Supplementary Figure 1. Dose-response curves of GFP vs. L-ara with the initial state ‘ON’ or ‘OFF’ shows no hysteresis. The steady-state level of GFP/OD as a function of L-ara concentration with initial state ‘ON’ (red curve) and ‘OFF’ (blue curve). The ‘ON’ cells were pretreated with a high dose of L-ara (2.5x10^{-3}%), and then diluted into fresh medium. Data indicate mean±s.d. (n=3).
Supplementary Figure 2. The activation dynamics of the SA-circuit. Dynamics of GFP/OD after 1:100 dilution of ‘OFF’ cells into fresh medium with various concentrations of L-ara. Data indicate mean±s.d. (n=3).
Supplementary Figure 3. Memory loss of the SA-circuit was confirmed with a dilution of cells in the exponential phase into fresh medium. a-b, The dynamics of cell growth (OD) and GFP/OD after dilution of cells that were activated to different levels at OD=0.3~0.7 as indicated into fresh medium with high-dose (2.5*10^{-3}%)(a) or without L-ara (b). Data indicate mean±s.d. (n=3).
Supplementary Figure 4. Loss of memory of the LuxR self-activation circuit. The dynamics of GFP/OD after dilution of ‘ON’ cells into fresh medium with high-dose or without 30C6HSL. Data indicate mean±s.d. (n=3).
Supplementary Figure 5. The effects of dilution frequency on memory loss of the SA-circuit. a-b, Dynamics of GFP/OD after dilution of ‘ON’ cells every 3 (a) or 6 (b) hours into the fresh medium with high-dose (2.5*10^{-3}\%) or without L-ara. Negative control (green lines) refers to the ‘OFF’ cells without L-ara. Data indicate mean±s.d. (n=3).
Supplementary Figure 6. Inhibition of cell growth by gene circuit expression. a-b, The growth curves of stains with or without the constitutive gene expression circuit in Fig. 1A and the estimated doubling time in the exponential growth phase based on the fitting growth curve. c-d, The growth curves of stains with or without the SA-circuit and the estimated doubling time in the exponential growth phase based on the fitting growth curve. Data indicate mean±s.d. (n=3).
Supplementary Figure 7. The contribution of growth rate heterogeneity in the subpopulations to the memory loss of the SA-circuit. a-c, The dynamics of memory loss at the single-cell level under three scenarios, including both the growth rate heterogeneity and growth-mediated dilution (a), growth-mediated dilution only (b), or growth rate heterogeneity only (c). 200 simulation cells were shown for a-b and 10,000 simulation cells were shown in (c) for demonstration. Initial 100 cells (10% at the ‘OFF’ state and 90% at the ‘ON’ state) growing up to 10,000 were considered for demonstration in all three scenarios. d, The dynamics of average AraC under three scenarios. Initial 1,000 cells (10% at the ‘OFF’ state and 90% at the ‘ON’ state) growing up to 100,000 were considered in simulation to calculate the average AraC. The growth rate constant at the population level is varied to ensure that the growth curve remains the same for all three scenarios. e-f, The dynamics of normalized GFP/OD under different ratios of mixed ‘ON’ and ‘OFF’ cells after dilution into fresh medium with high-dose L-ara (2.5·10⁻³%). Data indicate mean±s.d. (n=3). The boxed area in e is enlarged in f.
Supplementary Figure 8. The effects of growth rate on the bistability of the SA-circuit. a, Bifurcation diagram of AraC versus L-ara level under conditions with various constant growth rates. b, Dependencies of the thresholds for activation (blue) and deactivation (orange) of the switch on growth rate.
Supplementary Figure 9. Decoupling of growth feedback reveals the bistability of the SA-circuit. 

(a) Diagram of the new protocol decoupling of growth feedback. Instead of dilution into fresh medium, cells were diluted into conditioned medium, which is from a parallel culture without any inducers in the stationary phase. 

(b–c) The dynamics of growth (b) and GFP/OD (c) after 1:100 dilution of ‘ON’ cells into the conditioned medium with various doses of L-ara. Negative control (blue line) was ‘OFF’ cells grown in conditioned medium with no L-ara.
Supplementary Figure 10. Estimation of the GFP-Iva half-life in the conditioned medium.
The dynamics of GFP/OD (top) and OD (bottom) of cells carrying the circuit shown in Fig. 1a in
the conditioned medium without inducer L-ara. Data indicate mean±s.d (n=3). The estimated half-
life of GFP-Iva is 68.26±4.46 minutes with 6 replicates.
Supplementary Figure 11. Decoupling of growth-mediated feedback with low-nutrient medium maintains the memory of the SA-circuit. a-b, The dynamics of the GFP/OD in the SA-circuit after diluting ‘ON’ cells into mediums without (a) or with high-dose L-ara (b), and low-nutrient (blue lines) or rich-nutrient (red lines). Data indicate mean±s.d. (n=3).
Supplementary Figure 12. Toggle switch is refractory to memory loss from the growth-mediated feedback. a-b, Dynamics of OD and GFP/OD after 1:100 dilution of ‘ON’ cells into fresh medium with various doses of inducer aTc. c-d, Dynamics of OD and GFP/OD after 1:100 dilution of ‘ON’ cells into the conditioned medium with various doses of aTc. Negative control (blue line) was ‘OFF’ cells grown in conditioned medium without aTc. e-f, Time-lapse imaging of GFP (and brightfield overlay) showed that it decreased after several rounds of cell divisions with fast growth in fresh medium and then recovered in the conditioned medium under both conditions without (e) or with high dose aTc (f). Representative results from three replicates are shown.
Supplementary Figure 13. Mathematical simulation confirms that the toggle switch is refractory to growth feedback. **a**, Simulation with mathematical model shows the change of LacI as a function of time and dose of inducer aTc. The system was set to the activated (LacI high) state initially. **b**, The hysteresis curve for the toggle switch circuit coupled with or without growth feedback.
Supplementary Figure 14. The robustness analysis of the self-activation gene circuit and the toggle switch gene circuit to the cell division under different timescales. a, The dose-response curve of the self-activation gene circuit at different timescales of AraC. b, The dose-response curve of the toggle switch gene circuit at different timescales of LacI and TetR. c-d, The dose-response curve of the toggle switch gene circuit by varying the timescale of TetR (c) or LacI (d) while keeping the timescale of the other fixed. The cell is set to ‘ON’ state initially for all simulation and the dose-response curve without cell growth is shown at the top for comparison.
Supplementary Figure 15. Schematics of how the self-activation switch and toggle switch behave differently during the process of cell growth after diluting cells into fresh medium. Dilution of the gene expression significantly affects the production rate of the gene in the self-activation switch and thus leads to the inactivation of the switch. However, in the toggle switch, dilution of the gene expression does not affect relative expression levels of two mutual-inhibitive genes and thus leads to a robust memory.
Supplementary Video 1. A time-lapse video showing the dynamics of GFP in the AraC self-activation circuit under the condition without L-ara fresh medium for 14h and conditioned medium for 7 hours thereafter. Initially, the system was set in the high-GFP state. The photos were taken at 15 minutes intervals.

Supplementary Video 2. A time-lapse video showing the dynamics of GFP in the AraC self-activation circuit under the condition with a high dose of L-ara and fresh medium condition for 14h and conditioned medium for 7 hours thereafter. Initially, the system was set in the high-GFP state. The photos were taken at 15 minutes intervals.

Supplementary Video 3. A time-lapse video showing the dynamics of GFP in the toggle switch circuit under the condition without aTc and fresh medium for 16h and conditioned medium for 10 hours thereafter. Initially, the system was set in the high-GFP state. The photos were taken at 15 minutes intervals.

Supplementary Video 4. A time-lapse video showing the dynamics of GFP in the toggle switch circuit under the condition with 2ng/ml aTc and fresh medium for 16h and conditioned medium for 10 hours thereafter. Initially, the system was set in the high-GFP state. The photos were taken at 15 minutes intervals.
**SUPPLEMENTARY NOTE**

**Mathematical modeling**

**Self-activation Switch.**

The following mathematical model was used for the self-activation switch circuit without growth feedback,

\[
\frac{d[AraC]}{dt} = f([AraC]) - d \cdot [AraC]
\]

where \( f = k_0 + k_1 \cdot \frac{S_a[AraC]^2}{S_a[AraC]^2+1} \), \( S_a = C_{\text{min}} + (C_{\text{max}} - C_{\text{min}}) \cdot \frac{\text{Lara}^n}{\text{Lara}^n+K^n} \). \( k_0 \) is the basic production rate of AraC, \( k_1 \) is the maximum L-ara-induced production rate, \( S_a \) describes how the production rate is regulated by the inducer L-ara, \( C_{\text{max}} \) and \( C_{\text{min}} \) are the maximum and minimum affinities of AraC dimers to the binding sites on the promoter, \( n \) represents the nonlinearity of the promoter activation by L-ara, and \( d \) is the degradation rate. \([\text{AraC}]\) is the concentration of AraC, which is co-expressed with GFP and thus used interchangeably. The derivation of the equation for the circuit can be found in our previous work. The model is suited to analyze the steady-state behavior of the system under the no-growth condition or when the system reaches the stationary phase, where the AraC steady-state level is controlled only by the production and degradation rate and depends on the circuit itself and thus shows the true behavior of the circuit. The hysteresis curves in Fig. 1b and the rate-balance plot in Fig. 2e are based on this model.

In our system, the cell growth rate is dynamic in most cases as we diluted cells into the fresh medium at the beginning and cultured them overnight. Thus, the system covers all cell growth phases from exponential phase to stationary phase. To model the interplay between the cell growth and gene circuit, the mathematical model was revised by coupling cell growth.

\[
\frac{d[AraC]}{dt} = f([AraC]) - d \cdot [AraC] - \frac{1}{N} \cdot [AraC]
\]

\[
\frac{dN}{dt} = k_{\text{growth}} \cdot g([AraC]) \cdot \left(1 - \frac{N}{N_0}\right) \cdot N
\]

where \( N \) is the cell density, \( k_{\text{growth}} \) is the maximum growth rate, and \( N_0 \) is the maximum cell density. The logistic growth function is used to describe dynamics of the cell density, in which the growth rate depends on the circuit expression \( k_{\text{growth}} \cdot g([AraC]) = k_{\text{growth}} \cdot \frac{1}{[\text{AraC]}^{1/4+1}} \). Fig. 2f is based on this revised model. When the system reaches the steady-state, cells growth stops and thus there are no changes in cell density \( \left(\frac{dN}{dt} = 0\right) \); and as such, the third term in AraC equation is 0 and the system is the same as the one without growth feedback. That is, including the cell density equation does not affect the existence of bistability of the circuit but may change the dynamics of the circuit. Fitted parameters are, unless otherwise mentioned, \( C_{\text{min}} = 0.9 \), \( C_{\text{max}} = 3 \), \( n = 3 \), \( K = 1.92 \times 10^{-3} \), \( k_0 = 0.1 \), \( k_1 = 2 \), \( d = 1 \), \( f = 1 \), \( k_{\text{growth}} = 2.3 \), \( N_0 = 1.35 \), \( \text{Lara} = 0 \sim 2.5 \times 10^{-3} \).

For the theoretical analysis, we also considered a scenario of constant growth rate to study how the strength of growth feedback affects the gene circuits. Constant growth rate can be achieved with frequent dilutions to control the cell in the exponential growth phase. In this case, the model is simplified as follows:
\[
\frac{d[\text{AraC}]}{dt} = f(\text{AraC}) - (d + k_{\text{growth}}) \cdot [\text{AraC}]
\]

Supplementary Fig. 8 shows the simulation under several constant growth rates based on this model. With an increase in the growth rate constant, the steady-state level of AraC decreases and the activation threshold increases exponentially.

**Toggle Switch.**

The following equations were used for the toggle switch circuit without growth feedback,

\[
\frac{d[\text{LacI}]}{dt} = f1([\text{LacI}], [\text{TetR}]) - d \cdot [\text{LacI}]
\]

\[
\frac{d[\text{TetR}]}{dt} = f2([\text{LacI}], [\text{TetR}]) - d \cdot [\text{TetR}]
\]

where \( f1 = \text{crl} + \frac{1.0}{1.0 + [\text{TetR}] / k_t (1.0 + \frac{\text{ATc}}{\text{K}_{\text{ATc}}})^{-m}} \cdot (\text{cil} - \text{crl}) \), \( f2 = \text{crt} + \frac{1.0}{1.0 + [\text{LacI}] / k_l} \cdot (\text{cit} - \text{crt}) \), [LacI] and [TetR] are the concentration of LacI and TetR, respectively, crl and cil are the production rates of LacI when the promoter is repressed or induced, respectively, while crt and cit are the production rates of TetR when the promoter is repressed or induced. \( k_t \) is the active TetR concentration needed to make its inhibition on LacI 50% of the maximum and nt describes the nonlinearity of this inhibition. \( k_l \) is the active LacI concentration needed to make its inhibition on TetR 50% of the maximum, and nl describes the nonlinearity of this inhibition; d is the degradation, and m is the Hill coefficient of the Hill function to describe the relationship between the active ratio of repressor TetR and the aTc inducer concentration. LacI is co-expressed with GFP, and thus was used interchangeably. The derivation of the equation for the circuit can be found in our previous works\(^2,3\). The nullclines in Fig. 4e were based on this revised model.

The following mathematical model was used for the toggle switch circuit by coupling cell growth.

\[
\frac{d[\text{LacI}]}{dt} = f1([\text{LacI}], [\text{TetR}]) - d \cdot [\text{LacI}] - \frac{dN}{dt} \cdot \frac{1}{N} \cdot [\text{LacI}]
\]

\[
\frac{d[\text{TetR}]}{dt} = f2([\text{LacI}], [\text{TetR}]) - d \cdot [\text{TetR}] - \frac{dN}{dt} \cdot \frac{1}{N} \cdot [\text{TetR}]
\]

\[
\frac{dN}{dt} = k_{\text{growth}} \cdot g([\text{LacI}] + [\text{TetR}]) \cdot \left(1 - \frac{N}{N_0}\right) \cdot N
\]

where \( g = \frac{1}{([\text{LacI}]+[\text{TetR}])/j+1} \). Supplementary Fig. 11 was based on this revised model. Fitted parameters are, unless otherwise mentioned, \( \text{crl} = 0.5, \ \text{crt} = 0.5, \ \text{cil} = 21.25, \ \text{cit} = 21.25, \ d = 0.5, \ m = 3, \ nl = 3.5, \ nt = 1.5, \ k_l = 8, \ k_t = 6, \ k_{\text{ATc}} = 0.4, \ j = 20, \ k_{\text{growth}} = 2.3, \ N_0 = 1.35, \ \text{ATc} = 0-4.\)

**Stochastic simulation of cell division events at the single-cell level.**

To simulate the interplay between the circuit and cell growth at the single-cell level by considering the stochasticity from cell division before the system reaches the stationary phase, we developed the following algorithm.

1. Initialize the system: cell number \( N \), time \( t = 0 \), gene expression profile \( x_i \) for each cell.
2. Increase time by a small step \( t = t + \Delta t \).
3. Calculate the probability of division for each cell \( P_i \) according to
\[ P_i = \frac{\log(2) \cdot d_t}{DT} \cdot g(x_i) \left(1 - \frac{N}{N_0}\right) \cdot H(t_{i, \text{post div}} - DT) \cdot \frac{N}{N_{\text{div}}} \]

where \( DT \) is the cell doubling time, \( t_{i, \text{post div}} \) is the time after last division for each cell, \( N_{\text{div}} \) is the number of ready-for-division cells based on the condition \( t_{i, \text{post div}} > DT \), \( H \) is the Heaviside step function, and \( g(x) \) indicates on the effects of circuit expression on the growth rate.

4. Generate uniformly distributed random number \( r_i \) between [0 1] for each cell.

   If \( r_i > P_i \), the cell divides. Update \( x_i = x_i \cdot \lambda \cdot \alpha, t_{i, \text{post div}} = 0 \) and add a new cell \( x_{\text{new}} = x_i \cdot \lambda \cdot (1 - \alpha), \quad t_{\text{new}} = 0, \quad N = N + 1 \). \( \lambda \) represents the fold change of \( x_i \) (0.5~0.7) for each cell division by considering the expression changes before the cell division. \( \alpha \) is used for simulation of unsymmetrical cell division.

   Otherwise, \( t_{i, \text{post div}} = t_{i, \text{post div}} + dt \).

5. Simulate the dynamics of gene circuit for each cell with the current \( x_i \) according to the ODEs of the circuit without growth feedback.

6. Repeat 2~5 until \( t \) is more than maximum time \( T_{\text{max}} \).

Supplementary Fig. 7 was based on this stochastic simulation. The effects of the cell size on the concentration of variables were not considered in the model. Fig. 2e and Fig. 4e, four cell divisions at 0.7 hrs, 1.4 hrs, 2.1 hrs, 3.15 hrs were used as one example for demonstration and comparison in both the self-activation switch and the toggle switch circuits.

**Supplementary Note References**

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3. Wu, M. et al. Engineering of regulated stochastic cell fate determination. *Proc Natl Acad Sci U S A* 110, 10610-10615, doi:10.1073/pnas.1305423110 (2013).