Developmental Cell 12

## **Supplemental Data**

## A Morphologically Conserved Nonapoptotic

# Program Promotes Linker Cell Death

## in Caenorhabditis elegans

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Figure S1. EM Images Showing that Nuclear Invagination and U.l/rp Cell Vacuoles Accompany Linker Cell Death

(A) A highly invaginated nuclear membrane (arrow) of a dying linker cell. (B) Different section through linker cell of same animal as in (A). Two vacuoles containing floccular material (asterisks) are shown within the U.l/rp cell. Arrow, U.l/rp nucleus. Such vacuoles may be engulfed linker cell components that have been degraded. Vacuoles flank a disintegrating linker cell (arrowhead) also engulfed by U.l/rp. IG, intestinal granule. Scale bar, 1  $\mu$ m. (C) Dying linker cell in a *him-4(e1267)* male. Black arrows, swollen ER. White arrow, swollen mitochondrion surrounded by a membranous layer.

Figure S1



#### Figure S2. Linker Cell Death May Not Be Autophagic

(A) Table showing the number of LGG-1::GFP puncta at different stages of linker cell development. n, number of animals scored. (B) A migrating linker cell (outlined) expressing a transgene consisting of the *lgg-1* promoter driving expression of a LGG-1::GFP fusion protein. Top, DIC image. Bottom, fluorescence image. Autophagosomal puncta is marked with an arrowhead. (C) A linker cell (outlined) beginning to be engulfed by U.lrp (asterisk) in a late L4 stage animal expressing LGG1::GFP. Top, DIC image. Bottom, fluorescence image capturing most puncta (arrowhead) marking autophagosomes. Scale bar, 2 μm. (D) Same as (B), except in a fully engulfed linker cell.

Figure S2

linker cell stage	No. LGG-1 puncta (n)
migrating	3.8 ± 2.8 (8)
starting to be engulfed	$6.7 \pm 2.1$ (9)
corpse	$6.8 \pm 4.5$ (8)



Figure S3. lin-29 and let-7 Mutant Male Gonads Accumulate Sperm

(A) A *lin-29(n333*) adult male. Note accumulation of sperm in the male gonad (arrowhead). Arrow, linker cell. Scale bar, 10  $\mu$ m. (B) Same as (A) except in a *let-7(n2853*) animal. Scale bar, 10  $\mu$ m.

Figure S3



Figure S4. A Dying Linker Cell Can Be Engulfed by the Male Gonad

Image of a *him-4(e1267)* animals in which the linker cell migrated abnormally and was engulfed by the male gonad. Scale bar,  $5 \mu m$ .



Figure S4