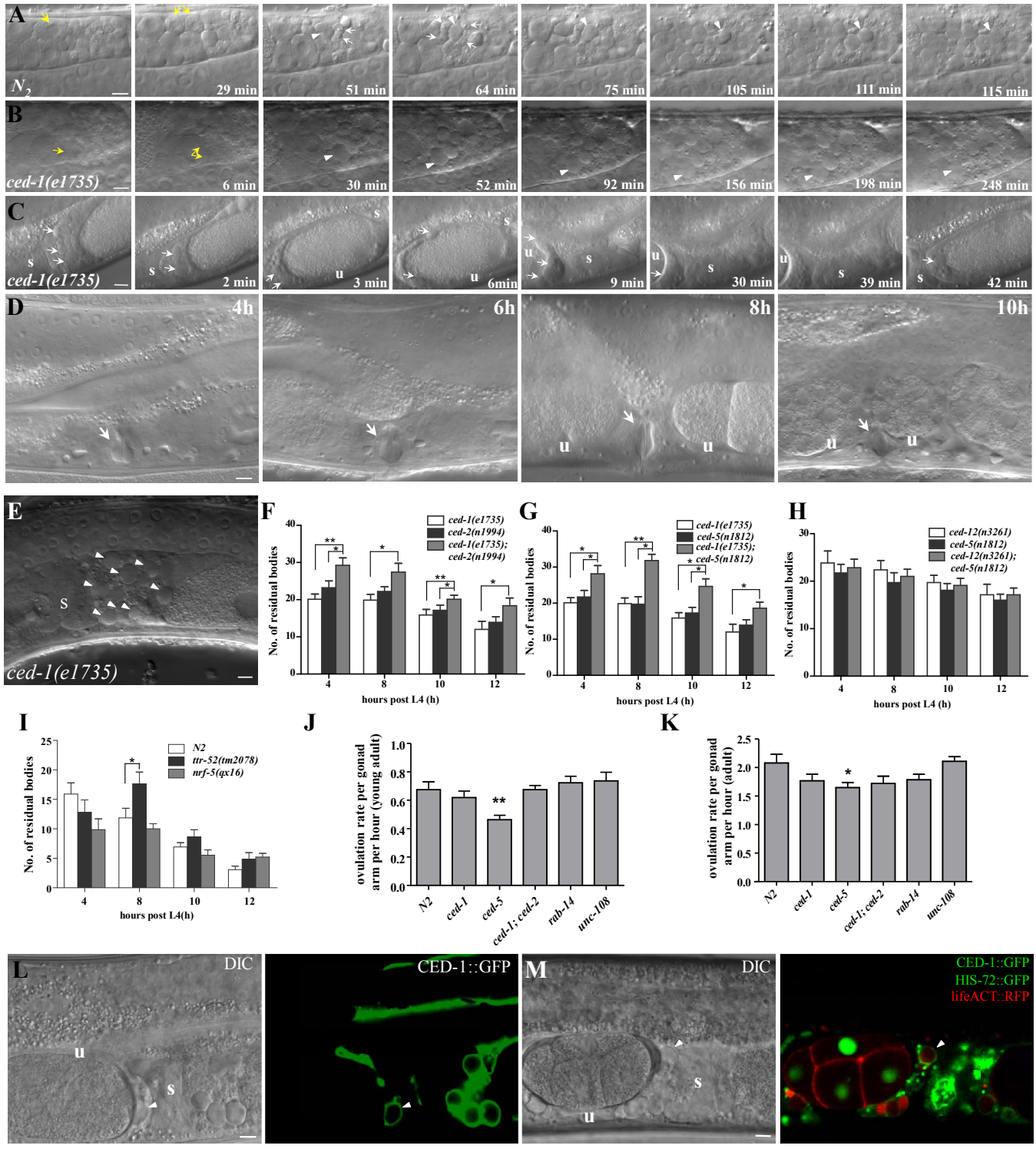


Fig. S1. Accumulation of residual bodies in engulfment mutants. (**A**,**B**) Time-lapse analysis of spermatogenesis in wild type (A) or *ced-1(e1735)* (B). The residual body persisted much longer in *ced-1(e1735)* mutants (218 minutes) than in wild type (64 minutes). Arrowheads indicate residual bodies; yellow arrows point to primary and secondary spermatocytes; white arrows designate spermatids. Residual bodies on the left arm of gonad are shown. (**C**) The residual bodies (arrows) are pushed into the uterus by a fertilized oocyte in a *ced-1(e1735)* hermaphrodite shown by a time-lapse recording. The right arm of the gonad is shown. s, spermatheca; u, uterus, where fertilized embryos are located. (**D**) The development of the vulva (arrows) was monitored to determine the time points (4, 6, 8, 10 hours post L4) at which residual bodies are quantified in different strains. (**E**) DIC view of the proximal gonad of a *ced-1* animal. Residual bodies in the right arm of the gonad (arrowheads) are identified by their button-like morphology under Nomarski optics. (**F-I**) Residual bodies were quantified in hermaphrodites of the indicated strains at different time points. Fifteen animals were examined for each genotype. Data are shown as mean ± s.e.m. $^{**}P<0.0001$, $^{*}P<0.05$. (**J**,**K**) The ovulation rate per gonad arm per hour of the indicated strains at young adult (2 hours post L4) (J) and adult (10 hours post L4) (K) stage. At least 20 animals were examined for each genotype. The worm stages were indicated in method. Data are mean ± s.e.m. $^{**}P<0.001$, $^{*}P<0.05$. (**L**,**M**) DIC and fluorescence images of wild-type animals carrying P_{lim-7}CED-1::GFP (L) or P_{ced-1}CED-1::GFP, P_{his-72}HIS-72::GFP and P_{pie-1}lifeACT::RFP (M). CED-1::GFP expressed specifically in sheath cells (L) or phagocytes (M) labeled residual bodies (arrowheads, lifeACT::RFP-positive and HIS-72::GFP-negative) in the uterus displaced by fertilization. The residual bodies without CED-1::GFP may have been engulfed. s, spermatheca; u, uterus. Scale bars: 5 μm.

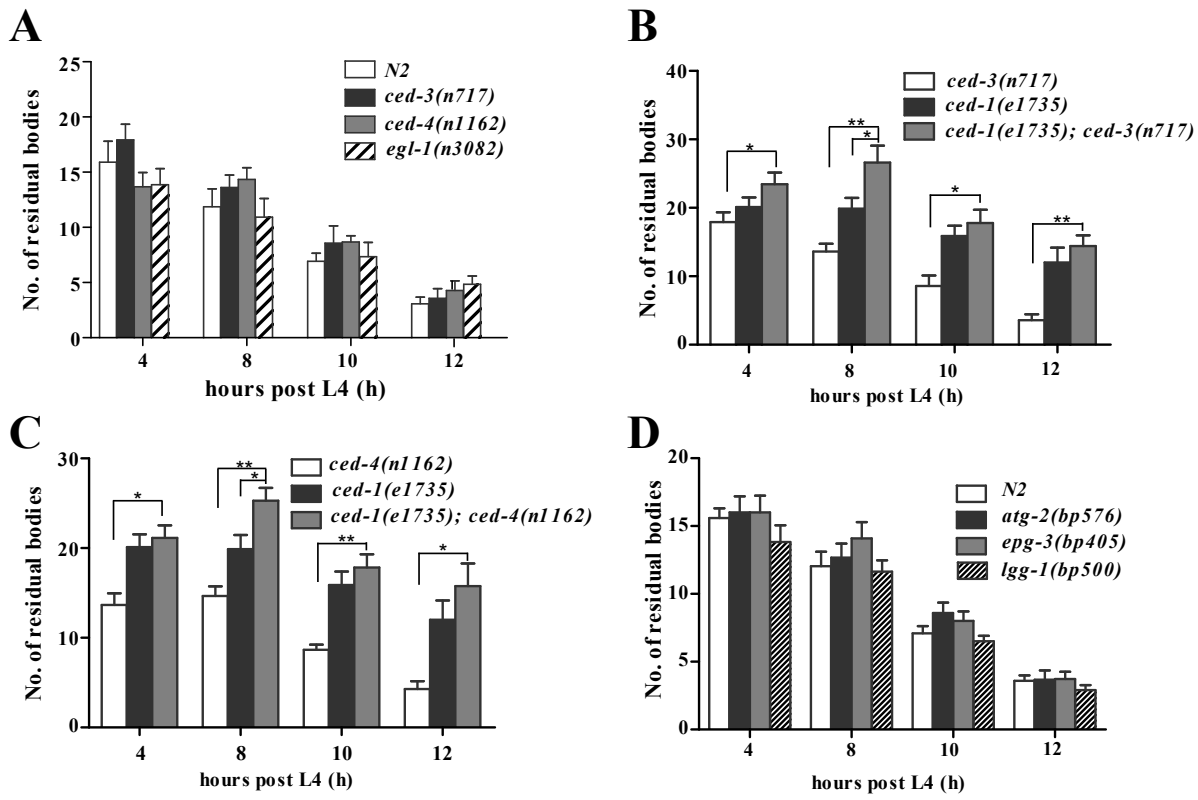


Fig. S2. Mutations in genes essential for programmed cell death or autophagy cause no defect in residual body removal. (**A-D**) Residual bodies were scored at different time points from one gonad arm in the indicated strains. At least 15 animals were scored in each strain at each time point. Data are mean ± s.e.m. Data derived from double mutants were compared with single mutant (B,C) by unpaired *t*-test, whereas cell death and autophagy mutants were compared with wild type (N2) (A,D). **P*<0.05, ***P*<0.0001.

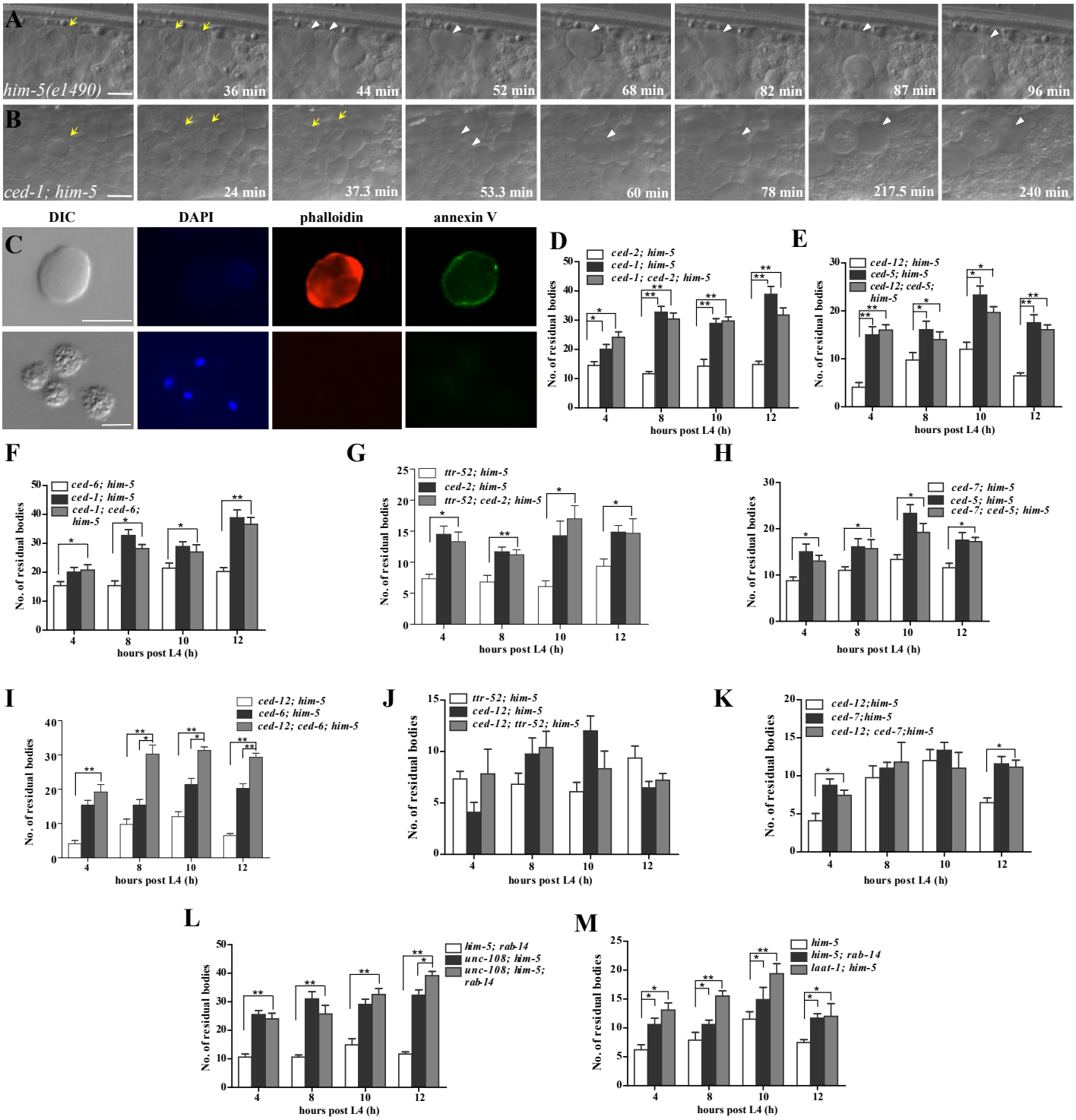


Fig. S3. Engulfment genes are differentially required for removing residual bodies in males. (**A**,**B**) Time-lapse recording of spermatogenesis in wild-type (A) and *ced-1(e1735)* (B) males. Residual bodies are cleared much faster in wild-type (30 minutes) than in *ced-1* males (162 minutes). Yellow arrows indicate spermatocytes and white arrowheads point to residual bodies. (**C**) The residual body from a wild-type male (upper panels) is stained by phalloidin and Annexin V but not DAPI, whereas spermatids (lower panels) are stained by DAPI but not phalloidin or Annexin V. (**D-M**) Residual bodies were quantified in males of the indicated strains at different time points. Fifteen animals were examined for each genotype. Data are mean ± s.e.m. ***P*<0.0001, **P*<0.05.

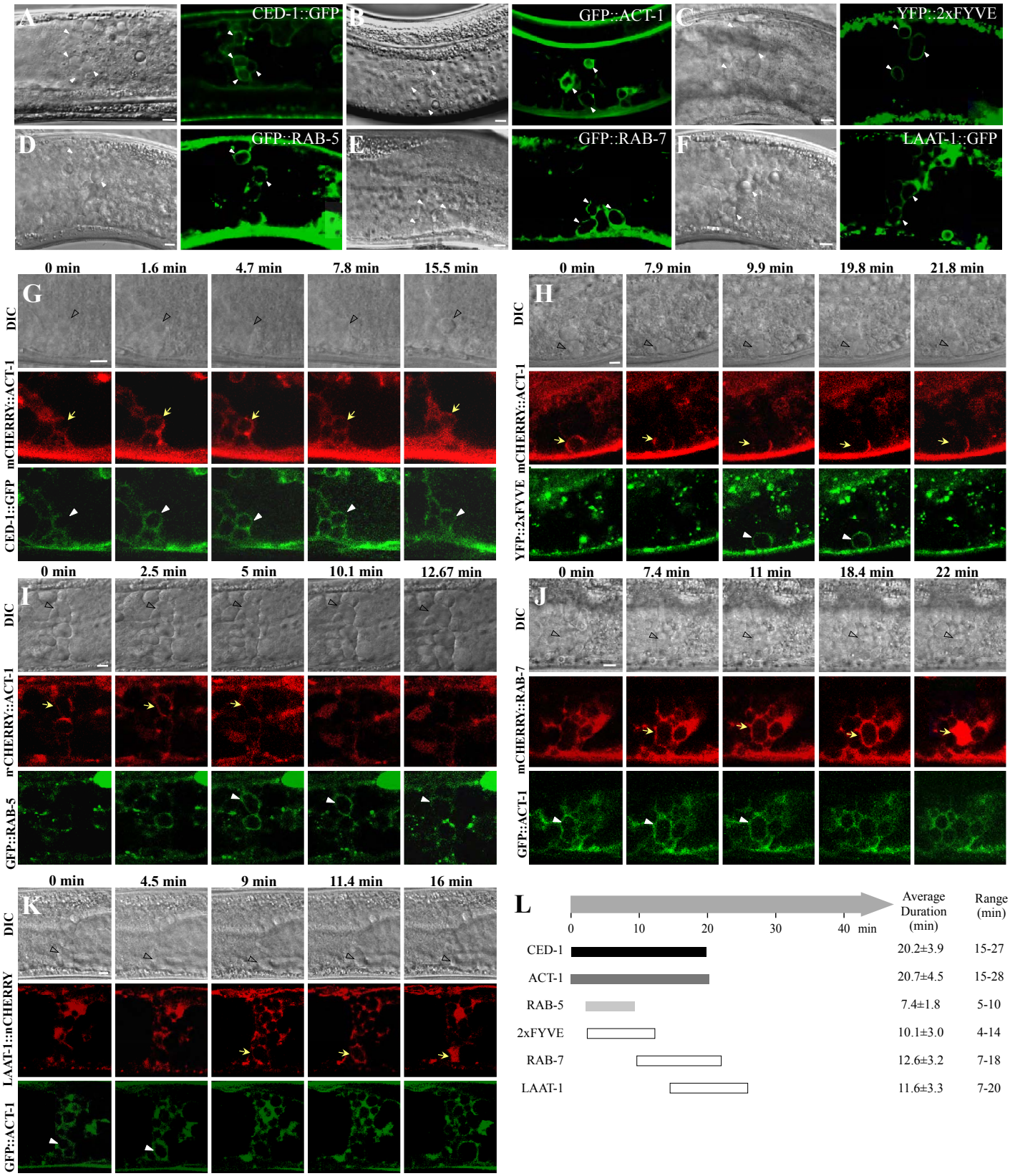
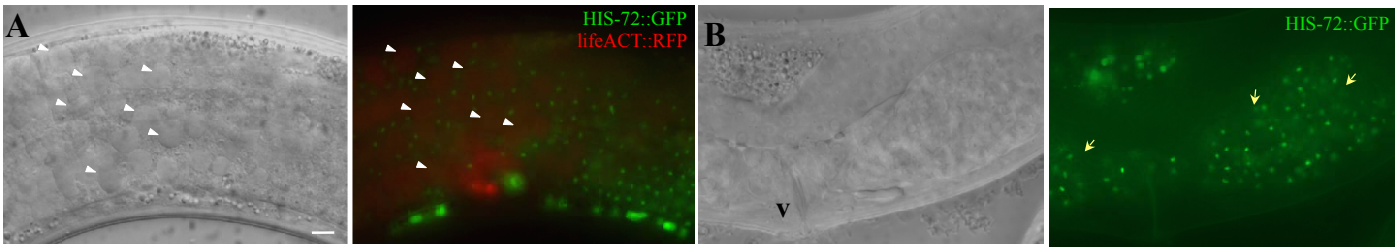
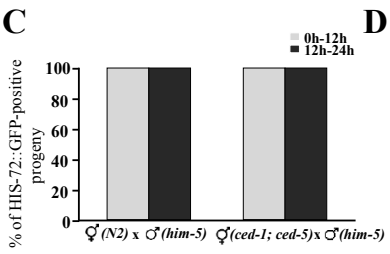
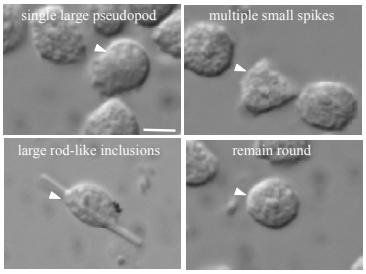


Fig. S4. Recruitment of maturation factors to the residual body-containing phagosome in males. (**A-F**) DIC and fluorescence images of wild-type males expressing CED-1::GFP (A), GFP::ACT-1 (B), YFP::2xFYVE (C), GFP::RAB-5 (D), GFP::RAB-7 (E) and LAAT-1::GFP (F). Residual bodies (arrowheads) are labeled by all six reporters. (**G-L**) DIC and fluorescent images of wild-type males expressing mCHERRY::ACT-1 and CED-1::GFP (G), YFP::2xFYVE (H) or GFP::RAB-5 (I), or GFP::ACT-1 and mCHERRY::RAB-7 (J) or LAAT-1::nCHERRY (K) at different time points. '0 min' indicates the time point when the mCHERRY::ACT-1 or GFP:::ACT-1 ring was just forming. Quantification is shown in L. At least 12 phagosomes were examined for each reporter. Among the 15 phagosomes examined, one showed two waves of 2xFYVE recruitment, but the rest only recruited 2xFYVE once. Yellow arrows, residual bodies labeled by red reporters; white arrowheads, residual bodies positive for green reporters; unfilled arrowheads, residual bodies observed by DIC. Data are mean ± s.e.m. Scale bars: 5 μm.

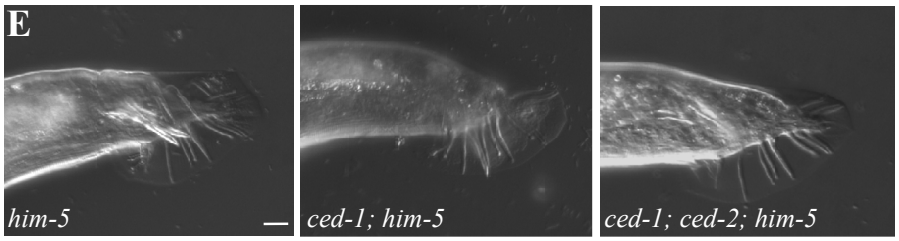


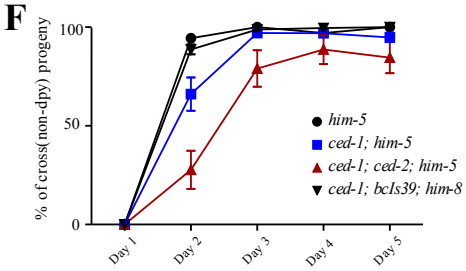




In vitro sperm activation assay

	single large pseudopod (%)	large rod-like inclusions (%)	multiple small spikes (%)	round (%)	n
him-5	92.6	2	2	3.4	253
ced-1; him-5	91.6	2.8	1.4	4.2	264
ced-1; ced-5; him-5	92.6	3	2.2	2.2	278
ced-1; ced-2; him-5	91.8	3	2.6	2.6	280





G

Genotype	Number of cell corpses 2f	4f
ced-1(e1735); him-5	31.18 ± 0.57	30.69 ± 0.56
ced-6(n2095); him-5	30.58 ± 0.81	27.89 ± 0.74
ced-7(n2094); him-5	32.37 ± 0.52	33.62 ± 0.46
ced-2(n1994); him-5	26.95 ± 0.59	26.86 ± 0.57
ced-5(n1812); him-5	33.09 ± 0.71	32.90 ± 0.81
ced-12(n3261); him-5	26.47 ± 0.71	26.13 ± 0.91
ttr-52(tm2078); him-5	20.19 ± 0.52	10.5 ± 0.63
ced-1(e1735); ced-5(n1812); him-5	38.84 ± 0.67	35.65 ± 0.79
ced-6(n2095);ced-5(n1812); him-5	43.37 ± 0.70	39.92 ± 0.77
ced-12(n3261); ttr-52(tm2078); him-5	41.43 ± 0.61	39.35 ± 0.68
ced-7(n2094); ced-12(n3261); him-5	37.35 ± 0.59	37.1 ± 0.64

Fig. S5. Engulfment mutant males show normal sperm activation and male tail development. (**A**,**B**) No residual bodies are transferred into hermaphrodites during mating. Wild-type males expressing HIS-72::GFP and lifeACT::RFP (A) contain a large number of residual bodies labeled by lifeACT::RFP but not HIS-72::GFP (white arrowheads). *unc-76* hermaphrodites (2 h post L4) mated with wild-type males (B) were immediately examined after mating for 30 minutes. Sperm (yellow arrows) but not residual bodies were observed. v, vulva. (**C**) The percentage of cross-progeny in wild-type and *ced-1(e1735); ced-5(n1812)* mutant hermaphrodites mated with wild-type males expressing HIS-72::GFP was quantified. At least five animals were scored in each strain. (**D**) In vitro sperm activation assay. The percentage of sperm with different morphologies (left panels, arrowheads) after activation was quantified (shown in the right panel). Mutations that cause defective engulfment do not affect sperm activation. Sperm with single pseudopods were activated and sperm with other morphologies were aberrant. (**E**) *ced* mutant males show normal development of rays, fan and spicules. Scale bar: 5 μm. (**F**) Percentage of cross-progeny (non-Dpy) generated by *dpy-5* hermaphrodites mated with males from the indicated strain for each day. Data are mean ± s.e.m. (**G**) Cell corpses were scored in the head region of 2.5-fold and 4-fold stage embryos as described in Materials and methods and are shown as mean ± s.e.m. At least 40 embryos were scored for each strain.