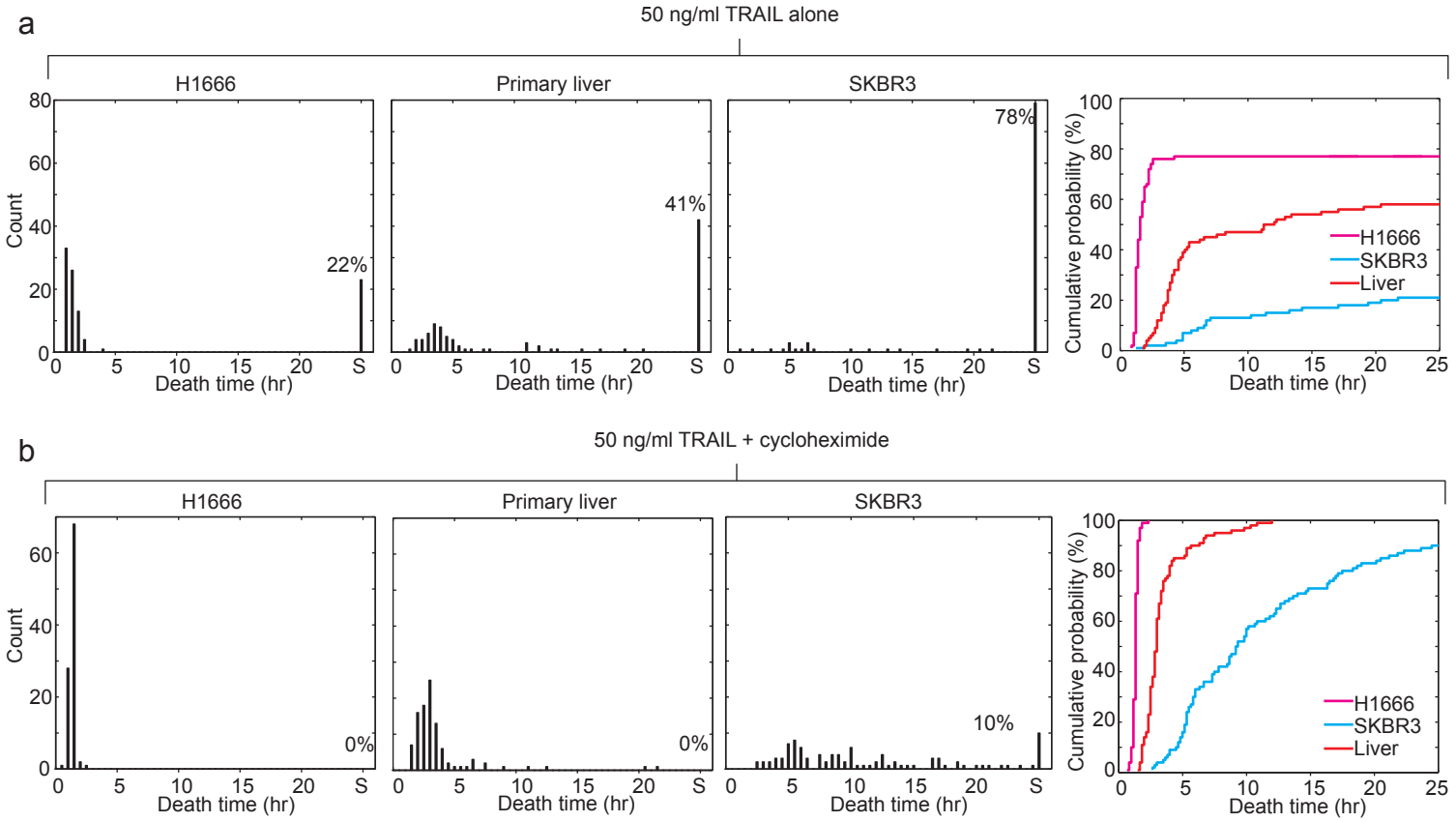
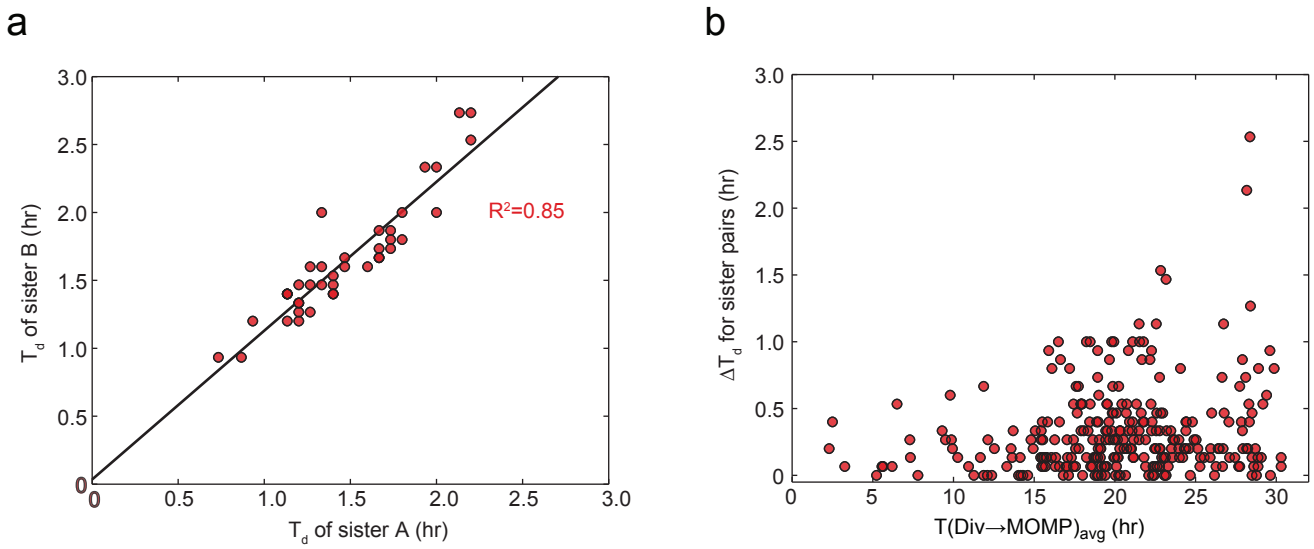


Supplementary Figure 1. Variability in death time with and without cycloheximide. The three cell types indicated were treated with 50 ng/ml TRAIL alone (top panels) or 50 ng/ml TRAIL + cycloheximide bottom panels) and imaged by brightfield microscopy every 10 minutes for 25.5 hr. One hundred cells were analyzed for each distribution. Death time was scored as the first frame of apoptotic morphology. Cells surviving this 25.5 hr movie are labeled with an "S" and the percentage of survivors is indicated. H1666 cells were the most sensitive line examined, while SK-BR-3 cells were the most resistant. However, even H1666 show variability in cell fate when treated with TRAIL alone. Cumulative count was calculated from the distributions.



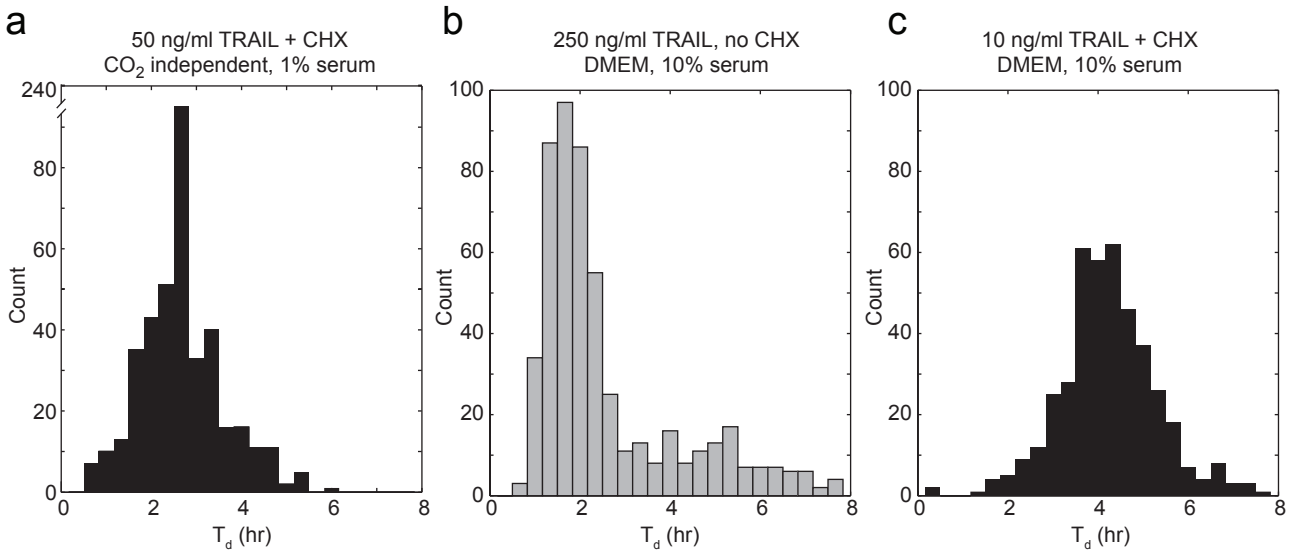
Supplementary Figure 2. Time of MOMP is correlated in sister MCF10A cells treated with TRAIL + CHX. The fluorescent reporter for MOMP (IMS-RP) was introduced into MCF10A cells. Cells were imaged with at 10x magnification for 28 hr (frames every 10 min) to determine division times and track pairs of sister cells. Subsequently, the growth media was replaced with media containing 50 ng/ml TRAIL plus 2.5 μg/ml cycloheximide and cells were imaged (frames every 4 min) until all had died. **a**, Correlation in death time (T_d) between 40 pairs of recently divided sister cells ($T(Div \rightarrow MOMP)_{avg}$< 15.4 hr). 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 (ΔT_d) as a function of $T(Div \rightarrow MOMP)_{avg}$.

Number of cells that died: 360
Number of cells that survived: 240
Total number of cells analyzed: 600
Probability of dying: 0.6
Probability of surviving: 0.4

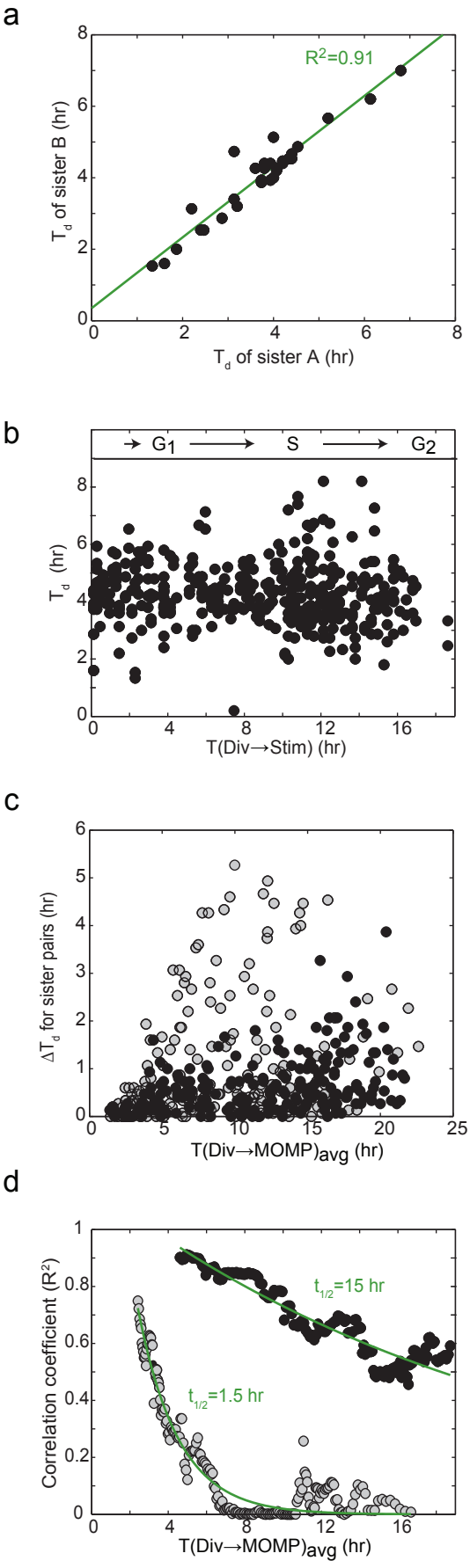
Fates of sister pairs:	Live-Live	Live-Die or Die-Live	Die-Die
Expected fraction:	0.16	0.48	0.36
Expected number:	48	144	108
Observed number:	86	68	146

Chi^2 value = 83.56
Degrees of freedom = 2
p-value = 7.16×10^{-19}

Supplementary Figure 3. Cell fate is correlated in sister HeLa cells treated with TRAIL alone. Table contains sister cells from the experiment depicted in Fig. 1f-i (250 ng/ml TRAIL). 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 random pair of cells would either both live, both die, or have disparate fates (one lives, one dies). A Chi^2 test was used to compare the expected and observed numbers of pairs in each category. The highly statistically significant p-value indicates that sister pairs are much more likely to share the same fate (both live or both die) than would be expected for random pairs of cells.

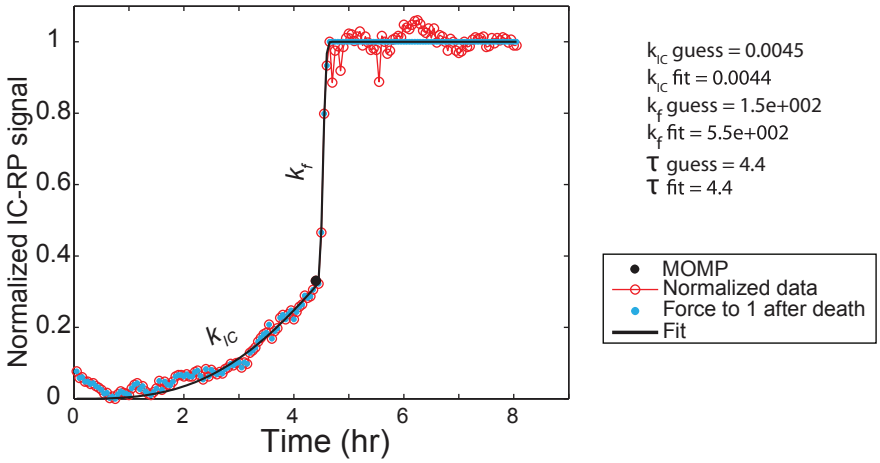


Supplementary Figure 4. Distributions of death time for HeLa sister cells, with and without cycloheximide. The distributions of MOMP times (T_d) of HeLa cells used for the sister cell experiments presented in Fig. 1 are plotted. **a**, Cells treated with 50 ng/ml TRAIL plus cycloheximide (CHX) in CO_2 independent medium with 1% serum (for Fig. 1b-e). **b**, Cells treated with 250 ng/ml TRAIL in the absence of CHX in DMEM with 10% serum (for Fig. 1f-i and Supplementary Fig. 5). **c**, Cells treated with 10 ng/ml TRAIL plus CHX in DMEM with 10% serum (for Fig. 1i and Supplementary Fig. 5).



Supplementary Figure 5. Comparison of sister cell experiments performed in parallel, with and without cycloheximide.

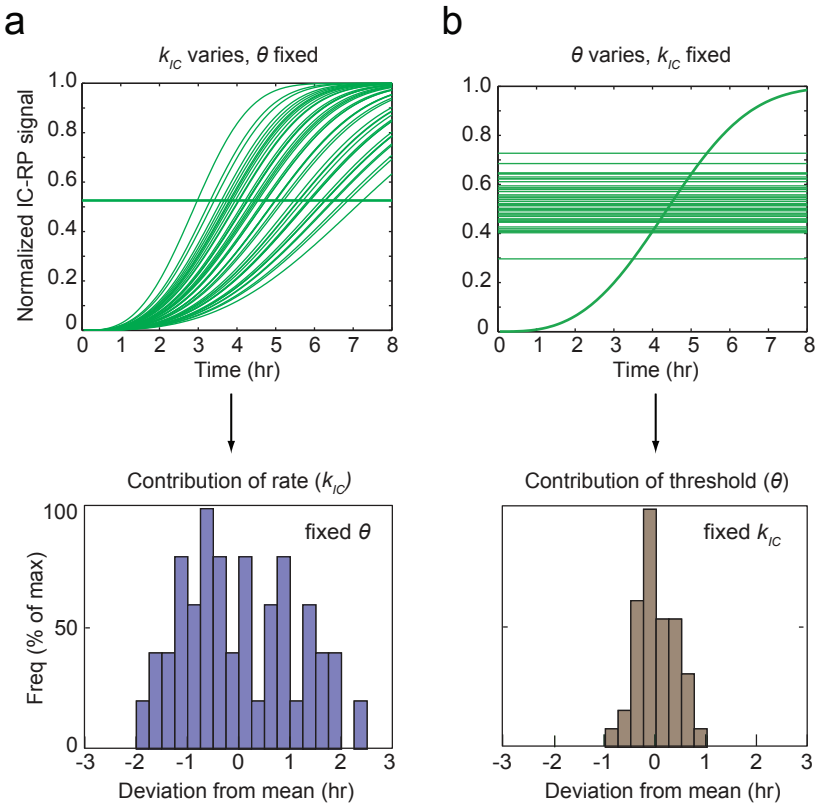
Comparison of sister cell decay times for cells imaged in DMEM with 10% serum and treated with 250 ng/ml TRAIL without cycloheximide (gray circles, data reproduced from Fig. 1f-i) or with 10 ng/ml TRAIL with 2.5 μg/ml cycloheximide (black circles, data plotted or reproduced from Fig. 1i); these TRAIL doses were chosen based on Supplementary Fig. 3 to create populations of cells with similar ranges of death times. **a**, Correlation of T_d among 30 pairs of recently divided sister cells ($T(Div \rightarrow MOMP)_{avg}$ < 5 hr) . Each circle denotes a pair of sister cells. b, Td as a function of time between division and stimulation, a proxy for cell cycle phase. Cell cycle phases are ndicated as G1, S, G2, as approximated from time since division. No correlation was observed between cell cycle phase and T_d (R^2 = 0.0008). **c**, Difference in T_d of sister cell pairs (ΔTd) as a function of $T(Div \rightarrow MOMP)_{avg}$. **d,** Decay in the correlation of sister cells for cells treated without cycloheximide (grey; reproduced from Fig. 1i) or with cycloheximide (black).



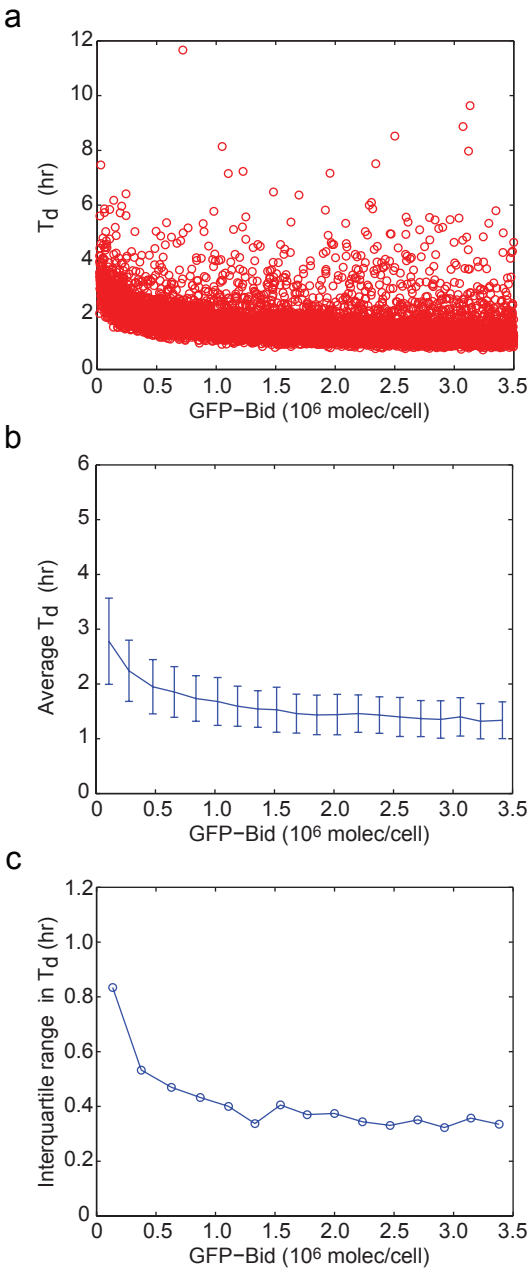
Supplementary Figure 6. Fitting of IC-RP trajectories. The cell shown was treated with 50 ng/ml TRAIL plus 2.5 μg/ml CHX and imaged as described in Methods. For all of the cells in Fig. 3c, background-subtracted CFP and YFP images were divided to create a ratiometric image using ImageJ and custom plug-ins. 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). To produce the trajectory in Fig. 3d, fitting was performed in MATLAB using the function 'nlinfit' 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).

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

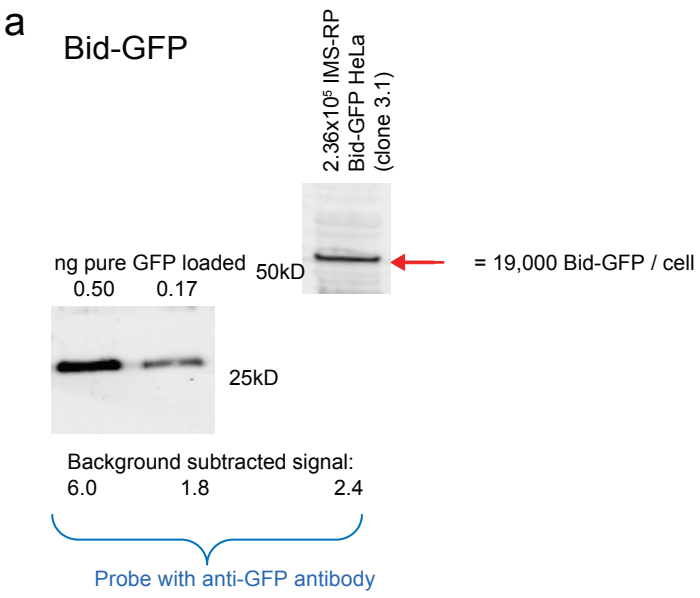
τ corresponds approximately to the time of MOMP. We found k_f to be invariant across dose and thus we focused only on k_{IC} in the body of the text.

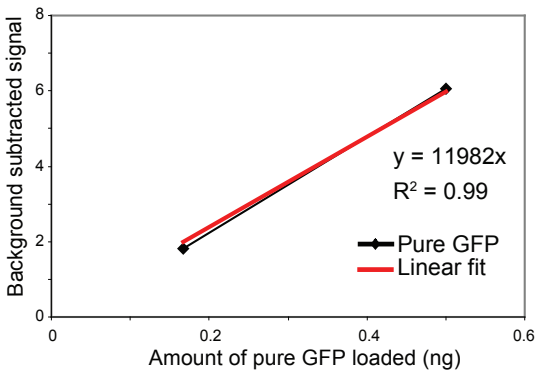


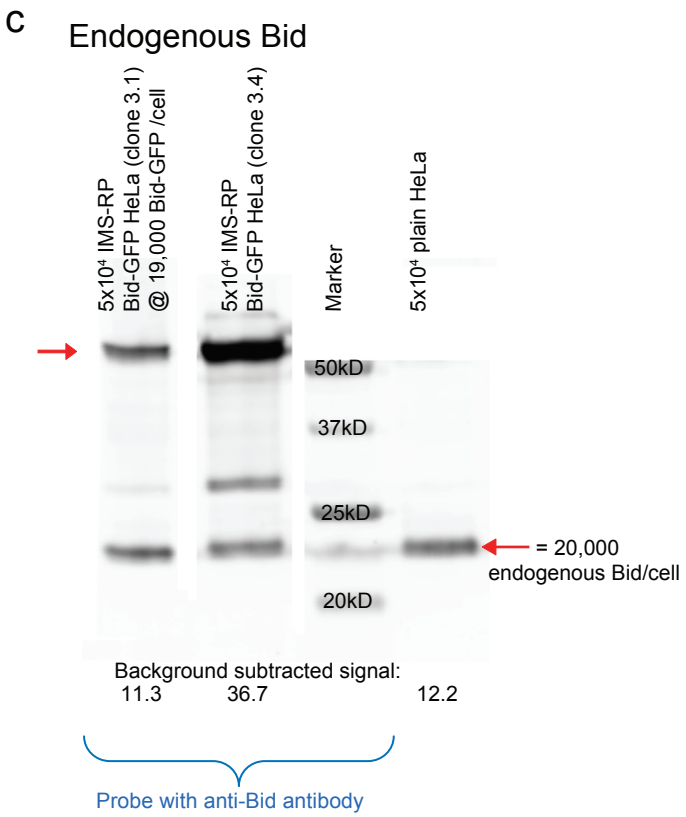
Supplementary Figure 7. Contribution of k_{IC} vs. θ. For distributions shown in Fig. 3f, the relative contribution of variability in k_{IC} and θ to variability in T_d was assessed computationally by fixing one parameter at its mean value, allowing the other to vary over the observed range, and assessing the resulting distribution of T_d. The distributions were then mean-centered at 0. The effects of the two parameters contribute non-linearly to time of MOMP.



Supplementary Figure 8. Computational analysis suggests that Bid overexpression reduces T_d mean and dispersion. Simulations were performed to mimic overexpression of GFP-Bid. All initial protein concentrations were sampled from lognormal distributions as described in Methods, but an additional amount of Bid was added (GFP-Bid), by sampling from a uniform distribution. **a**, T_d as a function of GFP-Bid amount per cell for 10,000 simulations. **b**, Average T_d plotted as a function of GFP-Bid levels for 15 bins showing that Bid overexpression reduces average T_d. Error bars represent the interquartile range (75th percentile value - 25th percentile value). **c**, Dispersion in the values of T_d as a function of GFP-Bid levels. The dispersion in the distribution of T_d in 15 equal intervals of GFP-Bid expression was measured using the interquartile range to minimize the effect of outliers in the distribution. The variability in T_d decreases as GFP-Bid levels increase.







Supplementary Figure 9. Quantitative immunoblots for Bid-GFP and endogenous Bid in HeLa cells. Pure GFP protein (Biovision, #4999-100) and HeLa cell lysate were separated by 10% Tricine SDS-PAGE as indicated and transferred to a PVDF membrane, 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. **b**, From the standard curve, we calculate that a single IMS-RP Bid-GFP clone 3.1 HeLa cell has 8.51×10^{-10} g Bid-GFP. Using 27 kDa 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 62,000 (calculated above; 70.9 RFU = 62,000 Bid-GFP/cell). This allows us to rescale the x-axis of Fig. 4d into units of Bid-GFP 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.