supplementary Figs. 3-4). The phase diagram, summarizing the population-level growth behaviors, consists of four phases - deterministic growth, random growth, no-growth, and no-growth due to insufficient nutrients - as a function of the initial population-density and temperature. It reveals that the conventional picture (Figs. 1b-c) mistakenly arises because one typically sets the initial population-density to lie within some narrow range when studying population growths. This leads to, for example, the growth rate *appearing* to decrease as the temperature increases within a given range (e.g., 36.5 ^{O}C ~ 39 ^{O}C) (Fig. 1b). But, in fact, for the same temperature range, we found that the populations' growth rates - when they

grew - were poorly correlated with temperature and could highly vary among populations even for the same temperature if we widely varied the initial population-density (Fig. 2e). The phase boundary between the deterministic-growth and random-growth phases (Fig. 2d) describes the minimum, initial population-density necessary to guarantee that a population grew at each temperature. Conversely, the phase boundary between the random-growth and no-growth phases (Fig. 2d) describes the maximum, initial population-density necessary to guarantee that a population never grew at each temperature. Both of these values are highly sensitive to temperature (e.g., a ~100-fold change when going from 39 ^{O}C to 40 ^{O}C (Fig. 2d)). The random-growth phase may be seen as a hybrid of the deterministic-growth and no-growth phases. A small change of either can transform a no-growth into a deterministic-growth and vice-versa (Fig. 2a). Intriguingly, all phase boundaries converge at a single point ("fold-bifurcation point") located at 40.3 ^{O}C, leading to only the no-growth phase at temperatures beyond 40.3 ^{O}C (Fig. 2d). Specifically, at temperatures higher than 40.3 ^{O}C, populations can grow but stop growing before reaching the carrying capacity, with their final population-densities depending on their initial population-densities. The term, "fold-bifurcation point" comes from dynamical systems theory and is the point in the phase diagram where a stable fixed point (carrying capacity) merges with an unstable fixed point (the upper boundary of the no-growth phase).

Expressing a superfluous gene reshapes phase diagram

We discovered that forcing yeasts to constitutively express the Green Fluorescent Protein (GFP), which serves no function for cell growth, shifts the phase boundaries (Fig. 2f and Supplementary Fig. 5). In particular, reducing the GFP expression could shift the phase boundaries by several degree Celsius, suggesting that the cost of expressing superfluous genes can markedly alter the phase diagram. In light of previous studies (*17,18*), this may be due to expressing GFP shifting the intracellular resources, which may be especially crucial for surviving high temperatures, away from performing roles that are for cell growth. While our study has revealed a genetic means for reshaping the phase diagram, the molecular mechanisms that underlie the reshaping are left for future studies.

A few cells initiate population growth in random-growth phase and transiently replicating sub-populations exist in non-growing populations

We turned to single-cell-level measurements for further insights. The wild-type strain has a mutated *ade2* gene that causes a cell to accumulate a red pigment, which can only be diluted by persistent cell divisions (*19*). Thus, we could use our flow cytometer's red-fluorescence detector

to determine which cells had been replicating and which cells had not (Extended Data Fig. 1). For a deterministically growing population, we discovered that, after a short transient growth associated with the transfer from 30 ^{O}C to the high temperature, the number of replicators exponentially increased over time up to the carrying capacity (Extended Data Fig. 1). In contrast, for random-growth and no-growth populations, the number of replicators typically decreased until very few cells (~1-5% of population) remained as replicators. Subsequently, the number of replicators either spontaneously increased by orders of magnitude after an unpredictable hours or days (random-growth) or remained sustainably low and fluctuated by few-folds over nearly a week (no-growth) (Extended Data Fig. 1). These fluctuations were sufficiently small that the total population-density remained nearly constant. These results establish that a small sub-population of transiently replicating cells exist and that the fraction of replicators in the population could stably remain in low numbers (e.g., ~1% of total population). We will later return to these features with a mathematical model that recapitulates them.

Cells collectively combat extinctions at high temperatures

We next asked whether cell death, like cell replication, also depends on the initial population-density. At several temperatures, we measured how the number of surviving cells changed over time for no-growth phase populations (Supplementary Fig. 6). Surprisingly, these measurements deviated qualitatively, not just quantitatively, from the textbook picture in which microbes such as yeasts autonomously die and that dictates that the number of survivors should exponentially decrease over time (*4*) (Fig. 3a - brown line). Yet, we discovered that the number of survivors decreases over time in a heavy-tailed (power-law-like) manner (Fig. 3a - blue curve). In other words, the population continuously decelerates, and eventually would cease, its approach to extinction. For example, after three days at 41 ^{O}C, the number of survivors in a population deviated by ~10^{7}-fold from the expected value dictated by the conventional theory (Fig. 3a - last time point; Supplementary Figs. 6). Moreover, we discovered that the rate at which cells die at high temperatures depends on the initial population-density (Fig. 3b and Supplementary Fig. 6). Specifically, the number of survivors appears to exponentially decrease during the first day before it noticeably enters a heavy-tailed decay regime on later days (Fig. 3b). Hence, we can assign a constant rate of decay to each population to describe how the number of survivors initially decreases (e.g., during the first day at a high temperature). We found that this rate ("initial death-rate") decreases as the initial population-density increases, meaning that the number of survivors decreases more slowly for higher initial population-densities (Fig. 3b - three dashed lines). These results suggest a highly non-linear, cooperative effect that cells have on each other's survival.

Temperature of the fold-bifurcation point separates two extinction-avoidance regimes

We next measured the initial death-rate at multiple temperatures for populations of differing initial densities. The population half-life, which is derived from the initial death-rate and is the time taken for the number of survivors to be halved, should be independent of the initial population-density, according to the conventional view in which yeasts autonomously die (Fig. 3c). Instead, we discovered that increasing the initial population-density always increases the population half-life and that the temperature determines how sensitively the population half-life depends on the initial population-density (Fig. 3d). Specifically, a population half-life has two regimes of sensitivities. Temperatures below 40.3 ^{O}C exhibit the first regime. Here the population half-life is highly sensitive to the initial population-density: it increases from hours to days if the initial population-density nearly doubles (Fig. 3d - yellow curves for 39 ^{O}C ~ 40 ^{O}C). Moreover, as the initial population-density keeps increasing, the population half-life keeps increasing and eventually becomes infinity. This is because a sufficiently high-density population grows at these temperatures (Fig. 2d). Temperatures above 40.3 ^{O}C exhibit the second regime: increasing the initial population-density above some value hardly changes the population half-life, which eventually plateaus at a finite value as the initial population-density keeps increasing (Fig. 3d - purple curves for 41 ^{O}C ~ 43 ^{O}C). This occurs because populations cannot grow regardless of their initial densities at these temperatures (Fig. 2d). At the fold-bifurcation point (Fig. 2d), the population-density can remain at a nearly constant value (i.e., population half-life is infinite because the initial death-rate is zero). The fold-bifurcation point is the only place in the phase diagram (at 40.3 ^{O}C with ~1 x 10^5 cells/mL) at which a non-growing population's half-life is infinite. In other words, a population at the fold-bifurcation point can constantly maintain its density, apparently indefinitely, unless fluctuations cause its demise. Taken together, our results establish that a yeast's death depends on the other cells in the population. Moreover, we determined that neither heat-resistant mutants nor “persister-like” cells such as those seen in antibiotic persistence (*20*) can explain our data on cell deaths (Extended Data Fig. 2).

Extracellular factor dictates cell replications at high temperatures

We next sought to uncover the mechanisms that underlie the density-dependent replications and deaths of yeasts. As a start, we determined that cells isolated from a growing culture and put into a fresh medium do not grow, whereas the liquid medium isolated from an exponentially growing population and transplanting a fresh population of cells into it causes that population to grow, even though the phase diagram indicated that the population initially had too few cells for it to grow (Fig.

4a-c, Supplementary Figs. 7-8). These results suggested that an “instruction” that dictates population growths resides in the extracellular - not intracellular - environment. Moreover, we confirmed that depletion of any of the nutrients does not instruct a population to grow (Supplementary Figs. 8-9), indicating that it is the secretion of some factor(s) at high temperatures that induces population growths.

Yeasts secrete glutathione to help each other replicate at high temperatures

By performing a transcriptome analysis (RNA-seq) on wild-type yeasts at different locations in the phase diagram (Supplementary Fig. 10), we uncovered gene-expression profiles that are similar to those of yeasts undergoing environmental stresses (*21,22*). We hypothesized that yeasts at high temperatures may be stressed due to reactive oxygen species, which are known to be abundant at high temperatures (*23-25*) and damaging for cells (*26-28*). Given that antioxidants inactivate reactive oxygen species, we further hypothesized that yeasts at high temperatures may be secreting antioxidants. Indeed, studies have found that heat-shocked yeasts produce and maintain elevated levels of intracellular glutathione (*23,24*), a tripeptide that is the yeast's primary antioxidant (*23,29,30*) besides having other essential roles (*31*). Although much is known about glutathione's intracellular roles in yeast (*29-34*), little is known about whether yeasts secrete glutathione and, if so, why and when they would do so aside from a few examples such as yeasts secreting glutathione to defend against harmful extracellular arsenite (*35*).

Supporting our hypothesis, is the fact that yeasts are known to secrete small amounts of glutathione in stationary-phase at 30 ^{O}C (after diauxic shift) *(36)*, and that we found that medium coming from such populations induces growth at high temperatures (Supplementary Fig. 8). Indeed, we discovered that adding high concentrations of either glutathione or ascorbic acid - both antioxidants (*29*) - to the growth medium caused growth of populations that, without the added antioxidants, could not have grown by themselves because they had too few cells (Fig. 4d). Hence, extracellular antioxidants - glutathione and ascorbic acids - are sufficient for inducing yeast-population growths at high temperatures. Focusing on glutathione, we found that random-growth phase and deterministic-growth phase populations continuously secreted and extracellular accumulated glutathione during log-phase growths and stationary-phase at high temperatures (Fig. 4e and Supplementary Fig. 11). But we detected only small concentrations of extracellular glutathione that barely increased over time for no-growth phase populations at high temperatures. Moreover, consistent with the population-density dependent growths only occurring for temperatures above ~36 ^{O}C being caused by glutathione, we found that yeasts secreted glutathione only at temperatures above ~36 ^{O}C but not below it (Fig. 2d and Supplementary Fig.

11). Furthermore, we had to add sufficiently high concentrations of glutathione to induce growth of a population that could not grow by itself (Fig. 4f). Specifically, if the extracellular glutathione concentration was below ~0.3 μM, populations hardly grew. But extracellular glutathione concentrations above ~0.3 μM induced population growths up to carrying capacity. Consistent with these findings, when we did not add any glutathione at high temperatures, no-growth populations had accumulated less than ~0.3 μM of extracellular glutathione whereas the growing population had accumulated more than ~0.3 μM of extracellular glutathione (Fig. 4d). In summary, we have now established that yeasts at high temperatures secrete and extracellularly accumulate glutathione that - above some threshold concentration (~0.3 μM) - induces population growths (Fig. 4g).

Mathematical model recapitulates experimental data

To explain our data, we developed a stochastic, mathematical model that contained just one free parameter (see Supplementary text). In this model, each alive cell secretes glutathione at a constant rate and in each time step, with some probability, takes one of three actions: replicate, die or stay alive without replicating (Fig. 5a). The probability of dying is fixed by and linearly increases with temperature. Given that yeast-populations require at least a threshold glutathione-concentration for growth (Fig. 4f), the probability of replicating non-linearly increases with extracellular glutathione concentration in our model (Fig. 5b). The only free parameter, which requires fitting to our data, is the extracellular glutathione concentration at which the probability of replicating is half its maximum (Fig. 5b - blue curve). All other parameters are directly read-off from our data (Supplementary text).

Our model recapitulates all the main experimental data (Figs. 5c-f and Extended Data Fig. 3). The model's main idea is that in order to avoid becoming extinct at a high temperature, the population - which initially lacks any extracellular glutathione and thus starts with a zero probability of a cell replicating - must keep accumulating extracellular glutathione to keep increasing the probability of replication up to and above the probability of a cell dying - which is fixed by the temperature (Extended Data Fig. 3). Populations achieve this if and only if they starts with sufficiently many cells. Populations with too few cells goes extinct and belong to the no-growth phase because they have insufficient time to accumulate enough extracellular glutathione: the probability of replicating increases until the last cell dies but always remains below the probability of dying. Populations with intermediate densities may grow or approach extinction (i.e., exhibits the random-growth phase) because the glutathione concentration nears the threshold concentration by the time there are very few surviving cells, whose stochastic replications or

deaths subsequently determine whether or not the probability of replicating exceeds that of dying. At temperatures above ~40.3 ^{O}C - where the fold-bifurcation is - the probability of dying exceeds the maximally allowed probability of replicating, meaning that only the no-growth phase is possible at these temperatures (Fig. 5b – grey dashed line). In the no-growth phase, the continuous accumulation of extracellular glutathione results in populations decelerating their approach to extinction over time, leading to the heavy-tailed function describing the number of survivors decreasing over time and populations with higher initial densities more slowly approaching extinction (see Supplemental Fig. 12). Taken together, our minimal model recapitulates all the main experimental data (Figs. 5c-f).

Extracellular glutathione is necessary and sufficient for surviving high temperatures

To address whether extracellular glutathione is necessary - not only sufficient (Fig. 4e-f) - for yeasts to survive high temperatures, we used a "masking reagent" (1-Methyl-2-vinylpyridinium, M2VP) that specifically inactivates extracellular glutathione only, without interfering with the intracellular glutathione and any other processes (Supplementary Fig. 13) (*37,38*). Adding the masking agent stopped deterministically growing populations at high temperatures (Fig. 6a). Thus, glutathione is both necessary and sufficient - glutathione is the only responsible molecule - for inducing cell replications at high temperatures (above ~36.7 ^{O}C).

Manipulating synthesis, import, and export of glutathione at high temperatures

To gain further insights, we constructed mutants that were either unable to synthesize glutathione (*gsh1Δ*-strain) (Fig. 6b) (*39*), or unable to import glutathione (i.e., *hgt1Δ*-strain) (*40*), or had severely reduced ability to secrete glutathione (*gex1,2Δ-adp1Δ*-strain) (*41,42*). We found that the mutants that cannot synthesize glutathione (*gsh1Δ*-strain) confirmed our earlier conclusion that the wild-type cells secrete glutathione only at high temperatures (above ~36 ^{O}C) (Extended Data Fig. 4). We also found that the mutants that cannot import glutathione (*hgt1Δ*-strain) have the same population-density-dependent growths at high temperatures as the wild-type strain (Fig. 6c). Thus, yeasts do not need to import extracellular glutathione in order to replicate at high temperatures. This, in turn, means that glutathione's extracellular action alone, not intracellular action, is responsible for promoting replications at high temperatures. Indeed, we found that the mutants with significantly reduced abilities to secrete glutathione (*gex1,2Δ-adp1Δ*-strain) are less able to replicate than the wild-type strain (Fig. 6d), thus requiring a higher initial population-density for growth. Consistent with glutathione's extracellular action – rather than intracellular action – promoting cell replications, we found that reducing the export of glutathione reduces the

populations' ability to grow at high temperatures. Strikingly, we found that the mutants with a reduced glutathione-export (*gex1,2Δ-adp1Δ*-strain) still secreted measurable amounts of glutathione at high temperatures, which is not due to glutathione passively leaking out through cell membranes, meaning that other glutathione exporters function at high temperatures.

Yeasts can replicate at "unlivable" temperatures

No population can avoid extinctions at temperatures higher than 40.3 ^{O}C (Fig. 2d) because the cells die too fast to accumulate enough extracellular glutathione. Hence, giving high concentrations of glutathione to populations at the start of incubation at extremely high temperatures may help them accumulate enough glutathione before extinction. Indeed, at 41 ^{O}C for example, we could rescue populations with as low as 400 cells/mL from extinction: they exponentially grow until reach a carrying capacity (Fig. 6e).

DISCUSSION

By showing that secreting and extracellularly accumulating glutathione is necessary and sufficient for yeasts and their future generations to survive and replicate at high temperature (Fig. 6f), our work revises the textbook view of cells autonomously combating heat-induced damages. A common explanation for why cells, including budding yeasts, cannot replicate at high temperatures is that essential proteins unfold at high temperatures *(6)*. Our work suggests that this explanation requires revisions. In fact, we found that yeasts with enough extracellular glutathione can replicate at extremely high temperatures for which such proteins would unfold (i.e. above 41 ^{O}C - Fig. 6e, Supplementary Fig. 12). Glutathione, an antioxidant essential for many organisms, including humans (*29*), is central to diverse processes (*30-35*). Our work extends the relatively little-known extracellular functions of glutathione in yeast (*35,36,43*) by showing that yeasts must secrete sufficient amounts of glutathione at high temperatures during log-phase growth and stationary phases. We found that the glutathione extracellularly accumulates and that either ~75% (for no-growth populations) or ~25% (for growing populations) of it exists in in the oxidized form (Supplementary Fig. 11). Both values, 75% and 25%, are higher than the previously reported values for oxidized glutathione that budding yeasts steadily maintain (*36*), suggesting that yeasts collectively "clean up" their environment by reducing harmful, extracellular reactive oxygen species and thereby help each other and their future generations replicate and survive high temperatures.

Researchers have observed fold-bifurcation points, like the one in our study (Fig. 2d), in other microbial populations on the verge of extinctions such as those in which yeasts collectively hydrolyze extracellular sucrose (*44-46*). These dynamical systems, including ours, typically exhibit features familiar from phase transitions such as “critical slowing down” (*45*) which, in our study, manifests as the yeast-population’s half-life being infinite at the fold-bifurcation point (Fig. 3d). By uncovering a phase diagram for cell replication, our work may aid in advancing theories of non-equilibrium statistical mechanics (*47*) that pertain to biologically realistic, self-replicating systems that drive and maintain themselves out of thermal equilibrium. Moreover, investigating how organisms can collectively combat high temperatures, as in our study, may suggest ways to help organisms combat climate change and help us understand how climate change impacts unicellular life and multicellular communities.

Methods

Growth media and strains. The "wild-type", haploid yeast strain that we used is from Euroscarf with the official strain name "20000A". It is isogenic to another laboratory-standard haploid yeast "W303a", and has the following genotype: *MATa*; *his3-11_15; leu2-3_112; ura3-1; trp1⊿2; ade2-1; can1-100*. We built the two strains that constitutively expressed GFP by first using PCR to insert a functional *ADE2* gene into the locus of the defective *ade2* gene in the wild-type strain, by a homologous recombination, so that the red pigments that would have accumulated without the *ADE2* insertion no longer existed (i.e., the strain can now synthesize adenine). We could thus detect their GFP fluorescence without interferences from the red pigments. After replacing the defective *ade2* locus with a functional *ADE2*, we constructed the 1x-GFP and 100x-GFP strains (see GFP-expression levels in Supplementary Fig. 5a) by integrating a single-copy of an appropriate, linearized yeast-integrating plasmid at the *his3* locus on the chromosome. Specifically, the 1x-GFP strain had its GFP expression controlled by the constitutive promoter of yeast's *KEX2* gene (621 bases upstream of its ORF) which was on a yeast-integration plasmid (*48*) that constitutively expressed *HIS3* (from *C. glabrata*) and integrated into the non-functional *his3* locus of the wild-type strain by a homologous recombination. The 100x-GFP strain had its GFP expression controlled by a strong constitutive promoter pGPD1 (*48*) which was on the same plasmid as the one for the 1x-GFP strain except that the *KEX2* promoter was swapped with the *GDP1* promoter. We cultured all yeasts in defined, minimal media that consisted of (all from Formedium): Yeast Nitrogen Base (YNB) media, Complete Supplement Mixture (CSM) that contained all the essential amino acids and vitamins, and glucose at a saturating concentration (2% = 2 g per 100 mL). The agar pads, which we used for growing yeast colonies, contained 2%-agar (VWR Chemicals), Yeast Extract and Peptone (YEP) (Melford Biolaboratories Ltd.), and a 2%-glucose.

Growth experiments. In a typical growth experiment, we first picked a single yeast colony from an agar plate and then incubated it at 30 ^{O}C for ~14 hours in 5 mL of minimal medium, which contained all the essential amino acids and a saturating concentration of glucose (2%). Afterwards, we took an aliquot of a defined volume from the 5 mL culture (typically 20 μL), and then flowed it through a flow cytometer (BD FACSCelesta with a High-Throughput Sampler) to determine the 5 mL culture's population-density (# of cells/mL). We then serially diluted the culture into fresh minimal media to a desired initial population-density for a growth experiment at various

temperatures. Specifically, we distributed 5-mL of diluted cells to individual wells in a "brick" with twenty-four 10 mL-wells (Whatman: "24-well x 10mL assay collection & analysis microplate"). This ensured that we had 8 identical replicate cultures for each initial population-density (e.g., in Figure 2a-c). We sealed each brick with a breathable film (Diversified Biotech: Breathe-Easy), covered it with a custom-made Styrofoam-cap for insulation, and incubated it in a compressor-cooled, high-precision thermostatic incubators (Memmert ICP260) that stably maintained their target temperature throughout the course of our growth-experiments, with a typical standard deviation of 0.017 ^{O}C over time (deviation measured over several days - see Supplementary Fig. 2). Throughout the incubation, the cultures in the brick were constantly shaken at 400 rpm on a plate shaker (Eppendorf MixMate) that we kept in the incubator. To measure their population-densities, we took a small aliquot (typically 50 μL) from each well, diluted it with PBS (Fisher Bioreagents) into a 96-well plate (Sarstedt, Cat. #9020411), and then flowed it through the flow cytometer which gave us the # of cells/mL. We determined the growth rates by measuring the maximum slope of the log-population-density after their initial, transient growths.

Flow cytometry. We used BD FACSCelesta with a High-Throughput Sampler and lasers with the following wave lengths: 405 nm (violet), 488 nm (blue), and 561 nm (yellow/green). We calibrated the FSC and SSC gates to detect only yeast cells (FSC-PMT=681V, SSC-PMT=264V, GFP-PMT=485V, mCherry-PMT=498V. As a control, flowing PBS yielded no detected events). The number of cells per mL that we plotted in our growth experiments is proportional to the number of events (yeast cells) that the flow cytometer measured in an aliquot of cells with a defined volume. We measured the GFP fluorescence with a FIT-C channel and the "red cells" (Extended Data Fig. 1) with a mCherry channel. We analysed the flow cytometer data with a custom MATLAB script (MathWorks).

Measuring number of surviving cells. For Figures 3a-b, Extended Data Fig. 2 and Supplementary Fig. 6, we prepared 250 mL cultures of wild-type cells in 500 mL Erlenmeyer flasks. We placed a constantly spinning magnetic stir-bar at the bottom of the flasks and placed each flask on top of spinning magnets (Labnet Accuplate - at 220 rpm) inside the thermostatic incubators (Memmert ICP260) that we set at desired high temperatures. For Figure 3d, we prepared a brick with cultures as described in the “Growth Experiments” section in order to have multiple replicate populations and to compare the different population-densities. For every time

point we ensured that these populations were not growing (i.e., all populations were in the no-growth phase after a transient growth) by using the flow cytometer to measure their population-densities over time to verify that their population-densities indeed remained constant over time. For the first 48 hours of incubation, we measured the number of Colony Forming Units (CFUs) by taking out a small volume aliquot from the liquid cultures at high temperatures and distributed droplets from a serial dilution of the aliquot across an agar pad (2% glucose with YEP) that we then incubated in 30 ^{O}C for several days until (no) colonies appeared. When there were few surviving cells per mL - especially for the last time points in each experiment - we determined, in parallel to the plating method, the number of CFUs by transferring an appropriate volume of the liquid cultures from the incubator to an Erlenmeyer flask and then diluting it with the same volume of fresh minimal media. We sealed this flask with a breathable film (Diversified Biotech: Breathe-Easy) and then left it still without stirring, on a benchtop at ~24-30 ^{O}C - we checked that slightly lower temperatures (e.g., room temperatures) did not affect colony-forming abilities - which allowed any surviving cells to settle down to the bottom of the flask and form colonies. We counted the number of colonies at the bottom of the flask - this is the value that we plotted as the last time points in each experiment (Fig. 3a, Extended Data Fig. 2 and Supplementary Fig. 6).

Cell-transfer experiments. We incubated a 24-well brick that contained liquid cultures, each of which were in a deterministic-growth phase, at a desired temperature (e.g., 10,000 cells/mL at 39.2 ^{O}C). We incubated the brick containing these liquid cultures in the thermostatic incubators (Memmert ICP260) as described in the "Growth experiments" section. About 48 hours after the incubation, we took aliquots from the cultures that were growing in mid-log phase (as checked by flow cytometry) and then diluted each of them into fresh 5 mL minimal media that were in 24-well bricks so that these newly created populations were in the no-growth phase at the same temperature as the original population that they came from (400 cells/mL at 39.2 ^{O}C). We sealed the 24-well brick with a breathable film (Diversified Biotech: Breathe-Easy) and then incubated them at the same temperature as the original population. We performed the growth experiments with these new populations as described in the "Growth experiments" section.

Medium-transfer experiments. Details are also in Supplementary Fig. 8. At a given temperature, we first grew populations in the deterministic-growth phase (e.g., initial population-density of 30,000 cells/mL at 39.2 ^{O}C). We used a flow cytometer to measure their growing population-

densities at different times so that we knew in which part of deterministic growth they were in (e.g., mid-log phase). We then transferred each liquid culture to a 50 mL tube (Sarstedt) and centrifuged it so that the cells formed a pellet at the bottom of the tube. We then took the resulting supernatant, without the cell pellet, and flowed it through a filter paper with 200 nm-diameter pores (VWR: 150 mL Filter Upper Cup) to remove any residual cells from the supernatant. After filtering, we flowed an aliquot of the filtered media through a flow cytometer to verify that there were no cells left in the filtered media. We incubated fresh cells into these filtered media (instead of into fresh minimal media) and proceeded with a growth experiment at a desired temperature as described in the "Growth experiments" section.

Measuring the depletion of extracellular nutrients. Details are also in Supplementary Fig. 9. We prepared various growth media by diluting the minimal media (SC media) by various amounts with water. These diluted SC-media were each supplemented with a 2% glucose. Next, we incubated fresh cells in these diluted SC-media at the desired temperature (e.g. 39.2 ^{O}C) as described in the “Growth experiments” section. We compared populations of cells that initially had 400 cells/mL (this corresponds to a no-growth phase, see Fig. 2d) with populations that initially had 10,000 cells/mL (this corresponds to a deterministic-growth phase, see Fig. 2d) in order to confirm that cells were still able to grow in these media. Similarly, we also varied the amounts of glucose that we supplemented to SC-media.

RNA-seq. For each temperature that we studied, we collected cells in 50 mL tubes and spun them in a pre-cooled centrifuge. We then extracted RNA from each cell-pellet with RiboPure Yeast Kit (Ambion, Life Technologies) as described by its protocol. Next, we prepared the cDNA library with the 3' mRNA-Seq library preparation kit (Quant-Seq, Lexogen) as described by its protocol. Afterwards, we loaded the cDNA library on an Illumina MiSeq with the MiSeq Reagent Kit c2 (Illumina) as described by its protocol. We analysed the resulting RNA-Seq data as previously described (49): We performed the read alignment with TopHat, read assembly with Cufflinks, and analyses of differential gene-expressions with Cuffdiff. We used the reference genome for *S. cerevisiae* from ensembl. We categorized the genes by the Gene Ontologies with AmiGO2 and manually checked them with the Saccharomyces Genome Database (SGD).

Measuring concentration of extracellular glutathione. To quantify the concentration of extracellular glutathione, cells were removed from their liquid media by flowing the liquid cultures that contained cells through a 0.45 μm pore filter (VWR, cellulose-acetate membrane). To ensure and verify that there were no cells remaining in the filtered media, we flowed the filtered media through a flow cytometer. The flow cytometer indeed did not detect any cells in the filtered media. We measured concentrations of glutathione in the filtered media as described in the manufacturers' protocol (38185 quantification kit for oxidized and reduced glutathione, 200 tests). We used "BMG Labtech Spectrostar Nano" to measure the optical absorbance at 415 nm. As a background subtraction (blank) for all absorbance measurements, we subtracted the absorbance that we obtained by applying the assay to fresh minimal medium which does not have any glutathione (the background absorbance could come from, for example, cysteine in the minimal media). We subsequently determined the concentrations of extracellular glutathione by using a calibration curve that we constructed by measuring the absorbance at 415 nm for known amounts of glutathione that we added by hand into a buffer provided by the manufacturer.

Glutathione masking experiment. We incubated a brick of liquid cultures that were in the deterministic-growth phase (20.000 cells/mL) at 39.2 ^{O}C. After some time (e.g., 8.5 hours afterwards), we added 750 μM of 1-Methyl-2-vinylpyridinium (M2VP, Sigma-Aldrich Cat. No. 69701), which is a thiol scavenging agent that rapidly masks reduced glutathione (*38*) and proceeded with the experiment as described in the “Growth experiments” section. Identical replicate cultures, that did not receive the M2VP, were used as a reference.

Mutant yeasts. We constructed mutant strains that could not synthesize glutathione or could not import or export glutathione. Primers were designed with a 50-60 bp sequence that was either homologous to the 50-60 bp that is upstream of the desired gene’s start codon or downstream of the desired gene’s stop codon. These primers were used to amplify a selection marker by PCR, resulting in a PCR product that contained a selection marker and whose ends were homologous to the flanking regions of the gene to be knocked out. The wild-type strain (W303) was grown overnight in a 5 mL YPD in a rotator (40 rpm) at 30 ^{O}C, and subsequently transformed with the PCR fragment using standard methods of yeast cloning. The biosynthesis mutant (*gsh1Δ*-strain) was constructed by removing the *GSH1* gene from W303. The import mutant (*hgt1Δ*-strain) was constructed by removing the *HGT1* gene. The export mutant (*gex1,2Δ-adp1Δ*-strain) was

512 constructed by removing, sequentially, the *GEX1* gene, then the *GEX2* gene, and then the *ADP1*
513 gene. The resulting transformants were grown on YPD selection plates, and knockouts were
514 verified by PCR.

515

516 **Measuring integrity of cell membrane.** Cells of the *gex1,2Δ-adp1Δ*-strain were incubated in
517 liquid media at 39.2 ^{O}C (3,000-10,000 cells/mL; corresponds to a random-growth phase). We took
518 aliquots of these cultures and then stained them with 1 μg/mL of propidium iodide (Thermo Fisher
519 Cat. No. P3566). We then flowed these stained aliquots through a flow cytometer. The flow
520 cytometer measured the number of cells that were unstained by the propidium iodide (i.e., cells
521 whose membranes were intact).

522

523 **Mathematical model.** Derivations of equations, a detailed description of the mathematical model,
524 and the parameter values used for simulations are in the Supplemental text.

525

526 **Code availability.** All scripts used for simulations in this work are publicly available (GitHub
527 diederiklt/YeastHighTemperatures).

528

529 **Data Availability.** The authors declare that all data supporting the findings of this study are
530 available within the paper and its supplementary information files. RNA-Seq data is available at
531 NCBI GEO (accession #137151). Source data for the main figures is provided. The data that
532 support the findings of this study are available from the corresponding author upon reasonable
533 request.
534

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Supplemental Information:

- Supplementary Figures 1-14
- Supplementary Text: Detailed description of the mathematical model

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Author contributions:

H.Y. initiated this research and performed the initial growth experiments. D.S.L.T. subsequently designed additional experiments with guidance from H.Y.. D.S.L.T. performed all the experiments, developed the mathematical model, and analysed the data with advice from H.Y.. D.S.L.T. and H.Y. discussed and checked all the data and wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Figure captions:

Figure 1. Conventional, cell-autonomous view of temperature-dependent cell-replications.
(**a**) The conventional view states that cells autonomously replicate at "habitable temperatures" (blue region) and that at sufficiently high temperatures (i.e., "unlivable temperatures"), cells fail to replicate and can eventually die (red region).
(**b**) Growth rate as a function of temperature for populations of wild-type yeast cells. Black data points in the blue region are for populations with sustained, exponential growth over time and white data points in the red region are for populations without sustained exponential growth (error bars represent the mean with s.e.m., n = 3 replicates per data point). 39 ^{O}C is near a boundary of blue and red regions. (Also see Supplementary Fig. 1).
(**c**) The conventional view (explained in (a)) applied to budding yeast, based on the data in (b).
(**d**) Question that we investigated in our study: can microbes collectively combat rising temperatures so that they can turn a temperature that is unlivable (e.g., 40 ^{O}C shown in (c)) into a habitable temperature?

Figure 2. Population-density determines replicability of cells and habitability of each temperature.
(**a-c**) Population-density (number of cells/mL) measured over time with a flow cytometer for populations of wild-type yeast of differing initial population-densities at (**a**) a conventionally-defined habitable temperature (38.4 ^{O}C), (**b**) near the edge of conventionally-defined habitable and unlivable temperatures (39.2 ^{O}C), (**c**) and at a conventionally-defined unlivable temperature (40.3 ^{O}C). Figure 1b sets the conventional definition of temperature's habitability. For (a-b): Each color shows n = 8 populations that start with the same density. Red curves show no growths beyond initial transient growths (i.e., "no growth"). Green curves show unpredictable growths (i.e., "random growth"). Blue curves show deterministic, exponential growths whereby all populations identically grow (i.e., "deterministic growth"). For (c): Each color shows n = 4 populations with the same initial population-density. All colors except the red show growths by ~10-fold.
(**d**) Phase diagram constructed from measurements. Colors of regions and triangles represent the behaviors mentioned in (b) - blue marks deterministic growth, green marks random-growth, red marks no-growth, and grey marks populations not growing as they have more cells than the carrying capacity. Each triangle represents an experiment of the type shown in (a-c). (Also see Supplementary Figs. 3-4 for details).

(**e**) Growth rates of populations in the no-growth phase (red), random-growth phase (green) and deterministic-growth phase (blue) as a function of temperature (error bars represent the mean with s.e.m., n = 6 or more replicates per data point for temperatures below 40 ^{O}C, and n = 3 for temperatures above 40 ^{O}C). Grey data are from Figure 1b.

(**f**) Phase diagrams constructed for engineered yeast strains that constitutively express *GFP* at the indicated levels (1x and 100x). Triangles indicate experimental data. Since the wild-type strain's genetic background is slightly different from that of GFP-expressing strains, compare the 1x-GFP strain with the 100x-GFP strain only. (Also see Supplementary Fig. 5).

Figure 3. Cells collectively combat death to avoid extinction high temperatures.

(**a**) The number of survivors/mL (circles) over time in a non-growing wild-type population at 41.0 ^{O}C. Brown line is an exponentially decaying function fitted to the first three data points (between 10 and 40 hours). Blue curve is a power-law function fitted to the same data points. (Also see Supplementary Fig. 6).

(**b**) Number of survivors/mL for three populations of differing initial population-densities at 41.0 ^{O}C measured as in (a). Initial population-densities, after transient growths, are 92,000 cells/mL (purple), 231,000 cells/mL (orange), and 312,000 cells/mL (blue). Dashed lines represent an exponentially decreasing function fitted to the first three time points. (Also see Supplementary Fig. 6).

(**c**) Cartoon illustrating the conventional view which states that cells autonomously die and that every cell has the same probability of dying per unit time. This means that the population half-life is independent of the initial population-density for every temperature. Different colors represent different temperatures as indicated.

(**d**) Population half-life as a function of initial population-density, based on fitting an exponentially decreasing function to the number of survivors/mL measured during the first 24 hours of incubation (after ~20 hours of transient growths due to cells coming from 30 ^{O}C and adjusting to the new temperature). Shown here are the half-lives of populations at 39.2 ^{O}C, 40 ^{O}C, 40.3 ^{O}C, 40.8 ^{O}C, 42 ^{O}C, and 43 ^{O}C, each in a different color as indicated (error bars represent the mean with s.e.m., n = 3 replicates per data point). Circles represent populations in the no-growth phase. The two squares (at 39.2 ^{O}C and 40 ^{O}C) represent populations that grew due to having sufficient population-densities to trigger their own growths (see Fig. 2d).

Figure 4. Cells secrete and extracellularly accumulate glutathione to help each other and future generations of cells replicate at high temperatures.

(**a**) Schematic description of experiments in (b) and (c) to determine whether ("1") intracellular or ("2") extracellular factors dictate population growth.

(**b**) At 39.2 ^{O}C. Labelled "1" in (a): wild-type cells were transferred (boxed data point) from log-phase populations (blue, initially ~10.000 cells/mL) to fresh media (green, initially ~400 cells/mL). Each color shows n = 4 replicate populations. (Also see Supplementary Fig. 7).

(**c**) At 39.2 ^{O}C. Labelled "2" in (a): fresh cells from 30 ^{O}C were incubated in a growth medium that previously harbored log-phase cells at ~39 ^{O}C for 0 hours (grey), 12 hours (red), or 16 hours (purple). Each color shows at least n = 6 replicate populations. (Also see Supplementary Fig. 8).

(**d**) No-growth populations (initially ~400 cells/mL) at 39.2 ^{O}C. Adding either ascorbic acid (5 mM - yellow) or glutathione (200 μM - green) to the growth media induces population growths. Without adding either one, populations do not grow (grey). Each color shows n = 4 replicate populations.

(**e**) At 39.2 ^{O}C. The measured concentrations of extracellular glutathione as a function of the population-density over time for no-growth (red, initially ~400 cells/mL), random-growth (light blue, initially ~2,000 cells/mL), and deterministic-growth populations (dark blue, initially ~10,000 cells/mL) (error bars represent the mean with s.e.m., n = 3 replicates per data point). The arrow shows both the population-density and concentration of extracellular glutathione increasing together over time. (Also see Supplementary Fig. 11).

(**f**) At 39.2 ^{O}C. Sensitivity of no-growth populations (initially ~400 cells/mL) to glutathione added into the growth medium, as a function of glutathione concentration (error bars represent the mean with s.e.m., n = 4 replicates per data point). Shown here is the fold-change in the population-density after two days of incubation.

(**g**) Cartoon illustrating the mechanism deduced in (a-f). Yeasts secrete and extracellularly accumulate glutathione at high temperatures, inducing population growth when its concentration reaches at least a threshold amount (~0.3 μM from (f)).

Figure 5. Mathematical model with one free parameter recapitulates all the main experimental data.

(**a-b**) Description of the mathematical model (see full description in the Supplementary text). (**a**) A cell (yellow circle) can be in three states. In each time step, any alive cell either stays alive without replicating, replicates, or dies. Alive cells constantly secrete glutathione (green circle). (**b**) Schematic description of the probabilities that describe each of the transitions between states

shown in (a). Left panel: probability of a cell dying (red line) is fixed by the temperature and does not change over time. It linearly increases with temperature and, beyond some temperature, it exceeds the maximum allowed value for the probability of a cell replicating (grey line). Right panel: probability of a cell replicating (blue curve) non-linearly increases with the concentration of the extracellular glutathione.

(**c-f**) Results generated by the model described in (a-b) with a single fixed set of parameters for all the panels. Model recapitulates: (**c**) the population-growth curves (compare with Fig. 2a), (**d**) the phase diagram (compare with Fig. 2d), (**e**) population-density dependent deaths (compare with Fig. 3b), (**f**) population half-life (based on cell deaths during the first day of incubation - compare with Figure 3d), the number of survivors decaying over time as a heavy-tailed function (see Supplementary Fig. 12), and single-cell-level data on growths (compare Extended Data Fig. 1 with Extended Data Fig. 3). The number of replicate simulations matches that of the respective experiments. (Also see Supplementary Fig. 12).

Figure 6. Budding yeast exports glutathione whose extracellular role – not intracellular roles - as antioxidant enables yeasts to survive high temperatures.

(**a**) At 39.2 ^{O}C. Wild-type populations (all initially ~20,000 cells/mL) that should deterministically grow if left alone. A masking agent (M2VP) that inactivates extracellular glutathione was added after 4.5 hours (dark brown) or 8.5 hours (light brown) of incubation. Grey curve shows populations that did not receive the masking reagent. Each color shows n = 4 replicate populations. (Also see Supplementary Fig. 13).

(**b**) Schematic showing how the budding yeast synthesizes, imports, and exports glutathione. Glutathione is intracellularly synthesized via an enzyme encoded by *GSH1*. Glutathione is imported by a proton-coupled glutathione-importer encoded by *HGT1*. Glutathione is exported by numerous exporters (not all shown), including proton antiporters encoded by *GEX1,2* and an ATP-dependent exporter encoded by *ADP1*.

(**c**) At 39.2 ^{O}C. Light blue curves show deterministically growing populations of a mutant strain (*hgt1*Δ-strain) that cannot import glutathione (initially ~10,000 cells/mL). Grey curves show mutant populations (initially ~400 cells/mL) incubated without any glutathione added. Dark blue curves show mutant populations (initially ~400 cells/mL) incubated with 250 μM of glutathione added to the media. Each color shows n = 4 replicate populations.

(**d**) At 39.2 ^{O}C. Populations of a mutant strain that lacks some of the main glutathione exporters (*gex1,2*Δ-*adp1*Δ-strain) (initially ~9,500 cells/mL (purple) or ~7,500 cells/mL (pink)). Wild-type

853 populations shown as a comparison (grey, initially ~9,500 cells/mL). Each color shows n = 4
854 replicate populations. (Also see Extended Data Fig. 4).
855 (**e**) At 41 ^{O}C. Wild-type populations of various initial densities (from ~400 cells/mL (lightest green
856 curves) to ~14,000 cells/mL (darkest green curves)) grown in media supplemented by 750 μM of
857 glutathione. Each color shows at least n = 3 replicate populations.
858 (**f**) Cartoon illustrating mechanisms deduced in (a-e). Exporting glutathione is necessary and
859 sufficient for yeasts to reshape the habitability of temperature. For yeasts to survive and replicate
860 at high temperatures, extracellular glutathione is (1) necessary since blocking glutathione or
861 blocking glutathione-export stops yeast's growths and is (2) sufficient since adding glutathione or
862 blocking glutathione-import enables yeasts to grow.
863
864

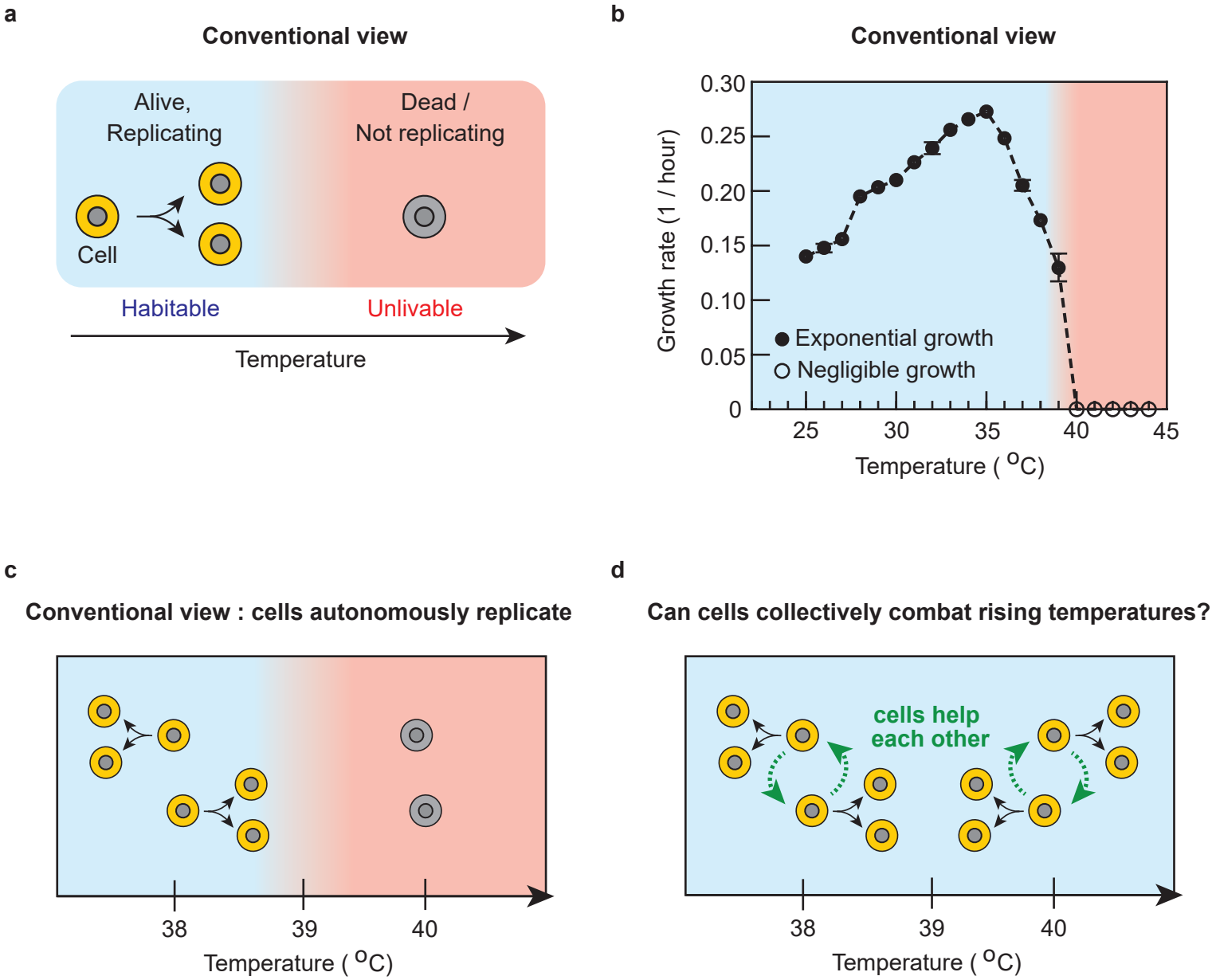

a
Conventional view
Alive,
Replicating
Dead /
Not replicating
Cell
Habitable
Unlivable
Temperature
b
Conventional view
Growth rate (1 / hour)
0.30
0.25
0.20
0.15
0.10
0.05
0
Exponential growth
Negligible growth
25
30
35
40
45
Temperature (°C)
c
Conventional view : cells autonomously replicate
38
39
40
Temperature (°C)
d
Can cells collectively combat rising temperatures?
cells help
each other
38
39
40
Temperature (°C)

Fig. 1

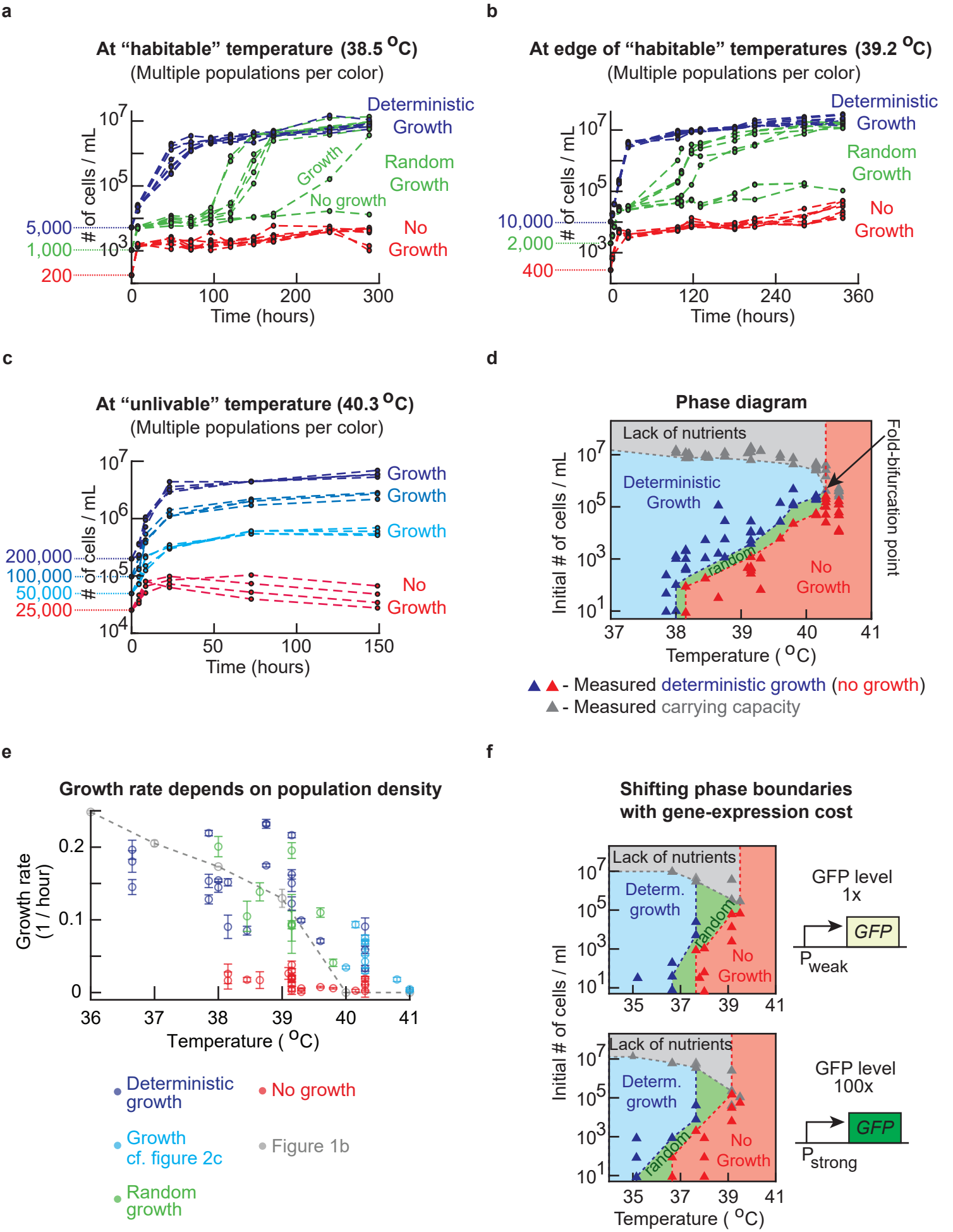
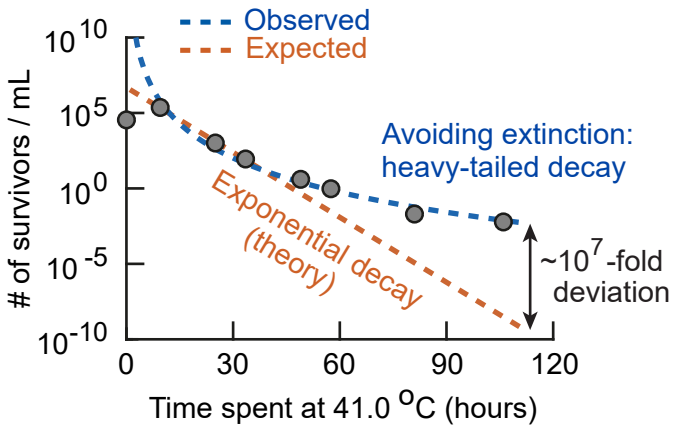
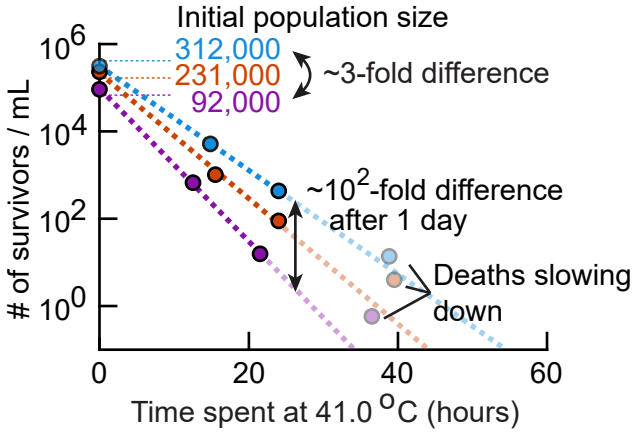

a
At "habitable" temperature (38.5 °C)
(Multiple populations per color)
Deterministic Growth
Random Growth
No Growth
Growth
No growth
5,000
1,000
200
# of cells / mL
Time (hours)
b
At edge of "habitable" temperatures (39.2 °C)
(Multiple populations per color)
Deterministic Growth
Random Growth
No Growth
10,000
2,000
400
# of cells / mL
Time (hours)
c
At "unlivable" temperature (40.3 °C)
(Multiple populations per color)
Growth
Growth
Growth
No Growth
200,000
100,000
50,000
25,000
# of cells / mL
Time (hours)
d
Phase diagram
Lack of nutrients
Deterministic Growth
random
No Growth
Fold-bifurcation point
Initial # of cells / mL
Temperature (°C)
▲▲ - Measured deterministic growth (no growth)
▲ - Measured carrying capacity
e
Growth rate depends on population density
Growth rate (1 / hour)
Temperature (°C)
Deterministic growth
No growth
Growth cf. figure 2c
Figure 1b
Random growth
f
Shifting phase boundaries with gene-expression cost
Lack of nutrients
Determ. growth
random
No Growth
GFP level 1x
GFP
Pweak
GFP level 100x
Pstrong
Initial # of cells / ml
Temperature (°C)

Fig. 2

a

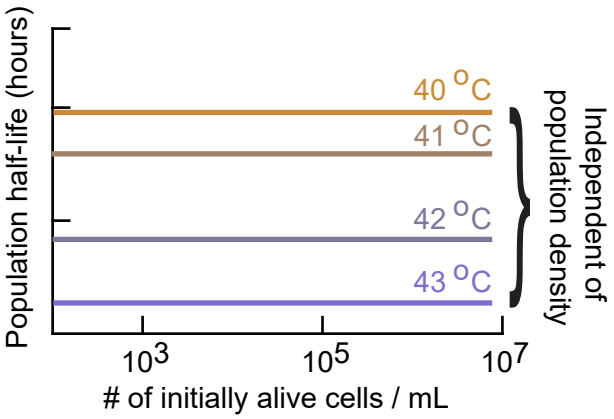


b



(c-d) Time taken for # of survivors to be halved

c



d

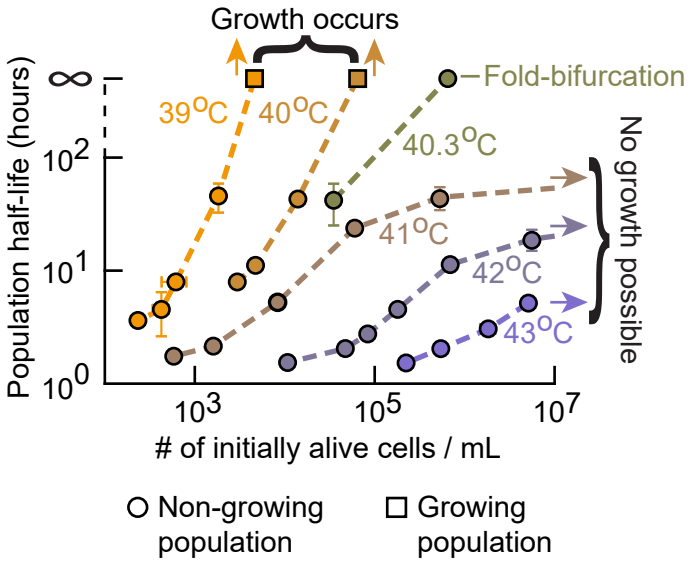


Fig. 3

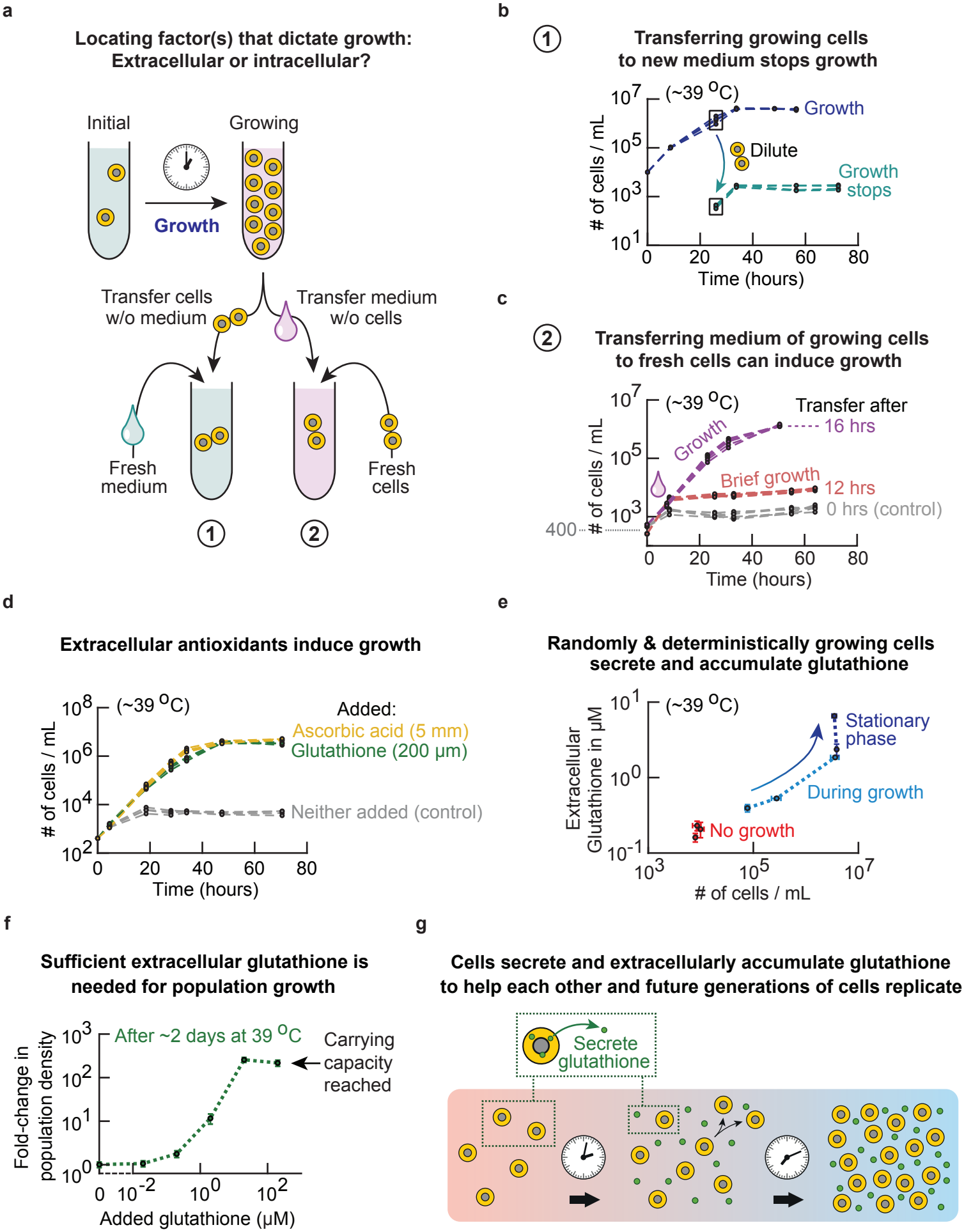

a
Locating factor(s) that dictate growth:
Extracellular or intracellular?
Initial
Growing
Growth
Transfer cells
w/o medium
Transfer medium
w/o cells
Fresh
medium
Fresh
cells
1
2
b
1
Transferring growing cells
to new medium stops growth
(~39 °C)
Growth
Dilute
Growth
stops
# of cells / mL
Time (hours)
c
2
Transferring medium of growing cells
to fresh cells can induce growth
(~39 °C)
Transfer after
16 hrs
Growth
Brief growth
12 hrs
0 hrs (control)
400
# of cells / mL
Time (hours)
d
Extracellular antioxidants induce growth
(~39 °C)
Added:
Ascorbic acid (5 mm)
Glutathione (200 µm)
Neither added (control)
# of cells / mL
Time (hours)
e
Randomly & deterministically growing cells
secrete and accumulate glutathione
(~39 °C)
Stationary
phase
During growth
No growth
Extracellular
Glutathione in µM
# of cells / mL
f
Sufficient extracellular glutathione is
needed for population growth
After ~2 days at 39 °C
Carrying
capacity
reached
Fold-change in
population density
Added glutathione (µM)
g
Cells secrete and extracellularly accumulate glutathione
to help each other and future generations of cells replicate
Secrete
glutathione

Fig. 4

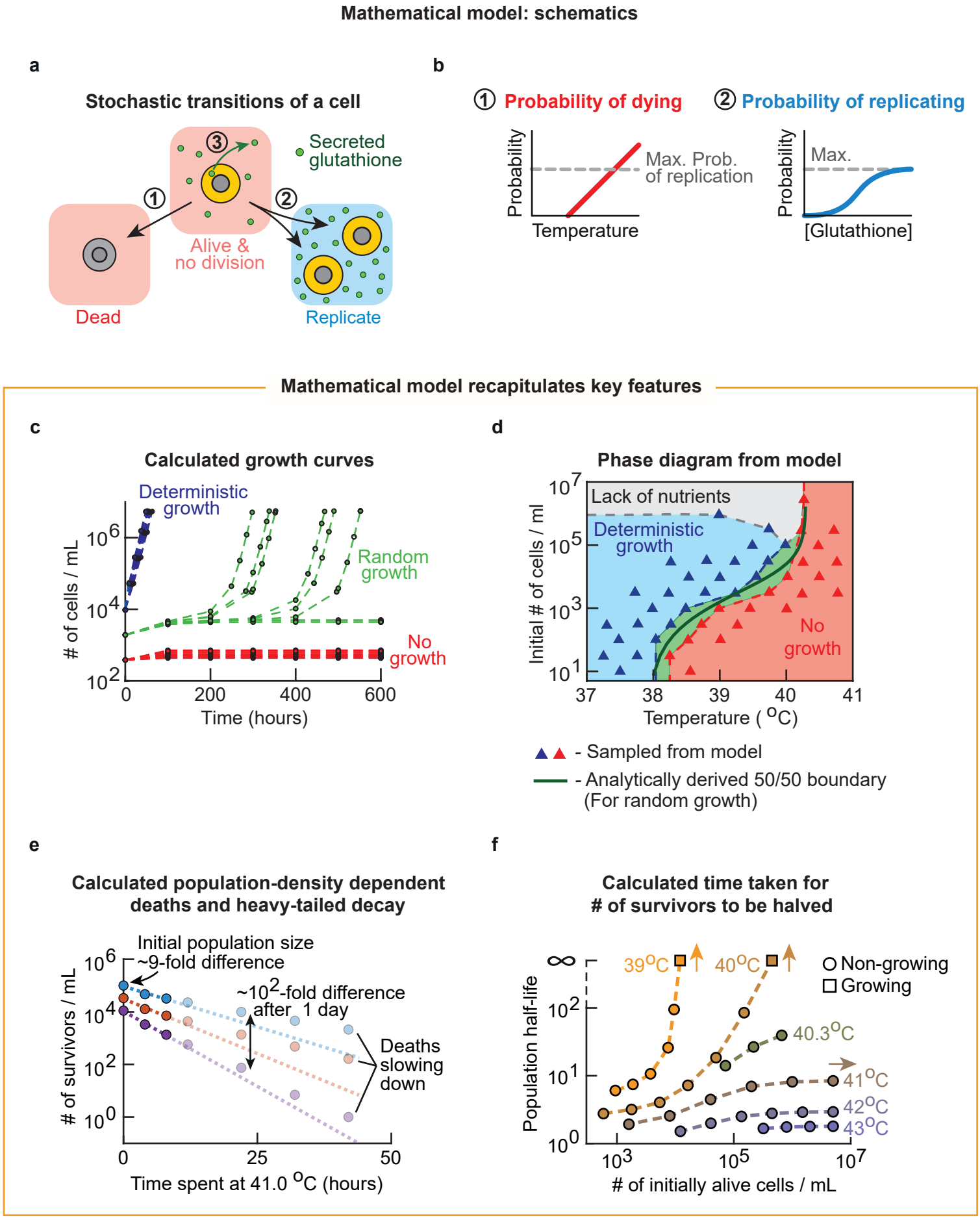
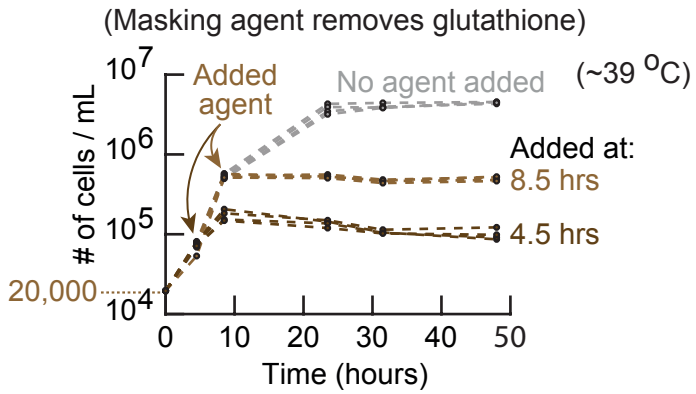

Mathematical model: schematics
a
Stochastic transitions of a cell
Secreted glutathione
Alive & no division
Dead
Replicate
b
① Probability of dying
② Probability of replicating
Probability
Max. Prob. of replication
Temperature
Max.
[Glutathione]
Mathematical model recapitulates key features
c
Calculated growth curves
Deterministic growth
Random growth
No growth
# of cells / mL
Time (hours)
d
Phase diagram from model
Lack of nutrients
Deterministic growth
No growth
Initial # of cells / ml
Temperature (°C)
▲▲ - Sampled from model
- Analytically derived 50/50 boundary (For random growth)
e
Calculated population-density dependent deaths and heavy-tailed decay
Initial population size ~9-fold difference
~10^2-fold difference after 1 day
Deaths slowing down
# of survivors / mL
Time spent at 41.0 °C (hours)
f
Calculated time taken for # of survivors to be halved
39°C
40°C
Non-growing
Growing
40.3°C
41°C
42°C
43°C
Population half-life
# of initially alive cells / mL

Fig. 5

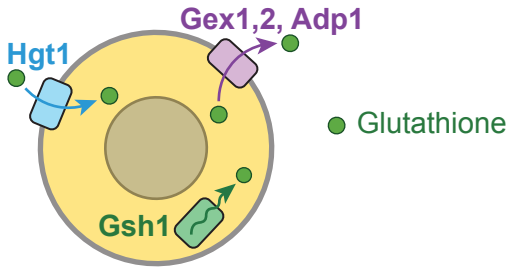
a

Removing extracellular glutathione stops growth



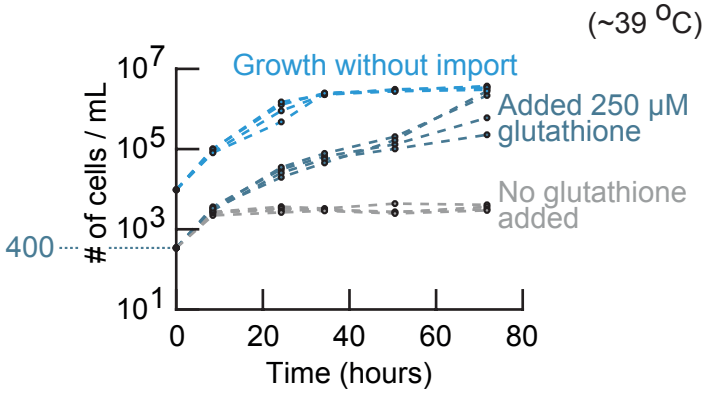
b

Schematic: glutathione synthesis & transport



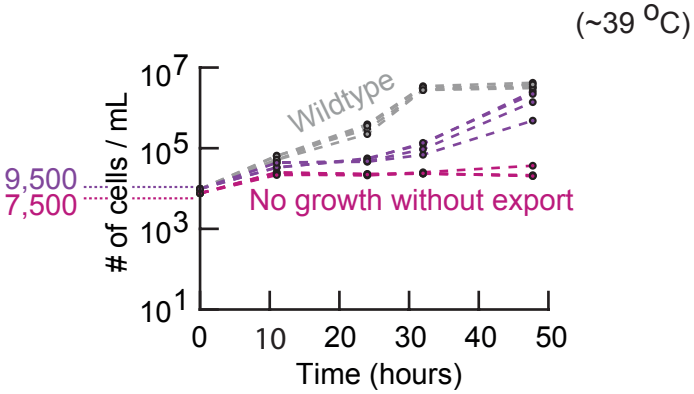
c

Populations that cannot import glutathione can grow (*hgt1Δ*)



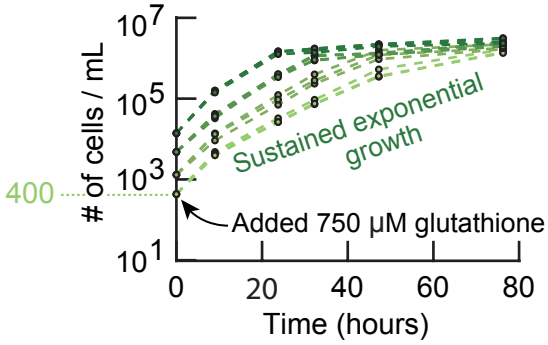
d

Populations that cannot export glutathione cannot grow (*gex1,2Δ*, *adp1Δ*)



e

Growth enabled at 'forbidden' temperature (41 °C) by adding extracellular glutathione



f

Exporting glutathione is necessary & sufficient for making temperature habitable

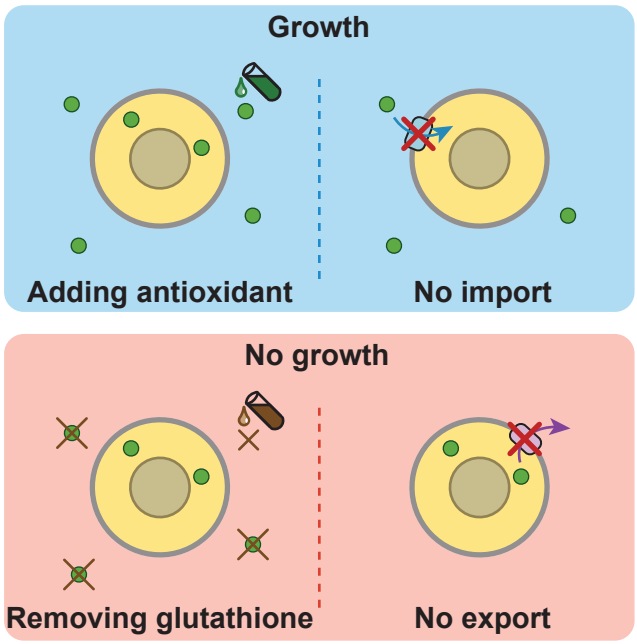


Fig. 6