**Supplementary Information**

**“A double negative feedback loop between mTORC1 and AMPK kinases**

**guarantees a precise autophagic response upon cellular stress”**

**I. Mathematical codes for computational simulations**

A biological regulatory network can be translated into a set of ordinary differential equation (ODE) to describe how the concentration/activity of each control element in the network changes with the time. A generic differential equation depicting the temporal changes of a regulatory element is composed of two parts: production and consumption terms. In a cellular protein-protein regulatory network the production can be given by protein synthesis (i.e. transcription and translation) and/or an activation (i.e. post-translational modification) term, while the consumption can be given by protein degradation and/or inactivation term. Usually synthesis and degradation reactions are described by mass action kinetics, whereas protein activity can be described either by mass action or Michaelis-Menten kinetics. Solving a set of non-linear ODEs gives the time evolution of the relative protein concentration/activity (time courses).

The temporal profiles were computed numerically using *XPP-AUT*. All the simulations presented in the text are based on the following XPP codes which contains ODEs. 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 code for simulating time series when mTOR ┤AMPK connection is not present*

# a model to simulate mTOR-AMPK-ULK1 regulatory triangle with XPP-AUT

# the code for simulating time series when mTOR ┤AMPK connection is not present

# initial conditions

init ULK1=0.0055, AMPK=0.1017, mTOR=0.4691, ATG=0.0214, starv=0, res=0, rap=0, cc=0

# differential equations

# ULK1 represents the active form of ULK1

ULK1' = (kaulk + kaulk"\*delay(AMPK,tau1))\*(Ulk1T-ULK1)/(Julk + Ulk1T-ULK1) - (kiulk + kiulk'\*mTOR)\*ULK1/(Julk + ULK1)

# AMPK represents the active, phosphorlyated form of AMPK

AMPK' = (kaak + kaak\*starv + kaak\*res)\*(AMPKT-AMPK)/(Jampk + AMPKT-AMPK) - (kiak + kiak\*cc + kiak'\*ULK1)\*AMPK/(Jampk + AMPK)

# mTOR represents the active form of mTORC1 complex

mTOR' = kamtor\*(mTORT-mTOR)/(Jmtor+mTORT-mTOR) - (kimtor + kimtor'\*AMPK + kimtor"\*ULK1 + kimtor\*res + kimtor\*rap)\*mTOR/(Jmtor+mTOR)

# ATG represents the active ATG genes during autophagy; when ATG is active we assumes that autophagy is also active

ATG' = (kaau + kaau'\*ULK1)\*(1-ATG) - (kiau + kiau'\*mTOR)\*ATG

# starv represents the increasing ratio of AMP/ATP during starvation

starv' = kistv\*(starvT-starv)/(Jstv + starvT-starv) - kostv\*starv/(Jstv+starv)

# res represents the resveratrol up-taken by the cell

res' = kires\*(resT-res)/(Jres + resT-res) - kores\*res/(Jres+res)

# rap represents the rapamycin up-taken by the cell

rap' = kirap\*(rapT-rap)/(Jrap + rapT-rap) - korap\*rap/(Jrap+rap)

# cc represents the Compound C up-taken by the cell

cc' = kicc\*(ccT-cc)/(Jcc + ccT-cc) - kocc\*cc/(Jcc+cc)

# parameters

# simulating starvation: starvT=2

# simulating rapamycin treatment: rapT=2.25

# simulating resveratrol treatment: resT=1.5

# simulating ULK1 silencing: ULK1T=0.001

# simulating TSC1/TSC2 silencing: kamtor=0.05

# simulating Compound C treatment: CCT=100

p kaulk=5, kaulk"=1, kiulk=0.1, kiulk'=30, julk=0.01, Ulk1T=1

p kaak=0.35, kiak=0.5, kiak'=150, AMPKT=1, Jampk=0.5

p kamtor=0.025, kimtor=0.0075, kimtor'=0.15, kimtor"=0.5, Jmtor=0.1, mTORT=1

p kaau=0.01, kaau'=3, kiau=0.75, kiau'=1

p starvT=0, kistv=0.105, Jstv=0.03, kostv=0.1

p resT=0, kires=0.0025, Jres=0.75, kores=0.00075

p rapT=0, kirap=0.125, Jrap=0.1, korap=0.1

p ccT=0, kicc=0.5, Jcc=0.1, kocc=0.1

p tau1=5

# numerics

@ TOTAL=1, METH=stiff, delay=50

done

*The code for simulating time series when mTOR ┤AMPK connection is present*

# a model to simulate mTOR-AMPK-ULK1 regulatory triangle with XPP-AUT

# the code for simulating time series when mTOR ┤AMPK connection is present

# initial conditions

init ULK1=0.0008, AMPK=0.0161, mTOR=0.7013, ATG=0.0568, starv=0, res=0, rap=0, cc=0

# differential equations

# ULK1 represents the active form of ULK1

ULK1' = (kaulk + kaulk"\*delay(AMPK,tau1))\*(Ulk1T-ULK1)/(Julk + Ulk1T-ULK1) - (kiulk + kiulk'\*mTOR)\*ULK1/(Julk + ULK1)

# AMPK represents the active, phosphorlyated form of AMPK

AMPK' = (kaak + kaak\*starv + kaak\*res)\*(AMPKT-AMPK)/(Jampk + AMPKT-AMPK) - (kiak + kiak\*cc + kiak'\*ULK1 + kiak"\*mTOR)\*AMPK/(Jampk + AMPK)

# mTOR represents the active form of mTORC1 complex

mTOR' = kamtor\*(mTORT-mTOR)/(Jmtor+mTORT-mTOR) - (kimtor + kimtor'\*AMPK + kimtor"\*ULK1 + kimtor\*res + kimtor\*rap)\*mTOR/(Jmtor+mTOR)

# ATG represents the active ATG genes during autophagy; when ATG is active we assumes that autophagy is also active

ATG' = (kaau + kaau'\*ULK1)\*(1-ATG) - (kiau + kiau'\*mTOR)\*ATG

# starv represents the increasing ratio of AMP/ATP during starvation

starv' = kistv\*(starvT-starv)/(Jstv + starvT-starv) - kostv\*starv/(Jstv+starv)

# res represents the resveratrol up-taken by the cell

res' = kires\*(resT-res)/(Jres + resT-res) - kores\*res/(Jres+res)

# rap represents the rapamycin up-taken by the cell

rap' = kirap\*(rapT-rap)/(Jrap + rapT-rap) - korap\*rap/(Jrap+rap)

# cc represents the Compound C up-taken by the cell

cc' = kicc\*(ccT-cc)/(Jcc + ccT-cc) - kocc\*cc/(Jcc+cc)

# parameters

# simulating starvation: starvT=0.75

# simulating rapamycin treatment: rapT=10

# simulating resveratrol treatment: resT=0.175

# simulating ULK1 silencing: ULK1T=0.001

# simulating TSC1/TSC2 silencing: kamtor=0.05

# simulating Compound C treatment: CCT=100

p kaulk=0.001, kaulk"=3, kiulk=0.1, kiulk'=0.75, julk=0.01, Ulk1T=1

p kaak=0.5, kiak=0.1, kiak'=1.5, kiak"=15, AMPKT=1, Jampk=0.5

p kamtor=0.015, kimtor=0.01, kimtor'=0.15, kimtor"=0.5, Jmtor=0.1, mTORT=1

p kaau=0.1, kaau'=3, kiau=1, kiau'=1

p starvT=0, kistv=0.1, Jstv=0.1, kostv=0.1

p rapT=0, kirap=0.15, Jrap=0.1, korap=0.1

p ccT=0, kicc=1, Jcc=0.5, kocc=0.001

p tau1=50

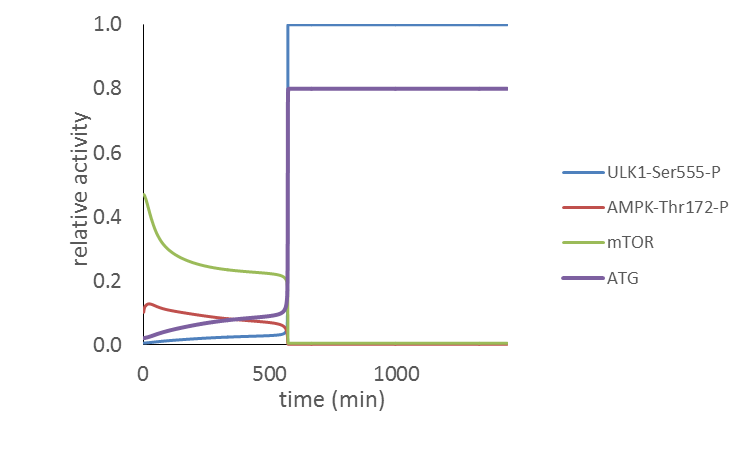
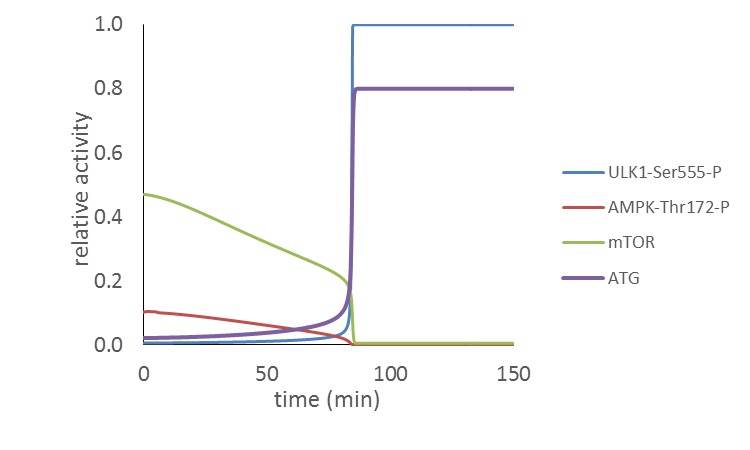
# numerics

@ TOTAL=1, METH=stiff, delay=50

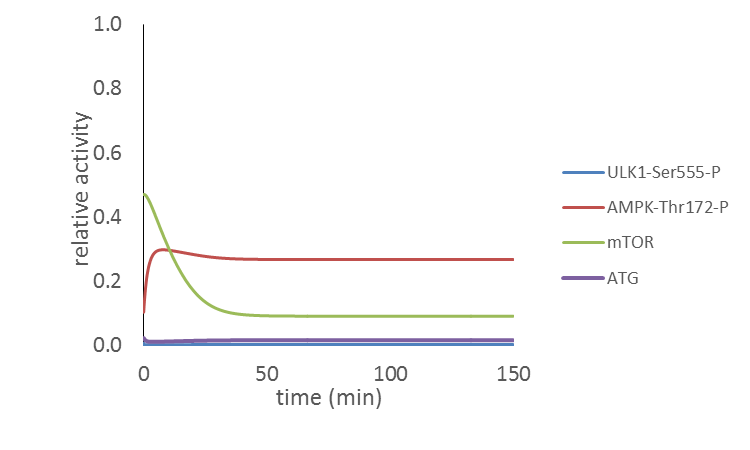
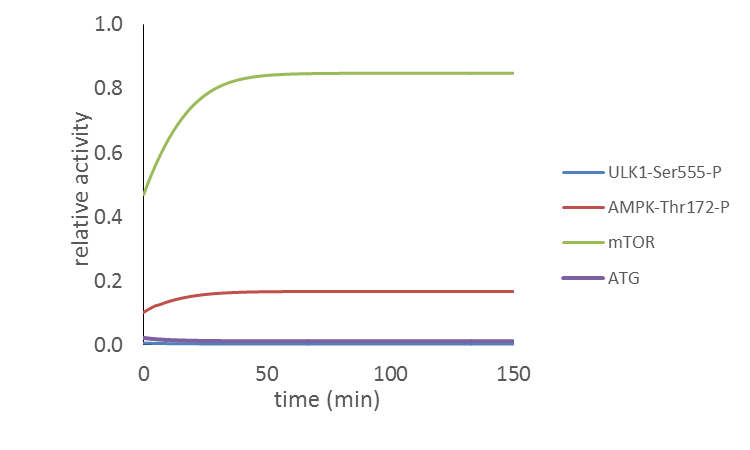
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**II. Figures**

1. **(B)**

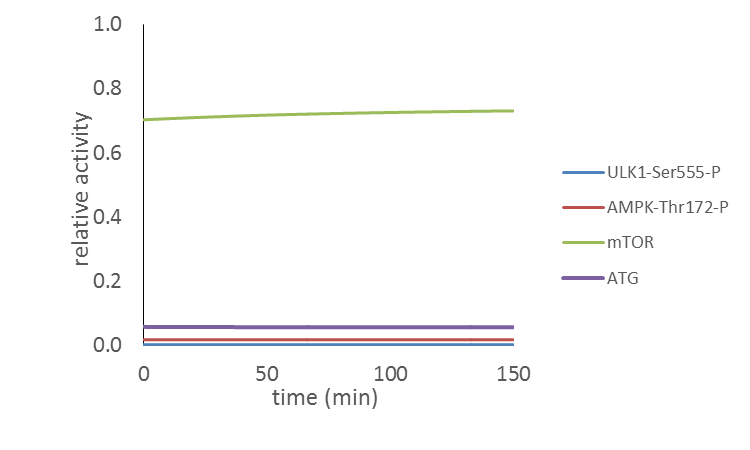
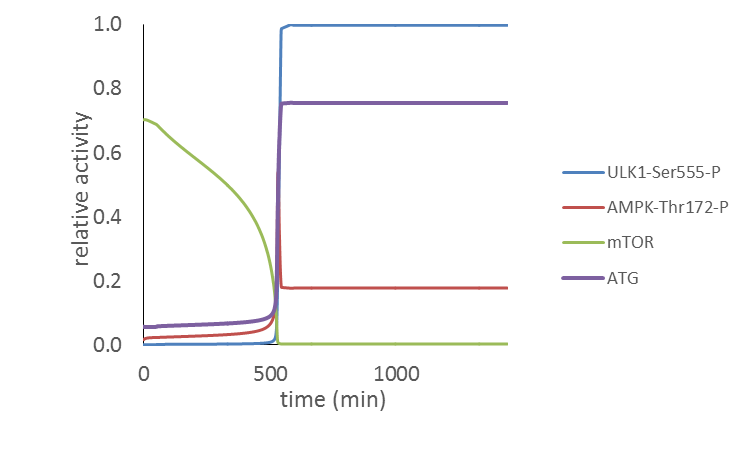
 

**(C) (D)**

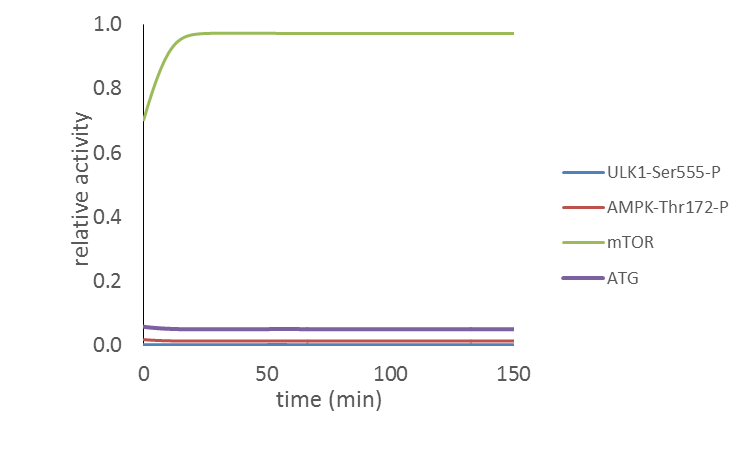
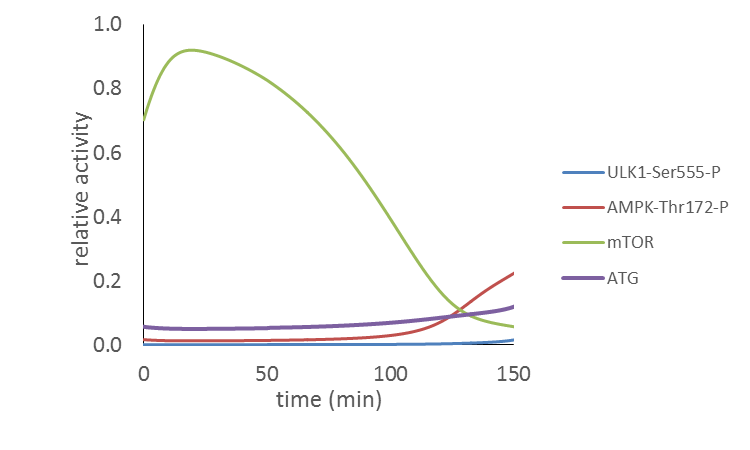
 

**Supplementary Figure 1.** Computer simulation of **(A)** starvation (starvT=2); **(B)** rapamycin treatment (rapt=2.25); **(C)** ULK1 silencing (ULK1T=0.001) and **(D)** mTOR hyperactivation with siTSC1/TSC2 (kamtor=0.05) when mTOR ┤AMPK connection is not present in the regulatory network. The relative activity of AMPK-Thr172-P, mTOR, ULK1-Ser555-P and autophagy (ATG) are plotted in time.

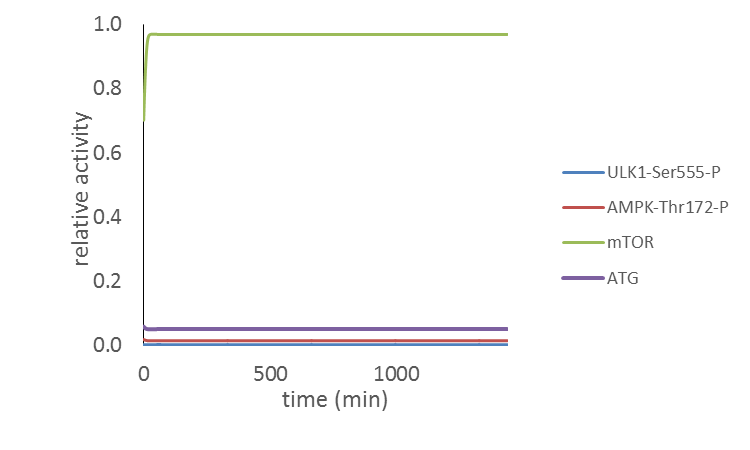
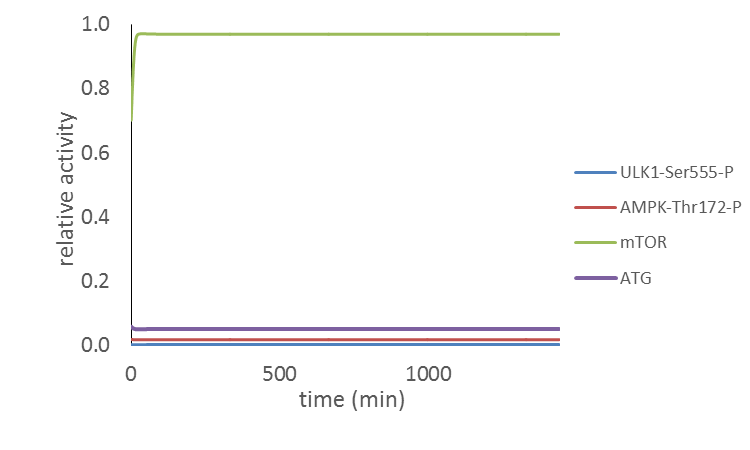
**(A) (B)**



**(C) (D)**

**(E) (F)**



**Supplementary Figure 2.** Computer simulation of AMPK-mTOR-ULK1 regulatory triangle when mTOR ┤AMPK connection is present in the control network. Time series data are plotted mimicking **(A)** starvation (starvT=0.75); **(B)** ULK1 silencing (ULK1T=0.001); **(C)** mTOR hyperactivation with siTSC1/TSC2 (kamtor=0.05); and combined treatment of TSC1/2 silencing **(D)** + addition of rapamycin (kamtor=0.05, rapT=10); **(E)** + starvation (kamtor=0.05, starvT=0.75) or **(F)** + addition of resveratrol (kamtor=0.05, resT=0.175). The relative activity of AMPK-Thr172-P, mTOR, ULK1-Ser555-P and autophagy (ATG) are plotted in time.

**III. Tables**

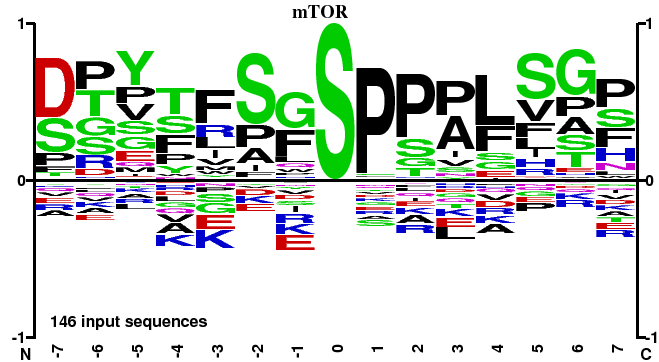
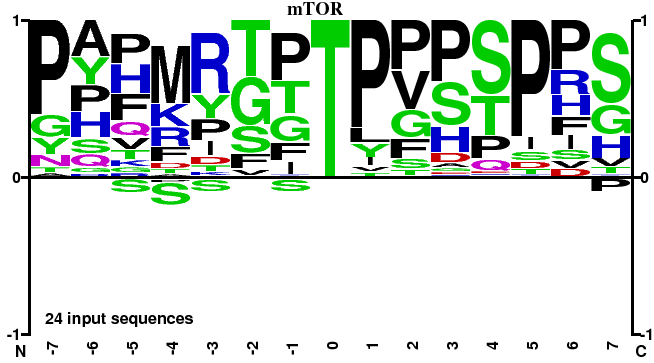


**Supplementary Table 1. Collecting data from literature about activity changes of AMPK, mTOR and ULK1 after various treatments.** ATG means active autophagy.

**Supplementary Table 2. Comparison of the two mathematical models i.e. mTOR inhibits AMPK directly or not by simulating various treatments.** The activity change of AMPK, mTOR and ULK1 is presented after the treatments. ATG means active autophagy. Red background refers that this computational result is not matched the data found in the literature, while red notes present our predictions since these treatments have not carried out yet experimentally.

**IV. Introducing the theoretical analysis of phosphorylation site search on AMPK**

PhosphoSite Plus (https://www.phosphosite.org/proteinAction?id=564&showAllSites=true) shows the preferred Ser and Thr phosphorylation sites of mTOR kinase:

Using NetPhos 3.1. (<http://www.cbs.dtu.dk/services/NetPhos/>) the potential serine and thr phosphorylation sites were searched in human AMPK protein sequence (the algorithm of this freely availably software can be found in Blom et al., 2014). The program identified four phosphorylation sites with the following amino acid sequences:

* Thr-232: DHVPTLFKK
* Ser-356: YLATSPPDS
* Thr-488: AKSGTATPQ
* Ser-496: QRSGSVSNY

where the yellow colour shows that this amino acid around the potential Ser and Thr resiudes (marked with red background) are preferred by mTOR kinase. According to analysis done by NetPhos 3.1., The-488 and Ser-496 residues are really close to each other further suggesting that these two might be regulated similarly by mTOR-dependent phosphorylation.

**V. References**

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