Electronic Supplementary Material (ESI) for Molecular BioSystems


# Supplementary Material
# "A cellular stress-directed bistable switch controls the crosstalk between autophagy and apoptosis"

In this section, we briefly describe the mathematical approach used to study the crosstalk between autophagy-apoptosis network. A system level view can be developed by bringing together the components and interactions reported in the literature. Such a network can be translated into a set of mathematical equations that describe how each component concentration/activity in the network changes with the time. The rate of change of a component is described by ordinary differential equation (ODE) based on biochemical reaction kinetics (see equation below). Each biochemical reaction is represented as a term on the right hand side of the ODE for a component participating in the reaction [1, 2]. Each reaction in the network can be described either by using law of mass action or Michaelis-Menten kinetics [3-5].

A generic differential equation describing the temporal changes of protein $X_a$ is composed of two parts: production and consumption terms.

$$dX_a/dt = k_s + k_{act}*(X_T-X_a) - (k_d + k_{in})*X_a$$

Where:

$X_a$ – concentration of active X
$X_T$ – total concentration of X
$k_s$ – synthesis rate constant of X
$k_{act}$ – activation rate constant of $X_a$
$k_d$ – degradation rate constant of X
$k_{in}$ – inactivation rate constant of $X_a$

The production can be given by protein synthesis and/or an activation term, while the consumption can be given by protein degradation and/or inactivation term. Usually synthesis, degradation, binding and dissociation reactions are described by mass action kinetics, whereas protein activity can be described either by mass action or Michaelis-Menten kinetics [3, 6]. For example, if the activity of protein is controlled by covalent modification involving multi-site phosphorylations, Michaelis-Menten kinetics provides a good approximation for the process [7, 8]. The value of parameters (rate constants, Michaelis constants) and initial conditions have to be specified in order to solve ODEs. The non-linear nature of biological processes makes it difficult to find the solution of ODEs analytically hence the equations has to be solved numerically. The equations can be solved using different numerical integration methods that are implemented as solvers in many of the freely available computer software.

Solving a set of non-linear ODEs gives the time evolution of the protein concentration/activity called **time courses**. Further, ODEs can be solved to obtain the input-output relationship called as **signal response curves** or as **one parameter bifurcation diagram** [2, 3, 9]. An input is the signal strength that is varied to obtain the steady state behaviour of the control system. This helps to capture the qualitative changes in the behaviour of the system. For example, the system behaviour can become abrupt and discontinuous when signal strength is increased from a low value to high value. A point at which such a qualitative change in the system occurs is defined as bifurcation point [2].

## Minimal model of the crosstalk between autophagy and apoptosis

In this work, the temporal profiles and signal response curves were computed numerical using *XPP-AUT*. All the simulations presented in the text are based on the following XPP code which contains ODEs that describe Bcl2-Beclin1-Casps minimal network. The rate constants (k) have the dimension of $min^{-1}$ and Michaelis constants (*J*) are dimensionless. The proteins levels/activities are

given in arbitrary units (a.u). The starting parameter set was obtained based on the dynamics of autophagy and apoptosis activation in response interleukin-3 (IL-3) depletion and starvation [10, 11]. The parameters values were perturbed to capture all the possible qualitative behaviours that the given network can exhibit.

```
# XPP code
# Model of autophagy-apoptosis crosstalk

# Bcl2 regulation by stress and Casps
Bcl2t' = ksb2 - (kdb2 + kdb2'*stress + kdb2"*Casp)*Bcl2t

# Bax-Bcl2 complex formation
Baxc' = kasbx*(Baxt-Baxc)*(Bcl2t-Baxc) - (kdsbx + kdb2 + kdb2'*stress +
kdb2"*Casp)*Baxc

# Casps regulation by Bax and Beclin1 (Beci, Beca)
Casp' = (kacp + kacp'*Beci + kacp"*(Baxt-Baxc))*(Caspt-Casp)/(Jcp + Caspt-Casp)
- (kicp + kicp'*Beca)*Casp/(Jcp + Casp)

# The active, free Beclin1 (Beca) regulation by Casps, Bcl2
Beca' = - kasbc*(Bcl2t - Becac - Becic)*Beca + (kdsbc + kdb2 + kdb2'*stress +
kdb2"*Casp)*Becac + kabc*Beci - (kibc + kibc'*Casp)*Beca

# The inactive, free Beclin1 (Beci) regulation by Casps, Bcl2
Beci' = - kasbc*(Bcl2t - Becac - Becic)*Beci + (kdsbc + kdb2 + kdb2'*stress +
kdb2"*Casp)*Becic - kabc*Beci + (kibc + kibc'*Casp)*Beca

# The active, Bcl2-bounded Beclin1 (Becac) regulation by Casps, Bcl2
Becac' = kasbc*(Bcl2t - Becac - Becic)*Beca - (kdsbc + kdb2 + kdb2'*stress +
kdb2"*Casp)*Becac + kabc*Becic - (kibc + kibc'*Casp)*Becac

# The algebraic equation inactive, Bcl2-bounded Beclin1 (Becic)
Becic = Bect - Beca - Beci - Becac

p stress=0
p ksb2=0.05, kdb2=0.01, kdb2'=0.4, kdb2"=0.1
p Baxt=1, kasbx=100, kdsbx=0.1
p kacp=0, kacp'=0.05, kacp"=0.4, kicp=0.1, kicp'=0.35, Caspt=1, Jcp=0.01
p kasbc=1, kdsbc=1, kabc=2, kibc=0.01, kibc'=5, Bect=1

done
```

| | **description** |
|---|---|
| `stress` | stress level |
| `ksb2` | synthesis constant of Bcl2 |
| `kdb2` | backgorund degradation constant of Bcl2 |
| `kdb2'` | stress-dependent degradation constant of Bcl2 |
| `kdb2"` | Casps-dependent degradation constant of Bcl2 |
| `Baxt` | total level of Bax |
| `kasbx` | association constant of Bcl2-Bax complex |
| `kdsbx` | dissociation constant of Bcl2-Bax complex |
| `kacp` | background activation constant of Casps |
| `kacp'` | cleaved Beclin1-dependent activation constant of Casps |
| `kacp"` | Bax-dependent activation constant of Casps |

| | description |
|---|---|
| kicp | background inactivation constant of Casps |
| kicp' | Beclin1-dependent inactivation constant of Casps |
| Caspt | total level of Casps |
| Jcp | Michaelis-constant of Casps |
| kasbc | association constant of Bcl2-Beclin1 complex |
| kdsbc | disociation constant of Bcl2-Beclin1 complex |
| kabc | activation constant of Beclin1 |
| kibc | background inactivation constant of Beclin1 |
| kibc' | Casps-dependent inactivation constant of Beclin1 |
| Bect | total level of Beclin1 |

Parameters different from the .ode file:

| | |
|---|---|
| Figure 3c | stress=0.5 |
| Figure 3d | stress=2 |
| Figure 4a | kibc'=0;5;10 |
| Figure 4b | kibc'=0;5;10 stress=2 |
| Figure 4c | kicp'=0;0.35;0.7 |
| Figure 4d | kicp'=0;0.35;0.7 stress=2 |
| Figure 5a | kdb2"=0;0.1;0.2 |
| Figure 5b | kdb2"=0;0.1 stress=2 until 20 hrs then 0 |
| Figure 5c | stress=2 until 7.5 hrs then 0 |
| Figure 5d | stress=2 until 10 hrs then 0 |
| Figure 6a | kicp'=0;0.15;0.35 kdb2"=0 kibc1=0 |
| Figure 6b | kicp'=0;0.15;0.35 kdb2"=0 kibc1=0 stress=2 |
| Figure 6c | kicp'=0 kdb2"=0 kibc1=0 kdb2'=0.8 kacp"=0.2 |
| Figure 6d | kicp'=0 kdb2"=0 kibc1=0 kdb2'=0.8 kacp"=0.2 stress=2 |
| Figure S3a | kibc'=0;5;10 |
| Figure S3b | kibc'=0;5;10 stress=2 |
| Figure S3c | kicp'=0;0.35;0.7 |
| Figure S3d | kicp'=0;0.35;0.7 stress=2 |
| Figure S5a | kdb2"=0;0.1;0.2 |
| Figure S5b | kdb2"=0;0.1 stress=2 until 20 hrs then 0 |
| Figure S6a | kicp'=0;0.15;0.35 kdb2"=0 kibc1=0 |
| Figure S6b | kicp'=0;0.15;0.35 kdb2"=0 kibc1=0 stress=2 |
| Figure S6c | kicp'=0 kdb2"=0 kibc1=0 kdb2'=0.8 kacp"=0.2 |
| Figure S6d | kicp'=0 kdb2"=0 kibc1=0 kdb2'=0.8 kacp"=0.2 stress=2 |

## References


1. J. J. Tyson, K. Chen and B. Novak, *Nature Rev. Mol. Cell Biol.*, 2001, **2**, 908-916.
2. S. H. Strogatz, *Nonlinear Dynamics and Chaos*, Addison-Wesley Co., Reading, MA, 1994.
3. J. J. Tyson, K. C. Chen and B. Novak, *Current Opinion in Cell Biology*, 2003, **15**, 221-231.
4. I. H. Segel, *Enzyme kinetics behavior and analysis of rapid equilibrium and steady state enzyme systems*, Wiley, 1975.
5. A. Goldbeter and D. E. Koshland, Jr., *Proc Natl Acad Sci U S A.*, 1981, **78**, 6840-6844.
6. J. J. Tyson, A. Csikasz-Nagy and B. Novak, *BioEssays*, 2002, **24**, 1095-1109.
7. J. E. Ferrell, Jr., *Trends Biochem Sci*, 1996, **21**, 460-466.
8. O. Kapuy, D. Barik, M. R. Sananes, J. J. Tyson and B. Novak, *Prog Biophys Mol Biol.*, 2009, **100**, 47-56. Epub 2009 Jun 2011.
9. D. Kaplan and L. Glass, *Understanding Nonlinear Dynamics*, Springer-Verlag, New York, 1995.
10. E. Wirawan, L. Vande Walle, K. Kersse, S. Cornelis, S. Claerhout, I. Vanoverberghe, R. Roelandt, R. De Rycke, J. Verspurten, W. Declercq, P. Agostinis, T. Vanden Berghe, S. Lippens and P. Vandenabeele, *Cell Death Dis.*, 2010, **1**, e18.
11. Y. Wei, S. Sinha and B. Levine, *Autophagy*, 2008, **7**, 9494-951. Epub 2008 Oct 14.

**Supplementary Figure 1**

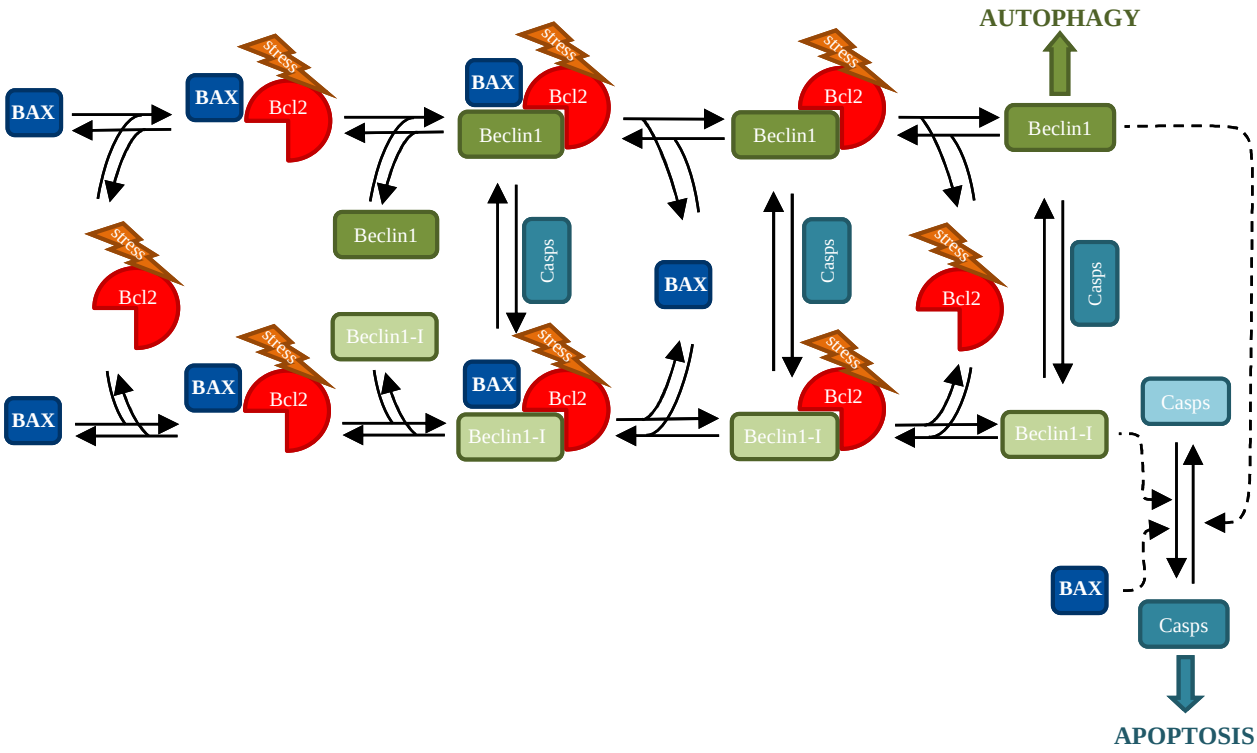


**Figure S1. The wiring diagram of the full model which describes the crosstalk between autophagy and apoptosis.** The active form of BAX, Beclin1 and Casps are depicted by isolated dark-coloured boxes. BAX and Beclin1 undergo reversible binding to all form of Bcl2. The dissociation of both components from Bcl2 results in their activation. Beside Bcl2, Beclin1 is also regulated by Casps. Casps cleave both free and Bcl2 bound forms of Beclin1. The Casps-cleaved forms of Beclin1 are depicted by light-coloured boxes. The free form of Beclin1 promotes autophagy and Casps inactivation, while the free form of cleaved Beclin1 promotes Casps activation. Casps activation is also promoted by free BAX. Active form of Casps induces apoptosis. Solid arrows represent biochemical reactions; dashed line shows how the molecules can influence each other.

**Supplementary Figure 2**

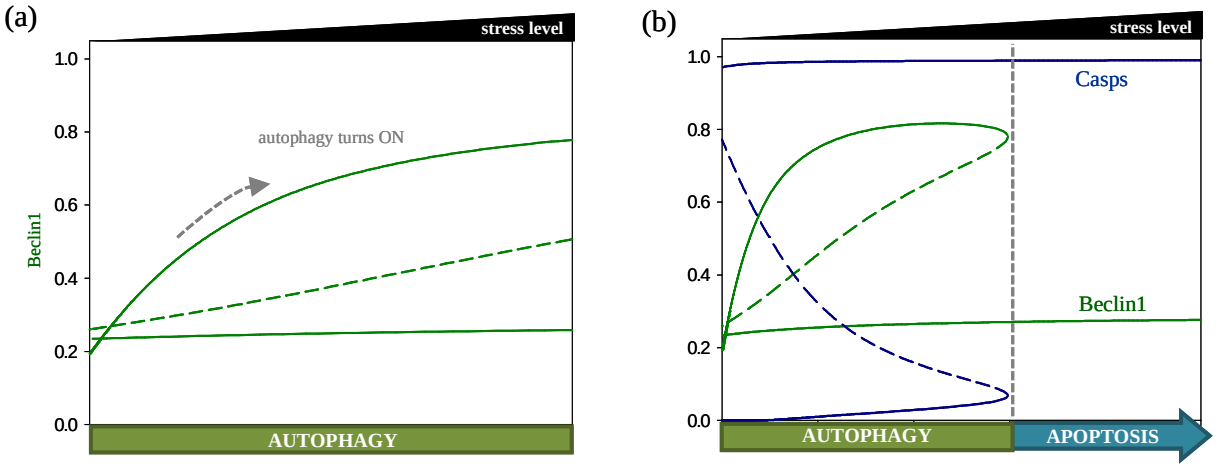


**Figure S2. The characteristic properties of Blc2-Beclin1-Casps regulatory network.** **(a)** A blow-up of the region of Beclin1 signal-response curve at low stress level. **(b)** Superimposition of Beclin1 signal-response curve on top of Casps signal-response curve. Solid lines denote stable states, while dashed line denotes the unstable state.

**Supplementary Figure 3**

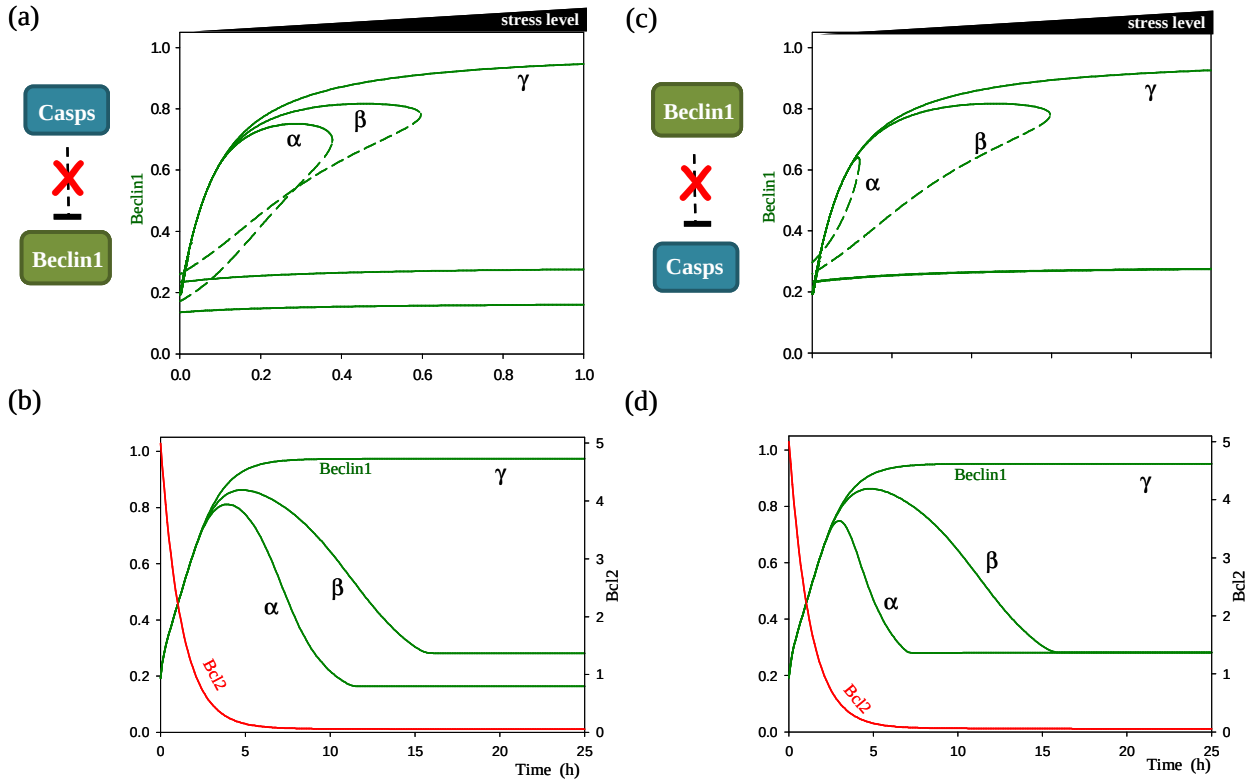


**Figure S3. The effect of feedback loops involving Beclin1 and Casps on the network properties.** **(a)** Signal response curve of active Beclin1 (free form) and **(b)** dynamics of the network are shown with different rates of inhibition of Beclin1 by Casps-dependent cleavage. Symbols γ: kibc' = 0, β: kibc' = 5 and α: kibc'=10 represent the situations without inhibition, control and higher rate of inhibition of Beclin1 by Casps, respectively. **(c)** Signal response curve of active Beclin1 (free form) and **(d)** dynamics of the network are shown with different rates of Casps inhibition by Beclin1. Symbols α: kicp' = 0, β: kicp' = 0.35 and γ: kicp' = 0.7 represent the situations without inhibition, control and higher rate of inhibition of Casps by Beclin1, respectively. The solid lines in the signal response curve denote stable states, while dashed line depicts the unstable state. The temporal dynamics is simulated for continuous high stress conditions (stress = 2). The dynamics of Bcl2 and active Beclin1 (free form) are shown.

**Supplementary Figure 4**

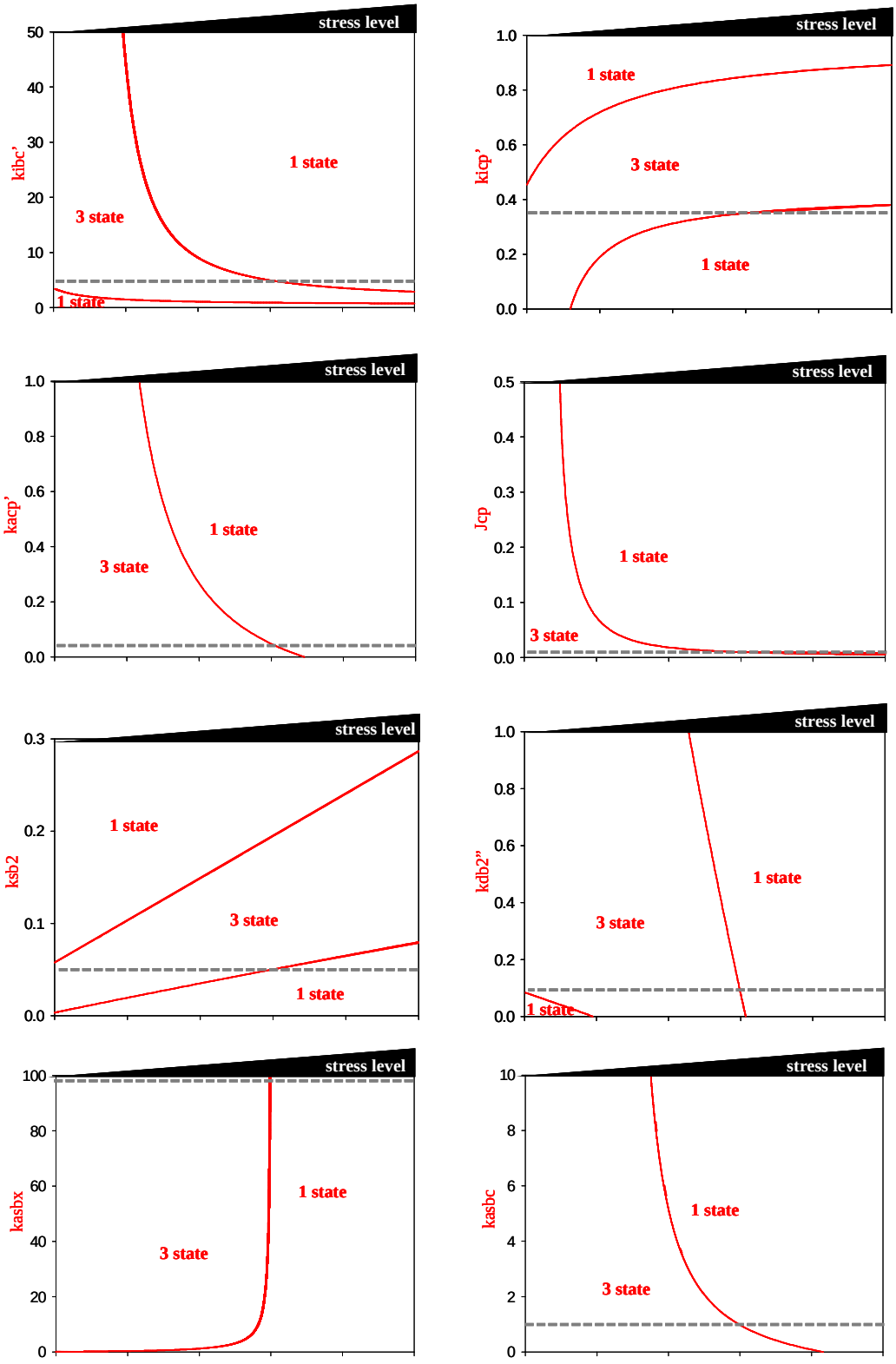


**Figure S4. Testing the robustness of the regulatory system using two parameter bifurcation diagrams.** The diagrams depict the changes to the two saddle node bifurcation points as function of the second parameter: kibc', kicp', kacp', Jcp, ksb2, kdb2", kasbx and kasbc. Red lines determine the region of bistability. "1 state" represents one stable state, while "3 states" denotes two stable states separated by an unstable one. Grey dotted lines depict the parameter values used in the simulations.

**Supplementary Figure 5**

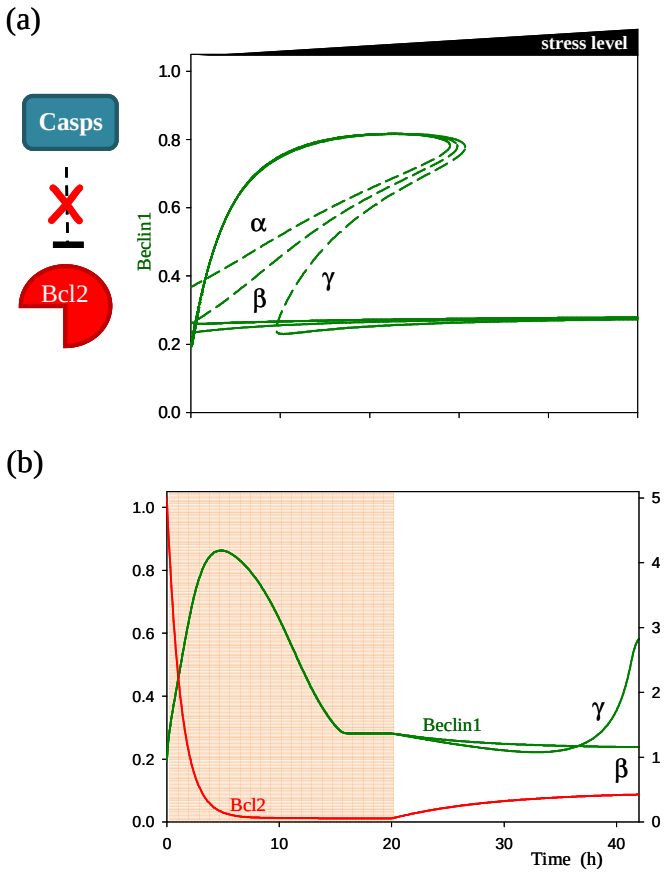


**Figure S5. Irreversible one-way switch of apoptosis induction. (a)** The response curve of active Beclin1 (free form) and **(b)** dynamics of the network are shown with different rates of Casps-dependent inhibition of Bcl2. The temporal dynamics is simulated for 20 hours long treatment with high stress (stress = 2) depicted by brown background. Symbols γ: kdb2" = 0, β: kdb2" = 0.1 and α: kdb2" = 0.2 represent the situations without inhibition, control and higher rate of inhibition of Bcl2 by Casps, respectively. The solid lines in the response curve denote stable states, while dashed line depicts the unstable state. The dynamics of Bcl2 and active Beclin1 (free form) are shown.

**Supplementary Figure 6**

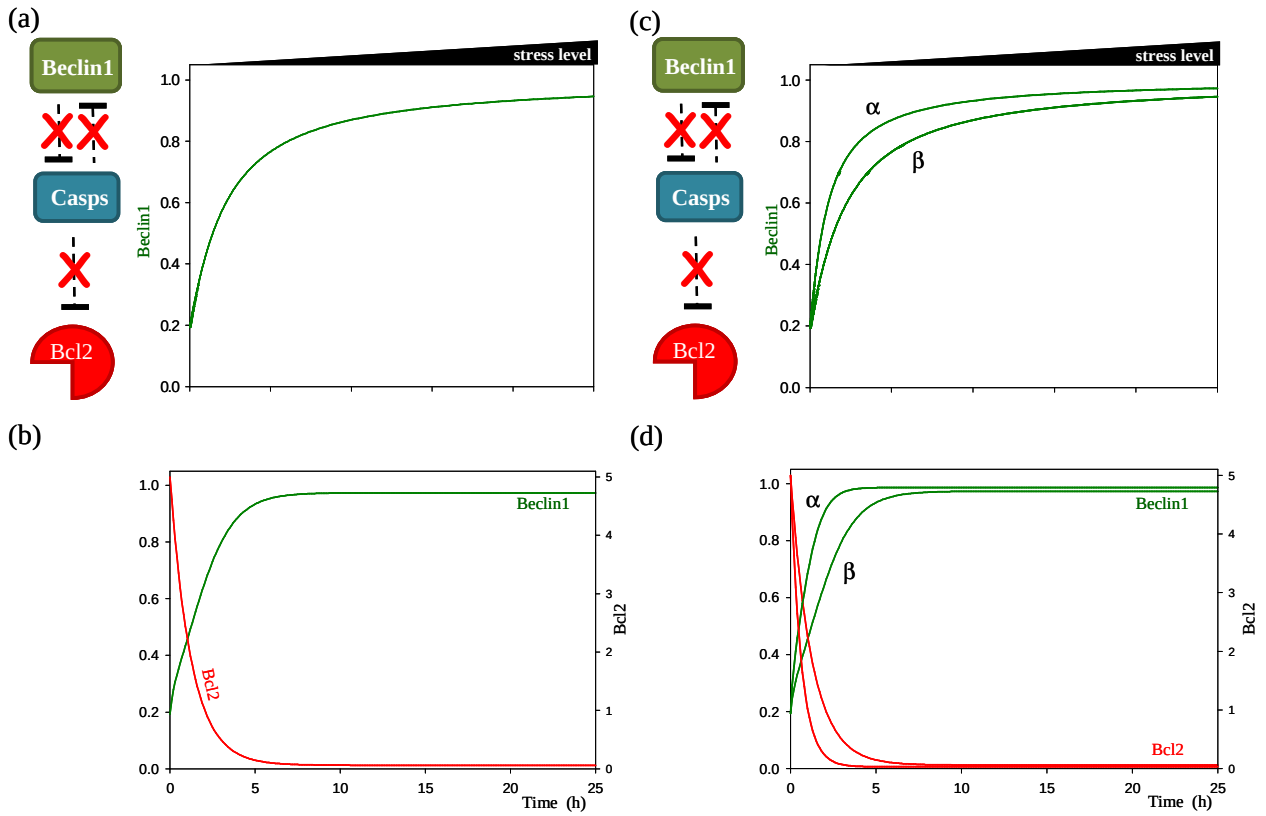


**Figure S6. The key roles of Beclin1 and Bcl2 in Casps activation, when all the feedback loops are compromised.** The effect of feedback loops is removed by setting the parameter values of kdb2" and kibc' to zero. **(a)** The response curve of active Beclin1 (free form) and **(b)** dynamics of network are shown with different rates of inhibition of Casps by Beclin1. Both the response curve and dynamics of Beclin1 are unaffected for kicp' = 0, kicp' = 0.15 and kicp' = 0.35. **(c)** The response curve of active Beclin1 (free form) and **(d)** dynamics of the network are shown in the absence of inhibition of Casps by Beclin1 (symbol β: kicp' = 0) with either increased rate of Bcl2 degradation (symbol α: kdb2' = 0.8) or decreased rate of Casps activation by Bax (symbol β: kacp" = 0.2). Both the response curve and dynamics of Beclin1 are unaffected by decreased rate of Casps activation. The solid lines in the signal response curve denote stable states, while dashed line depicts the unstable state. The temporal dynamics is simulated for continuous high stress conditions (stress = 2). The dynamics of Bcl2 and active Beclin1 (free form) are shown.

**Supplementary Table 1**

| | related figure | reference from the Literature | stress target | stress conditions | phenotype |
|---|---|---|---|---|---|
| low stress | Figure 3c | Pattingre et al., 2005, Cell, 122:927-939 | mTOR | starvation 4 h | autophagy |
| | | Maiuri et al., 2007, EMBO J, 26:2527-2539 | mTOR | starvation 6 h | |
| high stress | Figure 3d | Wei et al., 2008, Autophagy, 4:949-951 | mTOR | starvation 16 h | autophagy followed by apoptosis |

**Table S1. Starvation induced cellular responses**. The stress is categorized into low or high stress depending on its ability to induce autophagy or autophagy followed by apoptosis. Each phenotype is related to a figure in the main text. The stress target, duration of stress and evidences from the literature are also provided.

**Supplementary Table 2**

| | related figure | reference from the Literature | stress target | stress conditions | phenotype |
|---|---|---|---|---|---|
| low stress | Figure 3c | Ogata et al., 2006, Mol Cell Biol, 26:9220-9231 | ER | 0.5 ug/ml tunicamycin 1 h | autophagy |
| | | | ER | 400 nM thapsigargin 4 h | |
| | | | ER | 2 ug/ml tunicamycin 8 h | |
| | | Noda & Ohsumi, 1998, J Biol Chem, 273:3963-3966 | mTOR | 0.2 ug/ml rapamycin 3 h | |
| | | Rutkowski et al., 2006, Plos Biol, 4:e374 | ER | 25 ng/ml tunicamycin 24 h | |
| | | | ER | 2.5 nM thapsigargin 24 h | |
| high stress | Figure 3d | Wirawan et al., 2010, Cell Death & Differ, 1:e18 | growth factor | interleukin-3-depletion 24 h | autophagy followed by apoptosis |
| | | Zhu et al., 2010, Protein Cell, 1:468-477 | general | staurosporine 24 h | |
| | | Ogata et al., 2006, Mol Cell Biol, 26:9220-9231 | ER | 0.5 ug/ml tunicamycin 36 h | |
| | | | ER | 300 nM thapsigargin 36 h | |
| | | Abedin et al., 2007, Cell Death & Diff, 14:500-510 | DNA | 2 uM camptothecin 48 h | |

**Table S2. Different stress stimuli that are known to induce autophagy or autophagy followed by apoptosis.** The stress is categorized into high or low stress depending on the phenotype. Each phenotype is related to a figure in the main text. The stress target, stress levels/duration and evidences from the literature are also provided.