Katanin, kinesin-13 and ataxin-2 inhibit premature interaction between maternal and paternal genomes in *C. elegans* zygotes

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Abstract

Fertilization occurs before completion of oocyte meiosis in the majority of animal species and sperm contents move long distances within zygotes of mouse and *C. elegans*. If incorporated into the meiotic spindle, paternal chromosomes could be expelled into a polar body resulting in lethal monosomy. Through live imaging of fertilization in *C. elegans*, we found that the microtubule disassembling enzymes, katanin and kinesin-13 limit long range movement of sperm contents and that maternal ataxin-2 maintains paternal DNA and paternal mitochondria as a cohesive unit that moves together. Depletion of katanin or double depletion of kinesin-13 and ataxin-2 resulted in capture of the sperm contents by the meiotic spindle. Thus limiting movement of sperm contents and maintaining cohesion of sperm contents within the zygote both contribute to preventing premature interaction between maternal and paternal genomes.

Introduction

Reproduction by most animal species requires expulsion of chromosomes into polar bodies during oocyte meiosis to reduce chromosome number and fertilization by sperm to restore a diploid chromosome number. Because fertilization occurs during oocyte meiosis in most animal species, there is an inherent risk of sperm DNA being incorporated into the meiotic spindle and being expelled into a polar body. In some species, the first line of defense against this hypothetical calamity is ensuring that sperm does not fuse with the oocyte plasma membrane directly over the meiotic spindle. A Ran-GTP gradient emanating from the mouse metaphase II meiotic spindle excludes the fusion proteins Juno and CD9 from the oocyte plasma membrane over the spindle. When this mechanism was bypassed by injecting demembranated sperm adjacent to the spindle, the sperm DNA was ejected into a polar body (1). In *C. elegans*, the nucleus is positioned away from the site of future fertilization so that the meiosis I spindle assembles at the opposite end of the ellipsoid zygote from the site of fertilization (2-4).

However, simply controlling the site of fertilization is not sufficient to maintain a distance between the meiotic spindle and the sperm DNA because cytoplasmic streaming can move the sperm DNA long distances in both mouse (1) and *C. elegans* (5, 6). Meiotic cytoplasmic streaming in *C. elegans* embryos requires microtubules (7), kinesin-1 (3), and reticulons (8), but both mechanism and purpose are not completely understood. In addition to meiotic spindle microtubules, *C. elegans* meiotic embryos have cytoplasmic microtubules around the cortex and throughout the cytoplasm of the entire embryo (3). These microtubules are thought to drive meiotic cytoplasmic

streaming because depletion tubulin stops cytoplasmic streaming (7) and depletion of the microtubule-severing protein katanin by RNAi results in an increased mass of cortical microtubules and an increase in cytoplasmic streaming (8).

In addition to paternal DNA, *C. elegans* sperm introduce centrioles, SPE-11 protein, membranous organelles (MOs) and paternal mitochondria into the zygote. Paternal centrioles are silenced during meiosis by maternal KCA-1 (9), which is also required to pack yolk granules inward from the cortex (3) and the meiotic spindle outward toward the cortex (10). SPE-11 is an RNA-binding protein in sperm (11) that is required paternally for polar body extrusion (12) and proper embryonic development (13, 14). MOs are membrane vesicles in sperm that fuse with the sperm plasma membrane before fertilization during sperm activation (15). However, a subset of MOs that do not fuse with the sperm plasma membrane are introduced to the zygote at fertilization and are ubiquitinated with maternal ubiquitin (16). Paternal mitochondria are labelled with maternal autophagy machinery (17). Whereas both MOs and paternal mitochondria are eventually destroyed during embryogenesis, during meiosis they remain in a tight cloud around the sperm DNA (5, 16, 17). The mechanisms holding the sperm contents together in the zygote during cytoplasmic streaming have not been explored.

In this study we monitored the cloud of paternal mitochondria after increasing cytoplasmic streaming or disrupting the integrity of the cloud of paternal organelles. Our results suggest that both limiting cytoplasmic streaming and maintaining the integrity of the paternal organelle cloud contribute to preventing capture of the sperm contents by the meiotic spindle.

Results

Sperm derived DNA and mitochondria are maintained in a volume that excludes maternal mitochondria and yolk granules but allows penetration by maternal ER during meiosis.

Time-lapse in utero imaging of the meiotic spindle and an endoplasmic reticulum marker (ER) revealed that the ER is distributed throughout the zygote but appears as undulating lines during metaphase I (n=8), changes to a dispersed appearance during anaphase I (n=9), transitions back to undulating lines interspersed with large blobs during metaphase II (n=6), then changes to a dispersed pattern during anaphase II (n=5)(Fig. 1A; Video S1). Previous electron microscopy studies have indicated that the undulating lines correspond to sheet-like ER and the dispersed appearance corresponds to tubular ER (18); Gong et al., 2024). In striking contrast with the ER filling the entire zygote, yolk granules are packed inward, away from the cortex (n = 9 metaphase I; Fig. 1B) as previously described (3). When Mitotracker-treated males were mated to hermaphrodites expressing GFP-labeled yolk granules, the paternal mitochondria were found in a discrete cloud around the paternal DNA (n = 34; Fig. 1B-D) as previously described (17). Both the cloud of paternal mitochondria (n=19) and the meiotic spindle (n= 9 metaphase I) were observed in cortical regions that are free of yolk granules (Fig. 1B). Maternal mitochondria labeled with COX-4::GFP were also packed inward, away from the cortex, and were excluded from the cloud of paternal mitochondria and excluded from the spindle (n = 5 metaphase I; Fig. 1D). In contrast with maternal yolk granules and maternal mitochondria, maternal ER was observed

penetrating into the cloud of paternal mitochondria and enveloping the paternal DNA in a shell that appeared as a ring in confocal sections both live (Fig. 1A, arrow at 9:20) and fixed (n = 10; Fig. 1C). Time-lapse imaging (n=21) revealed that the cloud of paternal mitochondria remained together and separate from maternal yolk granules even when moving long distances with cytoplasmic streaming (Fig. 1E).

Because yolk granules and maternal mitochondria are packed inward during meiosis (Fig. 1B, D) and sperm must enter from the outside, it is possible that the exclusion of maternal yolk granules from the volume of paternal mitochondria might simply be a consequence of inward packing. In *kca-1(RNAi)* meiotic embryos, yolk granules (3) and mitochondria (Fig. 2A) do not pack and instead extend to the plasma membrane. Maternal mitochondria were still excluded from the volume of paternal mitochondria in *kca-1(RNAi)* embryos (n=11 control, 8 RNAi; Fig. 2A). This result indicated that the volume of paternal mitochondria excludes maternal mitochondria and yolk granules but not maternal ER. In addition, paternal mitochondria also formed a discrete cluster in meiotic embryos of a nematode species with giant sperm (19) (n=4; Fig. 2B) indicating that the unique properties of the ball of paternal organelles in the meiotic zygote is conserved.

Maternal ER invades the volume of paternal organelles shortly after fertilization.

To elucidate how the maternal ER enters the ball of paternal organelles, we monitored 7 instances of sperm egg fusion by time-lapse microscopy. Previous studies have demonstrated that *C. elegans* sperm fuse with the egg as opposed to being phagocytosed (20). Mitotracker-labeled males were mated with hermaphrodites with a maternally-expressed ER marker and a maternally-expressed plasma membrane

marker (mCherry::PH). 20 seconds after the apparent entry of paternal mitochondria into the egg (0:20 in Fig. 3A), a sperm-sized volume devoid of maternal ER was observed within the maternal plasma membrane. One minute after apparent sperm-egg fusion, the maternal ER had moved into the plasma membrane behind the sperm (1:25 in Fig. 3A; 1:00 in Fig. 3B). The maternal ER ring enveloping the sperm DNA was not discernible until 7 min after apparent sperm-egg fusion (7:24 in Fig. 3B), although it might form earlier because the zygote undergoes dramatic movement through two sequential sphincters into the spermatheca then into the uterus during this time. These results suggest that all maternal membranes are initially excluded from the sperm at fusion. The plasma membrane marker diffuses in behind the sperm first, followed by plasma-membrane associated ER, followed by envelopment of the sperm DNA by maternal ER by 7 min after fusion. Maternal yolk granules and maternal mitochondria, however, are excluded from the sperm volume for much longer (Fig. 1).

The maternal ER envelope around the sperm DNA is permeable to proteins.

If the maternal ER envelope around sperm DNA was sealed and impermeable during meiosis, this could both prevent the sperm DNA from inducing ectopic spindle assembly and prevent the sperm DNA from interacting with meiotic spindle microtubules. BAF-1 is a chromatin-binding component of the inner nuclear envelope (21). GFP::BAF-1 was not detected on maternal or paternal chromatin during metaphase I or metaphase II but labelled the surface of both maternal and paternal chromatin during anaphase I and anaphase II (Fig. 4 A, B, C). Because BAF-1 is a chromatin-binding protein, this result indicates that BAF-1 can pass freely through holes in the ER envelope surrounding the sperm DNA.

Movement of sperm contents within the zygote is limited by katanin and kinesin-13 and this limitation prevents capture of the sperm DNA by the meiotic spindle.

C. elegans meiotic cytoplasmic streaming (3, 5, 7, 8) has the potential to bring the sperm contents into close proximity with the meiotic spindle, however, a previous study found that the male pronucleus very rarely forms at the same end of the embryo as the female pronucleus (6). This indicates that limitations on cytoplasmic streaming might be involved in maintaining distance between the sperm contents and meiotic spindle. We therefore asked whether increasing cytoplasmic streaming would cause collisions between the meiotic spindle and sperm contents. Meiotic cytoplasmic streaming requires microtubules (7) and kinesin-1 (3). Depletion of katanin by *mei-1(RNAi)* increases the mass of cortical microtubules and the extent of yolk granule streaming (8) and depletion of kinesin-13 by *klp-7(RNAi)* also increases the mass of cortical microtubules (22). We found that *mei-1(RNAi)* or *klp-7(RNAi)* increased the maximum displacement of the sperm contents in both the long and short axes of the ellipsoid embryo relative to control L4440(RNAi) embryos (Fig. 5A, B, C, D; Videos S2, S3, S4).

Among 15 control L4440(RNAi) time-lapse sequences, the closest center to center distance between spindle and sperm contents was 18 um. In contrast, among 51 time-lapse sequences of *mei-1(RNAi)* meiotic embryos, the sperm came within 5.5 um center to center distance of the spindle in 12 cases and in 12/12 of these cases, the sperm stopped moving relative to the spindle indicating a capture event (Fig. 5B; Video S3). Among a subset that could be tracked through pronuclear stage, a single male

pronucleus formed adjacent to multiple small female pronuclei. Among 25 time-lapse sequences of *klp-7(RNAi)* embryos, the sperm DNA became stuck against the meiotic spindle (4.6 um center to center distance) in only one case. In all other cases, the sperm streamed past a stationary spindle. However, the closest sperm to spindle distance was 7.7 um in one case. These results indicate that limiting cytoplasmic streaming is important for maintaining a distance between spindle and sperm and thus preventing capture of the sperm by the meiotic spindle. However, *mei-1(RNAi)* spindles are apolar and do not undergo normal polar body extrusion and the single capture event in a *klp-7(RNAi)* embryo resulted in cell cycle arrest. These results therefore do not reveal what would happen to paternal DNA captured by a normal meiotic spindle during polar body extrusion.

An important question is why the sperm contents move more than the meiotic spindle. During metaphase I, when the ER is sheet-like, movement of the sperm contents is limited. When the ER disperses during anaphase I, the spindle rotates and one pole is moved closer to the cortex by cytoplasmic dynein (Video S1; (23)). In contrast, the distance of the sperm contents from the cortex (Fig. S1) and movement of the sperm contents both increase when the ER disperses (Fig. 5C; Video S5).

Ataxin-2 is required to maintain cohesion of paternal mitochondria

Because maternal ER penetrates the sperm contents and envelops the sperm DNA, and because the *C. elegans* ortholog of ataxin-2, ATX-2, affects ER organization (24), we analyzed the contribution of ATX-2 to cohesion of the sperm contents in meiotic embryos. We first introduced an auxin-induced degron and GFP tag to the 3'end of the

endogenous *atx-2* gene. Endogenously tagged ATX-2 was observed throughout oocytes and meiotic embryos without partial co-localization with ER. Dark holes were observed suggesting exclusion from the lumens of larger membranous organelles (Fig. 6A; Fig. S2). We then compared the intensity of the GFP signal after three different depletion treatments, 1 hour auxin, 24 hr *GFP(RNAi)* or 27 hr *atx-2(RNAi)*. All 3 methods resulted in significant reduction of the GFP signal (Figure 6B, 6C, 6D).

The extent of scattering of paternal mitochondria was analyzed in Z-stacks of fixed meiotic embryos depleted of ATX-2 by each of the 3 methods. This analysis was restricted to embryos from anaphase I through anaphase II because our streaming data and that of Kimura 2020 indicate that the sperm contents have not moved significantly before anaphase I. Example Z-projections are shown in Figure 7. Mitochondria can exist as a tubular network (26) and tubules in close proximity cannot always be resolved by light microscopy. To account for the apparent heterogenous sizes of foci of paternal mitochondria, the fluorescence intensity of larger foci was divided by the fluorescence intensity of the smallest foci such that a focus with 3 times the fluorescence intensity was counted as 3 “mitochondria”. ATX-2 depletion by each of the 3 methods resulted in a significantly increased mean distance of paternal mitochondria from the sperm DNA (Figure 8A) as well as significantly increased standard deviation of individual distances (Figure 8B). Standard deviation is an important measure because an increase in the number of mitochondria both closer and further from the sperm DNA would result in no change in the mean. It remains possible that paternal mitochondria scatter is caused by pressure applied during fixation, pressure resulting from ovulation through the spermatheca valves, or cytoplasmic streaming. We were not able to unambiguously

track scattering by live imaging because significant movement occurs during acquisition of a complete z stack and because the embryos cannot withstand the additional photodamage. These results still support the hypothesis that ATX-2 is required to maintain the integrity or cohesiveness of the ball of paternal mitochondria that surrounds the sperm DNA to resist external forces.

Double depletion of KLP-7 and ATX-2 results in capture of the sperm DNA by the spindle

Because we observed increased cytoplasmic streaming in *klp-7(RNAi)* and disruption of the integrity of the sperm contents after ATX-2 depletion, we hypothesized that double depletion of KLP-7 and ATX-2 would result in an increased frequency of spindle microtubules capturing the sperm DNA. Among 24 time-lapse sequences of *atx-2(AID+auxin) klp-7(RNAi)* meiotic embryos, the ER envelope around the sperm DNA moved extensively in all cases but came within a threshold distance of 5.5 um (center to center) of the meiotic spindle in only 5 cases. In 5/5 of these cases, a bundle of microtubules extended from the meiotic spindle into the ER envelope around the sperm DNA and the sperm DNA became stuck in this position (Fig. 9A, 9B). In one case, the ER envelope around the sperm DNA was transiently captured by the meiosis I spindle and stretched in the direction of streaming (Fig. 9C). The sperm DNA then released, continued streaming and was captured by the meiosis II spindle. All cases of stable capture caused a cell-cycle arrest so that the consequences on polar body extrusion could not be determined.

Because ATX-2 depletion alters ER morphology, we were not able to score cytoplasmic streaming with the cell-cycle accuracy shown in Fig. 5. However, the maximum long axis and short axis displacement at any cell-cycle time was increased in *atx-2(AID)* with auxin vs without auxin (Fig. S3). No spindle capture events were observed, however, among 15 ATX-2 single depletion time-lapse sequences and the closest distance between sperm and spindle was 8.2 um with 1 hr auxin and 19.3 um without auxin. These results overall indicate that limiting cytoplasmic streaming and maintaining the integrity of the ball of paternal mitochondria are both important for preventing capture events between the meiotic spindle and sperm DNA.

Discussion

The mechanism excluding maternal yolk granules and mitochondria from the volume of sperm cytoplasm introduced to the egg at fertilization is not clear. The simplest explanation is that cytoplasm does not mix during the 45 min from GVBD to pronucleus formation due to the high viscosity of cytoplasm. Attempts at measuring cytoplasmic viscosity of the *C. elegans* zygote have revealed values from 0.67 – 1.0 Pa s (27-29) which are similar to the viscosity of 100% glycerol. Alternatively, the sperm contents might be held together by a cytoskeleton-like matrix as proposed for the Balbiani body (30). In either case, an active process appears to allow the maternal ER to penetrate into the paternal cytoplasm to envelope the sperm DNA. The higher frequency of capture of the sperm DNA by the meiotic spindle in ATX-2 KLP-7 double depleted embryos compared with either single depletion suggests that the integrity of

the exclusion zone around the sperm DNA may insulate the sperm DNA from spindle microtubules.

ATX-2 is required to maintain the integrity of the ball of paternal mitochondria around the sperm DNA, but the mechanism is unknown. Because the paternal mitochondria observed to scatter are from wild-type males, the effect of ATX-2 depletion must be on the egg and not on the sperm. Although ATX-2 depletion alters ER morphology (24) we still observed a maternal ER envelope around the sperm DNA in all ATX-2-depleted embryos. ATX-2 also plays roles in translational regulation (31), germline proliferation (32), cytokinesis (33), centrosome size (34), and fat metabolism (35). Thus the effects of ATX-2 could be extremely indirect. Because *C. elegans* ovulate every 23 min (4), however, our rapid 1 hr depletion would only affect the three most mature oocytes.

In control embryos, the sperm contents rarely came near the meiotic spindle in agreement with a previous study that found that male and female pronuclei rarely form next to each other (6). Streaming of the sperm contents was most commonly restricted to a jostling motion with little net displacement, circular streaming in the short axis of the embryo, or long axis streaming in which the sperm turned away from the spindle before the halfway point of the embryo. Depletion of MEI-1 or KLP-7 resulted in longer excursions of the sperm contents in the long axis of the embryo toward the spindle but frequent capture of the sperm by the spindle was only observed in *mei-1(RNAi)*. This may be because *mei-1(RNAi)* affects positioning of the spindle within the embryo (7) or because the altered structure of the *mei-1(RNAi)* spindle allows spindle microtubules to capture chromosomes further from the spindle center. In capture events observed after

double depletion of ATX-2 and KLP-7, a bundle of microtubules was discernible extending from the spindle into the ER envelope surrounding the sperm DNA. Such bundles were not observed in *mei-1(RNAi)* capture events, likely because of the previously reported low density of microtubules in *mei-1(RNAi)* spindles (36, 37).

None of our time-lapse sequences of sperm capture by the meiotic spindle resulted in extrusion of paternal DNA into a polar body as was observed when sperm was injected next to the meiotic spindle of mouse oocytes (1), likely because of the pleiotropic affects of depleting MEI-1, KLP-7 or ATX-2. Future development of more specific perturbations of cytoplasmic streaming and the organelle exclusion zone around the sperm DNA should address this problem.

Materials and Methods

Live *in utero* imaging

Adult hermaphrodites were anaesthetized with tricaine/tetramisole in PBS as described (4, 38) and then placed on 2% agarose pads on slides. Extra anesthetic was gently pipetted onto the agarose pad and a coverslip was placed on top. The slide was inverted and placed on the stage of an inverted microscope. Meiotic embryos were identified by bright-field microscopy before initiating time-lapse fluorescence. For all live imaging, the stage and immersion oil temperature was 21°C-24°C. For all time-lapse data, single-focal plane images were acquired with a Solamere spinning disk confocal microscope equipped with an Olympus IX-70 stand, Yokogawa CSU10, either Hamamatsu ORCA FLASH 4.0 CMOS (complementary metal oxide semiconductor) detector or Hamamatsu ORCA-Quest qCMOS (quantitative complementary metal oxide semiconductor) detector, Olympus 100x UPlanApo1.35 oil objective, 100-mW Coherent

Obis lasers (405, 640, 488, 561 nm) set at 30% power, and MicroManager software control. Pixel size was 65nm for the ORCA FLASH 4.0 CMOS detector and 46nm for the ORCA-Quest qCMOS detector. Exposures were 200ms for the ORCA FLASH 4.0 qCMOS detector and 100ms for the ORCA-Quest qCMOS detector. Time interval between image pairs or trios was 5 seconds. Focus was adjusted manually during time-lapse imaging.

For the ATX-2 images in Fig. 6A, z-stacks of -1 oocytes of anesthetized live worms were captured with a Zeiss LSM 980 confocal microscope with Airyscan 2 and a Zeiss Objective LD LCI Plan-Apochromat 40x/1.2 Imm Corr DIC M27 for water, silicon oil or glycerine.

Fixed Immunofluorescence

C. elegans meiotic embryos were extruded from hermaphrodites in 0.8× egg buffer by gently compressing worms between a coverslip and a slide, flash frozen in liquid N2, permeabilized by removing the coverslip, and then fixed in ice-cold methanol before staining with antibodies and DAPI. The primary antibodies used in this work were mouse monoclonal anti-tubulin (DM1α; Thermo Fisher Scientific; 1:200) and rabbit anti-GFP (NB600-308; Novus Biologicals; 1:600). The secondary antibodies used were Alexa Fluor 488 anti-rabbit (A-21206; Thermo Fisher Scientific; 1:200), Alexa Fluor 488 anti-mouse (A-21202; Thermo Fisher Scientific; 1:200) and Alexa Fluor 594 anti-mouse (A-21203; Thermo Fisher Scientific; 1:200). Z-stacks were captured at 1-μm steps for meiotic embryos using the same microscope described above for live imaging.

Sperm ER Ring Filming

For sperm ER ring filming, the ring was observed throughout meiosis I and II and manually kept in the focal plane by adjusting the stage (z axis). Filming typically began at spermatheca exit or meiosis I and ended at pronuclear formation or cell arrest. Due to the rigors of filming an embryo with optimal orientation and positioning in the uterus, videos that started and ended mid phases were still filmed and included in measurements so long as a complete phase was included between starting and ending filming (e.g., AI and MII were measured in videos starting mid MI and ending mid AII). In these cases when filming started mid phase and/or ended prematurely mid phase, the incompletely filmed phases were not included in quantifications. The phases at which cell arrest occurred were not included in quantifications, but the phases prior were still used.

Sperm ER Ring Tracking

Measurements of the ER rings' dynamics in videos of embryos were tracked manually using Fiji plugin MTrackJ. All embryos quantified were rotated such that at the beginning of each video the pole containing the meiotic spindle was at the left. Only embryos measured at >35 um long were used for ER ring tracking. Embryos measured <35 um were tilted and not ideal for tracking. The starting and ending frames of each cell phase were then determined based on ER morphology. Individual tracks for each phase were manually made by clicking on the center of the ER ring, or in cases when it was briefly out of focus, the mitochondria that most closely followed where the ER ring was previously seen. The following criteria was used for determining phases to be quantified by tracks:

Metaphase I: Starts when embryo becomes stationary after exiting spermatheca into uterus and ends when ER reticulation becomes dispersed.

Anaphase I: Starts when ER becomes dispersed and ends when it begins to reticulate.

Metaphase II: Starts when ER begins to reticulate and ends when it becomes dispersed.

Anaphase II: Starts when ER becomes dispersed and ends at paternal pronuclear formation.

Maximum X-axis displacement was quantified by subtracting the minimum x-axis coordinate of a phase's track from the maximum x-axis coordinate.

Maximum Y-axis displacement was quantified by subtracting the minimum y-axis coordinate of a phase's track from the maximum y-axis coordinate.

Displacement was measured as the distance between the first point of a phase's track and the last point.

Distance Traveled was measured as the the total length of the track that the sperm ring traveled.

Maximum Average Velocity was measured by taking the maximum of 3 point moving averages of velocities in each phase. MTrackJ was used to measure the velocity of the ER ring between each video frame.

Duration of Phases were calculated by subtracting the first frame number of a phase from the last one and multiplying by 5 due to the 5 second intervals.

Paternal Mitochondria Scattering Quantifications

Paternal mitochondrial scattering was calculated by using a circular ROI to measure the mean value fluorescence of Deep Red MitoTracker FM or Red MitoTracker CMXros labeling paternal mitochondria in meiotic embryos. Mean pixel values were taken using a circle ROI with an 18-pixel diameter to cover the entire area of a region of fluorescence that appeared as a punctum (unless specified otherwise due to a different resolution). This was repeated until all puncta fluorescence in an embryo were measured. For every embryo, a region of cytoplasm without mitochondria had its fluorescence measured. This value was subtracted from every mitochondrial measurement to correct for noise. The distances between the center of each punctum and the center of the paternal DNA were then recorded with a line tool. The Pythagorean theorem was used to determine the distance when the mitochondria were in a different z-stack than the paternal DNA. These distances were recorded in column scatter graphs to observe the distribution of mitochondrial distance from sperm DNA quantitatively. To measure large amorphous masses of mitochondria, the same sized ROI measuring fluorescence in distinct puncta was used to cover a portion of the mass, make measurements, and then moved to another portion to make more measurements. This was repeated until the entire area of the mass had its fluorescence measured. The corresponding distances to the paternal DNA were recorded for every ROI used. The mean value fluorescence of the amorphous masses were typically much greater than the distinct puncta and as such presumably had a greater density of mitochondria per ROI than the puncta. In order to account for this density, the mean values measured from the ROI's over the masses were divided by the average of the mean values of all

of the puncta in an embryo. This resulting number was then used to determine how many times the distance between the paternal DNA and the ROI was recorded in the column scatter graphs (e.g., if the rounded value was 2 then the distance was recorded twice). The averages and standard deviations of each distribution of mitochondrial distances from paternal DNA in the embryos were then measured in order to compare the scattering of mitochondria in different experimental treatments. Quantifications were done in meiotic embryos of all phases except metaphase I. Since this phase is right after fertilization, we believe embryos do not have the time to exhibit scattering as a phenotype.

Paternal Mitochondria Labeling

L4 *fog-2(q71)* males were picked onto an OP50 plate and treated with 200 uL 0.05 mM working stock of MitoTracker Deep Red FM (Invitrogen) or MitoTracker Red CMXros (Invitrogen) in M9 overnight. Subsequently all the males were moved to a fresh OP50 plate and incubated for 15 minutes to “wash” away excess MitoTracker. This washing step was conducted three times to fully remove excess MitoTracker. After the third wash, the males were then moved to plates with hermaphrodites 24 hours before they were to be filmed or used for immunofluorescence. A minimum ratio of 1:1 males to hermaphrodites was used for matings.

L4 *sdhc-1::mCherry; him-5(e1490)* males were added to plates with hermaphrodites 24 hours before they were to be filmed. A minimum ratio of 1:1 males to hermaphrodites was used for matings.

HALO Ligand

At least 20 hrs before imaging, hermaphrodites expressing HaloTag were treated with 2.5 uM Janelia Fluor HaloTag Ligand 646 or 549 in M9 added to the bacterial lawn of an MYOB agar plate.

RNA interference

For RNA interference, L4 hermaphrodites were placed on RNAi plates with an RNAi bacterial lawn for a set period of time before being used for live imaging or fixed slides. In 48 hrs treatment, the worms were moved to a fresh RNAi plate after 24 hrs. RNAi plates were always seeded the day before adding worms. For *mei-1, atx-2, gfp,* and L4440 *(RNAi),* worms fed on RNAi bacterial lawns for 24-28 hrs. For *klp-7 (RNAi)* and its corresponding control L4440 *(RNAi),* worms fed on RNAi bacterial lawns for 48-52 hrs.

Auxin induced degradation

For auxin induced degradation, L4's of strains endogenously tagged with auxin-inducible degrons and a TIR1 transgene were placed on a fresh plate of OP50. After 24 hrs, the hermaphrodites were moved to auxin plates with lawns of OP50 for 1-3 hrs before use in live or fixed experiments. 4 mM auxin plates were made by adding 400 mM auxin (indole acetic acid) in ethanol to molten agar which was then poured and seeded with OP50 bacteria. Depletion of ATX-2::AID::GFP was confirmed by the reduction of of ATX-2::AID::GFP signal in -1 oocytes.

Double RNA interference and auxin induced degradation

In AID *klp-7 RNAi* experiments, L4's were placed on *klp-7 (RNAi)* and then transferred to a fresh *klp-7 (RNAi)* lawn after 24 hrs. After a total of 47 hrs of treatment,

the worms were moved to auxin/RNAi plates seeded *with klp-7 (RNAi).* 1 hr later the worms were then used in live or fixed experiments. Auxin/RNAi plates consisted of 4 mM auxin, 1 mM IPTG, and 200 ug/mL ampicillin in agar. Stocks of 400 mM auxin, 1 M IPTG, and 200 mg/mL ampicillin were added to molten agar and mixed to create the plates.

Fluorescence intensity measurements

The depletion of atx-2 was measured by taking the mean value of an ROI over the cytoplasm of -1 oocytes, taking caution to not include the nucleus. The z-stack at which the nucleus appeared most in focus was used for measurements in each embryo. The mean value of the cytoplasm was then subtracted by the mean value of an area not containing any part of the worm to correct for noise.

GFP::BAF-1 fluorescence was measured by manually tracing the outline of chromosomes during metaphase I and II and then taking the mean value fluorescence. The same ROI was also used to measure the mean value fluorescence of the middle of the embryo's cytoplasm. The mean value of the fluorescence over the chromosomes was then divided by the cytoplasmic mean value in order to measure and compare BAF-1 fluorescence in metaphase I vs anaphase I

Statistics

Shapiro-Wilks tests through GraphPad Prism were used to test for normality in all data in which statistical tests were used to compare means. If the test determined the data was normal, P values were calculated in GraphPad Prism using Welch's T tests for comparing means of only two groups and ANOVA tests for comparing means of three or more groups. If the data was not normal, P values were calculated in GraphPad Prism

using Mann-Whitney tests for comparing means of only two groups and Kruskal-Wallis tests for comparing means of three or more groups.

Acknowledgments

This work was supported by National Institute of General Medical Science grant R35GM136241 to FJM. We thank the Caenorhabditis Genetics Center, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440), for strains. We thank Marie Kim and Aastha Lele for assistance with mitochondrial scatter quantification.

Figure legends

Figure 1. Sperm contents exclude maternal yolk granules and mitochondria but not ER.

(A) Time-lapse in utero images of control embryo expressing GFP::SPCS-1 (signal peptidase) and mKate::TBA-2 (tubulin). ER morphology transitions from sheet-like during metaphase I and metaphase II to dispersed during anaphase I and anaphase II. (B) Image of a fixed metaphase I embryo expressing GFP::VIT-2 (maternal yolk granules), paternal mitochondria labelled with MitoTracker Deep Red FM, and stained with alpha tubulin antibody and DAPI. (C) Image of fixed late metaphase I embryo expressing GFP::SPCS-1 (maternal ER), paternal mitochondria labelled with MitoTracker Deep Red FM, and stained with alpha tubulin antibody and DAPI. (D) Image of a fixed metaphase I embryo expressing COX-4::GFP (maternal mitochondria), paternal mitochondria labelled with MitoTracker Deep Red FM, and stained with alpha tubulin antibody and DAPI. (E) Time-lapse in utero images of an embryo expressing HALO::ER (HALO tag with signal peptide and ER retention signal from HSP-3/BiP), VIT-2::GFP, paternal mitochondria labelled with SDHC-1::mCherry (succinate dehydrogenase). Images demonstrate sperm contents streaming in the short axis of the embryo. (A-E) Bars: (whole embryo) 10 um; (inset) 2 um.

Figure 2. Maternal mitochondria are still excluded from the sperm contents in *kca-1(RNAi)* embryos.

(A) Images of fixed meiotic embryos expressing COX-4::GFP (maternal mitochondria) that have been mated with MitoTracker Deep Red FM stained *fog-2*(q71) males. In the

control, maternal mitochondria are packed inward away from the cortex (n=11). Paternal mitochondria take up a volume excluding maternal mitochondria. In *kca-1(RNAi)* embryos, maternal mitochondria extend to the plasma membrane but paternal mitochondria still occupy a volume excluding maternal mitochondria (n=8). (B) Image of fixed *Caenorhabditis macrosperma* meiotic embryo mated with MitoTracker Deep Red FM stained males. The paternal mitochondria take up a larger volume than in *C. elegans* but the cohesion of mitochondria near paternal DNA is still conserved between species (n=4). (A-B) Bars: (whole embryo) 10 um; (inset) 5 um. White dotted boxes denote area of insets.

Figure 3. Maternal ER enters the sperm contents after a delay. (A and B) Two different time-lapse sequences of fertilization of oocytes expressing TMCO-1::GFP::SSPB (maternal ER) and mCherry::PH (maternal plasma membrane) by sperm labelled with MitoTracker Deep Red FM (paternal mitochondria). (A) Sperm-egg fusion occurs at 0:00. Maternal ER ER invades the sperm cytoplasm at 1:25. (B) The envelope of maternal ER around the sperm DNA is first visible at 7:24. Bars: (whole embryo) 10 um; (inset) 5 um. White dotted boxes denote area of insets.

Figure 4. Maternal BAF-1 associates with sperm DNA during anaphase I indicating that the ER envelope is not sealed. (A) Time-lapse in utero images of an embryo expressing mCherry::histone and GFP::BAF-1. Association of BAF-1 during anaphase indicates that maternal BAF-1 associates after assembly of the ER envelope. Bar, 5 um. (B) Fixed embryos during metaphase I and anaphase I. Maternal GFP::BAF-1 strongly localizes to both maternal and paternal chromosomes during anaphase I, but

not metaphase I. Bars: (whole embryo) 10 um; (inset) 2 um. (C) Fluorescence intensity of GFP::BAF-1 on maternal and paternal chromosomes. During anaphase I, there is an increase in GFP::BAF-1 on both the maternal and paternal chromosomes. ** $p<0.01$, *** $p<0.001$ by Mann-Whitney U Test.

Figure 5. MEI-1^{katanin} and KLP-7$^{kinesin\text{-}13}$ limit meiotic cytoplasmic streaming of the sperm contents. (A) Illustration of the types of movement that can be quantified from tracks of sperm contents. (B) Example time-lapse sequences showing movement of the maternal ER envelope around the sperm DNA and paternal mitochondria during meiosis. The bottom row shows MTrackJ tracks of the ER envelope around the sperm DNA over the length of meiosis. Cell cycle stage was determined from the morphology of the ER. Minimal displacement was observed during metaphase I (MI, red track). Most displacement was observed during anaphase I (AI, yellow track). In *klp-7(RNAi)*, movement continued through metaphase II (MII, green track) which was prolonged in *klp-7(RNAi)* embryos. Bar, 10 um. (C) Maximum 2D long axis displacement and maximum short axis 2D displacement during individual cell cycle stages. * $p< 0.05$, ** $p< 0.01$, ** $p< 0.001$ by Mann-Whitney U Test.

Figure 6. ATX-2 is depleted by 3 different methods. (A-C) Single plane images of living -1 oocytes. (A) Background autofluorescence of N2 (wild-type) with no GFP. Drawn dotted lines show the outline of oocytes and germinal vesicles (nuclei). (B-C) Live images of -1 oocytes in strain with endogenous ATX-2::AID::GFP treated with no auxin, 1 hr auxin, 27 hr control L4440(RNAi), 27 hr *atx-2(RNAi)* or 24 hr *gfp(RNAi)*.

Bars, 10 um. (D) Mean GFP fluorescence in the cytoplasm of -1 oocytes after each treatment. **** p< 0.0001 by Welch's t-test and Brown-Forsythe test.

Figure 7. Paternal mitochondria scatter during meiosis after ATX-2 depletion. (A-E) Maximum intensity projections of z-stacks of fixed meiotic embryos stained with tubulin antibodies and DAPI and with paternal mitochondria labeled by mating with MitoTracker Deep Red FM treated *fog-2(q71)* males. (A) N2 wild-type embryos treated with control *L4440(RNAi)* or *atx-2(RNAi)*. (B, C, E) Embryos expressing TIR1, GFP::SPCS-1, mKate::TBA-2, and with endogenously tagged ATX-2::AID::GFP. (B) 27 hr control *L4440(RNAi)* or *atx-2(RNAi)*. Arrows denote mitochondrial fluorescence from sperm outside the embryo overlapping with the embryo as a result of the maximum intensity projection. (C) No auxin or 1 hr auxin treatment. (D) *GFP(RNAi)* on strain with no tag on ATX-2 but expressing GFP::SPCS-1 and mKate::TBA-2. GFP::SPCS-1 fluorescence remains because SPCS-1 and ATX-2 are tagged with GFPs with different sequences. (E) Control *L4440(RNAi)* or *gfp(RNAi)* of ATX-2::AID::GFP strain. Bars, 10 um. White dotted outlines indicate the cortex of the cell.

Figure 8. Quantification of paternal mitochondrial scatter in ATX-2-depleted anaphase I meiotic embryos. (A) Mean and (B) standard deviation of the distance of individual paternal mitochondria from the sperm DNA determined from Z-stacks of fixed anaphase I embryos. Each dot represents one embryo. Distances for individual mitochondria are in Supplementary data file.

Figure 9. Capture of the sperm DNA by the meiotic spindle in KLP-7 ATX-2 double depleted meiotic embryos. (A) Low magnification and (B) high magnification

time-lapse images of 47 hr *klp-7(RNAi)* + 1 hr auxin meiotic embryo expressing GFP::SPCS-1 (ER), mKate::TBA-2 (Tubulin), ATX-2::AID::GFP and with paternal mitochondria labeled by mating MitoTracker Deep Red FM treated *fog-2(q71)* males. (C) Time-lapse images of a KLP-7 ATX-2 double depleted embryo in which the maternal ER envelope around the sperm DNA is transiently captured and stretched in the direction of cytoplasmic streaming. Bars: (whole embryo) 10 um; (inset) 5 um.

Supplemental Data

Supplemental data file

Excel spreadsheet of all numerical data values.

Figure S1. Distance of the sperm contents from the cortex of control embryos at metaphase I vs anaphase I.

Figure S2. Figure S2: Localization of ATX-2 in -1 oocyte and +1 meiotic embryo. Deconvolved single plane images from z- stacks acquired on a spinning-disk confocal. A. -1 oocyte (n=10). B. Metaphase meiotic embryo (n=10). C. Anaphase meiotic embryo (n=4). ER labeled with HALO-tag with the signal peptide and ER retention signal from HSP-3. Endogenous ATX-2::AID::GFP. mKate::Tubulin. Paternal mitochondria labeled with SDHC-1::mCherry. All Bars= 10um.

Figure S3. Cytoplasmic streaming after ATX-2 depletion. Maximum displacement of the sperm contents during any meiotic cell-cycle phase in ATX-2::AID::GFP embryos with or without 1 hr auxin.

Video S1. In utero time-lapse sequence of control embryo expressing GFP::SPCS-1 (ER in magenta) and mKate::tubulin (in green).

Video S2. Movement of sperm contents in control L4440(RNAi) meiotic embryo expressing GFP::SPCS-1 (ER in magenta), mKate::tubulin (not in focal plane), and paternal mitochondria labeled with Mitotracker Deep Rd (cyan). Track colors indicate cell-cycle phase as shown in Fig. 5B.

Video S3. Movement of sperm contents in *mei-1(RNAi)* meiotic embryo expressing GFP::SPCS-1 (ER in magenta), mKate::tubulin (green), and paternal mitochondria labeled with Mitotracker Deep Rd (cyan). Track colors indicate cell-cycle phase as shown in Fig. 5B.

Video S4. Movement of sperm contents in *klp-7(RNAi)* meiotic embryo expressing GFP::SPCS-1 (ER in magenta), mKate::tubulin (green), and paternal mitochondria labeled with Mitotracker Deep Rd (cyan).

Video S5. Movement of sperm contents, maternal ER and maternal yolk granules increases at anaphase onset when sheet-like ER disperses. In utero time-lapse sequence of meiotic embryo transitioning from metaphase I to anaphase I. ER labeled with HALO-ER (green), yolk granules labeled with GFP::VIT-2 (cyan), paternal mitochondria labeled with SDHC-1::mCherry (red).

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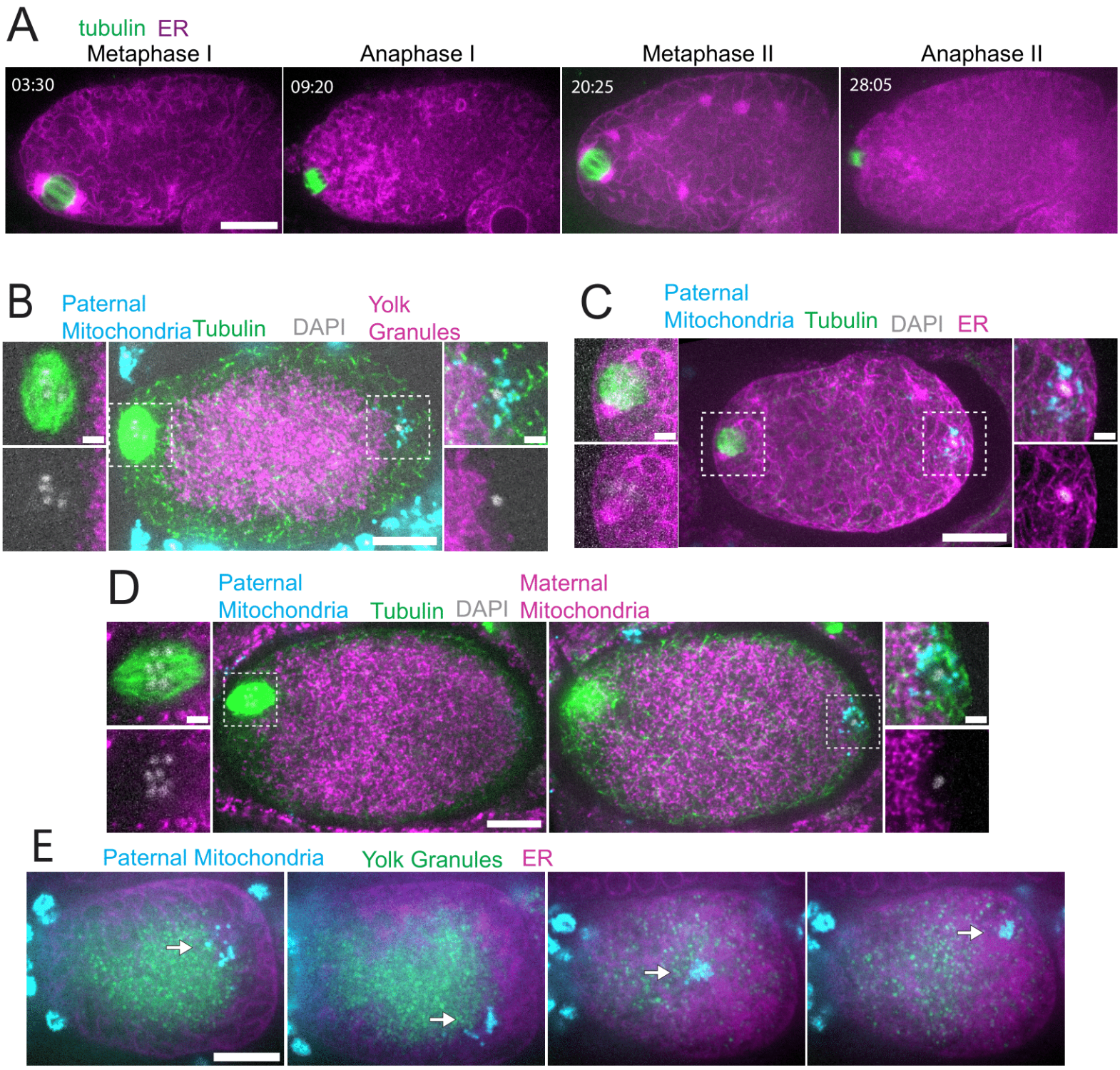


Figure 1. Sperm contents exclude maternal yolk granules and mitochondria but not ER.
(A) Time-lapse in utero images of control embryo expressing GFP::SPCS-1 (signal peptidase) and mKate::TBA-2 (tubulin). ER morphology transitions from sheet-like during metaphase I and metaphase II to dispersed during anaphase I and anaphase II. (B) Image of a fixed metaphase I embryo expressing GFP::VIT-2 (maternal yolk granules), paternal mitochondria labelled with MitoTracker Deep Red FM, and stained with alpha tubulin antibody and DAPI. (C) Image of fixed late metaphase I embryo expressing GFP::SPCS-1 (maternal ER), paternal mitochondria labelled with MitoTracker Deep Red FM, and stained with alpha tubulin antibody and DAPI. (D) Image of a fixed metaphase I embryo expressing COX-4::GFP (maternal mitochondria), paternal mitochondria labelled with MitoTracker Deep Red FM, and stained with alpha tubulin antibody and DAPI. (E) Time-lapse in utero images of an embryo expressing HALO::ER (HALO tag with signal peptide and ER retention signal from HSP-3/BiP), VIT-2::GFP, paternal mitochondria labelled with SDHC-1::mCherry (succinate dehydrogenase). Images demonstrate sperm contents streaming in the short axis of the embryo. (A-E) Bars: (whole embryo) 10 um; (inset) 2 um.

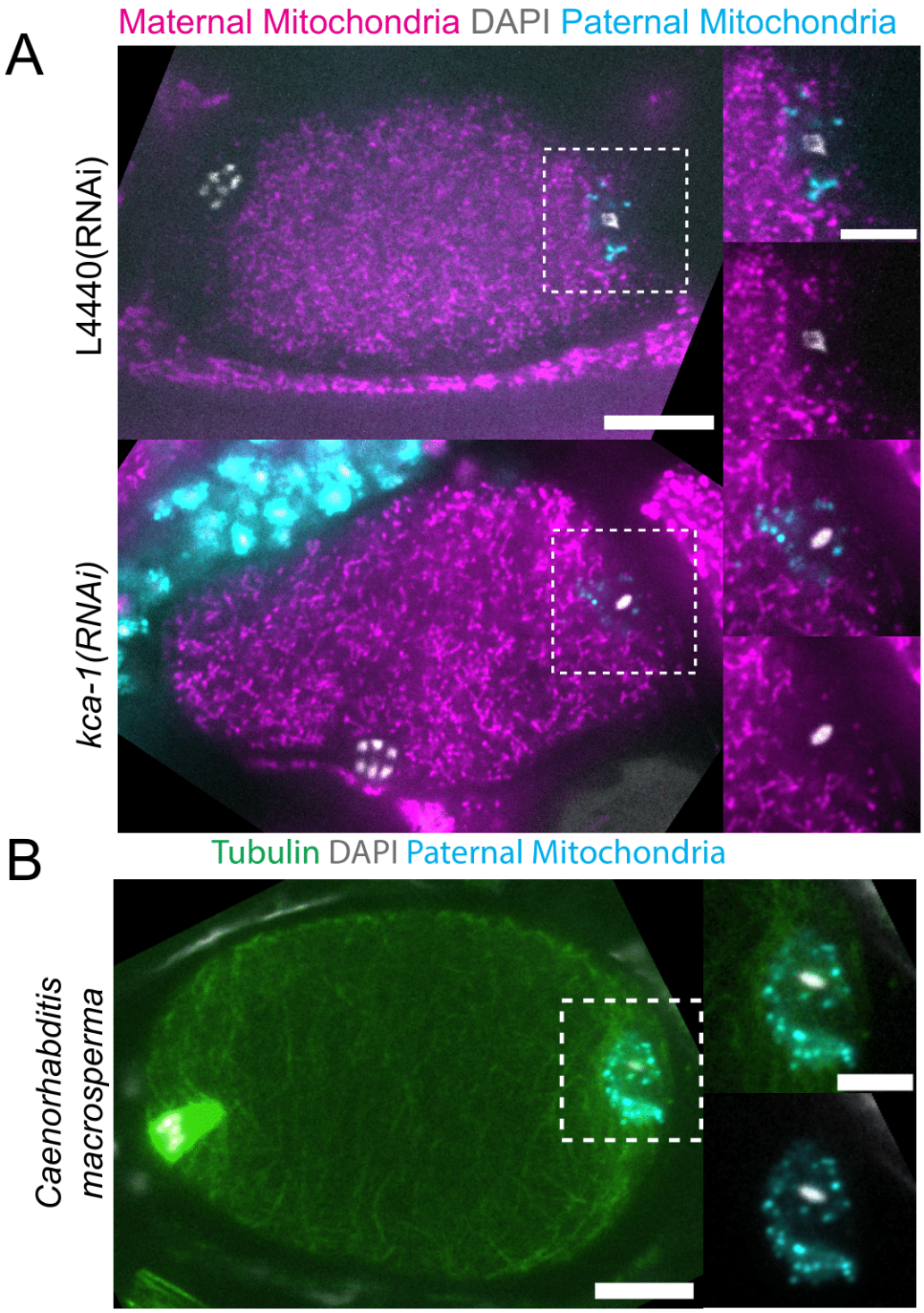
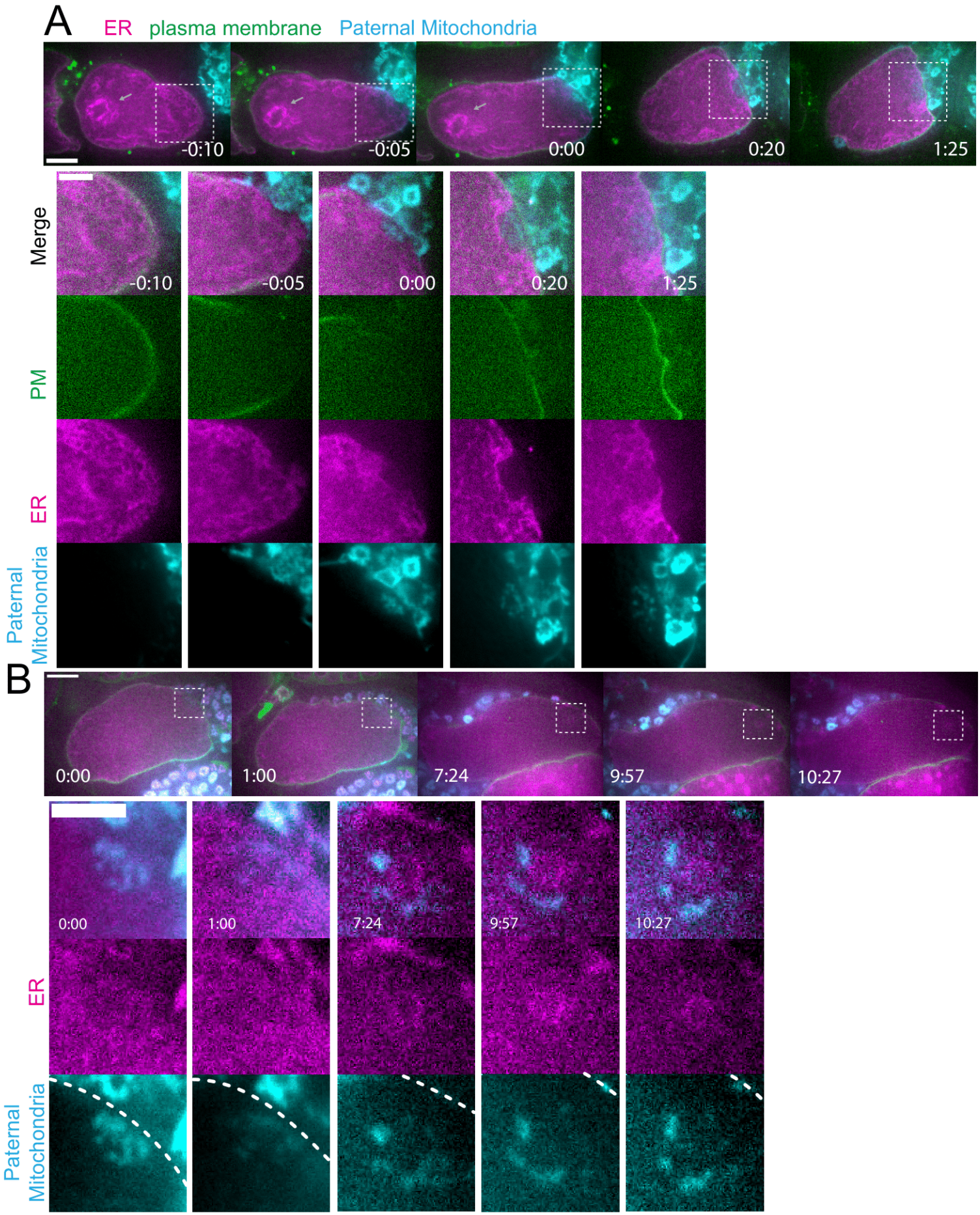


Figure 2. Maternal mitochondria are still excluded from the sperm contents in *kca-1(RNAi)* embryos.
(A) Images of fixed meiotic embryos expressing COX-4::GFP (maternal mitochondria) that have been mated with MitoTracker Deep Red FM stained *fog-2(q71)* males. In the control, maternal mitochondria are packed inward away from the cortex (n=11). Paternal mitochondria take up a volume excluding maternal mitochondria. In *kca-1(RNAi)* embryos, maternal mitochondria extend to the plasma membrane but paternal mitochondria still occupy a volume excluding maternal mitochondria (n=8). (B) Image of fixed Caenorhabditis macrosperma meiotic embryo mated with MitoTracker Deep Red FM stained males. The paternal mitochondria take up a larger volume than in C. elegans but the cohesion of mitochondria near paternal DNA is still conserved between species (n=4). (A-B) Bars: (whole embryo) 10 um; (inset) 5 um. White dotted boxes denote area of insets.



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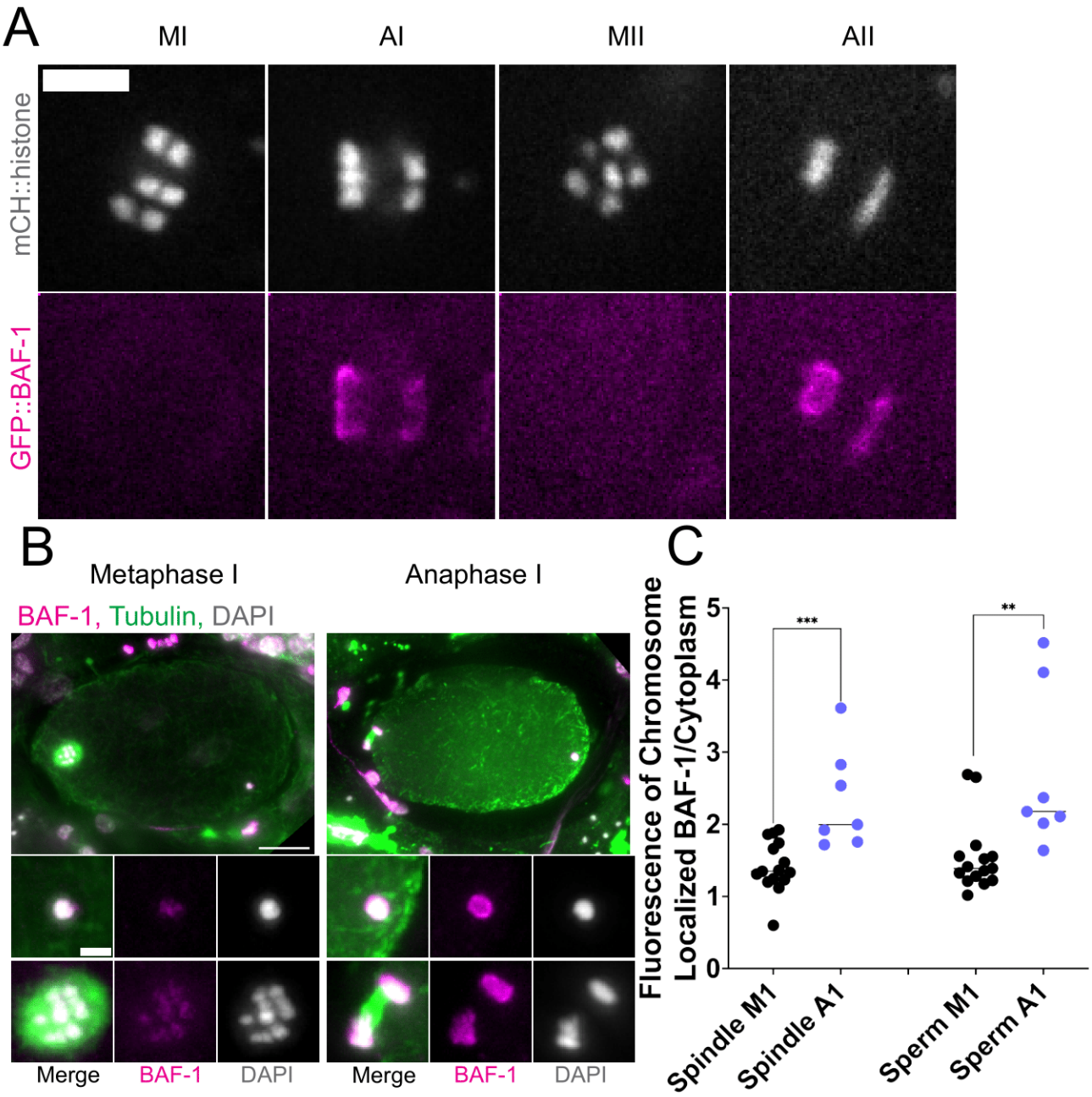

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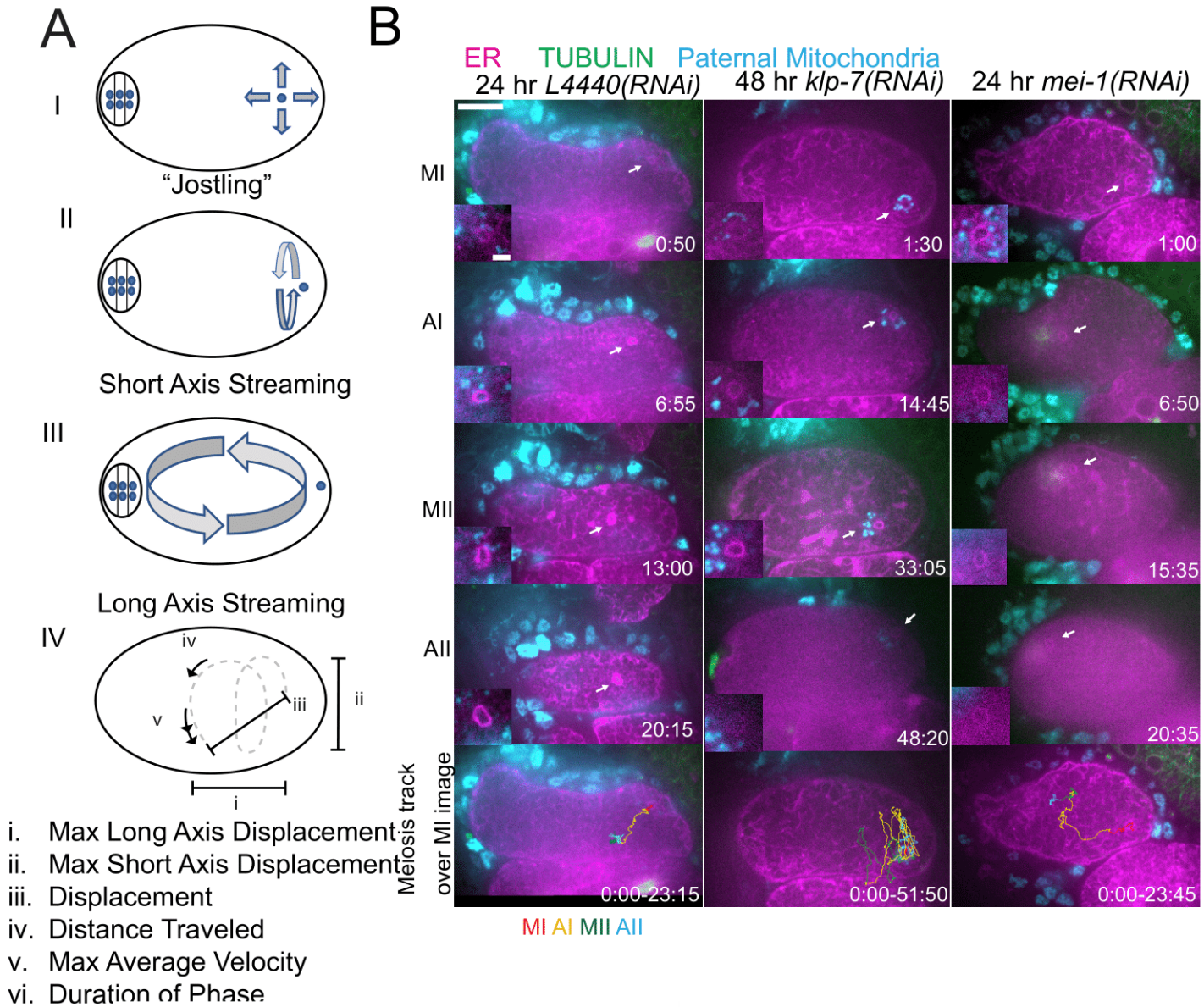

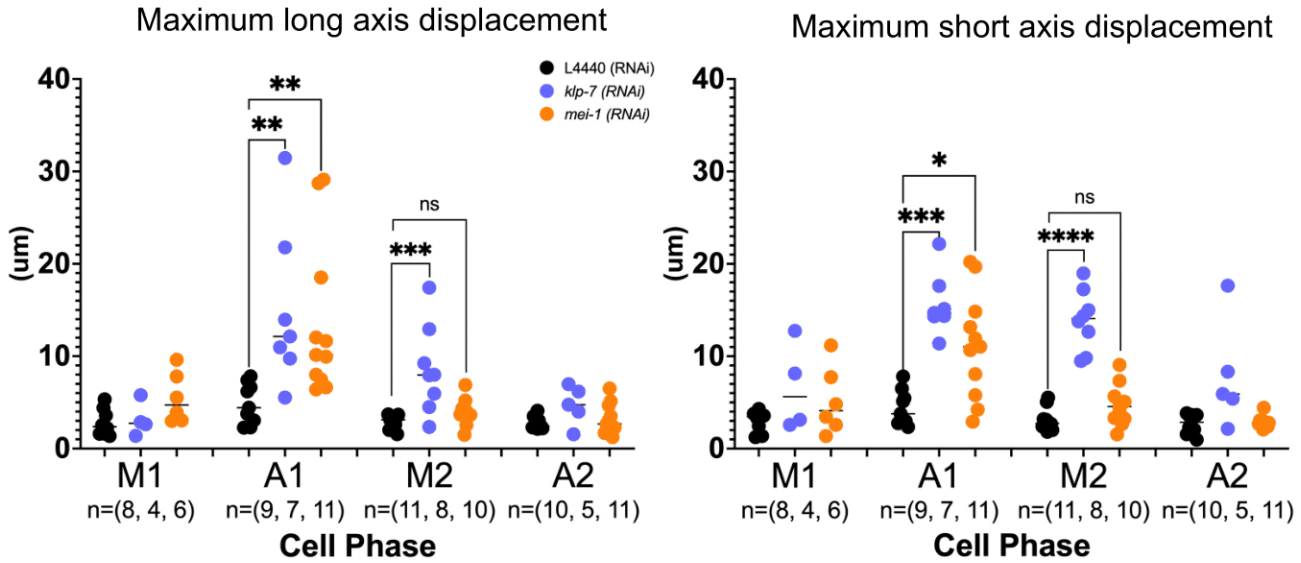

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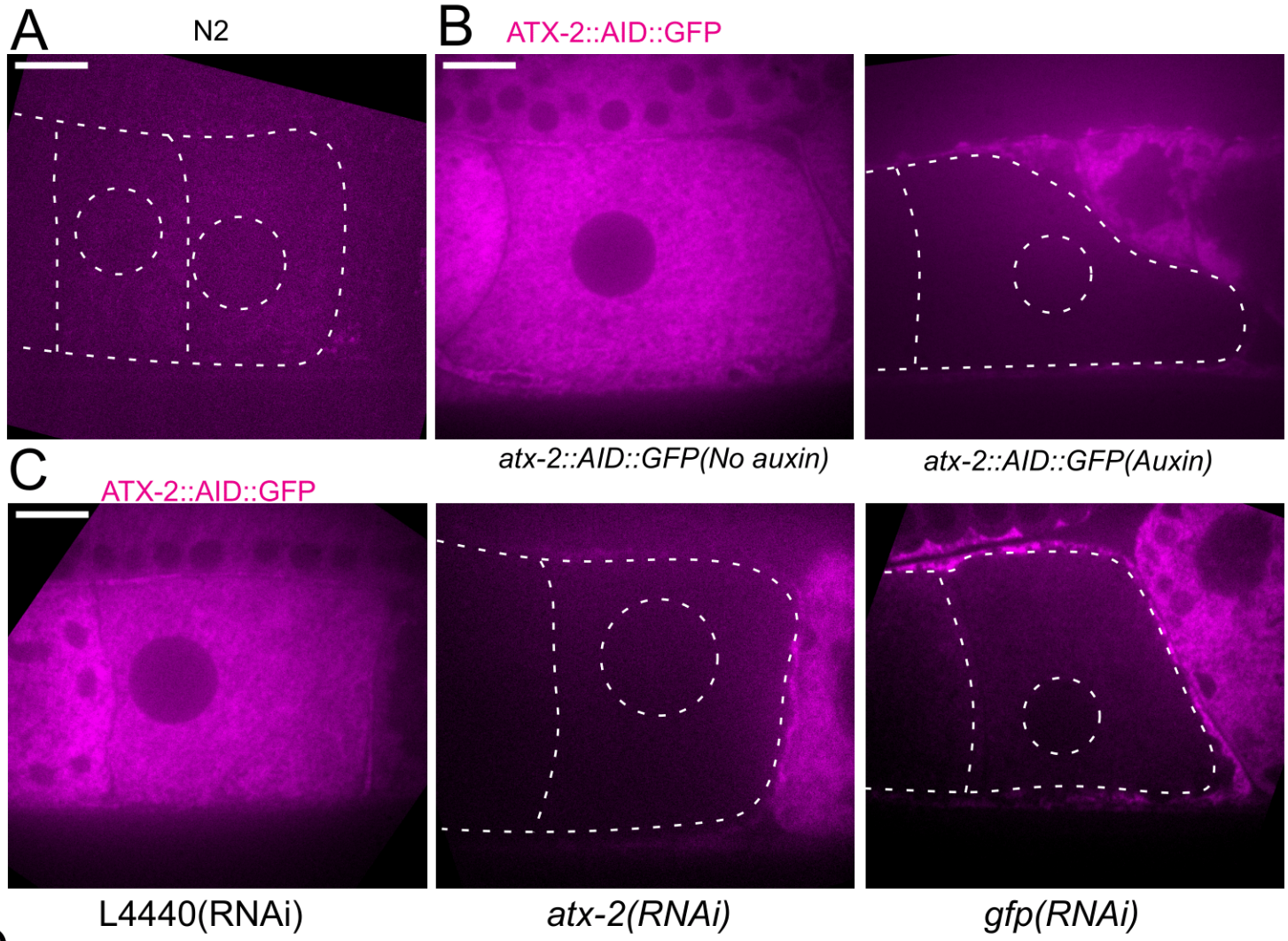

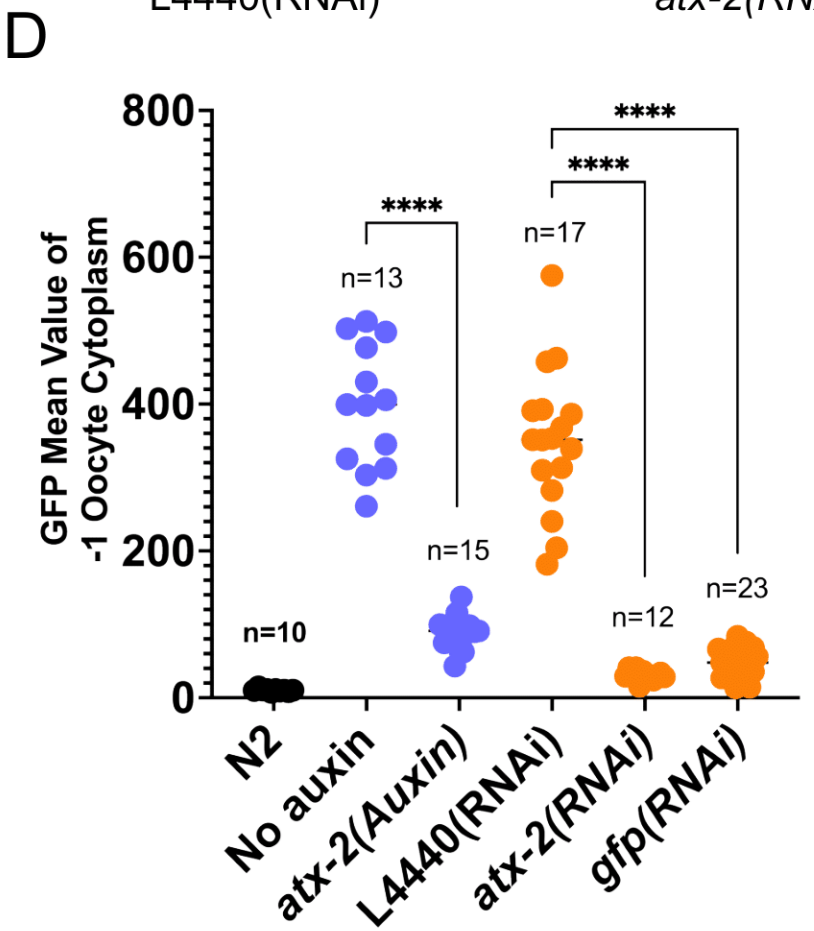

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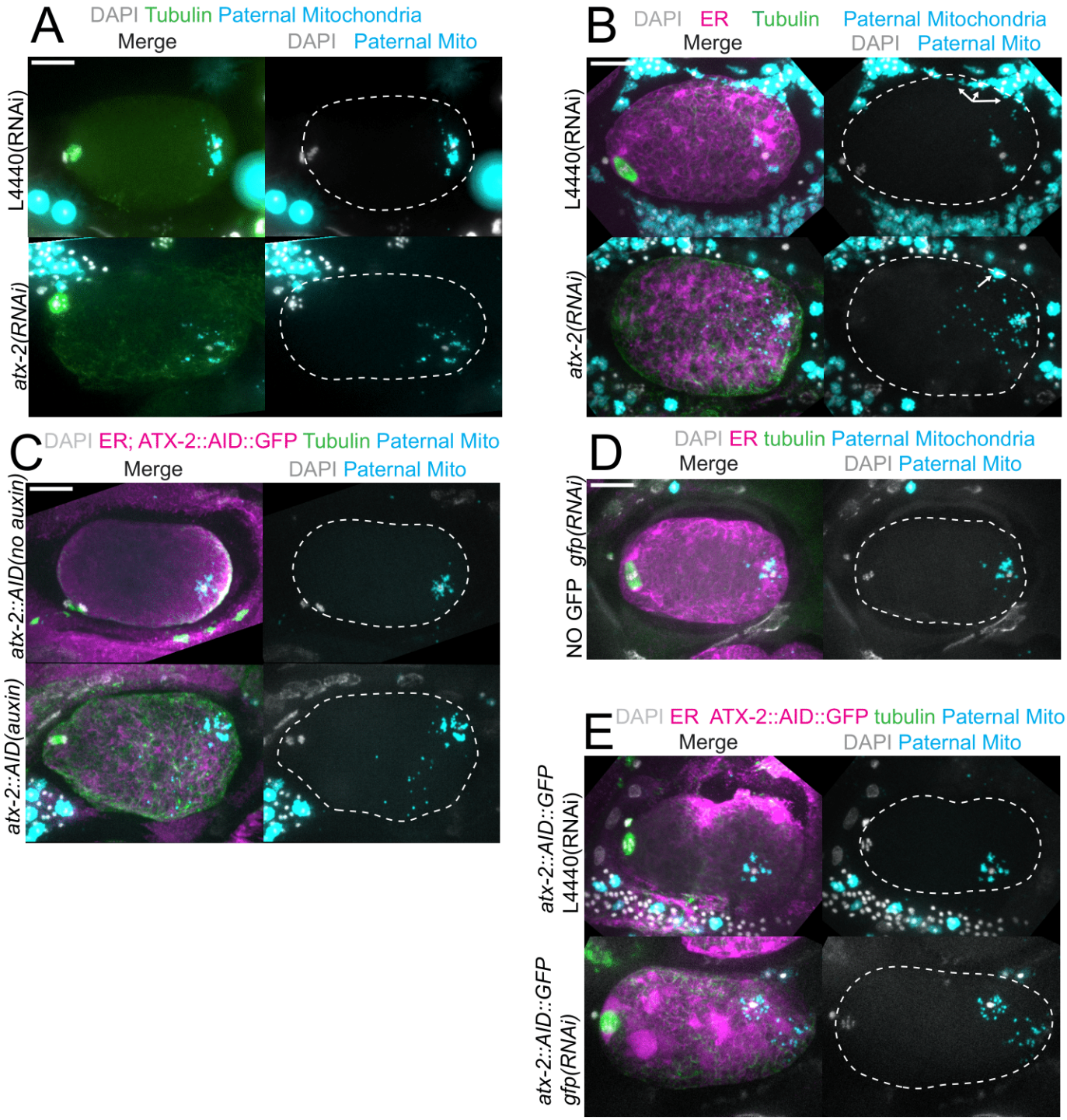

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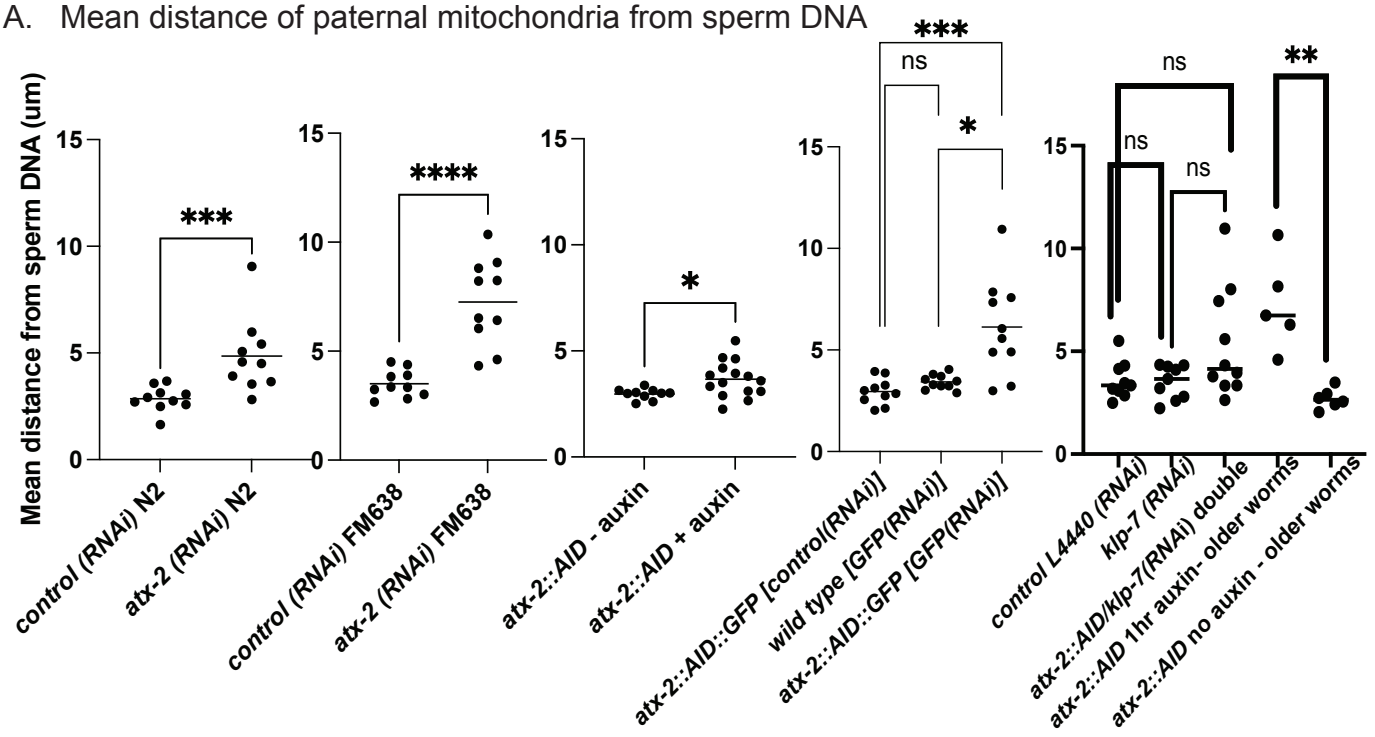
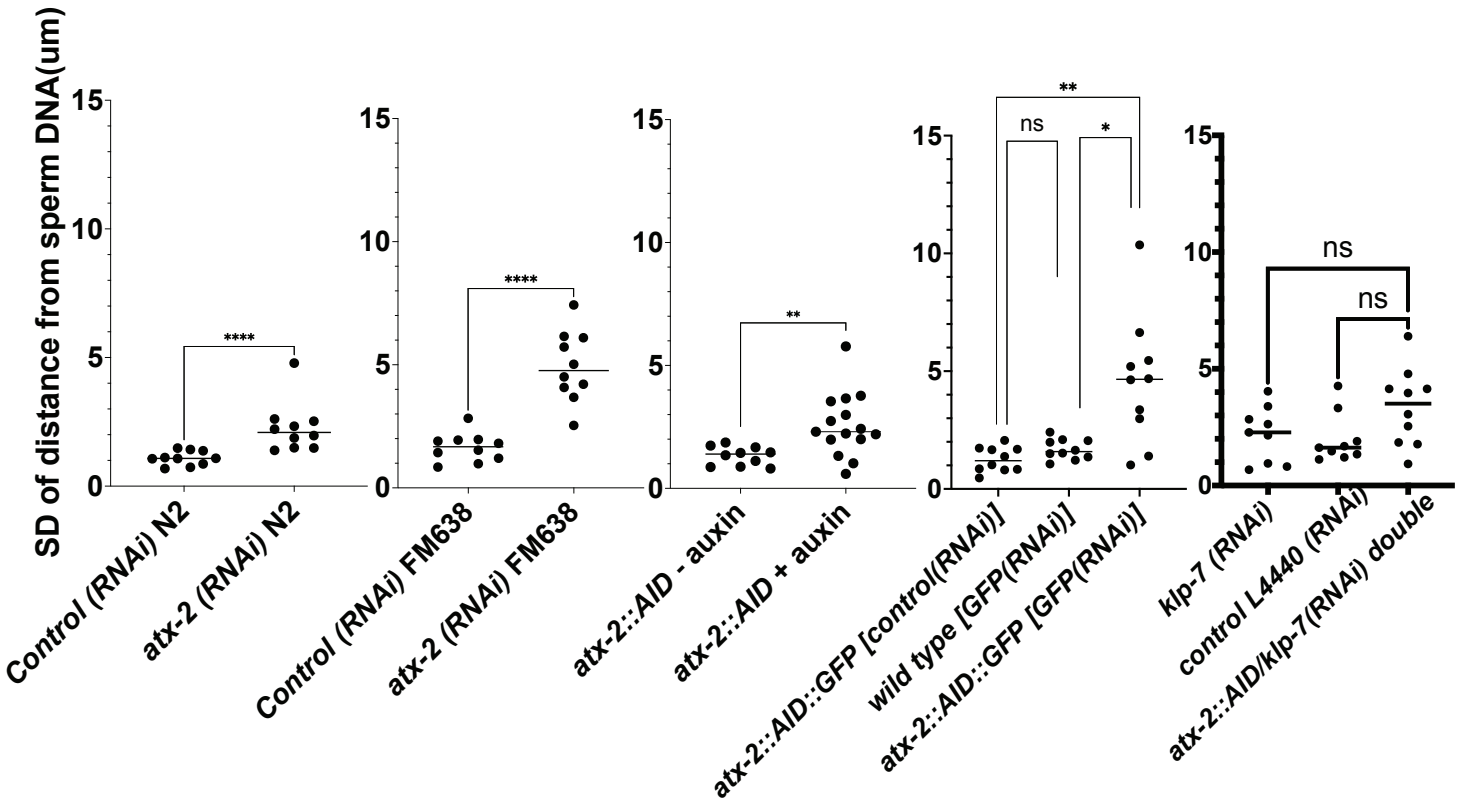
A. Mean distance of paternal mitochondria from sperm DNA



B. Standard Deviation of distance of paternal mitochondria from sperm DNA



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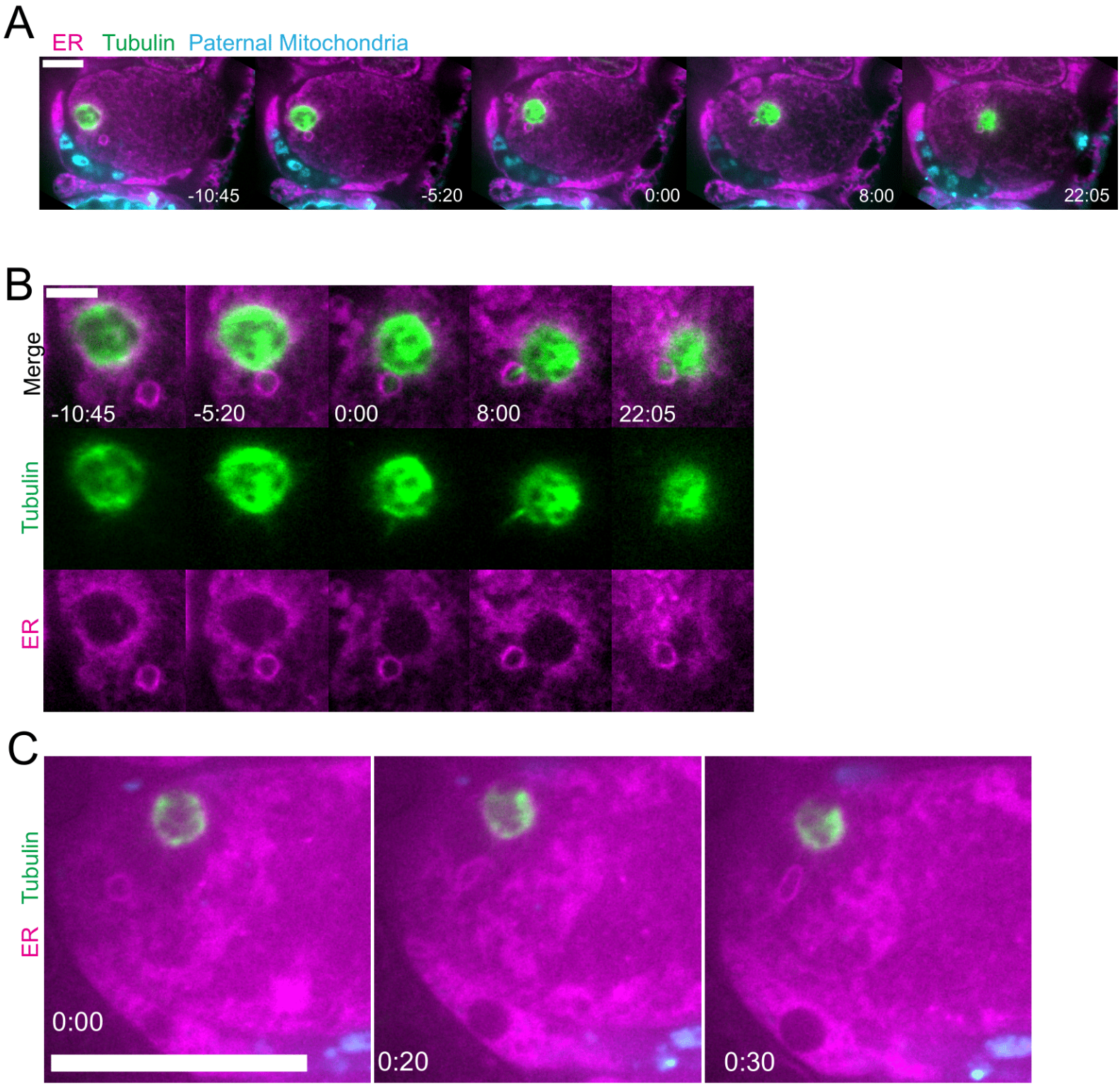

Figure 9. Capture of the sperm DNA by the meiotic spindle in KLP-7 ATX-2 double depleted meiotic embryos. (A) Low magnification and (B) high magnification time-lapse images of 47 hr klp-7(RNAi) + 1 hr auxin meiotic embryo expressing GFP::SPCS-1 (ER), mKate::TBA-2 (Tubulin), ATX-2::AID::GFP and with paternal mitochondria labeled by mating MitoTracker Deep Red FM treated *fog-2(q71)* males. (C) Time-lapse images of a KLP-7 ATX-2 double depleted embryo in which the maternal ER envelope around the sperm DNA is transiently captured and stretched in the direction of cytoplasmic streaming. Bars: (whole embryo) 10 um; (inset) 5 um.

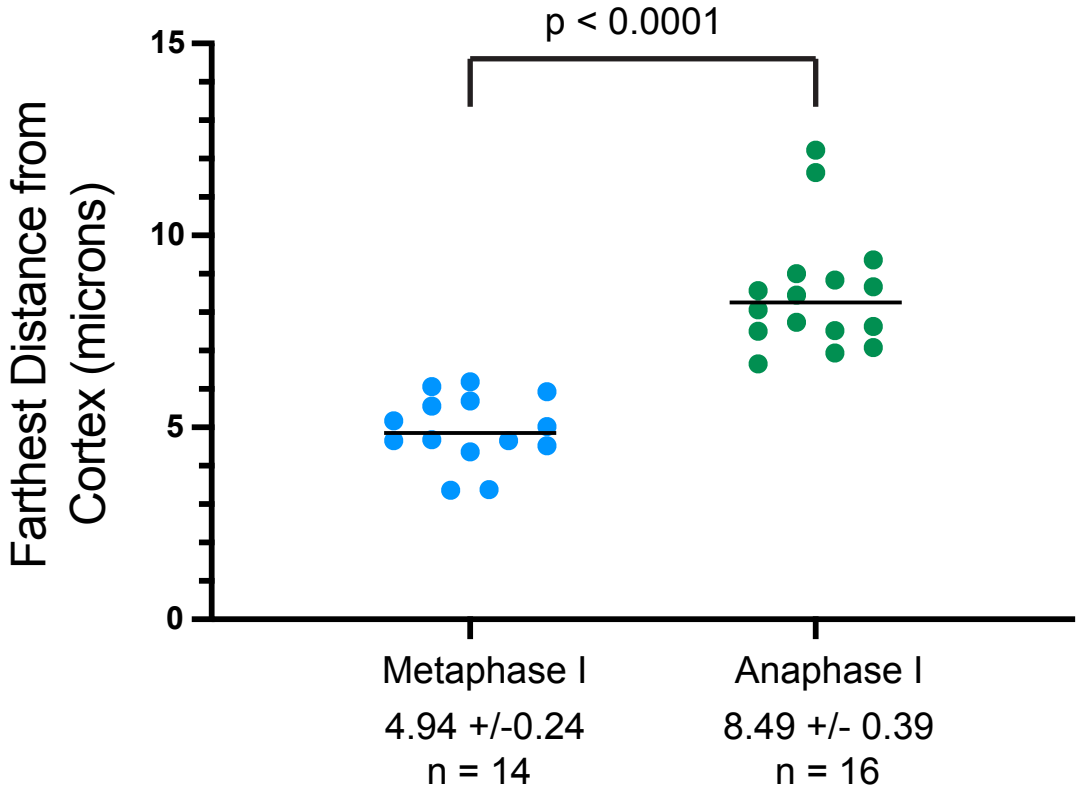

Figure S1. Distance of the sperm contents from the cortex of control embryos at metaphase I vs anaphase I.

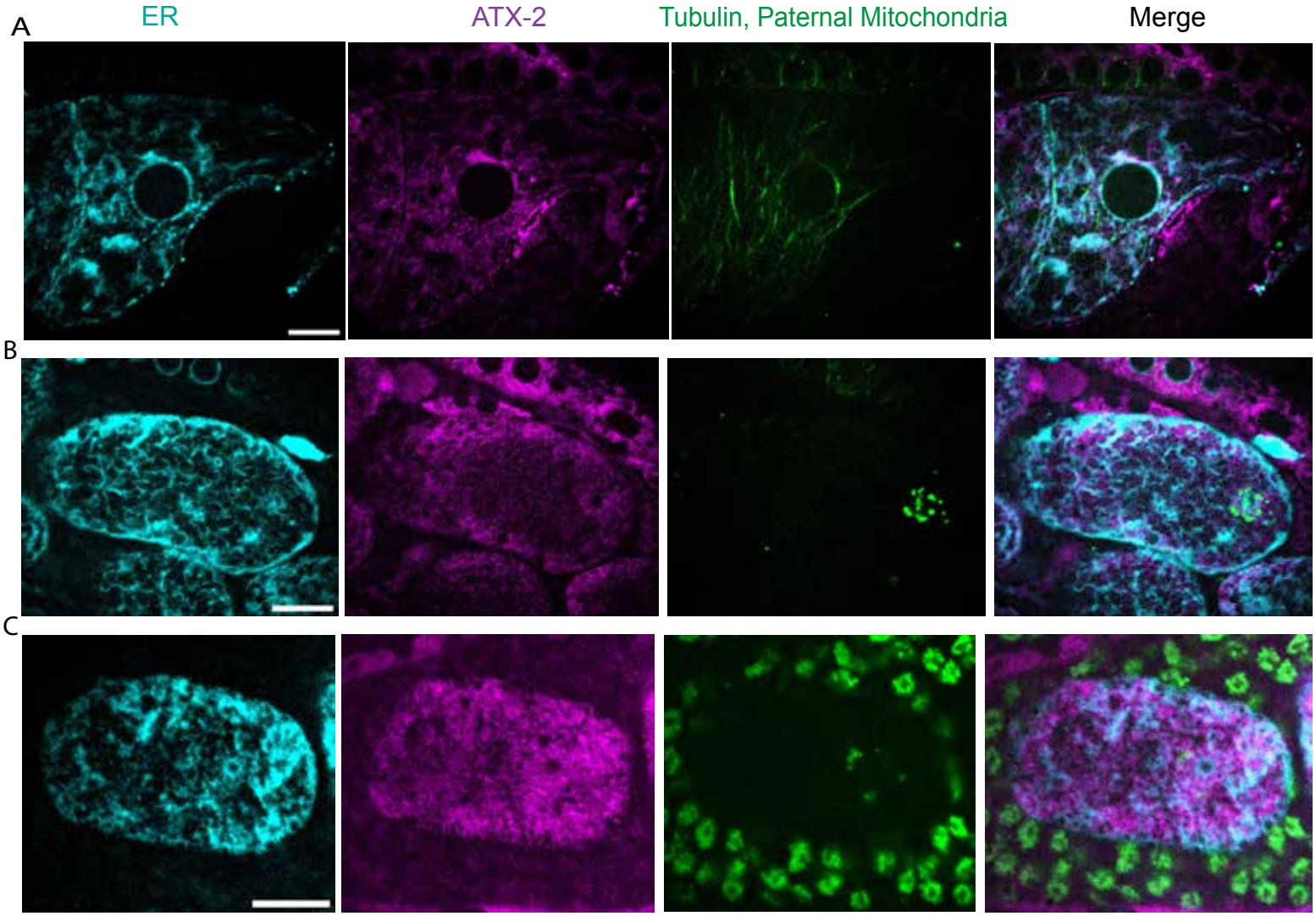

Figure S2: Localization of ATX-2 in -1 oocyte and +1 meiotic embryo. Deconvolved single plane images from z- stacks acquired on a spinning-disk confocal. A. -1 oocyte (n=10). B. Metaphase meiotic embryo (n=10). C. Anaphase meiotic embryo (n=4). ER labeled with HALO-tag with the signal peptide and ER retention signal from HSP-3. Endogenous ATX-2::AID::GFP. mKate::Tubulin. Paternal mitochondria labeled with SDHC-1::mCherry. All Bars= 10um.

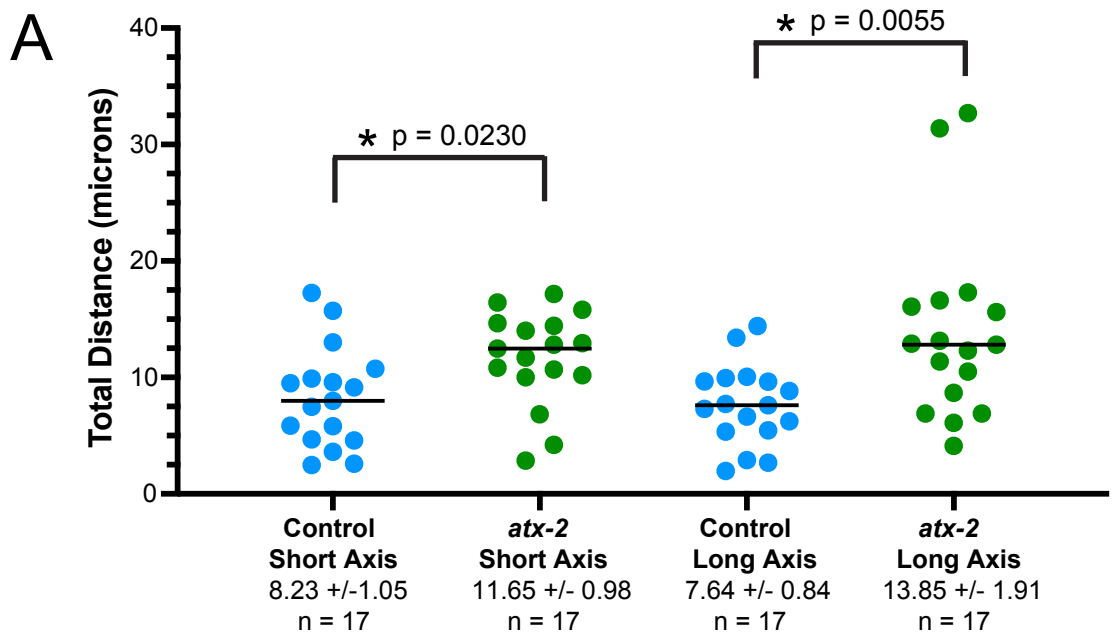

Figure S3. Cytoplasmic streaming after ATX-2 depletion. Maximum displacement of the sperm contents during any meiotic cell-cycle phase in ATX-2::AID::GFP embryos with or without 1 hr auxin.