# Cells collectively reshape habitability of temperature by helping each other replicate

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***Note:*** *Low resolution figures with captions are embedded within the main text. High resolution figures are at the end of the main text and preceded by their captions.*

**SUMMARY**

**How the rising global temperatures affect organisms is a timely question. The conventional view is that high temperatures cause microbes to replicate slowly or die, both autonomously. Yet, microbes co-exist as a population, raising the underexplored question of whether they can cooperatively combat rising temperatures. Here we show that, at high temperatures, budding yeasts help each other and future generations of cells replicate by secreting and extracellularly accumulating glutathione - a ubiquitous heat-damage-reducing antioxidant. Yeasts thereby collectively delay and can halt population extinctions 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. Despite the conventional theory stating that heat-shocked cells autonomously die and cannot stop population extinctions, we found that non-growing yeast-populations at high temperatures - due to cells cooperatively accumulating extracellular glutathione - continuously decelerate and can eventually stop their approach to extinction, with higher population-densities stopping faster. We show that exporting glutathione, but not importing, is required for yeasts to survive high temperatures. Thus, cooperatively eliminating harmful extracellular agents – not glutathione's actions inside individual cells – is both necessary and sufficient for surviving high temperatures. We developed a mathematical model - which is generally applicable to any cells that cooperatively replicate by secreting molecules - that recapitulates all these features. These results show how cells can cooperatively extend boundaries of life-permitting temperatures.**

**(238 words)**

Many model organisms are typically studied at a particular temperature that is deemed "optimal" for that organism's growth. For example, the budding yeast is often studied at 30 $^{O}$C while *E. coli* and mammalian cells are usually studied at 37 $^{O}$C. Yet, an organism can live and replicate at a range of "habitable temperatures" (*1-3*). Understanding why an organism cannot replicate and eventually dies for temperatures outside of the habitable range – at "unlivable temperatures" - may provide insights into living systems' vulnerabilities and how temperature, a fundamental physical quantity, drives processes of life. Moreover, given the rising global temperatures, a timely and practical question is how organisms do or can combat high temperatures to avoid extinction. For microbes and mammalian cells, the conventional view is that as the temperature increases above some optimal value within the habitable temperature regime and approaches unlivable temperatures, cells need more time to replicate and that once the temperature enters the unlivable temperature regime, cells fail to replicate and eventually die (*1-4*) (Figs. 1a-b). This view states that at sufficiently high temperatures, crucial proteins unfold and other heat-induced damages occur (e.g., damages by reactive oxygen species) (*5-7*), all of which disrupt cell replications. Moreover, it states that whether a cell can replicate or not at a high temperature depends on its autonomous ability to repair heat-induced damages by using its heat-shock response system, which is conserved across species (*8*). It is thought that while cells can autonomously repair heat-induced damages at moderately high, still-habitable temperatures that are just below the unlivable temperatures, they fail to do so at temperatures that are too high (i.e., at unlivable temperatures). Thus, this view states that one cell's ability to replicate and its lifespan are both independent of any other cell's lifespan and ability to replicate (*4*) (Fig. 1c). Yet a cell rarely exists alone - it lives within a population and can cooperate with other cells. Indeed, microbes can use mechanisms such as quorum sensing to coordinate their behaviors (*9*), share food (*10-12*), and collectively tune their extracellular pH (*13*). These findings motivated us to ask whether microbes use collective strategies to combat rising temperatures and if so, what such strategies are (Fig. 1d). These questions remain unaddressed for many microbial species. We sought to address them for the budding yeast, *Saccharomyces cerevisiae*. Surprisingly, we discovered that budding yeasts use a collective strategy to help each other replicate, help future generations of cells replicate, and thereby delay and even prevent population extinctions at high temperatures that one would conventionally define as being unlivable for the budding yeast.

As our starting point, we reproduced the well-known, textbook picture of how temperature affects the growth of yeast - and other microbes (*4,14-16*) - by using populations of a laboratory-standard ("wild-type") strain of haploid budding yeast (Fig. 1b and Supplementary Fig. 1).

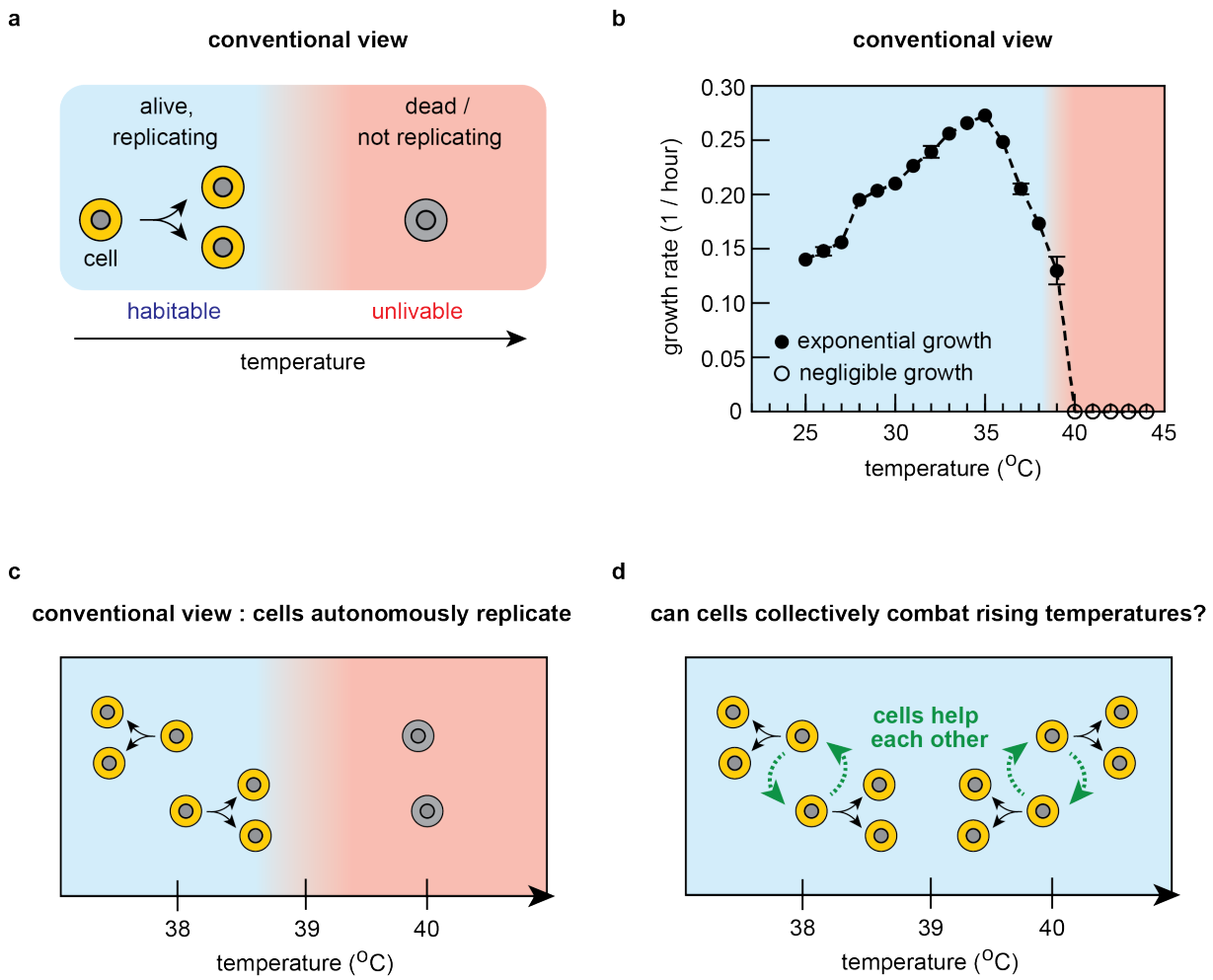


**Figure 1. Conventional, cell-autonomous view of temperature-dependent cell-replications.**

(**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). This view states that whether a cell can replicate or not at a given temperature depends on the cell's autonomous ability to use its heat-shock response system to sufficiently deal with various heat-induced damages.

(**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. 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 cells (budding yeasts) cooperatively combat rising temperatures so that they can turn a temperature that is unlivable for a few cells (e.g., 40 $^{O}$C shown in (c)) into a habitable temperature if there are sufficiently many cells working together to fight off extinction?

According to this picture, the yeasts' growth rate becomes zero - the population density negligibly changes - for temperatures of 40 $^{O}$C and higher, which would thus be defined as "unlivable temperatures" for budding yeast (Fig. 1b - dark red region; Supplementary Fig. 1). Despite being

evidently true - since we reproduced it here - we discovered that this cell-autonomous view of yeast replication (Fig. 1c) is misleading. For example, we discovered that yeast populations, depending on their population densities, can actually grow at "unlivable" temperatures (e.g., at 40 $^{O}$C) and not grow at "habitable" temperatures (e.g., at 38 $^{O}$C). As we will show, we discovered a picture that revises the cell-autonomous view: yeasts help each other and their future generations of cells replicate at high temperatures by secreting and extracellularly accumulating glutathione - a versatile antioxidant that is widely used by many species - which reduces damages caused by reactive oxygen species created by high temperatures. Thus, we found that yeasts collectively set the habitability of each temperature (Fig. 1d). A surprising consequence of this is that a temperature which is unlivable for relatively few yeasts becomes habitable if there are enough yeasts in a population that can help each other replicate. As another surprising consequence, we found that a yeast population - due to its cells gradually accumulating extracellular glutathione - decelerates and can eventually stop its approach to extinction at high temperatures. In fact, we found that a population of a higher density can more rapidly decelerate and halt its approach to extinction due to more cells cooperating through their secreted glutathione. This revises the prevalent theory which states that cells autonomously die at high temperatures. Intriguingly, we discovered that yeast populations at one particular temperature - a special temperature that places yeasts at a cusp of being able to replicate and unable to replicate - exhibit behaviors that are akin to those of biomolecular (*17,18*) and physical systems (*19*) that undergo phase transitions. We developed a mathematical model that recapitulates all the main results of our experiments. The model also quantitatively explains the origin of the phase-transition-like features by using, as the mechanism, cells cooperatively accumulating extracellular glutathione which promotes cell replications. Our paper ends by showing that glutathione's extracellular action as an antioxidant, not its intracellular action, is solely responsible for promoting cell replications, thus highlighting an underappreciated extracellular role of glutathione for yeast. Glutathione, antioxidants, and heat-induced reactive oxygen species are common to many organisms, including humans. Hence the cooperative mechanism for yeast that we uncovered here - involving glutathione's previously underappreciated role as a secreted factor at high temperatures – raises the possibility that cells of other species do or can also collectively combat rising temperatures in a similar manner. Taken together, our work shows how the habitability of a temperature emerges as a community-level property for a specie - determined by intraspecies interactions - rather than determined solely by the specie's autonomous features.

## RESULTS

### Population density determines replicability of cells and habitability of each temperature

We re-examined the conventional, cell-autonomous picture by incubating populations of wild-type yeasts 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). We first grew the yeasts at 30 $^{O}$C in 5 mL of standard minimal-media. We then transferred some of these cells to a fresh minimal media and then incubated them at a desired temperature, just as we did to obtain the conventional picture (Fig. 1b). This time, however, we took care to transfer a precise number of cells to the fresh minimal media so that we could vary the initial population-density (# of cells/mL) over a wide range. This is in contrast to how one obtains the conventional picture: one usually ignores the precise number of cells that a population begins with and, regardless of the temperature, keeps the initial population-density within a typical range of values that a plate reader can convert to an accessible range of optical densities (typically ~$10^6$ cells/mL in our experiments) (Supplementary Fig. 1). With a flow cytometer, we counted the integer numbers of cells per volume to determine the population density at each time point. For each temperature, we incubated multiple liquid cultures - these all started with the same population density – with cells that all came from a single liquid culture of cells that exponentially grew at 30 $^{O}$C. These experiments revealed surprising behaviors that deviated from the conventional picture. Specifically, at the supposedly-habitable temperature of ~38 $^{O}$C, none of the 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) than these non-growing populations 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 just five times larger (5,000 cells/mL) than these randomly-growing populations yielded populations that always grew exponentially over time, all in the same way, until they reached the carrying capacity (Fig. 2a - blue curves). Thus, among the three initial population densities at 38 $^{O}$C, only the largest one led to the deterministic, population-level growth that the conventional picture states should be exhibited by every initial population-density at habitable temperatures (*1,14-16*).

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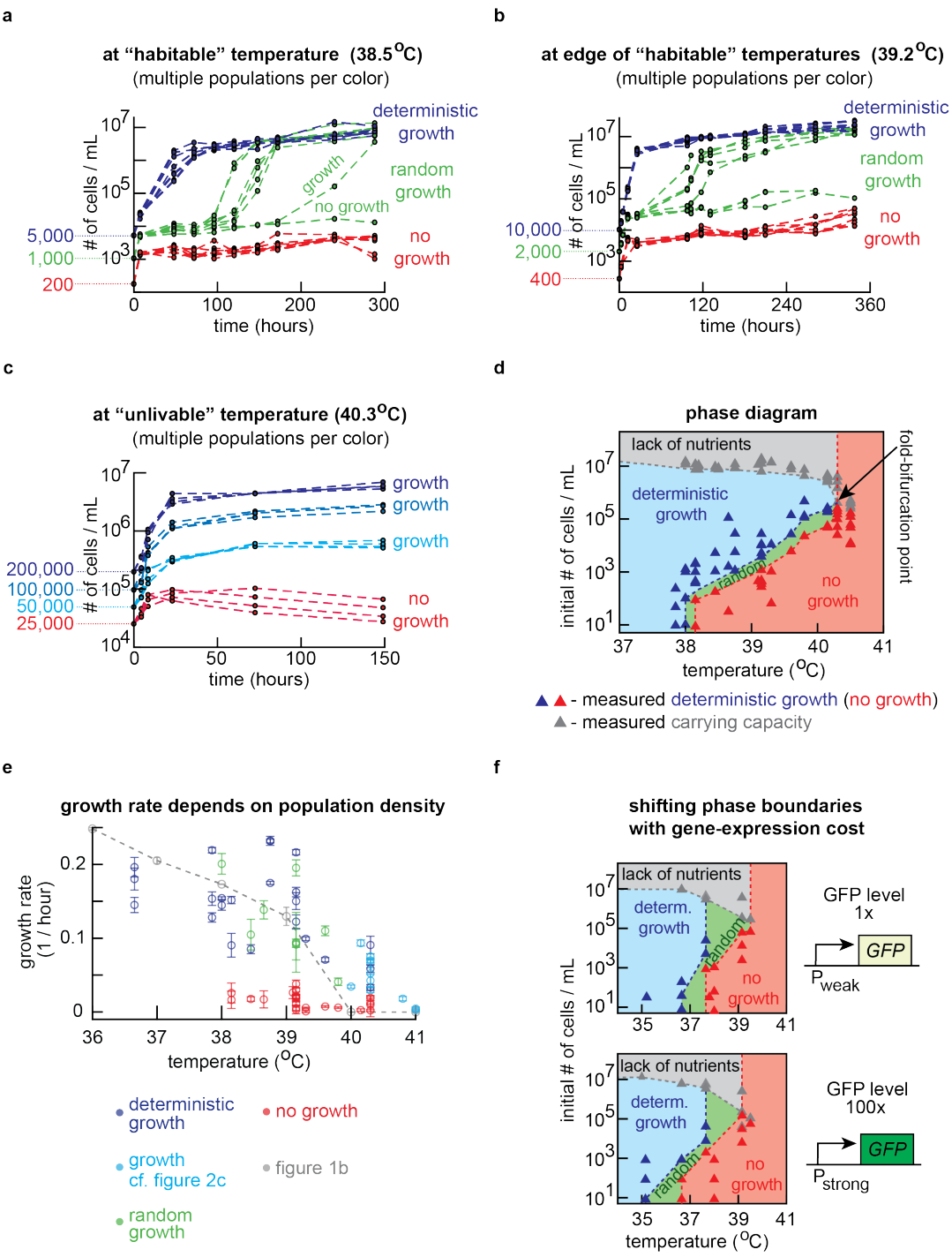


**Figure 2. Population density determines replicability of cells and habitability of each temperature.**

(**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 °C), (**b**) near the edge of conventionally-defined habitable and unlivable temperatures (39.2 °C), (**c**) and at a conventionally-defined unlivable temperature (40.3 °C). Figure 1b sets the conventional definition of temperature's habitability. For (a-b): Each color shows eight 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 four populations with the same initial population-density. All colors except the red show growths by ~10-fold.

(**d**) Phase diagram constructed from measurements. Colors represent the behaviors mentioned in (b) - blue region marks deterministic growth, green region marks random-growth, red region marks no-growth, and grey region 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), performed at a specific initial population-density and temperature (Supplementary Figs. 3-4). A triangle's color represents the phase exhibited by populations. See caption of Figure S4 for a detailed description of how we deduced the phase boundaries from the measurements.

(**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 are s.e.m., $n$ = 6 or more for temperatures below 40 $^{O}$C, and $n$ = 3 for temperatures above 40 $^{O}$C). Grey data are from Fig. 1b.

(**f**) Phase diagrams constructed for engineered yeast strains that constitutively express *GFP* at the indicated levels (1x and 100x - see Supplementary Fig. 5). Triangles indicate experimental data (samples of growth data represented by these triangles are in Supplementary Fig. 5). 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.

We also observed the same three population-level behaviors - no growth, random growth, and deterministic growth - as a function of the initial population-density for a temperature (~39 $^{O}$C) that is near the boundary of the "habitable" and "unlivable" regimes (Fig. 2b). Moreover, at a temperature that is supposedly unlivable (~40 $^{O}$C), according to the conventional picture (Fig. 1c), we found that a population with an initial density of at least 50,000 cells/mL did, in fact, grow by ~10-fold and then stably remained at a high density for several days without reaching the carrying capacity (Fig. 2c - non-red curves). In contrast, if the initial population-density was just half of this value (25,000 cells/mL), then the population density, instead of plateauing, continuously decreased over several days after an initial, transient growth (Fig. 2c - red curves). These results show that just a two-fold difference in the initial population density can determine whether there is a net cell-death or net cell-growth at ~40 $^{O}$C, which the conventional picture of autonomous cell-replications cannot explain.

**Phase diagram shows allowed population-level behaviors across temperatures**

Above results (Figs. 2a-c) show that in order to determine whether a population grows or not, one must know *both* the temperature and the initial population-density. We can summarize this in a "phase diagram" that we constructed by performing the above growth experiments at multiple, additional temperatures with multiple, initial population densities (Fig. 2d and Supplementary Figs. 3-4). The phase diagram consists of four phases - deterministic growth, random growth, no growth, and no-growth due to insufficient nutrients (i.e., the initial population-density is beyond the carrying capacity for a given temperature) - as a function of the initial population density and temperature. It shows that the conventional picture (Figs. 1b-c) mistakenly arises because one typically ignores

the initial population-density or sets it to be within some narrow range when studying population growth across temperatures. 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 in the deterministic-growth and random-growth phases - 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). In constructing the phase diagram, we determined at least how many cells per volume were necessary to guarantee that a population grew at each temperature (i.e., minimum number of cells per unit volume required for a deterministic growth at each temperature). A curve that represents how this value varies as a function of temperature forms the boundary between the deterministic-growth and random-growth phases in the phase diagram (Fig. 2d). We also determined at most how many cells per volume were necessary to guarantee that a population never grew at each temperature. A curve that represents how this value varies as a function of temperature forms the boundary between the random-growth and no-growth phases in the phase diagram (Fig. 2d). We found that both of these values - the minimum starting population-density required to guarantee that a population grows at a particular temperature and the maximum starting population-density required to guarantee that a population does not grow at a particular temperature - are extremely sensitive (i.e., "ultra-sensitive" (*20, 21*)) to temperature. For example, the phase diagram revealed that each of these two values change by ~100-fold as the temperature increases from 39 $^{O}$C to 40 $^{O}$C (Fig. 2d). The random-growth phase, in the phase diagram, lies between the no-growth and deterministic-growth phases - one might see it as a hybrid of the growth and no-growth phases - and is thin along the axis that represents the initial population-density. Its thinness reflects our previous observation that a small change (5-fold or less) in the initial population-density can transform either a no-growth or a deterministic growth into a random growth (Fig. 2a) (i.e., populations are ultra-sensitive to their initial densities). Intriguingly, the phase boundaries - the borders between the four different phases - all converge at a single point, a "fold-bifurcation point", whose coordinate is (40.3 $^{O}$C, 1 x $10^5$ cells/mL) in the phase diagram (Fig. 2d). This convergence of all four phases at the fold-bifurcation point leaves only the no-growth phase for temperatures higher than 40.3 $^{O}$C. Thus, according to the phase diagram, a population cannot grow to reach the carrying capacity regardless of its initial density for temperatures higher than 40.3 $^{O}$C. More precisely, at temperatures higher than 40.3 $^{O}$C, a population can grow but would eventually stop growing before reaching the carrying capacity, with the final population-density depending on the initial population-density. The term, "fold-bifurcation point", comes from dynamical systems theory and is appropriate here because, according to this

theory, this is the point in the phase diagram where a stable fixed point (carrying capacity) - this point "attracts" growing populations - merges with an unstable fixed point (the upper boundary of the no-growth phase) - this point "repels" populations towards extinction or the carrying capacity. The fold-bifurcation point is special for another reason which we will later turn to - one that is reminiscent of critical points in phase diagrams of physical systems in which temperature is one of the main parameters.

**Tuning the cost of expressing a single superfluous gene reshapes habitability of temperature**

To explore possible ways of manipulating the phase diagram (i.e., where the phase boundaries are), we genetically engineered the wild-type yeast so that it constitutively expressed the Green Fluorescent Protein (GFP), which serves no function for cell growth. We constructed two such strains - one expressing GFP at a relatively low level (defined as 1x) and another at a relatively high level (~100x) (Supplementary Fig. 5). We repeated the growth experiments with these two strains, for multiple temperatures and initial population-densities, and used these results to construct a phase diagram for each strain (Fig. 2f and Supplementary Fig. 5). Comparing the phase diagram of the 1x-GFP-expressing strain with that of the 100x-GFP-expressing strain revealed that increasing the GFP expression shifts the phase boundaries towards lower temperatures in such a way that a population of 100x-GFP-expressing strain need to have a higher initial population-density, compared to the 1x-GFP-expressing strain, in order to grow at a given temperature. Specifically, this means that the random-growth and no-growth behaviors are possible for the 100x-GFP-expressing strain at lower temperatures than for the 1x-GFP-expressing strain (Fig. 2f). For example, at ~36 $^{O}$C, a population with high GFP-levels (100x) can be in the random-growth and no-growth phases whereas the yeasts with the low GFP-levels (1x) can only deterministically grow regardless of how few cells there are. Therefore, a population that could grow at a given temperature can no longer grow at that same temperature because its cells express GFP. One needs to either increase the initial population-density or decrease the temperature to observe its growth. These results show that the cost of expressing superfluous genes can markedly alter the phase boundaries' locations and shapes. In particular, this means that reducing the cost of expressing a single, unnecessary gene can increase the life-permitting temperature by several Celsius degrees. In light of previous studies (*22,23*), this may be due to the fact that overexpressing a superfluous gene by a sufficiently large amount shifts the intracellular resources away from performing roles that are for cell growth.

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256 **Single-cell measurements reveal that a few "pioneer" cells initiate replications in randomly**
257 **growing populations and sustenance of transiently replicating sub-populations in non-**
258 **growing populations**
259 To gain further insights, we turned to single-cell-level measurements. Unlike the GFP-expressing
260 strains, the wild-type strain lacks a functional *ADE2* gene for synthesizing adenine. Since we
261 incubated yeasts in the minimal media with all the essential amino acids and nitrogenous sources
262 - including adenine that represses their adenine-biosynthesis - the wild-type cells were still capable
263 of growing. But, as is well-known, having a defective *ADE2* gene turns yeasts red if they have not
264 divided for some time because they have accumulated red pigments - these are by-products of
265 the not-fully-repressed and defective adenine-biosynthesis (*24*). The cells can only dilute away
266 the red pigments through cell divisions. Defective *ADE2* gene cannot be a reason for any of the
267 population-density-dependent growth behaviors that we observed because the GFP-expressing
268 strains do have the functional *ADE2* gene and yet exhibit the same, surprising behaviors as the
269 wild-type cells (Fig. 2f). But the red pigments were useful because, for the wild-type populations,
270 we could use our flow cytometer's red-fluorescence detector to count how many non-replicators
271 (red cells) and how many replicators (non-red, "white cells") co-existed in a population at each
272 time point (Supplementary Fig. 6). As a result, we discovered that, in every population and at every
273 temperature, nearly all cells were red at the start of each growth experiment due to the cells having
274 just been transferred from cultures that grew to saturation in 30 $^{O}$C to the higher temperature.
275 Subsequently, the number of replicators increased to about 10-30% of the population during the
276 initial, transient growth in which the cells from 30 $^{O}$C adjust to the higher temperature
277 (Supplementary Fig. 6). Afterwards, the number of replicators varied depending on which phase
278 the population has. For a deterministically growing population, the number of replicators kept
279 increasing over time until it reached the carrying capacity (Supplementary Fig. 6) whereas it
280 typically decreased until very few cells (~1-5 % of population) remained as replicators for random-
281 growth and no-growth populations (Supplementary Fig. 6). Subsequently, two behaviors were
282 possible depending on whether the population was in the no-growth or random-growth phase. For
283 populations in the random-growth phase, the number of replicators, after unpredictable hours or
284 days, suddenly started to increase by orders of magnitude until the population reached the
285 carrying capacity (Supplementary Fig. 6). On the other hand, for populations in the no-growth
286 phase, the number of replicators sustainably remained low (~1-5% of the population) and
287 fluctuated up and down by a few-fold during the incubation (~300 hours in our experiments)

(Supplementary Fig. 6). These fluctuations were too small to noticeably change the total population density over time. Yet, this result revealed that there is always a small sub-population of transiently replicating cells and that these transient replications are such 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 single-cell data when we introduce a mathematical model that recapitulates them.

**Cells collectively delay and prevent population extinctions at high temperatures**

We next asked whether cell deaths, like cell replication, also depend on the initial population density. At several temperatures, we measured how the number of surviving cells changed over time for a population that we kept in the no-growth phase (i.e., population with not enough cells to trigger its own random or deterministic growth). We counted the number of survivors by taking out an aliquot of cells from the population at different incubation times, spreading it onto an agar pad at 30 $^{O}$C, and then counting how many colonies formed (Supplementary Fig. 7). Surprisingly, these measurements deviated qualitatively - not just quantitatively - from the conventional theory of cell deaths which states that deaths of heat-shocked cells are autonomous. Specifically, the conventional theory says that the number of survivors should exponentially decrease over time with a constant rate until the population becomes extinct due to every cell having the same, fixed probability of dying per unit time regardless of the population density (*4*) (Fig. 3a - brown line). Yet, we discovered that the rate at which cells die continuously decreases over time. This leads to the number of survivors decreasing as a heavy-tailed (power-law-like) function (Fig. 3a - blue curve), instead of as an exponential decay. Consequently, the number of survivors appears to decrease exponentially at a constant rate during the first day of incubation but then, after a few days, decreases exceptionally slowly as a heavy-tailed decay. The heavy-tailed decay means that the population continuously decelerates and then eventually ceases its approach to extinction. This causes the number of survivors to plateau after some time. For example, the number of survivors at 41 $^{O}$C did not noticeably decrease after three days of incubation and deviated by ~$10^7$-fold from the number of survivors that the theory states one should have (Fig. 3a - last time point; Supplementary Figs. 7-8). Moreover, we discovered that the rate at which cells die during incubation at a high temperature depends on the initial population-density (Fig. 3b & Supplementary Fig. 7). After a transient growth of about one day of incubation at a high temperature - this arises from the cells being transferred from 30 $^{O}$C to the desired high temperature - the number of survivors seems to exponentially decrease over time (during the first

day of incubation at a high temperature) 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 in order to describe how the number of survivors initially decreases (e.g., during the first day of incubation at a high temperature). We found that this rate - which we will call the "initial death-rate" - depends on the initial population-density. Namely, we discovered that as the initial population-density increases, the initial death-rate decreases, meaning that number of survivors decreases more slowly during the first day for higher initial population-densities (Fig. 3b - three dashed lines with differing slopes). For example, after one day of incubation at 41.0 $^{O}$C, a population that started with ~92,000 cells/mL had about 100-fold less survivors than a population that started with about three times more cells (Figure 3b - compare blue and purple lines). Yet, the prevalent theory states that it should be 3-fold less, not 100-fold less. Taken together, these results establish that a population that starts with a higher density has a larger fraction of its cells remaining as survivors after one day and, due to the heavy-tailed decay, has vastly more survivors than a population that started with a lower density (Fig. 3b - last, faded data points for each color). This population-density dependent effect - a small initial difference in population density amplifying to a large, nonlinear change in the final population density - suggests a highly nonlinear cooperative effect that cells have on each other's survival.

**A special temperature that defines the fold-bifurcation point separates two extinction-avoidance regimes**

The conventional theory of cell death - which imposes a constant, exponential death rate - says that the time taken for the number of survivors to be halved decreases with increasing temperature and that it is independent of the initial population density (Fig. 3c). Yet, our experiments revealed a starkly different story (Fig. 3d). We can see this by extracting the initial half-life, which we define to be the amount of time taken for the number of survivors to be halved based on the initial death-rate (Fig. 3d). The initial half-life is inversely proportional to the initial death-rate. We discovered that while increasing the initial population density always increases the population's initial half-life, it is the temperature that determines how sensitively the initial half-life depends on the initial population-density. In particular, we found that a population's initial half-life has two regimes of sensitivities, depending on whether the temperature is below or above 40.3 $^{O}$C. Intriguingly, 40.3 $^{O}$C is the temperature where the fold-bifurcation point lies in the phase diagram (Fig. 2d). For temperatures below 40.3 $^{O}$C, we found that the initial half-life can increase from a few hours to a

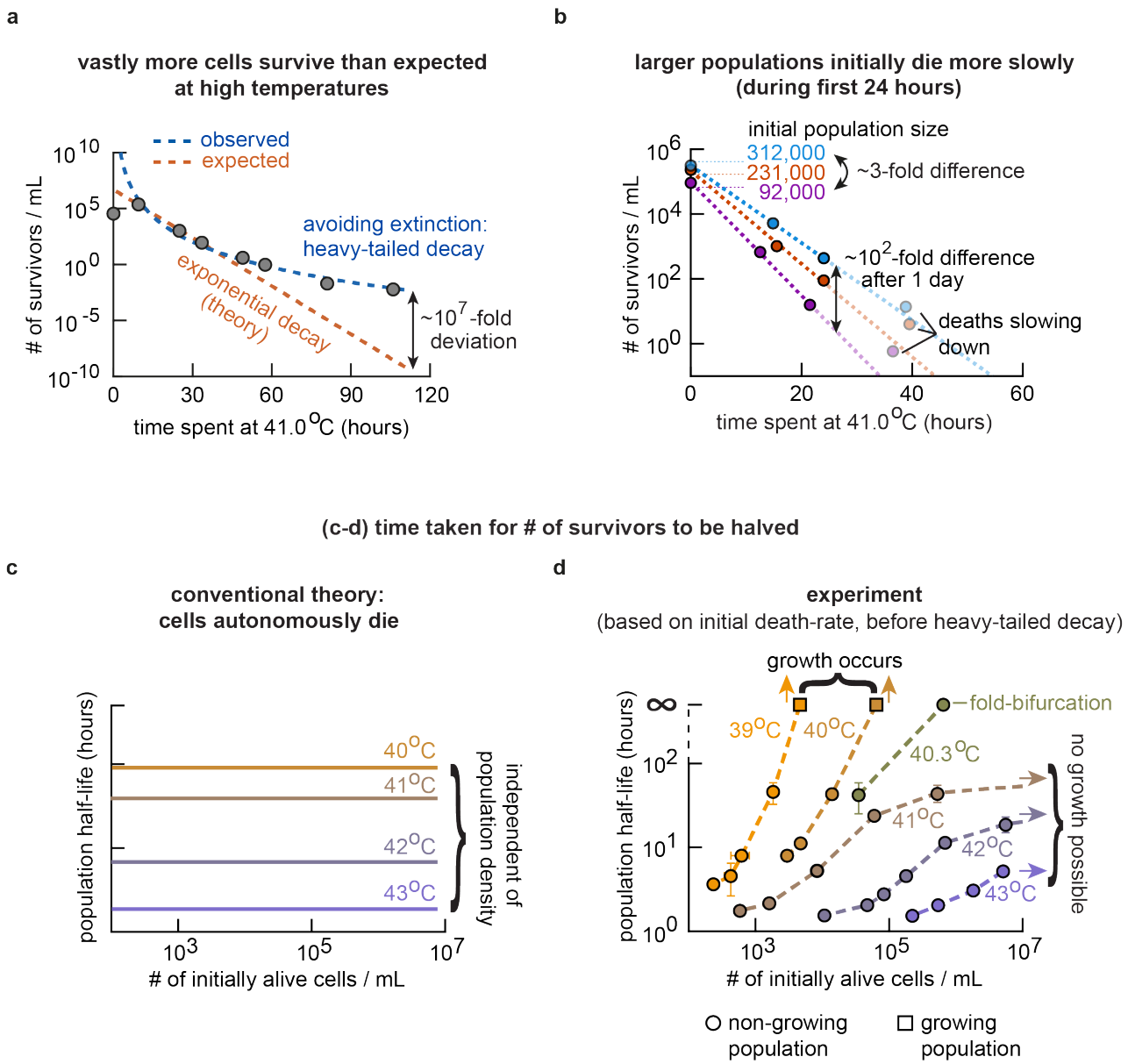


**Figure 3. Cell do not autonomously die and their deaths depend on initial population-density at high temperatures: populations of higher initial densities decelerate and halt their approach to extinction more rapidly.**

(**a**) We determined the number of survivors per mL over time in a non-growing, no-growth-phase population at 41.0 °C by transferring an aliquot of the liquid culture to an agar pad at different time points, incubating the agar pad at 30 °C, and then counting the number of colony forming units ("# of survivors/mL") (see Supplementary Fig. 7). Circles represent measured numbers of survivors per mL. 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 all the data points. Also see Supplementary Fig. 7.

(**b**) Number of survivors per mL for three populations of differing initial population-densities – all of them in the no-growth phase at 41.0 °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). For each color, a dashed line is an exponentially decreasing function that we fitted to the first three timepoints (i.e., deaths that occur during the first day of incubation after a transient growth). Also see Supplementary Fig. 7.

(**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 (i.e., time taken for a population density to be halved) is independent of the

initial population-density for every temperature. Different colors represent different temperatures as indicated.

(**d**) Population half-life, plotted here as a function of initial population-density, is defined as the time taken for a population density to be halved, based on fitting an exponentially decreasing function to the number of survivors / mL measured during the first 24 hours of incubation, which in turn occurs after a ~20-hours of transient growth that is due to the cells coming from 30 $^{O}$C and adjusting to the new temperature. Hence, we measured the number of survivors at approximately 20 hrs, 28 hrs, 36 hrs, and 44 hrs after we incubated the populations at the desired high temperature (these times are measured after the ~20 hours of transient growths ended). Usually, the 44-hr data point already deviated from exponential decay and thus we usually omitted them from the exponential fits. 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: s.e.m. ($n$ = 3 for all data points). 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 densities to trigger their own growths, in accordance with the phase diagram (Fig. 2d).

356 few days when the initial population density changes by just a few fold (e.g., 3-fold) (Fig. 3d -
357 yellow and brown curves for 39 $^{O}$C and 40 $^{O}$C respectively). Moreover, as we keep increasing the
358 initial population density, the initial half-life keeps increasing and eventually reaches infinity, due
359 to the fact that a sufficiently high-density population would grow at these temperatures (Fig. 2d).
360 For temperatures above 40.3 $^{O}$C, we discovered an opposite trend: increasing the initial
361 population-density above some value hardly changes the initial half-life, leading to the half-life
362 eventually plateauing at a finite value as we keep increasing the initial population density (Figure
363 3d - purple curves for 41 $^{O}$C ~ 43 $^{O}$C). Thus, after some amount, increasing the initial population
364 density does not yield much gain in the initial half-life. This occurs because a population can never
365 grow regardless of its density at these temperatures (Fig. 2d). Exactly at 40.3 $^{O}$C, we found that a
366 population whose initial density matches that of the fold-bifurcation point (~1 x $10^5$ cells/mL)
367 neither replicates nor dies. It is unable to grow yet can indefinitely maintain its number of survivors
368 at a nearly constant value (i.e., its initial half-life is infinite because its initial death-rate is zero).
369 The fold-bifurcation point is the only combination of temperature (40.3 $^{O}$C) and population density
370 (~1 x $10^5$ cells/mL) for which an infinite initial half-life is possible without the population growing.
371 This makes the fold-bifurcation point special: a population at the fold-bifurcation point initially can
372 - on average and subject to population-density fluctuations - maintain its cell numbers at a constant
373 value by having the number of births balanced by the number of deaths per unit time. Hence, a
374 population at the fold-bifurcation point can live for a very long time.
375
376 Taken together, the conventional theory of autonomous cell deaths (*4*) cannot explain the
377 features of cell deaths that we uncovered (Figures 3a-d). Likewise, mechanisms that are similar
378 to those known for yielding long-lived populations under other stresses - such as persistence to
379 antibiotics (*25*) - also cannot explain our data on cell deaths (Supplementary Fig. 8). There are

380 two reasons for their inability to do so. First, how fast cells die over time depends on the initial
381 population-density at high temperatures (rather than being independent of the initial population-
382 density as in the case of antibiotic persistence). Secondly, the rate at which cells die changes over
383 time at high temperatures (rather than remaining constant over time as in the conventional theory
384 of autonomous cell deaths). Thus, having “persistor-like” cells as a subpopulation that is more
385 tolerant of a high temperature than the rest of the population is inconsistent with above two
386 features in our data (see Fig. S8 for more in-depth explanations). Moreover, having more heat-
387 tolerant mutants forming a subpopulation - which may have formed during or before the first few
388 days of incubation at a high temperature – also cannot explain our data on cell deaths (Figs. 3a-
389 d and Supplementary Fig. 8). We verified this by taking the last remaining survivors of a high
390 temperature environment (i.e., cells at the tail-end of the heavy-tailed decay), growing them to a
391 high density at 30 $^{O}$C, and then subjecting the resulting population to the same, original high
392 temperature with the same initial population-density as their ancestral (the first) population. We
393 observed that this population approached extinction over time by following the same heavy-tailed
394 decay function as its ancestral population (Supplementary Fig. 8), which is inconsistent with
395 having more heat-tolerant mutants forming a subpopulation before or during a heat-shock – such
396 a subpopulation should lose viable cells over time as a slow, exponentially decaying curve or not
397 decay at all at the high temperature rather than as the same heavy-tailed function as the ancestral
398 population (Supplementary Fig. 8).
399
400
401 **Extracellular factor dictates cell replications at high temperatures**
402 Having established that replications and deaths of cells depend on the initial population-density,
403 we sought to uncover the underlying mechanisms. We first focused on finding a mechanism that
404 is responsible for cell replication. As we will show, this mechanism also explains the cell deaths.
405 Broadly, we can consider two classes of mechanisms. One is that the "factors" that dictate a cell's
406 replication, at high temperatures, resides purely within that cell (i.e., purely intracellular factors
407 dictate cell replications). In this case, cell replication would be a purely cell-autonomous process
408 and the reason that it depends on the initial population-density (Figs. 2a-c) would be that if we
409 have more cells, then it is more likely that at least one cell would manage to replicate. The other
410 possibility is that an extracellular factor dictates cell replications. In this case, a cell's replication
411 would depend on elements outside of that cell which may include the other cells in the population.
412 To distinguish these two classes of mechanisms, we performed experiments in which we
413 physically separated the cells from their extracellular environment. Specifically, in one experiment,

414 we took cells that were exponentially growing at a particular temperature and then, at that same
415 temperature, transferred them to a fresh medium that never harboured any cells before. This
416 experiment would reveal whether the cells can keep on growing which, if true, would mean that
417 purely intracellular factors dictate cell replications (Fig. 4a - bottom left tube). In another
418 experiment, we took away the growth medium from cells that were growing at a particular
419 temperature and then, at that same temperature, transplanted into the medium fresh, non-growing
420 cells whose initial population-density was too low for growth according to the phase diagram. This
421 experiment would reveal whether the transferred medium, taken from growing cells, can induce
422 growth of a population that should not grow which, if true, would mean that extracellular factors
423 dictate cell replications (Fig. 4a - bottom right tube). As an example of an experiment in which we
424 transferred the growing cells instead of their growth medium, we took some wild-type cells from a
425 population that was exponentially growing at a particular temperature (~39 $^{O}$C; Fig. 4b - blue
426 curves) and then transplanted them to a fresh medium at that same temperature so that this newly
427 created population started with a population density (400 cells/mL) that should not permit growth
428 according to the phase diagram (Fig. 2d). We found that these cells stopped growing in the fresh
429 medium soon after the transfer (Fig. 4b - green curves; also see Supplementary Fig. 9). This
430 strongly suggests that extracellular factors dictate cell replications (i.e., a cell does not have an
431 intrinsic ability to replicate that is independent of its extracellular environment).
432
433 To further test the idea that extracellular factors dictate cell replications, we transferred the
434 growth medium from cells that were growing at a particular temperature (~39 $^{O}$C) to fresh, non-
435 growing cells and tested whether these cells could also grow at that same temperature. Strikingly,
436 we found that these fresh cells could grow after being transplanted into the medium (Fig. 4c -
437 purple curves), even though they initially had a low population-density (400 cells/mL) that - without
438 the transplanted medium - prohibits growth according to the phase diagram. Crucially, whether
439 the transferred medium induced growth or not depended, in a highly sensitive manner, on the
440 amount of time that the medium harboured exponentially growing cells before the transfer (Fig. 4c
441 and Supplementary Fig. 10). For example, at ~39 $^{O}$C, if cells exponentially grew for 12 hours or
442 less before we transferred their growth medium to fresh cells, then the fresh cells did not grow in
443 the transferred medium at that same temperature (Fig. 4c - grey and red curves and
444 Supplementary Fig. 10). However, if cells had grown for 16 hours or more before we transferred
445 their growth medium to fresh cells, then the fresh cells grew in the transferred medium (Fig. 4c -
446 purple curves and Supplementary Fig. 10). These results support the following idea: for a
447 population to grow by an appreciable amount at high temperatures, the concentration(s) of growth-

448 inducing extracellular factor(s) must be above some threshold value(s). The next question then is
449 whether these extracellular factors must be secreted or depleted by cells – above or below some
450 threshold value(s) respectively - to induce population growths.
451
452 To test whether it is depletions of some extracellular factors that induce population
453 growths, we performed several experiments (Supplementary Figs. 10-11). In one experiment, we
454 took a fresh growth media that contained all the essential amino acids, diluted them with water by
455 different amounts, added a saturating level (2%) of glucose to them, and then incubated cells in
456 them at high temperatures (Supplementary Fig. 11). We found that a population could grow in any
457 of these diluted media only if their initial population-densities matched the values that, according
458 to the phase diagram (Fig. 2d), permit growth. The same was true if we decreased only the glucose
459 in fresh media by various amounts - without diluting other factors such as amino acids - and then
460 incubated cells in them (Supplementary Fig. 11). Thus, population growths are not dictated by
461 depletions of any of the resources that were already in the media before cells were incubated in
462 them. Instead, these results strongly suggest that a secretion of factor(s) induces population
463 growths.
464
465 As a complementary experiment, we took growth media that were harbouring growing cells
466 at 30 $^{O}$C for various amounts of time, from a few hours to 12 hours. We then transferred these
467 media, after flowing them through a filter membrane to eliminate any cells from them, to a fresh
468 cell-population and then incubating the resulting liquid culture a high temperature (39 $^{O}$C). These
469 newly created populations all had the same initial population-density that were too low for growth
470 at 39 $^{O}$C on their own (i.e., 400 cells/mL) (Supplementary Fig. 10). We found that the transferred
471 media taken from the log-phase cultures at 30 $^{O}$C did not cause the fresh cell-population to grow
472 at 39 $^{O}$C. This result further supports the idea that a secretion, not depletions, of factor(s) induces
473 population growths. Intriguingly, when we took a medium from a population that was in a stationary
474 phase at 30 $^{O}$C – this population underwent a diauxic shift after a log-phase growth – and then
475 transplanted a fresh cell-population into at 39 $^{O}$C, we found that this population grew at 39 $^{O}$C
476 (Supplementary Fig. 10). Hence certain factor(s) that yeasts secrete at 30 $^{O}$C when they are in a
477 stationary phase (following a diauxic shift) can induce population growths at high temperatures.
478 We reasoned that these secreted factor(s) may also be the same factor(s) that cells growing in
479 log-phase at high temperatures also secrete to induce population growths.
480

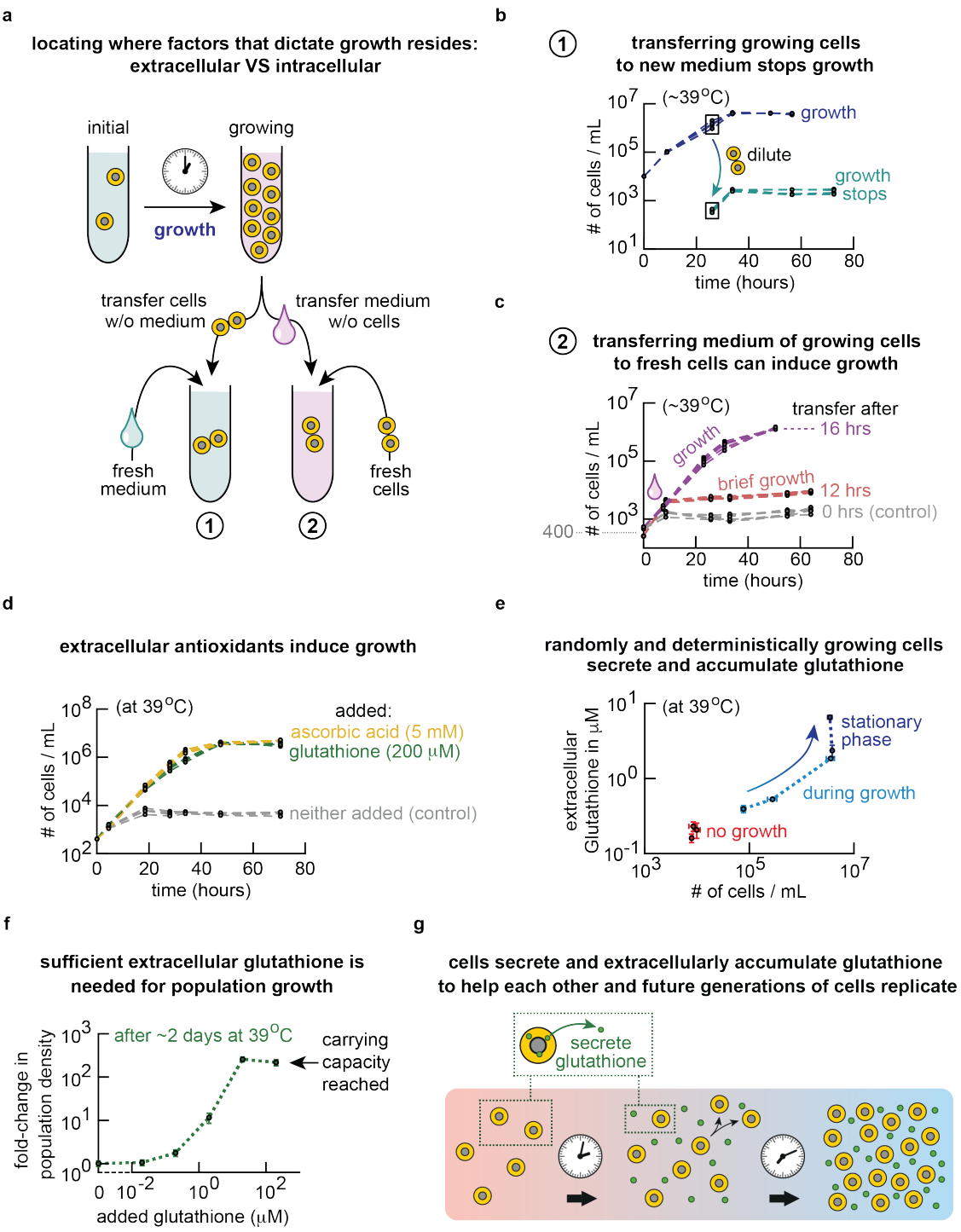


**Figure 4. Cells secrete and extracellularly accumulate glutathione to help each other and future generations of cells replicate at high temperatures.**

(**a**) Schematic description of experiments in (b) and (c) to distinguish two classes of mechanisms. Labelled "1': transfer growing cells into a fresh medium (to determine if intracellular factors dictate population growth). Labelled "2": take out only the liquid medium of a growing population and then incubate fresh cells in it (to determine if extracellular factors dictate population growth).

(**b**) Experiment labelled "1" in (a) at 39.2 $^{O}$C. Blue curves show populations of wild-type yeasts (initially ~10.000 cells/mL) that start to exponentially grow. Cells of these populations in exponential growth, marked by the boxed blue data point, were transferred without their

growth medium into a fresh medium. This resulted in ~400 cells/mL in the new culture, represented by the green curves (four replicate populations). Also see Supplementary Fig. 9.

(**c**) Experiment labelled "2" in (a) at 39.2 $^{O}$C. Wild-type population's density over time for cells that were incubated in a growth medium that previously harbored a population of exponentially growing wild-type cells at ~39 $^{O}$C for 0 hours (grey curves), 12 hours (red curves), and 16 hours (purple curves). Each color shows at least 6 replicate populations. Also see Supplementary Fig. 10.

(**d**) At 39.2 $^{O}$C. All wild-type populations start at 400 cells/mL, which is too low of a density to permit growths. 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 three replicate populations.

(**e**) At 39.2 $^{O}$C. Shown here are the concentrations of extracellular glutathione for wild-type populations in the no-growth phase (initially 400 cells/mL - red data), random-growth phase (initially 2,000 cells/mL), and deterministic-growth phase (initially 10,000 cells/mL). Three biological replicates are shown for each phase ($n$ = 3; error bars are s.e.m.). We measured three timepoints for each phase. Plotted here are the concentrations of extracellular glutathione as a function of the population density over time. The arrow shows both the population density and concentration of extracellular glutathione increasing together over time (see also Supplementary Fig. 13).

(**f**) Sensitivity of no-growth wild-type populations (initially ~400 cells/mL) at 39.2 $^{O}$C to glutathione that we added into the growth medium. Shown here is, as a function of glutathione concentration, the fold-change in the population density after two days of incubation with the added glutathione. Last two data points show ~200-fold change, which represents the populations having fully grown to reach the carrying capacity (see also Supplementary Fig. 13).

(**g**) Cartoon illustrating the mechanism deduced in (a-f). Yeasts secrete glutathione at high temperatures. The extracellular glutathione thus accumulates. When its concentration reaches at least a threshold amount (~0.3 μM from (f)), the population replicates at high temperatures by noticeable amounts.

482 **Extracellular antioxidants - glutathione and ascorbic acid - enable yeasts to replicate at**
483 **high temperatures**

484 To help us identify the secreted factor(s) responsible for cell replications at high temperatures, we
485 performed a transcriptome analysis (RNA-seq) on wild-type yeast at different parts of its phase
486 diagram (Supplementary Fig. 12). We found that deterministically growing cells at high
487 temperatures, compared to those growing at 30 $^{O}$C, downregulate genes involved in the central
488 carbon metabolism and majority of other genes in general. But we also found that they had,
489 compared to those growing at 30 $^{O}$C, upregulate genes that are associated with DNA damage
490 response, translation initiation, and biogenesis and assembly of cell membranes. These changes
491 in gene expressions are similar to those seen for yeasts experiencing environmental stresses in
492 general (*26*,*27*). We hypothesized that a cell upregulates these genes to repair damages to
493 various cellular components. Specifically, we hypothesized that reactive oxygen species may
494 cause these damages at high temperatures. Reactive oxygen species are known to damage
495 nucleic acids (*28*), proteins (*29*), and lipids in the cell membrane (*30*). Indeed, our transcriptome
496 analysis revealed that many genes associated with the mitochondria were downregulated in

497 replicating yeasts at high temperatures compared to yeasts at 30 $^{O}$C. Since respiration through
498 mitochondria creates reactive oxygen species, we reasoned that downregulating respiratory
499 mitochondrial genes may decrease the amount of reactive oxygen species that form and, as a
500 result, allow the yeast to replicate at high temperatures (*31*,*32*). Cells can combat reactive oxygen
501 species by producing antioxidants. Antioxidants reduce oxidative-stress-related damages by
502 capturing and then deactivating reactive oxygen species through redox reactions. We
503 hypothesized that budding yeasts may be secrete antioxidants at high temperatures. Moreover,
504 we reasoned that – as suggested by our media-transfer experiments (Fig. 4c and Supplementary
505 Fig. 10) – the secreted antioxidants must be present in high enough concentrations, meaning that
506 a population density must be high enough, in order for a population to grow at high temperatures.
507 Strengthening this hypothesis is that heat shocks are known to cause budding yeasts to produce
508 and maintain an intracellular – but not extracellular - pool of glutathione (*33*). As an antioxidant,
509 glutathione is widely used by many species, including humans. It is budding yeast's primary,
510 intracellular antioxidant (*32*,*34*). Also strengthening our hypothesis is the fact that after shifting
511 from a log-phase growth to a stationary phase at 30 $^{O}$C, budding yeasts are known to produce
512 glutathione – though most of the glutathione is known to be intracellularly kept and only low
513 amounts of it have been detected in their extracellular medium (*35*). Of particular interest to us is
514 the fact that in a few known instances, budding yeasts can secrete glutathione – they are known
515 to do so as an extracellular defense against arsenite that harms them (*36*). But for the budding
516 yeast, glutathione's extracellular role still remains underexplored and it is mainly viewed as an
517 intracellular agent with varied roles – for example, as a regulator of iron metabolism (*37*) or an
518 antioxidant (*38*). We reasoned that the very low concentration of extracellular glutathione, if it
519 indeed exists in the growth medium of the stationary-phase population at 30 $^{O}$C, may have induced
520 the growth of the fresh cell-population at 39 $^{O}$C that we previously observed (Supplementary Fig.
521 10). It is unclear, however, whether yeasts at high temperatures indeed secrete glutathione.
522
523 To first test whether antioxidants can even induce population growths at high
524 temperatures, we added either glutathione or ascorbic acid - two prominent antioxidants (*34*) - or
525 trehalose at various concentrations to the growth medium that harboured a low density of wild-
526 type cells. Strikingly, we found that both glutathione and ascorbic acid can induce population
527 growths as long as we add either one at high concentrations (e.g., glutathione at 200 μM and
528 ascorbic acid at 5 mM) (Fig. 4d). Furthermore, we determined that trehalose cannot induce
529 population growths at high temperatures (Supplementary Fig. 11). These results establish that at
530 high temperatures, glutathione and ascorbic acid are sufficient for inducing growths of low-density

531 populations that would not have grown on their own (e.g., 400 cells/mL, Fig. 2d). Moreover, these
532 results suggest that reactive oxygen species causing damages is the primary reason that yeasts
533 fail to replicate at high temperatures.
534
535
536 **Cells secrete glutathione to help each other replicate at high temperatures**
537 Although ascorbic acid, known as "erythroascorbate" for the budding yeast, acts as an antioxidant
538 in eukaryotes, its role in budding yeast remains unclear as researchers have not detected
539 appreciable amounts of it in yeast (*33*). Therefore, we reasoned that glutathione is a more likely
540 candidate as the secreted antioxidant. To test if yeast populations secrete glutathione at high
541 temperatures, we measured the glutathione concentration over time in the growth medium for
542 deterministically growing, randomly growing, and no-growth-phase populations at high
543 temperatures (Fig. 4e and Supplementary Fig. 13). For populations in the no-growth phase at high
544 temperatures (e.g., 39 $^{O}$C), glutathione concentration barely increased during ~50 hours of
545 incubation. The population did not grow during this time. In contrast, for populations in the random-
546 growth and the deterministic-growth phases at high temperatures, we detected a continuous
547 increase in the extracellular glutathione concentration over the ~50 hours of incubation, leading to
548 ~10-fold increases in the extracellular glutathione concentration. After these populations reached
549 their carrying capacities and stopped growing, their extracellular glutathione concentration still
550 continued to increase (Fig. 4e). These results establish that both log-phase and stationary-phase
551 yeasts at high temperatures secrete glutathione. On the other hand, at multiple temperatures
552 below 36.7 $^{O}$C, we found that only stationary-phase yeasts after a diauxic shift, but not during a
553 log-phase growth, secrete glutathione (Supplementary Fig. 13). Thus, in summary, budding yeasts
554 secrete glutathione while in log-phase growth and stationary phase at high temperatures (above
555 36.7 $^{O}$C) but they do not secrete glutathione during log-phase growth at lower temperatures.
556 Consistent with glutathione being responsible for the population-density-dependent growths, the
557 temperatures at which we detect secreted glutathione from log-phase growing yeasts – above ~36
558 $^{O}$C - coincide with the temperatures at which growth depends on the population density (Fig. 2d
559 and Supplementary Fig. 4).
560
561
562 **Extracellular glutathione concentration must be above a threshold concentration to induce**
563 **population growths**

564 We next addressed how sensitive yeasts are to extracellular glutathione at high temperatures. At
565 high temperatures (e.g., 39 $^{O}$C), we added various concentrations of glutathione to growth media
566 and then incubated a low density, no-growth-phase population in them. By measuring the fold-
567 change in the population density after two days of incubation, we obtained a highly non-linear
568 relationship between glutathione concentration and the fold-change in the resulting population
569 density (Fig. 4f). Namely, when the extracellular glutathione concentration was just above 0.3 μM,
570 the population grew by ~10-fold. But when the extracellular glutathione concentration was either
571 just below or much lower than 0.3 μM, the population hardly grew even after two days. Moreover,
572 when the extracellular glutathione concentrations were much higher than 0.3 μM (e.g., ~10 μM),
573 populations deterministically grew and reached their carrying capacities (i.e., ~200-fold growth).
574 These results establish that a population must accumulate enough glutathione in the extracellular
575 medium - above some threshold concentration (~0.3 μM) - in order to grow (Fig. 4g). Consistent
576 with glutathione being responsible for inducing population growths at high temperatures, this
577 threshold concentration of glutathione matches the secreted and accumulated glutathione
578 concentration that we measured in the growth media of populations that were able to grow at high
579 temperatures (Fig. 4d).
580
581
582 **Minimal mathematical model recapitulates all the experimental observations**
583 To test if glutathione secretion can tie together and quantitatively explain all our experimental data,
584 we developed a mathematical model. In this model, cells secrete glutathione at a constant rate -
585 this is the simplest possible scenario and we found that not assuming a constant secretion rate
586 does not qualitatively alter the model's outcomes (see Supplemental text). Moreover, aside from
587 always secreting glutathione, a cell in the model takes one of three actions: replicate, die, or stay
588 alive without replicating (Fig. 5a). A probability for each action determines what the cell does next
589 (i.e., a cell "rolls" a three-sided, loaded dice to determine its next action). Specifically, we assume
590 that the probability that a cell dies in the next time step to be fixed by temperature and thus remain
591 constant over time (Fig. 5b - left panel). Moreover, we assume that the probability of dying to
592 linearly increase with temperature. But we found that relaxing this assumption - having it non-
593 linearly increase with temperature - does not change the model's outcomes (see Supplementary
594 text). We let the probability of a cell replicating in the next time step to non-linearly (sigmoidally)
595 increase with glutathione concentration (Fig. 5b - right panel). This reflects our experimental
596 observation that glutathione concentration must be above some threshold value to appreciably
597 induce population growths (Fig. 4f). The probability of staying alive without dividing is then fixed

598 by the probabilities of dying and of replicating. This model contains four parameters, three of which
599 are rigidly fixed by (directly read-off from) the experimental data without any possibility of us
600 adjusting their values: (1) the maximum growth rate that a population can have (~0.25 / hour from
601 our experiments – Fig. 2e), (2) the temperature at which heat-induced deaths begin to be non-
602 negligible (i.e., temperature above which no-growth phase starts to exist - this is ~38 $^{O}$C according
603 to the wild-type's phase diagram (Fig. 2d) and it is also the temperature at which the red line in
604 Figure 5b starts to increase above zero), and (3) the temperature at which heat-induced deaths
605 are always dominant over cell replications (i.e., temperature above which only the no-growth
606 phase exists - this is ~40.3 $^{O}$C according to the wild-type's phase diagram (Fig. 2d) and it is also
607 the temperature at which the highest possible probability of replicating matches the probability of
608 dying (Fig. 5b - grey line)). The only free parameter that we can flexibly fit to our data (i.e., fit to
609 combinations of data rather than directly reading-off from a single measurement) is the glutathione
610 concentration at which the probability of replicating is half its maximum (Fig. 5b - blue curve).
611
612 Strikingly, after using our data for the wild-type strain to fit the one free parameter, our
613 highly constrained model qualitatively - and quantitatively - recapitulated all the main experimental
614 data (Figs. 5c-f and Supplementary Fig. 14). Specifically, the model recapitulates the population-
615 level growth kinetics with the distinct growth phases (Fig. 5c, compare with Fig. 2a), the single-
616 cell-level growth kinetics for each growth phase (Supplementary Fig. 14, compare with
617 Supplementary Fig. 6), the phase diagram (Fig. 5d, compare with Fig. 2d), the population-density
618 dependent deaths (including the number of survivors decreasing over time in a heavy-tailed
619 manner) (Fig. 5e, compare with Fig. 3b), and the relationship between temperature and the
620 population-density-dependent deaths (Fig. 5f, compare with Fig. 3d). Moreover, we used a
621 mathematical argument to establish that our model is the simplest possible class of model that
622 can explain our data (see Supplementary text). To intuitively see how the model reproduces all
623 the experimental data, note that the probability that a cell replicates is initially zero when the
624 population first enters a high-temperature environment, since the glutathione concentration is
625 initially zero. The probability increases over time as the glutathione concentration increases due
626 to the cells constantly secreting glutathione (Fig. 5b and Supplementary Fig. 14). However, the
627 probability for a cell to die starts at a nonzero value when the population first enters a high-
628 temperature environment, since it is set only by temperature. This probability remains constant
629 over time since its value is independent of the extracellular glutathione concentration (Fig. 5b and
630 Supplementary Fig. 14). Therefore, there exists a specific glutathione-concentration - a threshold
631 concentration - above which the probability of replicating exceeds the probability of dying, thereby

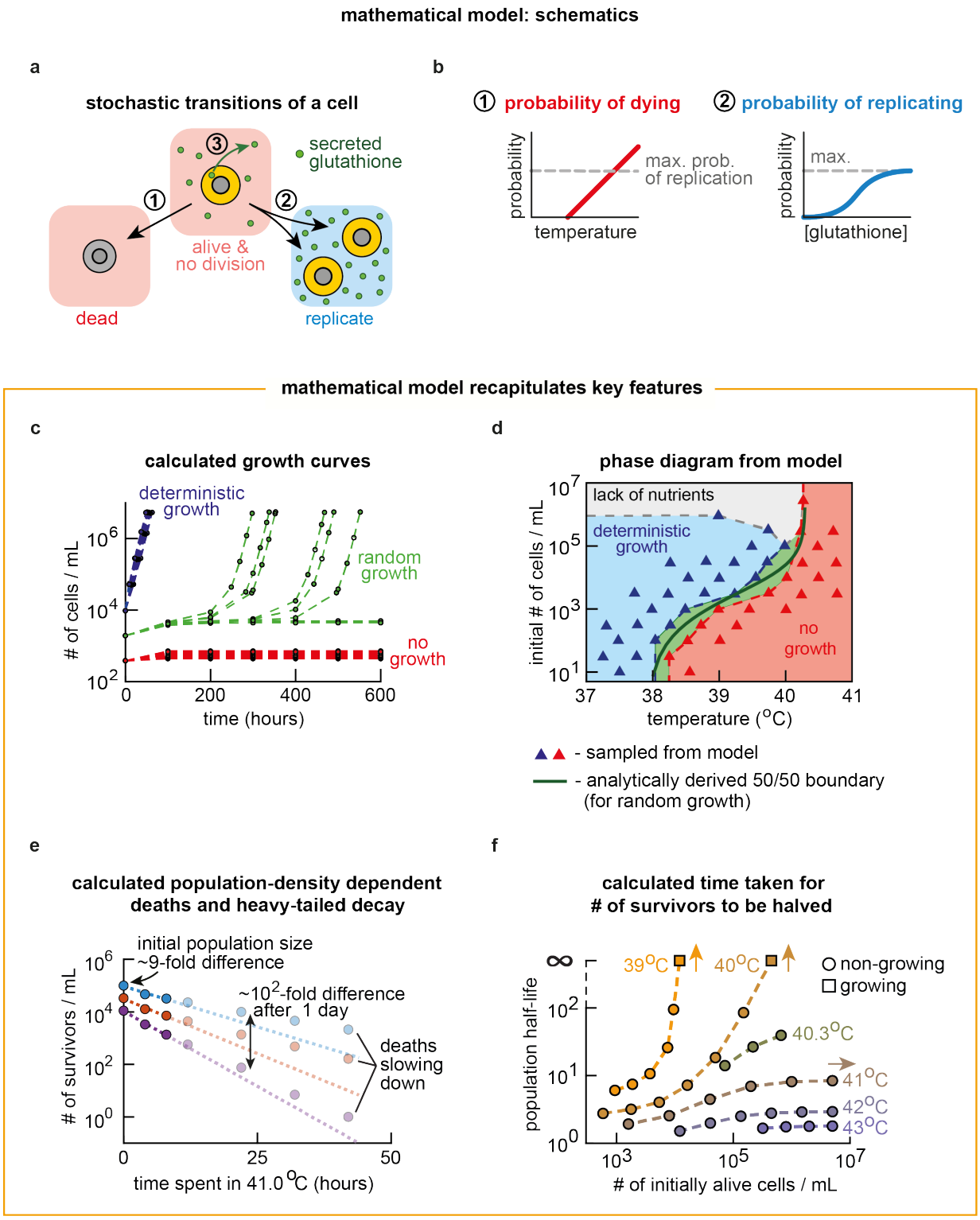


**Figure 5. Mathematical model with one free parameter recapitulates all the main experimental data.**

(**a-b**) Description of the mathematical model (see full description in the Supplemental 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) nonlinearly 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 Fig. 3d), the number of survivors decaying over time as a heavy-tailed function (see Supplementary Fig. 14), and single-cell-level data on growths (compare Supplementary Fig. 14 with Supplementary Fig. 6). Also see Supplementary Fig. 14.

resulting in a population growth. This sets up a "race" in which a population of cells, starting without any extracellular glutathione, must realize the threshold concentration before going extinct. There are initially more cell deaths than cell replications and thus there is a "ticking time bomb" until extinction. As a result, if the initial population-density is sufficiently high, then population growth wins the race - this occurs in the deterministic-growth phase (Supplementary Fig. 14). If the initial population-density is sufficiently low, then population extinction wins the race - this occurs in the no-growth phase (Supplementary Fig. 14). For a population that starts with an intermediate density, the glutathione concentration gets close to the threshold concentration by the time there are very few alive cells remaining. At this point in time, any subsequent, small changes in the number of alive cells determines whether the probability of a cell replicating exceeds the probability of a cell dying (Supplementary Fig. 14). Hence, when a population would grow and if it can even grow are both completely unpredictable because the population-level behavior here is highly sensitive to the stochastic behaviors of the few alive cells (*40*) - this occurs in the random-growth phase. In the model, at sufficiently high temperatures (i.e., above ~40.3 $^{O}$C), the probability of dying exceeds the highest probability that a cell can have for replicating (Fig. 5b - grey dashed line). This occurs precisely at the fold-bifurcation point in the phase diagram. In the no-growth phase, the model also reproduces the number of survivors decreasing over time in a heavy-tailed manner (Fig. 5e and Supplementary Fig. 14). The mechanism here is that cells keep dying as time passes - due to the probability of dying remaining constant over time - but, for the still-alive cells, the probability of replicating increases over time due to each surviving cell continuously secreting glutathione into the environment, making replication more likely for each alive cell as time passes. The competition between the two - a constant probability of dying and an initially lower probability of replicating that gradually approaches the probability of dying - results in a population whose approach to extinction continuously slows down over time, leading to the number of survivors decreasing over time in a heavy-tailed manner that we experimentally observed (see Supplemental text). Taken together, these results show that our relatively simple model, with the secreted and accumulated glutathione helping the current and future generations of cells replicate

660 as the primary ingredient, recapitulates all the main features of cell replications and deaths that
661 we experimentally observed (Figs. 5c-f).
662
663
664 **Extracellular glutathione is both necessary and sufficient for cooperative cell-replication**
665 **at high temperatures**
666 Our mathematical model did not require knowing exactly how secreting glutathione helps cells
667 replicate at high temperatures. We next sought to gain molecular insights into extracellular
668 glutathione promoting cell replications at high temperatures. To recap, we have shown so far that,
669 at high temperatures, yeasts secrete glutathione (Fig. 4e) and that a sufficient concentration of
670 extracellular glutathione is required to induce a population growth (Fig. 4f). These two results show
671 that extracellular glutathione is sufficient for the cells to help each other replicate at high
672 temperatures. Next, we sought to address if extracellular glutathione is also necessary - in addition
673 to being sufficient - for yeasts to help each other replicate. To test this, we inactivated ("removed")
674 all extracellular glutathione by adding a chemical that masked glutathione in the liquid media. This
675 "masking reagent" (called 1-Methyl-2-vinylpyridinium (M2VP)) rapidly scavenges all extracellular
676 glutathione, without interfering with the intracellular glutathione and any intracellular processes
677 (Supplementary Fig. 15) (*41,42*). As an example, at ~39 $^{O}$C, we added the masking agent to the
678 liquid media of deterministically growing populations (Fig. 6a). We found that these populations
679 stopped growing after we added the masking agent. Hence, we can conclude that glutathione is
680 not only sufficient (Fig. 4f) but is also necessary for cell replications at high temperatures. In other
681 words, glutathione is the only molecule that determines whether a population grows or not at high
682 temperatures. To see this, note that if any other molecules were necessary for growth at high
683 temperatures, then masking the glutathione should not have prevented the population growths.
684 Conversely, note that if any other secreted molecules besides glutathione were sufficient for
685 inducing population growth at high temperatures, then removing glutathione alone should not have
686 stopped the population growth. Finally, we found that adding the masking agent to the liquid media
687 of a growing population at 30 $^{O}$C did not affect the population growth - the population continued to
688 grow in the presence of the masking agent at 30 $^{O}$C (Supplementary Fig. 15). Taken together,
689 these results establish that extracellular glutathione is both necessary and sufficient for inducing
690 cell replications at high temperatures (above ~36.7 $^{O}$C) but that it is not necessary at temperatures
691 below ~36.7 $^{O}$C.
692
693

694 **Transport and function of glutathione at high temperatures**

695 Now that we have identified extracellular glutathione to be the sole factor that dictates whether a
696 population grows or not at high temperatures, we sought to gain molecular insights into how the
697 extracellular glutathione can promote cell replications at high temperatures. Myriad proteins
698 regulate the biosynthesis of glutathione and its transport in and out of the cell (Fig. 6b). The
699 enzyme Gsh1 is required for synthesizing glutathione and thus a strain lacking the *GSH1* gene
700 (i.e., *GSH1Δ*) cannot synthesize glutathione (*43*). The budding yeast requires the importer, Hgt1,
701 to import glutathione from outside and thus a strain lacking the *HGT1* gene (i.e., *HGT1Δ*) is unable
702 to import detectable amounts of glutathione (*44*). Finally, there are various glutathione exporters
703 that each contribute to the overall secretion of glutathione (*36*). Of these, Gex1 and Gex2 are
704 antiporters - they import protons while actively exporting glutathione - and knocking both the *GEX1*
705 and *GEX2* genes cause a reduction of about 40% in the rate of glutathione export (*45*). The
706 budding yeast also actively export glutathione via several ABC transporters such as Yor1 (*36*) and
707 Adp1 (*46*). To genetically manipulate the synthesis, import, and export of glutathione at high
708 temperatures, we constructed mutant strains that were either unable to synthesize glutathione
709 (*GSH1Δ*-strain), or unable to import glutathione (i.e., *HGT1Δ*-strain), or had severely reduced
710 ability to secrete glutathione (*GEX1,2Δ-ADP1Δ*-strain).

711

712 We first used the mutant strain that could not import glutathione (*HGT1Δ*-strain) to
713 determine whether yeasts need to import their secreted glutathione in order to grow at high
714 temperatures. We found that this mutant has the same population-density-dependent growths at
715 high temperatures, just like the wild-type strain (Fig. 6c). Similar to the wild-type strain at
716 sufficiently at low population densities, mutant populations cannot grow if they start with sufficiently
717 low densities. But they can grow if we add enough glutathione to their liquid media (Fig. 6c).
718 Together, these results establish that yeasts do not need to import secreted glutathione in order
719 to replicate at high temperatures. This, in turn, means that glutathione's extracellular action alone
720 must be responsible for promoting cells replications at high temperatures.

721

722 Since extracellular glutathione, without being imported, determines a population growth at
723 high temperatures, we reasoned that a mutant strain with a significantly reduced ability to secrete
724 glutathione (*GEX1,2Δ-ADP1Δ*-strain) may have a reduced ability to replicate at high temperatures.
725 Indeed, we found that while populations of this mutant can still grow at high temperatures (Fig.
726 6d), they have a reduced ability to replicate compared to the wild-type strain. Concretely, this
727 means that while a wild-type population can deterministically grow at a given high temperature, a

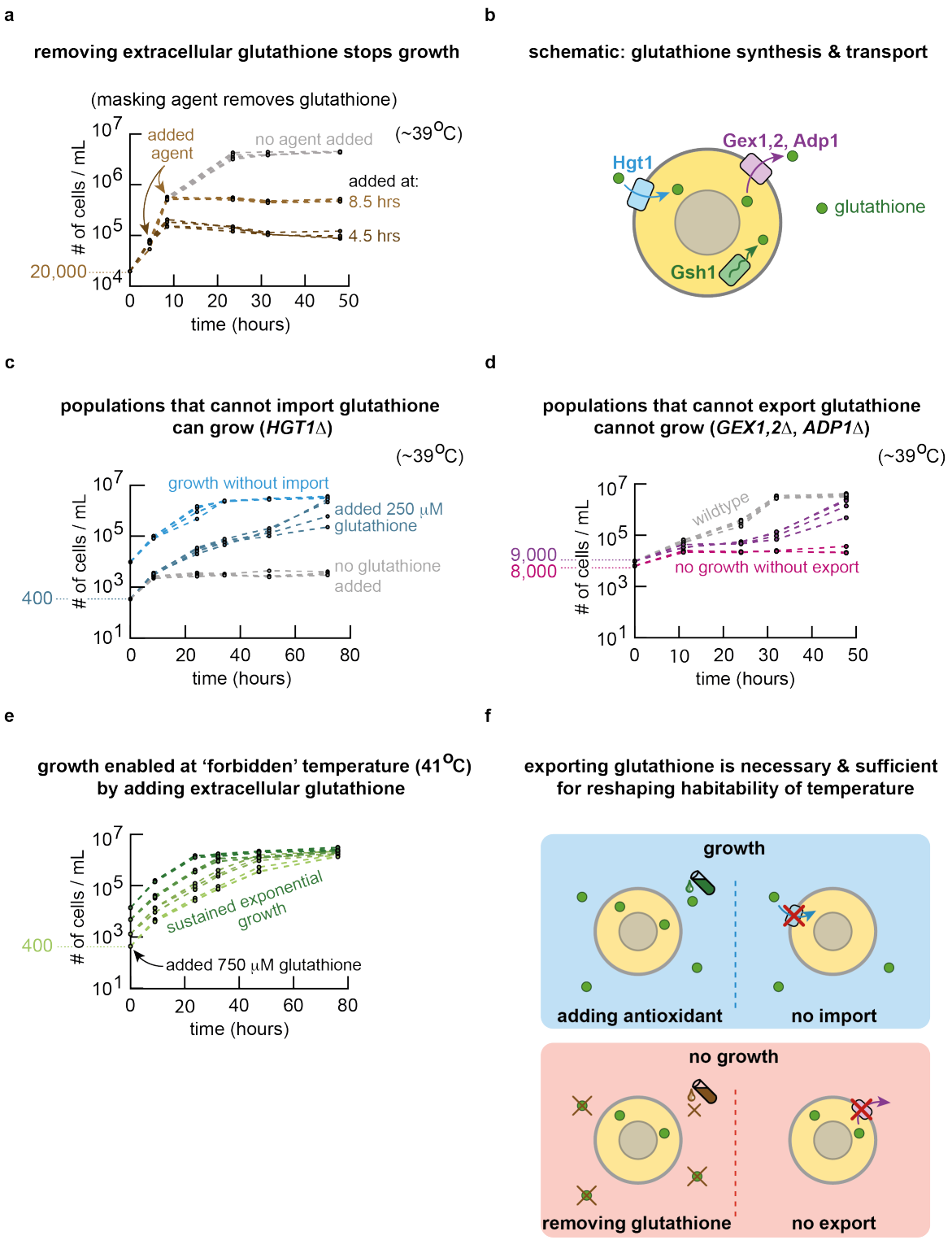


**Figure 6. Budding yeast exports glutathione whose extracellular role – not intracellular roles - as an antioxidant enables yeasts to survive high temperatures.**

(**a**) At 39.2 °C. Curves show wild-type populations (all initially at ~20,000 cells/mL) that should deterministically grow if left alone (as in grey curve). A glutathione masking agent (M2VP) – which inactivates extracellular glutathione - was added after 4.5 hours (dark brown) or 8.5 hours (light brown) of incubation (see Methods). Grey curve shows populations that did not receive the masking reagent.

Each color shows four replicate populations. Also see Supplementary Fig. 15.

(**b**) Schematic showing how the budding yeast synthesizes, imports, and exports glutathione. Glutathione is intracellularly synthesized via an enzyme (γ-glutamylcysteine synthetase) encoded by *GSH1*. Glutathione is imported by a proton-coupled glutathione-importer encoded by *HGT1*. Glutathione is exported by numerous exporters (not all shown here), 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 (*HGT1Δ*-strain) that cannot import glutathione (initially at ~10,000 cells/mL). Grey curves at the bottom show the mutant populations (initially ~400 cells/mL) incubated without any glutathione added. Dark blue curves in the middle show the mutant populations (initially ~400 cells/mL) incubated with 250μM of glutathione added to the media. Each color shows four replicate populations.

(**d**) At 39.2 $^{O}$C. Populations of a mutant strain that lacks some of the main glutathione exporters (*GEX1,2Δ-ADP1Δ*-strain) (initially ~9,000 cells/mL (purple curves in the middle) or ~8,000 cells/mL (pink curves at the bottom)). Grey curves at the top show, as a comparison, the wild-type populations (initially ~9,000 cells/mL). Each color shows at least four replicate populations.

(**e**) At 41 $^{O}$C. Wild-type populations of various initial densities (from ~400 cells/mL (lightest green curves) to ~14,000 cells/mL (darkest green curves)) grown in media supplemented by 750μM of glutathione. Each color shows four replicate populations.

(**f**) Cartoon illustrating mechanisms deduced in (a-e). Exporting glutathione is necessary and sufficient for budding yeasts to reshape the habitability of temperature. For yeasts to survive and grow at high temperatures, extracellular glutathione is (1) necessary since blocking glutathione or blocking glutathione-export stops yeast's growths and is (2) sufficient since adding glutathione or blocking glutathione-import enables the yeasts to grow at high temperatures.

729 mutant population that starts with the same density as the wild-type population can at most
730 randomly grow at the same temperature (i.e., the mutant achieves deterministic growth only with
731 higher initial population-densities). Likewise, while a wild-type population can grow randomly at a
732 given high temperature, a mutant population that starts with the same density cannot grow (i.e.,
733 the mutant strain needs to start with a higher population density to escape the no-growth phase).
734 Thus, consistent with glutathione's extracellular action – rather than intracellular action –
735 promoting cell replications, these results together show that reducing the yeast's ability to export
736 glutathione reduces the ability of yeast populations to grow at high temperatures.
737
738 The mutant strain with a reduced glutathione-export (*GEX1,2Δ-ADP1Δ*-strain) still secreted
739 measurable amounts of glutathione at high temperatures (Supplementary Fig. 16), which is
740 expected as it has other glutathione exporters (e.g., Yor1p). We wondered how much of this
741 secretion – and wild-type strain's glutathione secretion - was because glutathione simply leaked
742 out of the cells at high temperatures due to, for example, heat degrading the integrity of cell
743 membranes. To test this, we used a standard test – staining by propidium iodide (*47*) - to check
744 how many cells, in a mutant population (*GEX1,2Δ-ADP1Δ*-strain) that was growing at a high
745 temperature, allowed propidium iodide to diffuse through their potentially leaky (compromised)

746 membranes to stain their nucleic acids. We found that growing mutant populations at high
747 temperatures actually have intact membranes as almost none of the cells in the populations were
748 stained by propidium iodide (Supplementary Fig. 16). Thus, glutathione does not simply leak out
749 of the cells at high temperatures – cells must use exporters to secrete glutathione at high
750 temperatures.
751
752 Finally, we used the mutant strain that is unable to synthesize glutathione (*GSH1Δ*-strain)
753 to double check that the wild-type cells secrete glutathione at high but not at low temperatures.
754 We found this earlier by directly measuring the concentration of extracellular glutathione in growing
755 populations. As is well known, the *GSH1Δ*-strain cannot survive or grow at any temperature,
756 including at 30 $^{O}$C, unless one supplements its growth medium with glutathione (Supplementary
757 Fig. 17) (*43*). This is due to glutathione's essential intracellular roles which are not related to heat-
758 shock responses (*43*). As in our media-transfer experiments (Fig. 4b, Supplementary Fig. 10), we
759 took the liquid media of wild-type populations that were growing at a high temperature (e.g., at
760 ~39 $^{O}$C), then at 30 $^{O}$C, we transplanted fresh populations of the *GSH1Δ*-cells that were starved
761 of extracellular glutathione (Supplementary Fig. 17). We found that these mutant populations,
762 which cannot synthesize but need glutathione to survive, grew in the transferred media. Thus, the
763 media taken from the wild-type populations that were growing at high temperatures must contain
764 glutathione (i.e., wild-type cells indeed secrete glutathione at high temperatures). Moreover, we
765 found that the glutathione-starved *GSH1Δ*-cells did not grow in media that we took away from wild-
766 type populations that were growing at 30 $^{O}$C. This means that the wild-type cells indeed do not
767 secrete glutathione at 30 $^{O}$C. Taken together, these results again confirm our earlier conclusion:
768 wild-type yeasts secrete glutathione only at high temperatures (above ~36 $^{O}$C)
769
770 **Yeasts can replicate at "unlivable" temperatures**
771 Finally, since extracellular glutathione is both necessary and sufficient for population growths at
772 high temperatures, we reasoned that wild-type populations neither randomly nor deterministically
773 grow at temperatures above 40.3 $^{O}$C - according to the wild-type strain's phase diagram (Fig. 2d)
774 - because they cannot accumulate sufficient concentrations of extracellular glutathione at
775 temperatures beyond 40.3 $^{O}$C. Note that 40.3 $^{O}$C is where the fold-bifurcation point is located and
776 that beyond 40.3 $^{O}$C, only the no-growth phase exists in the wild-type strain's phase diagram (Fig.
777 2d). We therefore wondered if we could help wild-type populations grow at temperatures higher
778 than 40.3 $^{O}$C by supplementing their media with glutathione. We tested this idea by adding a
779 saturating amount of glutathione to the liquid media of wild-type populations at 41 $^{O}$C (Fig. 6e).

780 We found that these populations – nearly 1 $^{O}$C above the supposedly maximum growth-permitting
781 temperature - were now able to grow until they reached the carrying capacity. Thus, deterministic
782 growths are possible at a temperature that is nearly one degree Celsius higher than the maximum
783 temperature (40.3 $^{O}$C) for the wild-type strain, if we help them accumulate extracellular glutathione.
784
785 Taken together, our experiments with the three mutant strains above establish that budding
786 yeasts must secrete and extracellularly accumulate glutathione in order to replicate at high
787 temperatures and that glutathione's extracellular action alone – not its intracellular action - is
788 responsible for promoting cell replications at high temperatures (Fig. 6f). The latter, coupled with
789 the fact that adding another antioxidant (ascorbic acid) alone is sufficient for inducing population
790 growths at high temperatures (Fig. 4d), lends support to the conclusion that extracellular
791 glutathione promotes cell replications by reducing harmful extracellular agents such as reactive
792 oxygen species at high temperatures.

793

794 **DISCUSSION**

795 **Expanding the role of glutathione as mediator of cooperative cell-replication and extender**
796 **of population lifespan at high temperatures**

797 Glutathione is a well-known tri-peptide that is essential for many organisms, including humans
798 (*34*). It is central to diverse processes such as combating cancer (*48*) and neurodegenerative
799 diseases (*49*), slowing down aging (*50*), detoxifying the liver (*51*), and - relevant to our study -
800 protecting cells from heat-induced reactive oxygen species that damage cells (*28-30, 52*). For the
801 budding yeast, much of the focus on glutathione has been on its intracellular role. Intracellularly,
802 glutathione indeed has essential roles in iron metabolism (*37*), thiol-redox regulations (*53*),
803 protecting against reactive oxygen species as an antioxidant (*34,38*), detoxifying the environment
804 poisoned by xenobiotics (*54*) and heavy metals such as cadmium (*55*), and responding to sulphur
805 starvation (*56*) and nitrogen starvation (*57*). But glutathione's extracellular role for budding yeast
806 - both at high temperatures and in other contexts - remains poorly understood and has received
807 relatively little attention compared to its intracellular roles. Among the studies that examined
808 extracellular glutathione for yeasts are a recent study showing that extracellular glutathione can
809 protect yeasts from toxic arsenite (*36*) and a work showing how extracellular glutathione aids in
810 dealing with nutrient imbalance for certain mutant yeasts that have been evolved to secrete
811 glutathione at 30 $^{O}$C (*58*). Moreover, there have been some reports on how yeasts, during
812 stationary-phase that follows a diauxic shift at 30 $^{O}$C, uptake and secrete low amounts of

813 glutathione (*35*). Indeed, we confirmed that stationary-phase populations, after many hours, can
814 build up to ~2 μM extracellular glutathione at 30 $^{O}$C but that log-phase populations do not secrete
815 glutathione at 30 $^{O}$C. What was underappreciated before, which we have discovered here, is that
816 yeasts secrete glutathione at high temperatures - during log-phase growth and stationary phases
817 - and that this leads to their cooperative replications and an indefinite extension of a population's
818 lifespan (in the form of heavy-tailed population decay) at high temperatures.
819
820 Furthermore, we found that extracellular glutathione at high temperatures mirrors several
821 known features of intracellular glutathione at 30 $^{O}$C. Firstly, at 30 $^{O}$C, researchers have found that
822 yeasts maintain intracellular glutathione at high, millimolar concentrations and keep most of it in
823 its reduced form by recycling it in a NADPH-dependent manner (*34, 59*). This is because
824 glutathione in its reduced form, rather than its oxidized form, is essential for capturing reactive
825 oxygen species (*34*). Analogously, we found that, at high temperatures, log-phase wild-type yeasts
826 maintain ~77% of their extracellular glutathione in the reduced form and the rest in the oxidized
827 form (Supplementary Fig. 13). Secondly, we established that accumulating sufficiently large
828 amounts of extracellular glutathione is crucial to yeasts' survival at high temperatures, just as their
829 survival depends on building up of intracellular glutathione. Specifically, high temperatures cause
830 yeasts to respire, which in turn causes the mitochondria to produce reactive oxygen species (*33*).
831 Thus, it makes sense that - as previous studies established - high temperatures cause yeasts to
832 produce high amounts of intracellular glutathione to survive oxidative stress (*32*). Our work
833 extends this finding by revealing that the budding yeast must export their intracellular glutathione
834 in order to replicate and survive at high temperatures. Moreover, our work establishes that
835 accumulating extracellular glutathione, even by the alive cells in the no-growth-phase populations,
836 extends the population's lifespan in a manner that depends on the population's history - namely,
837 how many cells the population started with and how the extracellular glutathione has been
838 accumulating over time. In this way, glutathione extends population's lifespan in a manner that
839 depends on the population's history. Even if the population currently has very few alive cells
840 remaining, these cells benefit from the extracellular glutathione that the past generations of cells
841 had secreted and accumulated before they died. Given glutathione's utility and secretion in other
842 organisms - for example, human lung epithelial cells secrete it when they are exposed to asbestos
843 (*60*) - future studies may show that extracellular glutathione mediates cooperative survival in other
844 organisms at high temperatures or for other stresses.
845
846

**Why cooperate instead of autonomously combatting heat-induced damages?**

A natural question is why yeasts secrete glutathione at high temperatures to cooperatively survive instead of each yeast cell autonomously combating heat shocks by intracellularly keeping all the glutathione for itself. A major advantage of secreting glutathione is that glutathione can extracellularly accumulate, thereby allowing cells to help their future generations of cells to replicate, long after the current generation of cells have died off. Indeed, our model shows that it is the accumulation of extracellular glutathione that is responsible for a population to decelerate and eventually stop its approach to extinction.

**Relation to dynamical systems theory**

The term, fold-bifurcation, comes from dynamical systems theory – a branch of mathematics that focuses on explaining all possible ways that a set of mathematical variables can change over time and the qualitative features that describe them, often by invoking geometric depictions such as phase diagrams. This theory can describe many biological systems, from the classical predator-prey systems (*61-63*) to – as recently verified experimentally - microbial populations that are on the verge of extinction due to environmental perturbations (*64-68*). In the phase diagram that we uncovered (Fig. 2d), the shape of the phase boundaries that converge at the fold-bifurcation point mathematically resembles the shape of phase boundaries that merge into a fold-bifurcation point in another phase diagram - one that describes a yeast population for which one simulates the rate of cell deaths by periodically flushing out the liquid culture of cells and for which the replicability of cells depends on the yeasts collectively breaking down extracellular sucrose into glucose that they can import (*64*). A yeast population mathematically behaves in similar ways in both systems. In particular, both systems exhibit “critical slowing down” (*64,69,70*) which, in our case, manifests as the yeast-population’s initial half-life being infinite at the fold-bifurcation point (Fig. 3d) - this is a direct consequence the phase diagram having a fold-bifurcation point (Fig. 2d) according to dynamical systems theory (*69*). Crucially, the resemblance between the two dynamical systems – yeasts at high temperatures and yeasts that collective breakdown sucrose – arises because cells cooperate in both systems. In such populations of cells whose survival depend on cooperating amongst them, one typically observes minimal population-density that is required for survival. By providing another example of cooperation among microbes, our work thus adds to the on-going efforts by researchers to experimentally map phase diagrams that depict how populations of cooperating microbes behave when they are on the verge of going extinct (*64-68*).

881 **Summary and outlook**

882 With budding yeasts, we have shown that cells can avoid extinctions at high temperatures by
883 helping each other and their future generations replicate. As a consequence of this cooperative
884 behavior, a temperature which is unlivable for one population of cells can be habitable for another
885 population of cells of the same species, depending on whether there are sufficiently many cells
886 that are cooperating to keep the population alive. Specifically, we have shown that yeasts at high
887 temperatures export glutathione and that the extracellularly accumulating glutathione, in turn,
888 increases the probability of a cell replicating. By accumulating extracellular glutathione, yeasts
889 also aid their future generations of cells to replicate, long after their own deaths. Conversely, yeast
890 populations cannot grow if we remove extracellular glutathione. Thus, extracellular glutathione -
891 in fact, exporting of glutathione - is both necessary and sufficient for yeasts to grow at high
892 temperatures. Taken together, our experiments and mathematical model replaces the
893 conventional picture in which whether a yeast survives a high temperature or not depends on
894 whether it can autonomously use its heat-shock response system to combat heat-induced
895 damages. A surprising consequence of our model, which recapitulates all the main features of the
896 experimental data, is that yeasts can, in fact, replicate - albeit with a vanishingly low probability -
897 at extremely high temperatures (e.g., 45 $^{O}$C) for which the population cannot grow (due to only the
898 no-growth phase existing in the population-level phase diagram) (Supplementary Fig. 14).
899 Experimentally, we could indeed increase, by nearly one degree Celsius, the maximum
900 temperature at which yeast populations can grow to a carrying capacity by supplementing the
901 growth media with glutathione (Fig. 6e). Previous studies examined cell growth (*71-74*), gene
902 regulations (*75,76*), and metabolite exchanges among microbes (*77-80*) for various species at
903 their conventionally-defined habitable temperatures (i.e., habitability of temperature defined by a
904 cell-autonomous view). Our work encourages re-examining these features at high temperatures
905 that were previously dismissed as unlivable but which may, in fact, be habitable for sufficiently
906 large populations due to the cells cooperating in ways that may have been overlooked.

907

908 A common explanation for why cells cannot replicate at high temperatures has been that
909 a few, yet essential, proteins unfold at these temperatures and that the cells' autonomous heat-
910 shock response system fails to deal with these unfolded proteins. While crucial proteins do unfold
911 at certain high temperatures in budding yeast (*6*), our work shows that the reason for yeasts failing
912 to replicate at high temperatures is more intricate. For one, we find that if there is a sufficient
913 amount of extracellular glutathione then budding yeasts can, in fact, replicate at extremely high
914 temperatures (i.e., above 41 $^{O}$C) for which the essential proteins for yeast have been reported to

915 be unstable (Fig. 6e and Supplementary Fig. 14). Secondly, our work establishes that glutathione's
916 extracellular action as an antioxidant determines the replicability of yeasts and yeast population's
917 longevity at high temperatures. Simply put, yeasts fail to replicate at high temperatures because
918 there are not enough of them working together to help each other replicate and not enough of
919 them leaving behind extracellular glutathione to help their future generations of cells replicate.
920
921 From a standpoint of physics, we can interpret the random-growth phase in the phase
922 diagram as a boundary formed by a co-existence of the deterministic growth and no-growth
923 phases. A population that is at the endpoint of this boundary at the fold-bifurcation point can
924 indefinitely maintain a steady number of cells without either growing or becoming extinct - its
925 population density can fluctuate but does not change over time on average. The fold-bifurcation
926 point and the number of surviving cells decreasing over time as a heavy-tailed function for
927 populations that are near the fold-bifurcation point in the phase diagram, remind us of the power-
928 law functions that often describe the behaviors of non-living systems at critical points which are
929 associated with phase transitions (*17-19*). By exploiting these resemblances and using our data
930 and model, a future study might further advance theories of non-equilibrium phase transitions (*81*)
931 that pertain to biologically realistic, self-replicating systems that constantly drive and keep
932 themselves out of equilibrium by using thermal energy. Relatedly, a chronologically ageing
933 population of yeasts may also have the number of survivors decreasing over time as a heavy-
934 tailed function (*82*), like the number of survivors in non-growing populations incubated at high
935 temperatures. Thus, an improved understanding of how thermal energy reliably drives self-
936 replication of cells may provide insights into the dynamics of cellular ageing as well. Furthermore,
937 understanding how cells can work together to collectively combat extreme temperatures, as we
938 have done here, may suggest intervention mechanisms to combat climate change as well as
939 advance our understanding of how temperature and climate change can impact unicellular life and
940 multicellular communities.

941 ## Methods

942

943 **Growth media and strains.** The "wild-type", haploid yeast strain that we used is from Euroscarf
944 with the official strain name "20000A". It is isogenic to another laboratory-standard haploid yeast
945 "W303a", and has the following genotype: *MATa*; *his3-11_15; leu2-3_112; ura3-1; trp1Δ2; ade2-*
946 *1; can1-100*. We built the two strains that constitutively expressed GFP by first using PCR to insert
947 a functional *ADE2* gene into the locus of the defective *ADE2* gene in the wild-type strain, by a
948 homologous recombination, so that the red pigments that would have accumulated without the
949 *ADE2* insertion no longer existed (i.e., the strain can now synthesize adenine). We could thus
950 detect their GFP fluorescence without interferences from the red pigments. After replacing the
951 defective *ADE2* locus with a functional *ADE2*, we constructed the 1x-GFP and 100x-GFP strains
952 (see GFP-expression levels in Fig. S4a) by integrating a single-copy of an appropriate, linearized
953 yeast-integrating plasmid at the *HIS3* locus on the chromosome. Specifically, the 1x-GFP strain
954 had its GFP expression controlled by the constitutive promoter of yeast's *KEX2* gene (621 bases
955 upstream of its ORF) which was on a yeast-integration plasmid (*83*) that constitutively expressed
956 *HIS3* (from *C. glabrata*) and integrated into the non-functional *HIS3*-locus of the wild-type strain
957 by a homologous recombination. The 100x-GFP strain had its GFP expression controlled by a
958 strong constitutive promoter pGPD1 (*83*) which was on the same plasmid as the one for the 1x-
959 GFP strain except that the *KEX2* promoter was swapped with the *GPD1* promoter. We cultured
960 all yeasts in defined, minimal media that consisted of (all from Formedium): Yeast Nitrogen Base
961 (YNB) media, Complete Supplement Mixture (CSM) that contained all the essential amino acids
962 and vitamins, and glucose at a saturating concentration (2% = 2 g per 100 mL). The agar pads,
963 which we used for growing yeast colonies, contained 2%-agar (VWR Chemicals), Yeast Extract
964 and Peptone (YEP) (Melford Biolaboratories Ltd.), and a 2%-glucose.

965

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

974 twenty-four 10-mL-wells (Whatman: "24-well x 10mL assay collection & analysis microplate"). This
975 ensured that we had 8 identical replicate cultures for each initial population-density (e.g., in Figure
976 2a-c). We sealed each brick with a breathable film (Diversified Biotech: Breathe-Easy), covered it
977 with a custom-made Styrofoam-cap for insulation, and incubated it in a compressor-cooled, high-
978 precision thermostatic incubators (Memmert ICP260) that stably maintained their target
979 temperature throughout the course of our growth-experiments, with a typical standard deviation of
980 0.017 $^{O}$C over time (deviation measured over several days - see Supplementary Fig. 2).
981 Throughout the incubation, the cultures in the brick were constantly shaken at 400 rpm on a plate
982 shaker (Eppendorf MixMate) that we kept in the incubator. To measure their population densities,
983 we took a small aliquot (typically 50 μL) from each well, diluted it with PBS (Fisher Bioreagents)
984 into a 96-well plate (Sarstedt, Cat. #9020411), and then flowed it through the flow cytometer which
985 gave us the # of cells/mL. We determined the growth rates by measuring the maximum slope of
986 the log-population density after their initial, transient growths.

987

988 **Flow cytometry.** The flow cytometer that we used was a BD FACSCelesta with a High-
989 Throughput Sampler and lasers with the following wave lengths: 405 nm (violet), 488 nm (blue),
990 and 561 nm (yellow/green). We calibrated the FSC and SSC gates to detect only yeast cells (FSC-
991 PMT=681V, SSC-PMT=264V, GFP-PMT=485V, mCherry-PMT=498V. As a control, flowing PBS
992 yielded no detected events). The number of cells/mL that we plotted in our growth experiments is
993 proportional to the number of events (yeast cells) that the flow cytometer measured in an aliquot
994 of cells with a defined volume. We measured the GFP fluorescence with a FIT-C channel and the
995 "red cells" (Figure S6) with a mCherry channel. We analysed the flow cytometer data with a custom
996 MATLAB script (MathWorks).

997

998 **Measuring number of surviving cells.** For Figures 3a-b and Figures S7-S8, we prepared 250-
999 mL cultures of wild-type cells in 500-mL Erlenmeyer flasks. We placed a constantly spinning
1000 magnetic stir-bar at the bottom of the flasks and placed each flask on top of spinning magnets
1001 (Labnet Accuplate - at 220 rpm) inside the thermostatic incubators (Memmert ICP260) that we set
1002 at desired high-temperatures. For Figure 3d, we prepared a brick with cultures as described in the
1003 “Growth Experiments” section in order to have multiple replicate populations and to compare the
1004 different population densities. For every time point in Fig. 3 and Supplementary Figs. 7-8, we
1005 ensured that these populations were not growing (i.e., all populations were in the no-growth phase

1006 after a transient growth) by using the flow cytometer to measure their population-densities over
1007 time to verify that their population-densities indeed remained constant over time. For the first 48
1008 hours of incubation, we measured the number of Colony Forming Units (CFUs) by taking out a
1009 small-volume aliquot from the liquid cultures at high temperatures and distributed droplets from a
1010 serial dilution of the aliquot across an agar pad (2% glucose with YEP) that we then incubated in
1011 30 $^{O}$C for several days until (no) colonies appeared. When there were few surviving cells per mL
1012 - especially for the last time points in each experiment - we determined, in parallel to the plating
1013 method, the number of CFUs by transferring an appropriate volume of the liquid cultures from the
1014 incubator to an Erlenmeyer flask and then diluting it with the same volume of fresh minimal media.
1015 We sealed this flask with a breathable film (Diversified Biotech: Breathe-Easy) and then left it still
1016 without stirring, on a benchtop at ~24-30 $^{O}$C - we checked that slightly lower temperatures (e.g.,
1017 room temperatures) did not affect colony-forming abilities - which allowed any surviving cells to
1018 settle down to the bottom of the flask and form colonies. We counted the number of colonies at
1019 the bottom of the flask - this is the value that we plotted as the last time points in each experiment
1020 (Fig. 3 and Supplementary Figs. 7-8).

1021

1022 **Cell-transfer experiments.** We incubated a 24-well brick that contained liquid cultures, each of
1023 which were in a deterministic-growth phase, at a desired temperature (e.g., 10,000 cells/mL at
1024 39.2 $^{O}$C). We incubated the brick containing these liquid cultures in the thermostatic incubators
1025 (Memmert ICP260) as described in the "Growth experiments" section. About 48 hours after the
1026 incubation, we took aliquots from the cultures that were growing in mid-log phase (as checked by
1027 flow cytometry) and then diluted each of them into fresh 5-mL minimal media that were in 24-well
1028 bricks so that these newly created populations were in the no-growth phase at the same
1029 temperature as the original population that they came from (400 cells/mL at 39.2 $^{O}$C). We sealed
1030 the 24-well brick with a breathable film (Diversified Biotech: Breathe-Easy) and then incubated
1031 them at the same temperature as the original population. We performed the growth experiments
1032 with these new populations as described in the "Growth experiments" section.

1033

1034 **Medium-transfer experiments.** Details are also in Supplementary Fig. 10. At a given
1035 temperature, we first grew populations in the deterministic-growth phase (e.g., initial population-
1036 density of 30,000 cells/mL at 39.2 $^{O}$C). We used a flow cytometer to measure their growing
1037 population-densities at different times so that we knew in which part of deterministic growth they

1038 were in (e.g., mid-log phase). We then transferred each liquid culture to a 50-mL tube (Sarstedt)
1039 and centrifuged it so that the cells formed a pellet at the bottom of the tube. We then took the
1040 resulting supernatant, without the cell pellet, and flowed it through a filter paper with 200-nm-
1041 diameter pores (VWR: 150-mL Filter Upper Cup) to remove any residual cells from the
1042 supernatant. After filtering, we flowed an aliquot of the filtered media through a flow cytometer to
1043 verify that there were no cells left in the filtered media. We incubated fresh cells into these filtered
1044 media (instead of into fresh minimal media) and proceeded with a growth experiment at a desired
1045 temperature as described in the "Growth experiments" section.

1046

1047 **Measuring the depletion of extracellular nutrients.** Details are also in Supplementary Fig. 11.
1048 We prepared various growth media by diluting the minimal media (SC media) by various amounts
1049 with water. These diluted SC-media were each supplemented with a 2% glucose. Next, we
1050 incubated fresh cells in these diluted SC-media at the desired temperature (e.g. 39.2 $^{O}$C) as
1051 described in the “Growth experiments” section. We compared populations of cells that initially had
1052 400 cells/mL (this corresponds to a no-growth phase, see Fig. 2d) with populations that initially
1053 had 10,000 cells/mL (this corresponds to a deterministic-growth phase, see Fig. 2d) in order to
1054 confirm that cells were still able to grow in these media. Similarly, we also varied the amounts of
1055 glucose that we supplemented to SC-media.
1056

1057 **Measuring concentration of extracellular glutathione.** To quantify the concentration of
1058 extracellular glutathione, cells were removed from their liquid media by flowing the liquid cultures
1059 that contained cells through a 0.45–μm pore filter (VWR, cellulose-acetate membrane). To ensure
1060 and verify that there were no cells remaining in the filtered media, we flowed the filtered media
1061 through a flow cytometer. The flow cytometer indeed did not detect any cells in the filtered media.
1062 We measured concentrations of glutathione in the filtered media as described in the
1063 manufacturers' protocol (38185 quantification kit for oxidized and reduced glutathione, 200 tests).
1064 We used "BMG Labtech Spectrostar Nano" to measure the optical absorbance at 415-nm. As a
1065 background subtraction (blank) for all absorbance measurements, we subtracted the absorbance
1066 that we obtained by applying the assay to fresh minimal medium which does not have any
1067 glutathione (the background absorbance could come from, for example, cysteine in the minimal
1068 media). We subsequently determined the concentrations of extracellular glutathione by using a

1069 calibration curve that we constructed by measuring the absorbance at 415-nm for known amounts
1070 of glutathione that we added by hand into a buffer provided by the manufacturer.

1071

1072 **RNA-seq.** For each temperature that we studied, we collected cells in 50-mL tubes and spun them
1073 in a pre-cooled centrifuge. We then extracted RNA from each cell-pellet with RiboPure Yeast Kit
1074 (Ambion, Life Technologies) as described by its protocol. Next, we prepared the cDNA library with
1075 the 3' mRNA-Seq library preparation kit (Quant-Seq, Lexogen) as described by its protocol.
1076 Afterwards, we loaded the cDNA library on an Illumina MiSeq with the MiSeq Reagent Kit c2
1077 (Illumina) as described by its protocol. We analysed the resulting RNA-Seq data as previously
1078 described (*84*): We performed the read alignment with TopHat, read assembly with Cufflinks, and
1079 analyses of differential gene-expressions with Cuffdiff. We used the reference genome for *S.*
1080 *cerevisiae* from ensembl. We categorized the genes by the Gene Ontologies with AmiGO2 and
1081 manually checked them with the Saccharomyces Genome Database (SGD).

1082

1083 **Glutathione masking experiment.** We incubated a brick of liquid cultures that were in the
1084 deterministic-growth phase (20.000 cells/mL) at 39.2 $^{O}$C. After some time (e.g., 8.5 hours
1085 afterwards), we added 750μM of 1-Methyl-2-vinylpyridinium (M2VP, Sigma-Aldrich Cat. No.
1086 69701), which is a thiol scavenging agent that rapidly masks reduced glutathione (*42*) and
1087 proceeded with the experiment as described in the "Growth experiments" section. Identical
1088 replicate cultures, that did not receive the M2VP, were used as a reference.

1089

1090 **Mutant yeasts.** We constructed mutant strains that could not synthesize glutathione or could not
1091 import or export glutathione. Primers were designed with a 50-60bp sequence that was either
1092 homologous to the 50-60bp that is upstream of the desired gene's start codon or downstream of
1093 the desired gene's stop codon. These primers were used to amplify a selection marker by PCR,
1094 resulting in a PCR product that contained a selection marker and whose ends were homologous
1095 to the flanking regions of the gene to be knocked out. The wild-type strain (W303) was grown
1096 overnight in a 5-mL YPD in a rotator (40 rpm) at 30 $^{O}$C, and subsequently transformed with the
1097 PCR fragment using standard methods of yeast cloning. The biosynthesis mutant (*GSH1Δ*-strain)
1098 was constructed by removing the *GSH1* gene from W303. The import mutant (*HGT1Δ*-strain) was
1099 constructed by removing the *HGT1* gene. The export mutant (*GEX1,2Δ-ADP1Δ*-strain) was

1100 constructed by removing, sequentially, the *GEX1* gene, then the *GEX2* gene, and then the *ADP1*
1101 gene. The resulting transformants were grown on YPD selection plates, and knockouts were
1102 verified by PCR.

1103

1104 **Measuring integrity of cell membrane.** Cells of the *GEX1,2Δ-ADP1Δ*-strain were incubated in
1105 liquid media at 39.2 $^{O}$C (3,000-10,000 cells/mL; corresponds to a random-growth phase). We took
1106 aliquots of these cultures and then stained them with 1 μg/mL of propidium iodide (Thermo Fisher
1107 Cat. No. P3566). We then flowed these stained aliquots through a flow cytometer. The flow
1108 cytometer measured the number of cells that were unstained by the propidium iodide (i.e., cells
1109 whose membranes were intact).

1110

1111 **Mathematical model.** Derivations of equations, a detailed description of the mathematical model,
1112 and the parameter values used for simulations are in the Supplemental text.

1113

## Supplemental Information:

- Supplementary Figures 1-17
- Supplementary Text: Detailed description of the mathematical model


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## Author contributions:

H.Y. initiated this research and performed the initial experiments. D.S.L.T. subsequently developed the project with guidance from H.Y. D.S.L.T. performed 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.

**DATA AVAILABILITY**

The authors declare that all data supporting the findings of this study are available within the paper and its supplementary information files. RNA-Seq data is available at NCBI GEO (accession #137151). The data that support the findings of this study are available from the corresponding author upon reasonable request.

## References:


1. M. T. Madigan, J. M. Martinko, D. A. Stahl, & D. Clark. *Brock biology of microorganisms* (13th Ed.) (Pearson, 2011), pp. 162-163.

2. R. Milo, & R. Phillips. *Cell biology by the numbers* (1st ed.) (Garland Science, 2015), http://book.bionumbers.org/how-does-temperature-affect-rates-and-affinities/.

3. L. Bruslind. *Microbiology*. (Open Oregon State University, 2019), http://library.open.oregonstate.edu/microbiology/chapter/environmental-factors/

4. P. M. Doran. *Bioprocess Engineering Principles* (2nd ed.) (Academic Press, 2012), pp. 653-655.

5. K. Ghosh, & K. Dill. Cellular proteomes have broad distributions of protein stability. *Biophys J* **99**:3996-4002 (2010).

6. P. Leuenberger *et al.,* Cell-wide analysis of protein thermal unfolding reveals determinants of thermostability. *Science* **355,** eaai7825 (2017).

7. J. Verghese, J. Abrams, Y. Wang, & K. A. Morano. Biology of the heat shock response and protein chaperones: Budding yeast (*Saccharomyces cerevisiae*) as a model system. *Microbiol. Mol. Biol. Rev.* **76**:115-158 (2012).

8. K. Richter, M. Haslbeck, & J. Buchner. The heat shock response: life on the verge of death. *Mol. Cell* **40**:253-266 (2010).

9. M. B. Miller, & B. L. Bassler. Quorum sensing in bacteria. *Annu. Rev. Microbiol* **55**:165-199 (2001).

10. J. Gore, H. Youk, & A. van Oudenaarden. Snowdrift game dynamics and facultative cheating in yeast. *Nature* **459**:253-256 (2009).

11. J. H. Koschwanez, K. R. Foster, & A. W. Murray. Sucrose utilization in budding yeast as a model for the origin of undifferentiated multicellularity. *PLoS Biol.* **9**:e1001122 (2011)

12. J. H. Koschwanez, K. R. Foster, & A. W. Murray. Improved used of a public good selects for the evolution of undifferentiated multicellularity. *eLife* **2**:e00367 (2013).

13. C. Ratzke, J. Denk, & J. Gore. Ecological suicide in microbes. *Nat. Ecol. Evol.* **2**:867-872 (2018).

14. J. Postmus *et al.*, Quantitative analysis of the high temperature-induced glycolytic flux increase in Saccharomyces cerevisiae reveals dominant metabolic regulation. *J. Biol. Chem.* **283**:23524-23532 (2008).

15. R. M. Walsh, & P. A. Martin. Growth of saccharomyces cerevisiae and saccharomyces uvarum in a temperature gradient incubator. *J. Inst. Brew.* **83**:169-172 (1977).

16. D. A. Ratkowsky, R. K. Lowry, T. A. McMeekin, A. N. Stokes, & R. E. Chandler. Model for bacterial culture growth rate throughout the entire biokinetic temperature range. *J. Bacteriol.* **154**:1222-1226 (1983).

17. A. A. Hyman, C. A. Weber, F. Jülicher. Liquid-liquid phase separation in biology. *Annu. Rev. Cell. Dev. Biol.* **30**, 39-58 (2014).

18. Y. Shin, C. P. Brangwynne. Liquid phase condensation in cell physiology and disease. *Science* **357,** eaaf4382 (2017).

19. M. Kardar, *Statistical physics of fields* (1st ed.) (Cambridge University Press, 2007).

20. A. Goldbeter, D. E. Koshland Jr. Ultrasensitivity in biochemical systems controlled by covalent modification. Interplay between zero-order and multistep effects. *J. Biol. Chem.* **259**, 14441-14447 (1984).

21. G. J. Melen, S. Levy, N. Barkai, B. Z. Shilo. Threshold responses to morphogen gradients by zero-order ultrasensitivity. *Mol. Syst. Biol.* **1**:2005.0028 (2005).

22. E. Dekel, U. Alon. Optimality and evolutionary tuning of the expression level of a protein. *Nature* **436,** 588-592 (2005).

1205
1206 23. M. Scott, & T. Hwa. Bacterial growth laws and their applications. *Curr Opin Biotechnol.*
1207 **22**:559-565 (2011).
1208
1209 24. V. Bharathi *et al.* Use of *ade1* and *ade2* mutations for development of a versatile red/white
1210 colour assay of amyloid-induced oxidative stress in *Saccharomyces cerevisiae*. *Yeast* **33,**
1211 607-620 (2016).
1212
1213 25. N. Q. Balaban. Persistence: mechanisms for triggering and enhancing phenotypic variability.
1214 *Curr. Opin. Genet. Dev.* **21,** 768-775 (2011).
1215
1216 26. H. C. Causton *et al.* Remodeling of yeast genome expression in response to environmental
1217 changes. *Mol. Biol. Cell* **12**:323-337 (2001).
1218
1219 27. A. P. Gasch, *et al.* Genomic expression programs in the response of yeast cells to
1220 environmental changes. *Mol. Biol. Cell* **11**:4241-4257 (2000).
1221
1222 28. F. M. Yakes, & B. van Houten. Mitochondrial DNA damage is more extensive and persists
1223 longer than nuclear DNA damage in human cells following oxidative stress. *Proc. Natl. Acad.*
1224 *Sci. U.S.A.* **94**:514-519 (1997).
1225
1226 29. E. Cabiscol, E. Piulats, P. Echave, E. herrero, & J. Ros. Oxidative stress promotes specific
1227 protein damage in Saccharomyces cerevisiae. *J. Biol. Chem.* **275**:27393-27398 (2000).
1228
1229 30. T. Bilinski, J. Litwinska, M. Blaszczynski, & A. Bajus. SOD deficiency and the toxicity of the
1230 products of autoxidation of polyunsaturated fatty acids in yeast. *Biochim. Biophys. Acta.*
1231 **1001**:102-106 (1989).
1232
1233 31. A. Ayer, C. W. Gourlay, & I. W. Dawes. Cellular redox homeostasis, reactive oxygen species
1234 and replicative ageing in Saccharomyces cerevisiae. *FEMS Yeast Res.* **14**:60-72 (2014).
1235
1236 32. K. Sugiyama, A. Kawamura, S. Izawa, & Y. Inoue. Role of glutathione in heat-shock-induced
1237 cell death of Saccharomyces cerevisiae. *Biochem. J.* **352**:71-78 (2000).
1238

1239 33. K. Sugiyama, S. Izawa, & Y. Inoue. The Yap1p-dependent induction of glutathione synthesis
1240 in heat shock response of Saccharomyces cerevisiae. *J. Biol. Chem.* **275**: 15535-15540
1241 (2000).
1242
1243 34. D. J. Jamieson. Oxidative stress responses of the yeast Saccharomyces cerevisiae. *Yeast*
1244 **14**:1511-1527 (1998).
1245
1246 35. G. G. Perrone, C. M. Grant, & I. W. Dawes. Genetic and environmental factors influencing
1247 glutathione homeostasis in Saccharomyces cerevisiae. *Mol. Biol. Cell* **16**:218-230 (2005).
1248
1249 36. M. Thorsen *et al.* Glutathione serves an extracellular defence function to decrease arsenite
1250 accumulation and toxicity in yeast. *Mol. Microbiol.* **84**:1177-1188 (2012).
1251
1252 37. C. Kumar *et al.* Glutathione revisited: a vital function in iron metabolism and ancillary role in
1253 thiol-redox control. *EMBO J.* **30:**2044-2056 (2011).
1254
1255 38. B. Zechmann *et al.* Subcellular distribution of glutathione and its dynamic changes under
1256 oxidative stress in the yeast Saccharomyces cerevisiae. *FEMS Yeast Res* **11**:631-642 (2011).
1257
1258 39. M. C. Spickett, N. Smirnoff, & R. A. Pitt. The biosynthesis of erythroascorbate in
1259 Saccharomyces cerevisiae and its role as an antioxidant. *Free Radic. Biol. Med.* **28**:183-192
1260 (2000).
1261
1262 40. J. Coates *et al.* Antibiotic-induced population fluctuations and stochastic clearance of bacteria.
1263 *eLife*, **7**:e32976 (2018).
1264
1265 41. D. Giustarini *et al.* Pitfalls in the analysis of the physiological antioxidant glutathione (GSH)
1266 and its disulphide (GSSG) in biological samples: An elephant in the room. *J. Chromatogr B*
1267 *Analyt Technol Biomed Life Sci* **1019:**21-28 (2016).
1268
1269 42. A. R. Araujo, M. L. Saraiva, & J. L. Lima. Determination of total and oxidized glutathione in
1270 human whole blood with a sequential injection analysis system. *Talanta* **74**:1511-1519 (2008).
1271

1272 43. C. M. Grant, F. H. MacIver, & I. W. Dawes. Glutathione is an essential metabolite required for
1273 resistance to oxidative stress in the yeast Saccharomyces cerevisiae. *Curr Genet* **29**:511-515
1274 (1996).
1275
1276 44. A. Bourbouloux, P. Shahi, A. Chakladar, S. Delrot, & A. K. Bachhawat. Hgt1p, a high affinity
1277 glutathione transporter from the yeast Saccharomyces cerevisiae. *J. Biol Chem.*
1278 **275**:13259-13265 (2000).
1279
1280 45. M. Dhaoui *et al.* Gex1 is a yeast glutathione exchanger that interferes with pH and redox
1281 homeostasis. *Mol Biol Cell* **22:**2054-2067 (2011).
1282
1283 46. K. Kiriyama, K. Y. Hara, & A. Kondo. Extracellular glutathione fermentation using engineered
1284 Saccharomyces cerevisiae expressing a novel glutathione exporter. *Appl Microbiol Biotechnol*
1285 **96:**1021-1027 (2012).
1286
1287 47. C. Riccardi, & I. Nicoletti. Analysis of apoptosis by propidium iodide staining and flow
1288 cytometry. *Nat Prot* **1:**1458-1461 (2006).
1289
1290 48. J. Navarro *et al.* Changes in glutathione status and the antioxidant system in blood and in
1291 cancer cells associate with tumour growth in vivo. *Free Radic Biol. Med.* **26**:410-418 (1999).
1292
1293 49. J. B. Schulz, J. Lindenau, J. Seyfried, & J. Dichgans. Glutathione, oxidative stress and
1294 neurodegeneration. *Eur. J. Biochem.* **267**:4904-4911 (2000).
1295
1296 50. P. Maher. The effects of stress and aging on glutathione metabolism. *Ageing Res. Rev.* **4**:288-
1297 314 (2005).
1298
1299 51. Y. Honda *et al.* Efficacy of glutathione for the treatment of nonalcoholic fatty liver disease: an
1300 open-label, single-arm, multicenter, pilot study. *BMC Gastroenterol.* **17**:96 (2017).
1301
1302 52. J. F. Davidson, B. Whyte, P. H. Bissinger, & R. H. Schiestl. Oxidative stress is involved in
1303 heat-induced cell death in Saccharomyces cerevisiae. *Proc Natl Acad Sci USA* **93:**5116-5121
1304 (1996).
1305

1306 53. M. B. Toledano, C. Kumar, N. Le Moan, D. Spector, & F. Tacnet. The system biology of thil
1307 redox system in Escherichia coli and yeast: differential functions in oxidative stress, iron
1308 metabolism and DNA synthesis. *FEBS Lett* **581**:4549 (2007).
1309
1310 54. R. Zadzinski, J. Maszewski, & G. Bartosz. Transport of glutathione-S-conjugates in the yeast
1311 Saccharomyces cerevisae. *Cell Biol Int* **20**:325-330 (1996).
1312
1313 55. K. Polec-Pawlak *et al.* Investigation of Cd(II), Pb(II) and Cu(I) complexation by glutathione
1314 and its component amino acids by ESI-MS and size exclusion chromatography coupled to
1315 ICP-MS and ESI-MS. *Talanta* **72**:1564-1572 (2007).
1316
1317 56. M. T. Elskens, C. J. Jaspers, & M. J. Penninckx. Glutathione as an endogenous sulfur source
1318 in the yeast Saccharomyces cerevisiae. *J Gen Microbiol* **137**:637-644 (1991).
1319
1320 57. K. Mehdi, & M. J. Penninckx. An important role for glutathione and γ-glutamyltranspeptidase
1321 in the supply of growth requirements during nitrogen starvation of the yeast Saccharomyces
1322 cerevisiae. *Microbiology* **143**:1885-1889 (1997).
1323
1324 58. R. Green *et al.* Rapid evolution of an overt metabolic defect restores balanced growth. *BioRxiv*
1325 doi.org/10.1101/498543 (2018).
1326
1327 59. M. J. Penninckx. An overview on glutathione in Saccharomyces versus non-conventional
1328 yeasts. *FEMS Yeast Res.* **2**:295-305 (2002).
1329
1330 60. S. A. Golladay, S. H. Park, & A. E. Aust. Efflux of reduced glutathione after exposure of human
1331 lung epithelial cells to crocidolite asbestos. *Environ. Health Perspect.* **105**:1273-1277 (1997).
1332
1333
1334 61. A. J. Lotka. *Elements of physical biology*. (Williams and Wilkins Co., Baltimore, MD, 1925).
1335
1336 62. V. Volterra. Fluctuations in the abundance of a species considered mathematically. *Nature*
1337 **118**:558-560 (1926).
1338

63. R. Arditi, & L. R. Ginzburg. Coupling in predator-prey dynamics: ratio dependence. *J. Theor Biol* **139**:311-326 (1989).

64. L. Dai, D. Vorselen, K. Korolev, & J. Gore. Generic indicators for loss of resilience before a tipping point leading to population collapse. *Science* **336**:1175-1177 (2012).

65. L. Dai, K. S. Korolev, & J. Gore. Relation between stability and resilience determines the performance of early warning signals under different environmental drivers. *Proc Natl Acad Sci USA* **11:**10056-10061 (2015).

66. T. Artemova, Y. Gerardin, C. Dudley, N. M. Vega, & J. Gore. Isolated cell behavior drives the evolution of antibiotic resistance. *Mol Syst Biol* **11:**822 (2015).

67. E. A. Yurtsev, H. X. Chaio, M. S. Datta, T. Artemova, & J. Gore. Bacterial cheating drives the population dynamics of cooperative antibiotic resistance plasmids. *Mol Syst Biol* **9**:683 (2013).

68. L. Dai, K. S. Korolev, & J. Gore. Slower recovery in space before collapse of connected populations. *Nature* **496**:355-358 (2013).

69. S. H. Strogatz. *Nonlinear dynamics and chaos: with applications to physics, biology, chemistry, and engineering* (Westview, Boulder, CO, 1994).

70. M. Mojtahedi *et al.* Cell fate decision as high-dimensional critical state transition. *PLoS Biol* **14**:e2000640 (2016).

71. S. Iyer-Biswas *et al.,* Scaling laws governing stochastic growth and division of single bacterial cells. *Proc. Natl. Acad. Sci. USA* **111,** 15912-15917 (2014).

72. M. Scott, T. Hwa. Bacterial growth laws and their applications. *Curr. Opin. Biotechnol.* **22,** 559-565 (2011).

73. M. Kafri, E. Metzl-Raz, F. Jonas, N. Barkai. Rethinking cell growth models. *FEMS Yeast Research* **16,** fow081 (2016).

1373 74. A. R. Zomorrodi, D. Segrè. Genome-driven evolutionary game theory helps understand the
1374 rise of metabolic interdependencies in microbial communities. *Nat. Commun.* **8,** 1563 (2017).
1375
1376 75. J. Ihmels, R. Levy, N. Barkai. Principles of transcriptional control in the metabolic network of
1377 Saccharomyces cerevisiae. *Nature Biotechnol.* **22**, 86-92 (2004).
1378
1379 76. D. A. Charlebois, K. Hauser, S. Marshall, G. Balázsi. Multiscale effects of heating and cooling
1380 on genes and gene networks. *Proc. Natl. Acad. Sci. USA.* **115**, E10797-E10806 (2018).
1381
1382 77. J. E. Goldford *et al.* Emergent simplicity in microbial community assembly. *Science* **361**, 469-
1383 474 (2018).
1384
1385 78. J. Liu *et al.* Metabolic co-dependence gives rise to collective oscillations within biofilms.
1386 *Nature* **523**, 550-554 (2015).
1387
1388 79. A. Z. Rosenthal, Y. Qi, S. Hormoz, J. Park, S. H.-J. Li, M. B. Elowitz. Metabolic interactions
1389 between dynamic bacterial subpopulations. *eLife* **7,** e33099 (2018).
1390
1391 80. A. R. Pacheco, M. Moel, D. Segrè,. Costless metabolic secretions as drivers of interspecies
1392 interactions in microbial ecosystems. *Nat. Commun.* **10**, 103 (2019).
1393
1394 81. J. Garcia-Ojalvo, J. M. Sancho, L. Ramirez-Piscina. A nonequilibrium phase transition with
1395 colored noise. *Physics Letters A* **168**, 35-39 (1992).
1396
1397 82. L. Cao *et al.* Chronological lifespan in yeast is dependent on the accumulation of storage
1398 carbohydrates mediated by Yak1, Mck1, and Rim15 kinases. *PLoS Genet* **12**:e1006458
1399 (2016).
1400
1401 83. H. Youk, W. A. Lim. Secreting and sensing the same molecule allows cells to achieve versatile
1402 social behaviors. *Science* **343**,1242782 (2014).
1403
1404 84. C. Trapnell *et al*. Differential gene and transcript expression analysis of RNA-seq experiments
1405 with TopHat and Cufflinks. *Nat. Protoc.* **7**, 562-578 (2012).
1406

1407 ## Figure captions:

1408

1409 **Figure 1. Conventional, cell-autonomous view of temperature-dependent cell-replications.**
1410 (**a**) The conventional view states that cells autonomously replicate at "habitable temperatures"
1411 (blue region) and that at sufficiently high temperatures (i.e., "unlivable temperatures"), cells fail to
1412 replicate and can eventually die (red region). This view states that whether a cell can replicate or
1413 not at a given temperature depends on the cell's autonomous ability to use its heat-shock response
1414 system to sufficiently deal with various heat-induced damages.
1415 (**b**) Growth rate as a function of temperature for populations of wild-type yeast cells. Black data
1416 points in the blue region are for populations with sustained, exponential growth over time and
1417 white data points in the red region are for populations without sustained exponential growth. 39
1418 $^{O}$C is near a boundary of blue and red regions (also see Supplementary Fig. 1).
1419 (**c**) The conventional view (explained in (a)) applied to budding yeast, based on the data in (b).
1420 (**d**) Question that we investigated in our study: can cells (budding yeasts) cooperatively combat
1421 rising temperatures so that they can turn a temperature that is unlivable for a few cells (e.g., 40
1422 $^{O}$C shown in (c)) into a habitable temperature if there are sufficiently many cells working together
1423 to fight off extinction?

1424

1425

1426 **Figure 2. Population density determines replicability of cells and habitability of each**
1427 **temperature.**
1428 (**a-c**) Population density (number of cells / mL) measured over time with a flow cytometer for
1429 populations of wild-type yeast of differing initial population-densities at (**a**) a conventionally-defined
1430 habitable temperature (38.4 $^{O}$C), (**b**) near the edge of conventionally-defined habitable and
1431 unlivable temperatures (39.2 $^{O}$C), (**c**) and at a conventionally-defined unlivable temperature (40.3
1432 $^{O}$C). Figure 1b sets the conventional definition of temperature's habitability. For (a-b): Each color
1433 shows eight populations that start with the same density. Red curves show no growths beyond
1434 initial transient growths (i.e., "no growth"). Green curves show unpredictable growths (i.e., "random
1435 growth"). Blue curves show deterministic, exponential growths whereby all populations identically
1436 grow (i.e., "deterministic growth"). For (c): Each color shows four populations with the same initial
1437 population-density. All colors except the red show growths by ~10-fold.
1438 (**d**) Phase diagram constructed from measurements. Colors represent the behaviors mentioned
1439 in (b) - blue region marks deterministic growth, green region marks random-growth, red region
1440 marks no-growth, and grey region marks populations not growing as they have more cells than

1441 the carrying capacity. Each triangle represents an experiment of the type shown in (a-c),
1442 performed at a specific initial population-density and temperature (Supplementary Figs. 3-4). A
1443 triangle's color represents the phase exhibited by populations. See caption of Figure S4 for a
1444 detailed description of how we deduced the phase boundaries from the measurements.
1445 (**e**) Growth rates of populations in the no-growth phase (red), random-growth phase (green) and
1446 deterministic-growth phase (blue) as a function of temperature (error bars are s.e.m., $n$ = 6 or
1447 more for temperatures below 40 $^{O}$C, and $n$ = 3 for temperatures above 40 $^{O}$C). Grey data are from
1448 Figure 1b.
1449 (**f**) Phase diagrams constructed for engineered yeast strains that constitutively express *GFP* at
1450 the indicated levels (1x and 100x - see Supplementary Fig. 5). Triangles indicate experimental
1451 data (samples of growth data represented by these triangles are in Supplementary Fig. 5). Since
1452 the wild-type strain's genetic background is slightly different from that of GFP-expressing strains,
1453 compare the 1x-GFP strain with the 100x-GFP strain only.
1454
1455
1456 **Figure 3. Cell do not autonomously die and their deaths depend on initial population-**
1457 **density at high temperatures: populations of higher initial densities decelerate and halt**
1458 **their approach to extinction more rapidly.**
1459 (**a**) We determined the number of survivors per mL over time in a non-growing, no-growth-phase
1460 population at 41.0 $^{O}$C by transferring an aliquot of the liquid culture to an agar pad at different time
1461 points, incubating the agar pad at 30 $^{O}$C, and then counting the number of colony forming units
1462 ("# of survivors/mL") (see Supplementary Fig. 7). Circles represent measured numbers of
1463 survivors per mL. Brown line is an exponentially decaying function fitted to the first three data
1464 points (between 10 and 40 hours). Blue curve is a power-law function fitted to all the data points.
1465 Also see Supplementary Fig. 7.
1466 (**b**) Number of survivors per mL for three populations of differing initial population-densities – all
1467 of them in the no-growth phase at 41.0 $^{O}$C - measured as in (a). Initial population-densities, after
1468 transient growths, are 92,000 cells / mL (purple), 231,000 cells / mL (orange), and 312,000 cells /
1469 mL (blue). For each color, a dashed line is an exponentially decreasing function that we fitted to
1470 the first three timepoints (i.e., deaths that occur during the first day of incubation after a transient
1471 growth). Also see Supplementary Fig. 7.
1472 (**c**) Cartoon illustrating the conventional view which states that cells autonomously die and that
1473 every cell has the same probability of dying per unit time. This means that the population half-life

1474 (i.e., time taken for a population density to be halved) is independent of the initial population-
1475 density for every temperature. Different colors represent different temperatures as indicated.
1476 (**d**) Population half-life, plotted here as a function of initial population-density, is defined as the
1477 time taken for a population density to be halved, based on fitting an exponentially decreasing
1478 function to the number of survivors / mL measured during the first 24 hours of incubation, which
1479 in turn occurs after a ~20-hours of transient growth that is due to the cells coming from 30 $^{O}$C and
1480 adjusting to the new temperature. Hence, we measured the number of survivors at approximately
1481 20 hrs, 28 hrs, 36 hrs, and 44 hrs after we incubated the populations at the desired high
1482 temperature (these times are measured after the ~20 hours of transient growths ended). Usually,
1483 the 44-hr data point already deviated from exponential decay and thus we usually omitted them
1484 from the exponential fits. Shown here are the half-lives of populations at 39.2 $^{O}$C, 40 $^{O}$C, 40.3 $^{O}$C,
1485 40.8 $^{O}$C, 42 $^{O}$C, and 43 $^{O}$C, each in a different color as indicated. Error bars: s.e.m. ($n$ = 3 for all
1486 data points). Circles represent populations in the no-growth phase. The two squares (at 39.2 $^{O}$C
1487 and 40 $^{O}$C) represent populations that grew due to having sufficient densities to trigger their own
1488 growths, in accordance with the phase diagram (Fig. 2d).
1489
1490
1491 **Figure 4. Cells secrete and extracellularly accumulate glutathione to help each other and**
1492 **future generations of cells replicate at high temperatures.**
1493 (**a**) Schematic description of experiments in (b) and (c) to distinguish two classes of mechanisms.
1494 Labelled "1': transfer growing cells into a fresh medium (to determine if intracellular factors dictate
1495 population growth). Labelled "2": take out only the liquid medium of a growing population and then
1496 incubate fresh cells in it (to determine if extracellular factors dictate population growth).
1497 (**b**) Experiment labelled "1" in (a) at 39.2 $^{O}$C. Blue curves show populations of wild-type yeasts
1498 (initially ~10.000 cells/mL) that start to exponentially grow. Cells of these populations in
1499 exponential growth, marked by the boxed blue data point, were transferred without their growth
1500 medium into a fresh medium. This resulted in ~400 cells/mL in the new culture, represented by
1501 the green curves (four replicate populations). Also see Supplementary Fig. 9.
1502 (**c**) Experiment labelled "2" in (a) at 39.2 $^{O}$C. Wild-type population's density over time for cells that
1503 were incubated in a growth medium that previously harbored a population of exponentially growing
1504 wild-type cells at ~39 $^{O}$C for 0 hours (grey curves), 12 hours (red curves), and 16 hours (purple
1505 curves). Each color shows at least 6 replicate populations. Also see Supplementary Fig. 10.
1506 (**d**) At 39.2 $^{O}$C. All wild-type populations start at 400 cells/mL, which is too low of a density to
1507 permit growths. 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 three replicate populations.

(**e**) At 39.2 $^{O}$C. Shown here are the concentrations of extracellular glutathione for wild-type populations in the no-growth phase (initially 400 cells/mL - red data), random-growth phase (initially 2,000 cells/mL), and deterministic-growth phase (initially 10,000 cells/mL). Three biological replicates are shown for each phase ($n$ = 3; error bars are s.e.m.). We measured three timepoints for each phase. Plotted here are the concentrations of extracellular glutathione as a function of the population density over time. The arrow shows both the population density and concentration of extracellular glutathione increasing together over time (see also Supplementary Fig. 13).

(**f**) Sensitivity of no-growth wild-type populations (initially ~400 cells/mL) at 39.2 $^{O}$C to glutathione that we added into the growth medium. Shown here is, as a function of glutathione concentration, the fold-change in the population density after two days of incubation with the added glutathione. Last two data points show ~200-fold change, which represents the populations having fully grown to reach the carrying capacity (see also Supplementary Fig. 13).

(**g**) Cartoon illustrating the mechanism deduced in (a-f). Yeasts secrete glutathione at high temperatures. The extracellular glutathione thus accumulates. When its concentration reaches at least a threshold amount (~0.3 μM from (f)), the population replicates at high temperatures by noticeable amounts.

**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) nonlinearly 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**)

1542 the phase diagram (compare with Fig. 2d), (**e**) population-density dependent deaths (compare
1543 with Fig. 3b), (**f**) population half-life (based on cell deaths during the first day of incubation -
1544 compare with Figure 3d), the number of survivors decaying over time as a heavy-tailed function
1545 (see Supplementary Fig. 14), and single-cell-level data on growths (compare Supplementary Fig.
1546 14 with Supplementary Fig. 6). Also see Supplementary Fig. 14.
1547
1548
1549 **Figure 6. Budding yeast exports glutathione whose extracellular role – not intracellular**
1550 **roles - as an antioxidant enables yeasts to survive high temperatures.**
1551 (**a**) At 39.2 $^{O}$C. Curves show wild-type populations (all initially at ~20,000 cells/mL) that should
1552 deterministically grow if left alone (as in grey curve). A glutathione masking agent (M2VP) – which
1553 inactivates extracellular glutathione - was added after 4.5 hours (dark brown) or 8.5 hours (light
1554 brown) of incubation (see Methods). Grey curve shows populations that did not receive the
1555 masking reagent. Each color shows four replicate populations. Also see Supplementary Fig. 15.
1556 (**b**) Schematic showing how the budding yeast synthesizes, imports, and exports glutathione.
1557 Glutathione is intracellularly synthesized via an enzyme (γ-glutamylcysteine synthetase) encoded
1558 by *GSH1*. Glutathione is imported by a proton-coupled glutathione-importer encoded by *HGT1*.
1559 Glutathione is exported by numerous exporters (not all shown here), including proton antiporters
1560 encoded by *GEX1,2* and an ATP-dependent exporter encoded by *ADP1*.
1561 (**c**) At 39.2 $^{O}$C. Light blue curves show deterministically growing populations of a mutant strain
1562 (*HGT1Δ*-strain) that cannot import glutathione (initially at ~10,000 cells/mL). Grey curves at the
1563 bottom show the mutant populations (initially ~400 cells/mL) incubated without any glutathione
1564 added. Dark blue curves in the middle show the mutant populations (initially ~400 cells/mL)
1565 incubated with 250μM of glutathione added to the media. Each color shows four replicate
1566 populations.
1567 (**d**) At 39.2 $^{O}$C. Populations of a mutant strain that lacks some of the main glutathione exporters
1568 (*GEX1,2Δ-ADP1Δ*-strain) (initially ~9,000 cells/mL (purple curves in the middle) or ~8,000 cells/mL
1569 (pink curves at the bottom)). Grey curves at the top show, as a comparison, the wild-type
1570 populations (initially ~9,000 cells/mL). Each color shows at least four replicate populations.
1571 (**e**) At 41 $^{O}$C. Wild-type populations of various initial densities (from ~400 cells/mL (lightest green
1572 curves) to ~14,000 cells/mL (darkest green curves)) grown in media supplemented by 750μM of
1573 glutathione. Each color shows four replicate populations.
1574 (**f**) Cartoon illustrating mechanisms deduced in (a-e). Exporting glutathione is necessary and
1575 sufficient for budding yeasts to reshape the habitability of temperature. For yeasts to survive and

1576 grow at high temperatures, extracellular glutathione is (1) necessary since blocking glutathione or
1577 blocking glutathione-export stops yeast's growths and is (2) sufficient since adding glutathione or
1578 blocking glutathione-import enables the yeasts to grow at high temperatures.
1579

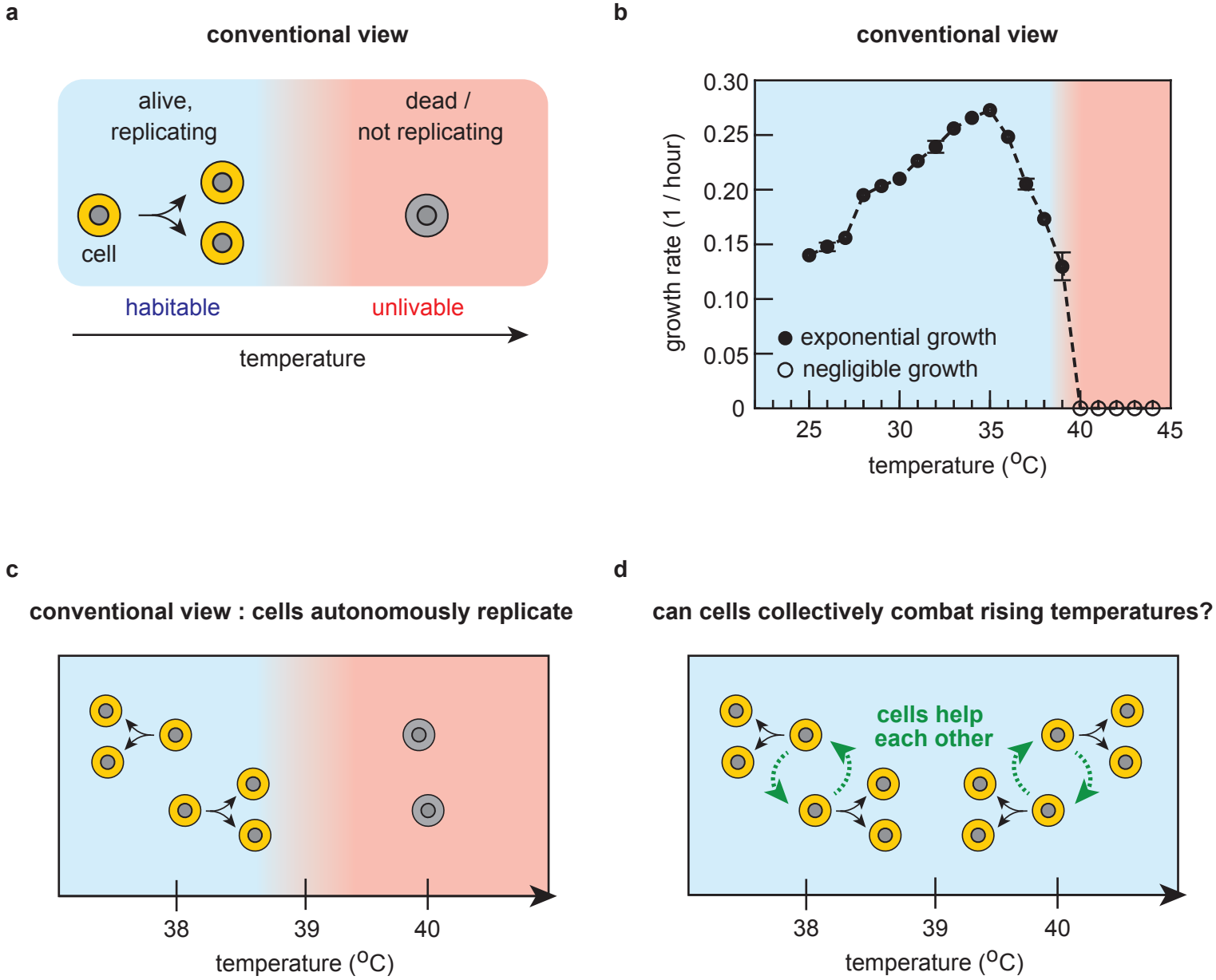

a
conventional view
alive,
replicating
dead /
not replicating
cell
habitable
unlivable
temperature
b
conventional view
growth rate (1 / hour)
0.30
0.25
0.20
0.15
0.10
0.05
0
exponential growth
negligible growth
25
30
35
40
45
temperature (°C)
c
conventional view : cells autonomously replicate
38
39
40
temperature (°C)
d
can cells collectively combat rising temperatures?
cells help
each other
38
39
40
temperature (°C)

Fig. 1

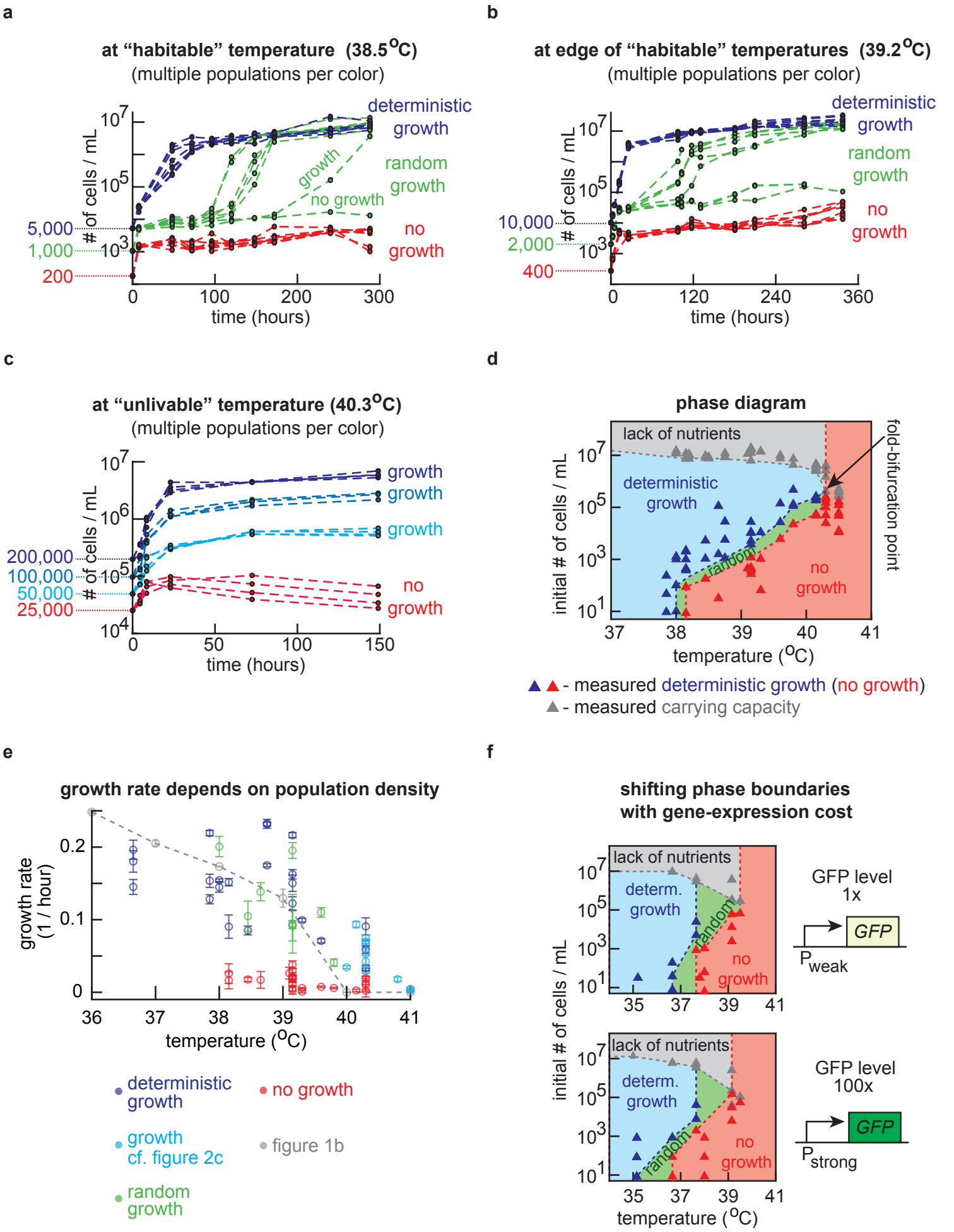

a
at "habitable" temperature (38.5°C)
(multiple populations per color)
deterministic growth
random growth
no growth
growth
no growth
5,000
1,000
200
# of cells / mL
time (hours)
b
at edge of "habitable" temperatures (39.2°C)
(multiple populations per color)
deterministic growth
random growth
no growth
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
P weak
lack of nutrients
determ. growth
random
no growth
GFP level 100x
GFP
P strong
initial # of cells / mL
temperature (°C)

Fig. 2

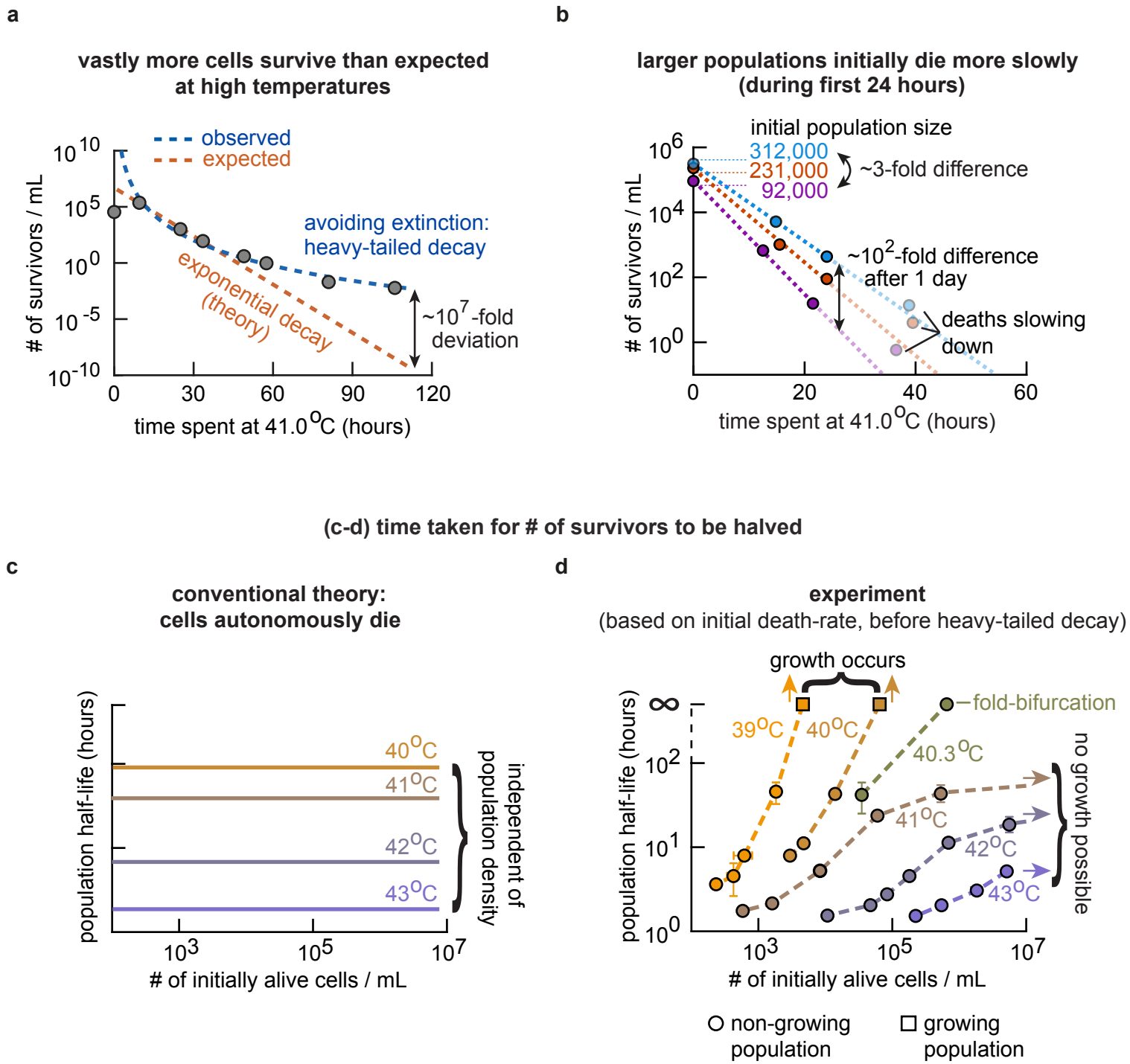

a
vastly more cells survive than expected at high temperatures
observed
expected
avoiding extinction: heavy-tailed decay
exponential decay (theory)
~10^7-fold deviation
# of survivors / mL
time spent at 41.0°C (hours)
b
larger populations initially die more slowly (during first 24 hours)
initial population size
312,000
231,000
92,000
~3-fold difference
~10^2-fold difference after 1 day
deaths slowing down
# of survivors / mL
time spent at 41.0°C (hours)
(c-d) time taken for # of survivors to be halved
c
conventional theory: cells autonomously die
40°C
41°C
42°C
43°C
independent of population density
population half-life (hours)
# of initially alive cells / mL
d
experiment
(based on initial death-rate, before heavy-tailed decay)
growth occurs
fold-bifurcation
39°C
40°C
40.3°C
41°C
42°C
43°C
no growth possible
population half-life (hours)
# of initially alive cells / mL
non-growing population
growing population

Fig. 3

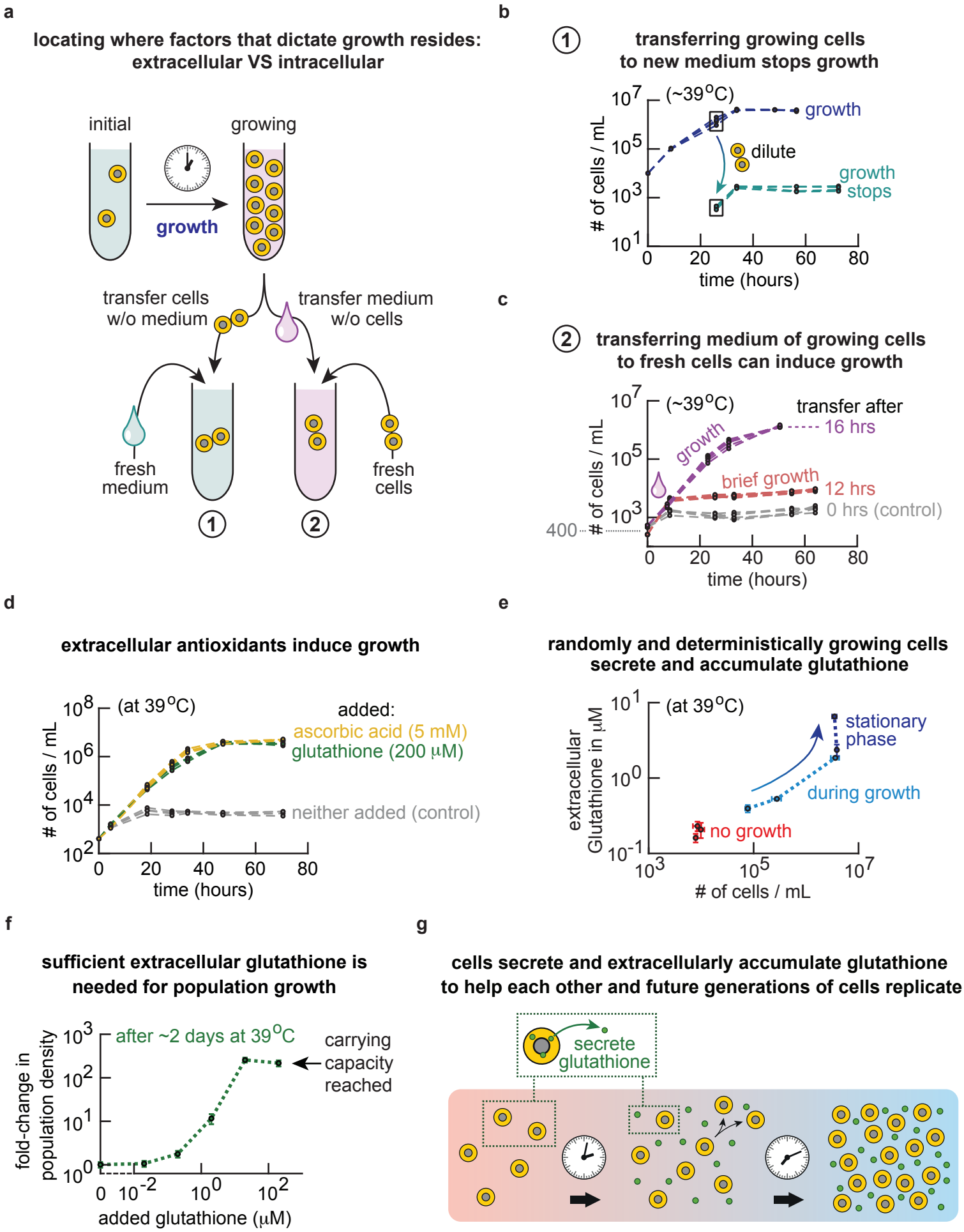
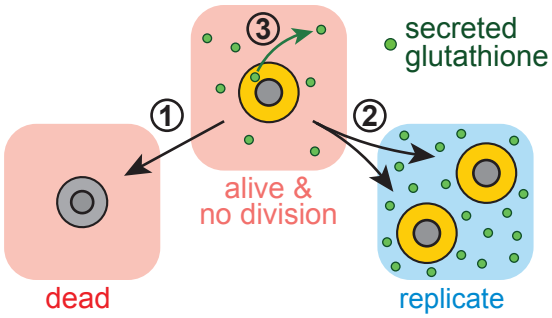

a
locating where factors that dictate growth resides: extracellular VS intracellular
initial
growing
growth
transfer cells w/o medium
transfer medium w/o cells
fresh medium
fresh cells
1
2
b
1
transferring growing cells to new medium stops growth
(~39°C)
growth
dilute
growth stops
# of cells / mL
time (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
(at 39°C)
added:
ascorbic acid (5 mM)
glutathione (200 μM)
neither added (control)
# of cells / mL
time (hours)
e
randomly and deterministically growing cells secrete and accumulate glutathione
(at 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

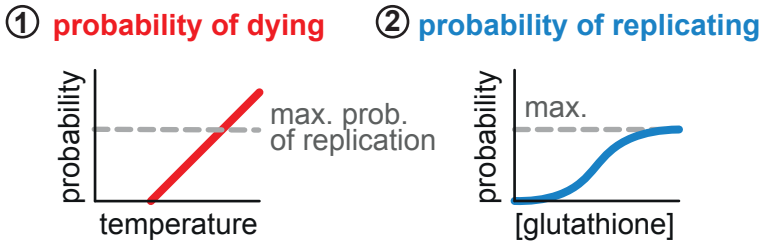
## mathematical model: schematics

**a**

**stochastic transitions of a cell**



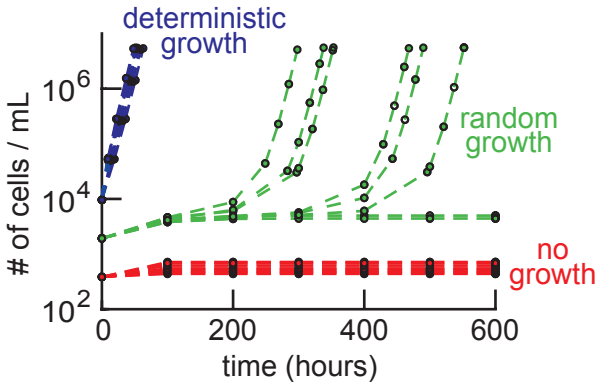

**b**




## mathematical model recapitulates key features

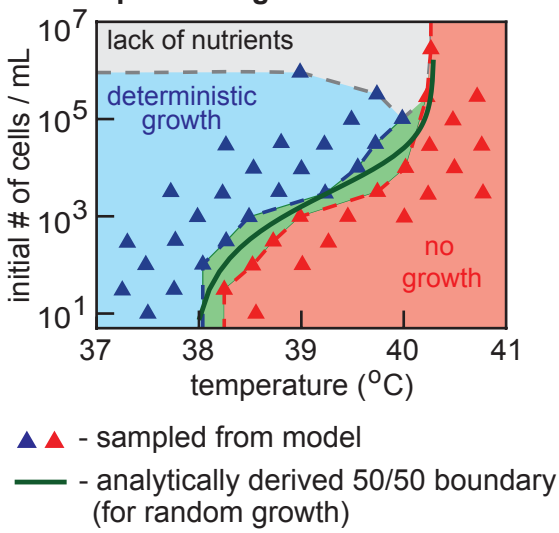
**c**

**calculated growth curves**



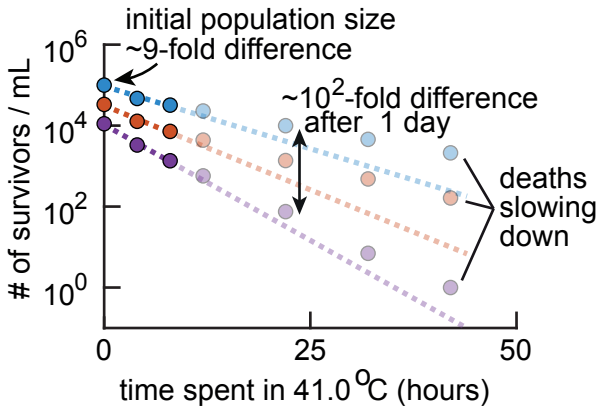

**d**

**phase diagram from model**




**e**

**calculated population-density dependent deaths and heavy-tailed decay**




**f**

**calculated time taken for # of survivors to be halved**

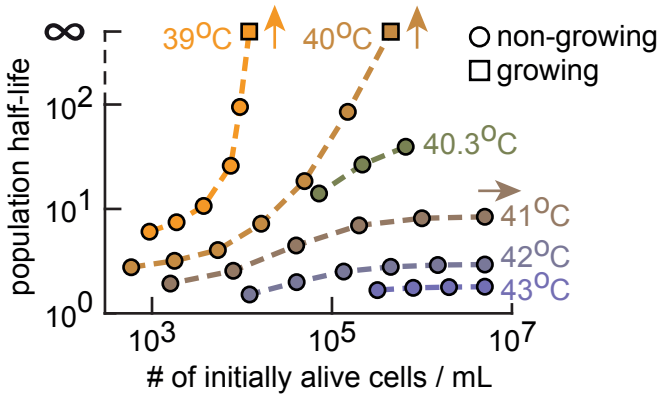



Fig. 5

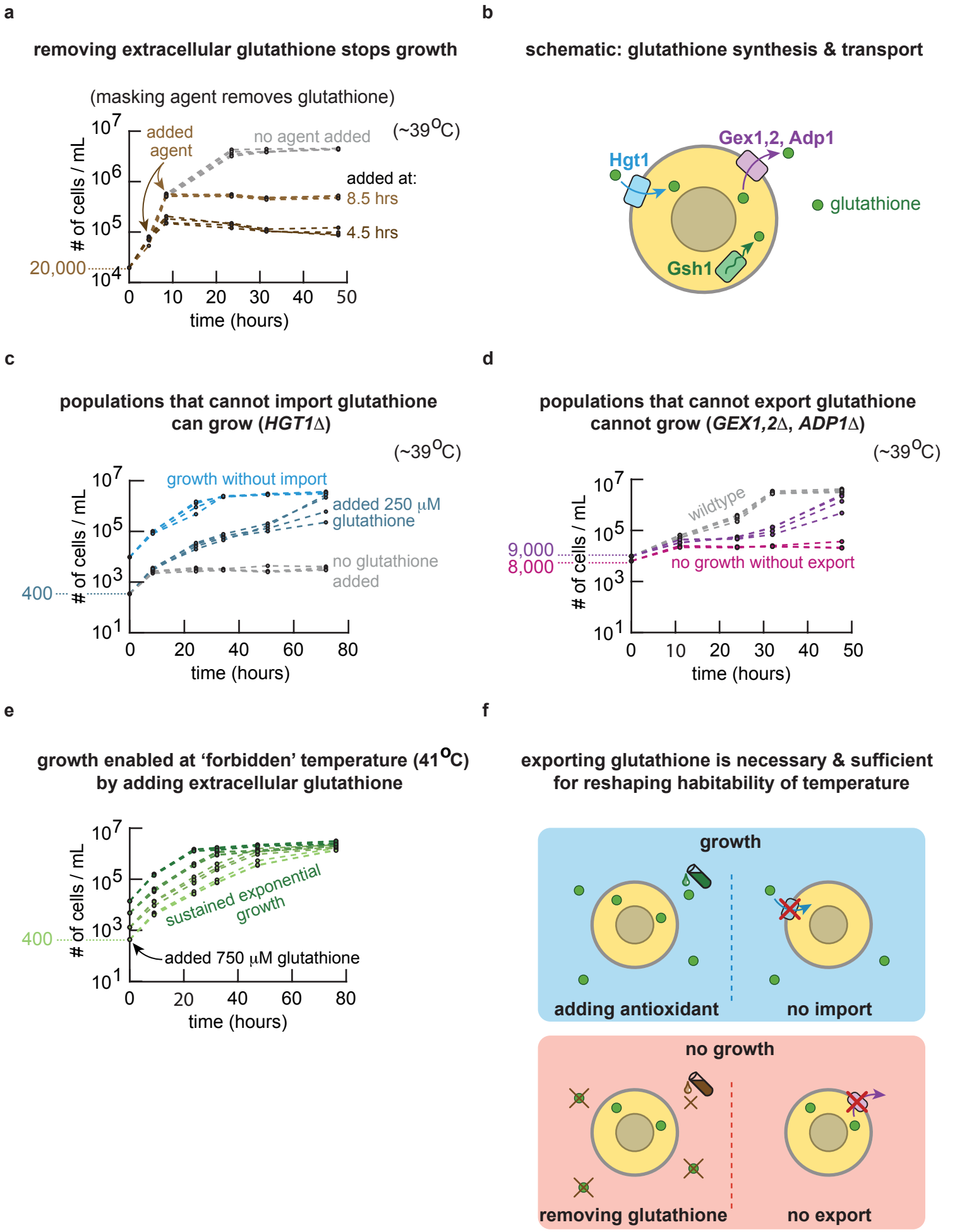

a
removing extracellular glutathione stops growth
(masking agent removes glutathione)
added agent
no agent added
(~39°C)
added at:
8.5 hrs
4.5 hrs
20,000
# of cells / mL
time (hours)
b
schematic: glutathione synthesis & transport
Gex1,2, Adp1
Hgt1
glutathione
Gsh1
c
populations that cannot import glutathione can grow (HGT1Δ)
(~39°C)
growth without import
added 250 μM glutathione
no glutathione added
400
# of cells / mL
time (hours)
d
populations that cannot export glutathione cannot grow (GEX1,2Δ, ADP1Δ)
(~39°C)
wildtype
9,000
8,000
no growth without export
# of cells / mL
time (hours)
e
growth enabled at 'forbidden' temperature (41°C) by adding extracellular glutathione
sustained exponential growth
400
added 750 μM glutathione
# of cells / mL
time (hours)
f
exporting glutathione is necessary & sufficient for reshaping habitability of temperature
growth
adding antioxidant
no import
no growth
removing glutathione
no export

Fig. 6