SUPPORTING INFORMATION (SI) APPENDIX:

Multiscale effects of heating and cooling genes and gene networks

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1. Models for Temperature-Dependent Expression of a Single Gene

To investigate the constitutive expression of a single reporter gene as a function of temperature, we calculate the effective activation energies for both protein synthesis and cellular growth rate in analogy to classical theories of reaction kinetics (Arnaud et al, 2015; van Kampen, 1992).

Consider a single gene that produces protein constitutively at rate k. The protein decays at rate g. The collapsed network of reactions is:

$$\emptyset \xrightarrow{k(T)} P \xrightarrow{g(T)} \emptyset$$
 (1)

implying the differential equation:

$$\frac{dP}{dt} = k(T) - Pg(T)$$
(2)

We develop two types of models:

(i) in "growth rate models" we only consider temperature-dependence of g

$$\frac{dP}{dt} = k - Pg(T)$$
, which implies at steady state: $P(T) = \frac{k}{g(T)}$.

 (ii) in Arrhenius models, we additionally assume Arrhenius-type temperature dependence (see SI section 1.3) of protein synthesis rates.

$$\frac{dP}{dt} = Ae^{-\frac{E}{T}} - Pg(T)$$
(3)

This implies that at steady state:

$$P(T) = \frac{Ae^{-\frac{E}{T}}}{g(T)}$$
(4)

Note that in these models we consider Boltzmann's constant $k_B = 1$.

1.1. <u>Growth rate models of constitutive gene expression.</u>

We observe that the experimentally measured growth rates of resistant cells have a biphasic dependence on temperature. Below the optimum 30 °C growth temperature, growth rates had Arrhenius-type temperature dependence (Fig. S2):

$$g_b(T) = \Gamma e^{-\frac{G}{T}}$$
(5)

with *G* given by the slope and $\ln(\Gamma)$ by the y-intercept of the Arrhenius plot. Although a monophasic temperature-dependence of growth rates was recently reported (Benet et al, 2017) in *S. cerevisiae*, this may be due to a lower maximum temperature of 37 °C considered in that study (compared to 40 °C in our case) or to strain-specific grow rate dependence on temperature.

If we consider only this Arrhenius scaling of the growth rates, then the solution of such a "growth rate model" below $30 \,^\circ$ C will be:

$$P(T) = \frac{k}{g(T)} = \frac{k}{\Gamma e^{-\frac{G}{T}}}$$
 (6)

Above 30 °C, the growth rates depend approximately linearly on temperature (Fig. S2):

$$g_a(T) = aT + b \tag{7}$$

with *a* given by the slope and $\ln(\Gamma)$ by the y-intercept of growth rate-temperature plots. Therefore, the growth rate models predict that the protein expression above 30 °C will be:

$$P(T) = \frac{k}{g(T)} = \frac{k}{aT+b}.$$
(8)

1.2. <u>Arrhenius models of constitutive gene expression.</u>

If we also include Arrhenius-type protein synthesis rates in addition to temperature-dependent growth-rates into the above models, then we obtain an Arrhenius model. The temperature-dependence of gene expression levels below 30 °C in this Arrhenius model can be expressed as another Arrhenius relationship:

$$P_{b}(T) = \frac{A_{b}e^{-\frac{E_{b}}{T}}}{g_{b}(T)} = \frac{A_{b}e^{-\frac{E_{b}}{T}}}{\Gamma e^{-\frac{G}{T}}} = Qe^{-\frac{(E_{b}-G_{b})}{T}} = Qe^{-\frac{F_{b}}{T}}$$
(9)

from where in general $A = Q\Gamma$ and E = F + G.

Likewise, the temperature-dependence of gene expression levels in Arrhenius models above 30 ℃ could be expressed as:

$$P(T) = \frac{Ae^{-\frac{E}{T}}}{g_a(T)} = \frac{Ae^{-\frac{E}{T}}}{aT+b}$$
(10)

For all models, see Table S2 for parameter values and for corresponding figures numbers.

1.3. <u>Population dynamics of temperature-arrest in growing cell populations.</u>

Consider a growing cell population where some cells randomly arrest due to nonoptimal temperatures. Two subpopulations will emerge: resistant cells (R) and arrested cells (A).

The population dynamics can be described by the following set of "reactions":

$$R \xrightarrow{g_R} R + R$$

$$R \xrightarrow{r} A$$
(11)

and the corresponding ordinary differential equations (ODEs):

$$\frac{dC}{dt} = g_C C - rC$$
$$\frac{dA}{dt} = rC$$
(12)

This implies the following growth equation for the total number of cells:

$$\frac{dN}{dt} = \frac{d(R+A)}{dt} = g_R R < g_R N$$
(13)

Importantly, we can immediately conclude that the growth rate of the whole population must be different (smaller) than the growth rate g_R of resistant cells. Let us denote by g the apparent growth rate of the full population. Our goal will be to estimate g_R and r from g, R and A, which we can experimentally measure. The first equation is analytically solvable:

$$\frac{dR}{dt} = (g_R - r)R \Longrightarrow R(t) = R(0)e^{(g_R - r)t}$$
(14)

Substituting into the second equation gives the number of arrested cells:

$$\frac{dA}{dt} = rR(0)e^{(g_R - r)t} \Longrightarrow A(t) = \frac{r}{g_R - r}R(0)e^{(g_R - r)t}$$
(15)

Therefore, the total number of cells will be given by:

$$N(t) = R(t) + A(t) = R(0)e^{(g_R - r)t} + \frac{r}{g_R - r}R(0)e^{(g_R - r)t} =$$
$$= R(0)e^{(g_R - r)t} \left[1 + \frac{r}{g_R - r}\right] = \frac{g_R}{g_R - r}R(0)e^{(g_R - r)t}$$
(16)

The exponential growth of the whole population can also be described by:

$$N(t) = N(0)e^{gt}$$
 (17)

This implies:

$$N(t) = \frac{g_R}{g_R - r} R(0) e^{(g_R - r)t} = N(0) e^{gt}$$
(18)

$$\ln\left[\frac{g_{R}}{g_{R}-r}R(0)\right] + (g_{R}-r)t = \ln[N(0)] + gt$$
. (19)

In the long-term limit $(t \rightarrow \infty)$, we get:

$$g_R = g + r \tag{20}$$

Therefore, the growth rate g_R of resistant cells is equal to the sum of the arrest rate *r* and the apparent growth rate *g* of the whole population.

The fraction of arrested cells will be given by the ratio:

$$f_{A} = \frac{A(t)}{N(t)} = \frac{\frac{r}{g_{R} - r} R(0) e^{(g_{R} - r)t}}{\frac{g_{R}}{g_{R} - r} R(0) e^{(g_{R} - r)t}} = \frac{r}{g_{R}}$$
(21)

where f_R is the fraction of resistance cells. From here, we obtain another relationship between r and g_R :

$$r = f_A g_R \tag{22}$$

Finally, we find for g_R :

$$g_R = g + f_A g_R \Longrightarrow g_R = \frac{g}{1 - f_A} = \frac{g}{f_R}$$
(23)

Likewise, the arrest rate *r* will be given by:

$$r = f_A g_R = \frac{f_A}{1 - f_A} g = \frac{f_A}{f_R} g$$
(24)

We note accounting for dying cells may result in higher R-cell growth rates.

2. Model of Temperature-Dependent Gene Expression for the NF Gene Circuit

To study the temperature-dependence of gene expression for NF cells, we developed growth rate models and Arrhenius models as described above. We found that growth rate models did not match the experimental data even for uninduced gene circuits. Therefore, we proceeded with the approach outlined in this section, to develop Arrhenius models of gene expression for synthetic gene circuits.

Starting with the established set of ODEs for the NF gene circuit:

$$\frac{dx}{dt} = aF(x) + l - bxy - g(T)x$$

$$\frac{dy}{dt} = C - bxy - g(T)y - fy$$

$$\frac{dr}{dt} = bxy - g(T)r$$

$$\frac{dz}{dt} = aF(x) + l - g(T)z$$
(25)

we described the temperature dependence of the reaction rates for protein synthesis *a*, leaky protein synthesis rate *l*, inducer/repressor-protein binding *b*, inducer influx *C*, and inducer degradation rate *f*, in addition to the dependence of growth rates g(T). For each parameter k_i obtained for 30 °C, we used the Arrhenius equation to scale its value as a function of temperature

$$k_i = A_i e^{\frac{-E_i}{RT}}$$
(26)

where A is the pre-exponential factor, E is the activation energy, R the gas constant, and T the temperature. As A and E were unknown for the reactions in the above systems, we adopted a

method proposed by Ruoff *et al.* (Ruoff et al, 1997) which successfully captured the effect of temperature on biological oscillators (Heiland et al, 2012; Ruoff et al, 1997). Thus, we normalized each k_i ($T \neq 303.15$ K) = $k_{i,2}$ by the corresponding reaction rate at 30 °C, k_i (T = 303.15 K) = $k_{i,1}$ to obtain:

$$\frac{k_{i,2}}{k_{i,1}} = \frac{A_{i,2}e^{\frac{-E_{i,2}}{RT_2}}}{A_{i,1}e^{\frac{-E_{i,1}}{RT_1}}}$$
(27)

If we assume that A and E are independent of temperature (Heiland et al, 2012; Ruoff et al, 1997) then the above expression becomes:

$$k_{i,2} = k_{i,1} e^{\frac{-E_i}{R} \left(\frac{1}{T_2} - \frac{1}{T_1}\right)}$$
(28)

This formula describes the reaction rates as a function of temperature. The activation energy is obtained by re-arranging Equation 28:

$$E_{i} = R \frac{\ln \binom{k_{i,2}}{k_{i,1}}}{(T_{2} - T_{1})/T_{1}T_{2}}$$
(29)

Which, when $T_2 = T_1 + 10^{\circ}C$ and $Q_{10,i} = k_{i,2}/k_{i,1}$, simplifies to:

$$E_{i} = R \frac{T_{1}T_{2}}{10} \ln \left(Q_{10,i} \right)$$
(30)

where $Q_{10,i}$ is the temperature coefficient, which is a measure of the fractional change of reaction rate k_i with a 10 °C increase in temperature. To avoid non-physiological activation energies, we constrained Q_{10} between 2 and 3 in each reaction, which is the accepted range of values for the temperature coefficient for most biological reactions (Heiland et al, 2012; Reyes et al, 2008). This corresponded to a range of activation energies between 55 kJ/mol and 87 kJ/mol. As a basal temperature, we used 303.15 K (30 °C) which corresponds to the control (or standard) temperature applied in this experimental investigation of the effect of temperature on synthetic circuit function.

3. Stochastic Simulations of the NF Gene Circuit

To model the effect of temperature on gene expression variability, we translated the deterministic rate equation model for the NF gene circuit (Eqn. 2 in the main text) into the stochastic framework. As in the corresponding modified rate equation model, the stochastic model incorporates the fraction of doxycycline bound TetR protein molecules *m* that can still bind to tetR and yEGFP::zeoR (GAL1-D12) promoters into the repressor-dependent protein synthesis function. Consequently, $F(x) = \theta^n / [\theta^n + (x)^n]$ becomes $F(x+mr) = \theta^n / [\theta^n + (x + mr)^n]$. We chose the value for *m* qualitatively for each temperature to reflect the fraction of tetR-Dox complex that could still bind DNA, as predicted from MD simulations (Fig. 4A and SI section 4). This model can be described by the following reactions:

where, $k_1 = l + aF(x+mr)$, $k_2 = g$, $k_3 = C[dox]$, $k_4 = g + f$, $k_5 = (b*10^9)/(V*N_A)$, $k_6 = g$, $k_7 = l + aF(x+mr)$, $k_8 = g$. The cell volume *V* was set to 2.16 × 10⁻¹³ L (Phillips et al, 2008) and N_A is Avogradro's number (6.022 × 10²³ mol/L). All other parameters were obtained from the modified rate equation model. Results for stochastic simulations were obtain using the Gillespie stochastic simulation algorithm (Gillespie, 1976) (20 realizations of 1000 runs, where each run represents one cell in the population).

4. Molecular Dynamics Simulations

We based the model of TetR (class D) bound to DNA on the X-ray crystal structure PDB ID: 1QPI (Orth et al, 2000). We used the biological unit to obtain the homodimer:DNA complex. Each monomer of TetR was missing an eight-residue loop (between Leu155 and Glu164). We reconstituted this missing loop using a homology model subsequently relaxed using minimization and MD, merging the loop with TetR without adding strain (Fig. S14). The inducer binding pocket was occupied by imidazole. We thus removed imidazole and docked doxycycline: Mg2+ instead by merging the coordinates with a second TetR structure (Hinrichs et al, 1994) containing Doxycycline: Mg2+ (see section 4.3 in SI Appendix). We added three base pairs of GCG to the termini of the DNA to reduce end-fraying (Galindo-Murillo et al,

2014) by RMS-aligning the phosphate linkages to those of the DNA in the above model and merging the coordinates. This resulted in a holo TetR model (+DNA/+dox, i.e. bound to DNA, "+DNA" and bound to doxycycline, "+dox"). The apoTetR model (-DNA/+dox) was the result of unmerging DNA from the above model; the holo TetR model (+DNA/-dox) was the result of unmerging dox from the above model. A truncated octahedron with 10 Å buffer of explicit water and (0.2 M) KCl ions solvated these two systems. We used TIP3P (Jorgensen et al, 1983) parameters for water, Joung-Cheatham (Joung & Cheatham, 2008) parameters for K+ Cl- ions, Li et al. (Li et al, 2013) parameters for Mg2+, ff99SB parameters (Hornak et al, 2006) for protein and parmBSC0 parameters (Perez et al, 2007) for DNA (Hauser et al, 2016). We used an 8 Å non-bonded cutoff; and PME (Essmann et al, 1995) to calculate long-range electrostatics. We performed equilibration using a previous ten stage approach (Hauser et al, 2016) (Table S1), with three independent runs at three temperatures (12°C, 30°C and 38°C). After equilibration, we performed 200 ns of production MD (3.6 μ s total MD). Based on previous work with MTERF1 (Hauser et al, 2016), we obtained TetR superhelical pitch from the coordinates of the C α atoms of conserved Pro residues tracking the DNA major groove. TetR superhelical pitch is equivalent to the linear distance between Pro36 (DBD of monomer A) and Pro239 (DBD of monomer B).

4.1 System preparation

The tetracycline repressor (TetR) protein is a homodimer. For DNA binding each monomer inserts its N-terminal 40-residue DNA-binding domain (DBD) into the major groove of the DNA operator. While an experimentally characterized crystal structure for TetR is available in the Protein Data Bank, it is missing an 8-residue loop gating the binding pocket into which inducer (doxycycline) would bind. An imidazole molecule is bound in the inducer-binding pocket of the crystal structure. The DBDs bind DNA in major groove sites separated by one helical turn of DNA (i.e. separated by ~360° revolution of the helix). To prepare this experimental structure for MD simulations the following had to be modeled: (i) Remove imidazole from the binding pocket and dock doxycycline, and (ii) Construct and insert the missing loops between amino acids Leu155 and Glu164.

4.2 Construction and insertion of the missing loop

We modeled the eight-residue loop missing from the crystal structure of the TetR:DNA complex (PDB ID: 1QPI (Orth et al, 2000)] by taking a loop from a second crystal structure of TetR [PDB ID: 4D7M (Werten et al, 2014)], and optimizing the ends of this loop to bring the position of the common atoms (atoms resolved in both structures; loop-adjacent atoms) into alignment with those of 1QPI. This ensured that inserting the atomic coordinates of the missing loop did not introduce strain. The loop-adjacent segments used in this optimization comprised amino acids 127 to 155 (N-terminal end of the loop) and amino acids 164 to 179 (C-terminal end of the loop). We forced the coordinates of the C α , N and C atoms of these residues to adopt the structure of the corresponding atoms in the 1QPI structure (since these loop-adjacent atoms were present in the structure) using positional restraints to the 1QPI structure. We

prepared this 51 amino acid segment utilizing a system preparation protocol previously established for protein structure sampling using implicit solvent (Nguyen et al, 2014), except that we used the force field ff99SB (Hornak et al, 2006) instead of ff14SB (Maier et al, 2015), to be consistent with our earlier protein-DNA simulation protocol (Hauser et al, 2016) which we also used in this work.

Next, we merged the coordinates of the eight-residue loop contained within the 51 amino acid peptide optimized above into the structure of TetR (1QPI). We chose the optimal set of coordinates for insertion from the final two stages of MD such that the resulting amide bond distances between the 1QPI recipient structure and the loop model would have unstrained values (1.31 Å between the C atom of Thr152 and the N atom of Ala153; 1.33 Å between the atom of C of Glu164 and the atom of N of Asn165). In this way, we were able to insert the coordinates of the optimized loop (Leu155 to Glu164) into both monomers of 1QPI. To check that insertion of this loop did not introduce strain, we performed MD simulations (methods detailed below) on the resulting model. We expected that the length of the loop should change in lock-step with a change in TetR structure if the loop was releasing strain. We monitored this distance by measuring the distance between the Ca atoms of the loop-adjacent amino acids Thr152 and Asp164 (Fig. S14A,B). Lack of a biased change in loop length, indicated that the loop had not introduced strain into the protein. As expected based on this result, we observed no correlation between the change in loop length and protein structure (RMSD) for the three independent runs (Fig. S14C-H). We obtained similar results for the simulations in which DNA was removed (apoTetR).

4.3 Building doxycycline into the model of TetR

We obtained the coordinates of doxycycline from a crystal structure of TetR (PDB ID: 1TRT (Hinrichs et al, 1994). We merged these coordinates into the TetR:DNA complex model prepared above (1QPI) using a structure-based approach, which was previously used to insert tetracycline and Mg²⁺ in the binding pocket of TetR (Seidel et al, 2007). To align the binding pockets, we used the backbone atoms Ca, N, C and O in the following four segments enclosing the binding pocket: Arg46 to Ala61, Trp72 to Glu90, Ala97 to Leu101 and Arg128 to Ala153. Once the binding pockets were aligned, we transferred the coordinates of doxycycline, along with a coordinated Mg²⁺ ion, from 1TRT into the TetR:DNA complex. We generated doxycycline partial atomic charges using am1bcc (Jakalian et al, 2002). We used the general amber force field (Wang et al, 2004) for the remaining parameters of doxycycline.

4.4 MD equilibration and production protocol

We equilibrated two systems at three temperatures. We simulated each of these six systems in triplicate (independent runs) by initializing dynamics with different randomized velocities subject to the Maxwell-Boltzmann distribution. We used a protocol identical to the one in our previous work with MTERF1 (Hauser et al, 2016) to equilibrate these systems (Table S1). The sole difference was restraining the atoms during equilibration. However, we did not restrain the sidechain of Trp40 due to improper rotamer assignment in the crystal structure. Likewise, we did not restrain the three base pairs of GCG added to

the DNA termini. We defined the DNA backbone as previously (Hauser et al, 2016) - atom names C1', C2', C3', C4', O3', O4', O5', OP1, OP2, P.

4.5 Geometric analysis of simulations

We determined the structural stability of the DBDs by calculating the RMSD of each DBD (separately to remove rotation and translation between the DBDs) using the Ca atoms of residues 1 to 39 (DBD in monomer A) or the Ca atoms of residues 204 to 242 (DBD in monomer B). The equilibrated structure served as the reference structure against which we compared each snapshot in the trajectory (after aligning first to minimize the RMSD). Figure S15 shows the time course of the RMSDs for the DBDs of apoTetR (-DNA/+dox) at three temperatures. At 12 °C and 30 °C the DBDs of each monomer remain very stable with rare excursions beyond 3 Å (monomer B / 12 °C / run1) and (monomer B / 30 °C / run3). However, the simulations at high temperature (38 °C) are skewed slightly to higher RMSDs, as one would expect for system with higher temperature. Despite this gradual increase in flexibility, the RMSDs remain low (< 3 Å) for the simulations at 38 °C, consistent with stable DBD structure observed during MD.

We calculated the RMSD of doxycycline (using scaffold atoms C11, C6A, C6B, C6, C5A, C5B) and the binding pocket of TetR (using the Ca atoms of Arg46 to Ala61, Trp72 to Glu90, Ala97 to Leu101 and Arg128 to Ala153) to show that the inducer remained bound to TetR. As above, we used the equilibrated structure as the reference for the RMSD analysis. Figure S16 shows the time course of the RMSDs of doxycycline in the binding pocket structure for the apoTetR (-DNA/+dox) simulations at the three temperatures. Because these MD simulations were unable to reach the ms-s timescales likely to be important for reaching the equilibrium of TetR-doxycycline binding up to unbinding. However, it is also possible that TetR-doxycycline binding is strengthened by permitting the ligand to skip between microstates in the pocket that are individually favorable enthalpically; since multitudes of readily accessible microstates are generally entropically favorable.

5. Supplemental Figures



Figure S1. The effect of temperature on growth of the NF0 and NF populations expressing yEGFP::zeoR.

(A) Growth rate of the full NF0 cell population as a function of temperature.

(B) Growth rate of the resistant NF0 (R-cell) subpopulation as a function of temperature.

(C) Growth rate of the full NF cell population as a function of temperature.

(D) Growth rate of the resistant NF (R-cell) subpopulation as a function of temperature.

Error bars are SEM (N = 3).



Figure S2. Temperature-dependence of resistant cell growth rates in the single-reporter NF0 and uninduced NF strains.

(A) The effect of temperature on growth rate of resistant NF0 cells.

(B) The effect of temperature on growth rate of resistant uninduced (doxycycline = 0 μ g/ml) NF cells. R^2 values for corresponding $\ln[g(T)]$ versus 1/T plots for NF0 and uninduced NF strains were 0.987 and 0.952, respectively. We used an Arrhenius model below (blue dashed line) and an linear model above (red dotted line) the optimal temperature (30 °C). We did not use the Arrhenius model above the optimal temperature (red dashed line) in our models due to the poor fit to experimental data (black circles). See Table S2 for parameters.



Figure S3. The effect of temperature on population-level gene expression the single reporter NF0 strain.

(A) Experimental and Arrhenius model of temperature dose response of the population-level gene expression (GFP) of yEGFP::ZeoR expression.

(B) Experimental temperature dose response of the population-level coefficient of variation (CV) of yEGFP::ZeoR expression.

Error bars are SEM (N=3).



Figure S4. Modeling the effect of temperature-dependent growth rates on the single reporter NF0 strains. All the results in this figure were generated using the growth rate model. (A) The effect of temperature on reporter-resistance protein expression in NF0 cells using the temperature-dependent dilution rate *g* determined from an Arrhenius fit to the growth rate data (Fig. S2A). (B) The effect of temperature on reporter-resistance protein expression in NF0 cells using the estimated temperature-dependent growth rates of resistant (R) cells g_R (Fig. S1B). See Table S2 for parameter values.

Figure S5. Doxycycline does not affect YPH500 cell growth. The YPH500 strain is the "ancestor" from which the NF0, NF, and PF strains were derived. The growth rates were normalized by the corresponding growth rates of replicates in the control condition (no doxycycline) for each experiment.

Figure S6. The effect of temperature and inducer on NF and PF strain growth.

(A) Growth rate as a function of varying inducer (doxycycline) concentrations at 12°C, 30°C, and 38°C for NF cells.

(B) Full PF population growth rate as a function of varying inducer concentrations at 12 °C, 30 °C, and 38 °C.

(C) Growth rate as a function of varying inducer concentrations at 12 $^{\circ}$ C, 30 $^{\circ}$ C, and 38 $^{\circ}$ C for individual NF replicates.

(D) Growth rate as a function of varying inducer concentrations at 12° C, 30° C, and 38° C for individual PF replicates.

Error bars are SEM (N = 3).

Figure S7. The effect of inducer at each temperature on NF and PF full population growth. (A) Growth rate of NF cells as a function of varying inducer (doxycycline) concentrations at 12° C, 30° C, and 38° C.

(B) Growth rate of PF cells as a function of varying inducer concentrations at 12° C, 30° C, and 38° C. For both NF and PF strains, growth rate at all inducer concentrations was normalized by the corresponding growth rate value at doxycycline = 0 µg/ml for each temperature condition. Error bars are SEM (*N* = 3).

Figure S8. The effect of inducer and temperature on NF population-level gene expression dose responses.

(A) Dose-response of the population average yEGFP::ZeoR expression. Inset shows the doxycycline concentration at which GFP half-saturation (K_m) occurs as a function of temperature.

(B) Dose-response of the population-level coefficient of variation (CV) of yEGFP::ZeoR expression. Error bars are SEM (N = 3).

Figure S9. Temperature-dependent dose-response curves for population-level expression from growth-arrest model for NF gene circuit.

(A) Dose-response curves from ODE model and corresponding stochastic simulations of the mean of yEGFP::ZeoR expression.

(B) Dose response of the coefficient of variation (CV) obtained from stochastic simulations of yEGFP::ZeoR expression.

Error bars are SEM (N = 3).

Figure S10. The effect of temperature on NF and PF gene circuit expression dose responses without normalization.

(A) NF dose-response of the population average (mean) of yEGFP::ZeoR expression.

(B) PF dose-response of the mean of yEGFP::ZeoR expression.

Data points from a subsequent dose-response experiment, performed to characterize the CV peak at 12 °C are shown in cyan. Error bars are SEM (N = 3).

Figure S11. NF0 and NF population-level GFP and growth rate changes resulting from exposure to high temperature are reversible.

(A) Growth rates of NF0 and NF cells induced with 2 μ g/ml of doxycycline and batch-cultured for 48 hours at 38 °C, followed by 48 hours of batch-culture at 30 °C match those of control replicates continuously cultured at 30 °C for 96 hours. Error bars are SEM (N = 3).

(B) yEGFP::ZeoR distributions from an NF0 cell population and an NF cell population induced with 2 μ g/ml of doxycycline and batch-cultured for 48 hours at 38 °C followed by 48 hours of batch-culture at 30 °C are indistinguishable from those of control replicates continuously cultured at 30 °C for 96 hours. Representative yEGFP::ZeoR distributions from control and experimental conditions are shown.

Figure S12. NF0 and NF GFP flow cytometry sorting experiments show that growth-arrested A-cells are viable.

(A) Growth rates for low-sorted (A-cells below the median of the low-expressing subpopulation) and unsorted NF0 cell populations at 30 °C after being cultured for 48 hours in 38 °C (transfer from 38 °C condition to 30 °C condition corresponds to time = 0 hrs in figure). Error bars are SEM (N = 3). (B) Growth rates for low-sorted and unsorted NF cell populations at 30 °C after being cultured for 48 hours in 38 °C (transfer from 38 °C condition to 30 °C condition to 30 °C condition to 30 °C condition corresponds to time = 0 hrs in figure). Error bars are SEM (N = 3).

(C) Representative steady-state yEGFP::ZeoR distributions for sorted and unsorted NF0 cell populations.

(D) Representative steady-state yEGFP::ZeoR distributions for sorted and unsorted NF cell populations. All sorted and unsorted NF replicates were induced throughout the entire experiment using 2 µg/ml of doxycycline.

Figure S14. Loop modeling did not affect the dynamics of holo TetR-dox (+DNA, +dox).

(A) The distance between the C α atoms of Leu155/Glu164 in monomer A.

(B) The distance between the C α atoms of Leu155/Glu164 in monomer B.

The X-axis shows MD time (10 ps^{-1}) in which the first data point is the X-ray structure for initializing the simulations. The red, blue, and black dots denote loop attachment distances for each snapshot of three independent MD simulations. Vertical lines: time when positional restraints were released. **(C,E,G)** The correlation between the RMSD of monomer A and the separation distance in **(A)** for three

independent MD simulations.

(D,F,H) The same as (C,E,G), but for monomer B.

Figure S15. RMSD of the DBDs in monomer A and B for the apoTetR (-DNA/+dox) simulations at three temperatures (12° C, 30° C and 38° C).

MD simulation time is plotted on the X axis; protein backbone RMSD is plotted on the Y axis. Three independent simulations were performed at each temperature; they are denoted run1 (black), run2 (blue) and run3 (green).

Figure S16. RMSD of the position of doxycycline in the TetR binding pocket of monomer A and monomer B for the apoTetR (-DNA/+dox) simulation at three temperatures (12°C, 30°C, and 38°C). MD simulation time is plotted on the X axis; RMSD is plotted on the Y axis. Three independent simulations were performed at each temperature; they are denoted run1 (black), run2 (blue) and run3 (green).

Figure S17. Induced gene expression histograms at high temperature after 48 hours from high doxycycline range experiment.

Many doxycycline (dox) concentrations between 4 μ g/ml and 50 μ g/ml failed to eliminate the lowexpressing subpopulation. Representative histograms from experiments performed in triplicate are shown.

Figure S18. PF yeast strain temperature-dependent growth-arrest fractions and R-cell growth rates.

(A) Fraction of growth-arrested low-expressing PF cells as a function of temperature. (B) Resistant cell (R-cell) subpopulation growth rates of PF cells as a function of temperature and doxycycline concentration calculated from data in (A) and Figure S6B. Error bars are SEM (N = 3).

Figure S20. The effect of temperature on PF and NF dose-response gene expression histograms after 24 hours.

Fluorescence histograms of yEGFP::ZeoR expression for the PF strain at increasing doxycycline concentrations at (A) 12 °C, (B) 30 °C, and (C) 38 °C. Fluorescence histograms of yEGFP::ZeoR expression for the NF strain at increasing doxycycline concentrations at (D) 12 °C, (E) 30 °C, and (F) 38 °C.

Figure S21. PF and NF low temperature (12 ℃) dose response after 72 hours. (A) Fluorescence histograms of yEGFP::ZeoR expression for the PF strain for a doxycycline concentration range of 0-4 µg/ml.

(B) Fluorescence histograms of yEGFP::ZeoR expression for the NF strain for a doxycycline concentration range of 0-6 μ g/ml.

6. Supplemental Tables

Stage	Ref	Group	Ensemble	Temp (K)	Force constant (kcal/mol Å ²)	EOM	Steps (x 10 ³)
1	xtal	Х	-	-	100	min	10
2	1	Х	NVT	100/300	100	MD	100
3	2	Х	NPT	300	100	MD	100
4	3	Х	NPT	300	10	MD	250
5	4	Y	-	-	10	min	10
6	5	Y	NPT	300	10	MD	100
7	6	Y	NPT	300	1	MD	100
8	7	Y	NPT	300	0.1	MD	100
9	-	-	NPT	300	0	MD	1000

Table S1. MD equilibration protocol.

Ref, reference coordinates (xtal: 1QPI). EOM: min is minimization; MD is molecular dynamics. Ensemble: NPT and NVT used Berendsen barostat and thermostat (Berendsen et al, 1984). Group, atoms that were restrained to the reference structure (Ref): X includes all heavy atoms in the xtal; Y includes only backbone atoms in the xtal (see Tables S3 and S4 for system-dependent details). Force constant, positional restraint force constant. 0.1 ps bath coupling constants were used in stage 2 and 3; 0.5 ps coupling constants were used in subsequent stages.

Model	Strain	Temperature	Parameter	Symbol	Value
Arrhenius/ linear growth rate models	NFO	≤ 30°C	Activation energy (growth rate)	G _{NF0}	9.0489 kK
			In(Pre- exponential factor) (growth rate)	$\ln(\Gamma_{NF0})$	28.3183
			Activation energy (protein synthesis rate)	E_{NF0}	12.4794 kK
			In(Pre- exponential factor) (protein synthesis rate)	$\ln(A_{NF0})$	-0.0550
		≥ 30°C	Activation energy (growth rate)	G_{NF0}	2.4005 kK
			In(Pre- exponential factor) (growth rate)	$\ln(\Gamma_{NF0})$	-7.9
			Slope of linear function (growth rate)	a _{NF0}	-0.0221 h ⁻¹ K
			y-intercept of linear function (growth rate)	$b_{\rm NF0}$	6.6480 h ⁻¹
	NF (uninduced)	≤ 30°C	Activation energy (growth rate)	G_{NF}	1.3472 kK
			In(Pre- exponential factor) (growth rate)	$\ln(\Gamma_{NF})$	4.3
		≥ 30°C	Slope of linear function (growth rate)	a_{NF}	-0.0280 h ⁻¹ K
			y-intercept of linear function (growth rate)	b_{NF}	8.7612 h ⁻¹
			Activation energy (growth rate)	G_{NF}	8.2648 kK
			In(Pre- exponential factor) (growth rate)	$\ln(\Gamma_{NF})$	-27.2

Growth-arrest	NF (induced)	12°C for [dox]	Resistant cell	g(T)	[0.0358.
Arrhenius	(= [0, 1.5 3 4.5]	(R-cell) growth	0()	0.0198.
		6] µg/ml	rates		0.0255.
		*Additional			0.0267.
		values via			0.02621
		"intern1"			h ⁻¹
		(linear) in			
		Matlab			
		30°C for [dox]			[0 2522
		$= [0 \ 1 \ 5 \ 3 \ 4 \ 5$			0 2494
		6] ug/ml			0.2434,
		*Additional			0 2339
		values via			0 2114]
		"intern1"			h^{-1}
		(linear) in			
		Matlab.			
		38°C for [dox]			[0.03683
		= [0, 1.5 3 4.5]			0.0263 0.0350
		6] µg/ml			0.0364
		*Additional			0.02761
		values via			h ⁻¹
		"interp1"			
		(linear) in			
		Matlab.			
		12°C and 38°C	Activation	Е	[-87 -86 -62 -
		for [dox] = [0,	energy		69 -61]
		0.25, 0.5, 1, 2,			kJ/mol
		4] μg/ml			
		30°C	Activated	а	11.6481 nM h ⁻
			protein		1
			synthesis rate		
			Repression	θ	0.4323 nM
			threshold		
			Hill coefficient	п	4
			dox-TetR	b	9.8173 nM ⁻¹ h ⁻
			binding rate		1
			Inducer	f	0.0042 h ⁻¹
			degradation		
			rate		
			doxycycline	С	2.8459 [dox]
			influx rate		h ⁻¹
			Leaky protein	l	0.3225 nM h ⁻¹
			synthesis rate		
Revised	NF (induced)	[12°C, 30°C,	Fraction of dox-	т	[0, 0, 0.01]
growth-arrest		38°C]	TetR that can		
Arrhenius			still bind GAL1-		
			D12 promoter		

 Table S2. Parameter values for models from main text and SI. Blank squares in table denote the same label or value as the square above. Parameter values for low and high temperatures were obtained by using the Arrhenius equation.

7. Supplemental Movie

Movie S1 Legend: Movie recorded on Nikon Ti-E a inverted fluorescence microscope by time-lapse imaging of NF yeast cells (bright field and FITC channels) induced with dox = 2 ug/ml in SDGal media, maintained at a constant temperature of 38°C in a modified "high-throughput yeast aging analysis" (HYAA) microfluidic chip (Ref. [49] in the Main Text). The following phenomena are visible in the movie: (i) R-cells expressing yEGFP::zeoR and actively dividing; (ii) R-cells converting into growth-arrested A-cells with expanding vacuoles and gradually diminishing fluorescence; (iii) dying A-cells with vanishing fluorescence.

8. Supplemental References

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