Supporting Information

Geva-Zatorsky et al. 10.1073/pnas.1001107107

SI Text

The p53–Mdm2 System Can Be Approximated by a Set of Linear Equations. The equations that we used for the system identification of the p53–Mdm2 are linear (see Eq. **1** of the main text). Here we check the validity of the linear approximation. For this we calculated the kurtosis, which is the fourth-order cumulant. We used the normalized kurtosis defined as:

$$\kappa = \frac{E\{x^4\}}{E\{x^2\}^2}$$

where $E\{f(x)\}$ is the expectation value of $f(x)$.

A general feature of the kurtosis function (as defined here) is that it can accurately differentiate between Gaussian and non-Gaussian distributions. For a Gaussian distribution $\kappa = 3$ and generically $\kappa \neq 3$ for non-Gaussian distributions.

Here we calculate the kurtosis (κ) for the experimental data of p53 and Mdm2. For each time point we calculate the kurtosis for the distribution of p53–CFP and Mdm2–YFP of about 100 individual cells (Fig. S1 *A* and *B* red curve).

The calculated kurtosis can be used for quantitative estimation of the linearity of the p53–mdm2 system. If the p53–mdm2 system is linear then the inherent noise in the system will result in a Gaussian distribution of the measured p53–CFP and mdm2–YFP, and will therefore show a kurtosis of $\kappa = 3$ at each time point. A nonlinear system will generally show a non-Gaussian distribution and a kurtosis that is different from 3.

As a control we also calculated the kurtosis from our stochastic simulations. The stochastic simulation uses a linear set of equations and is calibrated according to our method of system identification. The added noise in the stochastic simulation is also calibrated, and it results in p53 and Mdm2 responses that are similar to the measured ones. We calculated the kurtosis of the simulated responses exactly as it was calculated for the experimental data (Fig. S1 blue curve). We also used independent runs of the stochastic simulation to create about 100 independent responses (as the number of individual cells in our experiment). Because the stochastic simulation uses a linear set of equations, the kurtosis should be around 3, and any divergence from this value is accounted for by the noise in the stochastic simulation.

Comparing the kurtosis of the experimentally measured responses (Fig. S1 red curves) to the kurtosis of the simulated responses (Fig. S1 blue curves) shows that they are equal within their statistical error, and both the experimental and simulated kurtosis is located around a value of $\kappa = 3$, indicating that the system can be approximated with reasonable accuracy by a linear system.

The mean normalized kurtosis (over time) of the experimental and simulated data of p53 and Mdm2 is equal to 3 within its statistical error (see Fig. S1 legend). This further supports our view that the p53–Mdm2 system can be approximated by a linear system.

The Distribution of the Measured p53–mdm2 Amplitudes Is Well Explained by a Theoretical Model, Suggesting That the Oscillations Are Sustained by the Inherent Noise in the System. A support for our view that the observed oscillations of the p53–Mdm2 system are sustained by the inherent noise in the system comes from a theoretical model (1). The model analyzes the distribution of amplitudes of noise-driven oscillations. In the simplest case we consider a harmonic oscillator that gives rise to damped oscillations (1). Here we follow the model derived in ref. 1:

$$\dot{x}_1 = x_2 - kx_1 + \sigma\Gamma_1$$

$$\dot{x}_2 = -x_1 - kx_2 + \sigma\Gamma_2$$

where Γi are uncorrelated, statistical independent Gaussian white noise with zero mean and variance of one, and σ, k are constants. The deterministic system ($\sigma = 0$) shows damped oscillations upon perturbation from its steady state. However, the stochastic system ($\sigma > 0$) shows sustained oscillations. The amplitude distribution of this simple system was analyzed in ref. 1, which found the amplitude distribution of the system:

$$P_A(\chi) \propto \chi \exp\left(-\frac{\chi^2 k}{\sigma^2}\right)$$

where χ is the amplitude. Our linear model of the p53–Mdm2 system is close to a damped harmonic oscillator; we therefore compared our measured amplitude distribution to the predicted one (Fig. 5 *C* and *D* in the main text) and found our experimentally measured amplitude distribution to be well explained by the predicted one. This supports our view that the inherent noise in the system sustains otherwise damped oscillations.

1. Lang M, Waldherr S, Allgower F (2009) Amplitude distribution of stochastic oscillations in biochemical networks due to intrinsic noise. *PMC Biophys* 2(1):10.

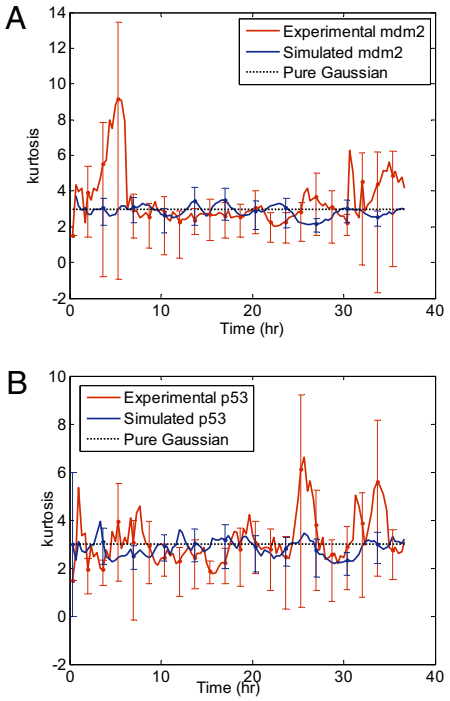

Fig. S1. The p53–Mdm2 system can be approximated by a linear system, with reasonable accuracy. Shown is the normalized kurtosis of the experimentally measured (*A*) Mdm2–YFP (red curve) (*B*) p53–CFP (red curve) and of the stochastically simulated (*A*) Mdm2 (blue curve) and (*B*) p53 (blue curve). The black dotted line is the kurtosis of a pure Gaussian distribution. The mean normalized kurtosis of the measured mdm2 response is $\kappa = 3.5 \pm 0.8$ and $\kappa = 3.2 \pm 0.7$ for the p53 response. The mean normalized kurtosis of the stochastic simulated mdm2 response is $\kappa = 2.9 \pm 0.2$ and $\kappa = 2.9 \pm 0.2$ for the p53 response. All of the error bars are statistically calculated by bootstrapping.