**Figure S1. Fitting of IC-RP trajectories.** The cell shown was treated with 50 ng/ml TRAIL plus 2.5 μg/ml CHX and imaged as described in Methods. For all cells in Fig. 1c-e, background-subtracted CFP and YFP images were divided to create a ratiometric image using ImageJ and custom plug-ins. For each cell, we then subtracted the minimum signal value across all time points. The cell was deemed "dead" at the first decrease in signal following MOMP. We normalized the trajectory by dividing by the signal's value at this point. As the cell dies and lifts off the plate, the signal becomes noisy. We therefore force the signal to a value of one from this point on and fit to this modified trajectory (blue). Fitting was performed in MATLAB with the following equation, mathematically derived to represent a single cell's IC-RP trajectory (J.M.B., J.G.A., S.L.S., D. Lauffenburger, P.K.S., manuscript in preparation).

$$y = \begin{cases} 1 - e^{-k_{IC}t^3} & t < \tau \\ 1 - e^{-k_{IC}t^3 - k_f(t-\tau)^3} & t \geq \tau \end{cases}$$

$\tau$ corresponds approximately to the time of MOMP. We found $k_f$ to be invariant across dose and thus we focused only on $k_{IC}$ in the body of the text.

**Figure S2. Contribution of $k_{IC}$ vs. $\theta$.** For cells shown in Fig. 1e, the relative contribution of variability in $k_{IC}$ and $\theta$ to variability in $T_d$ was assessed computationally by fixing one parameter at its mean value, allowing the other to vary over the observed range, and assessing the resulting distribution of $T_d$. At low doses of TRAIL, we observe a greater contribution from variability in $k_{IC}$. The effects of the two parameters contribute nonlinearly to variability in time of MOMP.

**Figure S3. Time of MOMP is highly correlated in sister MCF 10A cells treated with TRAIL + CHX.** The fluorescent reporter for MOMP (IMS-RP) was introduced into MCF 10A cells. The cells were imaged in the standard media described by ATCC in a humidified, 37ºC chamber with 5% $CO_2$. Cells were imaged with a 10x objective for 28 hr (frames every 10 min) to determine division times and track pairs of sister cells. Subsequently, the growth media was replaced with media containing 50 ng/ml TRAIL plus 2.5 μg/ml CHX and cells were imaged (frames every 4 min) until all had died. **a,** Correlation of $T_d$ among 40 pairs of recently divided sister cells ($T(\overline{Div \rightarrow MOMP})$ < 15.43hr). Each circle denotes a pair of sister cells. The correlation coefficient ($R^2$) was obtained by linear regression. **b,** Difference in $T_d$ of sister cell pairs ($\Delta T_d$) as a function of $T(\overline{Div \rightarrow MOMP})$. Each circle denotes a pair of sister cells.

**Figure S4. Cell fate is highly correlated in sister HeLa cells treated with TRAIL alone.** Cells were imaged for 30 hr (frames every 10 min) to determine division times and track pairs of sister cells. Subsequently, the growth media was replaced with media containing 50 ng/ml TRAIL (no CHX) and cells were imaged (frames every 3 min) for 13.5 hr. **a,** The number of cells that survived the treatment and the number that died were determined. The probability of surviving and the probability of dying were computed and used to determine the expected

probability that a pair of cells would either both live, both die, or have disparate fates (one lives, one dies), assuming independence of fate. A $Chi^2$ test was used to compare the expected and observed numbers of pairs in each category. The highly statistically significant *p*-value ($8x10^{-6}$) indicates that sister pairs are much more likely to share the same fate (both live or both die) than would be expected for independent pairs of cells. **b,** For the 76 sister pairs where both cells died, the difference in $T_d$ of sister cell pairs ($\Delta T_d$) was plotted as a function of $T(\overline{Div \rightarrow MOMP})$. Each circle denotes a pair of sister cells. The fact that sister cells have similar fates and that sister-to-sister correlation in $T_d$ decays as a function of time since division suggest that any induction of survival pathways (suppressed by addition of CHX in Fig. 2) does not override the effects of pre-existing variability in initial conditions.

**Figure S5. Quantitative immunoblots for endogenous Bcl-2 and GFP- Bcl-2 in HeLa cells.** Pure protein and HeLa cell lysate were loaded on a 10% Tricine SDS-PAGE gel as indicated. After transfer to a PVDF membrane, blots were probed, scanned on a LI-COR Odyssey scanner, and quantified digitally. **a,** The membrane was probed with rabbit anti-Bcl-2 (Santa Cruz Biotechnology SC783) followed by AF680-conjugated anti-rabbit. The pure Bcl-2 is a 46kD fusion protein (Santa Cruz Biotechnology SC4096). **b,** From the standard curve, we calculate that a single HeLa cell has $1.57x10^{-15}$ g Bcl-2. Using 26,135 as the molecular weight of Bcl-2, we find 36,000 Bcl-2/cell. The average of 14 such measurements yields 30,000 Bcl-2/HeLa cell; s.e.m = 10,000. **c,** The membrane was probed with mouse anti-GFP (Roche #11814460001) followed by IRDye 800-conjugated anti-mouse. The pure GFP was purchased from Biovision (#4999-100). **d,** From the standard curve, we calculate that a single HeLa cell has $5.68x10^{-15}$ g GFP-Bcl-2. Using 27,000 as the molecular weight of GFP, we find 127,000 GFP-Bcl-2/cell. The average of 5 such measurements yields 133,000 GFP-Bcl-2/HeLa cell s.e.m = 18,000. We set the average (background subtracted) GFP-Bcl-2 fluorescence intensity of the population of cells in the first frame of the movie used in Figure 3d equal to the average number of GFP-Bcl-2 in the HeLa cells used for the movie (calculated above) and rescaled the *x*-axis of Figure 3d into units of GFP-Bcl-2 proteins per cell.

**Figure S6. Quantitative immunoblots for endogenous Bcl-$X_L$ and GFP- Bcl-$X_L$ in HeLa cells.** Pure protein and HeLa cell lysate were loaded on a 10% Tricine SDS-PAGE gel as indicated. After transfer to a PVDF membrane, blots were probed, scanned on a LI-COR Odyssey scanner, and quantified digitally. **a,** The membrane was probed with rabbit anti- Bcl-$X_L$ (CST #2762) followed by AF680-conjugated anti-rabbit. The pure Bcl-$X_L$ is a gift from Emiko Fire (MIT). **b,** From the standard curve, we calculate that a single HeLa cell has $2.96x10^{-16}$ g Bcl-$X_L$. Using 25,918 as the molecular weight of Bcl-$X_L$, we find 6,900 Bcl-$X_L$ /cell. The average of 7 such measurements yields 7,700 Bcl-$X_L$ /HeLa cell; s.e.m = 1,300. **c,** The membrane was probed with mouse anti-GFP (Roche #11814460001) followed by IRDye 800-conjugated anti-mouse. The pure GFP was purchased from Biovision (#4999-100). **d,** From the standard curve, we calculate that a single HeLa cell has $1.99x10^{-14}$ g GFP- Bcl-$X_L$. Using 27,000 as the molecular weight of GFP, we find 445,000 GFP- Bcl-$X_L$ /cell. The average of 6 such measurements yields 579,000 GFP- Bcl-$X_L$ /HeLa cell; s.e.m = 200,000. We set the average (background subtracted) GFP- Bcl-$X_L$ fluorescence intensity of the population of cells in the first frame of the movie equal to the average number of GFP- Bcl-$X_L$ in the HeLa cells used for the

movie (calculated above) and rescaled the *x*-axis of Figure 3e into units of GFP- Bcl-$X_L$ proteins per cell.

**Figure S7. Quantitative immunoblots for Bid-GFP and endogenous Bid in HeLa cells.** Pure protein and HeLa cell lysate were loaded on a 10% Tricine SDS-PAGE gel as indicated. After transfer to a PVDF membrane, blots were probed, scanned on a LI-COR Odyssey scanner, and quantified digitally. **a,** The membrane was probed with mouse anti-GFP (Roche #11814460001) followed by IRDye 800-conjugated anti-mouse. The pure GFP was purchased from Biovision (#4999-100). **b,** From the standard curve, we calculate that a single IMS-RP Bid-GFP clone 3.1 HeLa cell has $8.51 \times 10^{-10}$ g Bid-GFP. Using 27,000 as the molecular weight of GFP, we find 19,000 Bid-GFP/cell. **c,** The membrane was probed with rabbit anti-Bid (Atlas Antibodies HPA000722) followed by AF680-conjugated anti-rabbit. Using the IMS-RP Bid-GFP clone 3.1 HeLa cells as a standard, we find that a single IMS-RP Bid-GFP clone 3.4 HeLa cell has 62,000 Bid-GFP/cell. We set the average (background subtracted) IMS-RP Bid-GFP clone 3.4 fluorescence intensity (70.9 relative fluorescence units, RFU) of the population of cells in the first frame of a movie equal to the average number of Bid-GFP per IMS-RP Bid-GFP clone 3.4 HeLa cell (calculated above). For movies of cells taken with the same microscope settings, we have the equality: 70.9 RFU = 62,000 GFP/cell. This allows us to rescale the *x*-axes of Fig. 3a and 3b into units of Bid-GFP proteins per cell. We also use this equality to rescale the *x*-axis of Fig. 3c into units of GFP-Bax proteins per cell. Finally, using the IMS-RP Bid-GFP clone 3.1 HeLa cells as a standard and 21,995 as the molecular weight of Bid, we find 20,000 endogenous Bid/HeLa cell. The average of 3 such measurements yields 28,000 endogenous Bid/HeLa cell; s.e.m. = 5,000.

**Figure S8. Quantitative immunoblots for GFP-Bax and endogenous Bax in HeLa cells.** Pure protein and HeLa cell lysate were loaded on a 10% Tricine SDS-PAGE gel as indicated. After transfer to a nitrocellulose membrane, blots were probed, scanned on a LI-COR Odyssey scanner, and quantified digitally. **a,** The membrane was probed with mouse anti-GFP (Roche #11814460001) followed by IRDye 800-conjugated anti-mouse. The pure GFP was purchased from Biovision (#4999-100). **b,** From the standard curve, we calculate that 9 µl of GFP-Bax HeLa lysate contains 0.43 ng of GFP. **c,** On a separate gel, a second 9 µl of GFP-Bax HeLa lysate was loaded along with a known number of plain HeLa cells. The membrane was probed with rabbit anti-Bax (Santa Cruz Biotechnology SC493) followed by IRDye 800-conjugated anti-rabbit. Since Bax is 1:1 with GFP, 9 µl of this lysate contains 0.43 ng Bax. Using the GFP-Bax HeLa lysate as a standard and 21,053 as the molecular weight of Bax, we find 176,000 endogenous Bax/HeLa cell. The average of 8 such measurements yields 224,000 endogenous Bax/HeLa cell; s.e.m. = 73,000.