Supplemental material

Saegusa et al., https://doi.org/10.1083/jcb.201708115

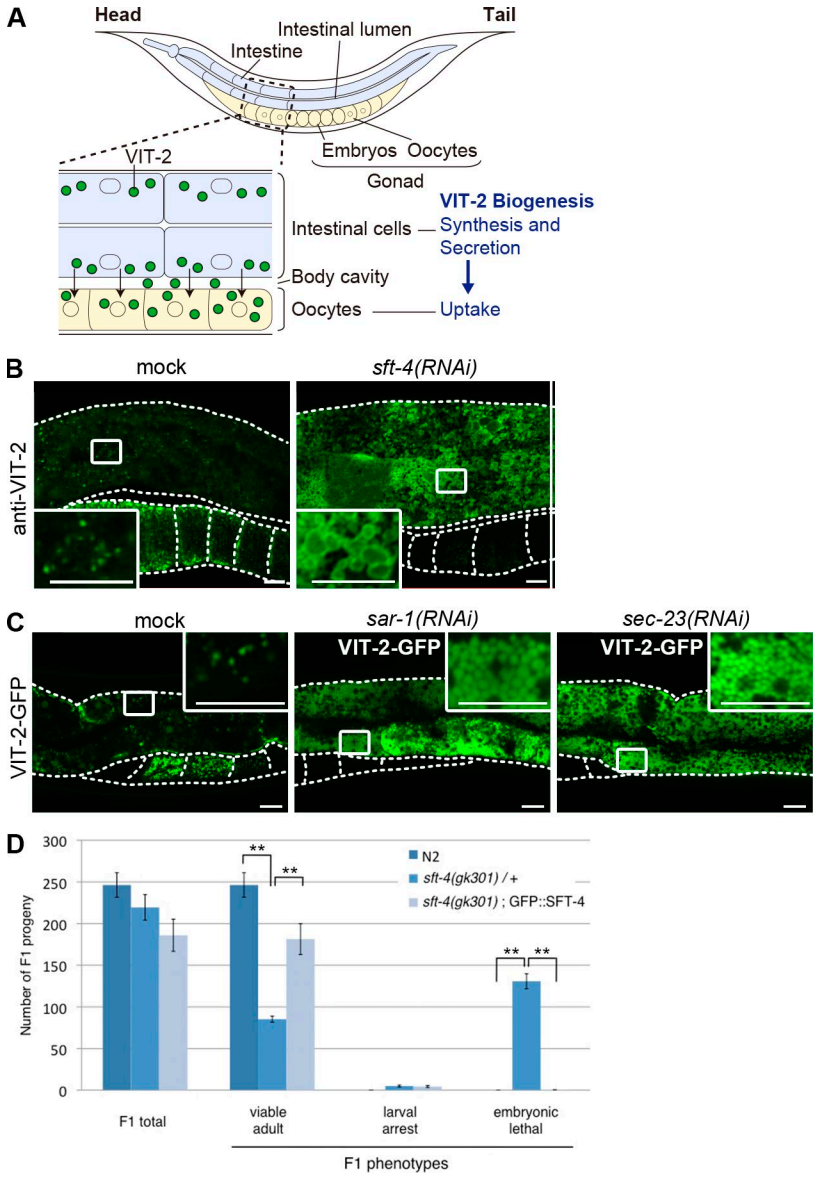

Figure S1. **SFT-4 is essential for yolk secretion and early development. (A)** Schematic presentation of VIT-2 trafficking in the *C. elegans* body. VIT-2 is initially synthesized in intestinal cells as a lipoprotein that contains neutral lipids such as triacylglycerol and cholesterol ester. VIT-2 is secreted from the intestinal cells into the body cavity and then taken up by oocytes through receptor-mediated endocytosis. **(B)** Immunostaining with an anti–VIT-2 antibody showing accumulation of VIT-2–containing vesicles in *sft-4(RNAi)* intestinal cells. Dotted lines indicate the outlines of intestines and oocytes. Regions surrounded by squares are enlarged (16×) in insets. Bars, 10 µm. **(C)** VIT-2–GFP accumulation was also observed when *sar-1* or *sec-23* was knocked down. L3 larvae were treated with RNAi for 2 d. Dotted lines indicate the outlines of intestines and oocytes. Regions surrounded by squares are enlarged (16×) in insets. Bars, 10 µm. **(D)** *C. elegans sft-4(gk301)* mutant showed severe embryonic lethality. L4 larvae from WT N2 (n = 5) or heterozygotes of *sft-4(gk301)* (n = 5) were placed on nematode growth medium plates, and the phenotypes of the F1 progeny were scored. The brood size of the *sft-4(gk301)* heterozygotes was comparable to that of N2, whereas their F1 progeny showed embryonic lethal phenotypes (60%), and only 39% of the progeny reached adulthood. GFP–SFT-4 expression under *sft-4* promoter rescued the embryonic lethality of the homozygotes of *sft-4(gk301)*, which indicates that the fusion protein was functional. Results were analyzed using Student's *t* test; **, P < 0.05; means ± SD.

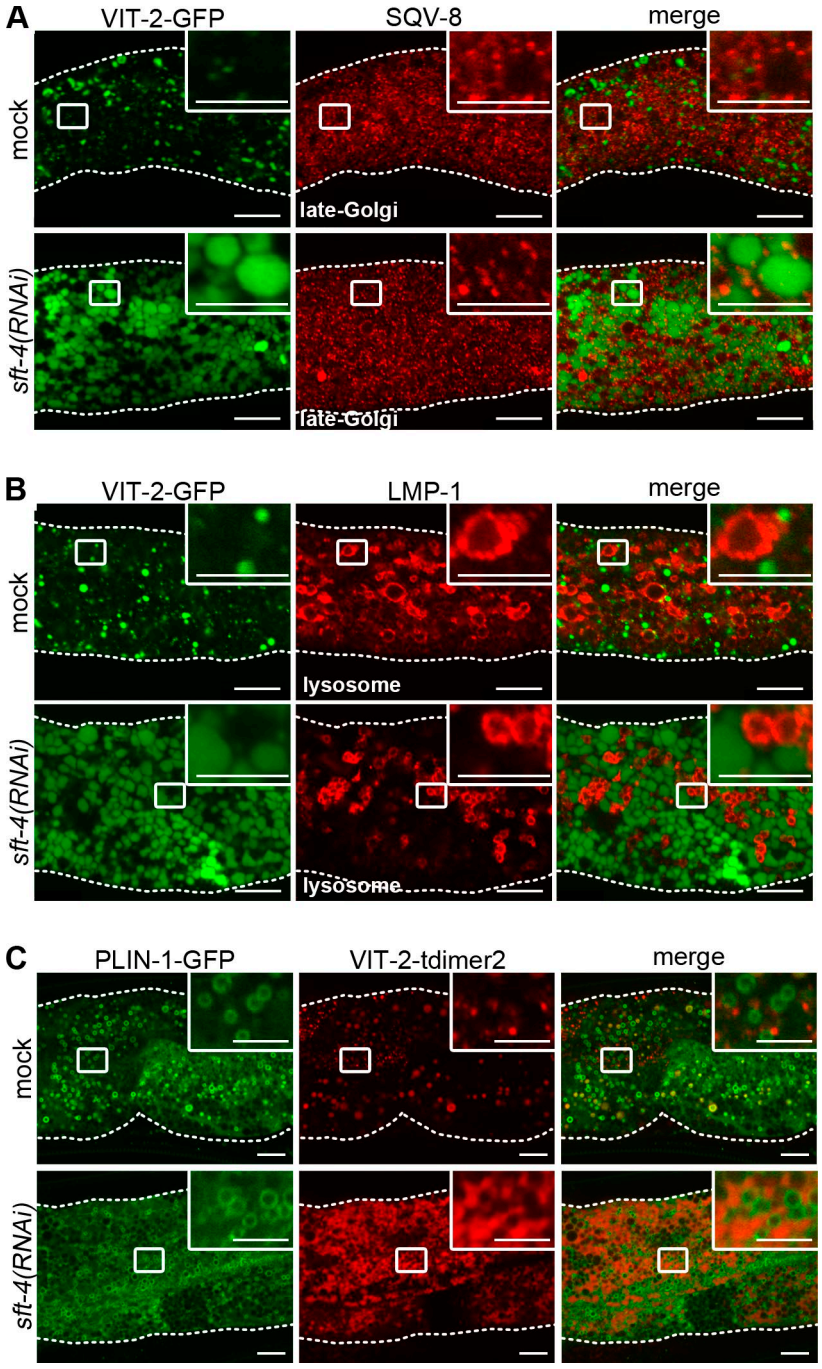

Figure S2. **VIT-2–GFP is not accumulated in late Golgi, lysosomes, and LDs in *sft-4(RNAi)* intestinal cells. (A and B)** Subcellular localization of VIT-2–GFP and SQV-8 (late Golgi; A) or LMP-1 (lysosome; B). Intestinal cells dissected from WT and *sft-4(RNAi)* animals were stained with anti–SQV-8 or anti–LMP-1 antibody. Knockdown of *sft-4* caused severe accumulation of VIT-2–GFP, whereas the localization of SQV-8 and LMP-1 was almost unchanged as compared with that in mock-treated cells. **(C)** The intestinal cells of transgenic worms coexpressing PLIN-1–GFP and VIT-2–tdimer2 were treated with mock or *sft-4(RNAi)*. Loss of SFT-4 caused VIT-2–tdimer2 accumulation in the ER but did not affect the localization or size of PLIN-1–GFP–positive LDs. Dotted lines indicate the outlines of intestines. Regions surrounded by squares are enlarged (16×) in insets. Bars: 10 µm; (insets) 5 µm.

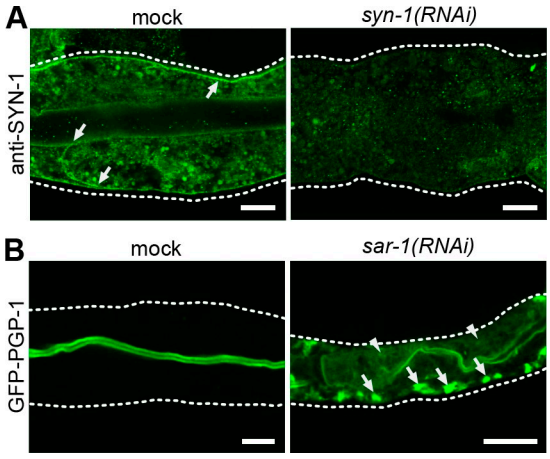

Figure S3. **Loss of SAR-1 affects the transport of plasma membrane protein. (A)** An anti–SYN-1 antibody recognizes endogenous SYN-1 proteins. Immunostaining using an anti–SYN-1 antibody mainly labeled the basolateral plasma membrane of intestinal cells in control animals (left; arrows) but not in *syn-1(RNAi)* animals (right). **(B)** SAR-1 is important for transport of GFP–PGP-1 to the apical membrane in intestinal cells. GFP–PGP-1 was mainly localized to apical plasma membrane in control intestinal cells, but it accumulated in reticular ER structures (arrowheads) and large punctate structures (arrows) near the plasma membrane in *sar-1(RNA)* intestinal cells. Dotted lines indicate the outlines of intestines. Bars, 10 µm.

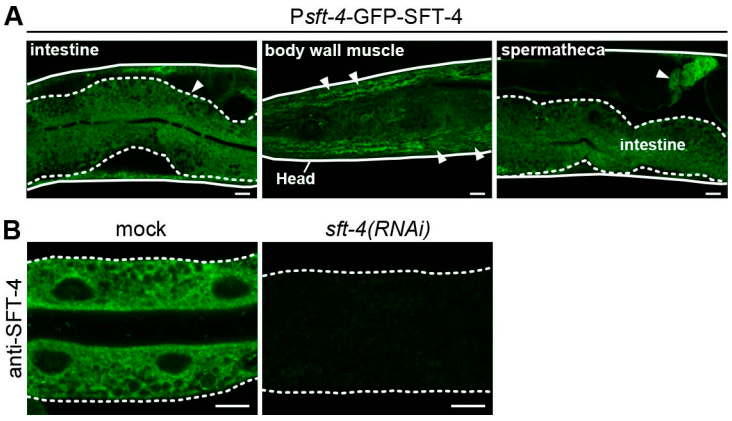

Figure S4. **Tissue-expression pattern of SFT-4. (A)** SFT-4 is highly expressed in the intestine, body wall muscle, and spermatheca. GFP–SFT-4 expressed under the control of the *sft-4* promoter (P*sft-4*–GFP–SFT-4) was detected in the intestine (left; arrowhead), body wall muscle (middle; arrowheads), and spermatheca (right; arrowhead). Dotted lines indicate the outlines of intestines, and lines indicate the outlines of the body wall. Bars, 10 µm. **(B)** An anti–SFT-4 antibody recognizes endogenous SFT-4 proteins. Immunostaining using an anti–SFT-4 antibody mainly labeled the ER reticular network and punctate structures in intestinal cells in control animals (left) but not in *sft-4(RNAi)* animals (right). Dotted lines indicate the outlines of intestines. Bars, 10 µm.