Supplemental information

Table S1

Lethality analysis of *Atg101^{6h}* mutant. All genetic crosses were performed as indicated. The number of progenies for each cross was counted. The percentage of *Atg101^{6h}* mutant females and males was reduced as compared to the controls (highlight in blue and red color). The reduction for *Atg101^{6h}* mutant males was restored when expressing *UAS-Atg101* by *Da-Gal4*.

Figure S1 Characterization of Atg101 mutant allele

(A) Relative S6KL expression levels in both wild type and *Atg101^{6h}* mutants. qPCR analysis for S6KL mRNA expression levels were carried out in both wild-type and *Atg101^{6h}* mutants. Data were normalized to rp49.

(B) Representative confocal images of *Drosophila* larval NMJ4 of wild type and *Atg101^{6h}* mutants labeled with anti-CSP. Scale Bar: 10 um.

(C) Quantification of synaptic bouton numbers in wild type and *Atg101^{6h}* mutants. n=8.

(D) Decreased hatching rate (200 embryos for each experiment, with three replicates), pupation rate (50 larvae for each experiment, with five replicates) and eclosion rate (at least 40 pupae for each experiment, with five replicates) in *Atg101^{6h}* mutants. Data are presented as mean±SD. Paired t-test was used for statistical analysis. ** indicates that $p<0.01$. * indicates that $p<0.05$.

(E) Lethality of Atg101 mutants during pupal stage.

(F) Lethality of Atg101 mutants during eclosion.

(G) A wild type male fly with normal wing posture.

(H) Wing posture defects of *Atg101^{6h}* mutants.

(I) A rescue male fly with normal wing posture.

(J) Delayed pupation in *Atg101^{6h}* mutants. Pupation rates of wild-type and *Atg101^{6h}* mutant animals at the indicated days after larval hatching.

(K) Delayed adult eclosion in *Atg101^{6h}* mutants. Eclosion rates of wild-type and *Atg101^{6h}* mutant animals at the indicated days after larval hatching.

(L) RT-PCR reveals the expression of *Atg101* mRNA at different developmental stages. E1, 0-12 hour embryo; E2, 12-24 hour embryo; L1, first instar larvae; L2, second instar larvae; L3, third instar larvae; P, pupae; A, adults. *rp49* serves as a control.

(M) RT-PCR reveals the expression of Atg101, Atg1, Atg3, Atg4a, Atg7 and Atg8a in 0-12 hour embryo. *rp49* serves as a control.

Figure S2 Atg101 mutant midguts display delayed cell death

(A)Morphology of midguts at +4 hour RPF for both wild-type and $Atg101^{6h}$ mutants. Less contracted gastric caeca (arrows) was seen in $Atg101^{6h}$ mutants. Scale Bar: 100 um. (B) Quantification of gastric caeca size in the control (n=14) and Atg101 mutants (n=8). Data are presented as mean±SD. *** indicates that $p<0.001$.

Figure S3 ***Partial colocalization between ubiquitinated proteins and Ref(2)p aggregates in Atg101 mutant brains***

(A-A'')Shown are confocal images of adult brain of a 7 day-old $Atg101^{6h}$ mutant fly. Ref(2)p protein aggregates partially colocalize with ubiquitin. (B-B'') Higher magnification images of the area surrounded by the white square in A''. Scale bar: 10 um.

Figure S4 ***Loss of Atg101 results in the enlarged abdomen***

(A) An image of a wild-type male abdomen.

(B) An image of an $Atg101^{6h}$ mutant male abdomen.

(C) An image of a rescue male abdomen.

Figure S1

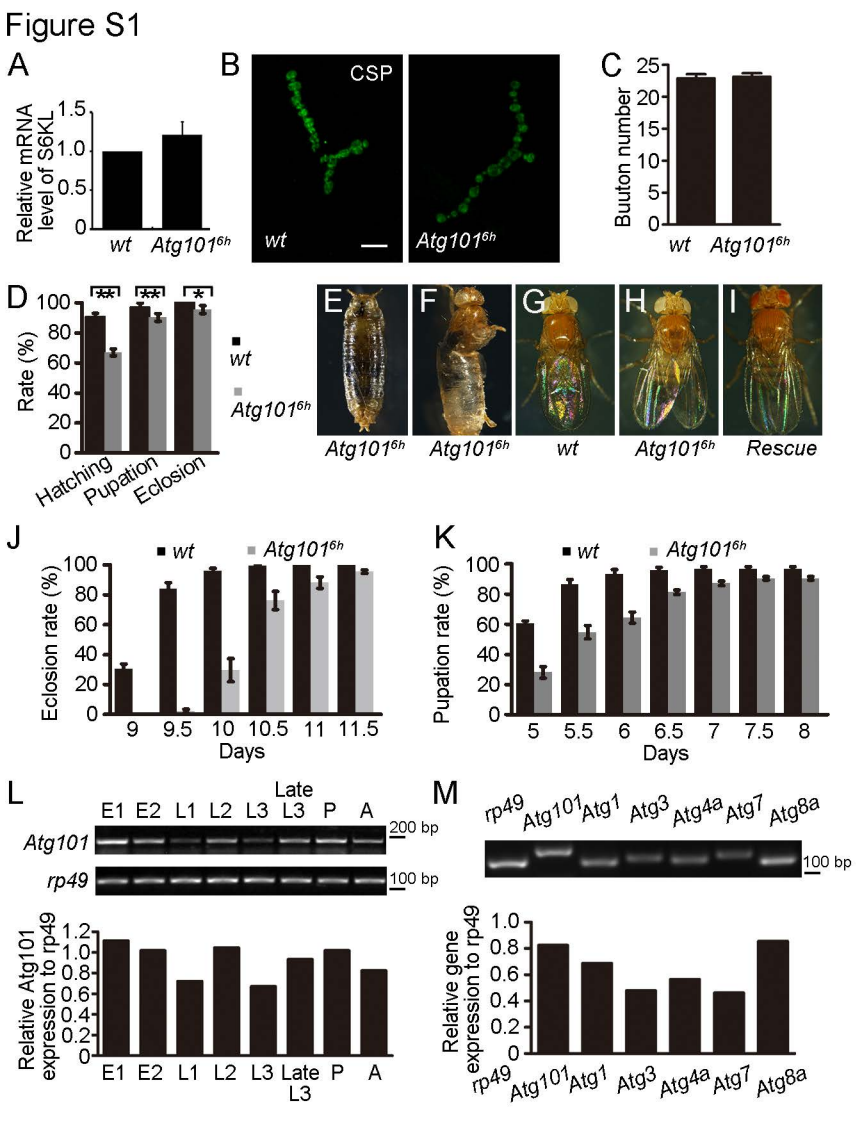
A
Relative mRNA level of S6KL
1.5
1.0
0.5
0
wt
Atg101^6h
B
CSP
wt
Atg101^6h
C
Buuton number
25
20
15
10
5
0
wt
Atg101^6h
D
Rate (%)
100
80
60
40
20
0
**
**
*
wt
Atg101^6h
Hatching
Pupation
Eclosion
E
F
G
H
I
Atg101^6h
Atg101^6h
wt
Atg101^6h
Rescue
J
wt
Atg101^6h
Eclosion rate (%)
100
80
60
40
20
0
9
9.5
10
10.5
11
11.5
Days
K
wt
Atg101^6h
Pupation rate (%)
100
80
60
40
20
0
5
5.5
6
6.5
7
7.5
8
Days
L
Late
E1 E2 L1 L2 L3 L3 P A
Atg101
200 bp
rp49
100 bp
Relative Atg101 expression to rp49
1.2
1.0
0.8
0.6
0.4
0.2
0
E1 E2 L1 L2 L3 Late L3 P A
M
rp49 Atg101 Atg1 Atg3 Atg4a Atg7 Atg8a
100 bp
Relative gene expression to rp49
1.0
0.8
0.6
0.4
0.2
0
rp49 Atg101 Atg1 Atg3 Atg4a Atg7 Atg8a

Figure S2

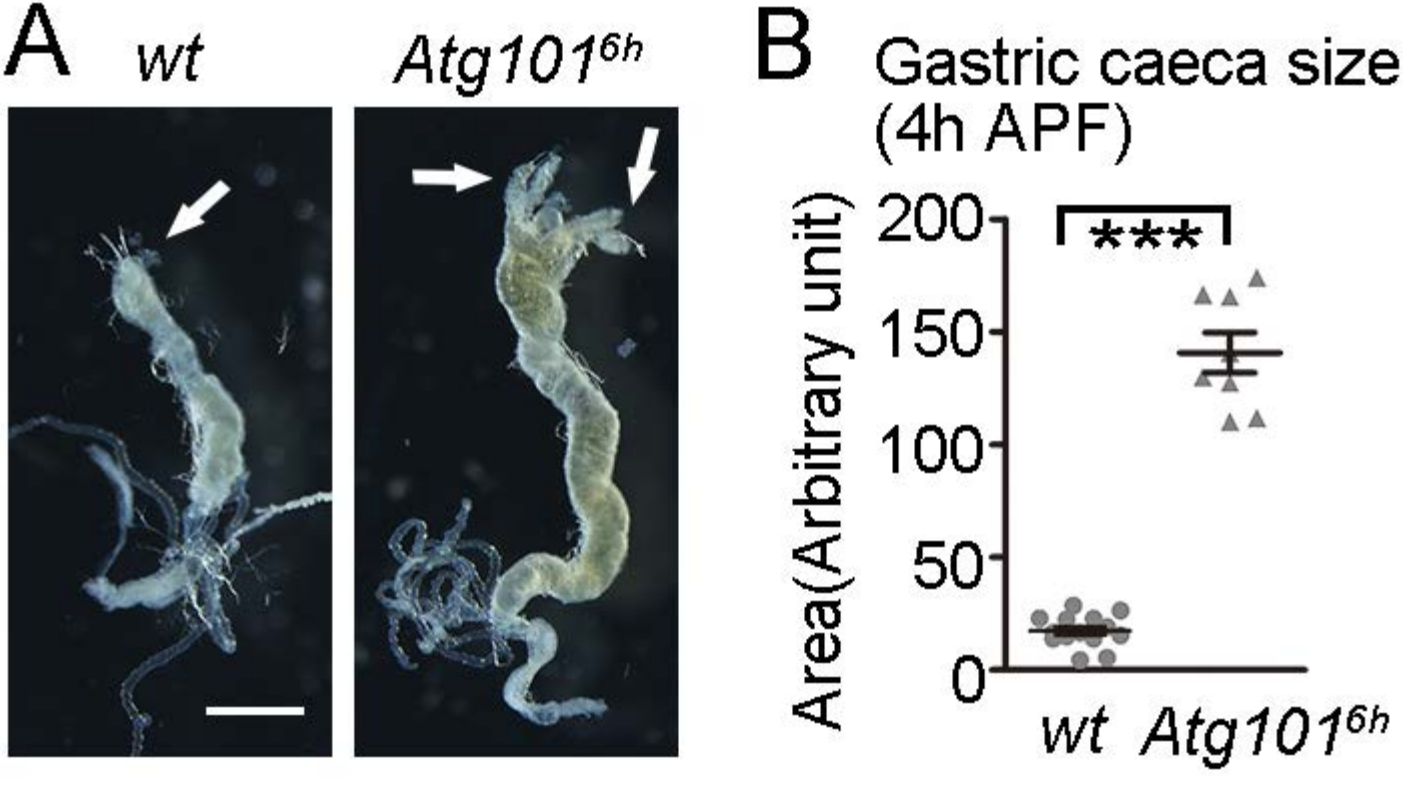
A
wt
Atg101^6h
B
Gastric caeca size
(4h APF)
Area(Arbitrary unit)
200
150
100
50
0
***
wt
Atg101^6h

Figure S3

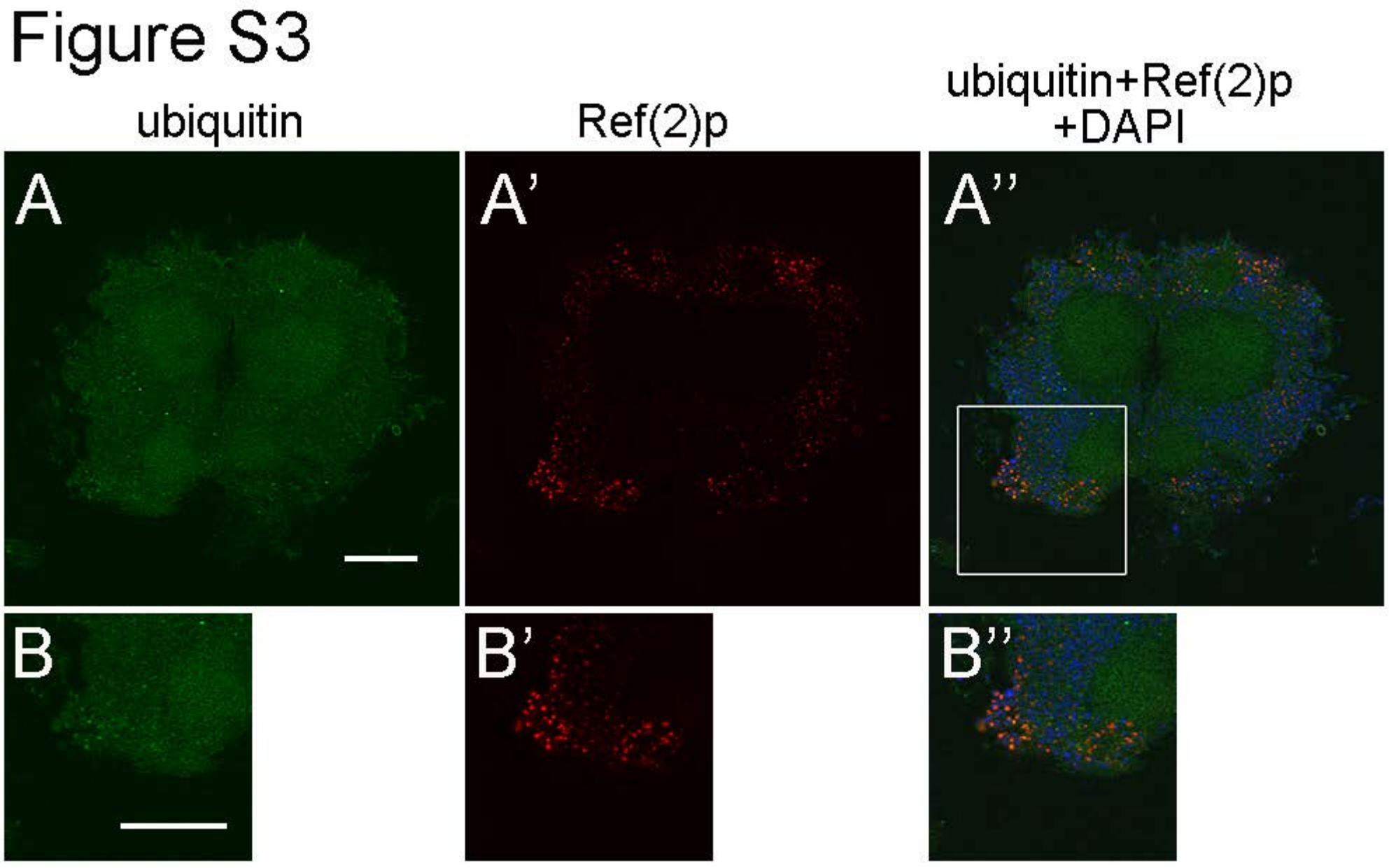

Figure S4

Table S1

Cross	Female		Male	
FM6/+ × +/Y	*+/+*	*FM6/+*	*+/Y*	*FM6/Y*
	96 **(54% of all females)**	83	70 **(74% of all males)**	24
Atg101 ^{6h} /FM6 × FM6/Y	*Atg101 ^{6h} /FM6*	*FM6/FM6*	*Atg101 ^{6h} /Y*	*FM6/Y*
	147	17	13 **(36% of all males)**	23
Atg101 ^{6h} /FM6 × Atg101 ^{6h} /Y	*Atg101 ^{6h} /Atg101 ^{6h}*	*Atg101 ^{6h} /FM6*	*Atg101 ^{6h} /Y*	*FM6/Y*
	31 **(21% of all females)**	118	34	46
Atg101 ^{6h} /FM6 ;UAS-Atg101/UAS-Atg101 × Da-GAL4/Da-GAL4	*Atg101 ^{6h} /+ ;UAS-Atg101/+;Da-GAL4/+*	*FM6/+ ;UAS-Atg101/+;Da-GAL4/+*	*Atg101 ^{6h} /Y ;UAS-Atg101/+;Da-GAL4/+*	*FM6/Y ;UAS-Atg101/+;Da-GAL4/+*
	122	104	113 **(80% of all males)**	28