Supporting Information

Toettcher et al. 10.1073/pnas.1005615107

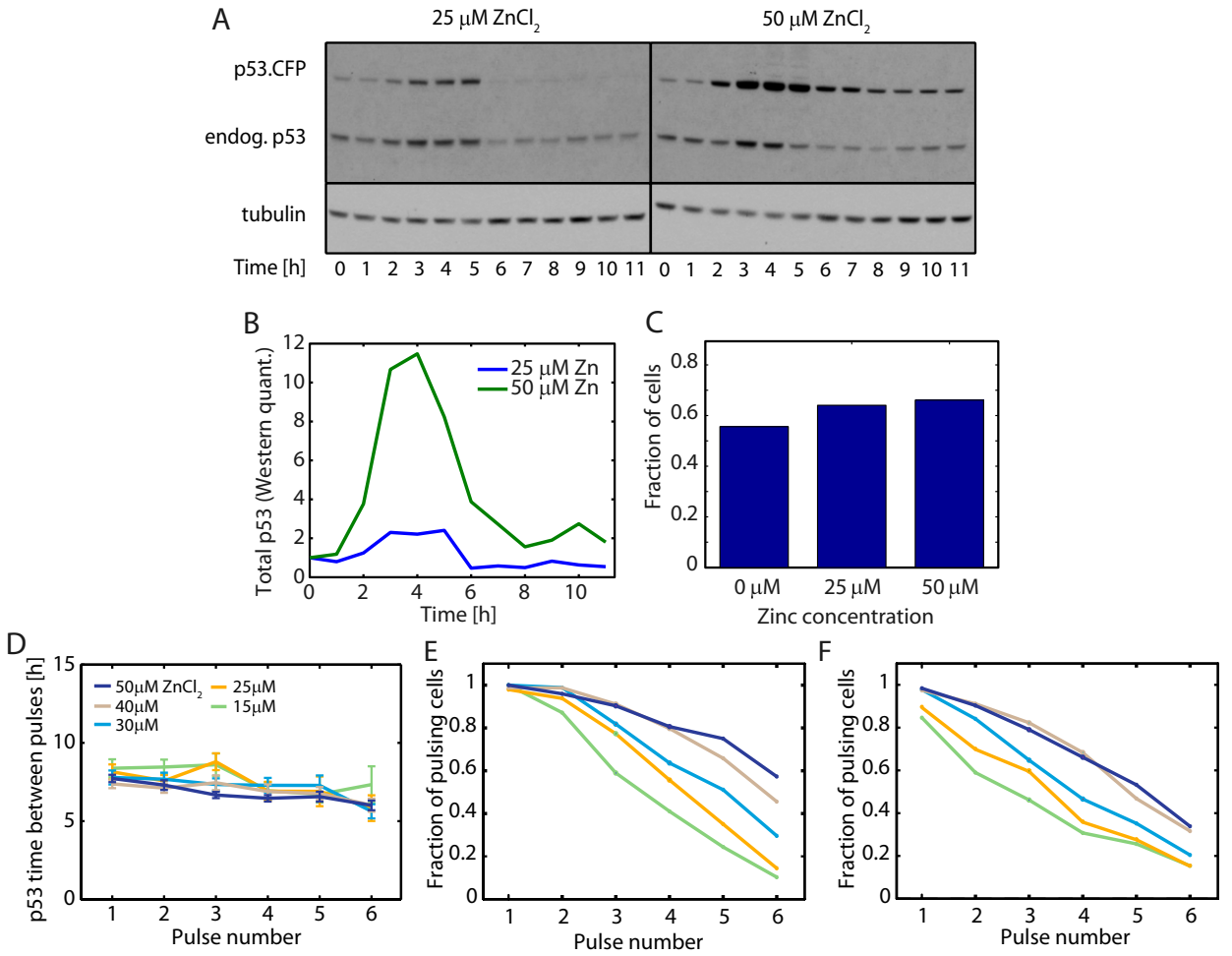

Fig. S1. Population and single cells data of p53 dynamics after zinc stimulation. (*A*) Time-course of endogenous p53 and exogenous p53–CFP levels in MCF7 cells after addition of 25 or 50 μM zinc. Cells were collected every hour and analyzed by Western blot (see *SI Appendix*, "Estimating pulse statistics from trajectories" for details regarding accounting for the endogenous protein level in our calculations of p53 pulse amplitude and damping). (*B*) Time-course of total p53 induction, quantified from the Western blot in *A*. All samples are normalized to tubulin and are shown relative to the value at $t = 0$. (*C*) Fraction of cells that divide within 24 h after zinc treatment. (*D*) Timing between successive p53 pulses, computed for the same cells shown in Fig. 1 *H–I*. Cells were treated with varying zinc doses; at least 50 cells were analyzed per condition. The fraction of cells observed undergoing each p53 pulse (*E*) or Mdm2 pulse (*F*) is plotted against pulse number from the same time-lapse microscopy experiments of Fig. 1 *F* and *G*. Colors representing each zinc dose are the same as shown in *D*.

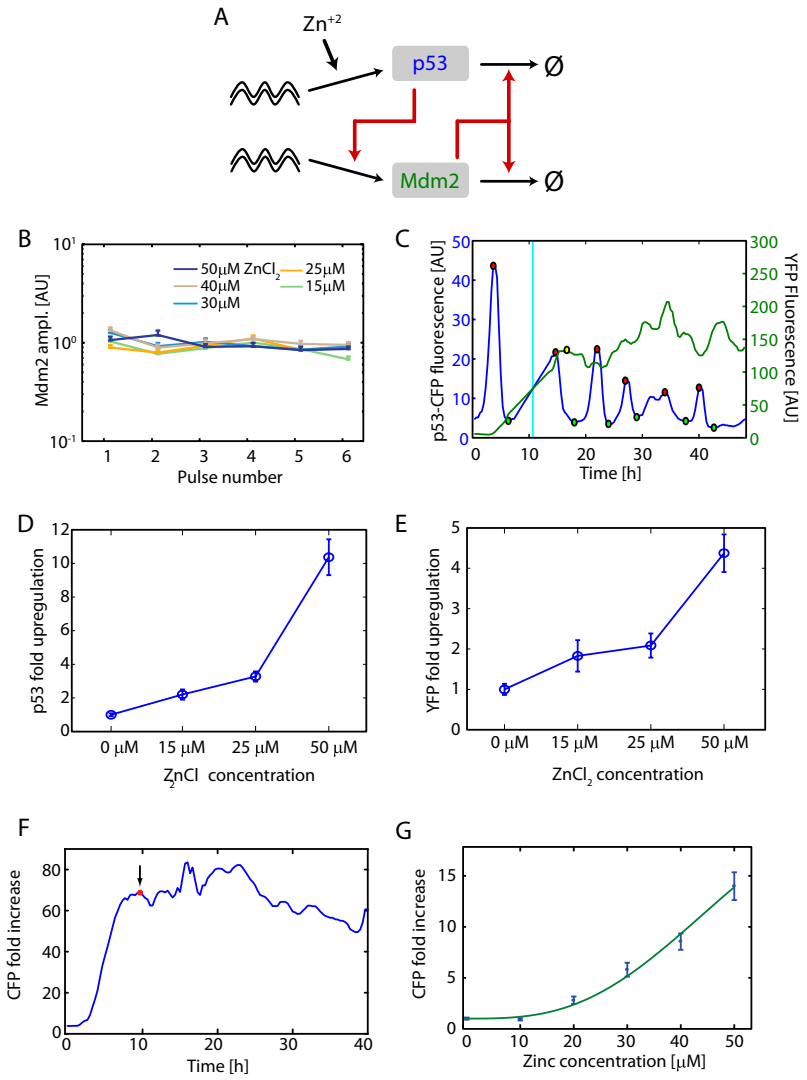

Fig. S2. Transfer functions of p53 and MTF in response to various zinc levels. (*A*) Detailed schematic diagram of the negative p53–Mdm2 feedback circuit, showing the three nonlinear reactions (red arrows) representing the action of p53 on the Mdm2 promoter, the ubiquitination of p53 by Mdm2, and Mdm2 autoubiquitination. (*B*) Mdm2–YFP amplitude is shown for each pulse after stimulation (mean + SEM). (*C*) Representative single-cell trajectory showing p53–CFP (blue curve) and YFP fluorescence (green curve) intensities over time, in a cell line in which YFP is driven by p53 from the Mdm2 promoter. Automatically identified maxima and minima are shown as points on each trajectory; vertical cyan bar represents time of cell division. (*D* and *E*) First pulse amplitude (mean + SEM) for p53 (*D*) and YFP (*E*) curves at three zinc concentrations. Datapoints are normalized to 0 μM condition. (*F*) Time-course of a typical cell in which CFP is driven by the metallothionein promoter, with the first CFP maximum identified shown as a red point. CFP expression reaches a plateau at ≈10 h. (*G*) The CFP maximum level at six zinc concentrations (points represent mean + SEM) are shown with the best-fit Hill equation (*SI Appendix*, Eq. **S7**) with parameters $a = 54.26$, $K_{zn} = 29.02$, and $n = 3$.

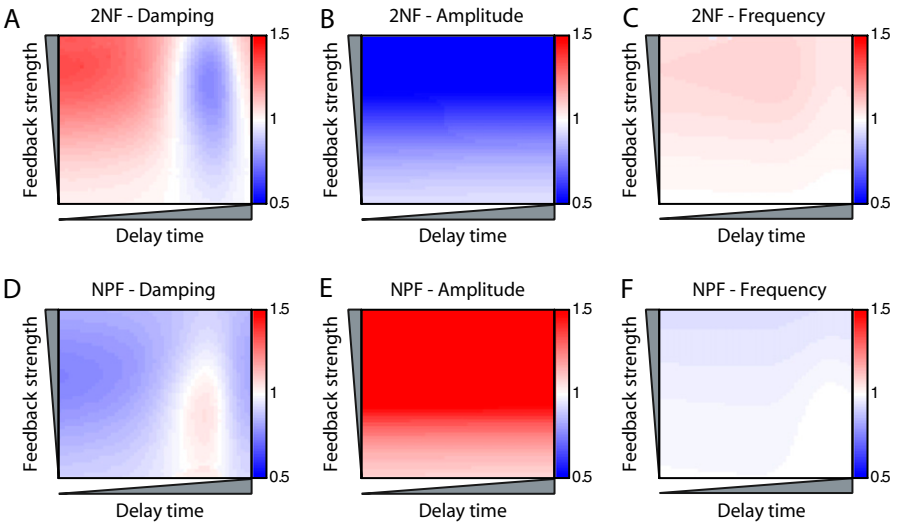

Fig. S3. Predictions for addition of synthetic feedback loops on p53. Prediction of p53 damping (*A*, *D*), p53 amplitude (*B*, *E*), and p53 frequency coefficient (*C*, *F*) for the model incorporating a second negative feedback in addition to the core p53–Mdm2 loop (*A*–*C*; 2NF model) and a synthetic positive feedback loop in addition to the core p53–Mdm2 loop (*D*–*F*; NPF model). The *x* and *y* axes represent 51 logarithmically spaced values from 0.1 to 10 times the nominal parameter value for the MTF1 protein production delay γ_{f0} and synthetic feedback strength β_{p0}, respectively.

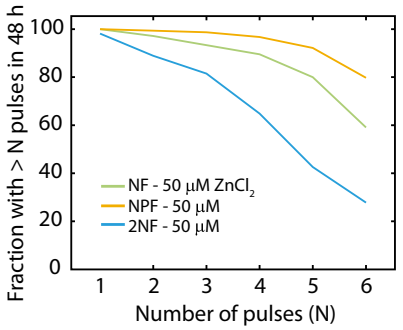

Fig. S4. Fraction of pulsing cells in the presence of additional synthetic feedback loops on p53. The number of cells observed undergoing each pulse are plotted against the number of pulses from the same single cell time-lapse microscopy experiments as Fig. 3 *E* and *F*.

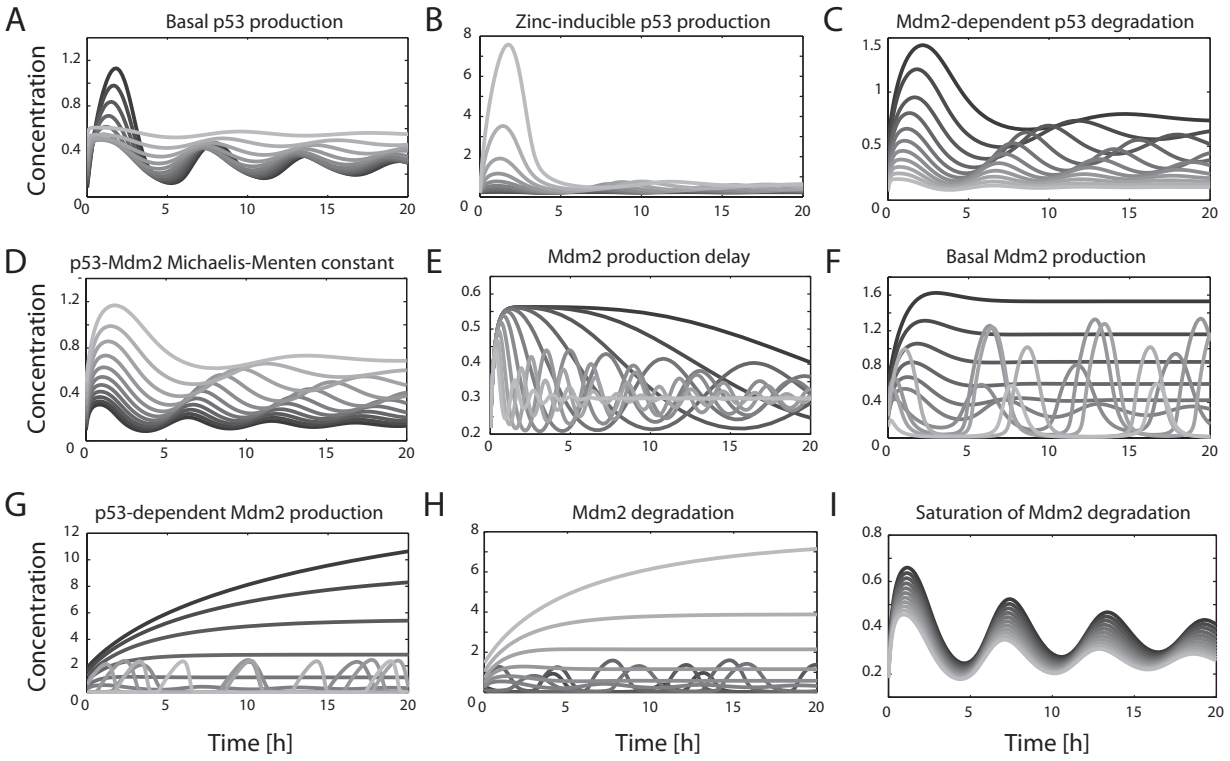

Fig. S5. Effect of parameter perturbations on oscillatory trajectories. Trajectories from the core negative-feedback model are shown for a range of values of key model parameters. In each panel, p53 concentration is plotted over time for 21 logarithmically spaced values of the variable parameter, ranging from 1.5 orders of magnitude below to 1.5 orders of magnitude above its nominal value. Parameters shown are (*A*) α_p, (*B*) p_z, (*C*) δ_p, (*D*) K_p, (*E*) γ_{m0}, (*F*) α_{m0}, (*G*) β_{m0}, (*H*) δ_m, and (*I*) K_m.

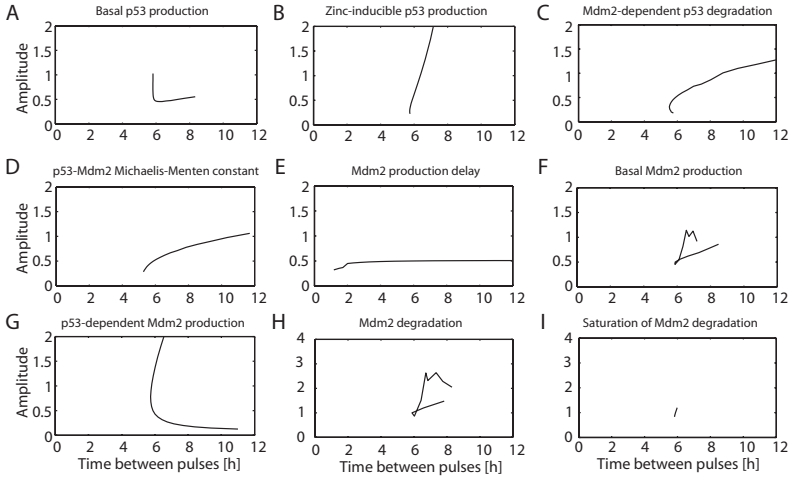

Fig. S6. Effect of parameter perturbations on oscillation amplitude and frequency. The variation in oscillation features are shown for a range of values of key model parameters. In each panel, curves trace the oscillation frequency and first-pulse amplitude at different logarithmically spaced values of an individual model parameter, ranging from 1.5 orders of magnitude below to 1.5 orders of magnitude above its nominal value. Parameters shown are (*A*) α_p, (*B*) p_z, (*C*) δ_p, (*D*) K_p, (*E*) γ_{m0}, (*F*) α_{m0}, (*G*) β_{m0}, (*H*) δ_m, and (*I*) K_m.

Table S1. Parameters for the core p53–Mdm2 negative feedback (1NF) model

Parameter name	Parameter value	Description	Units
p_z	0.527	Zinc-mediated p53 production rate	1/(Zn h)
α_p	1	p53 protein production rate	p53/h
δ_p	16,388	Mdm2-mediated p53 degradation rate	1/h
K_p	3.568	Saturation of Mdm2-mediated p53 degradation	p53
α_{m0}	0.069	Mdm2 mRNA basal transcription rate	*mdm2*/h
β_{m0}	0.058	p53-mediated Mdm2 mRNA transcription rate	*mdm2*/h
K_{m0}	0.117	Saturation of p53-mediated Mdm2 production	p53
γ_{m0}	1.854	Mdm2 production delay	1/h
γ_m	0	Mdm2 degradation rate	1/h
δ_m	0.128	Mdm2-mediated Mdm2 degradation rate	1/h
K_m	1.25×10^{-4}	Saturation of Mdm2-mediated Mdm2 degradation	Mdm2

The table indicates each parameter's name, its nominal value, a description of its effect, and the units in which it is measured. Italicized units are mRNA; otherwise units represent protein levels.

Table S2. Parameters for the models with additional synthetic feedback loops on p53

Parameter name	Parameter value	Description	Units
α_{p0}	0.040	P53 mRNA basal transcription rate	1/(Zn h)
$\beta_{p0,NPF}$	14.82	NPF-mediated *p53* mRNA transcription rate	*p53*/h
$\beta_{p0,2NF}$	0.317	2NF-mediated *p53* mRNA transcription rate	*p53*/h
K_f	2	Saturation of feedback-mediated p53 production	FB
γ_{p0}	3.293	p53 production delay	1/h
α_p	1	p53 production rate	p53/h
δ_p	16,388	Mdm2-mediated p53 degradation rate	p53
α_{m0}	0.069	*mdm2* mRNA basal transcription rate	1/h
K_p	3.568	Saturation of Mdm2-mediated p53 degradation	p53
β_{m0}	0.058	p53-mediated *mdm2* mRNA transcription rate	Mdm2/h
K_{m0}	0.117	Saturation of p53-mediated Mdm2 production	p53
γ_{m0}	1.854	Mdm2 production delay	1/h
γ_m	0	Mdm2 degradation rate	1/h
δ_m	0.128	Mdm2-mediated Mdm2 degradation rate	Mdm2/h
K_m	1.25×10^{-4}	Saturation of Mdm2-mediated Mdm2 degradation	Mdm2
α_{f0}	0.069	Feedback species mRNA transcription rate	*FB*/h
$\beta_{f0,NPF}$	0.396	NPF p53-mediated feedback species production	*FB*/h
$\beta_{f0,2NF}$	31.05	2NF p53-mediated feedback species production	*FB*/h
K_{f0}	0.117	Saturation of p53-mediated feedback production	p53
γ_{f0}	1	Feedback protein production delay	1/h
γ_f	1	Feedback protein degradation rate	1/h

The table indicates each parameter's name, its nominal value, a description of its effect, and the units in which it is measured. Dynamics of the core 1NF loop in the absence of feedback (obtained by setting β_{f0} to zero) are identical to those obtained from the original 1NF model. Italicized units are mRNA; otherwise units represent protein levels. For simplicity, units of the feedback species (either MTF1 or MTF1-KRAB) are shown as "FB."

Other Supporting Information Files

SI Appendix (PDF)