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Supplemental Information

Entosis Controls a Developmental Cell Clearance in *C. elegans*

Yongchan Lee, Jens C. Hamann, Mark Pellegrino, Joanne Durgan, Marie-Charlotte Domart, Lucy M. Collinson, Cole M. Haynes, Oliver Florey, and Michael Overholtzer

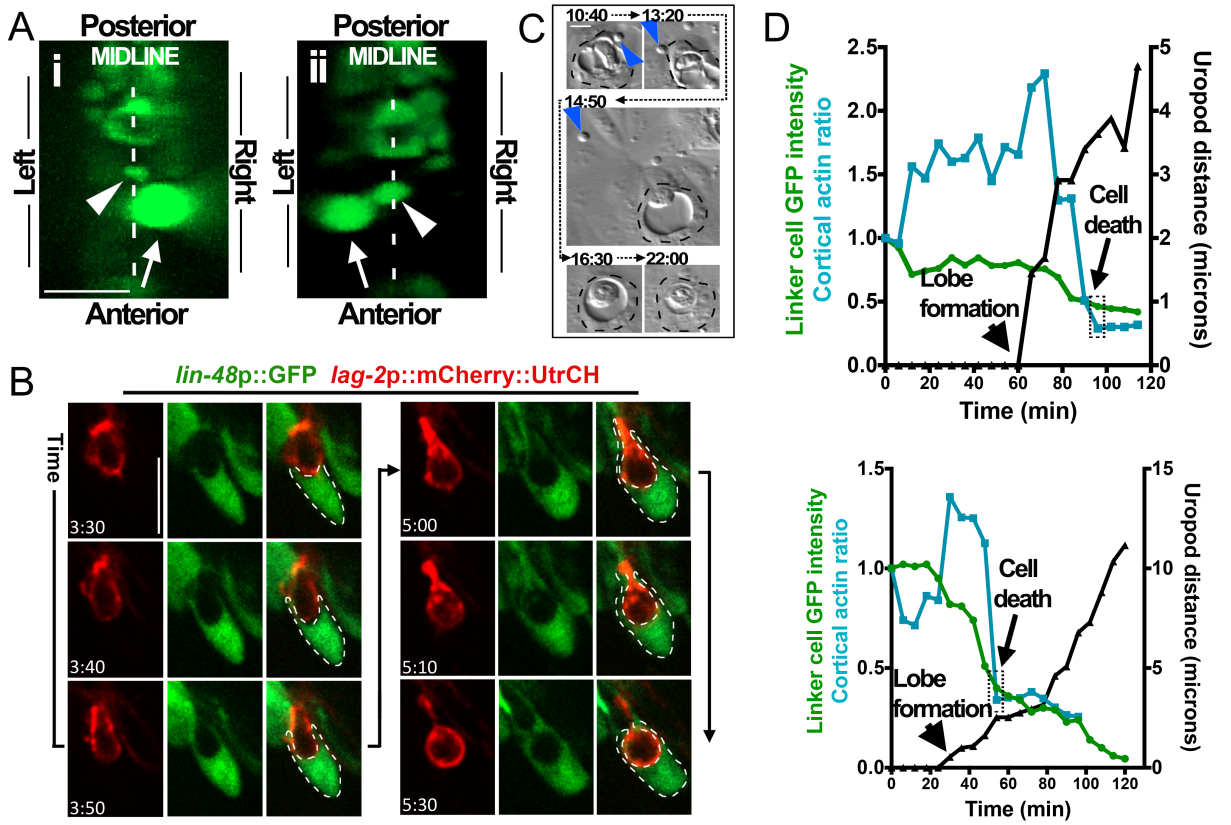

Figure S1

Figure S1 (related to Figure 1). Linker cell lobe forms during engulfment into U cell. (A) 3-dimensional reconstructions of linker cells show that lobe structures remain at the midline as linker cells move anterior and left or right. Anterior, posterior, left and right directions and the midline are indicated; linker cell bodies are labeled with arrows, lobes with arrowheads. (B) Lobe formation occurs as the linker cell is engulfed into a U cell. Images show linker cell expressing lag-2p::mCherry::UtrCH (red) and U cells expressing lin-48p::GFP (green) as the linker cell lobe is formed, during uptake into a U cell. Images are confocal x-y planes, times are shown as hours:minutes. Scale bar = 10mm. (C) Uropod cleavage precedes entotic cell death. Images show MCF-7 cells time-lapse imaged by DIC microscopy. Times are indicated as hours:minutes; uropod structure that undergoes cleavage is marked with blue arrowhead. Note that internalized cell undergoes death after uropod cleavage. See also Video S3B. (D) Graphs show cortical to cytoplasmic ratios of GFP::UtrCH fluorescence (blue lines, left y-axes) in linker cells prior to and after engulfment marked by lobe formation. Green lines show GFP intensities over time. Black lines (right y-axes) show distances of lobe separation from linker cells over time following lobe formation (black arrow). Hatched boxes on graphs represent timing of linker cell deaths determined by cortical actin ratios and GFP intensities (see also Figure 1D for additional example).

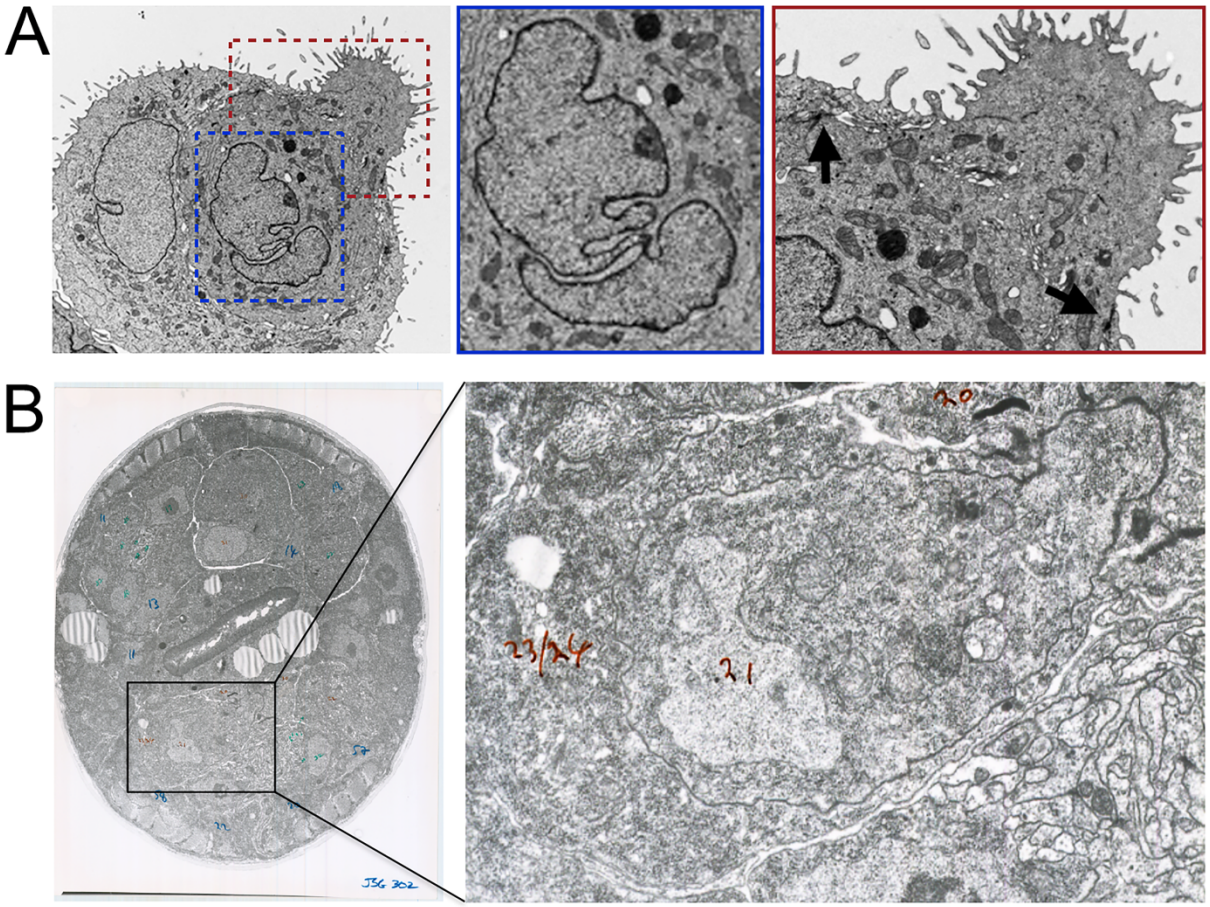

Figure S2

Figure S2 (related to Figure 3). Entotic cells exhibit nuclear crenellation and linker cells make junctions with U cells. (A) Transmission electron micrograph of MCF-7 cells undergoing entosis. Middle image: internalizing cell from left panel has a high degree of nuclear crenellation. Right image: cell junctions formed between internalizing and host cell from left panel are indicated with arrows. This electron micrograph was published previously in (Sun et al., 2014a). (B) Transmission electron micrograph of cross-section of tail region of late L4 stage *C. elegans*, from WormAtlas (www.wormatlas.org). Right image shows enlargement of boxed region in left image containing linker cell and U cell. Linker and U cell outlines are shown in Figure 3D.

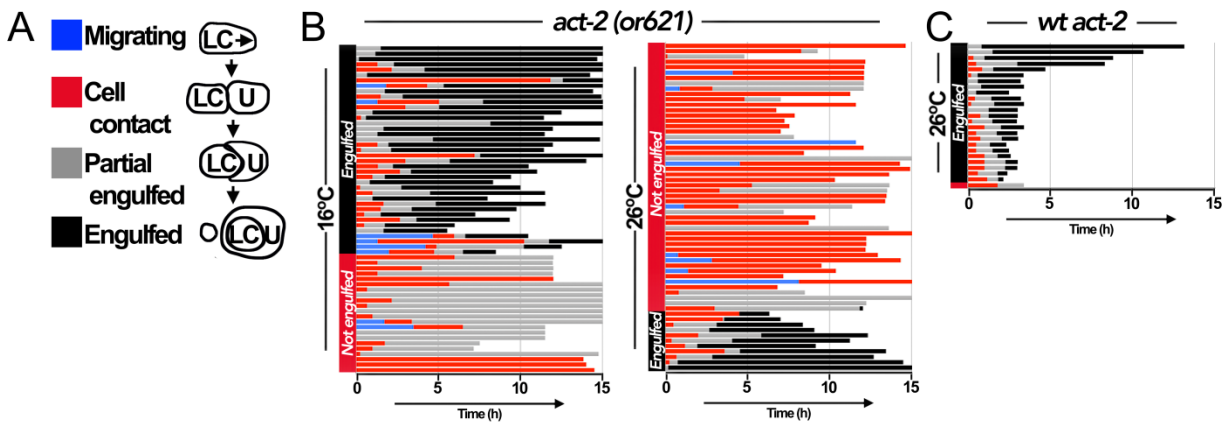

Figure S3

Figure S3 (related to Figure 3). Linker cells require actin for engulfment into U cells. (A-C) Graphs show all acquired imaging data summarized in Figure 5A, color-coded to indicate linker cell behavior over time as shown in part (A), from migration (blue bars), contact with a U cell (red bars), partial engulfment into a U cell (gray bars), and complete engulfment (black bars). (B-C) Time is indicated as hours (h); graphs start at the time of shift from permissive to restrictive temperature. Each horizontal bar represents a single 4D imaging experiment for one linker cell; bars terminate at the end of the imaging for each cell. Graphs in (B) show linker cells expressing act-2 (or621) at permissive (left) or restrictive (right) temperature. Graph in (C) shows linker cells expressing wild-type act-2.

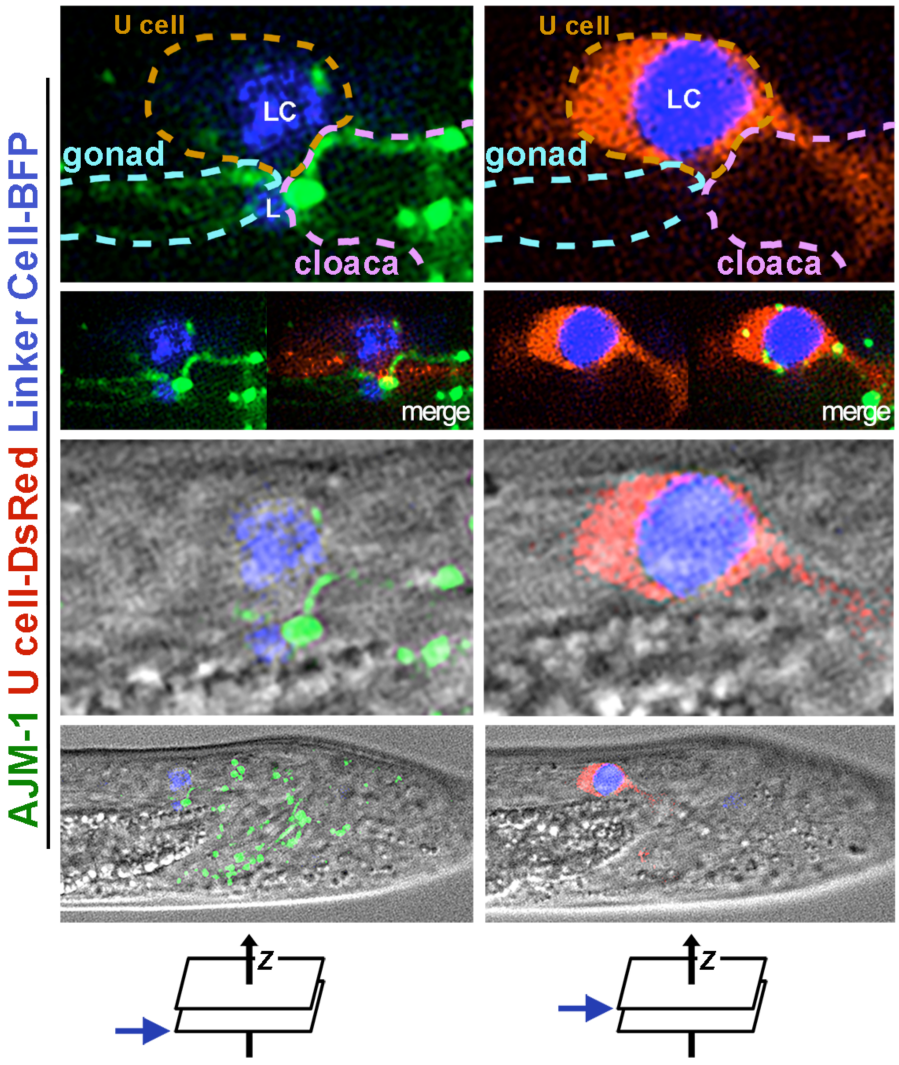

Figure S4

Figure S4 (related to Figure 4). The linker cell lobe is unengulfed and positioned between the gonad and cloaca. Confocal images were acquired from *C. elegans* expressing three fluorescent markers, AJM-1::GFP (green) that marks cell junctions in the tail region, lag2::tagBFP2 (blue) in the linker cell, and lin48::DsRed (red) in U cells. Confocal z-series shows that the linker cell lobe (L) is positioned between the gonad and cloaca, and is not engulfed by a U cell.