Supporting Information

Ma, Fig. S1

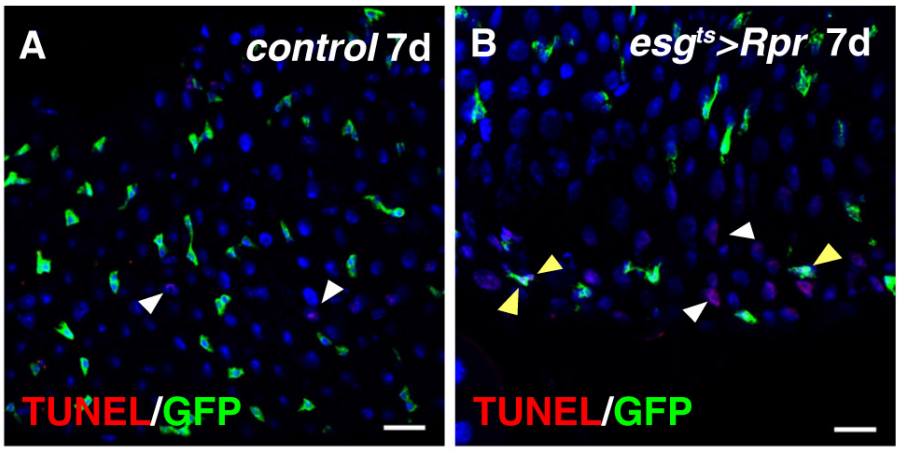

Fig. S1. Overexpression of Rpr causes progenitor cell death.

(A) TUNEL assay of control intestines. No progenitor cell death could be observed, except that some ECs are undergoing cell death (red, white arrowheads). (B) TUNEL assay of *esg*^{ts}>*Rpr* intestines. Some progenitors are undergoing cell death (red, yellow arrowheads). Dying ECs (red, white arrowheads) can also be observed. Blue indicates DAPI staining for DNA. Scale bars: 20 μm.

Ma, Fig. S2

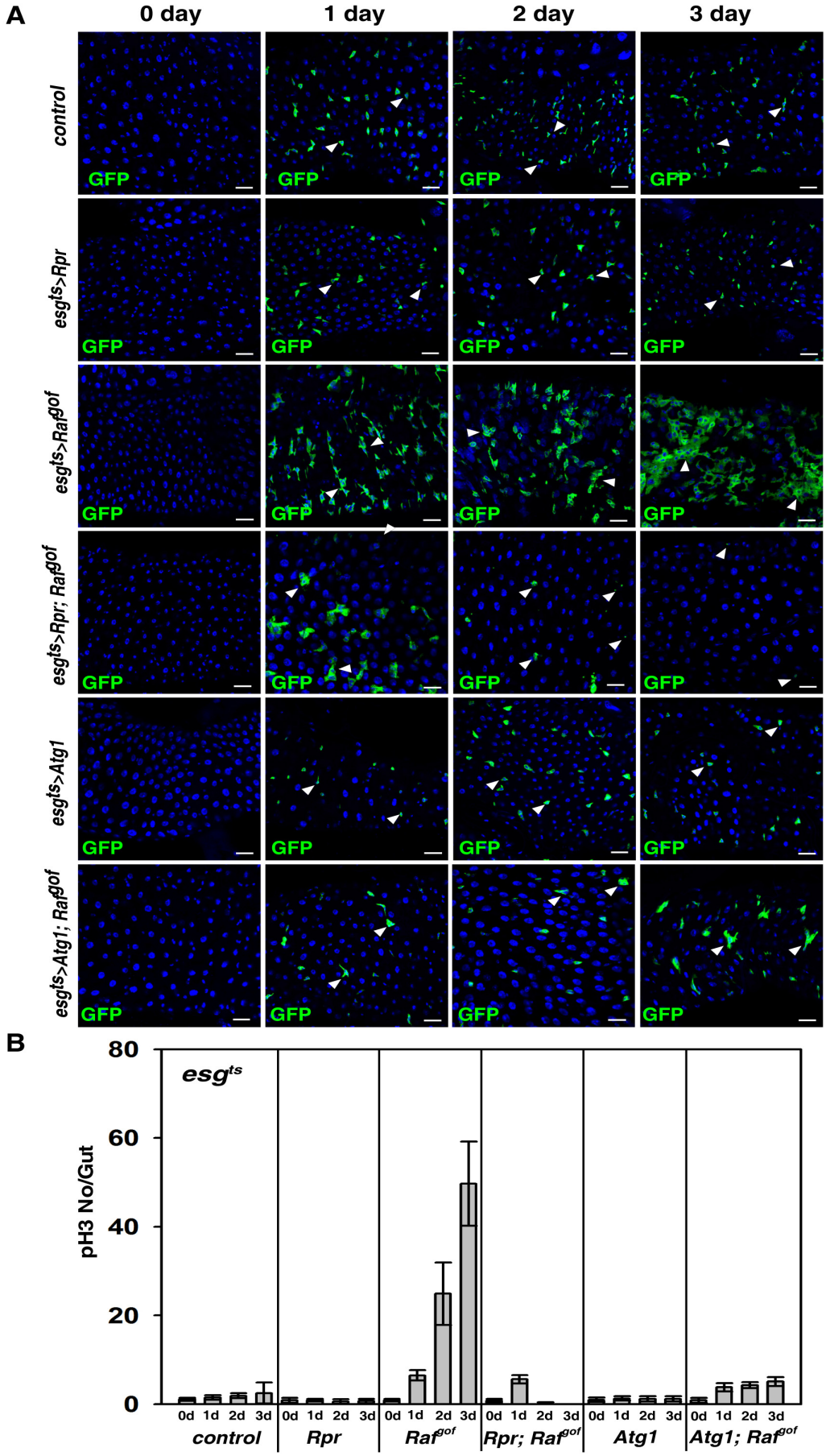
A
0 day
1 day
2 day
3 day
control
esgts>Rpr
esgts>Raf gof
esgts>Rpr; Raf gof
esgts>Atg1
esgts>Atg1; Raf gof
GFP
B
esgts
pH3 No/Gut
80
60
40
20
0
0d 1d 2d 3d
control
Rpr
Raf gof
Rpr; Raf gof
Atg1
Atg1; Raf gof

Fig. S2. Overexpression of Rpr ablates all Raf^{gof} tumor cells, while Atg1 induction inhibits Raf^{gof} tumor formation.

(A) A time course analysis in the intestines of different genotypes (from 0 to 3 days). 0 day: flies hatched from 18 ˚C incubator were dissected and fixed, thereby no GFP expression was observed. A transient increase of ISC number was detected in *esg^{ts}>Rpr; Raf^{gof}* intestines at day 1 (white arrowheads). Most of the *esg*+ cells expressing *Rpr* and *Raf^{gof}* at the 2nd day were morphologically abnormal and some cell debris were observed (white arrowheads), only some cell debris were observed at the 3rd day (white arrowheads). Raf^{gof} tumors were significantly suppressed by Atg1 co-induction (white arrowheads). GFP in green, blue indicates DAPI staining for DNA. Scale bars: 20 μm. (B) Quantification of pH3 staining per gut in the intestines of different genotypes at different time points. *n* = 10-15 intestines. Mean ± SD is shown.

Ma, Fig. S3

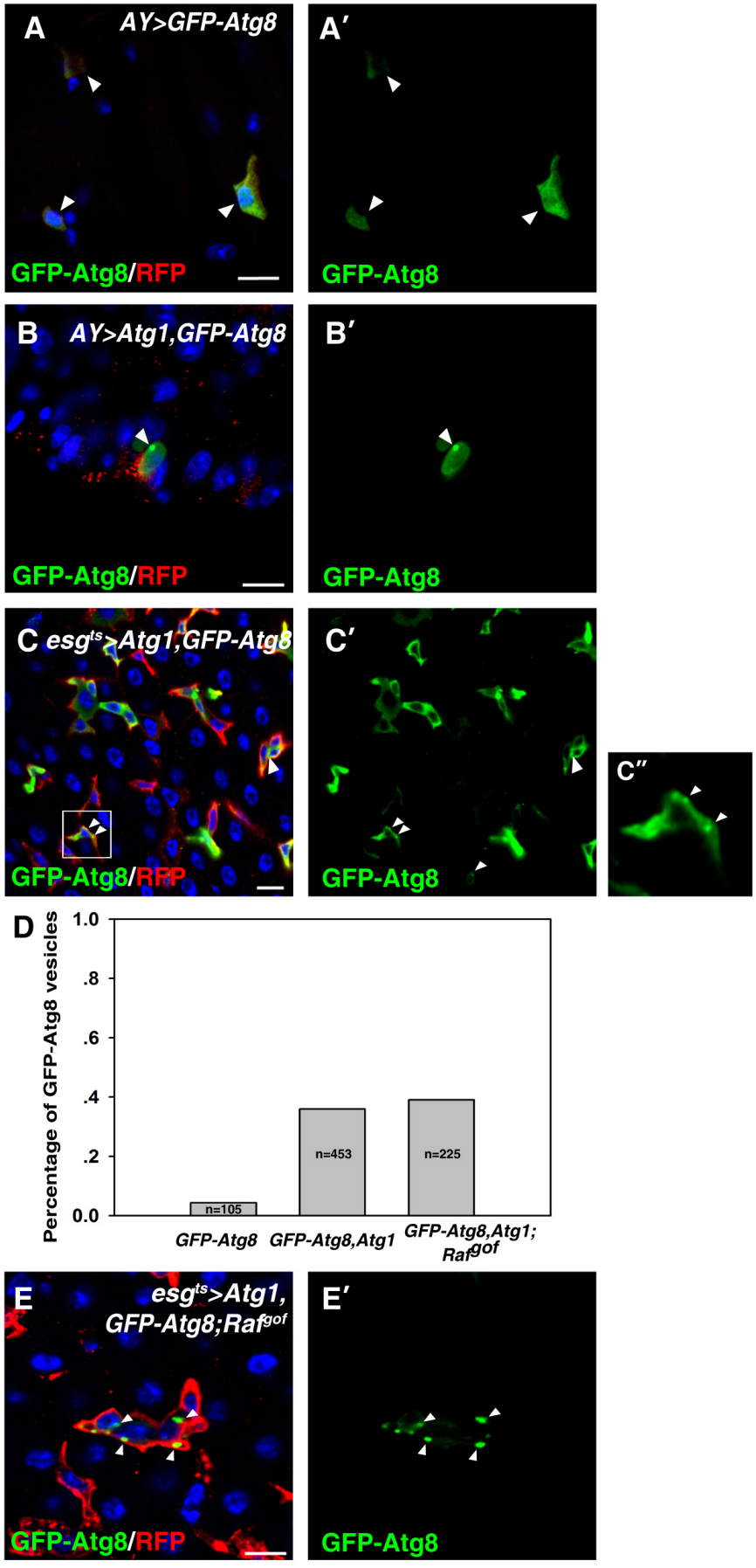
A
AY>GFP-Atg8
GFP-Atg8/RFP
A′
GFP-Atg8
B
AY>Atg1,GFP-Atg8
GFP-Atg8/RFP
B′
GFP-Atg8
C esgts>Atg1,GFP-Atg8
GFP-Atg8/RFP
C′
GFP-Atg8
C″
D
Percentage of GFP-Atg8 vesicles
1.0
.8
.6
.4
.2
0.0
n=105
n=453
n=225
GFP-Atg8
GFP-Atg8,Atg1
GFP-Atg8,Atg1; Rafgof
E
esgts>Atg1, GFP-Atg8;Rafgof
GFP-Atg8/RFP
E′
GFP-Atg8

Fig. S3. Overexpression of Atg1 induces autophagosome formation.

(A, A') Autophagosomes are rarely observed in *ActGal4* FLP-out clones (*AY*, red) expressing *GFP-Atg8* alone (white arrowheads). (B, B') Autophagosomes (green, GFP-Atg8/LC3) are readily observed in *AY* clones (red) expressing both *Atg1* and *GFP-Atg8* (white arrowhead). (C-C'') Autophagosomes (green, GFP-Atg8/LC3) are readily observed in progenitors (red) expressing both *Atg1* and *GFP-Atg8* using esg^{ts} (white arrowheads). (D) Quantification of the percentage of cells containing autophagosomes (GFP-Atg8 vesicles) in GFP-positive cells. For an unknown reason, when Atg1 and GFP-Atg8 were co-expressed, some of the RFP positive cells did not contain the GFP-Atg8 signal. (E, E') Large autophagosomes (green, GFP-Atg8/LC3) are observed in progenitors expressing *Atg1*, *GFP-Atg8,* and Raf^{gof} using esg^{ts} (white arrowheads). Blue indicates DAPI staining for DNA. Scale bars: 10 μm.

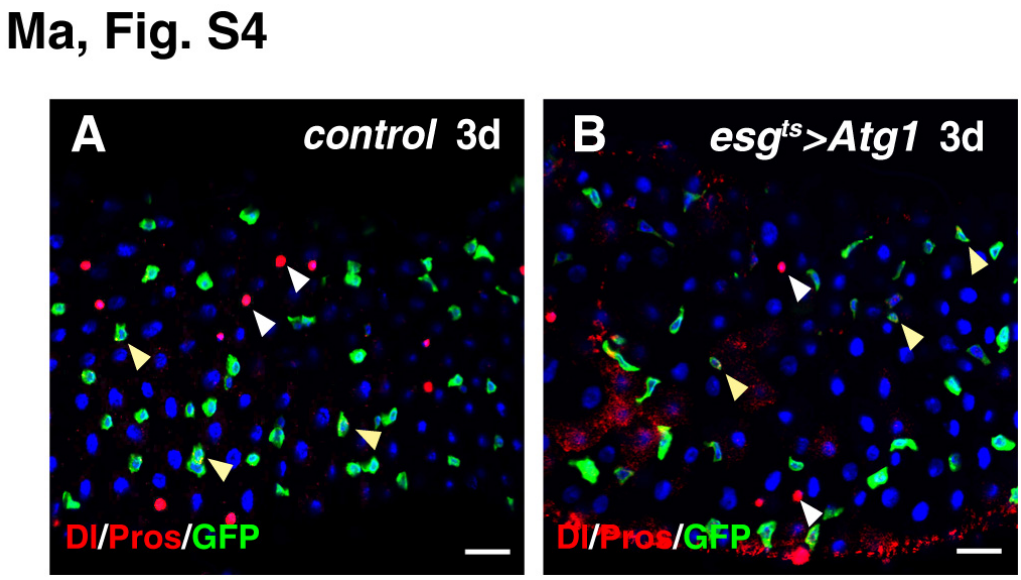

Fig. S4. Progenitor identity and progeny differentiation are not affected by the expression of Atg1 at 3 days.

(A) Progenitors (Dl in red, yellow arrowheads) and ee cells (Pros in red, white arrowheads) in control intestines. (B) Progenitor identity (Dl in red, yellow arrowheads) and ee cell differentiation (Pros in red, white arrowheads) in *esg*^{ts}>*Atg1* intestines. Blue indicates DAPI staining for DNA. Scale bars: 20 μm.

Ma, Fig. S5

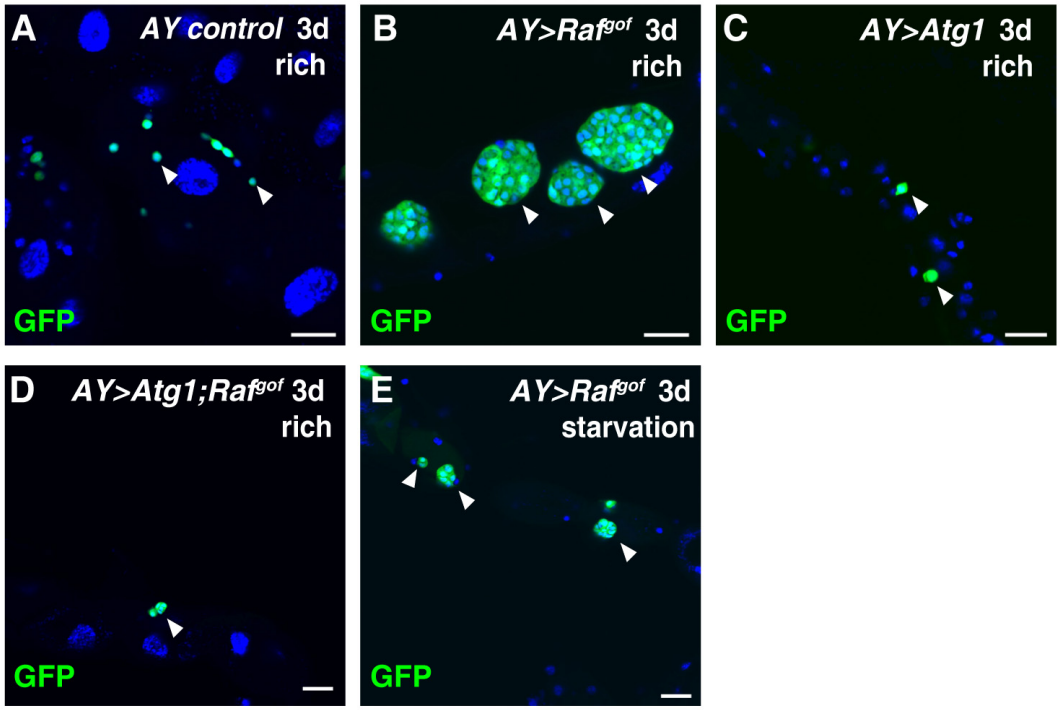

Fig. S5. Renal tumors induced by *Raf*^{gof} expression are inhibited by autophagy induction.

(A) Control *AY* renal clones grown in well-fed flies (rich medium) at 29 ºC for 3 days (white arrowheads). (B) Renal tumors are formed in well-fed animals after *AY* induction of *Raf*^{gof} at 29 ºC for 3 days (white arrowheads). (C) *AY* renal clones grown in well-fed flies expressing *Atg1* at 29 ºC for 3 days (white arrowheads). (D) Renal *Raf*^{gof} tumors are significantly inhibited by co-expressing *Atg1* (white arrowhead). (E)

Renal *Raf*^{gof} tumors are significantly inhibited under starvation (white arrowheads). Blue indicates DAPI staining for DNA. Scale bars: 20 μm.

Ma, Fig. S6

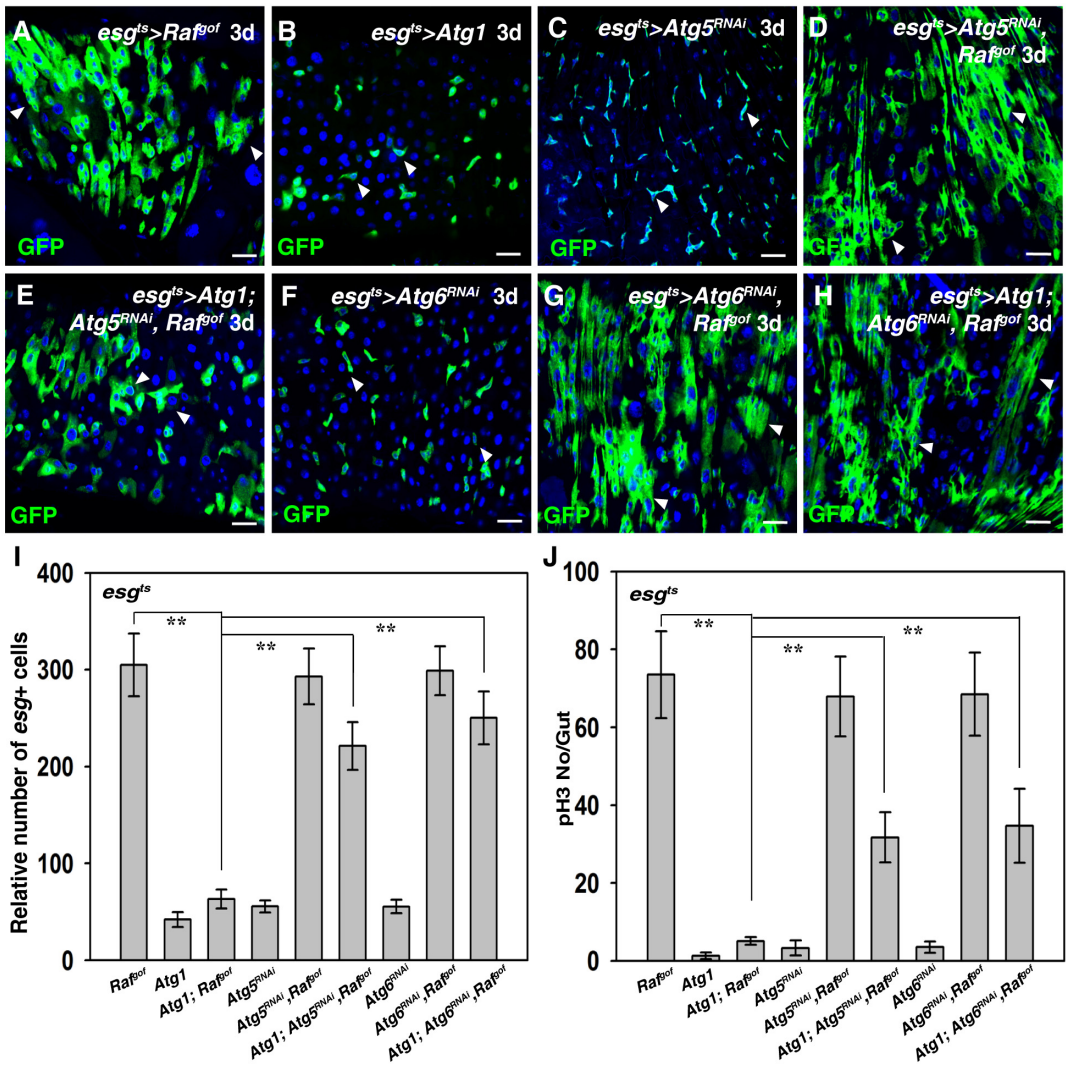

Fig. S6. Autophagy is required for the inhibition of *Raf*^{gof} tumors.

(A) Intestinal tumors are formed in *esg*^{ts}*>Raf*^{gof} intestines at 29°C for 3 days (white arrowheads). (B) No obvious defects are observed in *esg*^{ts}*>Atg1* intestines (white arrowheads). (C) Intestines with knockdown of *Atg5* (progenitors in green, white arrowheads). (D) Intestinal tumors are formed in *esg*^{ts}*>Atg5*^{RNAi}, *Raf*^{gof} intestines at 29°C for 3 days (white arrowheads). (E) *Atg5* RNAi significantly rescues the

inhibition of tumor formation observed in $esg^{ts}>Atg1,\ Raf^{gof}$ intestines (white arrowheads). (F) Intestines with knockdown of *Atg6* (progenitors in green, white arrowheads). (G) Intestinal tumors are formed in $esg^{ts}>Atg6^{RNAi},\ Raf^{gof}$ intestines at 29°C for 3 days (white arrowheads). (H) *Atg6* knockdown significantly rescues the inhibition of tumor formation observed in $esg^{ts}>Atg1,\ Raf^{gof}$ intestines (white arrowheads). (I) Quantification of the relative number of *esg*+ cells in the intestines of different genotypes. Note that because $esg^{ts}>Raf^{gof}$ intestines are highly deformed due to the formation of tumors, it is very difficult to accurately count the number of *esg*+ cells in these intestines. n = 10-15 intestines. Mean ± SD is shown. $^{**}p<0.001$. (J) Quantification of pH3 staining per gut in the intestines of different genotypes. n = 10-15 intestines. Mean ± SD is shown. $^{**}p<0.001$. Blue indicates DAPI staining for DNA. Scale bars: 20 μm.

Ma, Fig. S7

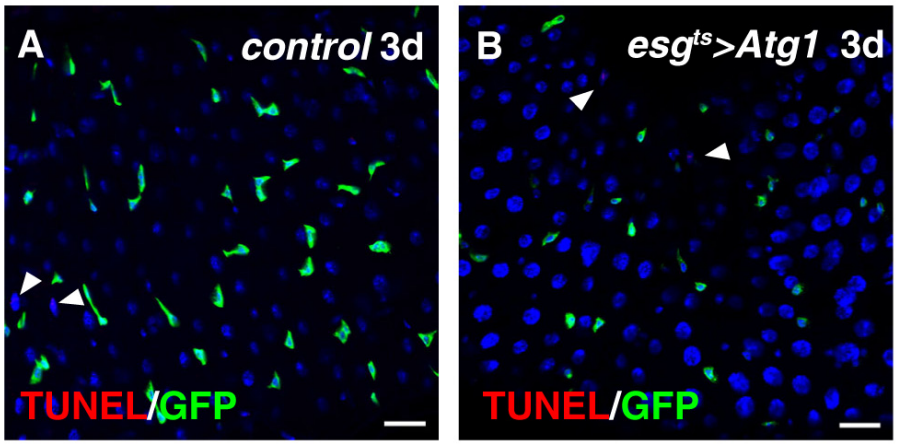

Fig. S7. Overexpression of Atg1 does not cause progenitor cell death.

(A) TUNEL assay of control intestines. No progenitor cell death could be observed, except that some ECs are undergoing cell death (red, white arrowheads). (B) TUNEL

assay of *esg*^{ts}*>Atg1* intestines. No progenitor cell death could be observed. Dying ECs (red, white arrowheads) can be observed. Blue indicates DAPI staining for DNA. Scale bars: 20 μm.

Ma, Fig. S8

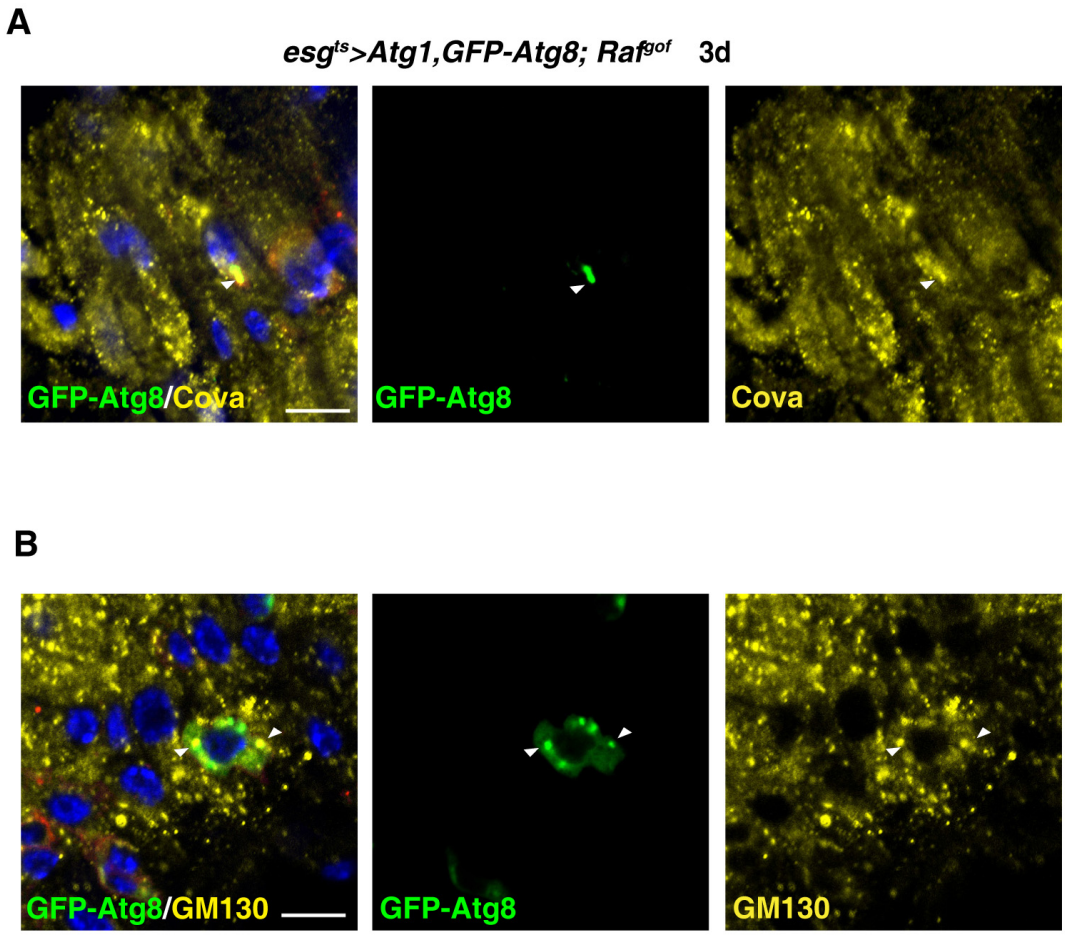

Fig. S8. Mitochondria and golgi apparatus are engulfed in autophagosomes in *esg*^{ts}*>Atg1, GFP-Atg8; Raf*^{gof}*/UAS-mRFP* cells.

(A) Mitochondria (Cova, yellow) are present within the autophagosomes (green, labeled with GFP-Atg8) (white arrowhead). (B) Golgi apparatus (GM130, yellow) are observed in the autophagosomes (green, labeled with GFP-Atg8) (white arrowheads).

For an unknown reason, when Atg1 and GFP-Atg8 were co-expressed, some of the RFP positive cells did not contain the GFP-Atg8 signal. mRFP in red. Blue indicates DAPI staining for DNA. Scale bars: 10 μm.