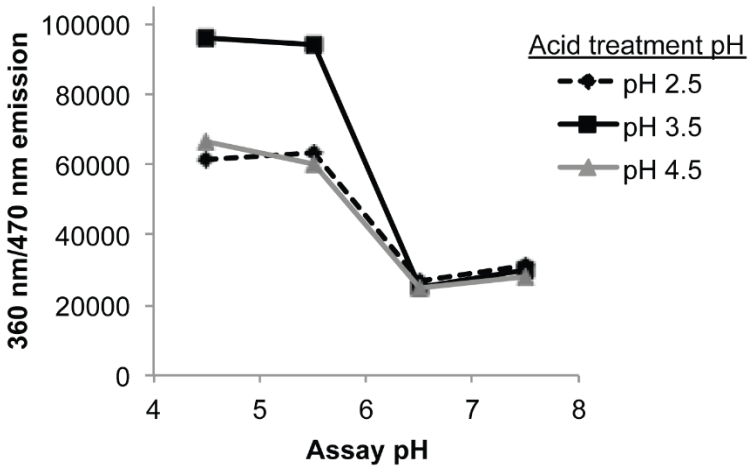


Fig. S1. Cathepsin B-like proteinase enzyme assay optimization. Embryo extracts from 0-2.5 HPF embryos were treated at pH 2.5-4.5 then assayed with a Cathepsin B-like proteinase reporter from pH 4.5-7.5. Cathepsin B-like proteinase is activated at a pH of 3.5 and has highest activity at a pH of 4.5-5.5. ~50-75 autophagosomes were measured for each replicate embryo.

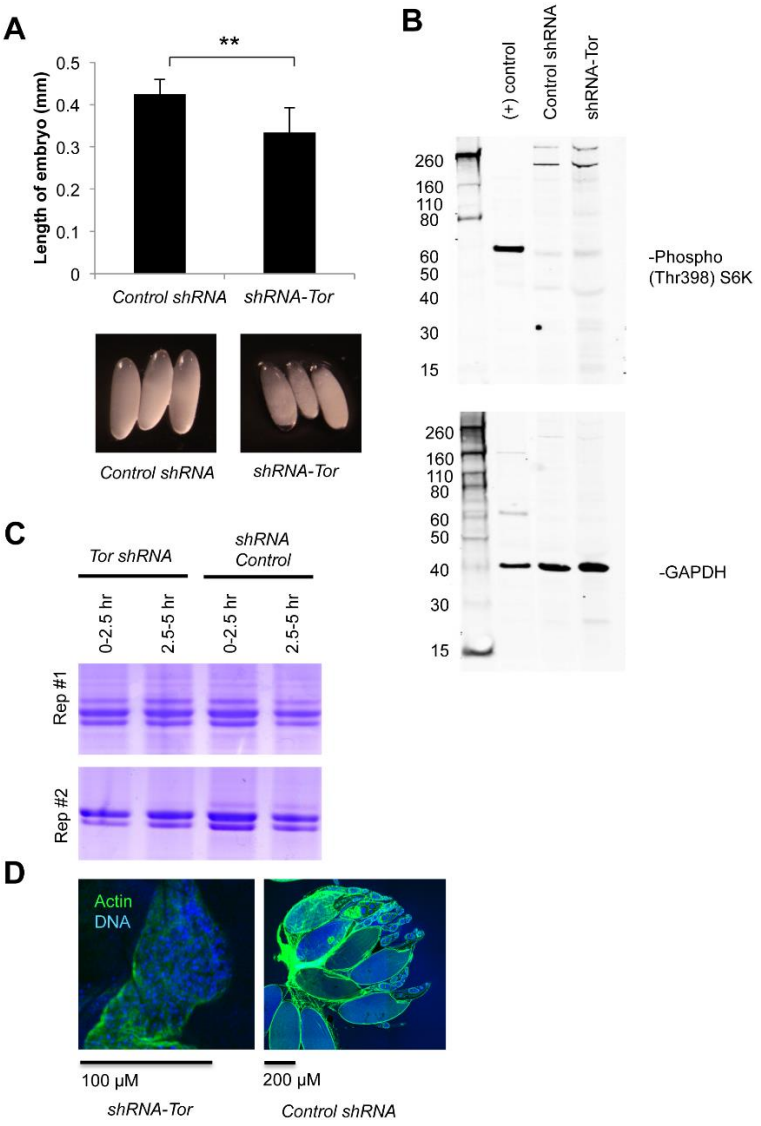


Fig. S2. Characterization of *shRNA-Tor* embryos. A. Length measurements of control shRNA and *shRNA-Tor* embryos. B. Western blot for phospho (Thr398) p70 s6K in control shRNA embryos and *shRNA-Tor* embryos. A decrease in phospho (Thr398) p70 s6K is used to detect decreases in Tor activity, however no phospho (Thr398) p70 s6K was detected in control-shRNA embryos. Lysate from *Drosophila* KC167 cells was used as a positive control. C. Coomassie staining of *shRNA-Tor* compared to control shRNA embryos between 0-2.5 and 2.5-5 HPF. 10 embryos were loaded per lane. Two replicates are shown. D. Oocyte development in control embryos compared to embryos expressing shRNA against *Tor* transcripts expressed in the germline using the MTD-Gal4 driver. No germarium forms when *shRNA-Tor* is expressed, only the terminal filament remains.

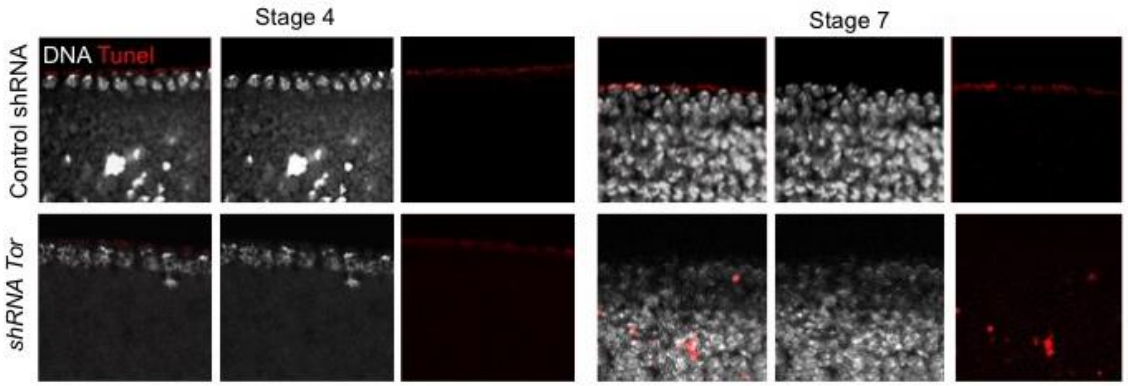


Fig. S3. TUNEL staining of control shRNA and *shRNA-Tor* embryos at stage 4 (pre-cellularization) and stage 7 (post-cellularization). *shRNA-Tor* embryos showed positive TUNEL staining post-cellularization.

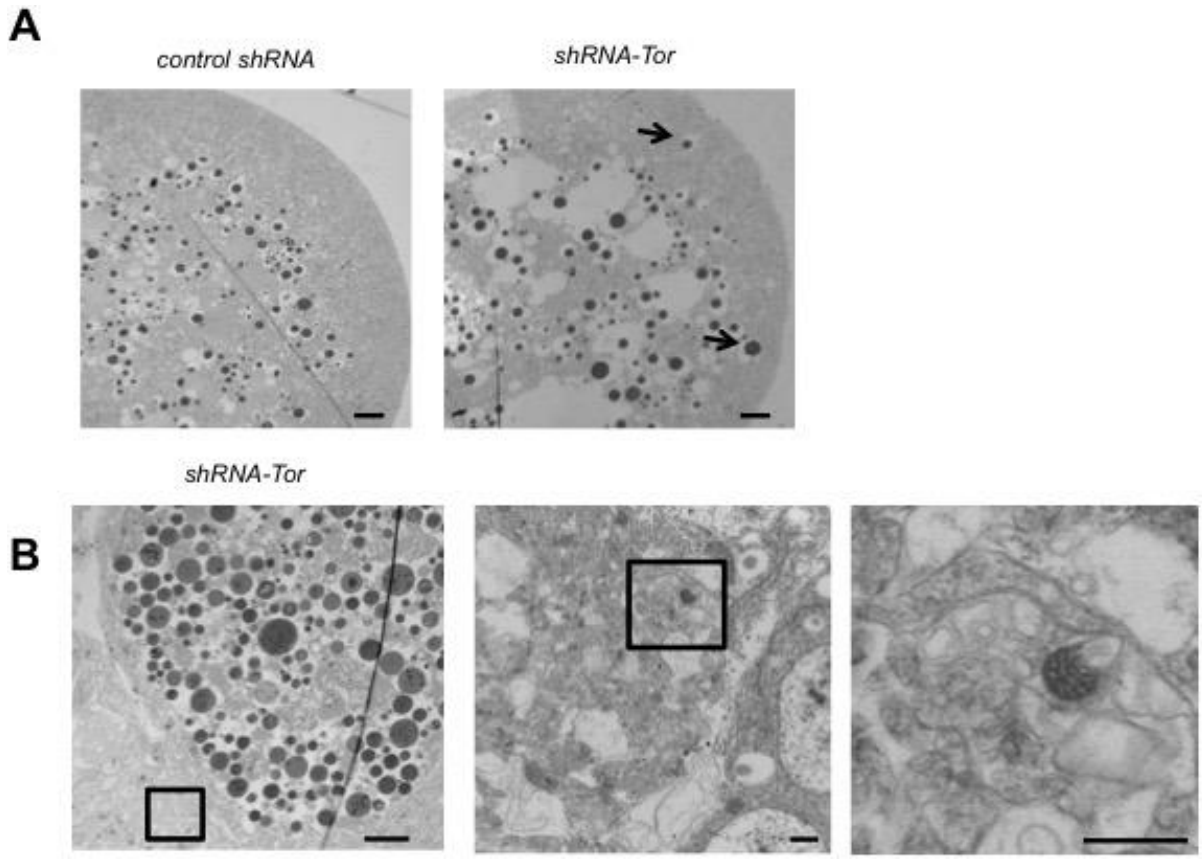


Fig. S4. EM of *shRNA-Tor* embryos pre-cellularization and post-cellularization multi-vesicular membranes. A. EM of (stage 4) control and *shRNA-Tor* embryos pre-cellularization. There are some mis-localized yolk platelets near nuclei *in shRNA-Tor* embryos (arrows). Scale bar = 10 μM. B. Multi-vesicular lakes of membrane observed by EM in *shRNA-Tor* embryos. Increasing magnification from left to right. Left scale bar = 10 μM. Middle and right scale bar = 500 nM.

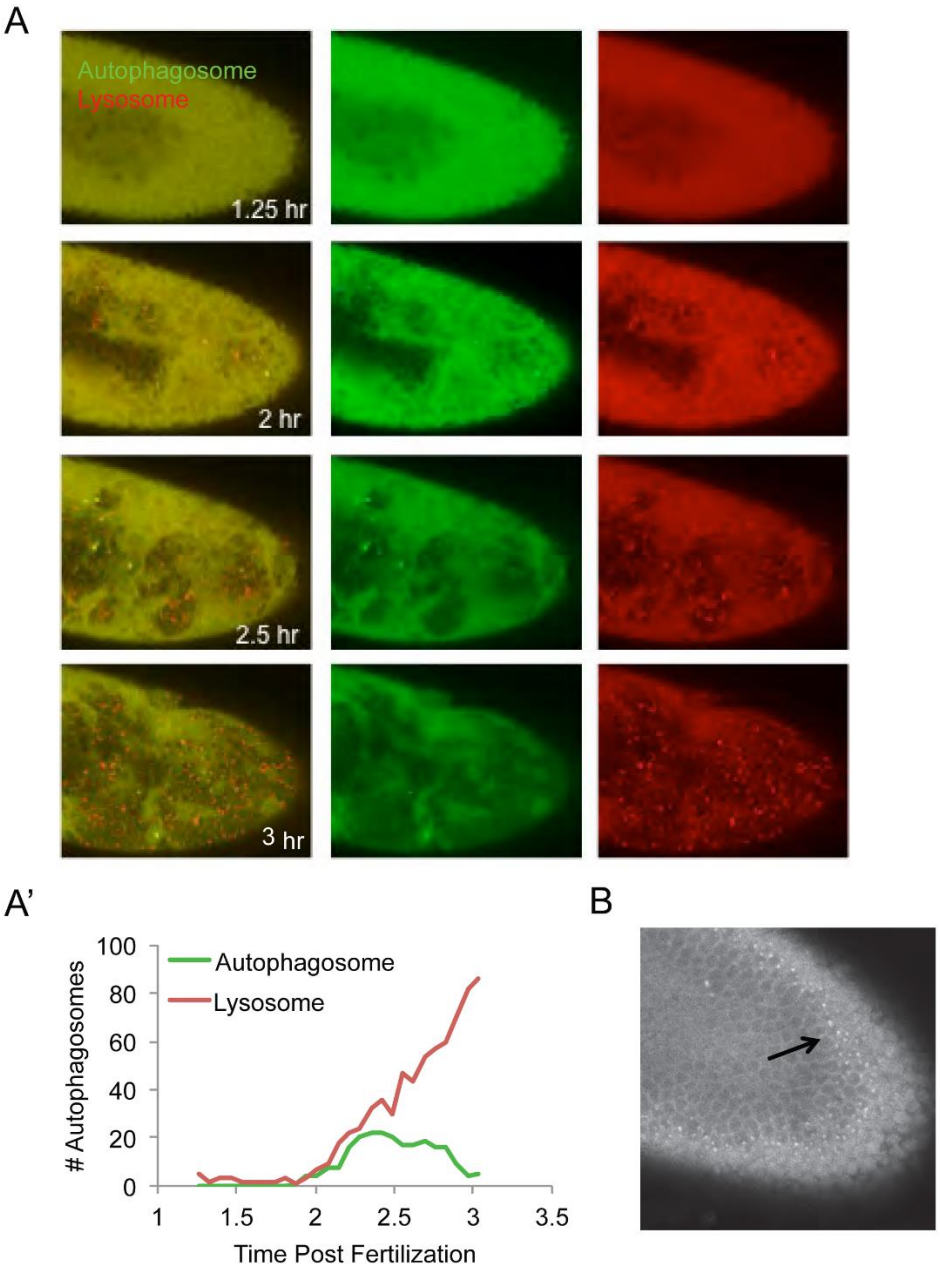


Fig. S5. Autophagosome formation at cellularization visualized using a GFP-Mcherry-Atg8a reporter. A. Autophagosome formation at cellularization visualized using a UAS-GFP-Mcherry-Atg8a reporter. A. Confocal imaging of UAS-driven GFP-Mcherry-Atg8a reporter using a maternal Gal4 driver showing yellow puncta (autophagosomes) followed by red puncta (lyososomes). A'. Quantification of autophagsosome and lysosome puncta in A. B. Live imaging resulted in high amounts of background and some photo-toxicity to the embryos not seen with fixed embryos expressing the reporter. A z-stack of a fixed embryo displays autophagosome puncta around the yolk-nuclei border.

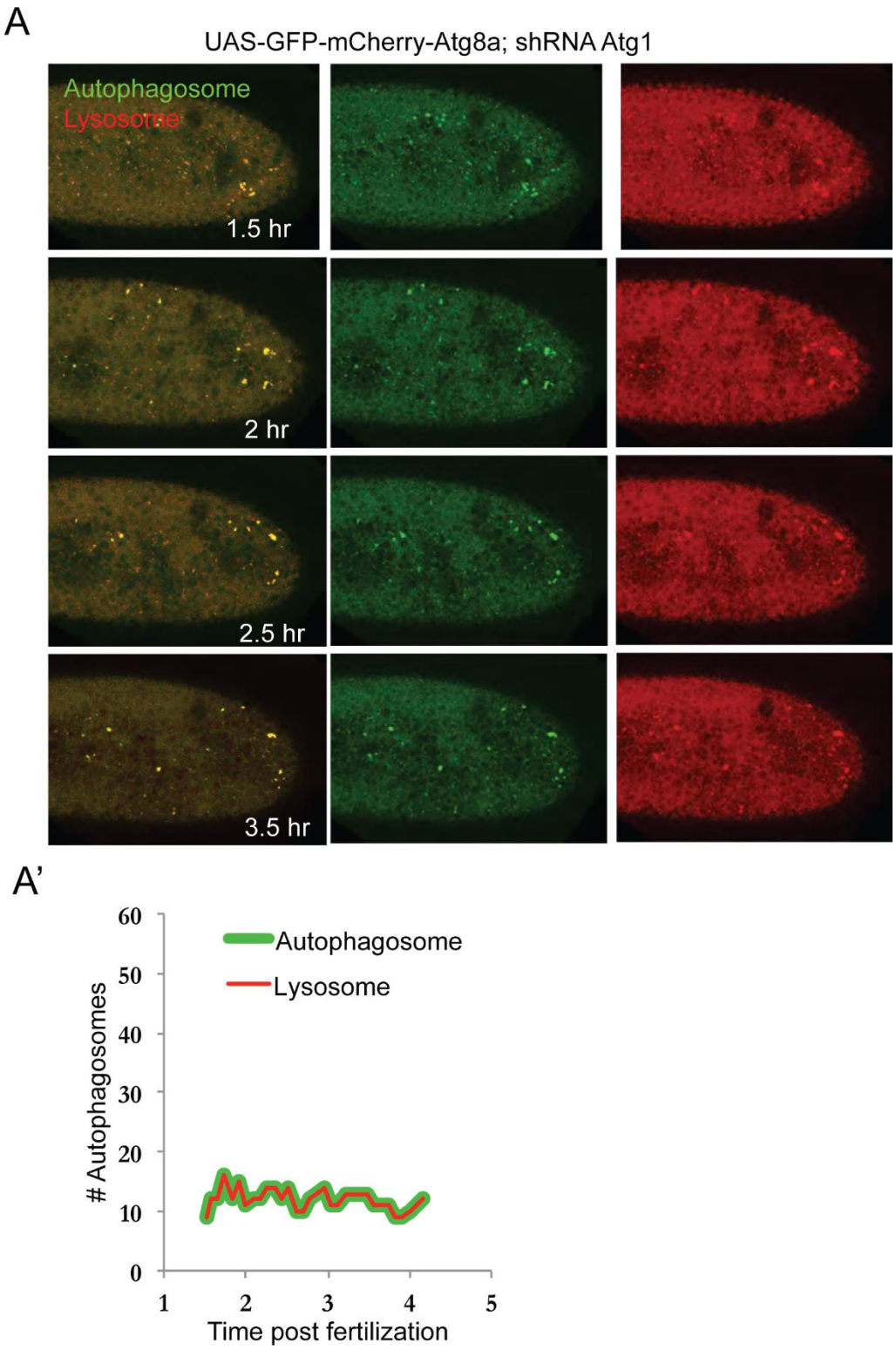


Fig. S6. Autophagosome formation at in *shRNA-Atg1* embryos visualized using a GFP-Mcherry-Atg8a reporter. A. Confocal imaging of UAS-driven GFP-Mcherry-Atg8a reporter using a maternal Gal4 driver. Some yellow puncta and red puncta appear but they do not change over time. A'. Quantification of puncta shown in A.

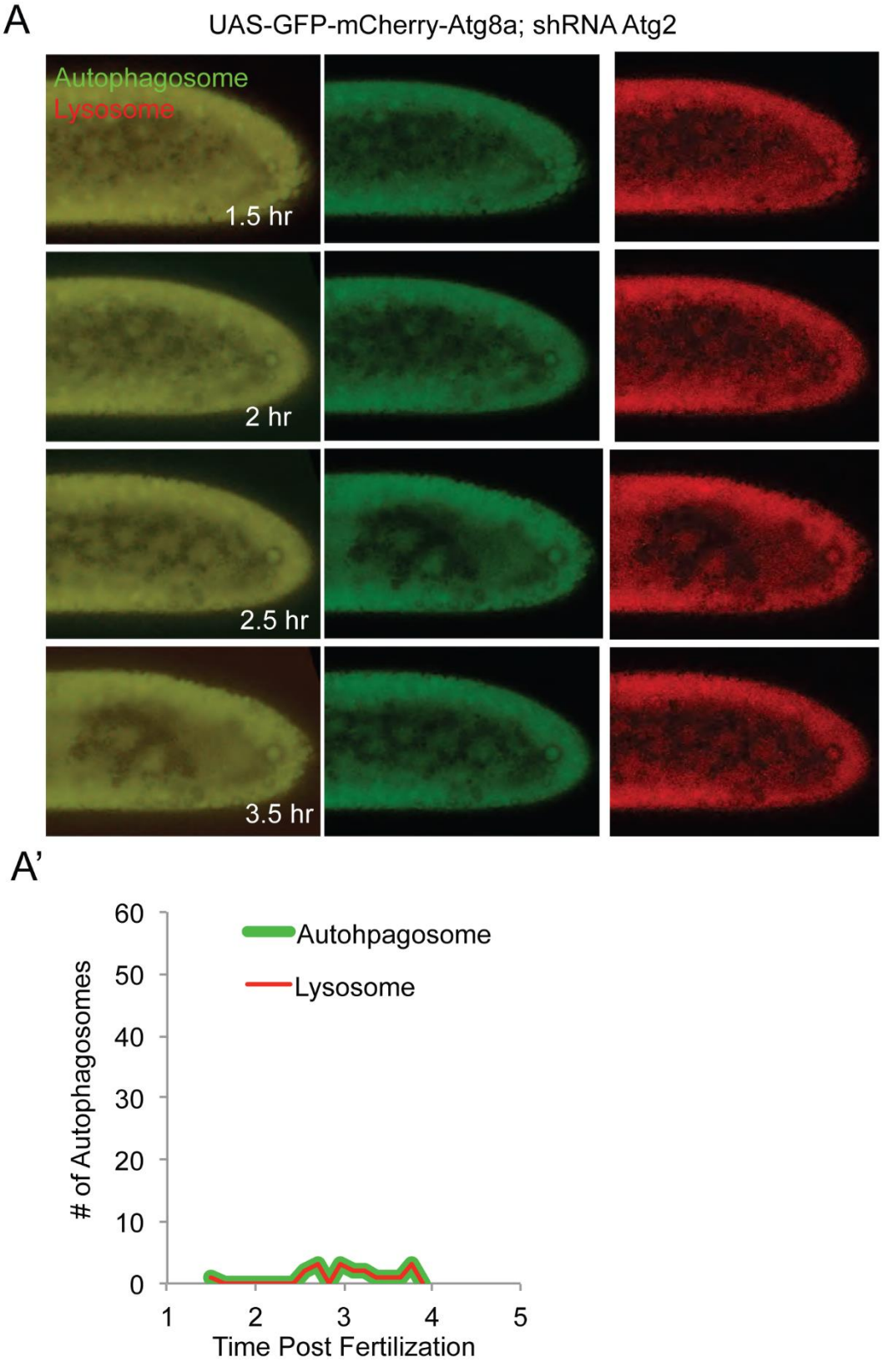


Fig. S7. Autophagosome formation at in *shRNA-Atg2* embryos visualized using a GFP-Mcherry-Atg8a reporter. A. Confocal imaging of UAS-driven GFP-Mcherry-Atg8a reporter using a maternal Gal4 driver. A'. Quantification of puncta shown in A.

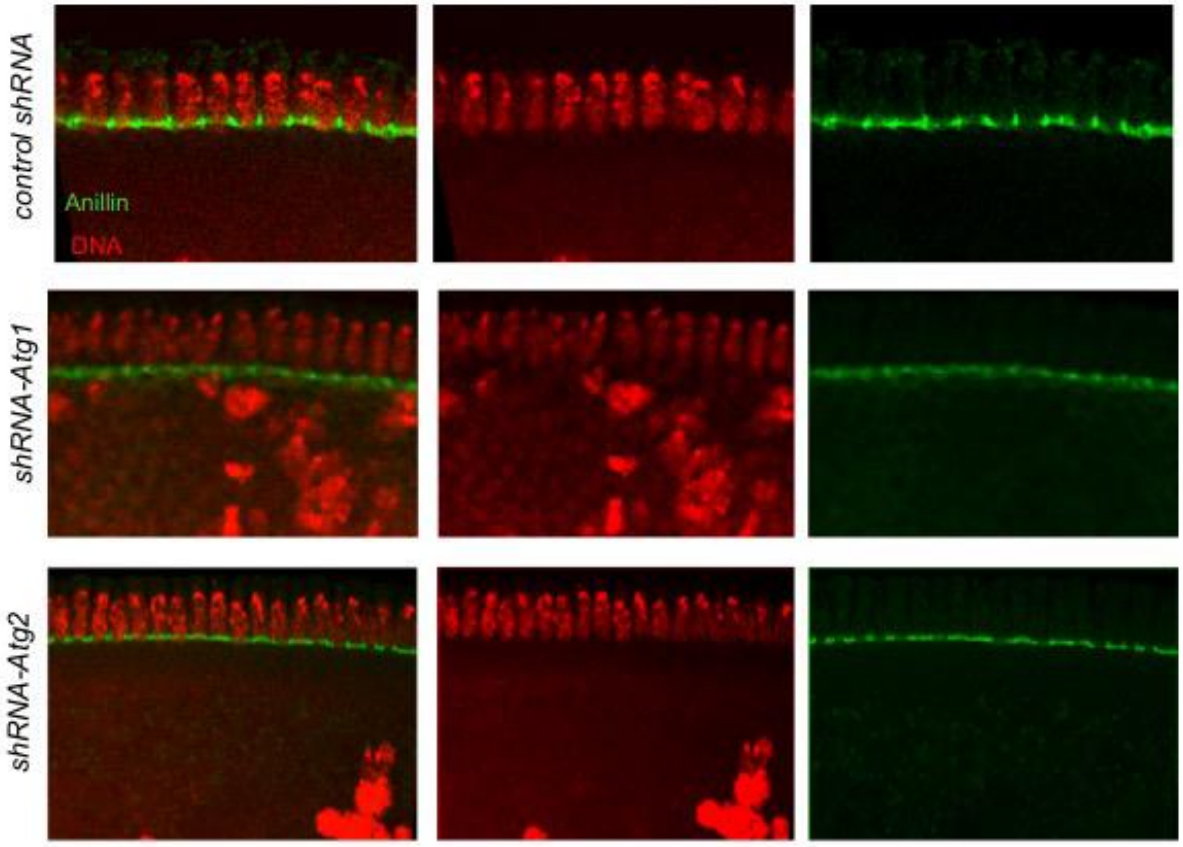


Fig. S8. Cellularization of *shRNA-Atg1* and *shRNA-Atg2* embryos. Immunofluorescence imaging of *Atg1* and *Atg2* depleted embryos during cellularization shows normal membrane ingression as monitored by staining for the contractile ring marker anillin.

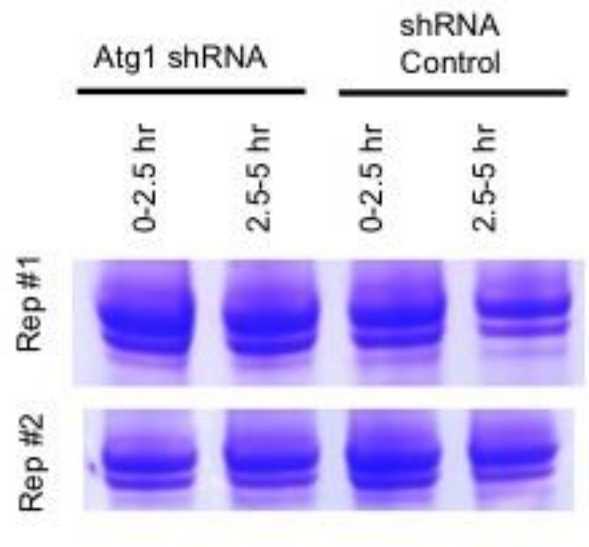


Fig. S9. Coomassie staining of *shRNA-Atg1* compared to control shRNA embryos between 0-2.5 and 2.5-5 HPF. 10 embryos were loaded per lane. Two replicates are shown.

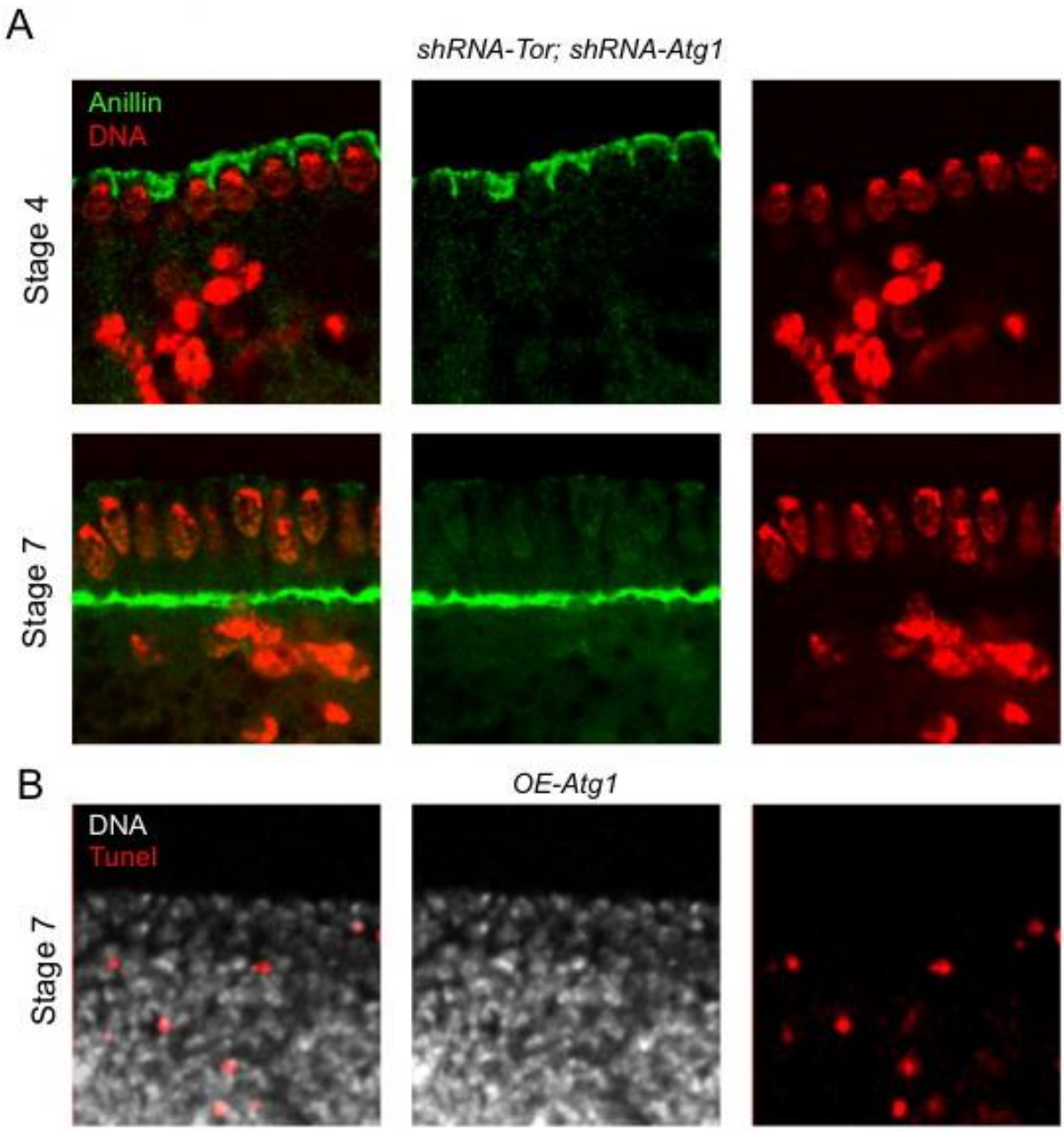
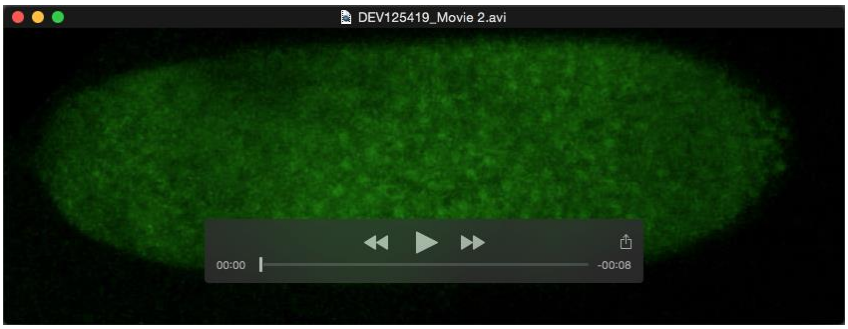


Fig. S10. Characterization of *shRNA-Tor; shRNA-Atg1* embryos and *OE-Atg1* embryos. A. Anillin staining of shRNA-Tor; shRNA-Atg1 embryos shows normal morphology pre- and post-cellularization. B. TUNEL staining of OE-Atg1 embryos is positive for nicked DNA post-cellularization.



Movie 1 and Movie 2

Live imaging of control and *shRNA-Tor* embryos expressing His2Av-EGFP. Time-lapse imaging of (Movie 1) control shRNA and (Movie 2) *shRNA-Tor* embryos expressing His2Av-EGFP during the first 1-3 hours of development. Imaging was carried out using a Nikon A1R point scanning confocal. Embryos were imaged at 27°C using a Tokai-Hit stage-top incubation system.

Table S1. Gal4 drivers and mRNA knockdown efficiencies. shRNAs were generated by the Transgenic RNAi Project and obtained from Bloomington Drosophila Stock Center (BDSC). Gal4 lines include maternal triple-driver MTD-Gal4 (MTD), a dual copy Maternal-tubulin-Gal4 driver (2xMat-Gal4), and a single copy Maternal-tubulin-Gal4 driver (1xMat-Gal4).

***shRNA* target**	**Hairpin ID**	**BDSC Stock #**	**Gal4 driver**	**% mRNA remaining**
Atg1	*GL00047*	35177	2xMat-Gal4	4%
Atg1	*HMS02750*	44034	2xMat-Gal4	7%
Atg2	*HMS01198*	34719	MTD	14%
Atg4a	HMS01482	35740	MTD	8%
Atg5	HMS01244	34899	MTD	29%
Atg10	HMS02026	40859	MTD	10%
Tor	*HMS00904*	33951	1xMat-Gal4	10%
Tor	*GL00156*	35578	1xMat-Gal4	17%
raptor	*HMS00124*	34814	2xMat-Gal4	13%
Rheb	*HMS00923*	33966	2xMat-Gal4	20%
RagA/B	*HMS01064*	34590	2xMat-Gal4	2%
Over-expression target				
OE-Atg1		See Methods	2xMat-Gal4	300%
Double *shRNA* targets				
Tor; Atg1	*HMS00904; HMS02750*	33951; 44034	2xMat-Gal4	Atg1: 6% Tor: 12%
Tor; Atg2	*HMS00904; HMS01198*	33951; 34719	2xMat-Gal4	Few eggs
Tor; shRNA Control	*HMS00904; VALIUM22-EGFP.shRNA.4*	33951; 41551	2xMat-Gal4	Few eggs
shRNA control; Atg1	*VALIUM22-EGFP.shRNA.4 HMS02750;*	41550; 44034	2xMat-Gal4	Atg1: 7%
shRNA control; Atg2	*VALIUM22-EGFP.shRNA.4 HMS01198*	41550; 34719	2xMat-Gal4	Atg2: 20%

Table S2. Primer sequences and efficiencies used for real-time quantitative PCR analysis.

shRNA target	Forward primer	Reverse primer	Primer Efficiency	R^2 Value
Atg1	CGTCAGCCTGGTCATG AGTA	TAACGGTATCCTCG TGAGCG	112.4%	0.996
Atg2	ATGCGCTGATGACC AACGA	CCGACGACCACA TGGACTC	93.7%	0.990
Atg4a	CTGTGGTCAGATGGT TCTCGC	TCAAAACGGTTC ACGATCTTGAG	102.9%	1.0
Atg5	GCCGAACACCAGGA TGGAG	AGCAGATCGTAT AGGACACCAAT	96.8%	0.995
Atg10	TCGGCTGAGTTGGTT AGTGTG	TTCCATCTGATCT GTGTGCCT	86.5%	0.990
Tor	TTGAGGAGCAAAACA GCTG	ATAAGCAGGCGCTC AATCAC	96.2%	0.978
raptor	TGAACGACCTGGGT AAGGTGA	AATGTCGGATATT TGCTCGATGT	95.4%	0.966
Rheb	AGTTCGTGGACTCCT ATGACC	ACGATGTAGTCTTG GACTTTAC	98.2%	0.999
RagA/B	TGGTGCTTAATCTCTG GACT	GGCTCTCCACATCA ACACATAA	99.6%	0.992

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