Supplementary Fig legends

Fig S1

(a) GFP-LC3 was co-transfected with increasing levels of Bax as indicated in duplicate. After transfection, one set (lanes 1-6) was treated with DMSO and the other set was treated with Bafilomycin A1 (Baf) (lanes 7-12). After 20 hours, cell lysates were subjected to western blot with anti-GFP (top) and anti-tubulin (bottom).

(b) Bax was transfected into HeLa cells as indicated in duplicate. After transfection, one set was treated with DMSO and the other set was treated with Baf. After 20 hours, cell lysates were subjected to western blot with anti-LC3 (top) to detect endogenous LC3-II and anti-tubulin (bottom). Note that the blots and exposures in the +Baf and –Baf conditions in Fig S2(a) and (b) are different, to allow us to measure differences without gel overexposure in the +Baf conditions.

Fig S2

HeLa cells were treated with media (lane 1) or with trehalose (lane 2) for 6 hours. Cell lysates were subjected to SDS-PAGE (15%) and probing with anti-LC3 and tubulin respectively. HeLa cells were transfected with empty vector (lanes 3, 5) or Bax (lanes 4, 6). After 18 hours, cells were treated with trehalose or trehalose+Baf for 6 hours. Cells were lysed and subjected to SDS-PAGE (12%) and blotting with anti-LC3 or tubulin respectively.

Fig S3

(a) Beclin 1-Flag was co-transfected with increasing levels of Bax as indicated, in duplicate, in HeLa cells. After transfection, one set (lanes 1-6) were treated with DMSO and the other set were treated with the caspase inhibitor, z-VAD-fmk (lanes 7-12). After 20 hours, cell lysates were subjected to western blots which were probed with anti-Beclin 1, anti-tubulin and anti-Bax antibodies. The Beclin 1 band intensities (as a function of tubulin) are shown for the 0 Bax lanes, to indicate similarities in control conditions.

(b) Bax was transfected as indicated, in duplicate, in HeLa cells. After transfection, one set (lanes 2) was treated with DMSO and the other set was treated with the caspase

inhibitor, z-VAD-fmk (lane 3). After 20 hours, cell lysates were subjected to western blots, which were probed with anti-Beclin 1 (to detect endogenous Beclin 1), anti-tubulin and anti-LC3 antibodies. LC3-II densitometry is indicated (versus tubulin).

Fig S4

(a) HeLa cells were treated with cycloheximide (CHX) for 4 hours (lane 1), CHX for 4 hours and TNF-alpha for 2 hours (lane 2), CHX for 4 hours and TNF-alpha for 4 hours (lane 3). Cell lysates were subjected to western blots which were probed with anti-Beclin 1 (N-terminal) (top) and anti-tubulin (bottom) antibodies. Note that the band around 64 kDa is full-length Beclin 1, which decreases in abundance with TNF alpha + CHX.

(b) HeLa cells were treated with CHX (lane 1), CHX+TNF-alpha (lane 2) and CHX+TNF-alpha+z-VAD-fmk (lane 3) for 5 hours. Cell lysates were subjected to western blots which were probed with anti-Beclin 1 (N-terminal) and anti-tubulin antibodies.

Fig S5

Atg5 wild-type and Atg5-/-MEFs were treated with trehalose (100mM) or media for 20 hours. Cell lysates were subjected to western blotting and probed with anti-Beclin 1 and anti-tubulin antibodies.

Fig S6

Myc-Beclin 1 (Myc-Bec), Myc-Beclin 1-N-terminus (Myc-Bec-N) and Myc-Beclin 1-C-terminus (Myc-Bec-C) were co-transfected with EGFP (control) into HeLa cells. After 24 hours, cell lysates were subjected to western blot, which was probed with anti-Myc, anti-EGFP and anti-tubulin antibodies.

Fig S7

(a) GFP-LC3 was co-transfected with vector and Bax, respectively, into HeLa cells. After 24 hours, one set of transfected cells were starved for 2 hours in HBSS media. Cells were then fixed. GFP dots were scored under fluorescent microscope.

(b) GFP-LC3/vector, GFP-LC3/Bax, GFP-LC3/Bax/Beclin 1-149E and GFP-LC3/Bax/Beclin 1 were transfected into HeLa cells. After 24 hours, one set of transfected cells were starved for 2 hours in HBSS media. Cells were then fixed. GFP dots were scored under fluorescent microscope.

Fig S8

(a) HeLa cells were transfected with vector, Bax, Bax/Beclin 1-149E and Bax/Beclin 1 respectively. After 20 hours, cells were harvested and caspase-3 activity assays were performed in triplicate, according to the manufacture's instructions.

(b) HeLa cells were transfected with vector, Bax, Bax/Beclin 1-149E and Bax/Beclin 1 respectively. After 20 hours, cell lystes were subjected to western blots and probed with anti-PARP p85 and anti-tubulin antibodies.

Fig S9

HeLa cells were treated with Beclin 1 siRNA (lanes 1, 2) and control siRNA with indicated concentration. After 48 hours, cells were lysed and subjected to SDS-PAGE and blotting with anti-Atg5 (top), anti-Beclin 1 (middle), anti-tubulin (bottom) respectively.

Fig S10

(a) HeLa cells were transfected with empty vector (lane 1), Bax (lane 2), Bax/Beclin 1 (lane 3), Bax/Beclin 1-149E (lane 4). After 20 hours, cells were lysed and subjected to SDS-PAGE and blotting with anti-Atg5 (top) and anti-tubulin (bottom), respectively. All Atg5 is in the Atg5-Atg12 conjugate.

(b) Quantification of Atg5-Atg12 levels between samples as indicated, using data from Fig S10 (a) and four other similar experiments. Y-axis: Atg5-Atg12/Tubulin. The ratio of Atg5-Atg12/tubulin where empty vector was transfected was set as 1. Data were from five independent experiments. Comparison was performed with T-test. Error bar is SD. ***: P1=0.0001; **: P2=0.0003; *: P3<0.05.

Fig S11

(a) Vps34/vector, vsps34/Beclin 1-Flag, vps34/Beclin 1-N-Flag, vps34/Beclin 1-C-Flag , vps34/p35-Flag (cdk5 activator, for control) were transfected into HeLa cells respectively. After 24 hours, cells were harvested and lysed. The cell lysates were subjected to anti-Flag antibody immunoprecipitation. The total lysates (lower panel) and immunoprecipitates (upper panel) were used for SDS-PAGE and western blotting with anti-vps34 antibody and anti-Flag antibody (middle panel) respectively.

(b) Vps34/vector, vps34/p35-Flag (negative control), vsps34/Beclin 1-Flag, vps34/Beclin 1-N-Flag, vps34/Beclin 1-C-Flag were transfected into HeLa cells respectively. After 24 hours, cells were harvested and lysed. The cell lysates were subjected to anti-Flag antibody immunoprecipitation. The total lysates (lower panel) and immunoprecipitates (upper panel) were used for SDS-PAGE and western blotting with anti-vps34 antibody and anti-Flag antibody (middle panel) respectively.

Fig S12

GFP-LC3 was transfected with empty vector, Bax, Bax-Bcl-xL or Bcl-xL into HeLa cells. After 20 hours, cells were fixed and cell death in GFP-positive cells was evaluated by scoring the cells for the presence of condensed or fragmented nuclei (apoptotic morphology) [1]. ***: P=0.

Fig S13

Models of reciprocal effects of Bax and Bcl-2/xL on autophagy

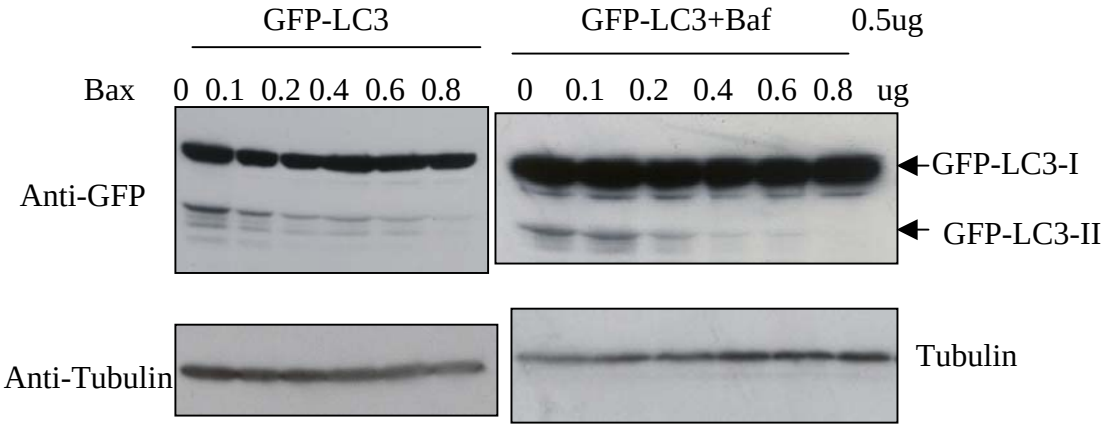
(a) Bcl-2 or Bcl-xL inhibit autophagy under normal conditions by binding Beclin 1. This effect is countered by Bax, which displaces the Bcl-2/Bcl-xL interaction with Beclin 1.

(b) Bax inhibits autophagy by activcating caspases, which cleave Beclin 1. This effect is countered by Bcl-2/Bcl-xL, which inhibit the ability of Bax to activate caspases.

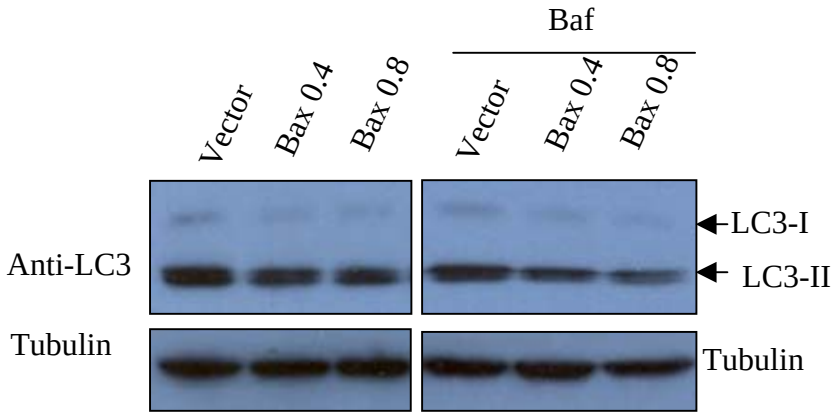
References

1. Ravikumar, B, Imarisio, S, Sarkar, S, O'Kane, CJ and Rubinsztein, DC, (2008) Rab5 modulates aggregation and toxicity of mutant huntingtin through macroautophagy in cell and fly models of Huntington disease. J Cell Sci 121: 1649-60.

Fig S1


GFP-LC3
GFP-LC3+Baf
0.5ug
Bax
0 0.1 0.2 0.4 0.6 0.8
0 0.1 0.2 0.4 0.6 0.8 ug
Anti-GFP
GFP-LC3-I
GFP-LC3-II
Anti-Tubulin
Tubulin

(a)


Baf
Vector
Bax 0.4
Bax 0.8
Vector
Bax 0.4
Bax 0.8
Anti-LC3
LC3-I
LC3-II
Tubulin
Tubulin

(b)

Fig S2

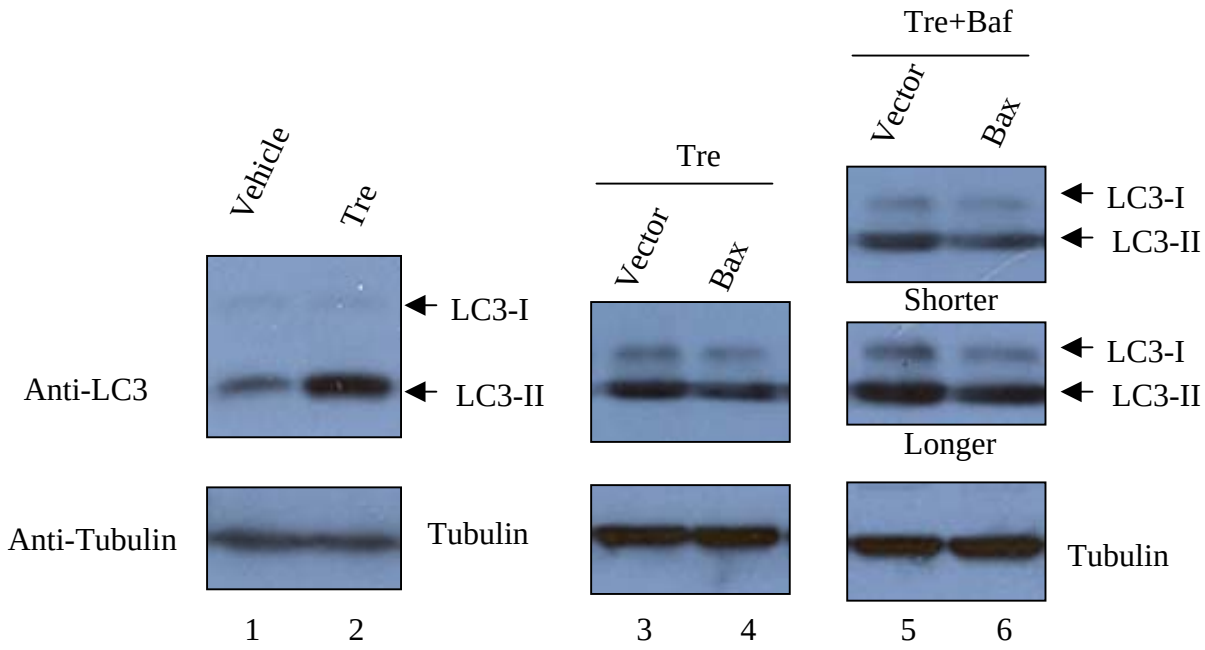
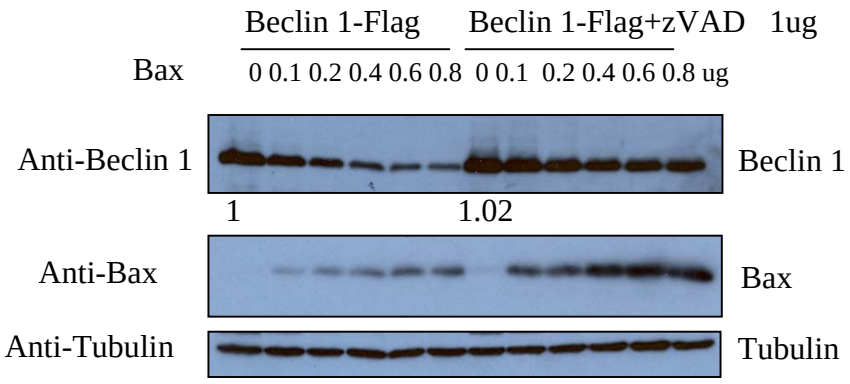
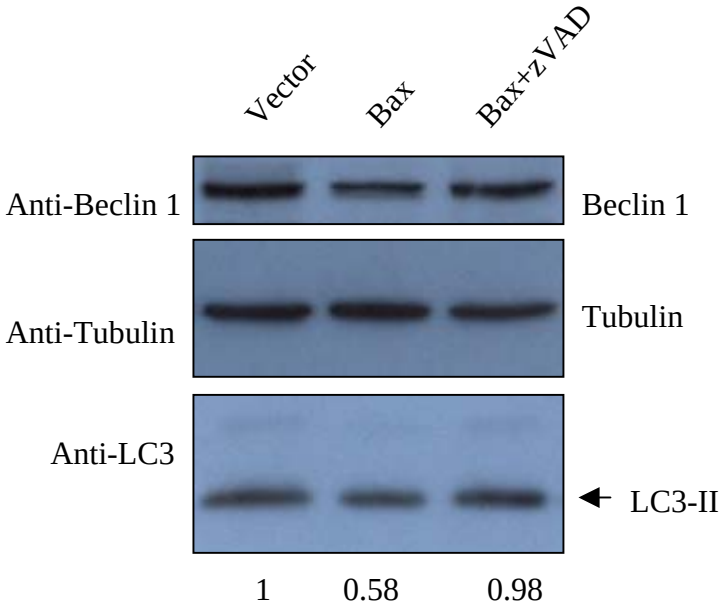

Tre+Baf
Vector
Bax
Vehicle
Tre
Tre
Vector
Bax
LC3-I
LC3-II
Shorter
LC3-I
LC3-II
Longer
Anti-LC3
Anti-Tubulin
Tubulin
Tubulin
1
2
3
4
5
6

Fig S3


Beclin 1-Flag
Beclin 1-Flag+zVAD 1ug
Bax
0 0.1 0.2 0.4 0.6 0.8 0 0.1 0.2 0.4 0.6 0.8 ug
Anti-Beclin 1
Beclin 1
1
1.02
Anti-Bax
Bax
Anti-Tubulin
Tubulin

(a)


Vector
Bax
Bax+zVAD
Anti-Beclin 1
Beclin 1
Anti-Tubulin
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LC3-II
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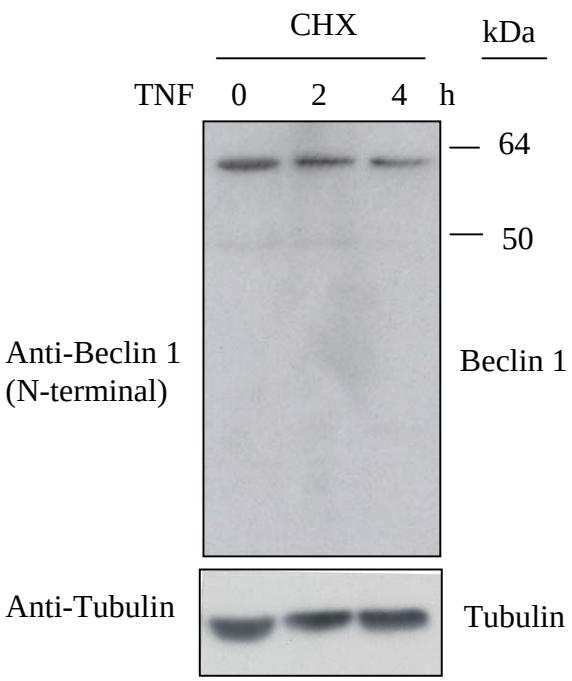
0.98

(b)

Fig S4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kDa
TNF 0 2 4 h
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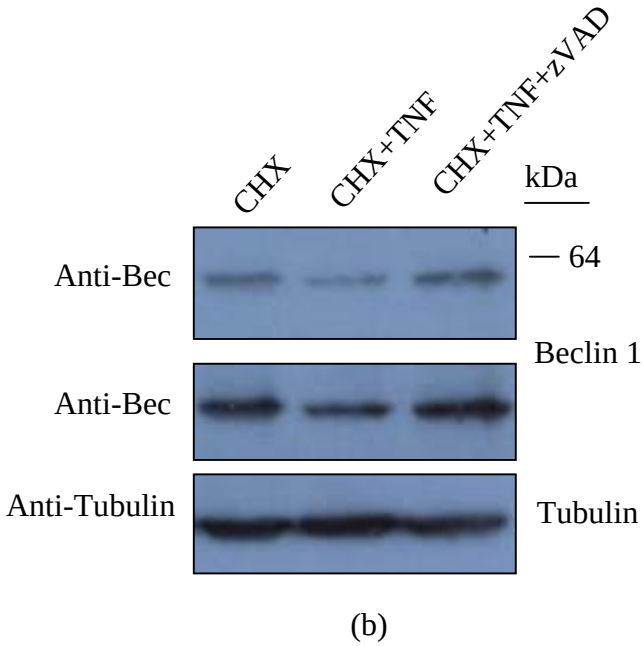
50
Anti-Beclin 1
(N-terminal)
Beclin 1
Anti-Tubulin
Tubulin

(a)


CHX
CHX+TNF
CHX+TNF+zVAD
kDa
Anti-Bec
64
Beclin 1
Anti-Bec
Anti-Tubulin
Tubulin

(b)

Fig S5

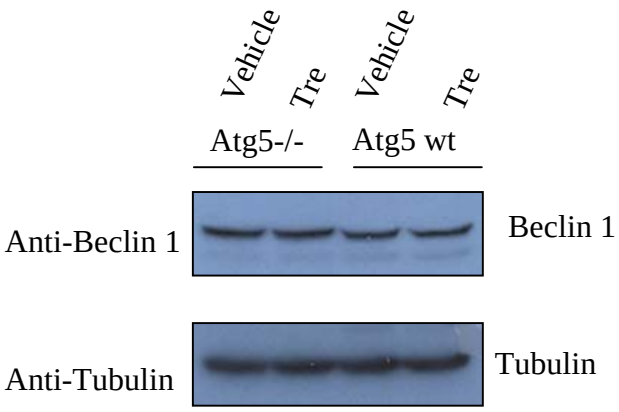

Vehicle
Tre
Vehicle
Tre
Atg5-/-
Atg5 wt
Anti-Beclin 1
Beclin 1
Anti-Tubulin
Tubulin

Fig S6

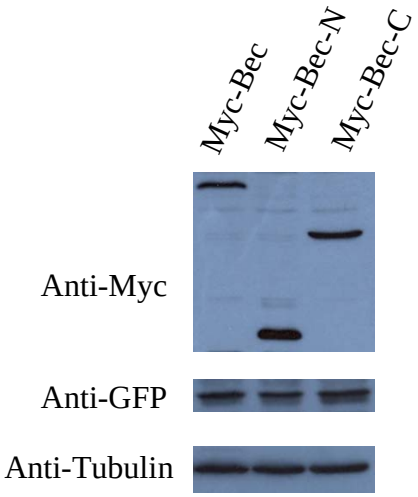
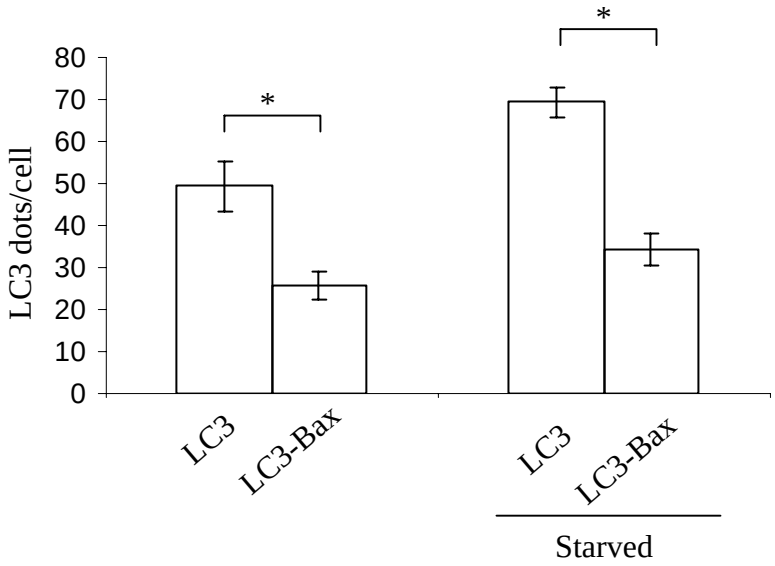
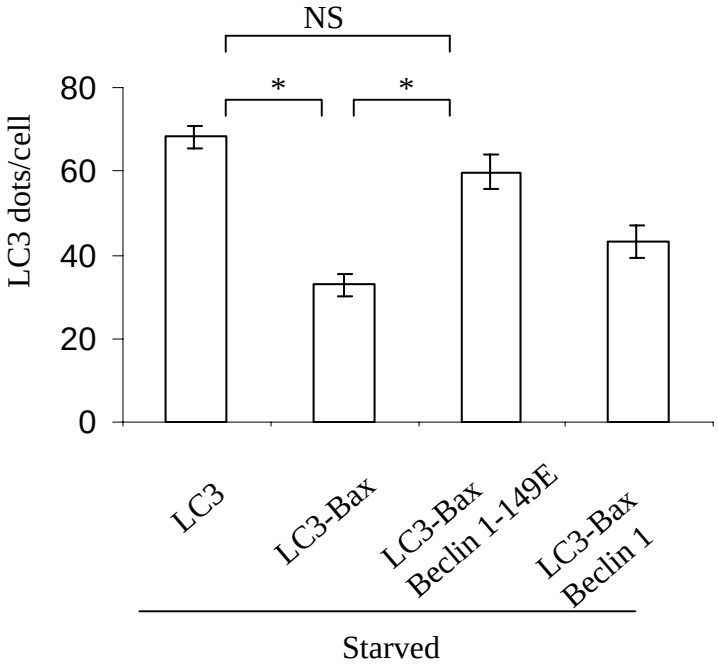


Fig S7

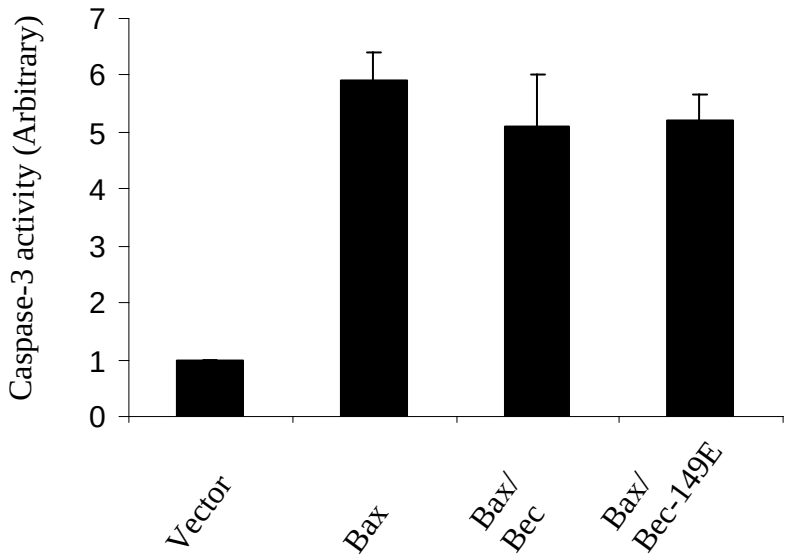

*
*
80
70
60
50
40
30
20
10
0
LC3 dots/cell
LC3
LC3-Bax
LC3
LC3-Bax
Starved

(a)


NS
*
*
80
60
40
20
0
LC3 dots/cell
LC3
LC3-Bax
LC3-Bax Beclin 1-149E
LC3-Bax Beclin 1
Starved

(b)

Fig S8


Caspase-3 activity (Arbitrary)
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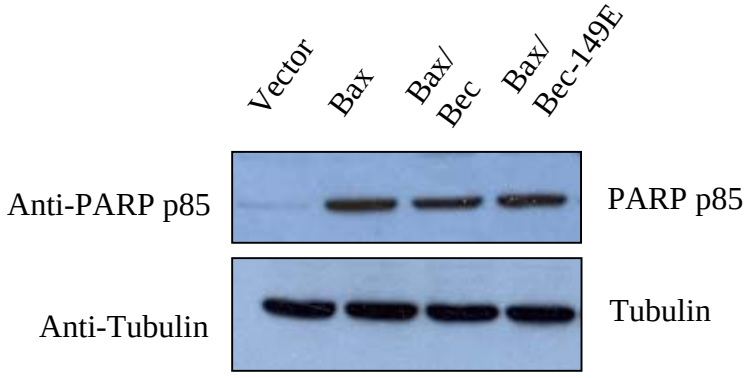
6
5
4
3
2
1
0
Vector
Bax
Bax/ Bec
Bax/ Bec-149E

(a)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Bax
Bax/ Bec
Bax/ Bec-149E
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PARP p85
Anti-Tubulin
Tubulin

(b)

Fig S9

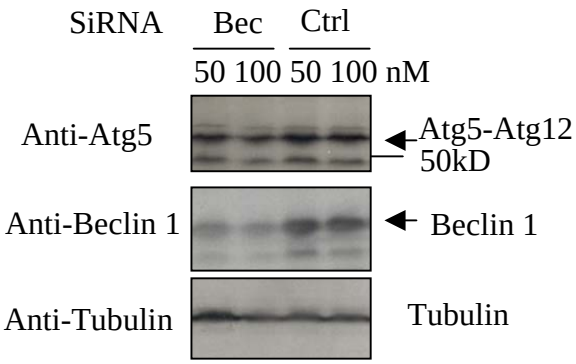
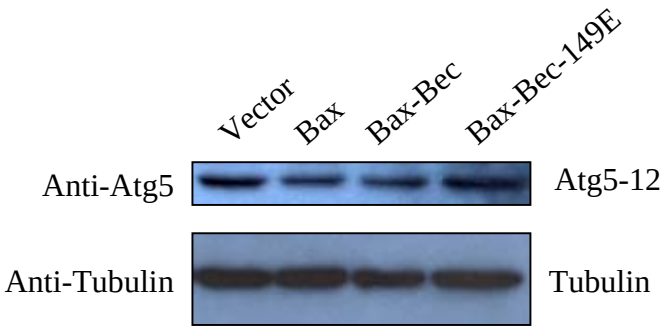

SiRNA
Bec
Ctrl
50 100 50 100 nM
Anti-Atg5
Atg5-Atg12
50kD
Anti-Beclin 1
Beclin 1
Anti-Tubulin
Tubulin

Fig S10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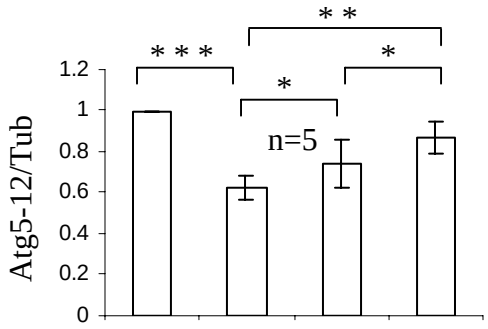
Bax-Bec
Bax-Bec-149E
Anti-Atg5
Atg5-12
Anti-Tubulin
Tubulin

(a)


* * *
* *
*
*
n=5
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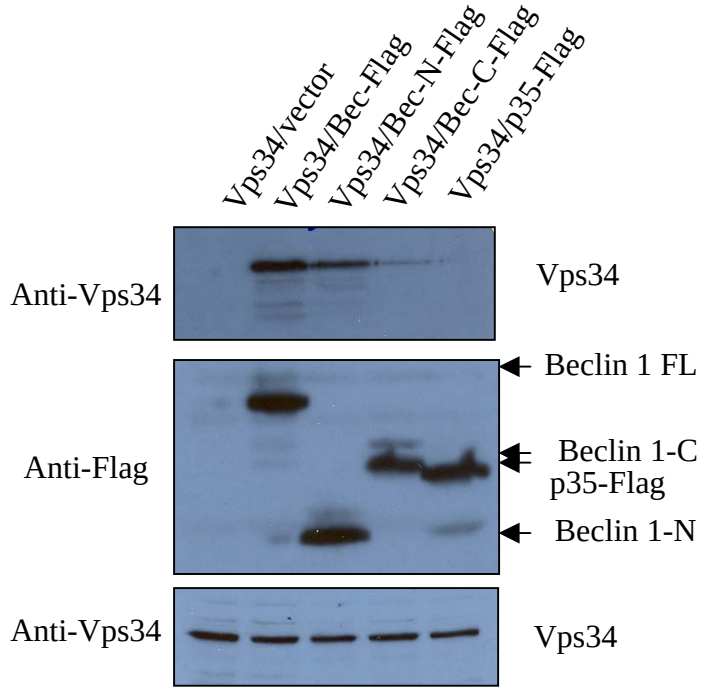
1
0.8
0.6
0.4
0.2
0

(b)

Fig S11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Vps34/Bec-N-Flag
Vps34/Bec-C-Flag
Vps34/p35-Flag
Anti-Vps34
Vps34
Beclin 1 FL
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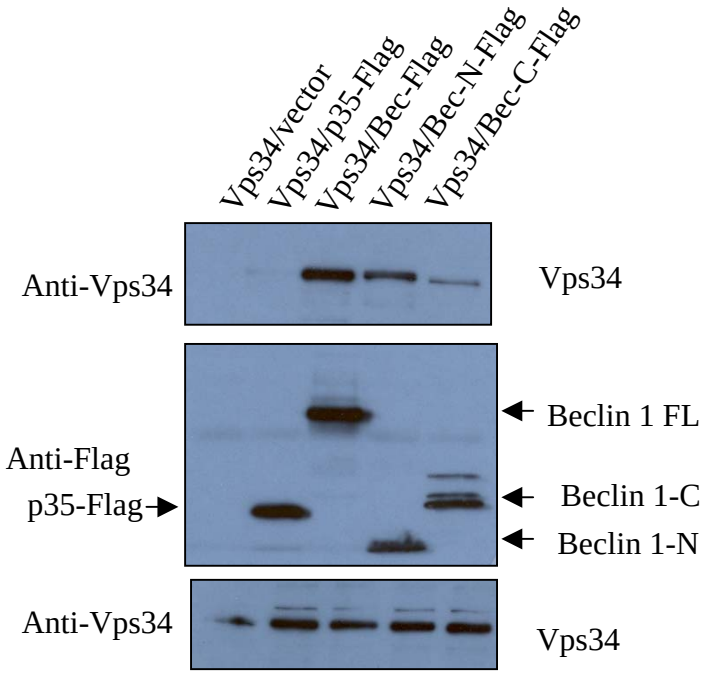
Beclin 1-C
p35-Flag
Beclin 1-N
Anti-Vps34
Vps34

(a)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Beclin 1-N
Anti-Vps34
Vps34

(b)

Fig S12

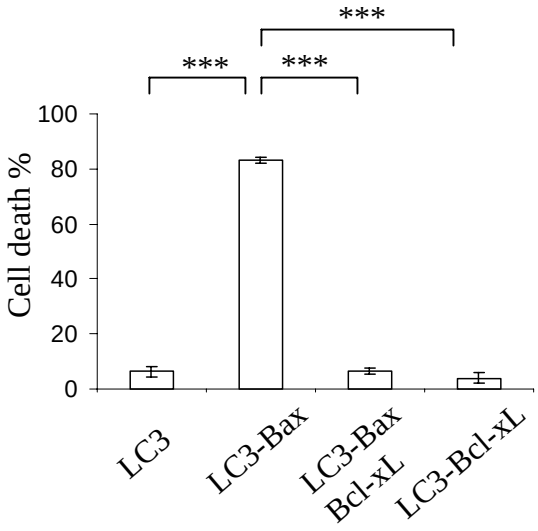
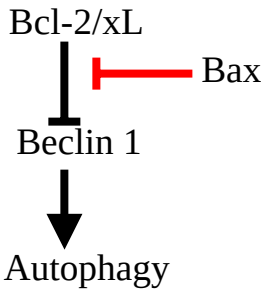
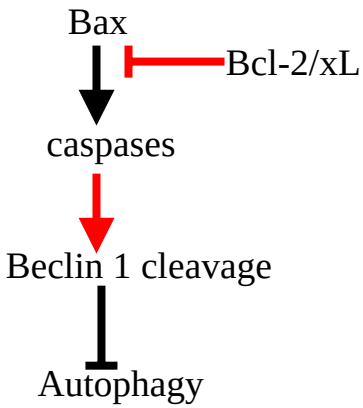


Fig S13



Bax rescuing autophagy inhibition by Bcl-2/xL

(a)



Bcl-xL rescuing autophagy inhibition by Bax

(b)