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Supplemental Information

Mitochondria transported by Kinesin-3 prevent localized calcium spiking to inhibit caspase-dependent specialized cell death

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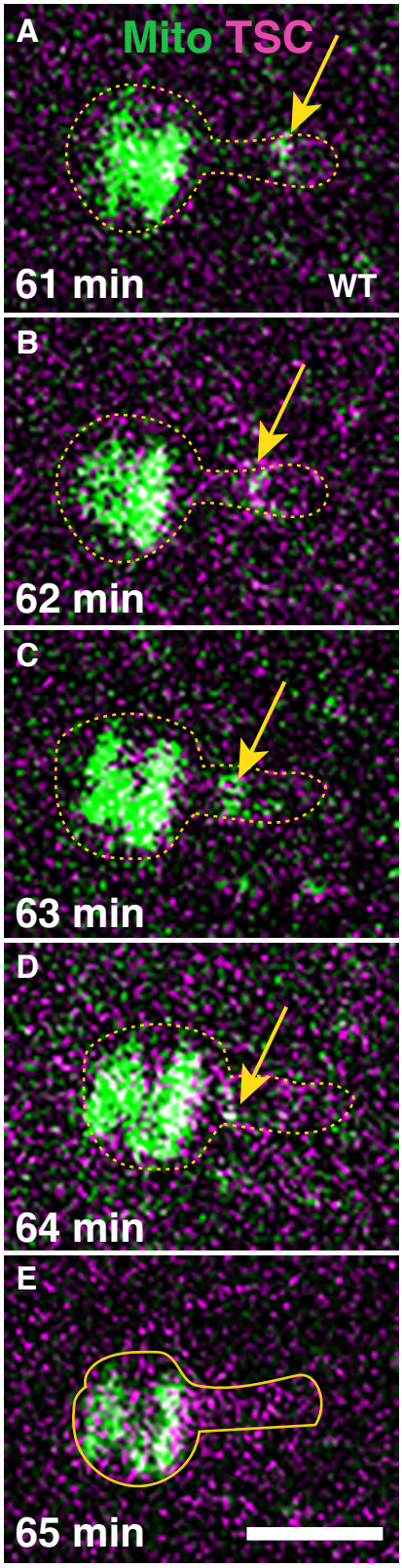

Figure S1. A second example of light-sheet acquired time-lapse of mitochondria entering the TSC soma from the process of a of a wild-type at the 1.5-fold embryo stage. Related to Figure 2. (A) Mitochondria distal in process. (B, C) Mitochondria progressed further towards soma. (D) Mitochondria at soma-process junction. (E) Mitochondria removed from process. Yellow arrow, mitochondria. (Video S3). Scale bar, 5μm. Images taken every 1 minute.

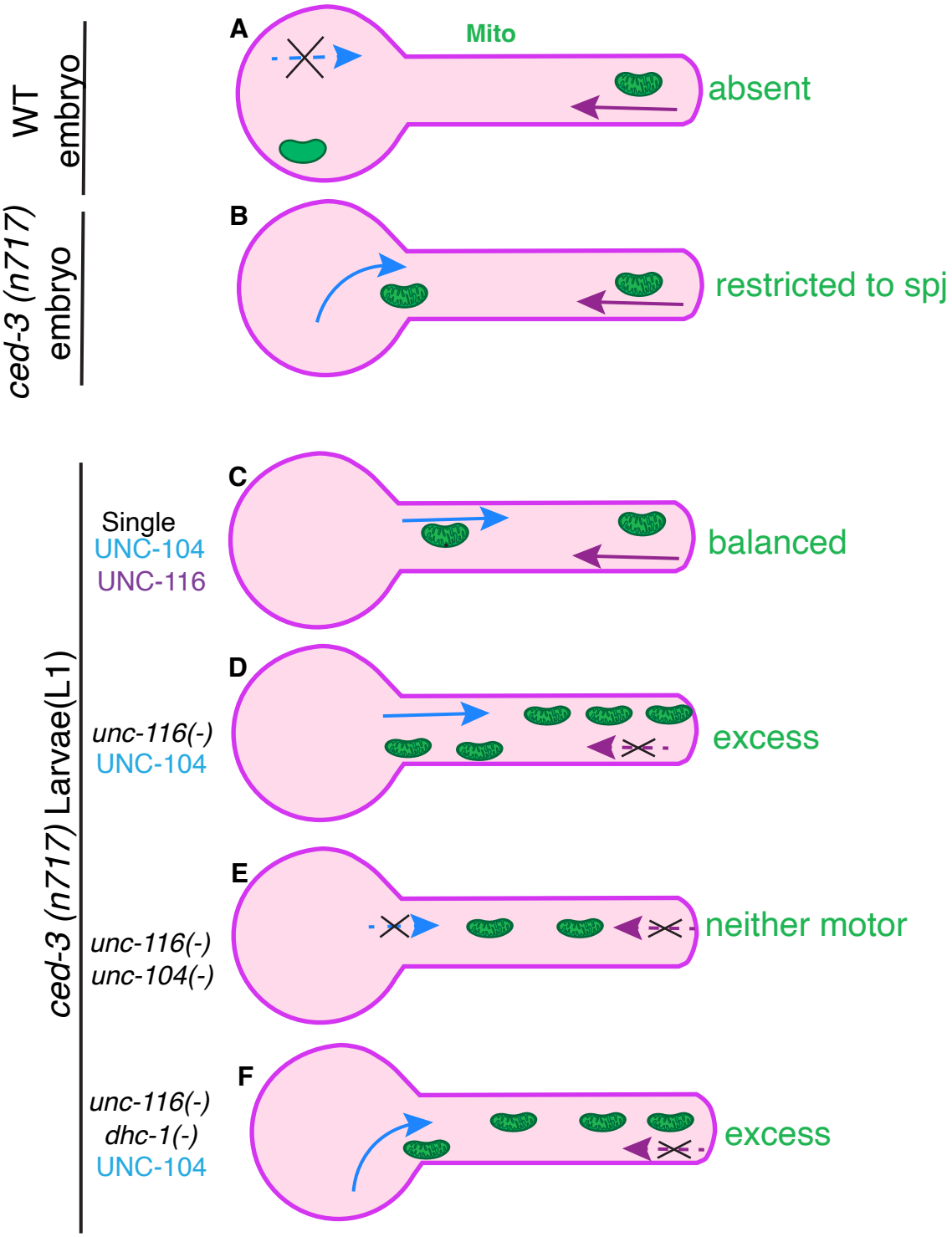

Figure S2. Schematic of proposed mitochondrial transport events in TSC. Related to Figure 2 and 5. (A) In wild-type embryos, UNC-104 is not present to transport mitochondria to the soma process junction. UNC-116 removes mitochondria from the process, soma-ward. (B) *In ced-3(n717)* embryos, UNC-104 transports mitochondria to the soma process junction. UNC-116 removes mitochondria from the process, soma-ward. (C) In *ced-3(n717)* L1 larva UNC-104 and UNC-116 act as two opposing motors. UNC-104 transports mitochondria to the soma process junction. Mitochondria can progress farther into the process. UNC-116 removes mitochondria from the process, soma-ward. (D) In *ced-3(n717);unc-116(ns827)* L1 larva, UNC-104 transports mitochondria to the soma process junction and mitochondria can progress farther into the process. In absence of UNC-116, mitochondria already present or newly entering cannot exit the process. (E) In *ced-3(n717);unc-116(ns827);unc-104(e1265)* L1 larva, due to absence of UNC-104, mitochondria cannot reach to the soma-process junction or progress farther into the process. In absence of UNC-116, mitochondria already present cannot exit the process. (F) *ced-3(n717);unc-116(ns827);dhc-1(js319)* L1 larva, UNC-104 transports mitochondria to the soma process junction and mitochondria can progress farther into the process. In absence of UNC-116, mitochondria already present or newly entering cannot exit the process. Absence of DHC-1 does not appear to influence how these motors function.

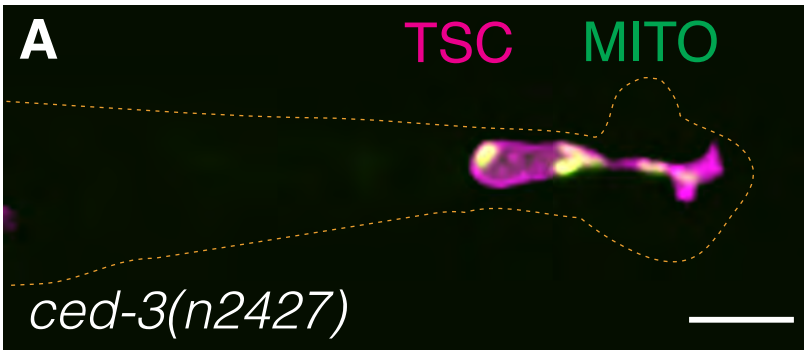

Figure S3. Mitochondria (green) seen in persisting process (magenta, TSC membrane marker) of *ced-3(n2427)* hypomorph. Related to Figure 6. These persisting mitochondria are hypothesized from this study to be qualitatively different (take up Ca2+ to preserve cell fragment) from those seen in the distal process remnant of *unc-116(ns827)* mutant (FIGURE 2A-C; FIGURE 6M). Scale bar, 5μm.

Gibson Cloning							
Plasmid name	**Plasmid Description**	**Insert Forward Primer**	**Insert Reverse Primer**	**Backbone Forward Primer**	**Backbone Reverse Primer**	**Insert Template**	**Backbone Template**
pPG294	TSCp::unc-104 cDNA (cell specific rescue)	tctagaggatc cccgggattgg ccaaag	GGCGCGC Cgttccgaata ttatgaaatg	tccatactttctca tttcataatattcg gaacGGCGC GCCaaaATG TCATCGGT TAAAGTAG CTGTACG	tacctttgggtc ctttggccaat cccggggatc ctctagaTTA TGAAGCA GCAATTG AAGATGA TGATG		pSM::SL2::mCherry
pPG331	TSCp::unc-104GFP_tomm7 (mito rescue)	tccatactttctc atttcataatatt cggaacGGC GCGCCaaa ATGTCATC GGTTAAAG TAGCTGTA CG	TACTgcttcc gccggtacctc cactgccaccg ctagtactTGA AGCAGCA ATTGAAGA TGATGATG TTG	agtactagcggt ggcagtggagg tac	GGCGCG CCgttccga atattatgaaa tg	unc-104 cDNA	pPG174 (below)
pPG445	TSCp::mcu-1 cDNA (cell specific rescue)	tccatactttctc atttcataatatt cggaacGGC GCGCCaaa ATGAGGAA TGGCCGA TGCTTGGT G	tacctttgggtc ctttggccaatc ccggggatcct ctagaTTAC TTTTCAGC TTCCAAAT TGGATAAA TAG	GGCGCGC Cgttccgaatatt atg	tctagaggat ccccgggatt ggc	mcu-1 cDNA	pSM::SL2::mCherry
pPG245	unc-116p::mKate2 (expression)	GATCCTCT AGAGTCG ACCTGCA GGC	GCTTCATA TGCATGTT TTCgTTAAT GAGCTCG GAGACCAT tttcagagaata atgatcaccag ttggc	ATGGTCTC CGAGCTCA TTAAcGAAA AC	GATCCTC TAGAGTC GACCTGC AGGC	unc-116 genomic DNA	pPG146=cdh-3p::mKate2
pPG246	TSCp::unc-116 cDNA (cell specific rescue)	tccatactttctc atttcataatatt cggaacGGC GCGCCaaa ATGGAGC CGCGGAC AGACGGA G	Taccttgggtc ctttggccaatc ccggggatcct ctagacgatga gttgatgttgttg ttgggagac				pSM::SL2::mCherry
pPG401	TSCp::UNC-104::mCherry	ATTGAAAT CTCCAACA TCATCATC TTCAATTG CTGCTTCA	caagttggtaat ggtagcgacc ggcgctcagtt gGAATTCT TActtgtacag	ctctattctcaca aaaaacatatg	ggagaaaga gcatgtagg	pPG331 (above)	pPG270=TSCp::lnp-1 cDNA_SL2::mCherry

		GGAGCATCGGGAGCCTCAGGAGCATCGATGGTGagcaagggcgaggaggataaca	ctcgtccatgccgc				(unpublished)
pPG274	TSCp::EBP-2::GFP	tccatactttctcatttcataatattcggaacGGCGCGCCttcagaaaccgaggcaaaATGGTCG	caagttggtaatggtagcgaccggcgctcagttgGAATTCctatttgtatagttcatccatgccatgt	GGCGCGCCCgttccgaatattatg	GAATTCcaactgagcgccggtc	Pklp-6::epb-2::gfp (A gift grom Maureen Barr)	pPG270
pPG482	TSCp_unc-116 motor_CC1_GFP-TOMM7	tccatactttctcatttcataatattcggaacGGCGCGCCATGggtgtccaggtattttgtcgtatc	TACTgcttccgccggtacctccactgccaccgctagtactagcacgccagcgtgaaagttccaaa	agtactagcggtggcagtggaggtacc	GGCGCGCCgttccgaatattatgaaatg	pPG174	Truncated UNC-116 synthesized by gene universal
Restriction Digest Based Cloning							
Plasmid name	**Plasmid Description**	**Insert Forward Primer**	**Insert Reverse Primer**	**Vector**		**Insert Template**	
pPG174	TSCp::unc116GFP_tomm7	cccccGGCCGGCCctattgacgataaccaccttacaaac	ccaatcccgggatcctctagaGGCGCGCCcggggg	Punc-47::unc-116_GFP::tomm-7		aff-1 promoter 1393 bp (Fse-1, AscI)	
pPG115	TSCp::GCaMP5a_SL2_myrmcherry	ccccccGGCCGGCCctattgacgataaccaccttacaaacacc	ccccccGGCGCGCCgttccgaatattatgaaatgagaaagtatgG	pSM::SL2::mCherry		GCaMP5a synthesized	
pPG113	TSCp::GFP::C3Ai_SL2::myrmCherry	ccccccGGCGCGCCaaaATGATCAAGATTGCCACACGGAAATAT	gggggGTTAACTTAATTCGGCAGATTGTCGACGCGCAT	pSM::SL2::mCherry		GFP::C3Ai synthesized	
pPG112	TSCp::mitoGFP	ccccccGGCGCGCCaaaATGGCACTCCTGCAATCACGTCTCC	gggggGTCGACTTTGTATAGTTCATCCATGCCATGTGTAATC	pglr-1::mitoGFP (Ghose et al, 2013)		aff-1 promoter 1391 bp (AscI, SalI)	

pPG114	TSCp::mitoGFP_SL2_myrmcherry	ccccggcgcgccaaaATGGCACTCCTGCAATCACGTC	ccccccagttaactaggtgaaagtaggatgagacaggatatc	pSM::SL2::mCherry	pPG112 (AscI, EcoRV)

Table S1. Plasmids used in this study. Related to STAR Methods.