
Supplementary information

The optimal strategy balancing risk and speed predicts DNA damage checkpoint override times

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Supplementary Information

Supplementary theoretical results

Supplementary note 1 – Outcomes survival or death

Focusing only on the possibilities that cells either die or survive was supported by our observations in all DNA break experiments that the surviving cells grew into colonies of roughly the same size at about the same speed. Thus, we did not observe two different colony sizes, which would suggest healthy and sick survivors.

Similarly, other researchers have scored viability by counting the number of colonies that have been formed from single surviving cells without noting sick colonies.^{1,35}

Galgoczy and Toczyski⁶ observed colonies growing at different speeds after exposing diploid cells that could not repair DSBs by homologous recombination (*rad52Δ*) to X-rays. Diploid cells can survive the loss of a chromosome and become aneuploid. Thus, sickness may have been a more common outcome for diploid cells than for haploid cells.

Furthermore, in reality, sick cells would be outcompeted exponentially quickly in time and thus would be effectively dead except in special circumstances, e.g., spatial isolation or immediate post-arrest mating and random assortment of the chromosomes generating a subset of healthy offspring. These more complicated scenarios may be considered for further developments of the theory but we did not find those to be necessary because of the excellent match between theory and experiment.

In any case, the theory can be easily extended by introducing additional parameters describing the statistics of the occurrence and degree of sick cells.

Supplementary note 2 – Arrest penalty in fixed-size populations

In a fixed-size population of N cells reproducing according to the Wright-Fisher model⁴⁶, the survival probability of an arrested cell is $\left(1 - (1 - 1/N)^N\right)$ per generation. This factor represents the probability that the arrested cell is not lost from the population due to drift, which would occur if the $N - 1$ other cells in the population were selected to reproduce N times by

chance. The probability of survival decreases from $3/4$ for $N = 2$ to $1 - e^{-1} = 0.63\dots$ as the population becomes larger ($N \rightarrow \infty$). Since in this model, the checkpoint-arrested cell either dies or survives and then rejoins the regular population dynamics as before the arrest, the expected number of progeny is reduced by $P_1 \left[1 - (1 - 1/N)^N \right]^{t/T}$ for a checkpoint arrest of t/T generations.

In a Moran process, the survival probability due to random drift is $(1 - 1/N)$ for a checkpoint-arrested cell, which is the probability that the cell is not killed with probability $1/N$. However, in a Moran process, it takes N generations on average for each cell to have its turn in reproduction. Thus, adjusted for the smaller time steps in this model, the survival probability in the presence of drift is $(1 - 1/N)^N$ per N generations in this model, which increases from $1/4$ for $N = 2$ to $e^{-1} = 0.37\dots$ for $N \rightarrow \infty$. The expected number of progeny after checkpoint arrest is thus $P_1(1 - 1/N)^{Nt/T}$.

Thus, in all models, the expected number of progeny of a checkpoint-arrested cell is reduced by the product of a temporal penalty for arrest, which is exponential in time, and a probability of proliferation. In exponential growth, the fitness penalty due to checkpoint arrest ($2^{-t/T}$) is exponential in time with base $1/2$. In the Wright-Fisher model, the base of the exponential penalty depends on the size of the population; in each generation, the probability of survival decreases by a factor of $1 - (1 - 1/N)^N$. In a Moran process, the equivalent factor is $(1 - 1/N)^N$. Interestingly, while these processes are very different, the loss of expected progeny per generation due to waiting is roughly similar numerically.

We represent the time penalty by a factor $e^{-t/\tau}$, which applies in all three cases and where τ absorbs the logarithm of the base, that is, $\tau = T/\log 2$ for exponential growth, $\tau = -T/\log \left(1 - (1 - 1/N)^N \right)$ for the Wright-Fisher model, and $\tau = -T/\log \left((1 - 1/N)^N \right)$ for the Moran model.

Supplementary note 3 – Fitness: arithmetic vs. geometric mean

To illustrate that with random checkpoint arrests, the average of the offspring numbers is an

appropriate fitness function, we consider the following simple exponential growth model:

1. Starting with one cell, every cell produces two offspring with probability $(1 - q)$ in every generation.
2. With probability q , a cell suffers damage, which is either lethal with probability $(1 - p)$ or which is repaired with probability p and delays reproduction by g generations.

The expected number of offspring $\langle \text{offspring} \rangle$ for this model after G generations is approximately:

$$\langle \text{offspring} \rangle = \left(2(1 - q) + 2^{-g}pq \right)^G, \quad (\text{S1})$$

where $2(1 - q)$ is the expected number of new cells due to normal divisions (model step 1) and $2^{-g}pq$ is the expected number of cells which resolve the damage after arresting at the checkpoint for g generations (model step 2).

As the simulations of the model in Fig. S1 show, the above equation for the average number of offspring ($\langle \text{offspring} \rangle$) captures the main peak of the distribution well. Thus, the average is a useful indicator for the behavior of the population. Given that the incidence of damage q is random and set by processes other than checkpoint control, we can only increase the average number of offspring by maximizing the product $2^{-g}p$, as we did to find the optimal checkpoint strategy.

If, by contrast, we consider a fluctuating environment model in which a whole generation – not random cells – suffers damage with probability q which is lethal with probability $(1 - p)$, then in each generation, the chance of survival goes down by a factor of $(1 - q(1 - p))$. The survival probability thus goes to zero with time, and the average number of offspring after many generations would be dominated by unlikely scenarios where cells survive for long times and with many offspring. In these types of models the more appropriate fitness function to maximize would be the expected number of offspring for a typical (average) scenario, which

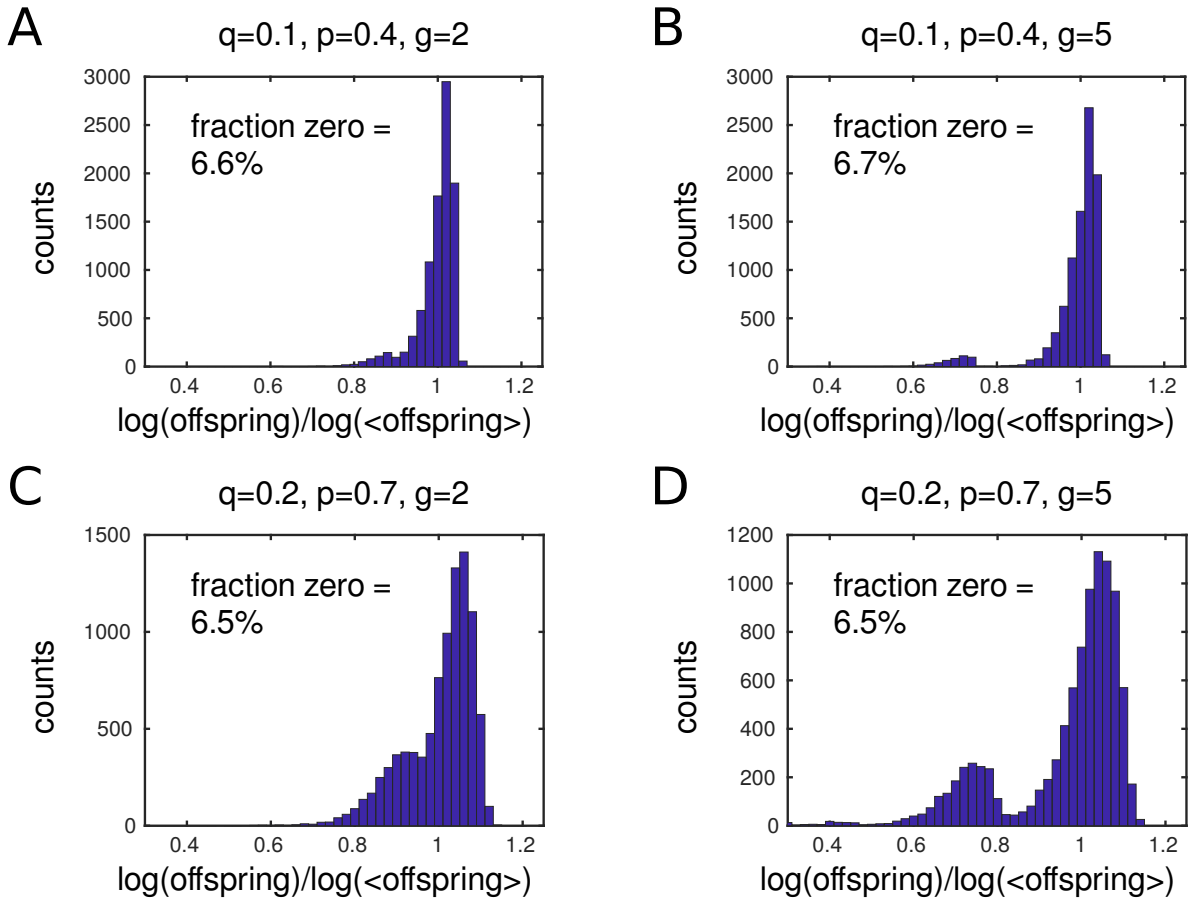


Fig. S1: Distribution of offspring numbers for a simple population model of damage and checkpoint arrest, as explained in Supplementary note 3, given the parameters listed on top of the histograms. As g increases, the first peak separates from the main peak. This small first peak represents trials where the very first cells in the simulation receive damage but arrest for g generations and repair the damage. The trials in which the first cell dies immediately ('fraction zero') are not indicated in the histogram because the logarithm of zero is undefined, but the fraction is noted in each panel. The simulation was run for twenty generations $G = 20$ and repeated 10^4 times.

leads to bet hedging.⁴⁸

Supplementary note 4: General fitness functional

To make the mathematical presentation simpler, we assume in Eq. (1) and in the subsequent calculations that if cells survive after cell cycle division, it is either always one of the two progeny that survives, in agreement with experimental data for DSBs [2], or always both progeny that survive. This assumption can be removed in the theory by introducing parameters for the relative likelihoods that one offspring survives versus both offspring survive.

Supplementary note 5: Timer strategy

A simple checkpoint strategy could be to wait for a fixed time t' , and then advance with the

cell cycle. Mathematically, this means setting $a(t|\{t_i\}) = \delta(t - t')$:

$$f(t') = e^{-t'/\tau} \int_0^\infty \prod_{E=1}^{E_0} dt_E r(\{t_i\}) s(t'|\{t_i\}) \quad . \quad (\text{S2})$$

Assuming that each error is repaired independently according to the cumulative probability function $\rho(t_i)$ and each unrepaired error reduces the probability of survival by a factor σ , we obtain:

$$\begin{aligned} f(t') &= e^{-t'/\tau} \sum_{E=0}^{E_0} \binom{E_0}{E} \rho^{E_0-E}(t') (1 - \rho(t'))^E 1^{E_0-E} \sigma^E \\ &= e^{-t'/\tau} [\rho(t') + \sigma(1 - \rho(t'))]^{E_0} \\ &= e^{-t'/\tau} [\rho(t')(1 - \sigma) + \sigma]^{E_0} \end{aligned} \quad (\text{S3})$$

The best timer strategy is obtained by maximizing $f(t')$ with respect to t' ,

$$\tau E_0 \frac{\partial_{t'^*} \rho(t'^*)}{\rho(t'^*) + \frac{\sigma}{1-\sigma}} = 1 \quad . \quad (\text{S4})$$

Assuming that repairs follow a Poisson process, $\rho(t_i) = \rho_{\max} (1 - e^{-t_i/\tau_r})$, we obtain for the optimal waiting time in the timer model:

$$\frac{t'^*}{\tau_r} = \log \frac{1 + E_0 \frac{\tau}{\tau_r}}{1 + \frac{1}{\rho_{\max}} \frac{\sigma}{1-\sigma}} \quad , \quad (\text{S5})$$

which implies that unless

$$E_0 \frac{\tau}{\tau_r} > \frac{1}{\rho_{\max}} \frac{\sigma}{1-\sigma} \quad (\text{S6})$$

is satisfied, it is not worth waiting a fixed time for repair at a checkpoint at all ($t'^* = 0$), for example, if survival is too likely (σ close to 1).

Supplementary note 6: Error-threshold strategy

In an alternative model, a fixed amount $(E_0 - E')$ of the total damage (E_0) is repaired before

continuing with the cell cycle. Here,

$$a(t | \{t_i\}) = \sum_{\text{perm.}} \underbrace{\delta(t - t_{i_1})}_{1 \text{ repaired at } t} \underbrace{\theta(t - t_{i_2}) \cdots \theta(t - t_{i_{E_0 - E' + 1}})}_{E_0 - E' - 1 \text{ repaired before } t} \underbrace{\theta(t_{i_{E_0 - E' + 1}} - t) \cdots \theta(t_{i_{E_0}} - t)}_{E' \text{ errors remaining}} \quad , \quad (\text{S7})$$

the cell cycle advancement probability, is equal to the sum of all permutations where damage i_1 is repaired at time t , before which $E_0 - E' - 1$ errors were repaired, leaving E' errors unrepaired.

Assuming as above that repair events are independent and survival for each error that remains at override is reduced by a factor σ , we obtain:

$$f(E') = \int_0^\infty dt E_0 \frac{d\rho(t)}{dt} \binom{E_0 - 1}{E'} \rho^{E_0 - E' - 1}(t) (1 - \rho(t))^{E'} \sigma^{E'} e^{-t/\tau} \quad . \quad (\text{S8})$$

Assuming a Poisson process as above but with $\rho_M = 1$ to simplify the equations, we obtain:

$$f(E') = \frac{E_0! (E' + \tau_r/\tau)!}{(E_0 + \tau_r/\tau)! E'!} \sigma^{E'} \quad . \quad (\text{S9})$$

Maximizing $f(E')$ with respect to E' , we obtain:

$$\psi_0(1 + E'^* + \tau_r/\tau) - \psi_0(1 + E'^*) = -\log \sigma \quad , \quad (\text{S10})$$

where ψ_0 is the polygamma function of order zero.

The left-hand side is a decreasing function of E'^* , so the larger the survival probability σ , the more errors E'^* can be tolerated for proceeding with the cell cycle.

E'^* is generally not an integer, and, therefore, $f(E'^*)$ has to be evaluated at the two nearest integers to E'^* to find the maximally tolerable amount of damage.

Supplementary note 7: The optimal strategy is deterministic

The idea of the proof is as follows: To evaluate whether the optimal checkpoint strategy is stochastic or deterministic, we take a particular checkpoint strategy and increase or decrease the

advancement probability $a(t, \{t_i\})$ at one point $(t, \{t_i\})$ in that strategy. Since the advancement probability must be normalized, that is, the integral or sum over all advancement probabilities must be equal to one, we also multiply all the other advancement probabilities that branch off from that point and that describe future advancement decisions by a compensatory factor. While satisfying the normalization constraint, we show that the fitness coefficient of the strategy $f[a]$ can be increased unless the advancement probability density at that point was zero or infinity, that is, a Dirac delta (δ) function. A Dirac delta (δ) function advancement probability means that the checkpoint strategy is deterministic. Thus, only a deterministic strategy can be optimal.

To begin the proof, we assume that time is discretized with time points spaced Δt apart to replace integrals by sums. Consider a checkpoint strategy with advancement probability $a(t, \{t_i\})$ and a particular point in the strategy, that is, a damaged cell is arrested for time t , has $E(t)$ errors, and has repaired $E_0 - E(t)$ errors at times $\{t_j\}$ which are smaller than t . The value of $a(t | \{t_i\})$ of course only depends on t and the values of the repair times $\{t_j\}$ before t , since the repair times greater than t lie in the future. Therefore, $a(t | \{t_i\}) = a(t | \{t_j\})$ holds. The contribution of this advancement probability to the fitness coefficient is:

$$\Delta f_{\text{point}} = \Delta t r(\{t_i\}) s(t | \{t_i\}) a(t | \{t_i\}) e^{-t/\tau} \quad , \quad (\text{S11})$$

according to Eq. (1).

Next, we consider the later advancement probabilities $a(t' | \{t'_i\})$ where $t' > t$ in which additional errors may have been repaired after t (the repaired errors before t are obviously the same $\{t_j\}$), see Fig. S2 for an illustration. These advancement probabilities make the following contributions to the fitness coefficient:

$$\Delta f_{\text{branch}} = \Delta t \sum_{t'} r(\{t'_i\}) s(t' | \{t'_i\}) a(t' | \{t'_i\}) e^{-t'/\tau} \quad . \quad (\text{S12})$$

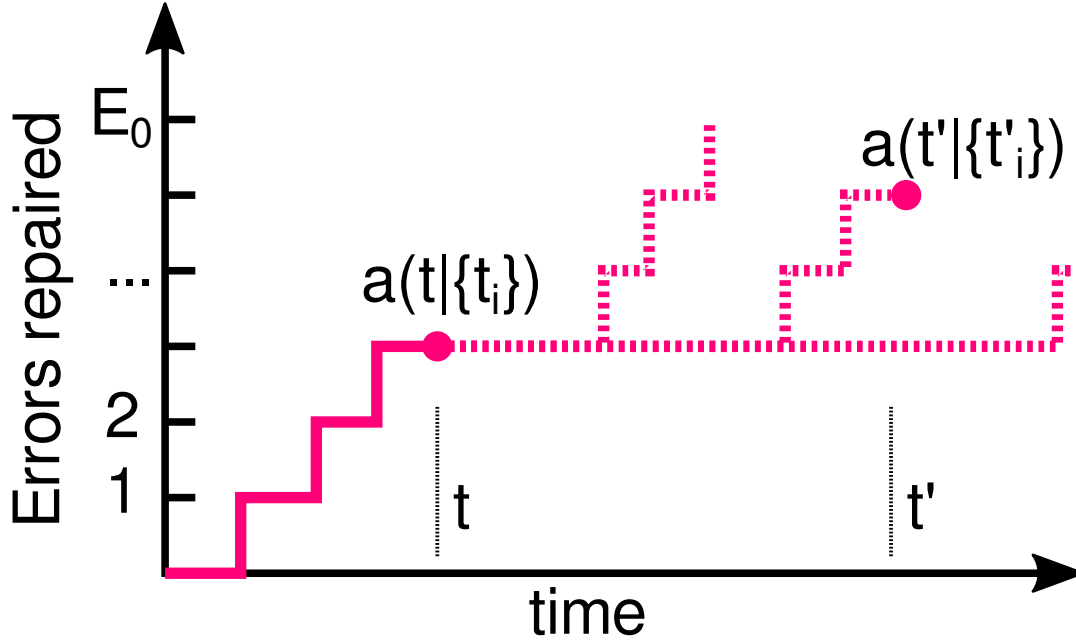


Fig. S2: Illustration of trajectories in a checkpoint strategy. Solid lines indicate the realized trajectory (sequence of repairs) of the cell until time t . Dashed lines indicate possible future trajectories. The advancement probabilities $a(t|\{t_i\})$ at time t and $a(t'|\{t'_i\})$ at a later time t' are indicated.

Suppose that the advancement probabilities of the checkpoint strategy are normalized such that:

$$\text{const.} = a(t|\{t_i\}) + \sum_{t'} a(t'|\{t'_i\}). \quad (\text{S13})$$

We create a new strategy in which we multiply $a(t|\{t_i\})$ by a factor α , yielding $\alpha a(t|\{t_i\})$, and we also multiply each of the later advancement probabilities $a(t'|\{t'_i\})$ by α' :

$$\alpha' = 1 + (1 - \alpha) \frac{a(t|\{t_i\})}{\sum_{t'} a(t'|\{t'_i\})}. \quad (\text{S14})$$

We see that in the new checkpoint strategy,

$$\text{const.} = \alpha a(t|\{t_i\}) + \sum_{t'} \alpha' a(t'|\{t'_i\}) \quad , \quad (\text{S15})$$

the advancement probability continues to be correctly normalized.

Plugging in the scaled advancement probabilities, we see that in this new strategy, the

modified point contributes:

$$\alpha \Delta f_{\text{point}} = \alpha \Delta t r(\{t_i\}) s(t | \{t_i\}) a(t | \{t_i\}) e^{-t/\tau} \quad (\text{S16})$$

to the fitness coefficient and the contribution of the future points is:

$$\begin{aligned} \alpha' \Delta f_{\text{branch}} &= \alpha' \Delta t \sum_{t'} r(\{t'_i\}) s(t' | \{t'_i\}) a(t' | \{t'_i\}) e^{-t'/\tau}, \\ &= \left[1 + (1 - \alpha) \frac{a(t | \{t_i\})}{\sum_{t'} a(t' | \{t'_i\})} \right] \Delta t \sum_{t'} r(\{t'_i\}) s(t' | \{t'_i\}) a(t' | \{t'_i\}) e^{-t'/\tau}, \end{aligned} \quad (\text{S17})$$

which is linear in α . Since the contributions to the fitness coefficient are linearly dependent on the parameter α ,

$$\begin{aligned} \alpha \Delta f_{\text{point}} + \alpha' \Delta f_{\text{branch}} &= O(\alpha^0) + \alpha \Delta t a(t | \{t_i\}) \\ &\times \left[r(\{t_i\}) s(t | \{t_i\}) e^{-t/\tau} - \sum_{t'} r(\{t'_i\}) s(t' | \{t'_i\}) e^{-t'/\tau} \frac{a(t' | \{t'_i\})}{\sum_{t''} a(t'' | \{t''_i\})} \right] \end{aligned} \quad (\text{S18})$$

the fitness coefficient of this strategy could not be maximal, unless either $a(t | \{t_i\})$ is zero or all future $a(t' | \{t'_i\})$ are zero. (Here, we assume that the slope of α , which is proportional to the term in square brackets in Eq. (S18), is not zero. This term is the difference between the contribution to the fitness coefficient if the advancement took place at t and the average contributions of all future divisions, which are weighted by their conditional advancement probability $\frac{a(t' | \{t'_i\})}{\sum_{t''} a(t'' | \{t''_i\})}$. Generically, it will not be zero.)

This argument applies for every point in the error-time plane, such that at points where $\Delta t a(t, \{t_i\})$ is nonzero, it must be equal to one. In the limit $\Delta t \rightarrow 0$ as the discretization becomes smaller, $a(t, \{t_i\})$ becomes a Dirac delta (δ) function.

Supplementary note 8 – Calculating the optimal strategy

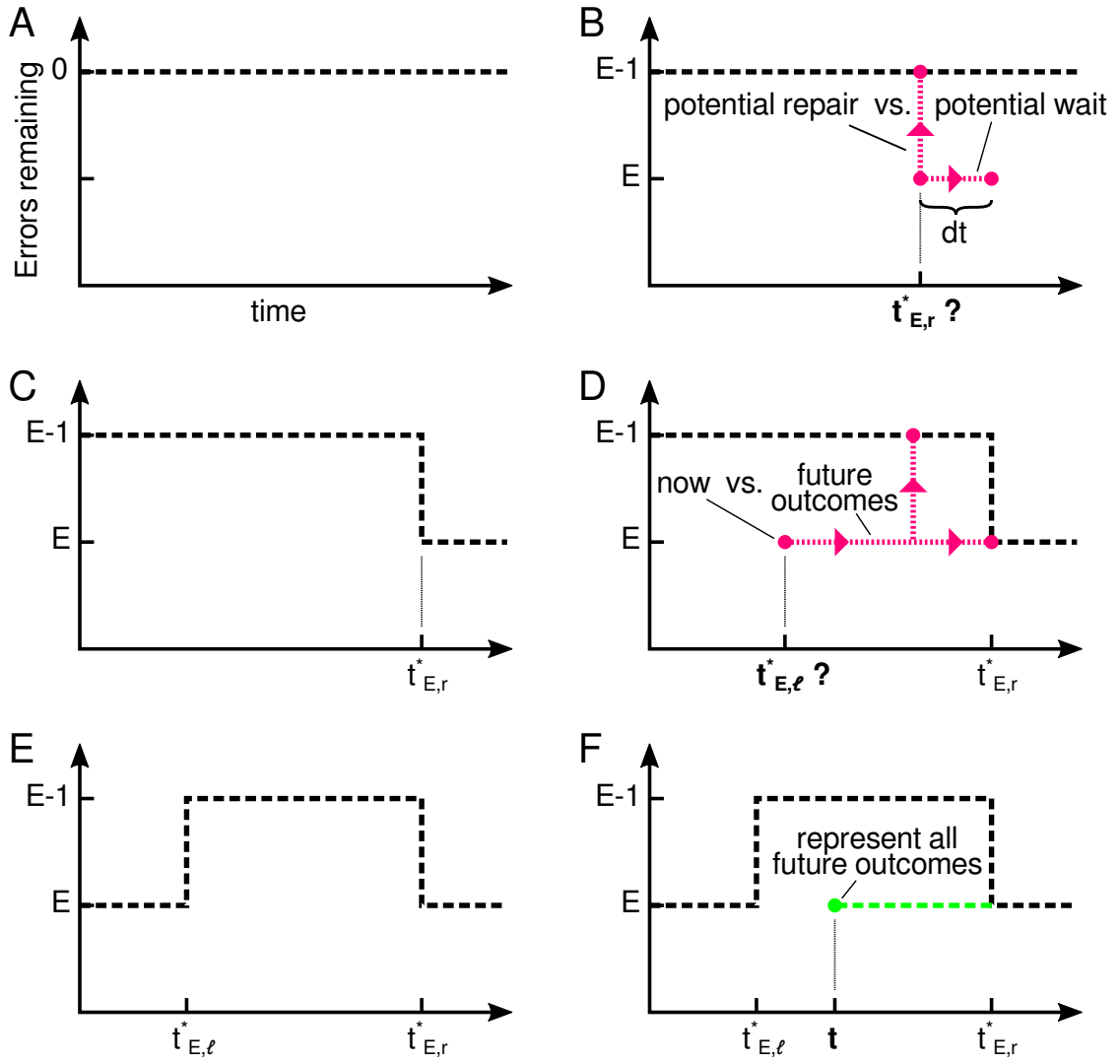


Fig. S3: Steps for computing the optimal advancement boundary. To make the labels on the y-axis simpler, we indicated the number of errors remaining E instead of the errors repaired $E_0 - E$. However, every step in the positive y-direction continues to indicate one repair event.

In the following steps, illustrated in Fig. S3, we start with the trivial case where all errors have been fixed ($E = 0$, Step 1) and calculate the advancement boundary for E errors provided we have carried out the analysis for $E - 1$ errors already (Steps 2-5):

1. The survival probability is equal to one when all E_0 errors are repaired. It is obviously optimal to advance with the cell cycle when there are no errors ($E = 0$). Therefore, we draw an advancement boundary line at $E = 0$ (Fig. S3 A). Next, we iterate the following Steps 2-5:
2. For states with E unrepaired errors at time t and an advancement boundary line at

$E - 1$, we consider what happens when we increase t , keeping E fixed. At some time $t_{E,r}^*$, it may cease to be worth waiting for another repair event. This time is reached when the conditional repair probability times the gain in fitness for making one more repair, $\frac{dt\partial_t\rho}{1-\rho}E(s(E-1)-s(E))e^{-t/\tau}$, is outweighed by the loss of fitness if another repair does not occur, $-s(E)dt\partial_t e^{-t/\tau}$, as depicted in Fig. S3 B. Equating these two terms, we obtain,

$$\left. \frac{\partial_t\rho}{1-\rho} \right|_{t=t_{E,r}^*} \tau E \left(\frac{s(E-1)}{s(E)} - 1 \right) = 1 \quad . \quad (\text{S19})$$

Next, we use $s(E) = \sigma^E$ and only keep the linear term in σ , neglecting the $O(\sigma^2)$ terms. This is justified, for example, for an unrepaired DSB since the survival probability is very small ($\sigma \ll 1$) (Fig. 5 D). This yields Eq. (2), which is reproduced here:

$$\boxed{\left. \frac{\partial_t\rho}{1-\rho} \right|_{t=t_{E,r}^*} \approx \frac{\sigma}{E\tau}} \quad . \quad (\text{S20})$$

This simple relation allows the right advancement boundary $t_{E,r}^*$ at E (Fig. S3 C) to be computed.

3. After computing the right advancement boundary $t_{E,r}^*$ in Step 2, we next find whether the wait up to the right boundary is preceded by any times $t_{E,\ell}^*$ that should also trigger cell cycle advancement for the same number of E errors (Fig. S3 D, E). For the left advancement boundary at $t_{E,\ell}^*$, we need to compare the instantaneous fitness $s(E)e^{-t/\tau}$ with the expected future fitness. To calculate the latter, one needs to take into account the possibility that another repair happens before reaching the right boundary at $t_{E,r}^*$ as well as the possibility that no other such repair occurs before time $t_{E,r}^*$ (Fig. S3 D). This

leads to the following condition:

$$s(E)e^{-t_{E,\ell}^*/\tau} = \int_{t_{E,\ell}^*}^{t_{E,r}^*} dt' (-\partial_{t'}) \left[\frac{1 - \rho(t')}{1 - \rho(t_{E,\ell}^*)} \right]^E s(E-1)e^{-t'/\tau} + \left[\frac{1 - \rho(t_{E,r}^*)}{1 - \rho(t_{E,\ell}^*)} \right]^E s(E)e^{-t_{E,r}^*/\tau} . \quad (\text{S21})$$

This sets the left advancement boundary $t_{E,\ell}^*$ at E (Fig. S3 E). (Equations (S21) and (S19) are related. Eq. (S21) simplifies to Eq. (S19) by taking the derivative with respect to $t_{E,r}^*$ and setting $t_{E,\ell}^* = t_{E,r}^*$.)

4. It is, in principle, possible for there to be alternating segments of advancement boundaries and segments where it is best to wait (Fig. S4 A). Additional boundary segments can be found by solving for the right-most $t_{E,r}^*$, then the preceding $t_{E,\ell}^*$, and then for a possible right boundary further to the left of $t_{E,\ell}^*$, and so on. Thus, in this step, we find whether there are a series of such advancement-boundary—wait-segments before (to the left of) $t_{E,\ell}^*$, repeating Steps 2 and 3 for the same number of errors E from right to left.
5. Thus far, all calculations assumed that with one fewer error ($E - 1$), it is optimal to advance and that there is a continuous advancement boundary line, as for $E = 0$. This assumption explicitly entered the right-hand sides of Eqns. (2), (S19), and (S21) which refer to the survival probability if one more error is fixed ($s(E - 1)$) at the advancement boundary at $E - 1$. However, if at the previous step, that is, at some time point t and for $E - 1$ errors, the optimal decision is to wait, it would not be correct to assess the consequence of reaching that point by the survival probability $s(E - 1)$ since upon arriving at t and $E - 1$, the cell cycle should continue to arrest. So, before moving down in the plane and repeating the calculation of the advancement boundary for $E + 1$ errors in the recursive computation, the time points inside the gaps (wait times) between $t_{E,\ell}^*$ and $t_{E,r}^*$ must be filled with predictions of the expected number of progeny if these time points are

reached (green dashed line in Fig. S3 F). This is because the formulas in Steps 2-4 rely on the survival probability for cell cycle advancement with $E(t) - 1$ errors but if there is no advancement boundary at $E(t) - 1$ and time t , the expected number of progeny is the quantity that has to replace $s(E - 1)$ in the relevant formulas. So, in this step, the gaps between advancement boundaries for E errors are filled with the expected number of progeny once a particular time point in the gap is reached (Fig. S4 B). To calculate the optimal checkpoint decision for $E + 1$ errors after the advancement boundary for E has been computed, for all times between the advancement boundaries, e.g., between $t_{E,\ell}^*$ and $t_{E,r}^*$ in Fig. S3 F, the expected number of offspring $f_{\text{wait}}(t, E)$ needs to be computed. The formula for $f_{\text{wait}}(t, E)$ has to be adapted to the specific situation but can be based on the following example:

$$f_{\text{wait}}(t, E) = \int_t^{t_{E,r}^*} dt' (-\partial_{t'}) \left[\frac{1 - \rho(t')}{1 - \rho(t)} \right]^E f_{\text{wait}}(t', E - 1) e^{(t-t')/\tau} + \left[\frac{1 - \rho(t_{E,r}^*)}{1 - \rho(t)} \right]^E s(E) e^{(t-t_{E,r}^*)/\tau}, \quad (\text{S22})$$

which describes a strategy in which the cell will wait at $E - 1$ (because there is also a $f_{\text{wait}}(t', E - 1)$ term on the right-hand side). $f_{\text{wait}}(t, E)$ replaces $s(E)$ in the above formulas for calculating the advancement boundary for $E + 1$ errors (Fig. S4 B).

The strategy encompasses two possible actions: i) wait for more potential repairs or ii) advance because waiting any longer brings down the expected offspring number. The reasons for advancement (ii) can be subtly different: Hitting a right border as in Fig. 2 E means that any further waiting is disadvantageous (waiting is hopeless); arriving at a left border, before it is optimal to wait, a number of repairs had to have been performed quickly, and any further improvements are unlikely to occur soon enough to be worth the wait (cash in a lucky draw); advancement at a top border can be interpreted either way.

The strategy is optimal by construction. Given that we start in the future and work backward, at each step we calculate the optimal decision based on having calculated the best decision for all accessible future time points.

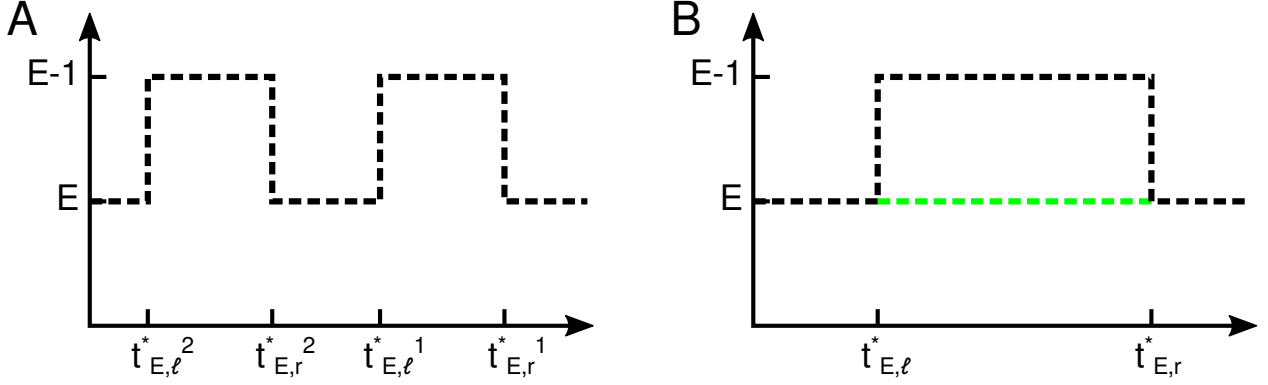


Fig. S4: Illustration of the recursive solution to the checkpoint optimization problem. A: Boundary with alternating advancement boundaries ($t_{E,r}^{*,i+1}$ to $t_{E,\ell}^{*,i}$) and wait segments ($t_{E,\ell}^{*,i}$ to $t_{E,r}^{*,i}$). B: After all points for E have been analyzed, there are either advancement segments (black) or wait segments (green) with the expected numbers of progeny computed at each point of the wait segments. The calculations can then be repeated for $E + 1$ errors.

For a Poisson process $\rho(t) = 1 - e^{-t/\tau_r}$, one can see by plugging into Eq. (2) that the boundaries are always horizontal, thus the optimal strategy reduces to the error-threshold strategy. (The resulting condition, $\tau/\tau_r D(1/\sigma - 1) = 1$, is not obviously equivalent to Eq. (S10), but we checked numerically, scanning a large range of parameters, that they both give the same optimal horizontal boundary.)

Supplementary experimental results

Supplementary note 9 – Characterization of DSB repair kinetics by microscopy

At the switch to Glu-Met (time point 0'), all cells showed low fluorescence due to the induced DSB and low ADH1 expression in raffinose and galactose (Fig. 3 C). Subsequently, fluorescence shot up in all cells that divided their nuclei (Figs. 3 C, S5). Thus, only cells where the DSB was repaired (or had never been induced in the first place, discussed below) divided. This underscores the effectiveness of override suppression by the *cdc5-ad* mutation since we saw no YFP-negative (YFP-) cells that performed nuclear division. Some cells did not divide their nuclei but showed a jump in fluorescence, indicating a failure to complete the cell cycle in the absence of a DSB. We investigated the four cells that divided but showed low fluorescence after 5 hrs in Fig. 3 C (dashed lines) and found that they did not grow noticeably, suggesting that they were dead. Thus, there was a clear gap in fluorescence levels between cells that did not divide and had an unrepaired DSB, and the rest (Fig. 3 C, D). Furthermore, we compared the time when fluorescence increased and when nuclear division occurred and found that in all cells with dividing nuclei, fluorescence increased earlier than anaphase (examples of large differences between the two shown in Fig. 3 E). Thus, the DSB sensor provided direct information about whether cells had a DSB or not, which complements information provided by nuclear division. In the cells observed, anaphase was always preceded by rising YFP levels but YFP could increase without anaphase, i.e., repair could occur but the cell cycle fail, nevertheless.

As a first estimate of repair times, we recorded the time from budding to nuclear division by fluorescence microscopy with 10 min time resolution (Fig. S6). We verified that the *cdc5-ad* mutation neither affected repair timing nor repair efficiency negatively: We compared the division times of *cdc5-ad* cells with wild-type CDC5 cells in which YFP turned on, i.e., did not override, and found that they were comparable, 61% versus 51% (Fig. S6 A, B).⁹

A substantial fraction of *cdc5-ad* cells ($39\% = 100\% - 61\%$) did not divide compared to 3% of cell cycles failing under these conditions in a no-cut-site control strain (Fig. S6 A).

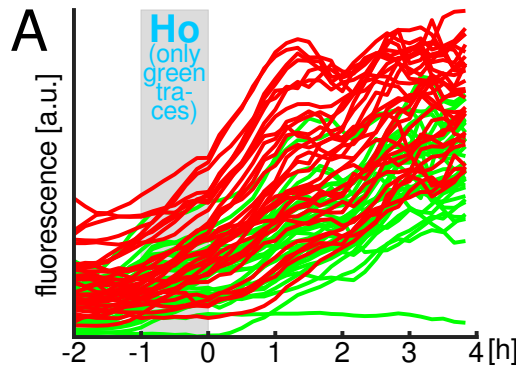


Fig. S5: A: Time course measurement performed as in Fig. 3 C but comparing fluorescence levels with and without HO induction. In addition to the cells that have been exposed to Raff+Gal+Met and perform nuclear division on time (green plots), we also show a control (red plots), in which cells were exposed to Raff+Met instead of Raff+Gal+Met in order to observe YFP dynamics in the absence of HO induction. Ignoring the plots in red that already start with higher fluorescence values before time point -1 h, the fluorescence time courses are very similar between the two groups. Unlike in Fig. 3 C, for this plot, we did not investigate the fate of the one low-fluorescence cell that did perform nuclear division on time.

Thus, approximately 36% (= 39% - 3%) failed to repair the DSB. (In agreement with these no-cut-site controls, we observed a division rate of 97% and mean bud-to-nuclear-division time of 64' (n=31) if we did not induce HO in the strain with the ADH1pr-HOcs-yEVenus-ADH1 construct, data not shown.)

The fraction of *cdc5-ad* cells that divided (61%) (Fig. S6 A) did not simply represent inefficient cutting by Ho. This is because only 31% of cells that additionally carried the *yku70Δ* (= *hdf1Δ*) deletion, which blocks the non-homologous end-joining (NHEJ) repair pathway⁵, divided, showing that 30% (= 61% - 31%) of cells repaired the DSB by a YKU70-dependent

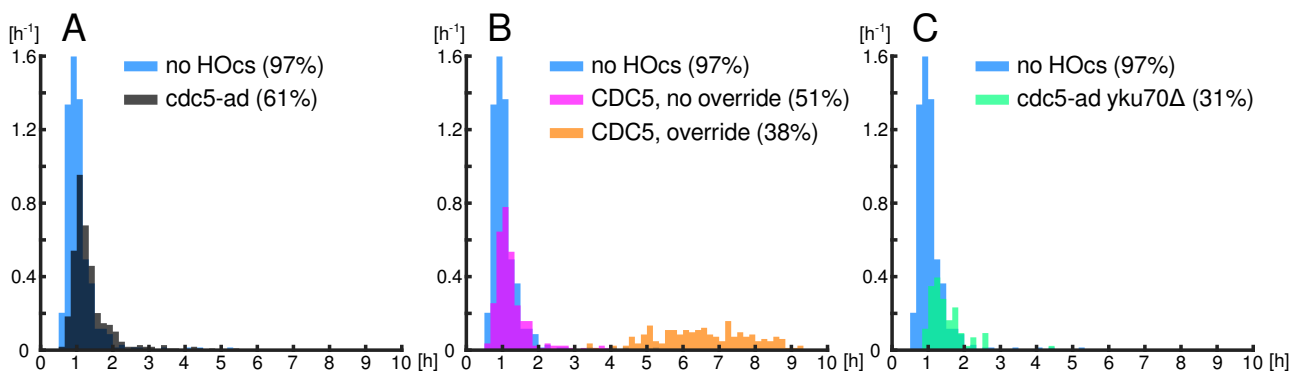


Fig. S6: Rate of nuclear division with respect to budding scored by fluorescence microscopy. The rate is calculated as the probability of anaphase within a particular time window divided by the size of the time bin. (Division by the size of the bin removes the arbitrary choice of bin size.) All divisions were accompanied by YFP increases except in panel B where dividing CDC5 cells could fail to turn on YFP (checkpoint override). Percentage in parentheses indicates the fraction of the budding cells of each genotype that divided nuclei. These cells' budding-to-nuclear-division times are shown in the histograms. The same no-HOcs control cells are shown in all three panels for comparison. n = 656 (*cdc5-ad*), 493 (wt-CDC5), 413 (no HOcs), 259 (*cdc5-ad yku70Δ*).

mechanism (Fig. S6 A, C).

The fraction of cells carrying a *yku70Δ* deletion that went through anaphase was higher than in previous studies^{35,45}. We speculated that this was due to a lower efficiency with which Ho cut the ADH1pr-HOcs-yEVENUS-ADH1 construct, possibly due to strong transcriptional activity at the ADH1 promoter. To investigate this hypothesis, we placed an HO cut site at URA3 instead. Indeed, only 1 in 200 cells with a *yku70Δ* deletion went through anaphase after 1 hour GAL1-HO induction, suggesting a high cut efficiency in line with values reported in the literature. Note, however, that cells with uncut DNA or early break repairs at the ADH1 locus, in any case, did not affect our measurements of late break repairs or of survival after override since we filter these cells out by flow cytometry, as discussed in the main text.

As expected, in the *cdc5-ad* population (Fig. S6 A) there were cells that divided late, i.e., later than 2 hours after budding, 6.1%, which was significantly ($p = 2 \cdot 10^{-3}$, one-tailed) higher than when there was no cut site, 2.7%, presumably representing late repair events. However, most YKU70-dependent repairs (NHEJ) clearly took place very quickly (Fig. S6 A, C).

Supplementary note 10 – Experiment – Differences with the results of Lee et al.¹³

We verified our override time measurements with two different DSB pairs, at ADH1 and URA3 or at MIC60 and DLD2 (Fig. 5 B, C). We pursued multiple explanations for the discrepancy between our results and those of Lee et al.¹³ First, we note the possibility that the fraction of post-override (1C DNA content) cells, which is small at any particular time point, was simply missed in past bulk culture DNA content measurements because the sampled time points were far apart, override is noisy for ≥ 2 DSBs (compare distributions for 1 DSB in Fig. 5 A and ≥ 2 DSBs in Fig. 5 B-E), and cells re-replicate DNA after override and remain arrested for long times. However, Lee et al.¹³ also measured microcolony formation, not just nuclear division. So, we performed the same assay with our strains. We observed that with one DSB at ADH1, 90% of cells that budded after HO induction at least budded one more time. In contrast, with two DSBs at ADH1 and URA3, only around 30% of cells with two DSBs rebudded on

galactose agar plates ($n = 30$ for both). Thus, microcolony formation with two DSBs is less frequent than nuclear division, suggesting that the discrepancies between our and previous¹³ data are not large according to this assay. To examine the potentially large discrepancy with respect to nuclear division experimentally, we focused on the strain YSL53 which was used in the 2-DSB measurements previously¹³. With our strains used in this study, we could induce cell cycle arrest and DSBs independently and then measure budding-to-nuclear-division times using fluorescent markers. To pursue the same experiments with the YSL53 genotype, we introgressed one of our *clnΔ* MET3pr-CLN2 HTB2-mCherry strains partially into YSL53. This was done by crossing our strain with YSL53 and crossing the progeny again into YSL53 for a total of four crosses. Then, we picked four different progeny strains, in which the cell cycle could be arrested, two DSBs induced, and budding and nuclear division monitored, as with our strains. Indeed, we found in all four progeny that checkpoint override (anaphase) did occur with two DSBs, even when partially introgressed into YSL53. This suggests that either nuclear division occurs in YSL53 in the absence of rebudding or, possibly, that YSL53 has an override-blocking property that is relieved after crossing. Finally, to eliminate most other differences between our and past experiments, we crossed the 1-cut-site strain JKM139 and the 2-cut-site strain YSL53 from the same past publication¹³. We selected progeny with two cut sites and of the same mating type as YSL53. Since these strains again lacked fluorescent markers, we only tested rebudding. In microfluidic chips, we observed rebudding in galactose medium in three of the seven progeny strains (25% ($n=20$), 15% ($n=41$), 10% ($n=20$)) with two DSBs but not in the parental YSL53 strain (0%, $n=23$). (As already mentioned, rebudding was generally weak with 2 DSBs, including in our strains.) We conclude that the failure of the YSL53 strain to rebud after checkpoint arrest is highly specific to that particular strain, and not generally true even in the same strain background as YSL53.

Our detection of override with at least up to four DSBs also brings the basic observations

about DNA damage checkpoint override more in line with each other. Override has been documented with difficult-to-quantify but plausibly large amounts of DNA damage due X-rays⁶ or telomere dysfunction in *cdc13-1*³⁷ or *tlc1 Δ* ⁹ cells. Our results revise the current view¹⁰ that different types of DSBs have different effects on override; in our measurements, varying numbers of DSBs led to quantitatively but not qualitatively different override times.

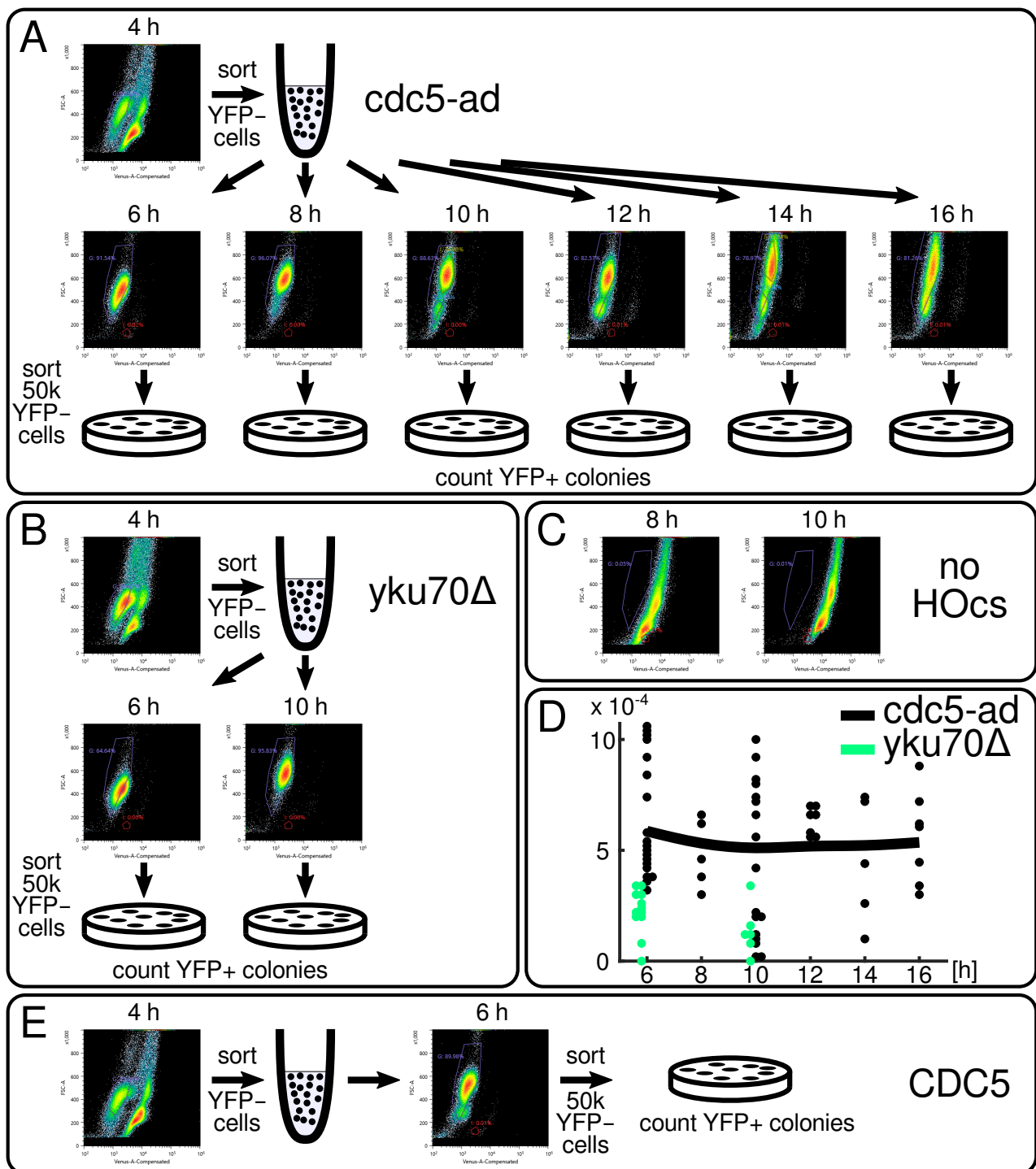


Fig. S7: Supplementary data for the FACS experiments. A-C, E: FACS gates and protocols for the indicated strains. D: Total fraction of colonies (YFP- and YFP+ together) relative to 50 000 YFP- cells that were plated. Same experiments as shown in Fig. 4 B but only the fraction of YFP+ colonies is shown in Fig. 4 B.

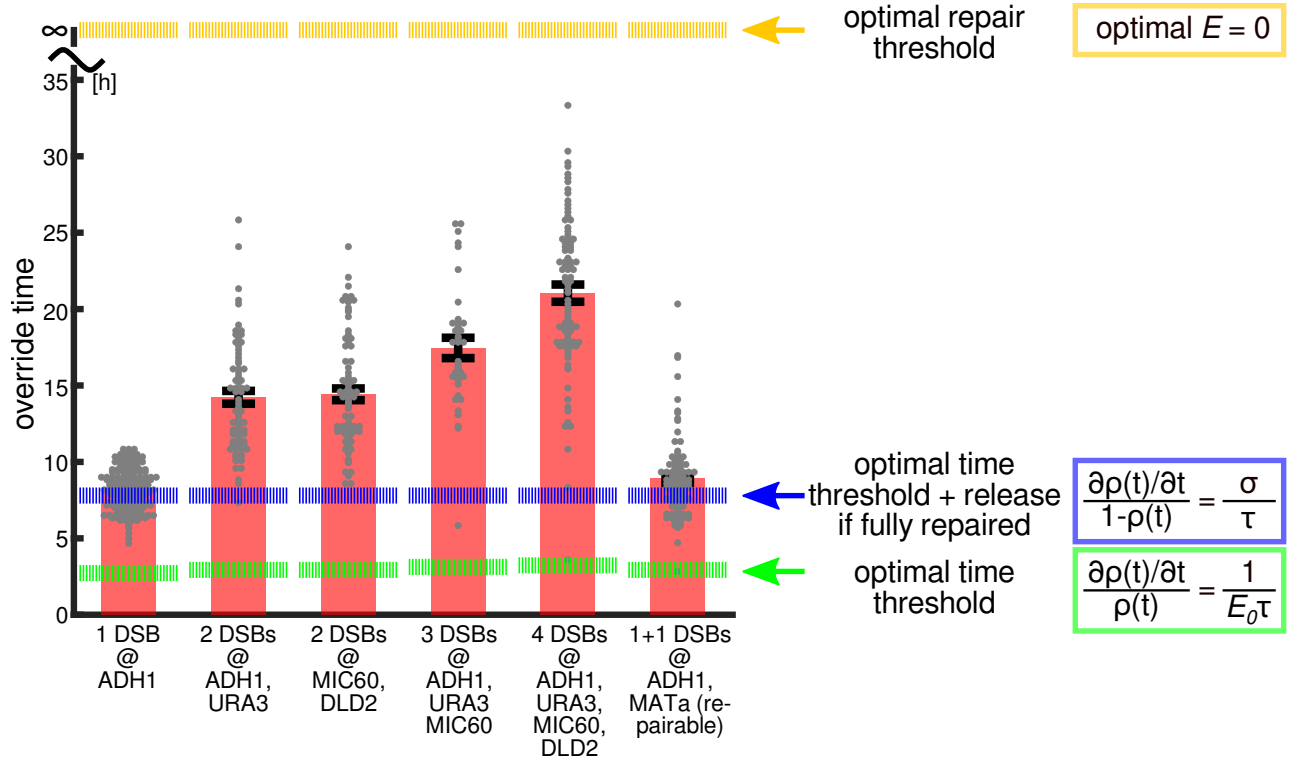


Fig. S8: Comparison of observed override times with alternative, non-optimal strategies. All experimental data as in Fig. 5 G. Dashed horizontal lines in different colors indicate the predictions of the various strategies. All models were solved to lowest order in the probability of survival after override $\sigma = 3.4 \cdot 10^{-5}$. In a time threshold strategy (Fig. 2 B), the cell waits a fixed amount of time before continuing with the cell cycle without feedback from repairs. To lowest order in σ , the optimal time threshold (green dashed lines) does not depend on σ (Eq. (S4)). Because the right-hand side of the equation in the green box is large, we had to use the repair statistics from microscopy data for early repairs (Fig. S6) for $\rho(t)$ to solve for the optimal time threshold. We augmented the optimal time threshold strategy in the “optimal time threshold + release if fully repaired” strategy (blue dashed lines) by allowing the cell cycle to continue if all errors are fixed. To lowest order in σ , this model does not depend on the initial number of errors E_0 . In a repair threshold strategy (Fig. 2 C), the cell waits for a fixed number of repairs but does not count time. Because the survival probability σ is small for DSBs, the optimal threshold (Eq. (S8)) evaluates to being at $E = 0$, when all errors are fixed. This means that in our experiments where at least one DSB always remained unrepaired, the cell should wait forever.

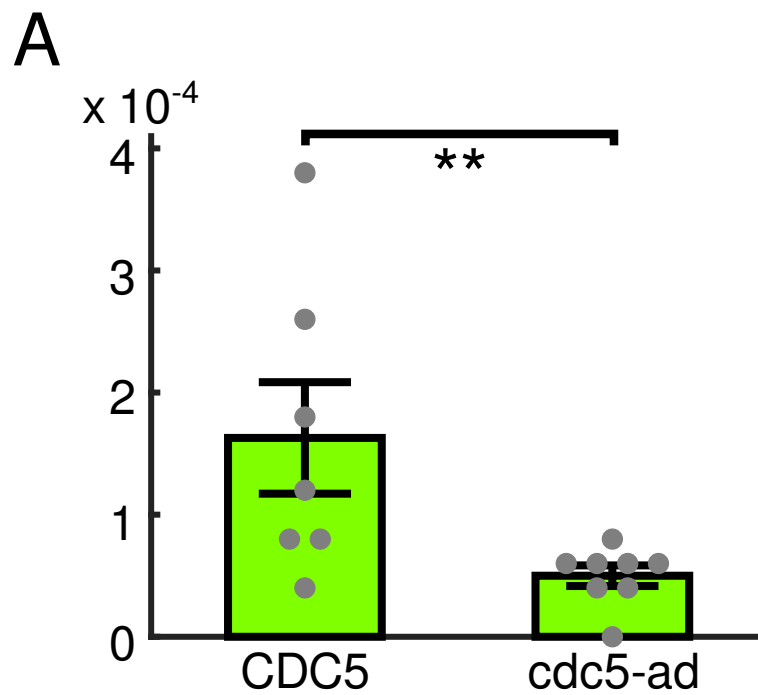


Fig. S9: A: Checkpoint override is an advantageous strategy, as determined by a direct comparison of survival probabilities for override-competent (CDC5) versus override-incompetent (cdc5-ad) cells carrying 1 DSB at the ADH1pr-HOcs-yEVENUS-ADH1 locus. Each grey circle represents the fraction of YFP+ colonies emerging after sorting for 50 000 YFP- cells at the 6 h time point, and plating on Glu-Met medium plates. Mean $\pm$ SEM shown. $p = 0.0075$, one-tailed. $n = 7$ (CDC5), 8 (cdc5-ad).

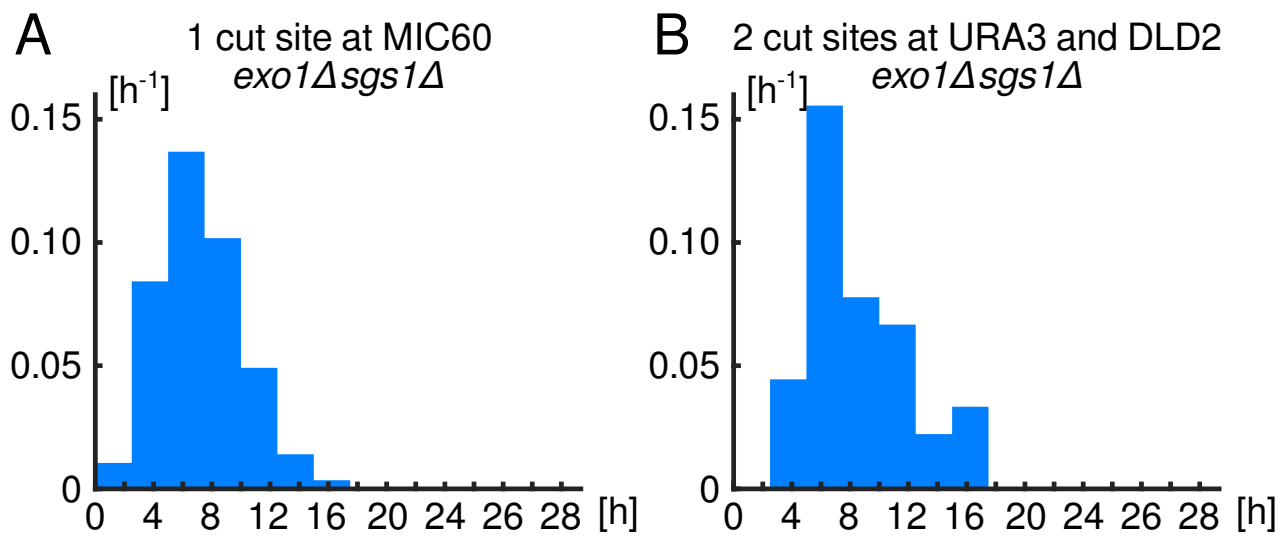


Fig. S10: Distribution of budding-to-nuclear-division times in Gal-Met medium in *exo1Δ sgs1Δ* cells. A: $n = 114$. B: $n = 36$.

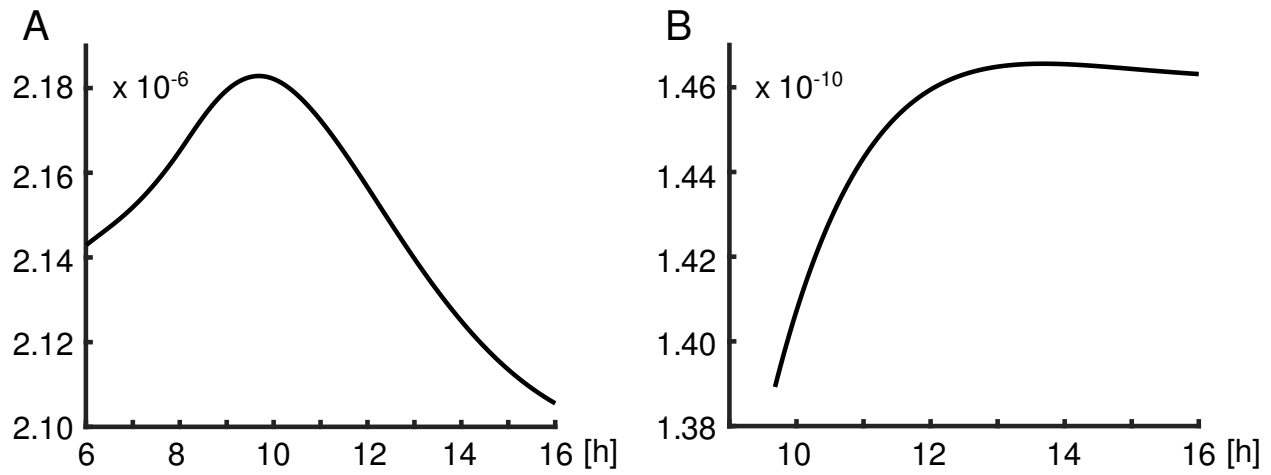


Fig. S11: The fitness (Eq. (1)) was evaluated for 1 (panel A) and 2 (panel B) DSBs using the experimental repair and survival probabilities. To evaluate the fitness function for 2 DSBs, we used the optimal time for 1 DSB. All integrals were evaluated starting at 6 h, which was the first time point of our flow cytometry data (Fig. 4).

Supplementary information references

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