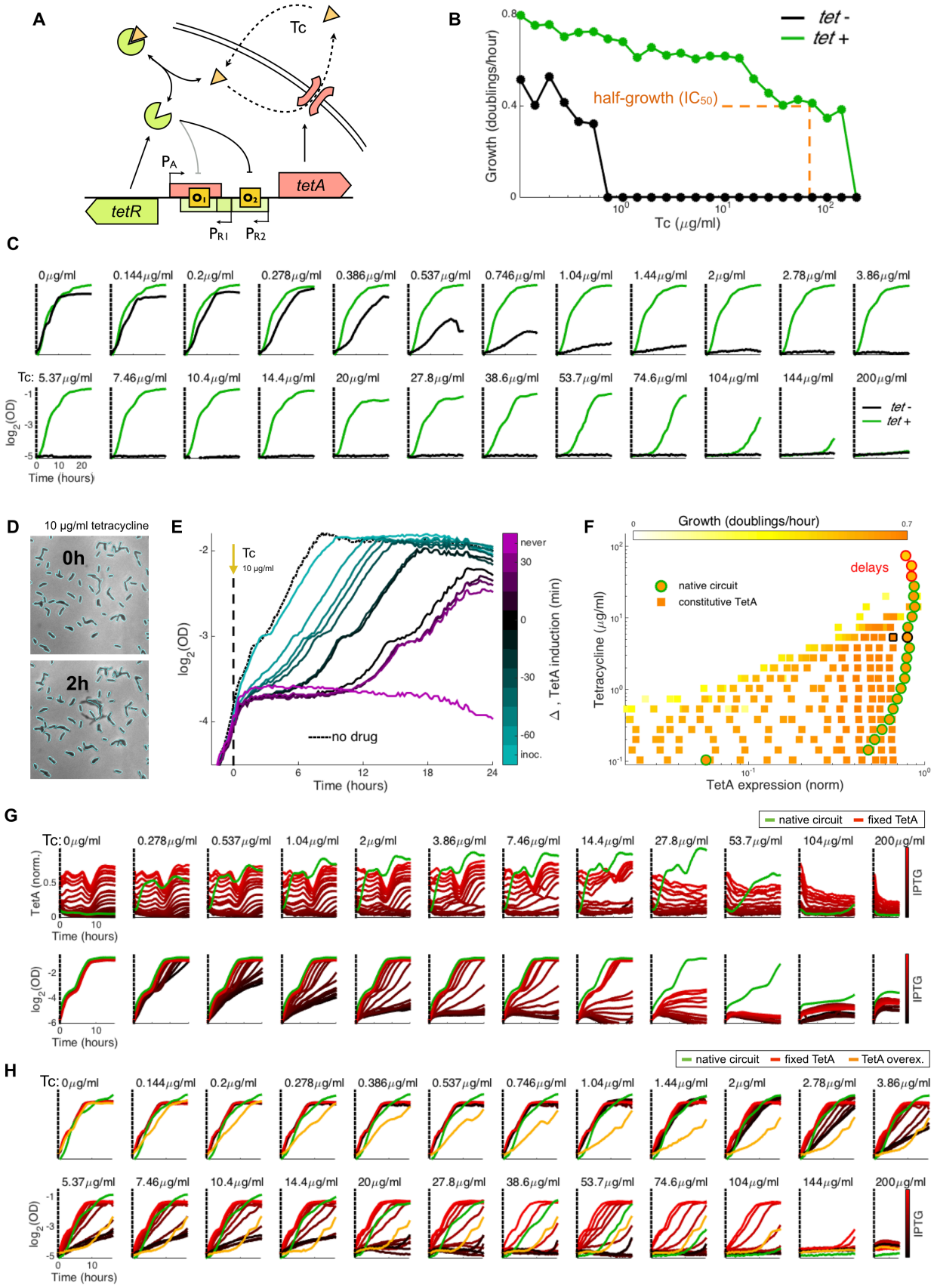
A
Tc
PA
tetA
tetR
O1
O2
PR1
PR2
B
tet -
tet +
half-growth (IC50)
Growth (doublings/hour)
Tc (μg/ml)
C
0μg/ml
0.144μg/ml
0.2μg/ml
0.278μg/ml
0.386μg/ml
0.537μg/ml
0.746μg/ml
1.04μg/ml
1.44μg/ml
2μg/ml
2.78μg/ml
3.86μg/ml
Tc: 5.37μg/ml
7.46μg/ml
10.4μg/ml
14.4μg/ml
20μg/ml
27.8μg/ml
38.6μg/ml
53.7μg/ml
74.6μg/ml
104μg/ml
144μg/ml
200μg/ml
log2(OD)
Time (hours)
tet -
tet +
D
10 μg/ml tetracycline
0h
2h
E
Tc
10 μg/ml
log2(OD)
never
30
0
-30
-60
inoc.
Δ, TetA induction (min)
------no drug
Time (hours)
F
Growth (doublings/hour)
delays
native circuit
constitutive TetA
Tetracycline (μg/ml)
TetA expression (norm)
G
native circuit
fixed TetA
Tc: 0μg/ml
0.278μg/ml
0.537μg/ml
1.04μg/ml
2μg/ml
3.86μg/ml
7.46μg/ml
14.4μg/ml
27.8μg/ml
53.7μg/ml
104μg/ml
200μg/ml
TetA (norm.)
log2(OD)
Time (hours)
IPTG
H
native circuit
fixed TetA
TetA overex.
Tc: 0μg/ml
0.144μg/ml
0.2μg/ml
0.278μg/ml
0.386μg/ml
0.537μg/ml
0.746μg/ml
1.04μg/ml
1.44μg/ml
2μg/ml
2.78μg/ml
3.86μg/ml
5.37μg/ml
7.46μg/ml
10.4μg/ml
14.4μg/ml
20μg/ml
27.8μg/ml
38.6μg/ml
53.7μg/ml
74.6μg/ml
104μg/ml
144μg/ml
200μg/ml
log2(OD)
Time (hours)
IPTG

Figure S1. Resistance in the wild-type *tet* operon and in cells with inducible TetA expression. Related to Figure 1
(A) The *tet* operon from the Tn10 transposon expresses TetR and TetA divergently and incorporates multiple overlapping promoters. TetA is expressed from promoter P_A and TetR is expressed from two promoters P_{R1} and P_{R2}. Two operator sites O_1 and O_2 bind TetR, blocking translation. TetR bound to any of the two operators blocks expression from P_A and P_{R2}, but TetR can be expressed from P_{R1} when operator O_1 is unbound. Operator O_1 has lower affinity to TetR, so TetR expression precedes expression of TetA upon drug exposure. (B) Maximum growth rates as a function of tetracycline concentration for liquid cultures of sensitive cells (black) and cells with the *tet* operon (green). The yellow line indicates the IC_{50} tetracycline concentration, where the growth rate is reduced to half. (C) Growth curves of sensitive and *tet*-resistant cells following exposure to a gradient of tetracycline. (D) Imaging of single cells with high constitutive TetA expression from an IPTG-inducible promoter upon exposure to 10 μg/ml Tc, showing that only a small fraction of the population recovers growth. Contour of cells at the time of exposure is overlaid on the image taken after two hours, to highlight cell growth. (E) Growth curves of liquid cultures where 200 μM IPTG was used to induce TetA expression at different times (Δ) in relation to the exposure to 10 μg/ml tetracycline. (F) Steady-state growth of cells carrying the native circuit and of cells constitutively expressing a range of TetA from an inducible promoter. Both strains have the resistance genes integrated in the chromosome and matching fluorescent reporters measuring TetA expression. The native circuit, expressing TetA from its native promoter, is able to achieve higher levels of expression and higher resistance. Markers with red edges indicate conditions where the native circuit showed delays in recovery larger than 2 hours. Markers with black edges indicate the curves shown in Figure 1G in the main text. (G) TetA expression and growth for the liquid cultures in (F), following exposure to a gradient of tetracycline at time zero. Colors indicate cells carrying the native *tet* operon (green) and cells with fixed TetA expression (red). The red color gradient indicates different levels of constitutive TetA expression obtained by IPTG induction. (H) Growth curves of the liquid cultures if Figures 1H-1J in the main text, following exposure to a gradient of tetracycline at time zero. Colors indicate cells carrying the native *tet* operon (green), cells expressing TetA from a low-copy vector (red) and cells with high expression of TetA from a high-copy vector (orange). The red color gradient indicates different levels of constitutive TetA expression obtained by IPTG induction.

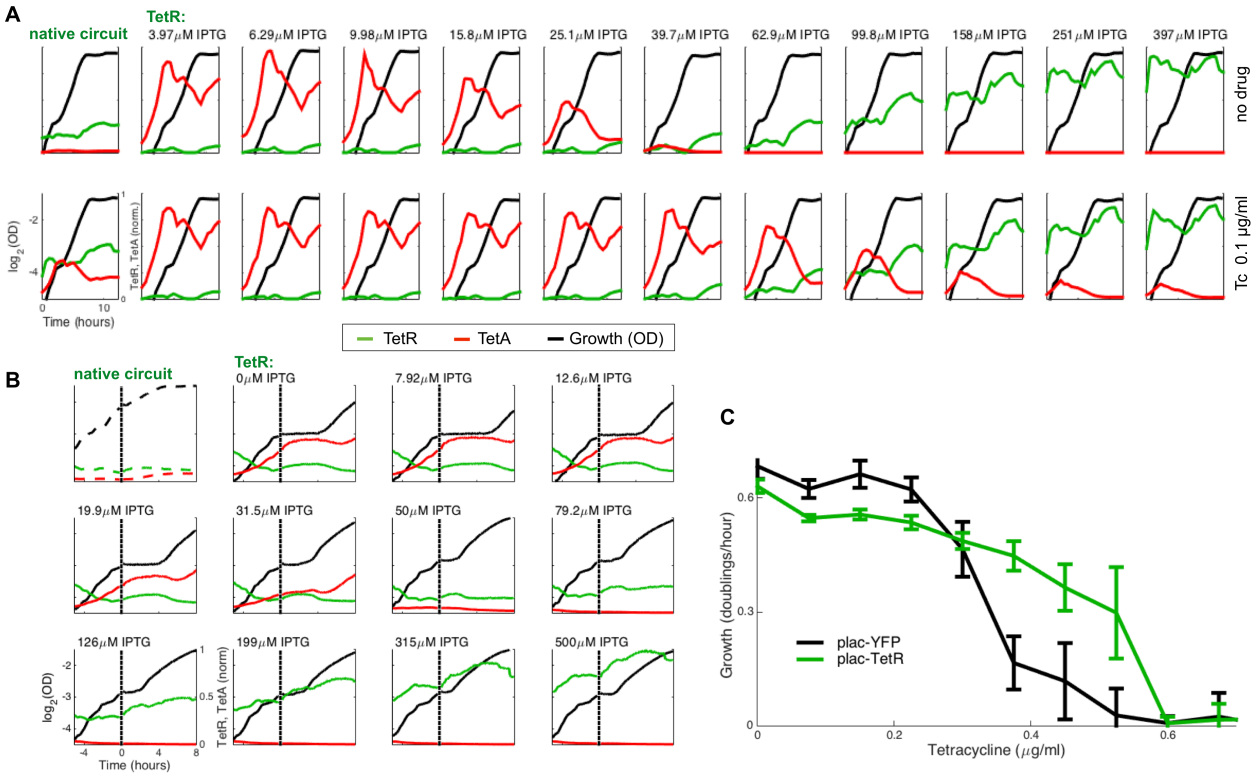

Figure S2. Resistance in cells with constitutive TetR expression. Related to Figure 2
(A) Growth curves and gene expression of liquid cultures from figure 2B in the main text (cells expressing TetA from its native promoter and expressing TetR constitutively from an IPTG-inducible promoter). Cells were grown across a range of IPTG for different TetR expression levels, with and without the presence of tetracycline. Fluorescent reporters show expression of TetR (green) and TetA (red). (B) Growth and gene expression in the liquid cultures from Figure 2C-2F in the main text. Cells were grown with a range of IPTG for different TetR expression levels and exposed to a step increase of 10μg/ml Tc during mid-log phase (dotted line). (C) Growth of cells overexpressing TetR across a gradient of tetracycline, showing mild resistance due to drug sequestration when compared to cells overexpressing YFP from the same promoter.

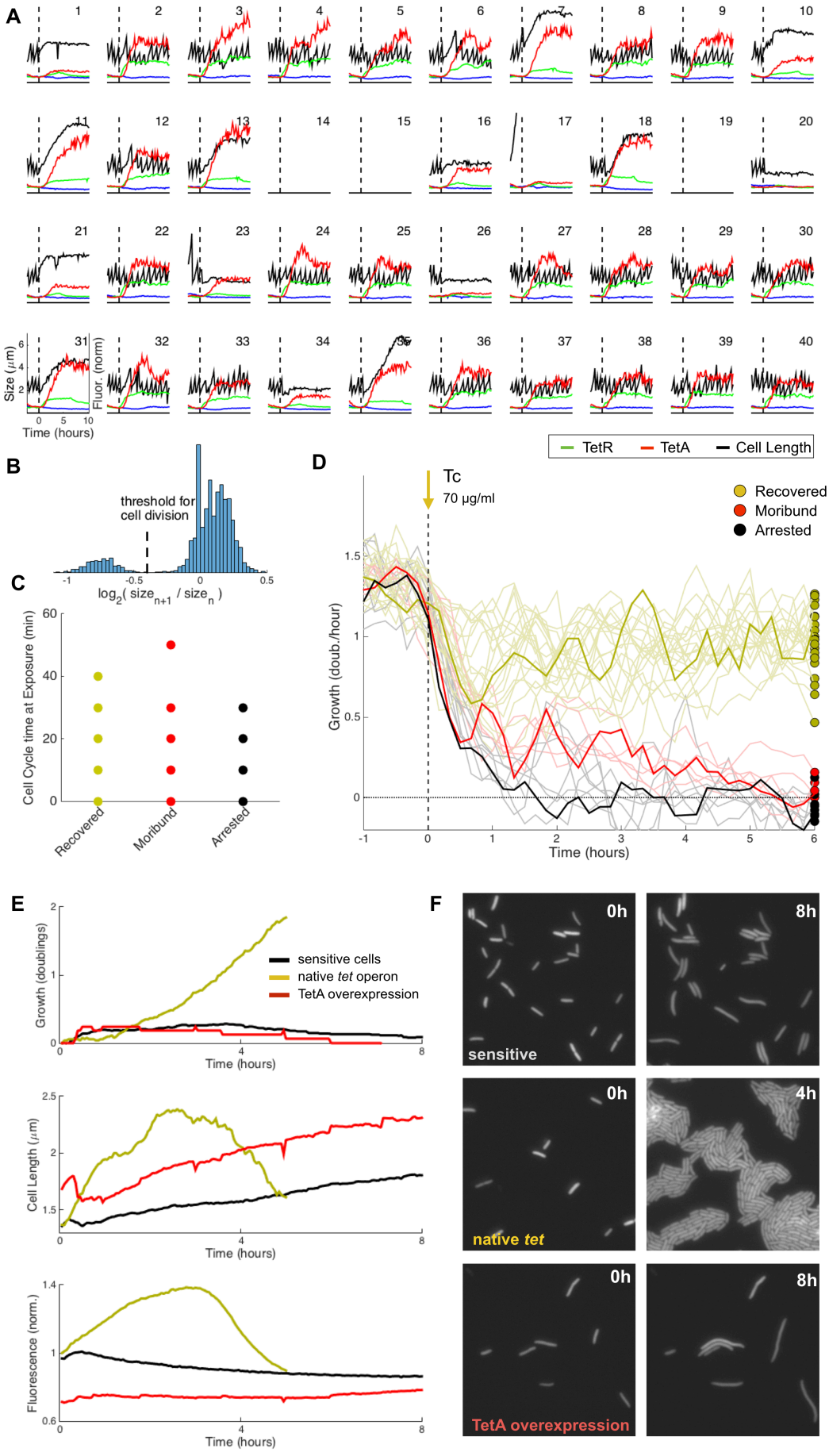
A
Size (μm)
Fluor. (norm)
Time (hours)
TetR
TetA
Cell Length
B
threshold for cell division
log2(sizen+1 / sizen)
C
Cell Cycle time at Exposure (min)
Recovered
Moribund
Arrested
D
Tc
70 μg/ml
Recovered
Moribund
Arrested
Growth (doub./hour)
Time (hours)
E
Growth (doublings)
sensitive cells
native tet operon
TetA overexpression
Time (hours)
Cell Length (μm)
Time (hours)
Fluorescence (norm.)
Time (hours)
F
0h
8h
sensitive
0h
4h
native tet
0h
8h
TetA overexpression

Figure S3. Growth and gene expression in single cells. Related to Figure 3

(A) Cell size and gene expression for each cell followed in the microfluidic device. The black, red, green and blue lines indicate cell size and expression of TetA, TetR and constitutive CFP, respectively. The vertical dashed line indicates the moment of exposure to 70μg/ml of tetracycline. At the moment of exposure, three cells had escaped their channels (14, 15 and 19) and one cell was already filamentous (17). (B) Histogram of cell sizes relative to their size at the previous time step (5 minutes earlier), throughout the whole experiment. The dotted line depicts the threshold used to identify division events. (C) Position along the cell cycle at the moment of exposure to the drug (measured as the time since the last cell division), separated by cell fate. The cell cycle does not influence outcome upon drug exposure. (D) Instant growth rates of all cells, obtained from the derivative of the accumulated growth (main text). (E, F) Imaging of sensitive cells, cells carrying the native *tet* operon and cells with high constitutive TetA expression upon exposure to 2 μg/ml tetracycline (a moderate concentration of drug). These expression patterns correspond to the three cell fates observed experimentally in cells carrying the native *tet* operon: sensitive cells arrest growth exclusively due to the presence of drug (Arrested), cells carrying the native circuit recover growth (Recovered) and cells overexpressing TetA arrest growth due to excessive expression of the efflux pump (Moribund). (E) Growth, cell length and fluorescence (CFP expressed from a constitutive promoter) for all three cell types. (F) Images of the three cell types upon exposure and at a later time.

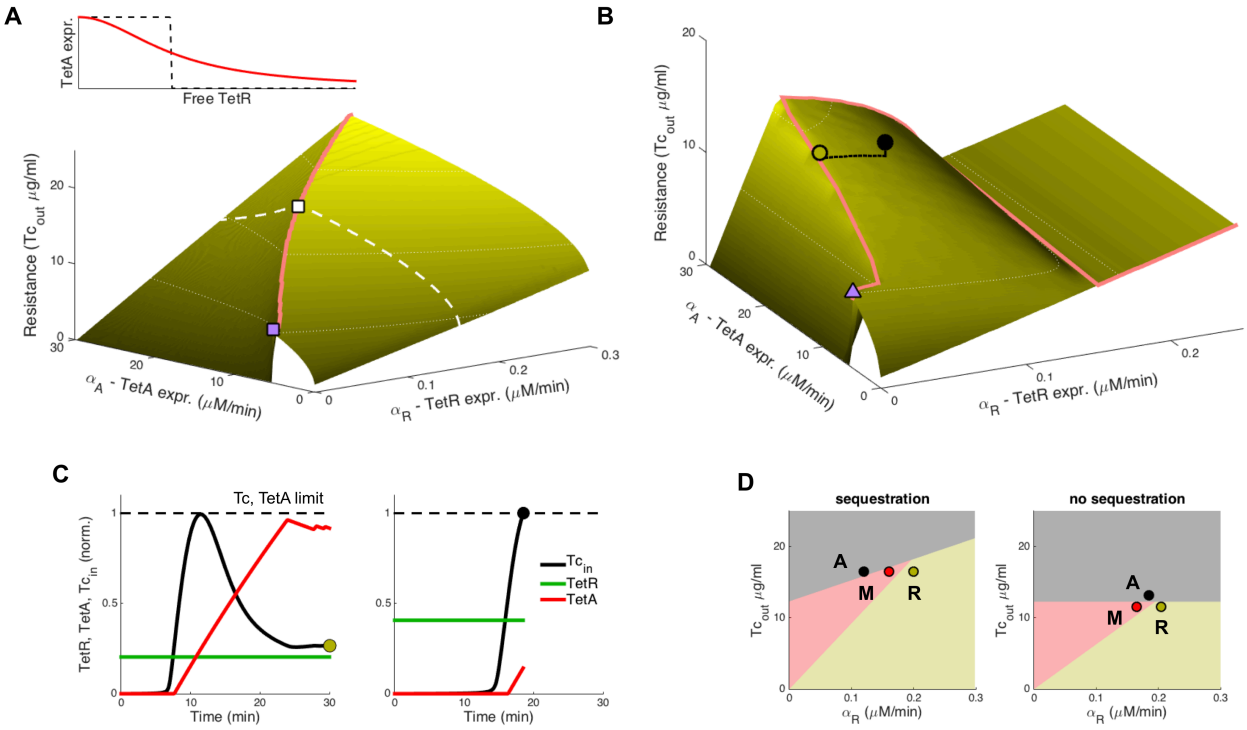

Figure S4. Simulation of alternative circuit designs. Related to Figure 4

(A) Resistance obtained by different combinations of TetR and TetA expression rates in the case where a gradual regulation of TetA by TetR (Hill function with a coefficient of 2) is used instead of an on/off regulation (inset). The results are qualitatively similar for both types of regulation. The red line in the surface indicates the optimal TetA expression for each value of TetR expression and the purple square shows the maximum resistance in the absence of TetR. The white dotted line and square show the TetR expression levels analyzed in the main text. (B) Resistance obtained by different combinations of TetR and TetA expression rates in the case where TetR expression is fixed from before exposure. The red line indicates the optimal TetA expression for each value of TetR expression and the purple triangle shows the maximum resistance in the absence of TetR. (C) Intracellular concentrations of TetR, TetA and free tetracycline for the yellow and black points indicated in (B), showing that increased TetR expression lowers resistance by delaying the induction of TetA. Sequestration of incoming drug molecules by TetR mitigates this loss of resistance. (D) Phase diagram showing cell fates (Recovered, Moribund and Arrested) of the native circuit in simulations with and in the absence of sequestration of incoming tetracycline molecules by TetR, showing the extra resistance gained with increased TetR expression. The gain in resistance increases linearly with the rate of TetR expression. The optimal TetA concentrations are the same in both cases, and all three cell fates are still obtained with slight variations around the optimized expression level.

Table S1. Parameters used in the mathematical model. Related to Figure 4

K_i	Tc permeability through cell membrane	0.015 min^{-1}	half-equilibration of 45 min (Sigler et al. 2000)
λ	growth rate of the cell	0.015 min^{-1}	doubling time of 45 min
K_b	dissociation constant of TetR + Tc	1 nM	(Takahashi et al. 1986)
K_a	maximum rate of TetA export	0.05 mol Tc/mol TetA/min	(McMurry et al. 1980)
k_a	Michaelis constant of TetA	10 μM	(McMurry et al. 1980)
a_{max}	TetA threshold for toxicity	300 μM	maximal resistance with constitutive TetA expression
x_{max}	Tc threshold for toxicity	1 μM	IC_{50} of sensitive cells