

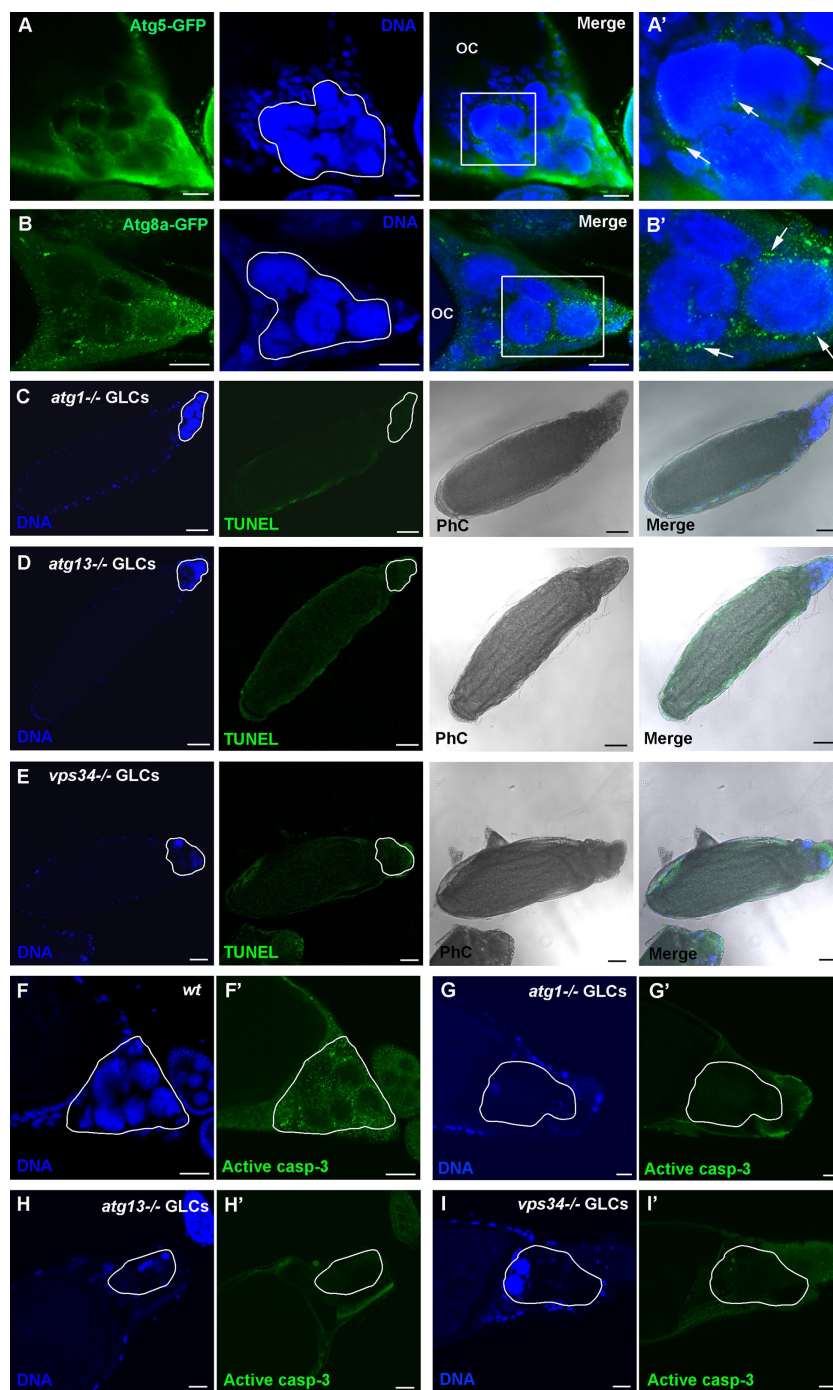
Nezis et al., <http://www.jcb.org/cgi/content/full/jcb.201002035/DC1>

Figure S1. **Genetic inhibition of autophagy in the germline prevents DNA fragmentation and results in reduced expression of cleaved caspase-3.** (A and B) Atg5-GFP and Atg8a-GFP expression pattern during late oogenesis in *D. melanogaster*. Confocal micrographs of the anterior pole of early stage 12 and early stage 13 egg chambers showing the nurse cell cluster. The autophagic markers Atg5-GFP (A) and Atg8a-GFP (B) exhibit a punctuate staining pattern in the nurse cells (arrows). A' and B' are high magnification images of the boxed areas shown in A and B, respectively. (C–I) Genetic inhibition of autophagy in the germline prevents DNA fragmentation and results in reduced expression of cleaved caspase-3 (casp-3). (C–E) Confocal micrographs of stage 12/13 autophagy germline mutant egg chambers stained with TUNEL. Outlined nurse cells are TUNEL negative. (F–I) Confocal micrographs of stage 12/13 autophagy germline mutant egg chambers stained for cleaved caspase-3. These egg chambers exhibit significantly reduced staining for cleaved caspase-3. Nurse cells are outlined with a white line. Draq5/Hoechst staining (blue) was performed to visualize the nuclei. PhC, phase contrast. Bars: (A, B, and F–I) 20  $\mu$ m; (C–E) 50  $\mu$ m.

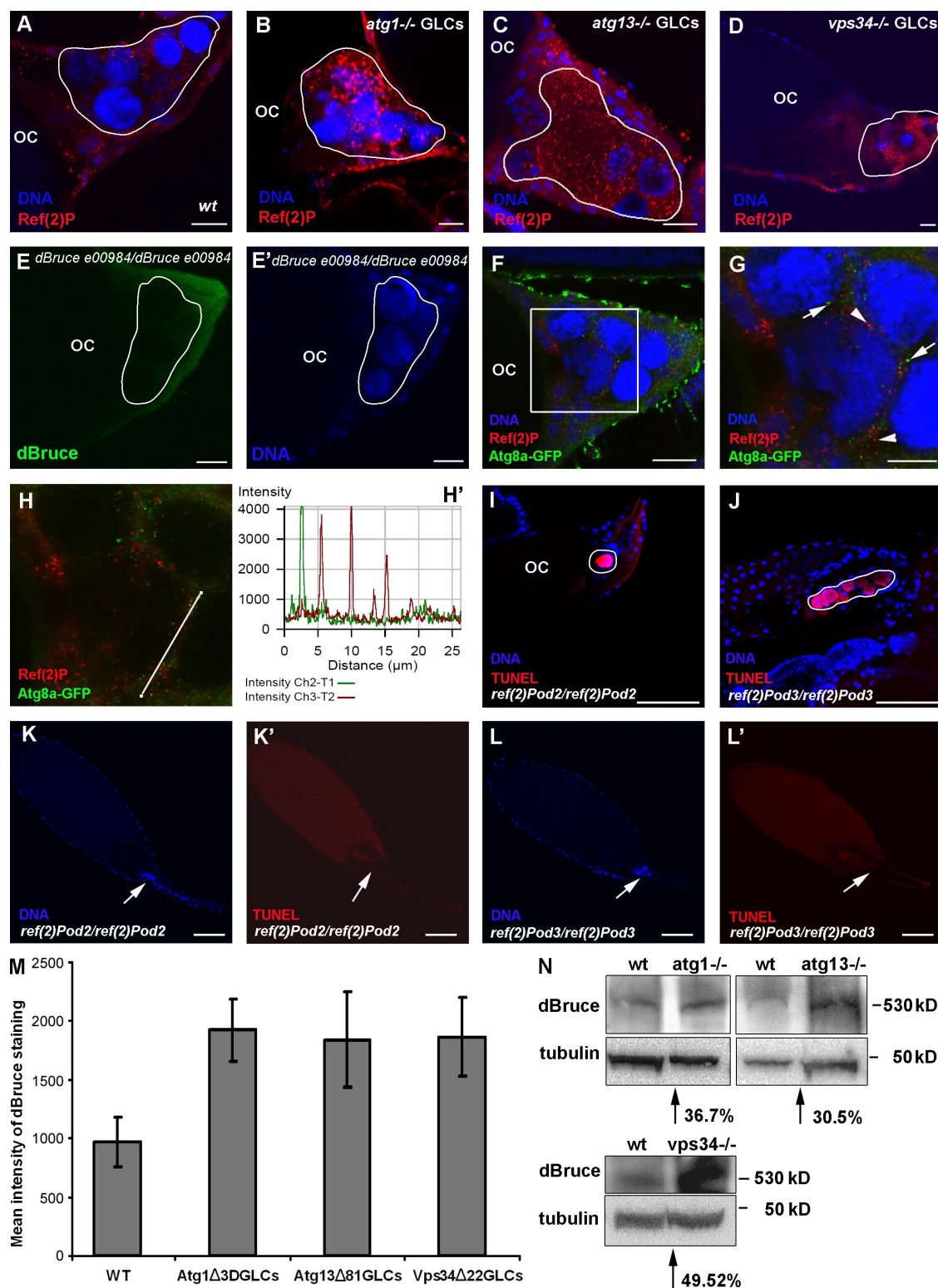


Figure S2. **Genetic inhibition of autophagy in the germline causes accumulation of Ref(2)P and dBruce in the nurse cells.** (A–D) Genetic inhibition of autophagy in the germline causes accumulation of Ref(2)P. (A) Ref(2)P staining in a wild-type (wt) stage 13 egg chamber. (B–D) Ref(2)P accumulates in stage 14 *atg1*<sup>-/-</sup> (B), *atg13*<sup>-/-</sup> (C), and *vps34*<sup>-/-</sup> (D) germline mutant egg chambers compared with wild type. (E and E') Confirmation of specificity of anti-dBruce antibody. The piggyBac insertion in *dBruce*<sup>e00984</sup> mutants results in premature termination of transcription and is predicted to produce a truncated protein of 1346 aa lacking the critical UBC domain (Sathyanarayanan et al., 2008). The antibody against dBruce was raised against the 446 aa at the C terminus (Arama et al., 2007). Consistent with the aforementioned information, *dBruce*<sup>e00984</sup> homozygous mutant egg chambers show no staining for dBruce. (F–H) Ref(2)P does not colocalize with the autophagic marker Atg8a-GFP in late stage egg chambers. G is a high magnification of the boxed area shown in F. Arrows point to Atg8a-GFP puncta, and arrowheads point to Ref(2)P-positive puncta. (H and H') Colocalization analysis of G. The white line in H is the linear area where the intensity plot presented in H' was calculated. (I–L) Ref(2)P mutant late stage egg chambers exhibit a normal pattern of TUNEL staining (I and J) and nurse cell elimination (K and L; arrows). DraQ5/Hoechst staining (blue) was performed to visualize the nuclei. Nurse cells are out-

Table S1. Quantification of nurse cell death during late oogenesis

Genotype	Normal	Persisting nurse cell nuclei	Number of persisting nurse cell nuclei			Dumpleless	n
			1–5	5–10	10–15		
	%	%	%	%	%	%	
<i>w<sup>1118</sup></i>	94.5 ± 3.4	5.4 ± 2.2 <sup>a</sup>	100	0	0	0	312
<i>atg1<sup>Δ3D</sup></i> GLCs	37.7 ± 6	62.2 ± 5.9	61 ± 5.7	37.1 ± 5.3	1.8 ± 1.4	15 ± 1.9	702
<i>atg13<sup>Δ81</sup></i> GLCs	39.9 ± 4.6	60 ± 4.6	70.8 ± 6.5	28.1 ± 6.1	0.98 ± 1.1	17 ± 3.2	508
<i>vps34<sup>Δm22</sup></i> GLCs	41.1 ± 3.3	58.6 ± 3	70.4 ± 4.6	29.1 ± 5.2	0.4 ± 0.8	20 ± 1.4	411
<i>dBruce<sup>E81</sup>/dBruce<sup>E81</sup></i>	85.2 ± 3.9	14.8 ± 3.1 <sup>a</sup>	100	0	0	0.95 ± 1	125
<i>vps34<sup>Δm22</sup></i> GLCs; <i>dBruce<sup>E81</sup>/dBruce<sup>E81</sup></i>	26 ± 3.2	74 ± 2.4 <sup>a</sup>	60 ± 5.4	35 ± 1.8	5 ± 2.1	22 ± 3.9	130
<i>atg1<sup>Δ3D</sup></i> GLCs, <i>dBruce<sup>E81</sup>/dBruce<sup>E81</sup></i>	42 ± 3.9	58 ± 5.4 <sup>a</sup>	56 ± 6.7	39 ± 3.8	5 ± 4.4	19 ± 4.1	82

Cell death was examined using the TUNEL assay to detect DNA fragmentation in the nurse cells of stage 14 egg chambers of the indicated genotypes. Numbers indicate TUNEL-negative nurse cell nuclei except from wild-type (*w<sup>1118</sup>*) and *dBruce<sup>E81</sup>* single mutant and double mutant (*vps34<sup>Δm22</sup>*GLCs; *dBruce<sup>E81</sup>/dBruce<sup>E81</sup>* and *atg1<sup>Δ3D</sup>*GLCs, *dBruce<sup>E81</sup>/dBruce<sup>E81</sup>*) egg chambers, where numbers indicate TUNEL-positive nurse cell nuclei. The total number of late stage 14 egg chambers per ovary in *dBruce<sup>E81</sup>/dBruce<sup>E81</sup>* flies is 42.5% reduced compared with the total number of late stage 14 egg chambers per ovary in control flies (*dBruce<sup>E81</sup>/+* or *w<sup>1118</sup>*). This is consistent with the increased number of degenerating egg chambers during mid-oogenesis in *dBruce* single mutants compared with control flies. Data are presented as mean ± SD. Number of persisting nurse cell nuclei and Dumpleless are a further breakdown of the Persisting nurse cell nuclei category. Dumpleless refers to egg chambers where nurse cell cytoplasm failed to be transferred to the oocyte. *n* indicates the number of egg chambers. 1–5, 5–10, and 10–15 refer to the number of persisting nurse cell nuclei.

<sup>a</sup>TUNEL-positive nurse cell nuclei.

Table S2. Quantification of nurse cell death during mid-oogenesis

Genotype	Normal	Degenerating	n
	%	%	
<i>dBruce<sup>E81</sup>/dBruce<sup>E81</sup></i>	46.8 ± 2.9	53.2 ± 3.2	250
<i>vps34<sup>Δm22</sup></i> GLCs; <i>dBruce<sup>E81</sup>/dBruce<sup>E81</sup></i>	45.1 ± 2.2	54.9 ± 1.4	111
<i>atg1<sup>Δ3D</sup></i> GLCs, <i>dBruce<sup>E81</sup>/dBruce<sup>E81</sup></i>	49 ± 3.5	51 ± 2.1	74

Cell death was examined using the TUNEL assay to detect DNA fragmentation in the nurse cells of stage 7–9 egg chambers of the indicated genotypes. Numbers indicate TUNEL-positive nurse cell nuclei. Data are presented as mean ± SD.

## References

- Arama, E., M. Bader, G.E. Rieckhof, and H. Steller. 2007. A ubiquitin ligase complex regulates caspase activation during sperm differentiation in *Drosophila*. *PLoS Biol.* 5:e251. doi:10.1371/journal.pbio.0050251
- Sathyanarayanan, S., X. Zheng, S. Kumar, C.H. Chen, D. Chen, B. Hay, and A. Sehgal. 2008. Identification of novel genes involved in light-dependent CRY degradation through a genome-wide RNAi screen. *Genes Dev.* 22:1522–1533. doi:10.1101/gad.1652308

lined with a white line. OC, oocyte. (M) Quantification of mean intensity of *dBruce* staining observed in the nurse cells of stage 12–14 egg chambers in wild-type and germline autophagy mutant egg chambers. Wild type (WT): three independent experiments, *n* = 30 egg chambers; *atg1<sup>-/-</sup>* GLCs: three independent experiments, *n* = 30 egg chambers; *atg13<sup>-/-</sup>* GLCs: three independent experiments, *n* = 30 egg chambers; *vps34<sup>-/-</sup>* GLCs: three independent experiments, *n* = 30 egg chambers. Data are presented as mean ± SD. Difference was significant with *P* < 0.001 for all values versus wild type. (N) Western blot analysis of cell lysates from wild-type and autophagy germline mutant egg chambers probed with anti-*dBruce* antibody, demonstrating a significant increase in *dBruce* protein levels in autophagy mutants. Bars: (A–F and I–L) 50 μm; (G and H) 20 μm.