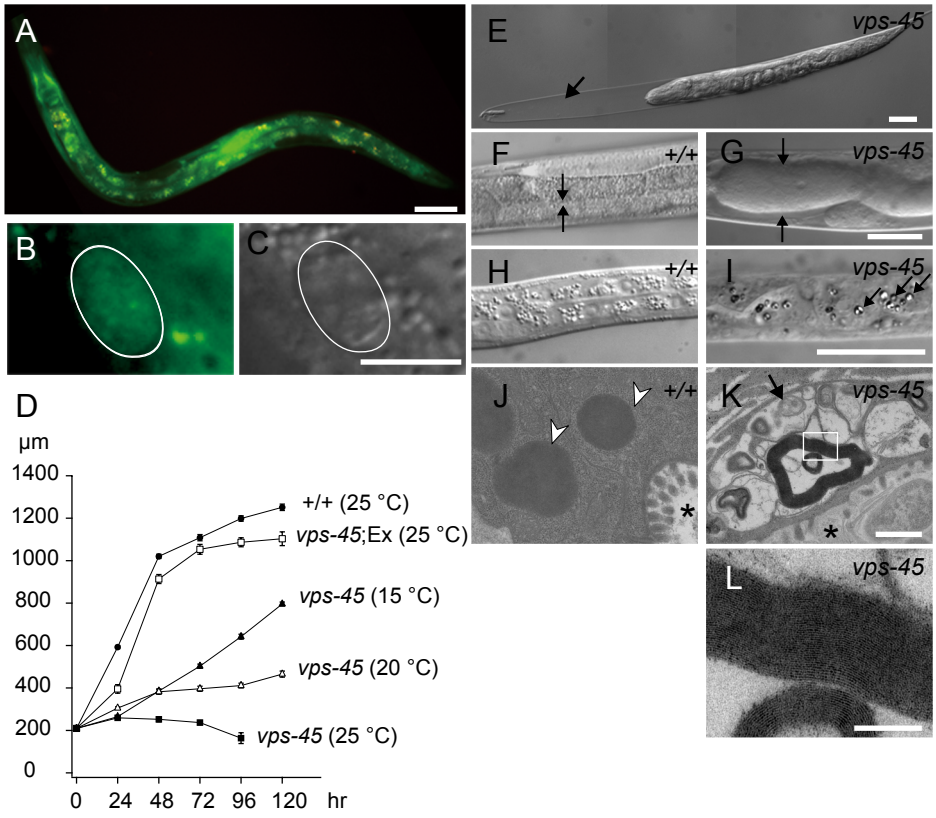

M

			Number of organelle per section [mean+/-SD (n)]			
strain	temp	stage	Nucleus	Yolk gr	Loose MLB	Dense MLB
+/+	25	L1	0.69+/-0.70 (16)	1.1+/-1.3 (16)	1.1+/-1.3 (16)	0 (16)
tm246	15	24h	0.89+/-0.57 (45)	7.4+/-8.2 (42)	1.1+/-1.6 (36)	0.05+/-0.21 (43)
tm246	25	24h	0.87+/-0.78 (38)	0.2+/-1.0 (38)	5.1+/-3.1 (38)	0.24+/-0.59 (38)
tm246	25	96h	0.83+/-0.98 (6)	0.3+/-0.8 (6)	5.3+/-2.9 (6)	1.3+/-1.8 (6)

Supplementary Figure 2

Supplementary Figure 2. *vps-45* is a ubiquitous gene and essential for viability. (A-C) Expression analysis of *vps-45* gene. (A) *vps-45::EGFP* is ubiquitously expressed in all major tissues, such as neuron, muscle, hypodermis and intestine. (B) *vps-45::EGFP* is also expressed in coelomocytes (outlined in white). (C) Differential interference contrast (DIC) image of the same field in B. Lethal phenotype of *vps-45 (tm246)* is rescued by expression of this fusion gene. (D) Growth curves of the *vps-45 (tm246)* grown on NGM at 15°C, 20°C or 25°C. The length of twenty animals was measured for each time point, and the mean values are plotted. Bars indicate SEM. The mutant worms grown at 15°C reach adulthood and are fertile, but a significant proportion of the larvae grown at 25°C arrest at the L1 stage. This *ts* lethal phenotype of *vps-45* was rescued by transgene containing the *vps-45* genomic sequence (*vps-45;Ex*). The *vps-45* worms grown at 15°C also exhibited weaker phenotypes in both Rme and Cup assays (data not shown). (E-I) DIC images of wild type (F, H) and *vps-45* mutant (E, G and I) animals at 25°C. (E) Double cuticle phenotype. Unshed cuticle (arrow) is surrounding the anterior region of the mutant worm. (F-I) Intestinal phenotypes. (F, G) The intestinal lumen is enlarged in the *vps-45* mutant. Arrows indicate the intestinal lumens. (H, I)

Gut granules of the intestines. The gut granules (arrows) are fewer and larger in the arrested mutant at early larval stage (I) than those in wild type larva at the same stage (H). (J-L) Electron micrographs of the intestines. Asterisks indicate the intestinal lumens. (J) Intestine of wild type at L1 stage. Arrowheads indicate yolk granules. (K, L) Intestine of the arrested L1 mutant which was incubated at 25°C for 96 hrs. Dense multilamellar body (MLB) and loose MLB (arrow) are seen in the mutant intestine. Dense MLB surrounded by white square in (K) is shown in higher magnification (L). (M) Number of organelle per section in electron micrographs. The *vps-45* mutant were grown at 15 °C and embryos were incubated at 15°C or 25°C for the indicated time (stage). The mutant intestine often contained the dense and loose MLBs, but these organella were rarely detected in the wild type intestine. The endocytic defect was also observed in the intestine of the *vps-45* mutant when TR-BSA (Texas-Red-labeled BSA) was fed (data not shown).