The tumor suppressor LKB1 regulates starvation-induced autophagy under systemic metabolic stress

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Supplementary Figures

Supplementary Figure 1

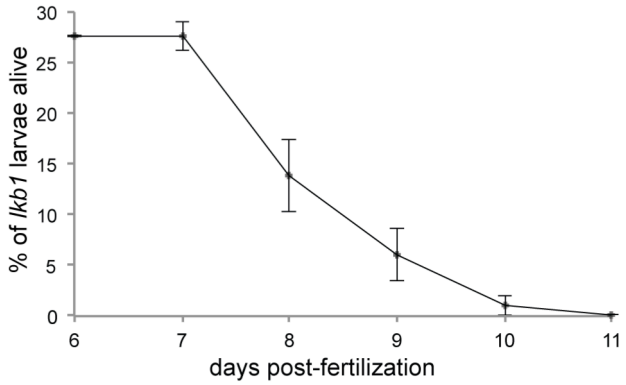


Figure S1. The majority of *lkb1* mutants die from premature starvation at 7-8 dpf. Larvae were collected from pairings of *lkb1* heterozygotes. 100 alive larvae/time-point were collected and genotyped for the *lkb1* mutation at 6, 7, 8, 9 and 10 dpf. The percentage of *lkb1* mutants alive in the total larvae population is plotted on each time-point. Error bars represent the means ± standard errors of the means (SEM) and are pooled from three independent experiments. The graph depicts that *lkb1* mutants start dying at 7 dpf, the majority of *lkb1* mutants die at 8 dpf and very few "escapers" are alive at 9 dpf.

Supplementary Figure 2

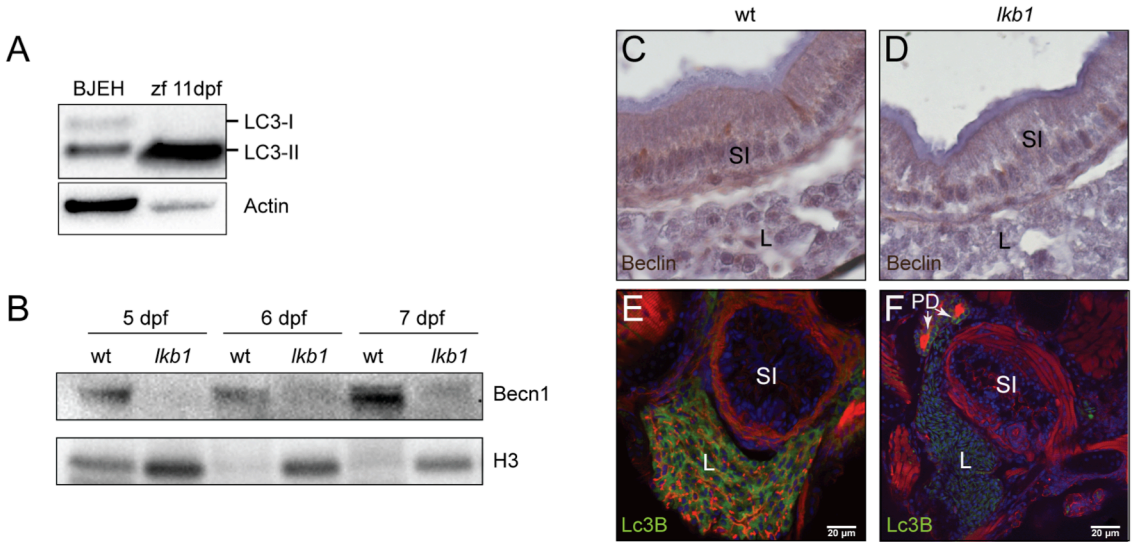


Figure S2. Autophagy markers Lc3B and Beclin are lower in *lkb1* mutants. (A) The LC3B antibody recognizes the cleaved Lc3-II in zebrafish. Western blot analysis using antibodies against LC3B and beta-actin (loading control) on total protein lysates from human BJEH cells that were serum-starved overnight and wt zebrafish larvae at 11 dpf. The LC3B antibody recognizes the uncleaved and cleaved forms of LC3B in the human sample, but predominantly the cleaved Lc3-II in the zebrafish sample. Uncropped images of the blots are presented in Supplementary Fig. S13. (**B)** Western blot analysis of Beclin (Becn1) and Histone H3 (loading control) in total protein lysates of wt and *lkb1* trunks between 5-7 dpf. Larvae were treated with chloroquine (2.5 µM) for 14 h prior to processing. Becn1 levels are lower in the *lkb1* mutants at all time-points. **(C-D)** Immunohistochemical analysis of transverse paraffin sections (5 µm) of intestine of 7 dpf wt and *lkb1* larvae shows very low levels of Becn1 expression in the *lkb1* intestine. Magnification: 100X. **(E-F)** Transverse vibratome sections (150 µm) of liver of 7 dpf wt and *lkb1* mutants stained with anti-LC3B antibody (green), rhodamine-phalloidin to detect F-actin (red), and DAPI to detect nuclei (blue). Lc3B staining in the *lkb1* liver is greatly reduced. PD: pronephric ducts; L: liver; SI: intestine.

Supplementary Figure 3

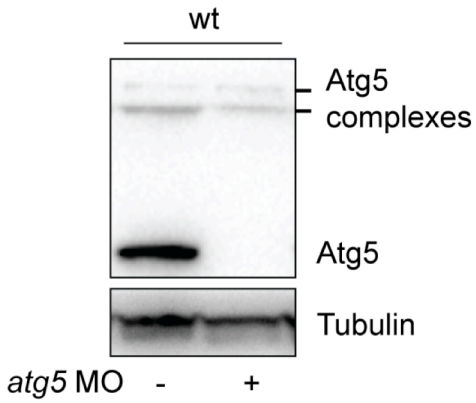


Figure S3. The *atg5*MO efficiently inhibits Atg5 translation. Western blot analysis of wt larvae at 4 dpf, with an antibody against Atg5. Tubulin is used as a loading control. Atg5 expression is almost undetectable in larvae injected with 0,5 mM *atg5*MO at the one-cell stage, showing efficient inhibition of Atg5 translation. Uncropped images of the blots are shown in Supplementary Figure S14.

Supplementary Figure 4

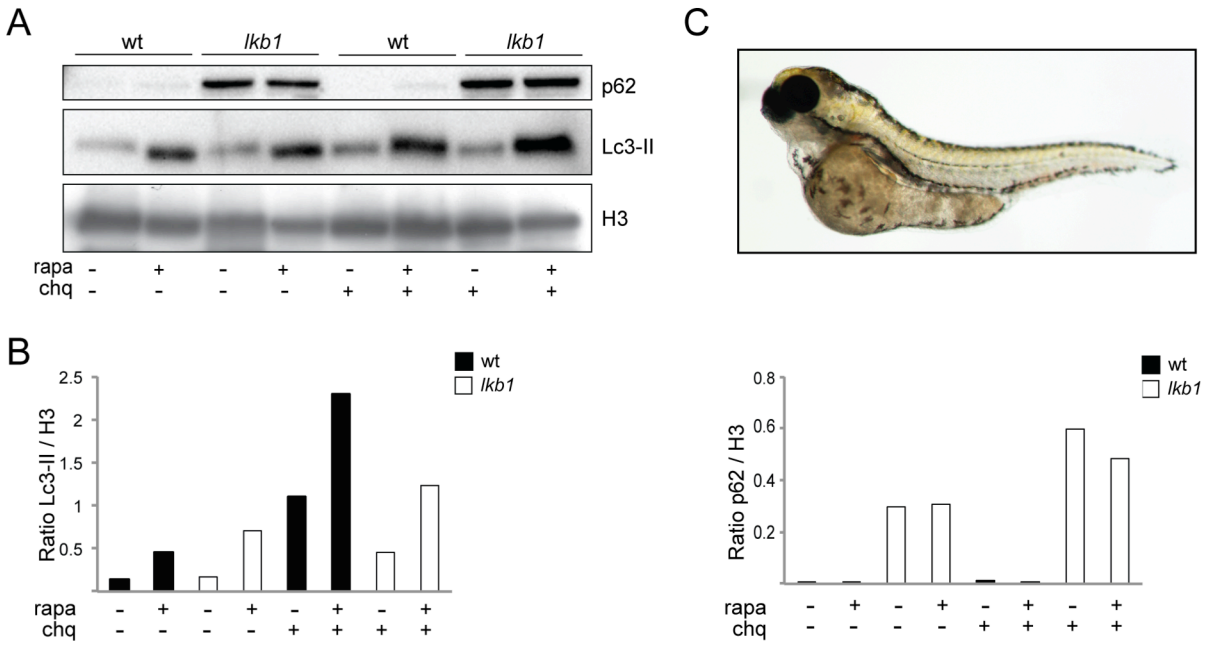


Figure S4. Rapamycin treatment leads to increased Lc3-II accumulation but does not restore p62 degradation in *lkb1* mutants. (A) Western Blot analysis of p62, Lc3-II, and Histone H3 (loading control) in total protein lysates of wt and *lkb1* trunks at 6 dpf that were treated with either 10 µM rapamycin from 1 dpf or DMSO (negative control). To detect the autophagic flux the larvae were treated or not with 2.5 µM chloroquine for 14 h prior to processing. Rapamycin treatment leads to increased Lc3-II levels in both wt and *lkb1* larvae, but p62 levels remained high in the *lkb1* mutants. Uncropped images of the blots are shown in Supplementary Figure S9B. **(B)** Graphical representation of the data shown in (A), depicting the densitometric p62/H3 and Lc3-II/H3 ratios. **(C)** Representative image of a wt larva at 6 dpf that has been treated with 10 µM rapamycin from 1 dpf onwards. Note the high amount of yolk still present indicating developmental delay.

Supplementary Figure 5

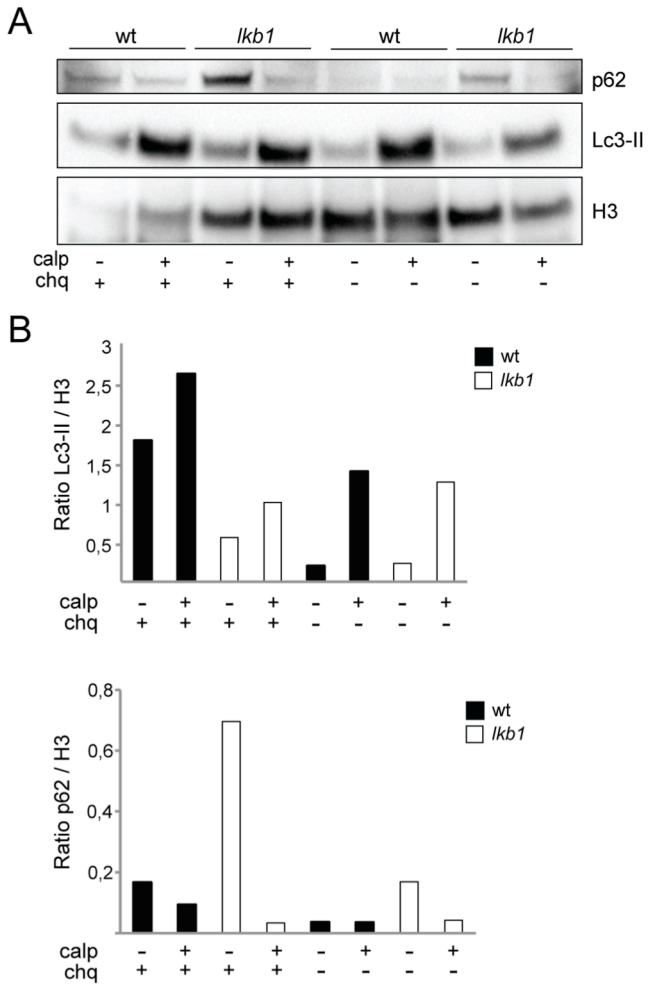


Figure S5. Calpeptin-mediated activation of autophagy restores p62 degradation in *lkb1* mutants. (A) Western blot analysis of Lc3-II, p62 and Histone H3 (loading control) in total protein lysates of wt and *lkb1* trunks at 6 dpf. The embryos were treated with 50 µM calpeptin or DMSO (negative control) from 1 dpf onwards. To detect the autophagic flux, larvae were treated or not with 2.5 µM chloroquine for 14 h prior to lysing. Calpeptin treatment leads to upregulation of Lc3-II levels in both wt and *lkb1* larvae. Induction of autophagy by calpeptin also leads to robust downregulation of p62 accumulation in *lkb1* larvae. Uncropped images of the blots are shown in Supplementary Figure S11A. **(B)** Graphical representation of the data shown in (A), depicting the densitometric p62/H3 and Lc3-II/H3 ratios.

Supplementary Figure 6

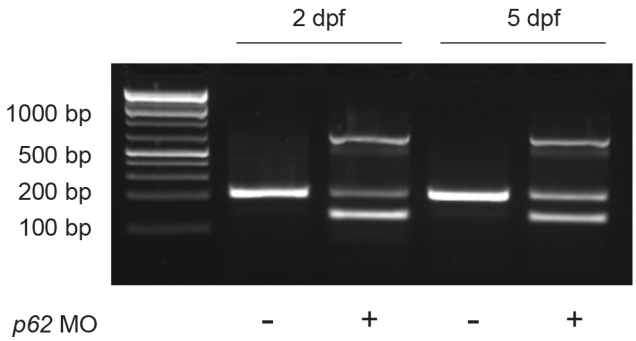
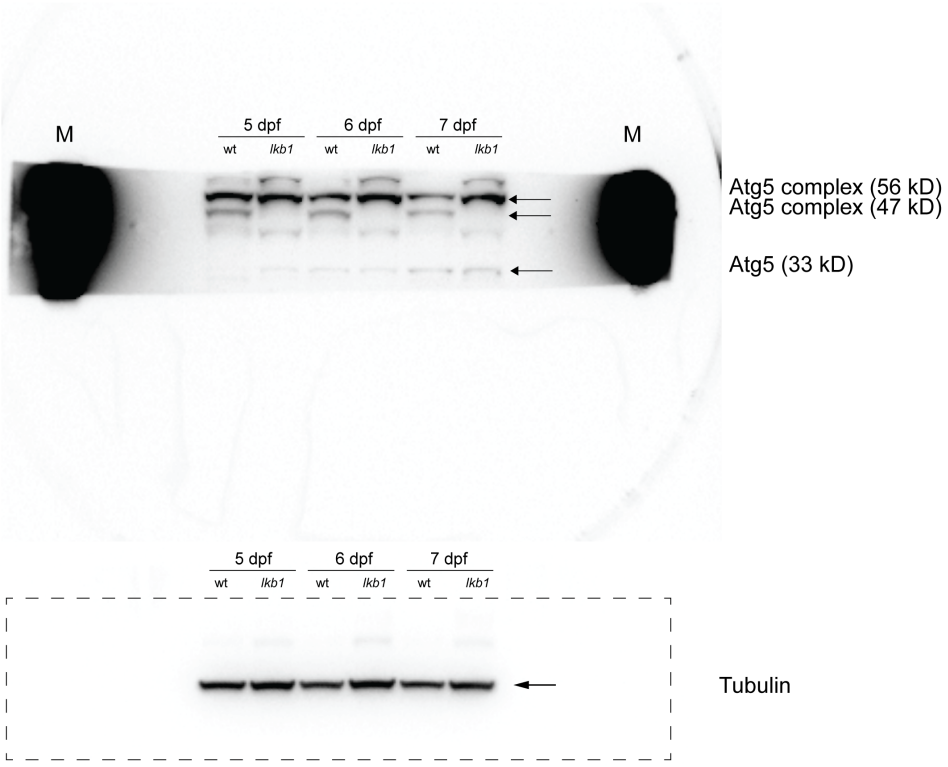


Figure S6. The *sqstm1/p62*MO efficiently blocks splicing of *sqstm1/p62* mRNA. Reverse transcription polymerase chain reaction (RT-PCR) was used to confirm antisense morpholino blocking of intron-exon splicing events in zebrafish *sqstm1/p62* mRNA at 2 and 5 dpf. Upon injection of 0,5 mM *sqstm1/p62* MO (i1e2, targeting splicing between the first intron and the second exon), the 200 bp RT-PCR product is disrupted.

Supplementary Figure 7

A Uncropped images of the blots presented in Figure 1 (Atg5, Tubulin)



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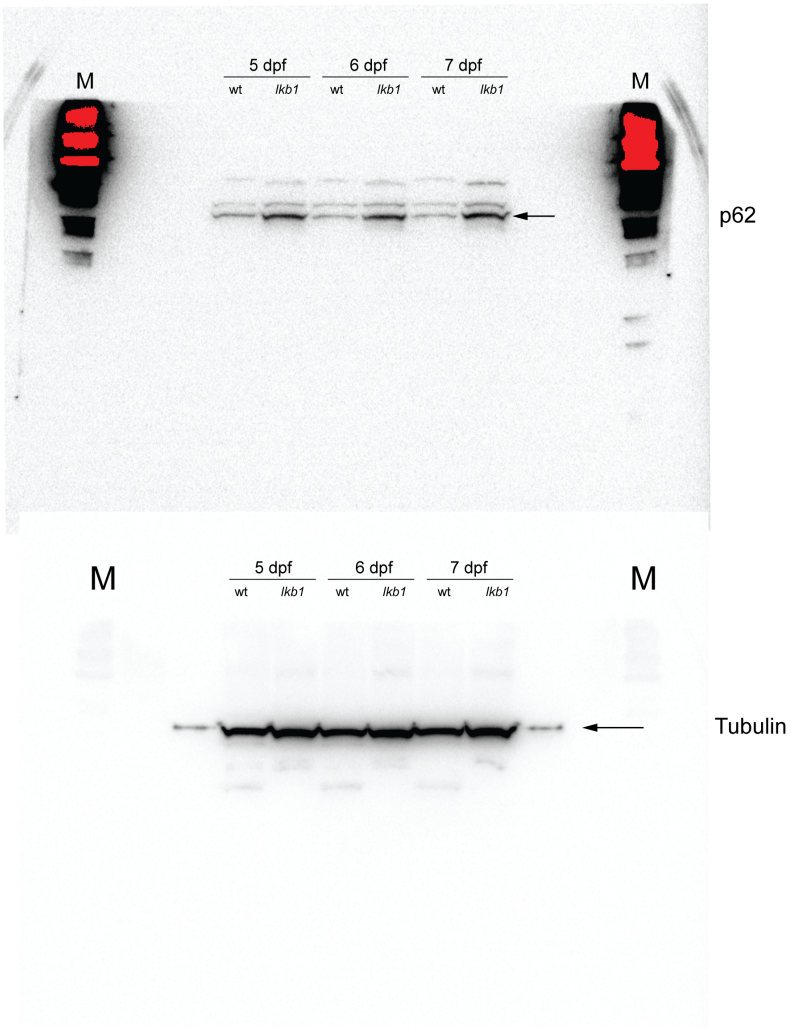
This tubulin blot was used in the final cropped image

Supplementary Figure 7

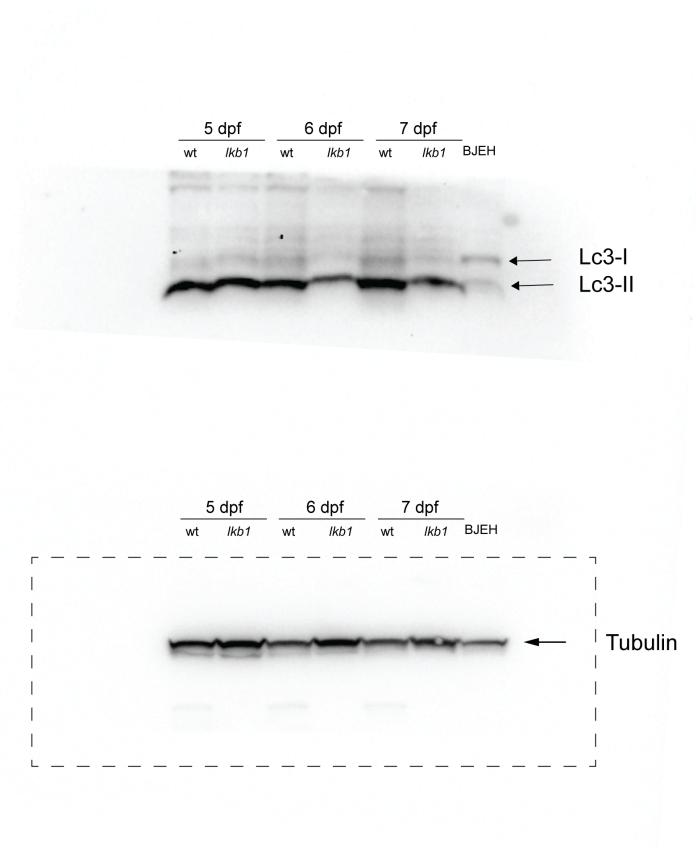
B Uncropped images of the blots presented in Figure 1 (p62, Tubulin)



Supplementary Figure 7

C

Uncropped images of the blots presented in Figure 1 (Lc3, Tubulin)



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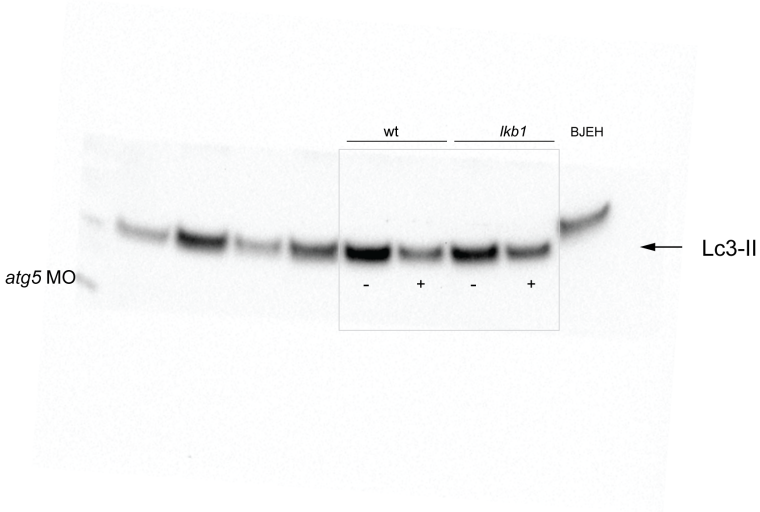
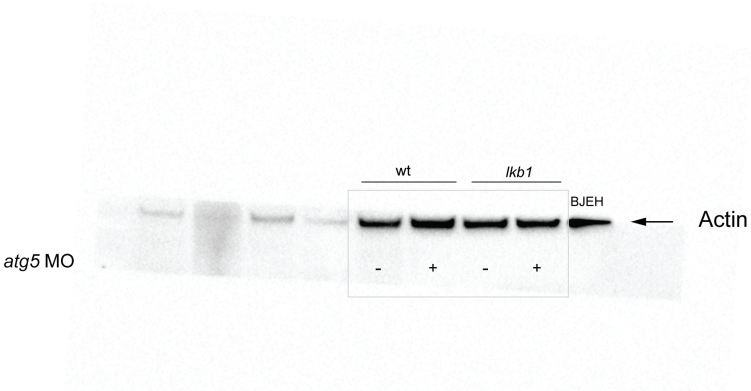
Supplementary Figure 8

Uncropped images of blots presented in Figure 2

Lc3 II, Actin, upon *atg5*MO-injection





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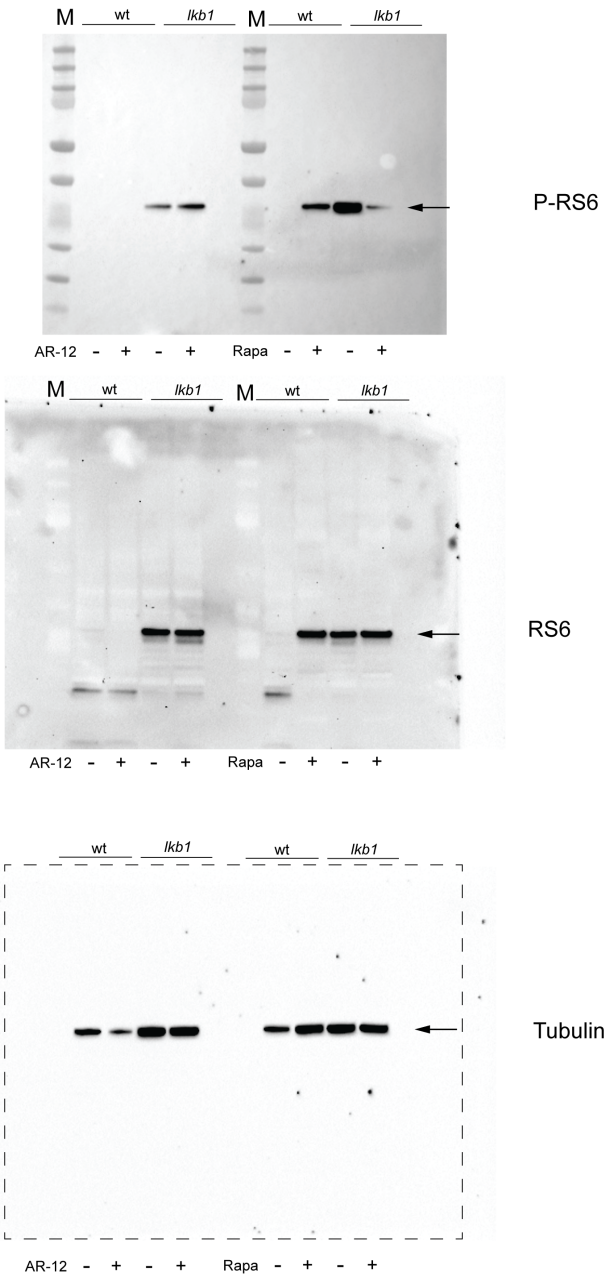
Supplementary Figure 9

A Uncropped images of blots presented in Figures 3A and Figure 4C

RS6, P-RS6 and Tubulin upon AR-12 and Rapamycin treatments



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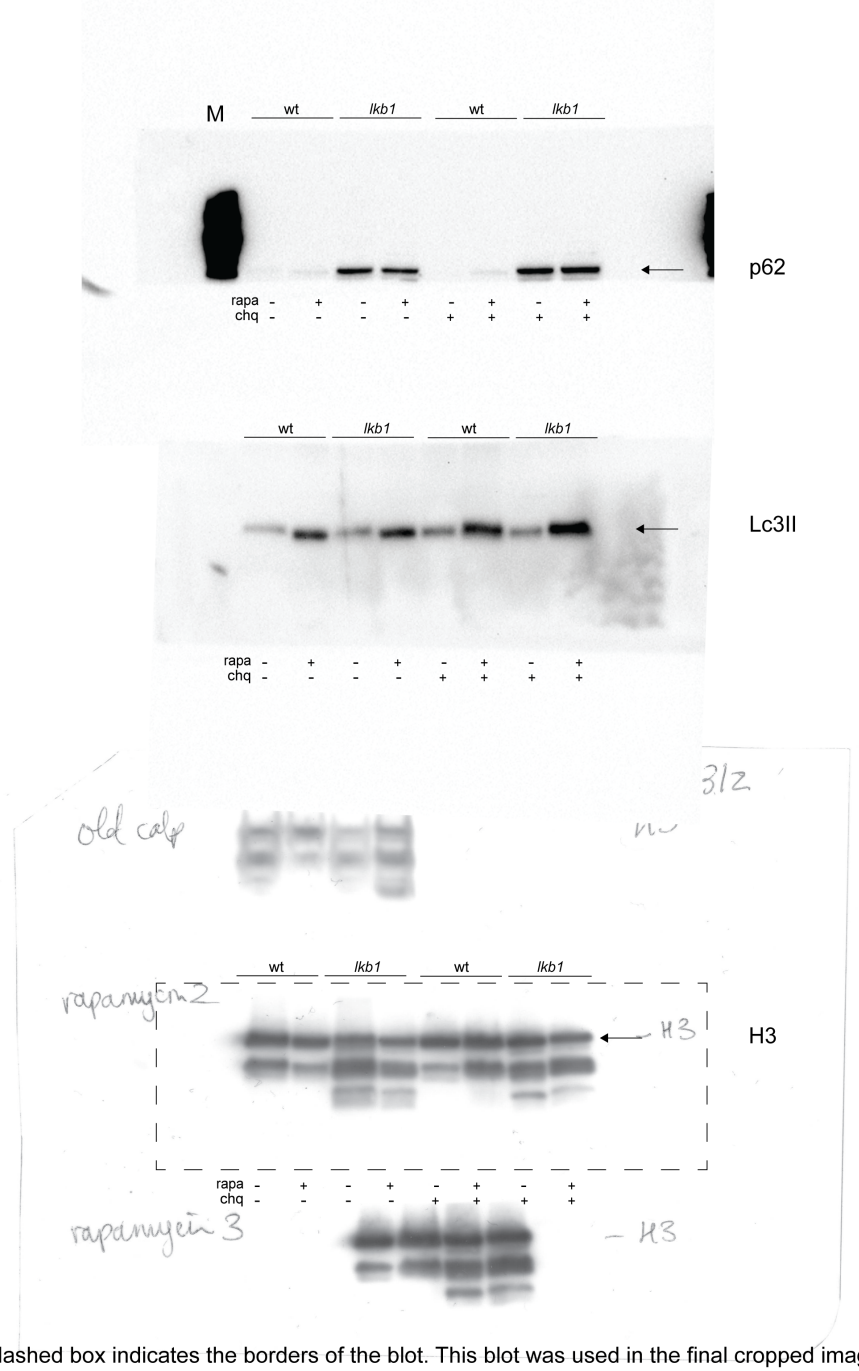
Supplementary Figure 9

B

Uncropped images of blots presented in Figure 3B and Supplementary Figure S4A

p62, Lc3 II and H3 upon Rapamycin treatment, +/- chloroquine



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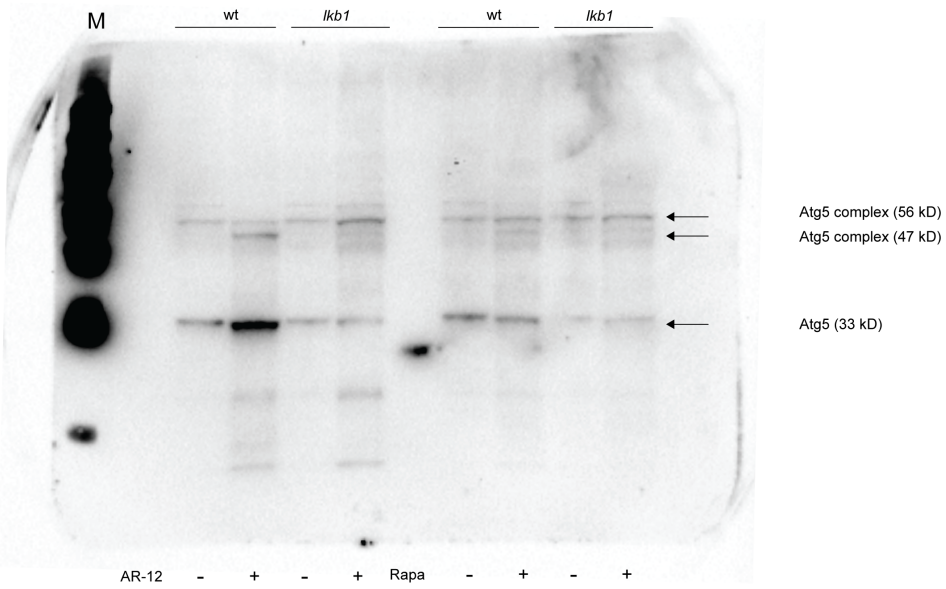
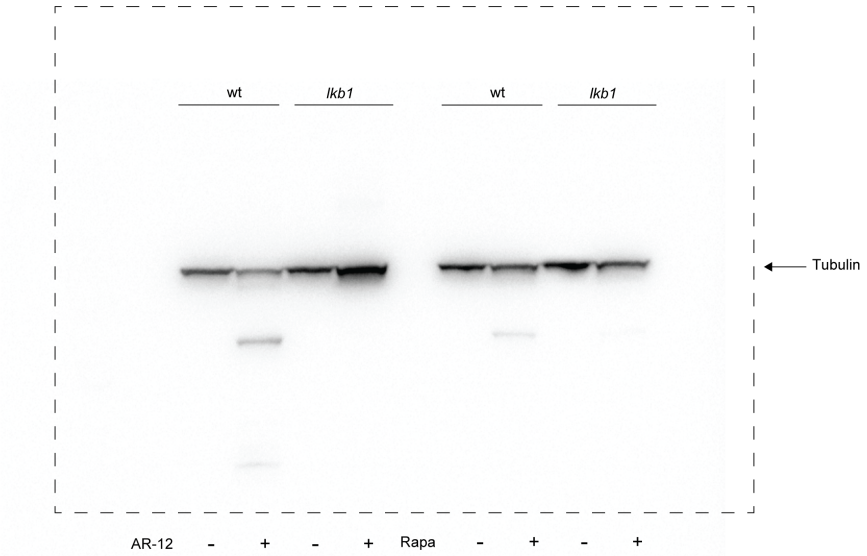
Supplementary Figure 9

C Uncropped images of blots presented in Figures 3C and 4B

Atg5 and Tubulin upon AR-12 and Rapamycin treatments





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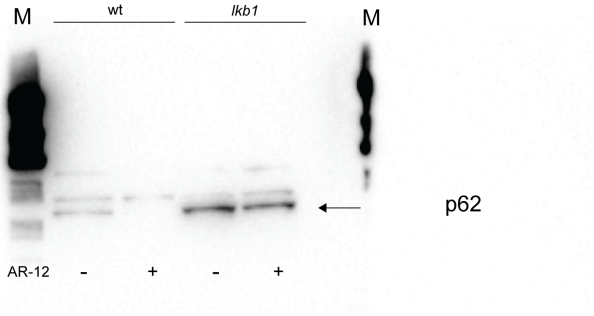
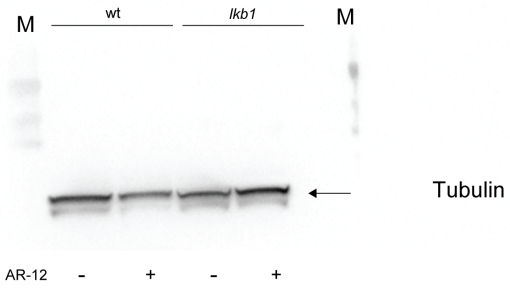
Supplementary Figure 10

A Uncropped images of blots presented in Figure 4A

p62 and tubulin upon AR-12 treatment



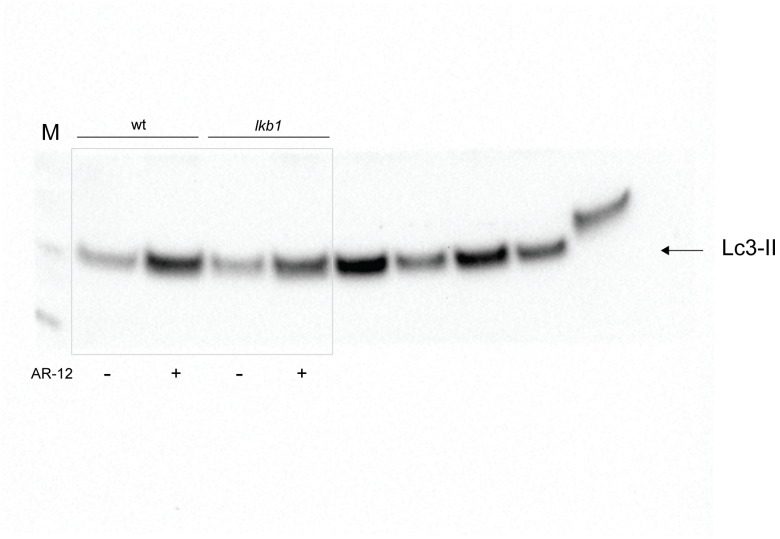


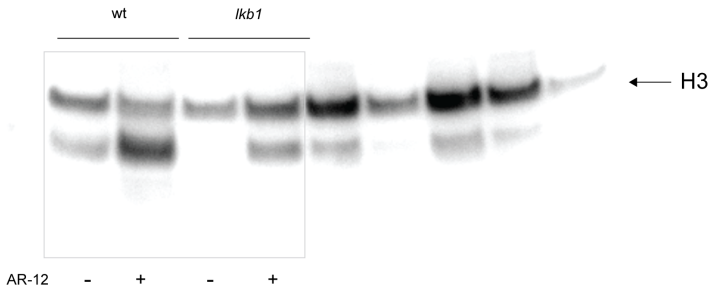
Supplementary Figure 10

B

Uncropped images of blots presented in Figure 4A

Lc3 II and H3 upon AR-12 treatment



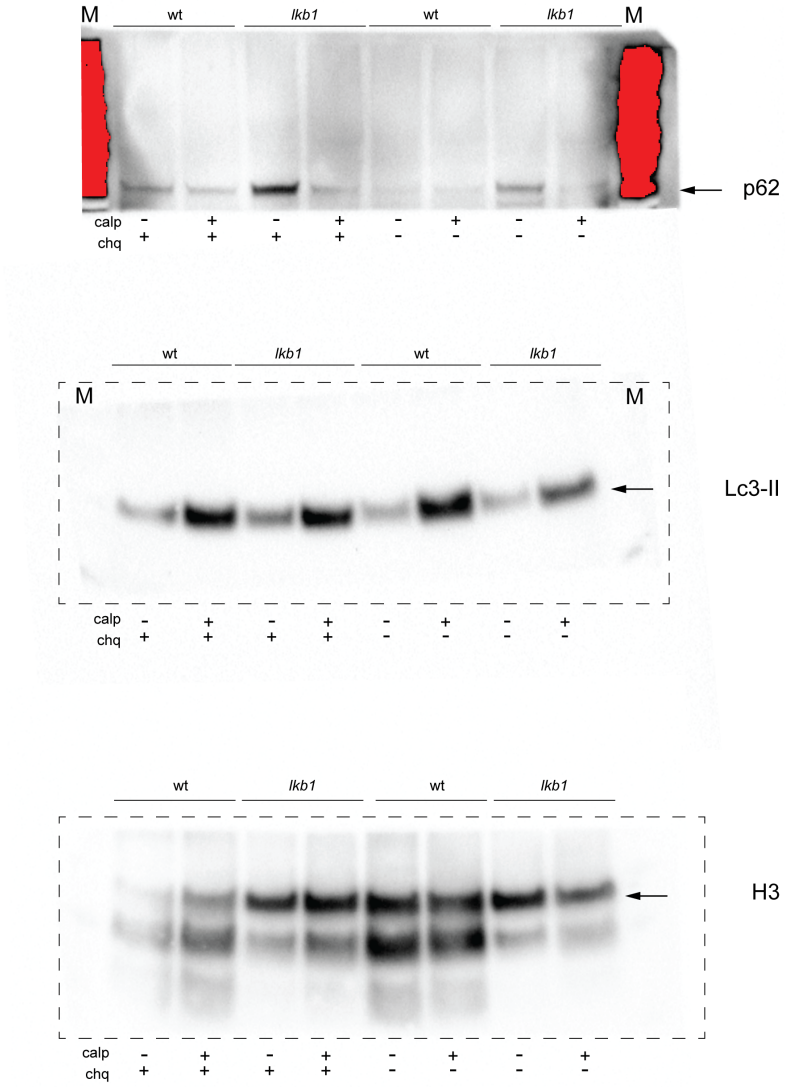


The boxes indicate the parts of the blots that were used in the final cropped image

Supplementary Figure 11

A Uncropped images of blots presented in Figure 5A and Supplementary Figure S5A

p62, Lc3 II, H3 upon calpeptin treatment, +/- chloroquine



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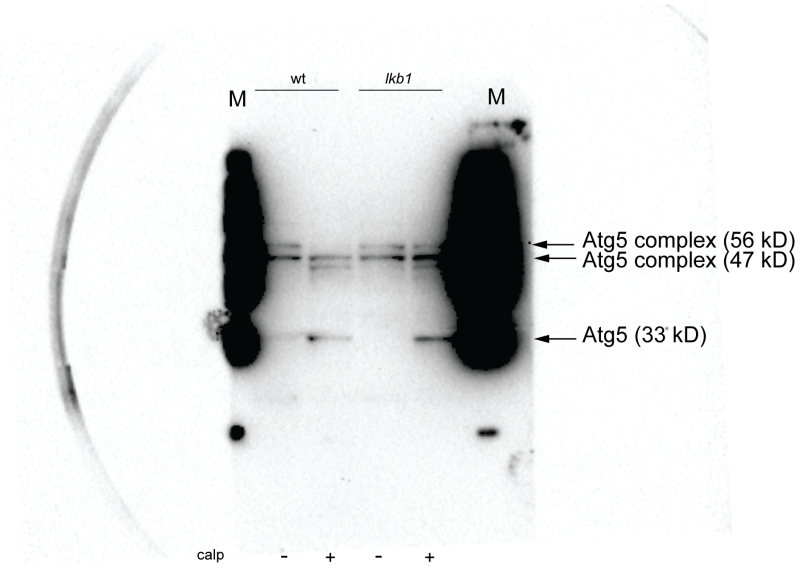
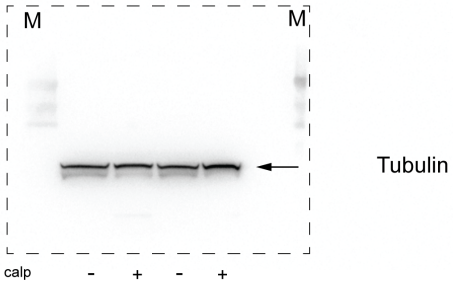
Supplementary Figure 11

B

Uncropped images of blots presented in Figure 5B

Atg5, Tubulin upon calpeptin treatment





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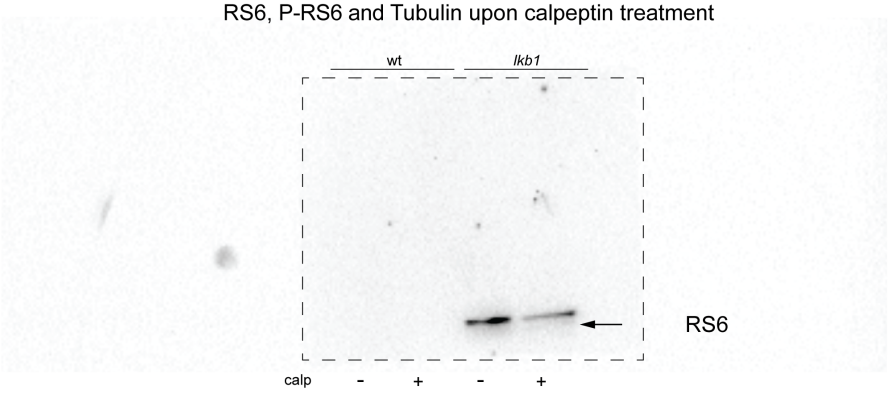
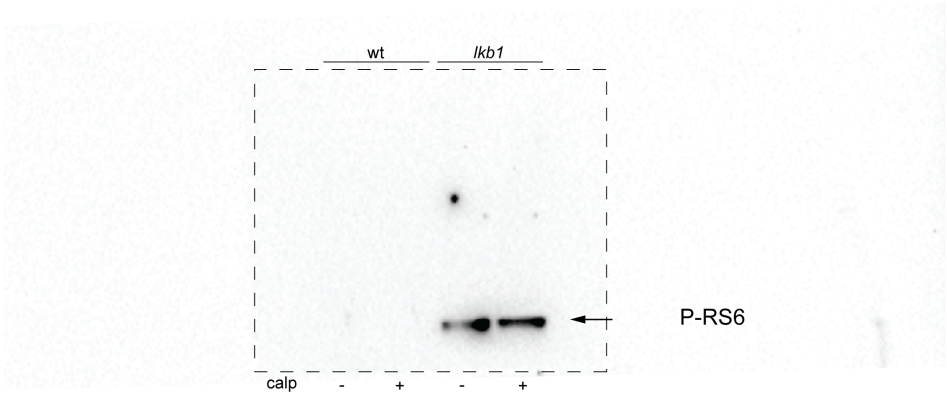
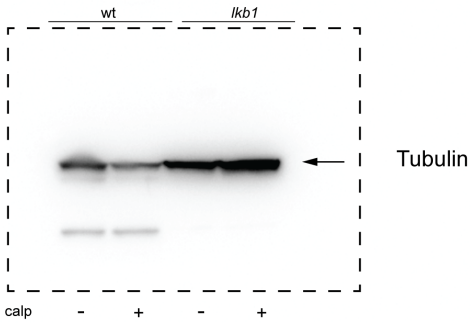
Supplementary Figure 11

C Uncropped images of blots presented in Figure 5C

RS6, P-RS6 and Tubulin upon calpeptin treatment







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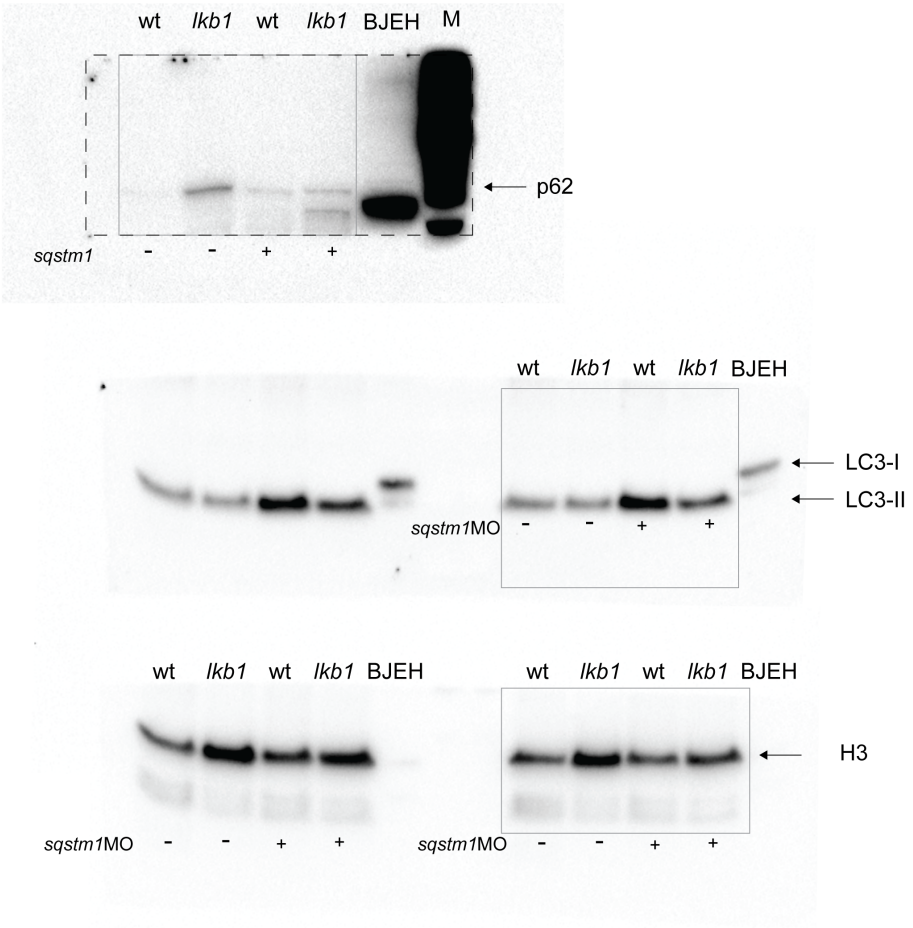
Supplementary Figure 12

Uncropped images of blots presented in Figure 6A

p62, Lc3 II, H3 upon *sqstm1*MO-injection



The dashed boxes indicate the borders of the blots

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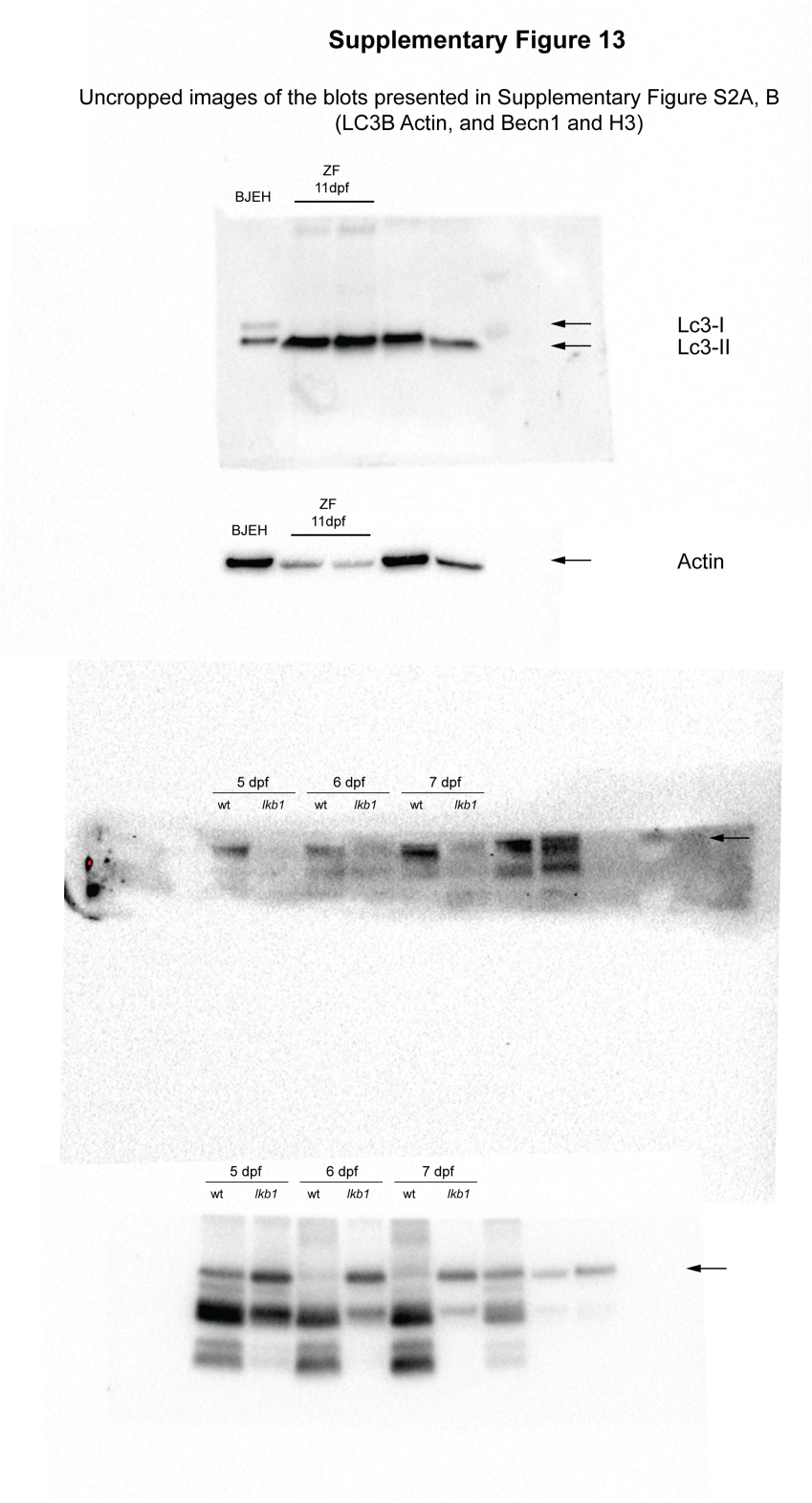
Supplementary Figure 13

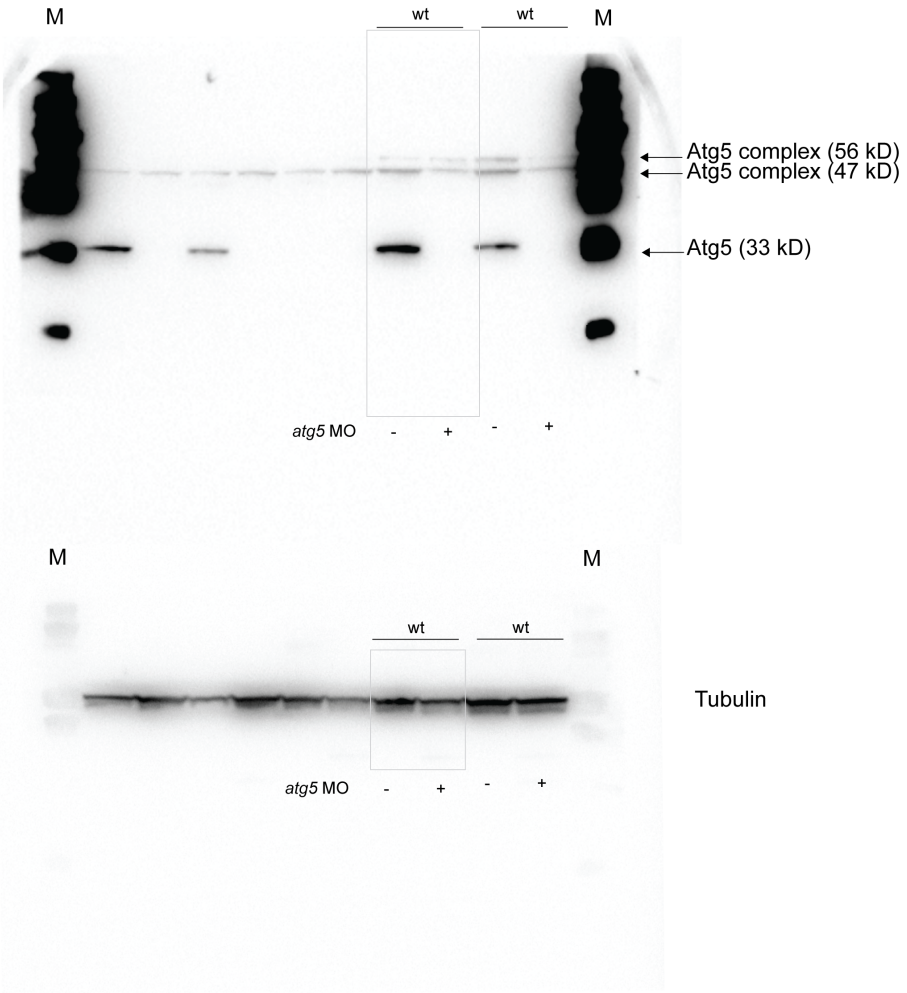
Uncropped images of the blots presented in Supplementary Figure S2A, B (LC3B Actin, and Becn1 and H3)



Supplementary Figure 14

Uncropped images of the blots presented in Supplementary Figure S3

Atg5, Tubulin upon *atg5*MO injection



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