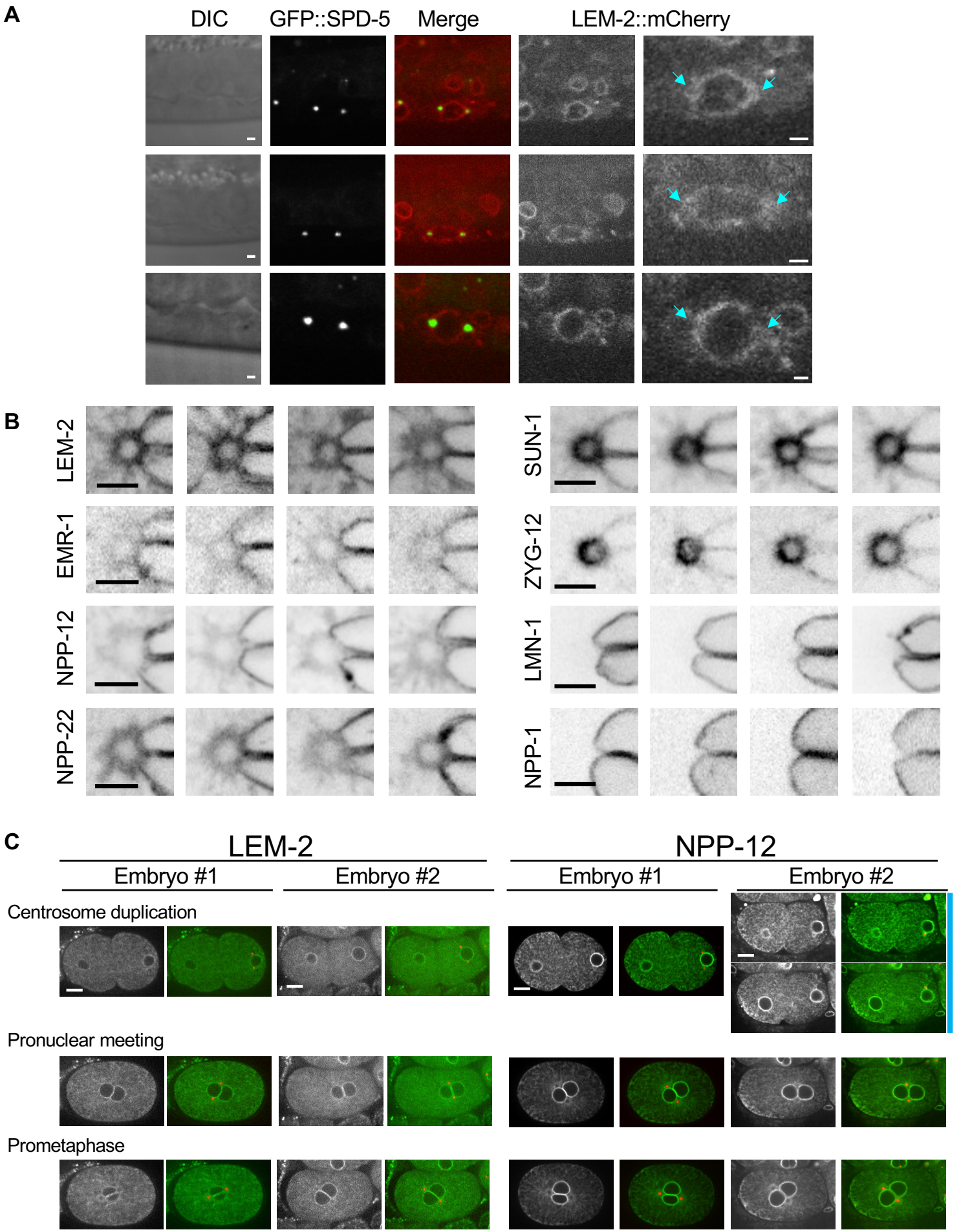

A
DIC
GFP::SPD-5
Merge
LEM-2::mCherry
B
LEM-2
EMR-1
NPP-12
NPP-22
SUN-1
ZYG-12
LMN-1
NPP-1
C
LEM-2
NPP-12
Embryo #1
Embryo #2
Embryo #1
Embryo #2
Centrosome duplication
Pronuclear meeting
Prometaphase

Figure S1: The presence of nuclear envelope proteins around centrosomes, related to Figure 1 (A) Vulval precursor cell from a L3 stage C. elegans larvae expressing endogenously tagged NE/ER protein LEM-2 fused to mCherry and PCM protein SPD-5 fused to GFP (strain OCF173). The images on the far right are an enlargement of the area around the nucleus. Arrows point to the membrane in the vicinity of the centrosome. Unlike the centrosomes in early embryo (Figure 1), which are surrounded by ER membrane, in the vulval precursor cells the centrosomes are associated with ER membrane, but may not be fully surrounded by it. Scale bars= 10 µm. (B) Additional examples to those shown in Figure 1C of NE protein localization relative to the membrane around the centrosome. Scale bars= 5µm. (C) Two examples of the distribution of LEM-2::GFP (OCF174) and NPP-12::mNeonGreen (OCF179) (green in the merged image) during the indicated stages of the first embryonic division. Embryos also express SPD-2::mCherry to label the centrosome (red in the merged image). The grey scale images are of the NE protein alone. The top two images in the right-most column (vertical blue line) are of the same embryo but in two different focal planes. A time course of SUN-1 at around centrosomes is shown in Figure 1D. Scale bar = 10 µm.

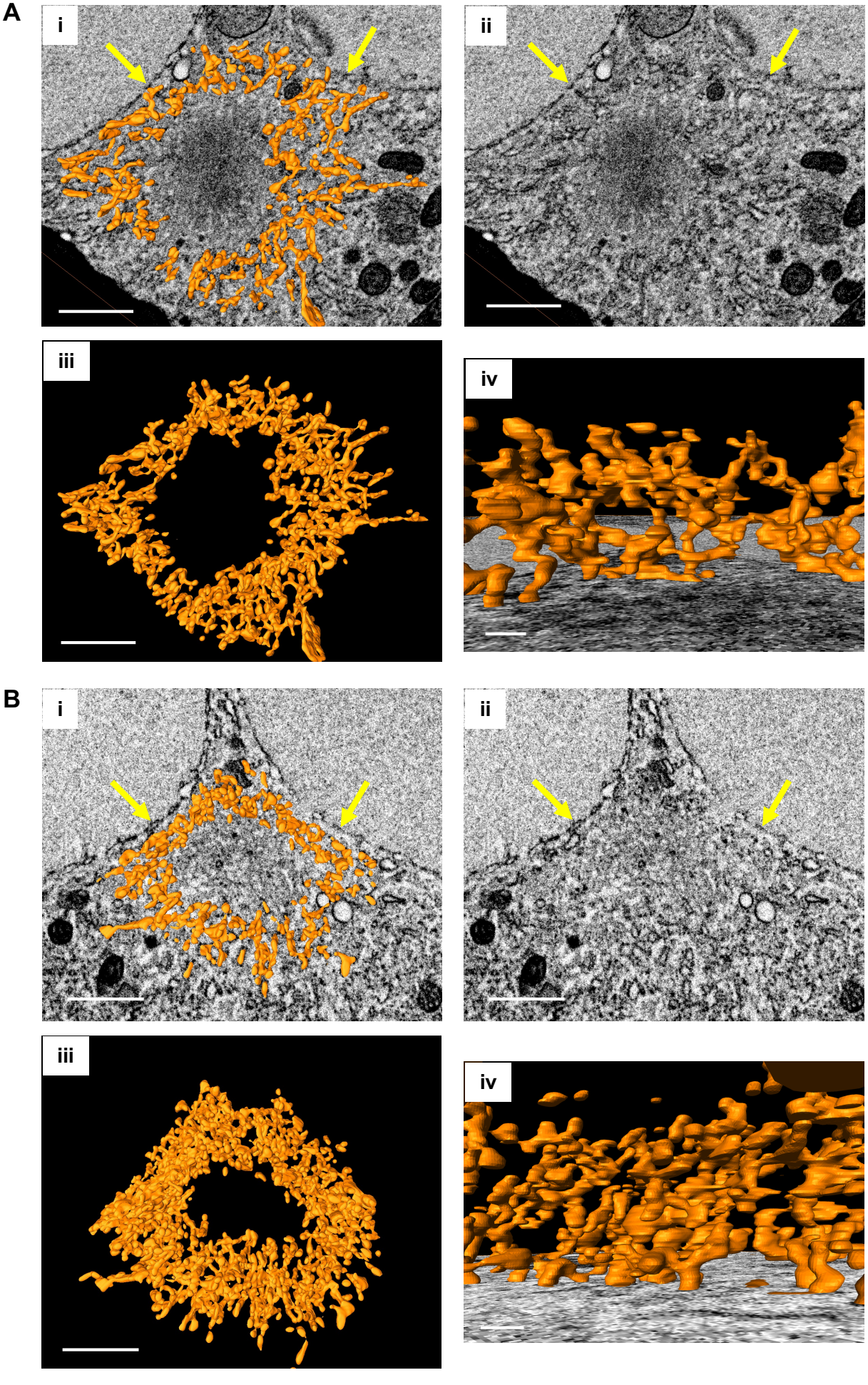

A
i
ii
iii
iv
B
i
ii
iii
iv

Figure S2: Centricula in prophase embryos, related to Figure 2. (A, B) Two centricula were segmented (orange) from 1-cell embryos at prophase as described in the main text and in the legend to Figure 2. (i, ii) a single SEM plane with (i) or without (ii) a superimposed 200 nm segmented centriculum section from planes above the SEM image shown. Arrows point to the nuclear envelope. (iii) An 800 nm slice of the centriculum. (iv) A view through the entire centriculum wall between the centrosome (forefront) and the pronuclei (background). Scale bar = 1 μm except panel iv, where scale bar = 100 nm.

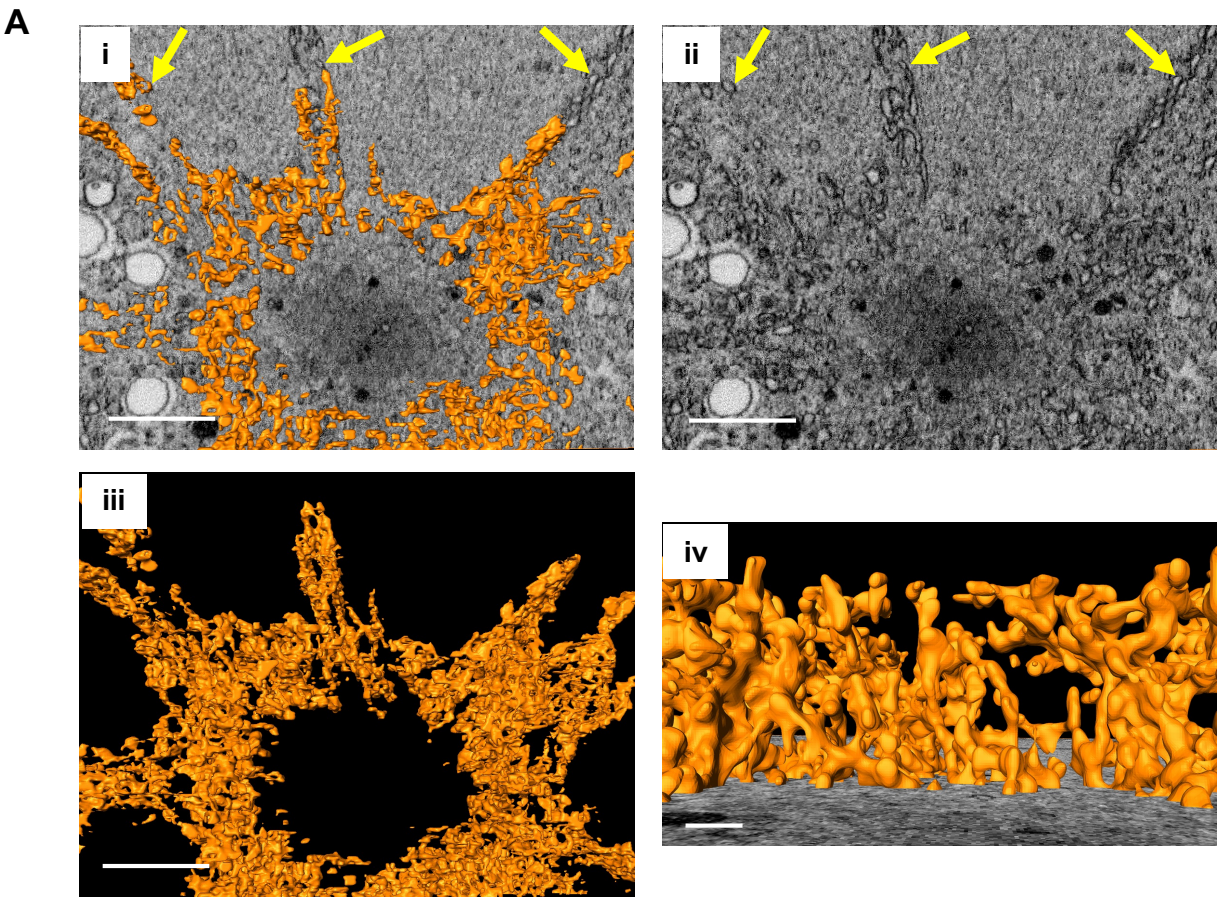

A
i
ii
iii
iv
B
i
ii
iii
iv

Figure S3: Centricula in metaphase embryos, related to Figure 2. (A, B) Two centricula were segmented (orange) from 1-cell embryos at metaphase as described in the main text and in the legend to Figure 2. The panels are as described in the legend for Figure S2. Scale bar = 1 μm except panel iv, where scale bar = 100 nm.

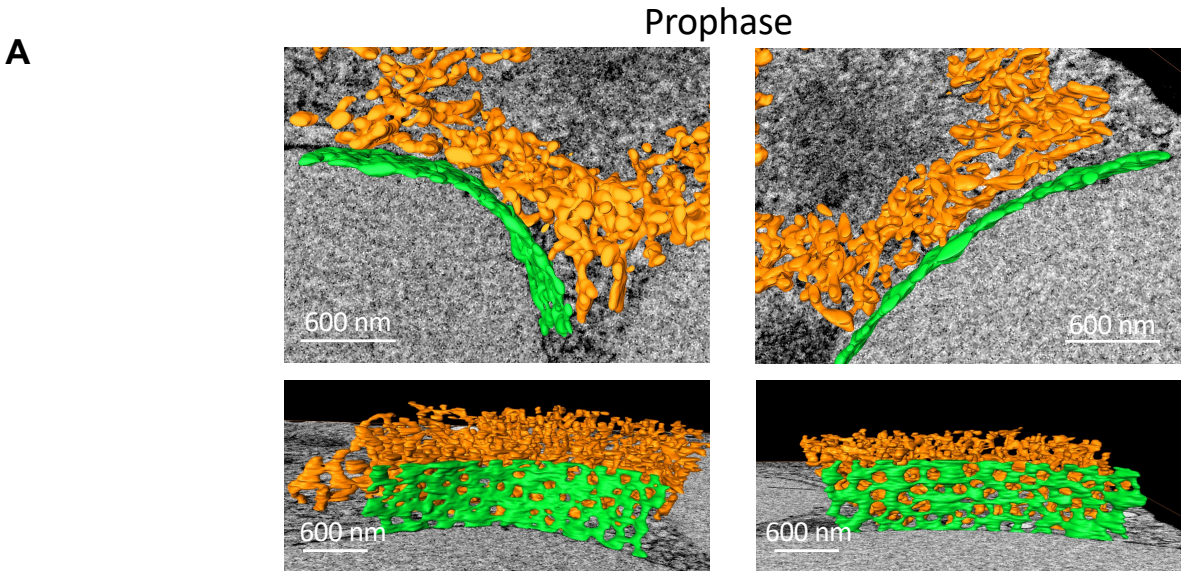

A
Prophase
600 nm
600 nm
600 nm
600 nm
B
Metaphase
i
ii
1000 nm
C
i
ia
150 nm
ii
iia
iib
200 nm
D
ia
50 nm
iia
50 nm
iib
50 nm

Figure S4: The centriculum at metaphase is continuous with the pronuclear membrane, related to Figure 2. (A) Segmentation of two centricula (in orange) and their adjacent pronuclear membranes (green) from embryos at prophase viewed from a cross section through the centricula and pronuclei (top row) and from inside the pronuclei (bottom row). (B-D) A representative example of a centriculum (orange) and two adjacent sections of the pronuclear membranes (green) from an embryo at metaphase viewed from the pronuclear interface. The centriculum was segmented starting from the centrosome and moving outwards. The pronuclear membranes were segmented starting at a distance from the centriculum and moving towards it. The locations of the junctions between the pronuclear membrane and centriculum are ambiguous because the membranes are continuous. Insets i and ii (enlarged in panel C) show locations where the inner and outer nuclear membranes run into the centriculum membrane. (D) Enlargement of the insets ia, iia and iib shown in panel C.

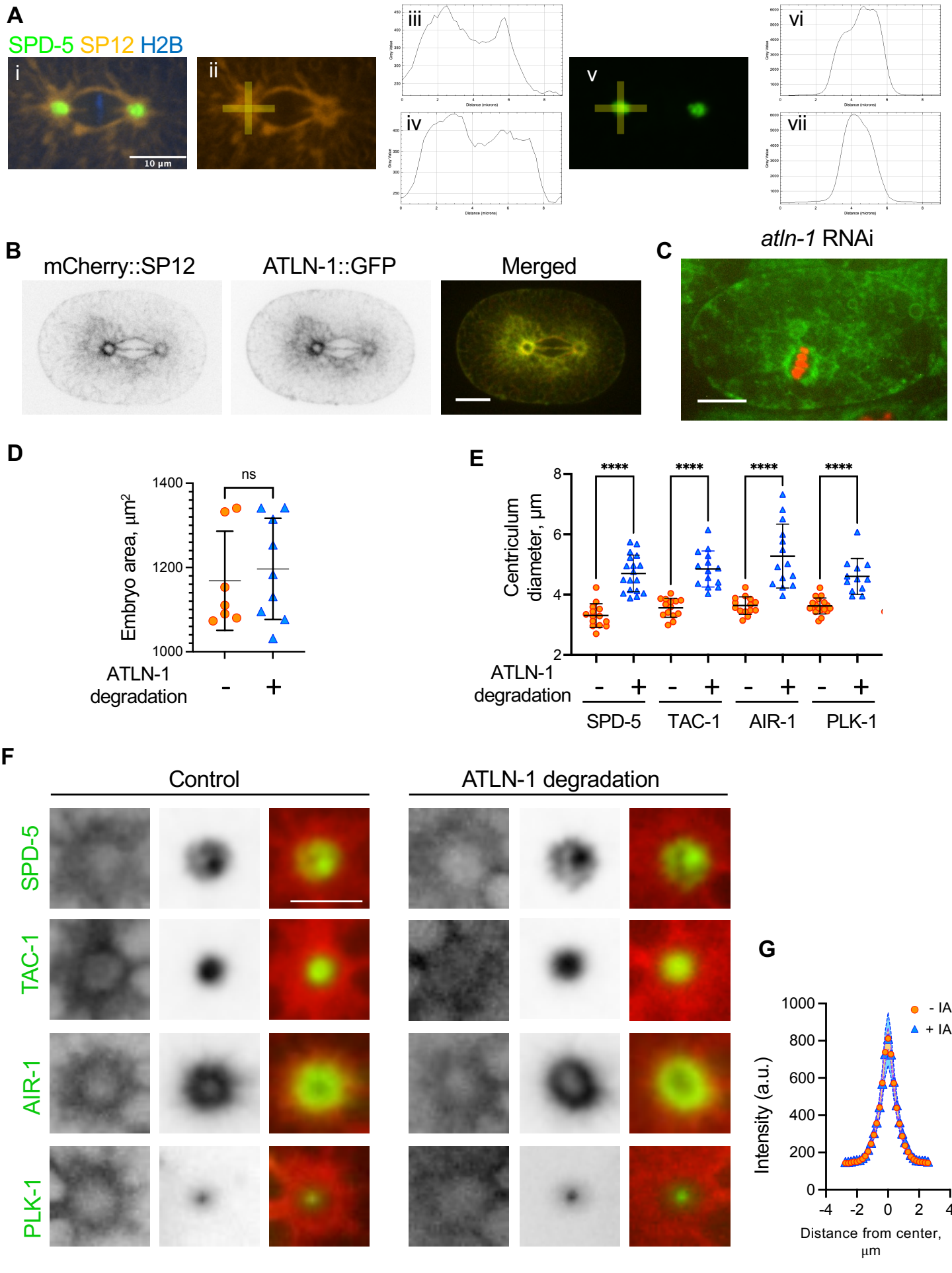
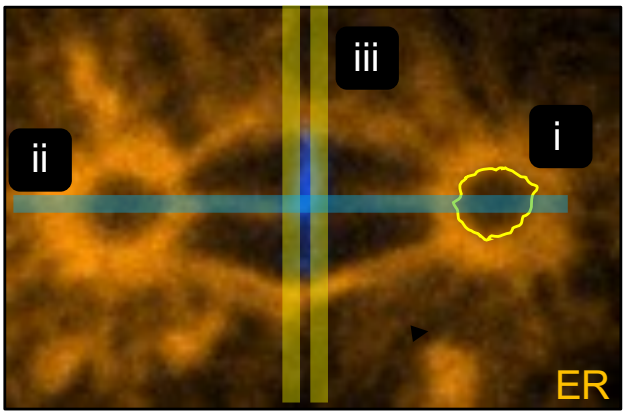
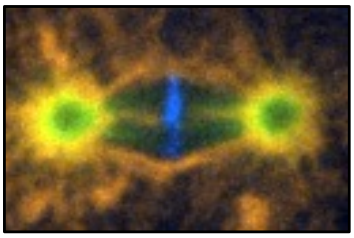
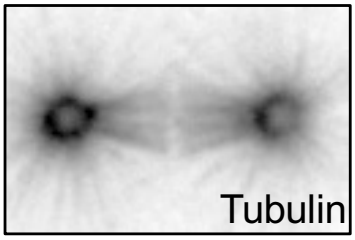

A
SPD-5 SP12 H2B
i
ii
iii
iv
v
vi
vii
10 µm
B
mCherry::SP12
ATLN-1::GFP
Merged
C
atln-1 RNAi
D
ns
Embryo area, µm²
1400
1200
1000
ATLN-1 degradation
-
+
E
****
Centriculum diameter, µm
8
6
4
2
ATLN-1 degradation
SPD-5
TAC-1
AIR-1
PLK-1
F
Control
ATLN-1 degradation
SPD-5
TAC-1
AIR-1
PLK-1
G
- IAA
+ IAA
Intensity (a.u.)
1000
800
600
400
200
0
-4
-2
0
2
4
Distance from center, µm

Figure S5: The centriculum affects centrosome structure, related to Figures 3 and 5. (A) Quantification of centriculum and centrosome protein fluorescence using line scanning: in the example shown, a 1-cell embryo at metaphase expressing the centrosomal protein GFP::SPD-5, the ER marker mCherry::SP12 and mTurquoise2::H2B was imaged by confocal microscopy (panel i, OCF176). Analyses were done using Fiji software as described under STAR Methods. Two perpendicular lines were drawn at the center planes of the mCherry::SP12 image (panel ii) or the GFP::SPD-5 image (panel v), with the horizontal line parallel to the interface between the two pronuclei, and traced for fluorescence intensities (panels iii, iv, vi and vii; top and bottom panels are the traces of the vertical and horizontal lines, respectively). The final traces, such as the ones in Figure 3, are the means and 95% confidence intervals of vertical and horizonal traces from the indicated number of centrosomes/centricula (for example, 12 traces from n=6 centrosomes). (B) A 1-cell embryo (strain OCF108) expressing mCherry::SP12 (left panel) and endogenously tagged ATLN-1::GFP (center panel), which co-localizes with SP12 (merged image, right). Scale bar= 10 µm. (C) The effect of ATLN-1 downregulation by feeding RNAi. Worms expressing SP12::GFP (green) and histone H2B fused to mCherry (red; strain OCF5) were exposed to RNAi against atln-1 for 48 hours and embryos were imaged. The effect of mild RNAi treatment is shown in Figure 5F and G. Scale bar = 10 µm. (D) The sizes of 1-cell embryos taken from worms expressing GFP::SPD-5, mCherry::SP12, mTurquoise2::H2B and atln-1::degron (strain OCF164), without (-) and with (+) IAA treatment, were measured as described in under STAR methods. n= 7 and 9 embryos for – and + IAA treatment, respectively. p=0.6065, as determined by Mann Whitney test. (E) Centriculum diameter in 1-cell metaphase embryos expressing atln-1::degron and the indicated fluorescently-tagged centrosomal proteins, without (orange) or with (blue) IAA treatment. The number of centricula analyzed (-/+ IAA) were as follows: SPD-5 (OCF164): 14/18; TAC-1 (OCF167): 14/14; AIR-1 (OCF172): 14/14; and PLK-1 (OCF166): 18/12. These data are from the same experiments described in Figure 5K and L. Error bars here and in all subsequent graphs in this figure indicate mean and standard deviation. All p values <0.0001, as determined by one-way ANOVA with multiple comparisons. (F) Distribution of the indicated centrosomal proteins fused to GFP in 1-cell metaphase embryos also expressing atln-1::degron in the presence or absence of IAA. The quantification of the area occupied by these proteins and their fluorescence intensity are shown in Figure 5K and L. (G) Fluorescence intensity of PLK-1::GFP along a line that traverses the center of the centrosome in strain OCF166, also expressing atln-1::degron, without (orange) or with (blue) IAA. The size of the area occupied by PLK-1::GFP in this experiment is shown in Figure 5K. Dashed lines indicate 95% confidence interval.

A

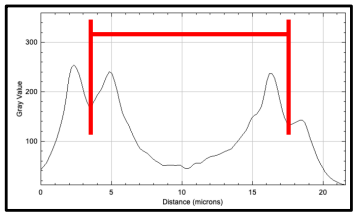




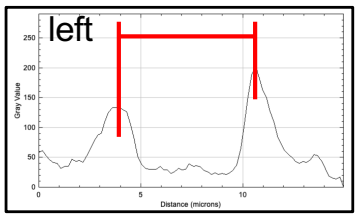


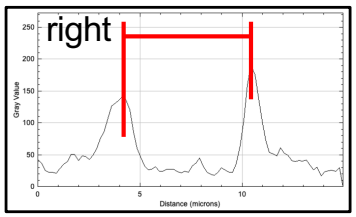
i. Void area

ii. Spindle length

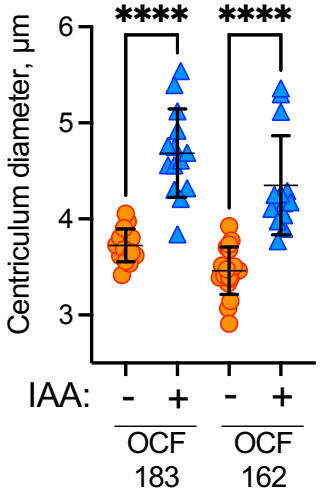


iii. Spindle width (0-0.5 µm from chromosomes)

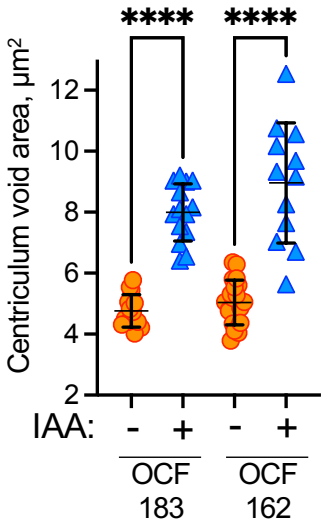




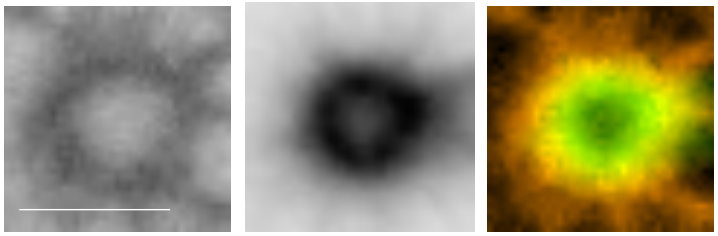
B



C



D



E

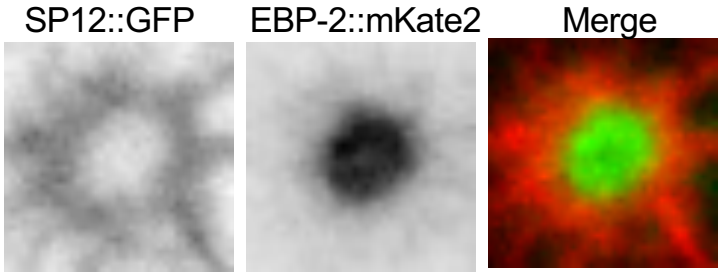


Figure S6: The centriculum affects microtubule nucleation and spindle width, related to Figure 6. (A) Quantification of centriculum void (i), spindle length (ii) and spindle width (iii) using fluorescence intensity and line scanning shown in Figure 6. (i) The centriculum void is defined as the area inside the centriculum perimeter, as determined by a fluorescent EM membrane marker (in this case mCherry::SP12). The area of void was defined using Fiji, eliminating any pixels with fluorescent intensity values above the average intensity in the center of the void. The area defined by the void was used to measure the area confined by the centriculum, as well as the total fluorescence of proteins that are not exclusively at the centrosome (e.g. AIR-1, TBA-2 and EBP-2). (ii) Spindle length was defined as the distance between the center of the two centrosomes. A line spanning both centrosome (in light blue) was traced for fluorescence intensity of mCherry::SP12, revealing the minima between the two peaks of centriculum intensity. Spindle length was defined as the distance between the minima. (iii) Spindle width was measured at 0-0.5 µm to the left and right of the metaphase chromosomes, as visualized using mTurquoise2::H2B. We found that determining spindle width based on tubulin or EBP-2 fluorescence was ambiguous due to the subjective nature of defining the low threshold value. We noticed, however, that the spindle filled the volume that is demarcated by the nuclear membrane and remnants thereof in mitosis (see imaged to the right). Therefore, a fluorescence intensity profile was generated based on mCherry::SP12 fluorescence, and spindle width was defined as the distance between the mCherry::SP12 peaks at the “top” and “bottom” of the chromosome. The images shown are of a 1-cell embryo from strain OCF183. (B, C) Quantification of centriculum diameter (panel B) and centriculum void area (panel C) for embryos analyzed in the experiment described in Figure 6C and D. (D, E) The localization of TBA-2 (panel D, strain OCF183) and EBP-2 (panel E, strain OCF162) in 1-cell metaphase embryos relative to the centriculum (Figure 6), visualized with either mCherry::SP12 (D) or SP12::GFP (E). Scale bar= 5 µm.

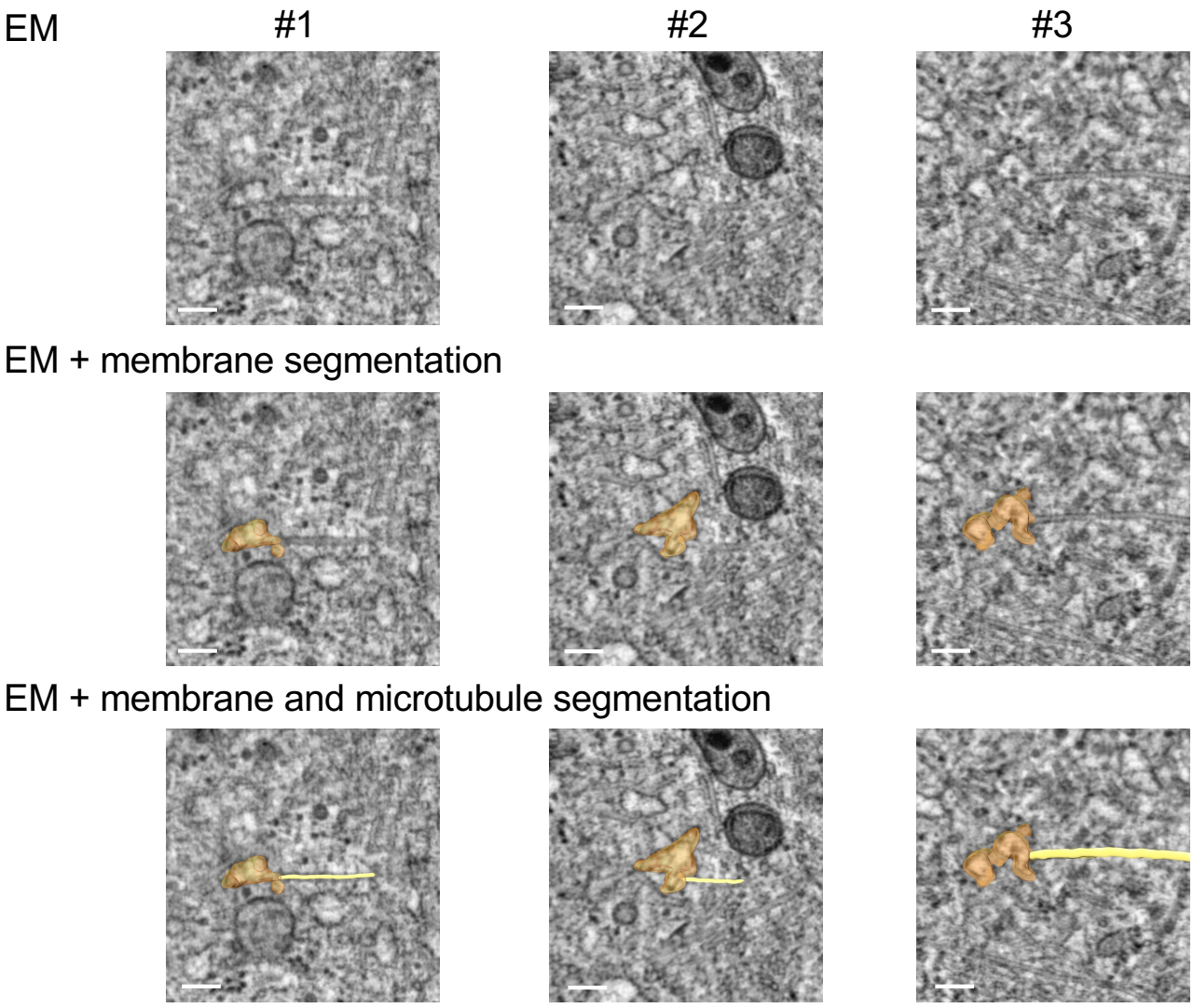


Figure S7: The centriculum serves as a microtubule filter, related to Figure 7. Three additional examples (Figure 7C) of microtubules (in yellow) terminating at membranes (orange). Top row: a single TEM image. Middle row: 3D reconstruction of a membrane segment where a microtubule terminates. Bottom row: 3D reconstructions of both the microtubule and the membrane. Note that the reconstructions use data from tomography images adjacent to the ones shown in the top row. Scale bar= 100 nm.

Table S1: Oligonucleotides use in this study, related to STAR methods

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Oligonucleotides		
Forward primer for *atln-1* RNAi vector tctggtaccCACCGAATACGGACGTTTGG; Template N2 cDNA	IDT	RM19
Reverse primer for *atln-1* RNAi vector tcttctagaCTCGCGAATCGCGATATTCT; Template N2 cDNA	IDT	RM20
Forward primer for spd-5 RNAi vector tctggtaccGAGAACTGCAAATGTATCAG; Template N2 genomic DNA	IDT	RM145
Reverse primer for spd-5 RNAi vector tcttctagaCATTCAATTCACCGTTCACC; Template N2 genomic DNA	IDT	RM146
Forward primer for sense strand of dsRNA of *smd-1* GTAAAACGACGGCCAGTGAG; Template Open Biosystems *E. coli* clone F47G4.7	IDT	RM155
Reverse primer for sense strand of dsRNA of *smd-1* TCTTCTAGACGGAGCGTTCGCCGTCGTCC; Template Open Biosystems *E. coli* clone F47G4.7	IDT	RM150
Forward primer for synthesis of reverse strand of dsRNA of *smd-1* TCTCCCGGGATGTCTGCCACGTCTGCCAC; Template Open Biosystems *E. coli* clone F47G4.7	IDT	RM149
Reverse primer for synthesis of reverse strand of dsRNA of *smd-1* GGAAGCAACCTGGCTTATCG; Template Open Biosystems *E. coli* clone F47G4.7	IDT	RM156
sgRNA sequence for *atln-1::3xflag::degron* CCGCTGATGGACTCAGAAAA	Horizon Discovery	RMRNA2
Forward primer for *atln-1::3xflag::degron* CRISPR tag at C-terminus: CGGCGGCCCCCGGAGCCGCTGATGGACTCAGAAA ACGTCATGACTACAAAGACCATGACGG Template plasmid pKO132	IDT	RM31
Reverse primer for *atln-1::3xflag::degron* CRISPR tag at C-terminus: GGAGAAATTCAGTGGAAAAAGCAATAATTGATCTAC TTCACGAACGCCGCCGCCT Template plasmid pKO132	IDT	RM32
sgRNA sequence for *atln-1::GFP::3xflag* CCGCTGATGGACTCAGAAAA	Horizon Discovery	RMRNA1
Forward primer for *atln-1::GFP::3xflag* CRISPR tag at C-terminus: CGGCGGCCCCCGGAGCCGCTGATGGACTCAGAAA ACGTCATGAGAACCTCTACTTCCAAGG Template plasmid pAP973	IDT	RM24
Reverse primer for *atln-1::GFP::3xflag* CRISPR tag at C-terminus: GGAGAAATTCAGTGGAAAAAGCAATAATTGATCTAC TTGTCATCGTCATCCTTGT Template plasmid pAP973	IDT	RM25
sgRNA sequence for *sun-1::GFP::3xflag* GTTGGGCAGGAACAATAATA	Horizon Discovery	RMRNA7

Forward primer for *sun-1::GFP::3xflag* CRISPR tag at C-terminus: TATTTGATTCGTGTTTACGGGGAACCAGTCGATCCA CCAAAGGAAACGCAACCAATGACGGATAATGGAAC TGAATCAAAGTTGGAATCTGCAATTGTTAACTCTGTA TCAGAAACCGCAGAGAACCTCTACTTCCAAGG Template plasmid pAP973	IDT	RM131
Reverse primer *sun-1::GFP::3xflag* CRISPR tag at C-terminus: ACAAACGGTAACGTGGATAGTAAAGCGGGACAAAA CATTTGCAAACGATTTCAAAGAAGCAATATTGAGATT CATAGTCAGTGGGGAACGTGAAAATGATATAAAAAC AAGTTGGGCAGGGACGATGATGCGTCGCTTTTACTT GTCATCGTCATCCTTGT Template plasmid pAP973	IDT	RM132