SI Appendix

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Estimating pulse statistics from trajectories

We used frequency, amplitude and rate of damping to quantitatively characterize oscillatory trajectories. For both experimental and computational data, we estimated these three characteristics from measurements of the steady state concentration (x_{ss}) and the times $t_{max}^{(i)}$ and amplitudes $x_{max}^{(i)}$ of the i$^{\text{th}}$ maxima during oscillation. For experimental data, where autofluorescence near the time of cell division often obscured dynamics, we split trajectories before and after cell division events and computed frequency and damping independently for each.

We estimated the frequency of oscillation by computing the differences in timing between successive peaks using the equation

$$\omega = \left\langle \frac{2\pi}{t_{max}^{(i+1)} - t_{max}^{(i)}} \right\rangle, \tag{1}$$

where the average is taken over all pairs of pulses throughout the population of cells. We ensured that this approach yielded similar results to those obtained by pitch analysis as in [1]. To estimate the amplitude of the first pulse, we computed the ratio of the steady state concentration to the peak concentration. This measurement represents the fold upregulation of p53 from its basal level.

$$a_1 = x_{max}^{(1)}/x_{ss}. \tag{2}$$

To estimate the rate of oscillatory damping, one must take into account both the relative amplitude of subsequent pulses as well as the time elapsed between them. We assumed that damping between successive extrema occurred *via* an exponential decay, and that this decay is offset from the origin by the steady state level of protein:

$$x(t_{max}) - x_{ss} = (x_0 - x_{ss})e^{-rt_{max}}. \tag{3}$$

We verified that these assumptions accurately fit the behavior of successive peaks obtained from

simulation. From measurement of maxima of successive pulses, we computed the decay constant of this exponential decay for each trajectory according to the formula

$$r = \left\langle \frac{ln\left(x_{max}^{(i)} - x_{ss}\right) - ln\left(x_{max}^{(i+1)} - x_{ss}\right)}{t_{max}^{(i+1)} - t_{max}^{(i)}} \right\rangle, \tag{4}$$

where the average is taken over all pairs of pulses throughout the population of cells.

Our live cell microscopy experiments measured levels of exogenous p53-CFP fusion protein in individual cells. In order to correctly account for the endogenous protein level in our calculations of p53 pulse amplitude and damping, we performed Western blotting to measure the induction of total protein levels during the first p53 pulse after 25 and 50 μM zinc stimulation (Fig. S1A-B). We observed a slight increase in endogenous p53 levels, although this change was much smaller than the increase in exogenous p53-CFP protein levels. We suspect the elevation of endogenous p53 levels is due to competition for Mdm2 binding between exogenous and endogenous p53, leading to indirect stabilization of the endogenous protein. Induction of Mdm2 by the p53-CFP protein led to a decrease in both p53-CFP and endogenous p53.

By summing the total protein in both the endogenous and exogenous bands, we found that zinc induction leads to an approximately 2-fold (11-fold) activation after zinc treatment at 25 (50) μM. We used these points to compute the equivalent fluorescence intensity expected from endogenous p53 using the equation

$$\frac{(p53_{max}^{exog} + p53^{endog})}{(p53_{0}^{exog} + p53^{endog})} = f \tag{5}$$

where f denotes the total p53 pulse amplitude after zinc treatment (as measured by Western blot), $p53_{max}^{exog}$ is the mean first-amplitude fluorescence observed by live-cell microscopy, $p53^{endog}$ is the unknown equivalent fluorescence of endogenous p53, and $p53_{0}^{exog}$ is mean initial level of exogenous p53 observed by live-cell microscopy. For our initial experiment of zinc stimulation, we obtained a least-squares solution using our data at 25 and 50 μM to solve for $p53^{endog}$, and computed the p53 amplitudes at each zinc concentration using this value and the corresponding $p53^{exog}$

values determined by microscopy. In subsequent experiments, we included a 50μM zinc treatment control to compute the corresponding value of $p53^{endog}$ and obtain an estimate for the true amplitudes associated with p53 dynamics.

Model construction and parameterization

Our model of the core p53-Mdm2 negative feedback loop was based on the topology of Model IV from Ref. 1. The model represents the production and degradation of p53 (*p*) and Mdm2 (*m*), as well as three nonlinear interactions: a Michaelis-Menten term representing p53's ubiquitination by Mdm2 and subsequent degradation, a Hill term representing p53 binding the Mdm2 promoter leading to Mdm2 production, and a saturation term representing the autocatalytic degradation of Mdm2. Each of these interactions incorporates saturation of its effect at high p53 and Mdm2 concentrations, and the p53-dependent Mdm2 transcription term is implemented with a Hill coefficient of 2 to reflect p53 oligomerization on the Mdm2 promoter [2]. Rather than a stiff delay, we implemented a boxcar procedure of five linear ODEs representing the combined delay of Mdm2 transcription, RNA export, translation, protein maturation and nuclear translocation (*i.e.* m_i; $0 \leq i \leq 4$ in Eq. (6)). The perfectly stiff delay is recovered in the limit of an infinite number of boxcar steps, and as we assume equally distributed boxcar steps, our approach does not add any additional parameters. Finally, our model represents fluctuations in protein levels due to transcriptional noise by multiplying protein production terms by noise terms $\xi_p(t)$ and $\xi_m(t)$. For noiseless simulations, these functions are set to 1 (the "Noise Simulations" section below describes simulations incorporating noise). The full model is shown in Eq. (6), and was fit to data as described in

the next section. All results were generated using the parameters listed in Table S1.

$$
\begin{aligned}
\dot{p} &= \xi_p(t)(\alpha_p + p_z(TF(Z(t)) - 1)) - \delta_p m \frac{p}{K_p(1 + N(t)) + p} \\
\dot{m}_0 &= \gamma_{m0}(\alpha_{m0} + \beta_{m0}\frac{p^2}{K_{m0}^2 + p^2} - m_0) \\
\dot{m}_i &= \gamma_{m0}(m_{i-1} - m_i) \\
\dot{m} &= \xi_m(t)m_4 - \delta_m \frac{m}{K_m + m} - \gamma_m m
\end{aligned}
\tag{6}
$$

Surprisingly, and in contrast to what we observed for p53-CFP (Fig. 1I), Mdm2 amplitude was independent of zinc concentration and pulse number (Fig. S2B). We reasoned that this distinction might result from two processes (Fig. S2A; red arrows originating from Mdm2): (1) saturation of Mdm2 promoter activity, or (2) Mdm2 autoubiquitination and subsequent degradation ([3, 4]). To investigate the roles of these potential control mechanisms, we used a cell line containing the same zinc-inducible p53-CFP construct together with the Mdm2 promoter driving expression of YFP alone. In this cell line, YFP induction should be subject only to the saturation of p53-dependent transcription. Stimulating these cells with zinc led to a slow increase in YFP levels (Fig. S2C). As opposed to Mdm2-YFP, YFP levels depended on zinc concentration, indicating that Mdm2 autoregulation is important for the invariance of Mdm2 levels (Fig. S2D-E). However, since YFP induction depended less strongly on zinc concentration than did p53-CFP amplitude, we concluded that transcriptional saturation of the Mdm2 promoter also plays a partial role in regulating Mdm2 dynamics. We therefore included both Mdm2 autoregulation and promoter saturation in our model of the p53-Mdm2 circuit.

To compare the negative feedback model with data collected after zinc induction, we needed to measure MTF1's transfer function from zinc concentration to the stimulation of p53 transcription. We measured this transfer function using a cell line where the metallothionein production drives CFP alone. In this cell line, we observed a plateau in fluorescence after 10 h, and used this steady

state level as a measure of transcriptional induction (Fig. S2F). Thus, we computed the peak CFP fluorescence attained during 48 h in single cells at 0, 10, 20, 30, 40, and 50 μM zinc, and normalized the results to the uninduced (0 μM) data. We fit a Hill-model saturating transfer function (Eq. (7)) with $n = 3$ to the experimentally observed CFP induction data (Fig. S2G), and used this transfer function to map specific zinc doses to the model input for all subsequent analyses.

$$TF(z) = 1 + a\frac{z^n}{K_z^n + z^n} \tag{7}$$

Parameter fitting

We performed a local optimization procedure on our model of the p53-Mdm2 negative feedback loop. The aim of this procedure was to find a set of parameters that would allow our model to simultaneously match (i) the p53 and Mdm2 first pulse amplitude (Equation 2), (ii) the frequency of pulses (Equation 1), and (iii) the damping rate of pulses at five zinc concentrations (Equation 4). In our fitting procedure, all parameters in the model were allowed to vary.

At each parameterization, we ran five simulations. For each, the model was allowed to evolve in the absence of zinc for 100 h to ensure steady state was reached, and then transcriptionally simulated with 15, 25, 30, 40, or 50 μM zinc for an additional 100 h. We measured amplitude, frequency, and damping using the same procedure as described for our data trajectories. For frequency and damping calculations, we used the mean frequency and damping computed for the first five pulses. We used the sum of the objective functions for the i^{th} zinc dose shown in Equation 8. Each represents a weighted sum of squares error between model and data, in which weights were chosen so that all terms would contribute with similar magnitude to the final error. The terms $\overline{a}$, $\overline{\omega}$, and $\overline{r}$ represent the measured amplitude, frequency and damping to which the modeled values are

compared.

$$O_i(\mathbf{p}) = 5\left(\omega_{p53,i} - \overline{\omega}_{p53,i}\right)^2 + 50\left(r_{p53,i} - \overline{r}_{p53,i}\right) + 0.1\left(a_{p53,i} - \overline{a}_{p53,i}\right)^2 + 0.5\left(a_{Mdm2,i} - \overline{a}_{Mdm2,i}\right)^2 \quad (8)$$

To determine how to update parameters at each fitting step, we efficiently computed the sensitivities of each parameter to these quantities using an adjoint method [5]. To calculate the sensitivity of objective function to parameters, it was necessary to compute sensitivities to both the *concentration* and *timing* of pulses. We used an adjoint formulation to compute both efficiently, as described in Refs. 6 and 7. We supplied these sensitivities and the objective function value to the MATLAB function `fmincon` configured to use Quasi-Newton with BFGS in the MATLAB Optimization Toolbox version 4.2.

Noise simulations

Noise in protein synthesis rates constitutes a major source of variability in mammalian cells (even when the number of protein molecules is high), and varies with a correlation time on the order of hours [1, 8]. Following 1, we modeled variability between cells using our core 1NF model by implementing the transcriptional noise terms $\xi_p(t)$ and $\xi_m(t)$ modifying protein production for p and m, respectively (see Eq. (6)). To incorporate a nonzero correlation time without driving the system with a strongly periodic signal, we implemented a simple iterative algorithm to generate Gaussian colored noise as described in Ref. 9. In applying this algorithm, we chose a correlation time τ of 5 hours and a standard deviation σ = 0.1, and a sampling time of 10 minutes. To numerically integrate the ODE system in the presence of this noise function, we assumed that the value of the noise term was constant between sampling times.

Modeling synthetic feedback

For predictions and analyses of additional synthetic negative and positive feedback loops, we constructed an augmented model in which we also included boxcar equations for p53 and MTF1 (or MTF1-KRAB) protein production (the full models are shown in Eq. (9), where $1 \leq i \leq 4$). As in the model represented by Eq. (6), p and m represent p53 and Mdm2, respectively. In this model, the action of an additional feedback species (either MTF1 or MTF1-KRAB) is represented by an additional state variable f. Because these models add additional positive and negative feedback loops to the original negative feedback (1NF) circuit, we refer to them as NPF and 2NF systems, respectively.

We used three strategies to constrain the parameter values in the NPF and 2NF models. First, the parameters governing the p53-Mdm2 interaction from the original negative feedback model were unchanged. Second, because the Mdm2 promoter was used to drive MTF1 expression, the same parameter values were used to represent p53 induction of MTF1 as used for Mdm2. Finally, we ensured that in the absence of feedback (*i.e.* setting $\beta_{f0} = 0$), the NPF and 2NF models generated identical p53 and Mdm2 dynamics to the original 1NF model. Table S2 lists the parameter values used for these models.

$$
\begin{aligned}
\dot{p}_0 &= \begin{cases} \gamma_{p0}(\alpha_{p0} + \beta_{p0}(TF(Z(t)) - 1)\frac{f^2}{K_f^2+f^2} - p_0) & \text{(NPF model)} \\ \gamma_{p0}(\alpha_{p0} + \beta_{p0}(TF(Z(t)) - 1)\frac{K_f^2}{K_f^2+f^2} - p_0) & \text{(2NF model)} \end{cases} \\
\dot{p}_i &= \gamma_{p0}(p_{i-1} - p_i) \\
\dot{p} &= \alpha_p p_4 - -\delta_p m \frac{p}{K_p(1 + N(t)) + p} \\
\dot{m}_0 &= \gamma_{m0}(\alpha_{m0} + \beta_{m0}\frac{p^2}{K_{m0}^2 + p^2} - m_0) \\
\dot{m}_i &= \gamma_{m0}(m_{i-1} - m_i) \\
\dot{m} &= m_4 - \delta_m \frac{m}{K_m + m} \\
\dot{f}_0 &= \gamma_{f0}(\alpha_{f0} + \beta_{f0}\frac{p^2}{K_{f0}^2 + p^2} - f_0) \\
\dot{f}_i &= \gamma_{f0}(f_{i-1} - f_i) \\
\dot{f} &= f_4 - \gamma_f f
\end{aligned}
\tag{9}
$$

After this procedure, two sources of parameters remained unconstrained: the delay through the synthetic feedback loop represented by p53 and MTF1 protein synthesis, and the feedback strength represented by the effect of variable MTF1 levels on p53 production. We did not know *a priori* which values these processes would take, and chose to query oscillatory characteristics over a wide range of parameter values. For this analysis, we sampled the parameters γ_{f0} and β_{p0} (to change delay time and feedback strength, respectively) at 51 points one order of magnitude above and below their nominal parameterization, and computed the resulting p53 amplitudes, frequencies and damping coefficients; the full results are shown in Fig. S3.

For the circuit with negative feedback loops, we observed damped oscillation with a frequency that remained consistent across delay times and feedback strengths (Fig. S3A-C). The damping results varied widely, and trajectories were both stabilized and destabilized for some values of the

parameters being varied. Both long and short delay times stabilized the system by increasing the oscillation damping rate. Examination of the corresponding trajectories showed that for many of these, a single first pulse dominates the dynamical response. However, we found that the addition of a synthetic negative feedback loop on p53 could destabilize oscillation if its delay time is tuned near that of the corresponding parameter in the core p53-Mdm2 loop (Fig. S3A; blue region).

The circuit with a negative and positive feedback loop did not have a pronounced effect on frequency and amplitude except at strong feedback strengths (Fig. S3E-F). Like the 2NF system, the NPF system is predicted to have a more pronounced effect on damping, but with opposite effects (Fig. S3A,D). For a broad range of parameter values, addition of a synthetic positive feedback loop is predicted to destabilize oscillation.

Parameter perturbation analysis

To map the effect of parameter variation on oscillation amplitude and frequency, we simulated our model after individually varying each parameter at 50 logarithmically spaced points between one order of magnitude below and above its nominal value, and computed the oscillation frequency and amplitude at each point. Simulations were run until the 5000 h elapsed or five maxima were observed; simulations that were not seen to oscillate by undergoing at least three pulses were discarded. The resulting trajectories are shown in Fig. S5, and are colored lighter for higher parameter values. The corresponding points in amplitude–frequency space are shown in Fig. S6. We found that parameters affecting the negative feedback loop's delay (*e.g.* γ_{m0}, affecting the delay in Mdm2 protein maturation; δ_p and K_p, affecting the rate of Mdm2-mediated p53 degradation; see Fig. S6C,D,E) had the largest effect on frequency, while parameters affecting protein production rates (*e.g.* α_p and p_z; see Fig. S6A,B,F,G) had a smaller effect on frequency.

Supplementary experimental methods

Immunoblots

Western blots were performed as described previously [10]. Antibodies used were anti-p53 (DO-1, Santa Cruz Biotechnology) and anti-β-tubulin (E7, Developmental Studies Hybridoma Bank).

Cell lines and expression constructs

Mdm2p-YFP was constructed previously as described in Ref. 11. MCF7 cells were transfected (FuGene6, Roche) and stable clones were selected by flow cytometry for expression of YFP.

Time-lapse microscopy and fluorescence quantification

MCF7 Mdm2p-YFP cell line was imaged using FMS2 for 48 hours every 20 min after addition of differing concentrations of $ZnCl_2$. Cells were analyzed as described in Materials and Methods.

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