

Figure 1

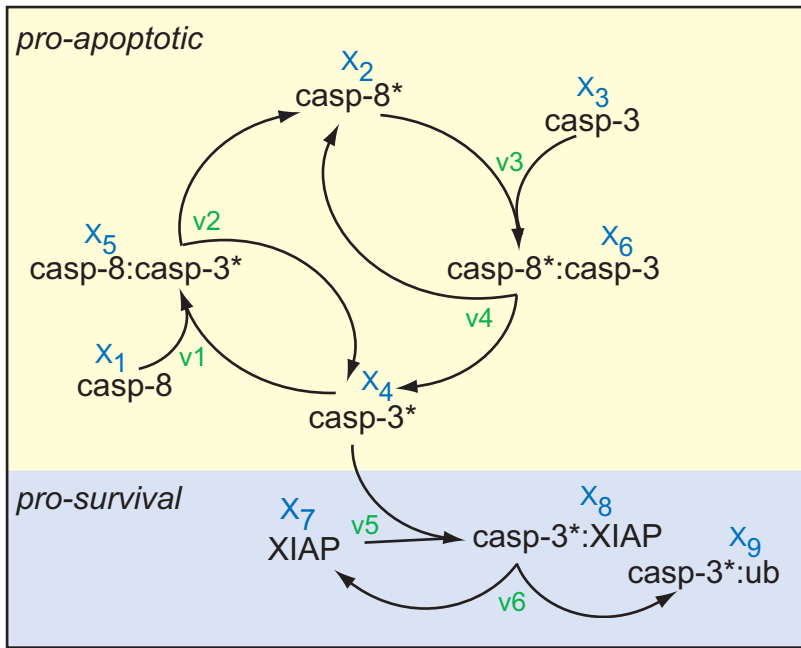


Figure 2

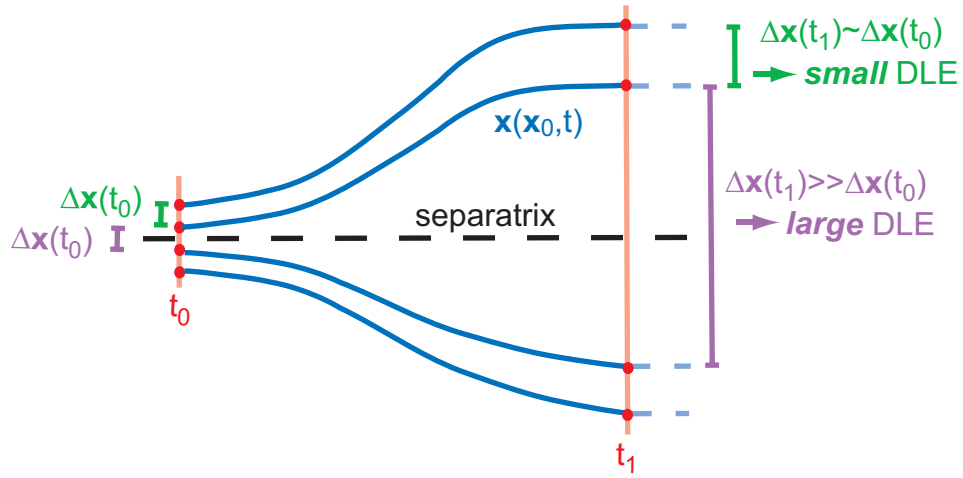
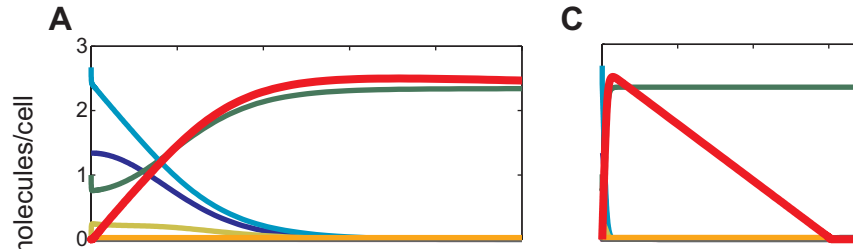


Figure 3

Low initial condition of XIAP: death



High initial condition of XIAP: survival

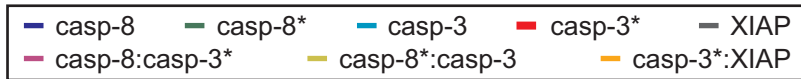
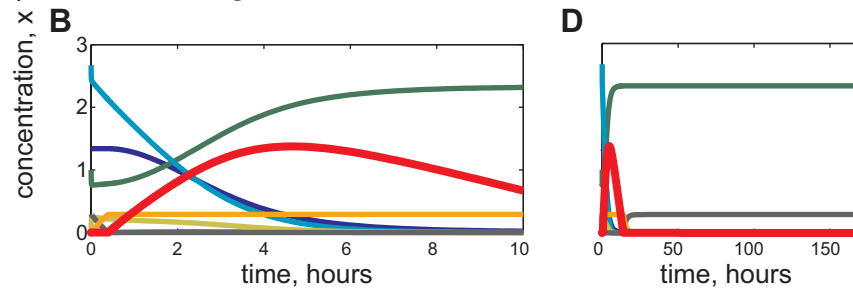


Figure 4

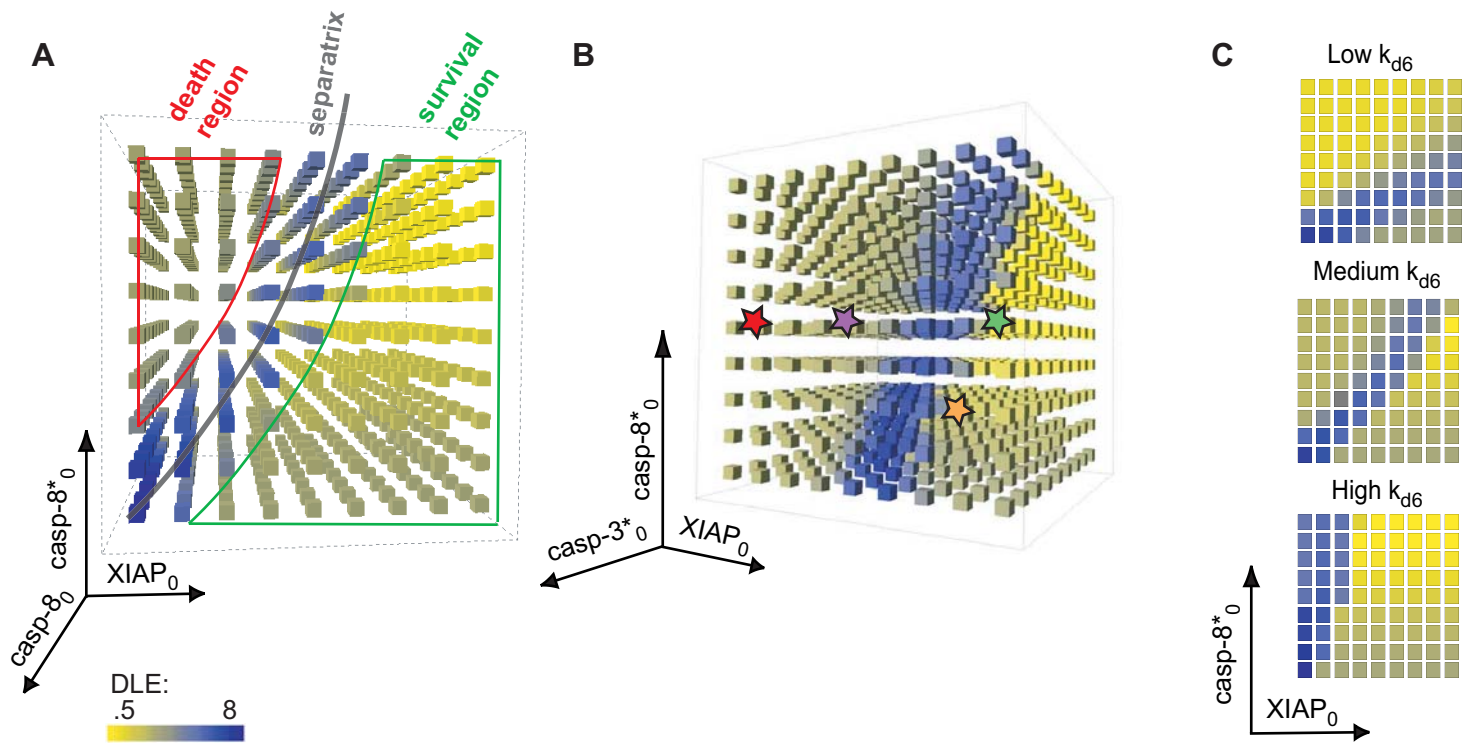


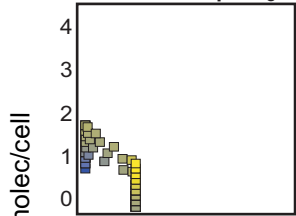
Figure 5

A

Non zero species: \ Fixed point type:	1	2	3	4
	U	U	U	S
casp-8			●	●
casp-8*	●			●
casp-3		●	●	
casp-3*	●	●		
casp8:casp3*				
casp8*:casp3				
XIAP			●	●
casp-3*:XIAP				

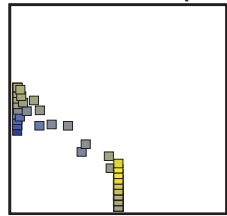
B

Low casp-8₀



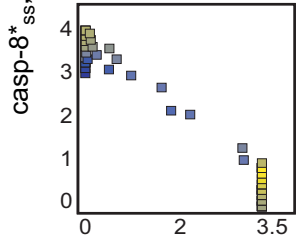
C

Medium casp-8₀



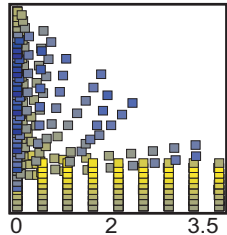
D

High casp-8₀



E

Mixed casp-8₀

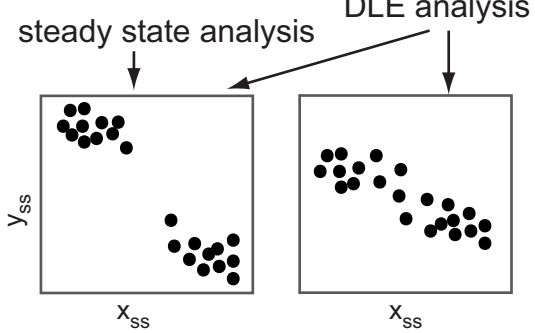


casp-8*_{ss}, x 10⁵ molec/cell

casp-8_{ss}, x 10⁵ molec/cell



F



9 SUPPLEMENTARY MATERIALS

9.1 Fixed point criterion

To solve for the fixed points, we note that the listed rate parameters are nonzero. By definition of a fixed point, we set derivatives of each species to zero:

$$0 = -k_1 x_4 x_1 + k_{d1} x_5 \quad (17)$$

$$0 = k_{d2} x_5 - k_3 x_2 x_3 + k_{d3} x_6 + k_{d4} x_6 \quad (18)$$

$$0 = -k_3 x_2 x_3 + k_{d3} x_6 \quad (19)$$

$$0 = k_{d4} x_6 - k_1 x_4 x_1 + k_{d1} x_5 - k_5 x_7 x_4 + k_{d5} x_8 + k_{d2} x_5 \quad (20)$$

$$0 = -k_{d2} x_5 + k_1 x_4 x_1 - k_{d1} x_5 \quad (21)$$

$$0 = -k_{d4} x_6 + k_3 x_2 x_3 - k_{d3} x_6 \quad (22)$$

$$0 = -k_5 x_7 x_4 + k_{d5} x_8 + k_{d6} x_8 \quad (23)$$

$$0 = k_5 x_7 x_4 - k_{d5} x_8 - k_{d6} x_8 \quad (24)$$

Substituting (17) into (21):

$$k_{d2} x_5 = 0 \Rightarrow x_5 = 0. \quad (25)$$

Substituting (19) and (25) into (18):

$$k_{d4} x_6 = 0 \Rightarrow x_6 = 0. \quad (26)$$

Substituting (25) into (17):

$$x_4 x_1 = 0. \quad (27)$$

Substituting (25), (26), and (27) into (20):

$$k_5 x_7 x_4 = k_{d5} x_8 \quad (28)$$

Rewriting (24):

$$k_5 x_7 x_4 = (k_{d5} + k_{d6}) x_8. \quad (29)$$

Comparing (28) and (29),

$$(k_{d5} + k_{d6}) x_8 = k_{d5} x_8. \quad (30)$$

Because we have assumed that the rate constants are nonzero,

$$x_8 = 0 \quad (31)$$

Substituting (15) into (12)

$$x_7 x_4 = 0 \quad (32)$$

From equations (9), (10), (11), (15), and (16) we can derive steady-state conditions:

$$x_5 = x_6 = x_8 = 0 \quad (33)$$

$$x_4 OR(x_1 AND x_7) = 0, \quad (34)$$

$$x_2 OR x_3 = 0 \quad (35)$$

Together, these define conditions for the four types of fixed points in the system (Figure 5A).

9.2 Determination of stability

The stability of each type of fixed point (listed in Figure 5A) was deduced using conservation relations and type-specific steady state conditions (equations 33-35). This is convenient since traditional stability analysis would have been extremely difficult for this 8-dimensional system. At each fixed point, there are 2-4 zero eigenvalues, making stability analysis methods based on linearization inconclusive. The number of zero eigenvalues was determined symbolically using Maple 9 (Waterloo Maple Inc., Ontario, Canada).

- Fixed point type 1: All species must have zero concentrations except active caspase-8 and active caspase-3. Since XIAP concentration is zero (there is no turnover of XIAP in the model and XIAP is not initially present), active caspase-3 cannot form complexes with XIAP. Therefore, the concentration of caspase-3 and caspase-8 will not change. These fixed points are unstable because perturbations of XIAP concentrations to nonzero values send these trajectories to fixed point type 4.

Biologically, these fixed points would represent cells signaling for death because all of the caspases are active. These fixed points are attracting in some directions because the pulse peaks approach these fixed points before declining towards fixed point type 4 (Figure 3). In comparing trajectories from above and below the separatrix, we noticed that the peak of caspase-3 activation corresponds to this type of unstable fixed point. The height of the pulse corresponds with its width. For example, in Figure 3C, the active caspase-3 pulse is tall and wide while in Figure 3D, the pulse is short and narrow. The unstable fixed point is attracting in some directions; therefore, the closer the trajectory moves towards this unstable fixed point, the stronger the pro-apoptotic pull. This attraction to the unstable fixed point is what resulted in higher and wider active caspase-3 pulses.

We therefore defined a pulse metric to explore the relationship between the pulse and the network outcome. Since we hypothesized that the pulse magnitude and width corresponded with its proximity to these unstable fixed points, we defined a pulse metric as the shortest distance between the trajectory and fixed point 1. For the time courses shown in Figures 3A-B, the pulse metrics are 1.3×10^4 and 1.4×10^5 for low and high initial conditions of XIAP, respectively. In Figure S1, the pulse metric is plotted in the same phase-space slice displayed for the DLEs in Figure 4A. This metric divides the phase space similar to the separatrix defined by high DLEs. Above the separatrix, the pulse metric is small, indicating that those trajectories are strongly attracted to these fixed points. In being so strongly pulled away from the steady state by this unstable fixed point, these systems have made a death decision. For the initial conditions where the pulse metric is large (under the separatrix), the trajectories are far enough away from the unstable fixed point to avoid being pulled away from the steady state. As a result, caspase-3 is not strongly activated and these systems avoid apoptosis.

- Fixed point type 2: All species must have zero concentrations except caspase-3 and active caspase-3. Since there is no synthesis of caspase-8 and XIAP in this model any species containing a form of caspase-8 or XIAP (including all intermediates complexes) will be absent. These fixed points are unstable because perturbations of caspase-8 and XIAP concentrations to nonzero values send these trajectories to fixed point type 4. This type of fixed point would represent an extremely rare (or knock-out) cell with no caspase-8 or XIAP.
- Fixed point type 3: All species have zero concentrations except caspase-3, caspase-8, and XIAP. Since there are no active caspases, intermediate complexes cannot be formed. These fixed points are unstable because perturbations in the active caspases or intermediate complexes to nonzero values send these trajectories to fixed point type 4. Biologically, this type of fixed point represents a cell that has not received a death signal.
- Fixed point type 4: All species have zero concentrations except caspase-8, active caspase-8, and XIAP. This fixed point is the most general, and almost all biologically significant initial conditions will fall under this scenario: caspase-8 is converted into active caspase-8 while caspase-3 is converted into active caspase-3 until XIAP causes all of the active caspase-3 to be degraded. Given ample time, all of the caspase-3 in the system is degraded and nonzero concentrations for caspase-8, active caspase-8, and XIAP may remain. This type of fixed point is stable because under perturbations to the caspases, intermediate complexes, or XIAP, the network will return to this type of fixed point. Biologically, this type is fixed point would

represent the state of the network after it is responded (with a transient behavior) to a death-signal before the system is reset (not modeled here).

9.3 Caspase-3 activation model with protein turnover

Our model was simplified to by omitting protein synthesis and degradation. A similar analysis was performed with a model extended to include turnover and cleavage of XIAP by active caspase-3. This extended model yielded similar results to the simplified model (Figures S2 and S3). A constitutive degradation rate, k_{deg} , was assumed for all species; it was estimated as $4.63 \times 10^{-5} \text{ (s}^{-1}\text{)}$ using the half-life of pro-caspase-3 [S1]. The synthesis rate was assumed to be the product of k_{deg} and the initial concentration of the protein for pro-caspase-3, pro-caspase-9, and XIAP. The model is specified below (equations 36-43). The rate of XIAP cleavage by active caspase-3, k_{clv} , is $5 \times 10^{-6} \text{ (s}^{-1}\text{)}$ [S2]. Species numbers and non-turnover parameters are identical to the basic model described in Figure 1 and Table 1.

$$\dot{x}_1 = -k_1 x_4 x_1 + k_{d1} x_5 - k_{deg} x_1 + k_{deg} x_1 (t = 0) \quad (36)$$

$$\dot{x}_2 = k_{d2} x_5 - k_3 x_2 x_3 + k_{d3} x_6 + k_{d4} x_6 - k_{deg} x_2 \quad (37)$$

$$\dot{x}_3 = -k_3 x_2 x_3 + k_{d3} x_6 - k_{deg} x_3 + k_{deg} x_3 (t = 0) \quad (38)$$

$$\dot{x}_4 = k_{d4} x_6 - k_1 x_4 x_1 + k_{d1} x_5 - k_5 x_7 x_4 + k_{d5} x_8 + k_{d2} x_5 - k_{deg} x_4 \quad (39)$$

$$\dot{x}_5 = -k_{d2} x_5 + k_1 x_4 x_1 - k_{d1} x_5 - k_{deg} x_5 \quad (40)$$

$$\dot{x}_6 = -k_{d4} x_6 + k_3 x_2 x_3 - k_{d3} x_6 - k_{deg} x_6 \quad (41)$$

$$\dot{x}_7 = -k_5 x_7 x_4 + k_{d5} x_8 + k_{d6} x_8 - k_{deg} x_7 - k_{clv} x_7 x_4 + k_{deg} x_7 (t = 0) \quad (42)$$

$$\dot{x}_8 = k_5 x_7 x_4 - k_{d5} x_8 - k_{d6} x_8 - k_{deg} x_8 \quad (43)$$

10 SUPPLEMENTARY FIGURE LEGENDS

Figure S1. A distance metric defines a separatrix.

A phase-space subplot of the distance metric (shortest distance to type 1 fixed points) is shown on a linear scale under the conditions shown for the DLE-defined separatrix in Figure 4A. The separatrix matching the DLE-defined separatrix is between small (blue) and large (red) distances. The distance is large below the separatrix (survival) and small above the separatrix (death).

Figure S2. Time-course simulations show transient death and survival responses under two different initial conditions of XIAP when turnover is introduced to the model.

In these time courses, the initial conditions matched those in Figure 3A-B, respectively. Under a low initial concentration of XIAP (A), the transient caspase-3 pulse is taller and wider than the pulse under a higher initial concentration of XIAP.

Figure S3. The six-hour DLE defines a separatrix separating phase-space into pro- and anti-apoptotic decisions in a model including turnover.

The six-hour DLEs are plotted under the conditions in Figure 4A. The introduction of protein turnover to the caspase-3 activation model did not significantly change the shape or the location of the separatrix.

11 SUPPLEMENTARY REFERENCES

- S1. DiPietrantonio, A.M., et al., *Fenretinide-induced caspase 3 activity involves increased protein stability in a mechanism distinct from reactive oxygen species elevation*. *Cancer Res*, 2000. **60**(16): p. 4331-5.
- S2. Eissing, T., et al., *Bistability analyses of a caspase activation model for receptor-induced apoptosis*. *J Biol Chem*, 2004. **279**(35): p. 36892-7.

Figure S1

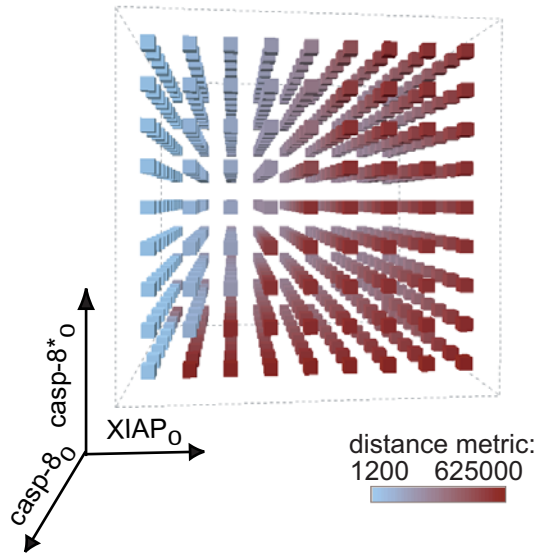


Figure S2

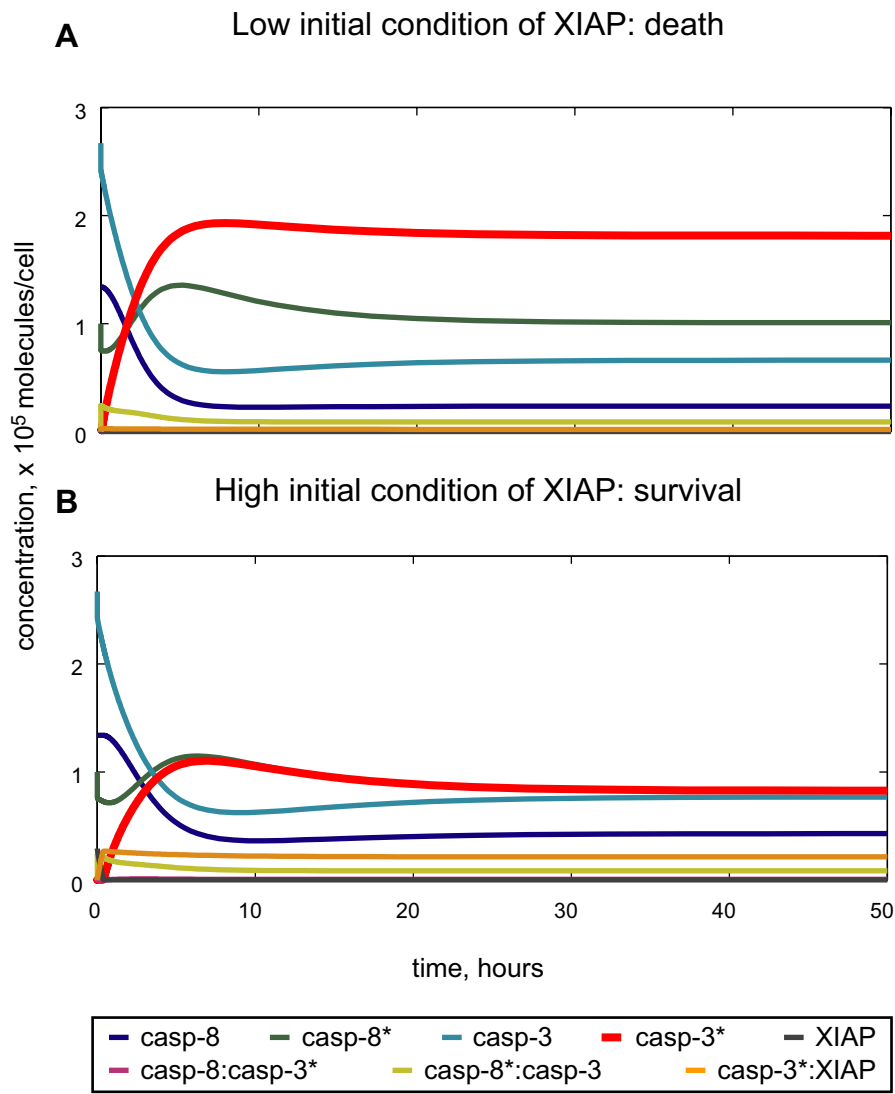


Figure S3

