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Supplementary Materials for

Initial spindle positioning at the oocyte center protects against incorrect kinetochore-microtubule attachment and aneuploidy in mice

Jessica N. Kincade *et al.*

Corresponding author: Ahmed Z. Balboula, abalboula@missouri.edu

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The PDF file includes:

Figs. S1 to S8
Legends for movies S1 to S3

Other Supplementary Material for this manuscript includes the following:

Movies S1 to S3

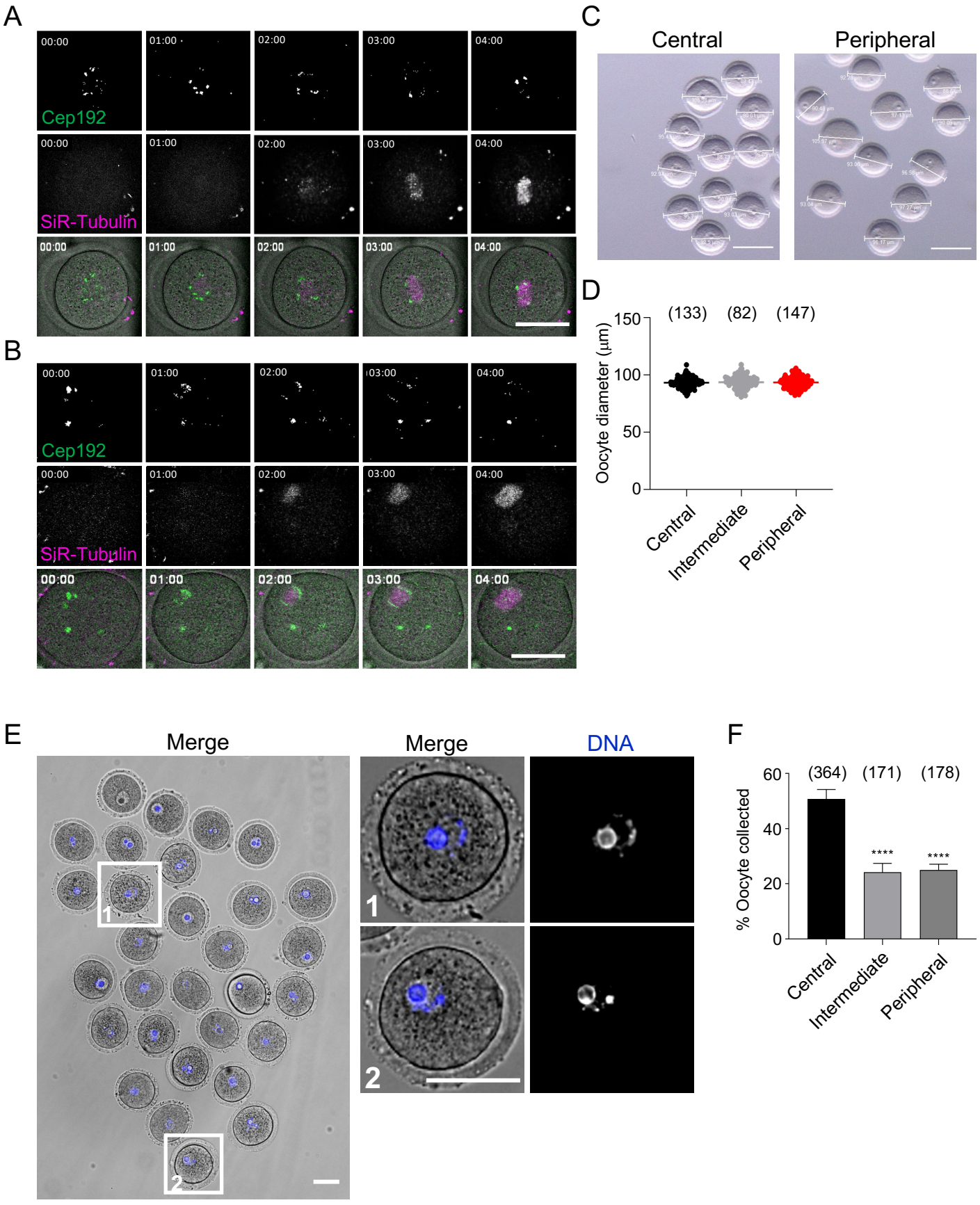

A
00:00
01:00
02:00
03:00
04:00
Cep192
SiR-Tubulin
B
C
Central
Peripheral
D
(133)
(82)
(147)
Oocyte diameter (µm)
150
100
50
0
Central
Intermediate
Peripheral
E
Merge
Merge
DNA
1
2
F
(364)
(171)
(178)
% Oocyte collected
60
40
20
0
****
****
Central
Intermediate
Peripheral

fig. S1

Fig. S1: Full-grown prophase I-arrested oocytes were collected from *Cep192-eGfp* reporter mice, sorted into central (A) and peripheral (B) groups based on the positioning of their nucleus and *in vitro* matured in CZB medium containing SiR-tubulin (to label microtubules, magenta in merge). Spindle poles were labeled by CEP192-EGFP (microtubule organizing centers, green in merge). Oocytes were imaged live using time-lapse confocal microscopy. Shown are representative fluorescence and bright-field image sequence depicting the location of NEBD and consequent spindle formation. Images were taken every 1 h (Z-projection of 13 sections every 5 μm). The scale bar represents 50 μm. (C) Oocytes were sorted based on location of their nucleus into central or peripheral groups, imaged and quantified according to their diameter to ensure similarity between groups. The overall diameter of oocytes included the zona pellucida. Please note that the large oocyte (105.97 μm, peripheral group) was undergoing cell death and excluded from further experiments. (D) Quantification of the average diameter of oocytes collected for experimentation. (E) Representative fluorescent image of Hoechst staining (to label DNA) in group of selected, but not sorted, oocytes. Representative images of an oocyte with a peripherally located nucleus and a surrounded nucleolus are featured in the right panel. The scale bar represents 50 μm. (F) Quantification of nucleus position in oocytes. One-way ANOVA and Tukey's post hoc test were performed to analyze the data. Data are displayed as mean ± SEM. Values with asterisks vary significantly, **** $P < 0.0001$. The total number of analyzed oocytes is specified above each graph.

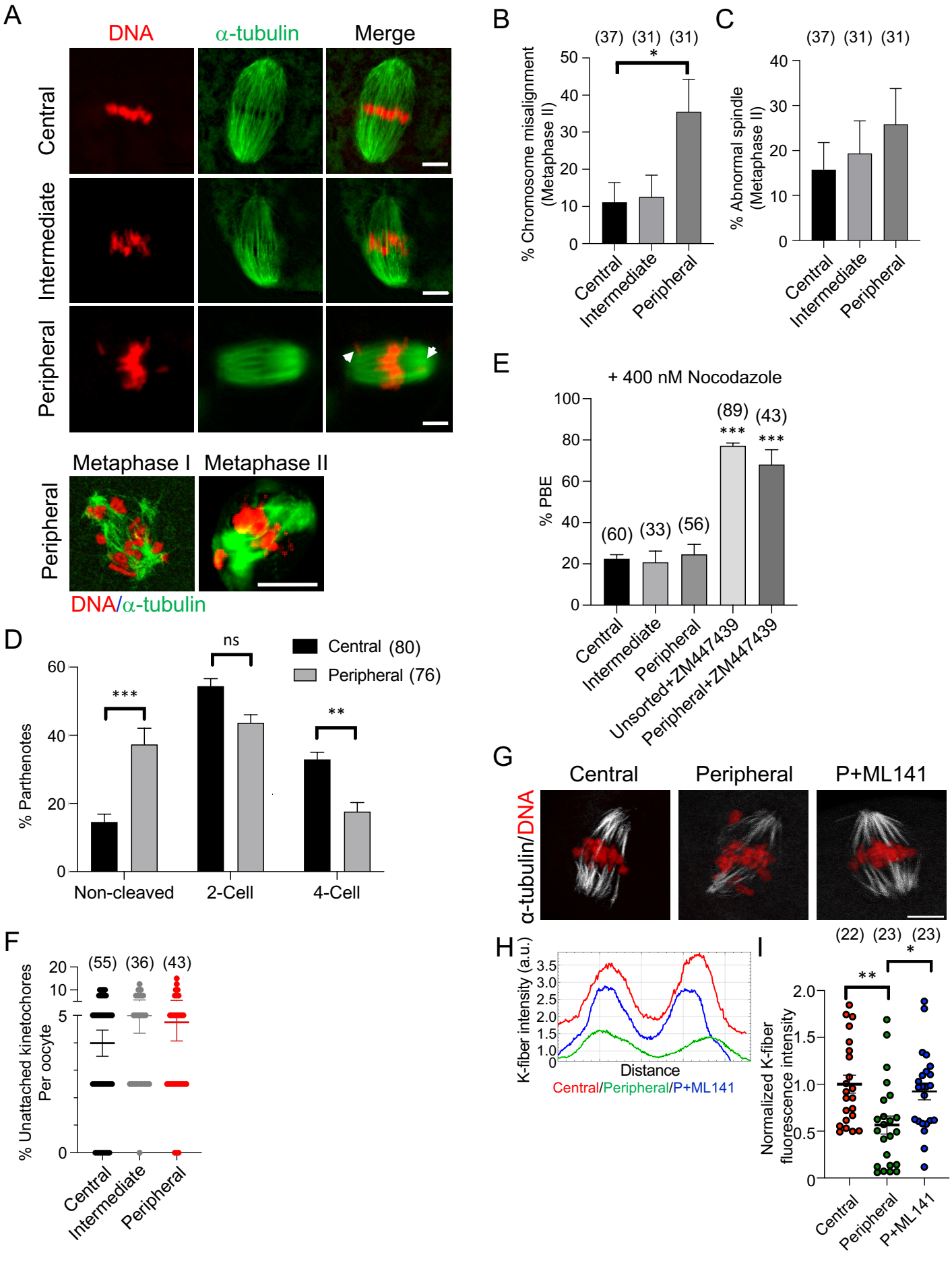

A
DNA
α-tubulin
Merge
Central
Intermediate
Peripheral
Metaphase I
Metaphase II
Peripheral
DNA/α-tubulin
B
(37) (31) (31)
*
% Chromosome misalignment (Metaphase II)
Central
Intermediate
Peripheral
C
(37) (31) (31)
% Abnormal spindle (Metaphase II)
Central
Intermediate
Peripheral
D
Central (80)
Peripheral (76)
ns
***
**
% Parthenotes
Non-cleaved
2-Cell
4-Cell
E
+ 400 nM Nocodazole
(89) (43)
***
***
(60) (33) (56)
% PBE
Central
Intermediate
Peripheral
Unsorted+ZM447439
Peripheral+ZM447439
F
(55) (36) (43)
% Unattached kinetochores Per oocyte
Central
Intermediate
Peripheral
G
Central
Peripheral
P+ML141
α-tubulin/DNA
H
K-fiber intensity (a.u.)
Distance
Central/Peripheral/P+ML141
I
(22) (23) (23)
**
*
Normalized K-fiber fluorescence intensity
Central
Peripheral
P+ML141

fig. S2

Fig. S2: (A) Representative confocal images of metaphase I and metaphase II oocytes. Full-grown prophase I oocytes were collected and sorted into central, intermediate and peripheral groups based on nucleus positioning and *in vitro* matured for 16 h (metaphase II). Oocytes were fixed and immunostained with α-tubulin to label the microtubules (the spindle). DNA was labeled by DAPI. Lower panels represent examples of abnormal spindle in metaphase I and metaphase II peripheral oocytes. (B) Quantification of the percentage of chromosome misalignment in metaphase II oocytes. (C) Quantification of the percentage of abnormal spindle morphology in metaphase II oocytes. Spindle morphology was assessed using the criterion that a normal meiotic spindle is barrel shaped with two clearly defined spindle poles. (D) Quantification of the percentage of embryonic development following parthenogenetic activation of metaphase II oocytes obtained from central and peripheral GV oocytes. (E) Quantification of successful polar body extrusion (1^{st}) in oocytes treated with nocodazole. ZM447439-treated oocytes served as controls. (F) Quantification of the percentage of unattached kinetochores in central, intermediate, peripheral oocytes from Fig. 2A. (G) Representative confocal images of central, peripheral and ML141-treated peripheral metaphase I oocytes (ML141 was added at NEBD) exposed to a brief cold shock (10 minutes) and immunostained with α-tubulin to label microtubules (K-fibers). DNA was labeled by DAPI. (H) Examples of fluorescence intensity of K-fibers in G, using the 'plot profiles' function in ImageJ. (I) Quantification of K-fiber fluorescence intensity from G. The scale bar represents 10 μm. One-way ANOVA and Tukey's post hoc tests were performed to determine statistical significance among groups. Data are displayed as mean ± SEM. Values with asterisks vary significantly, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. The total number of analyzed oocytes (from at least 3 independent replicates) is specified in each graph.

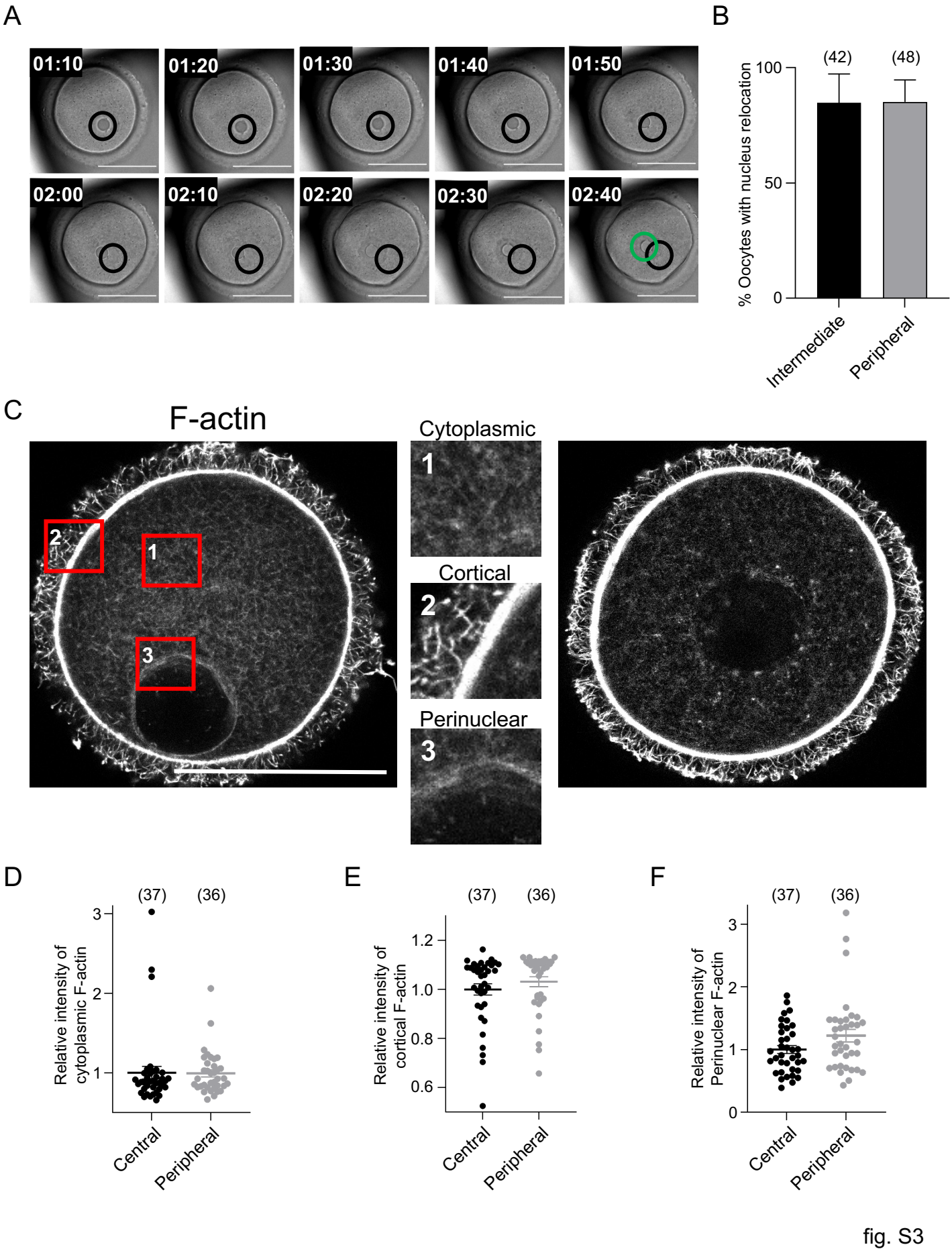

A
01:10
01:20
01:30
01:40
01:50
02:00
02:10
02:20
02:30
02:40
B
% Oocytes with nucleus relocation
100
50
0
(42)
(48)
Intermediate
Peripheral
C
F-actin
1
2
3
Cytoplasmic
Cortical
Perinuclear
D
Relative intensity of cytoplasmic F-actin
(37)
(36)
Central
Peripheral
E
Relative intensity of cortical F-actin
(37)
(36)
Central
Peripheral
F
Relative intensity of Perinuclear F-actin
(37)
(36)
Central
Peripheral

fig. S3

Fig. S3: (A) Representative bright-field images of time-lapse microscopy of the movement of a peripherally positioned nucleus during prophase I arrest in oocytes incubated with milrinone. Images were taken every 10 minutes using a Z-interval of 5 μm. The black circle identifies the initial positioning of the nucleus, while the green circle denotes the final positioning of the nucleus. The scale bar represents 50 μm. (B) Quantification of the percentage of peripheral and intermediate GV oocytes achieving full relocation of the nucleus to a central position within 3 h of incubation with milrinone. (C) Representative fluorescent image of F-actin populations within fixed oocytes labeled with phalloidin. Enlarged panels show, from top to bottom, areas containing cytoplasmic, cortical, and perinuclear F-actin. The scale bar represents 50 μm. (D-F) Quantification of the relative intensity of cytoplasmic, cortical, and perinuclear F-actin in oocytes with either a centrally or peripherally positioned nucleus. Student t-test was performed to determine statistical significance. Data are displayed as mean ± SEM. The total number of analyzed oocytes (from at least 3 independent replicates) is specified above each graph.

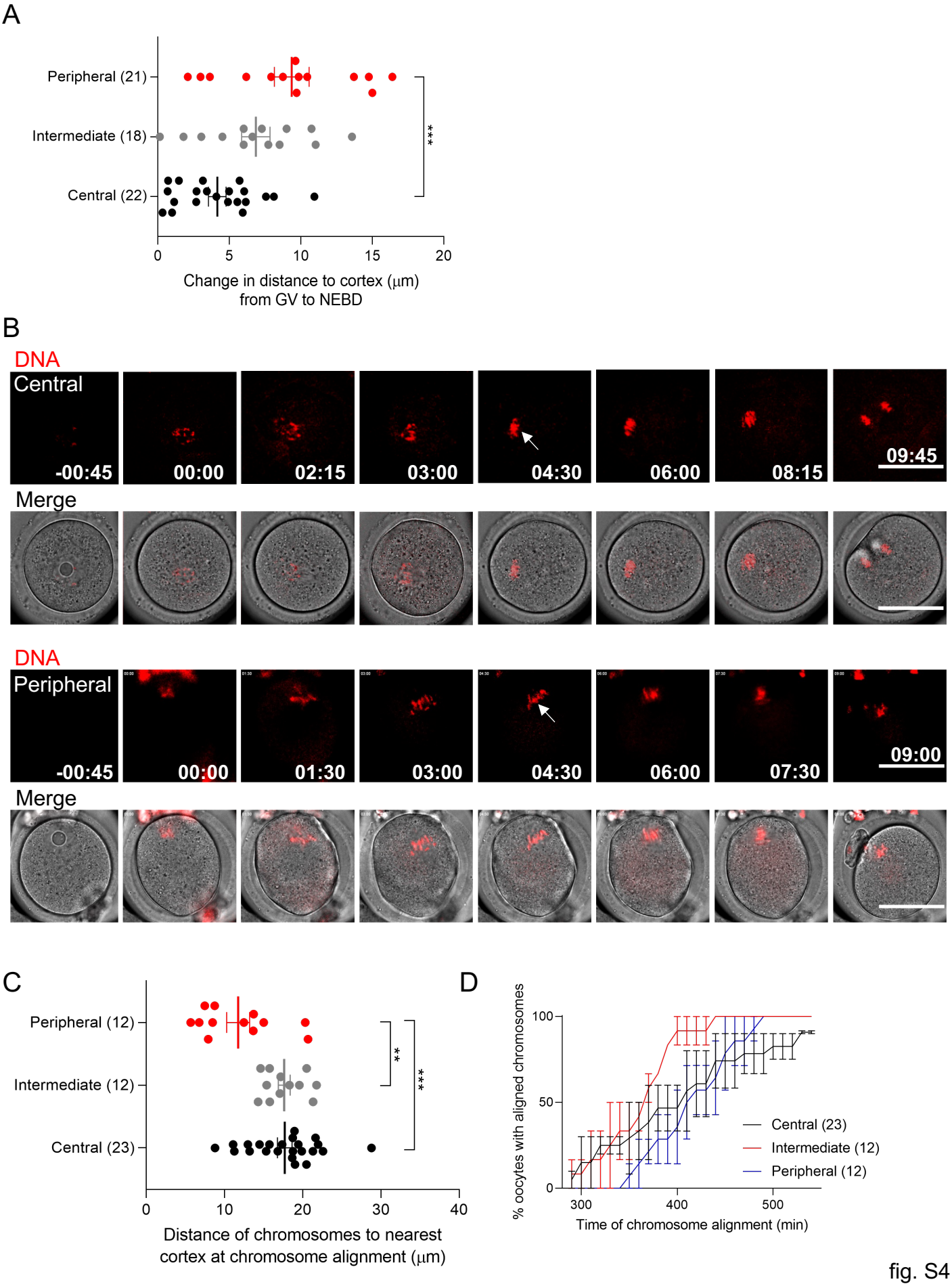
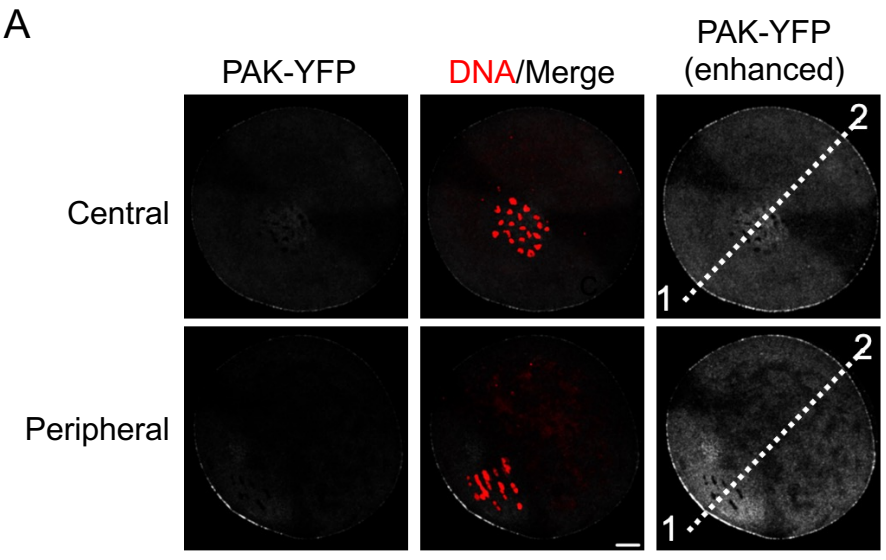
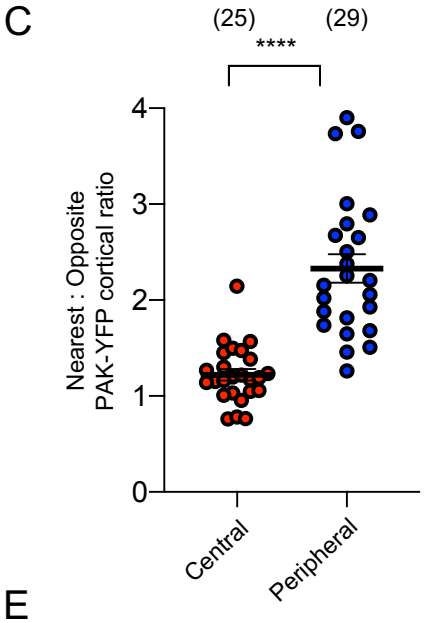
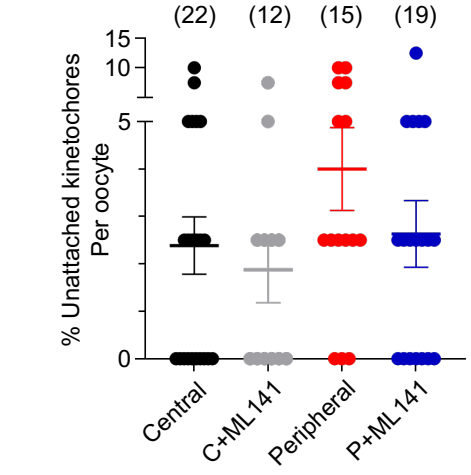

A
Peripheral (21)
Intermediate (18)
Central (22)
***
0
5
10
15
20
Change in distance to cortex (μm)
from GV to NEBD
B
DNA
Central
-00:45
00:00
02:15
03:00
04:30
06:00
08:15
09:45
Merge
DNA
Peripheral
-00:45
00:00
01:30
03:00
04:30
06:00
07:30
09:00
Merge
C
Peripheral (12)
Intermediate (12)
Central (23)
**
***
0
10
20
30
40
Distance of chromosomes to nearest
cortex at chromosome alignment (μm)
D
% oocytes with aligned chromosomes
100
50
0
300
400
500
Time of chromosome alignment (min)
Central (23)
Intermediate (12)
Peripheral (12)

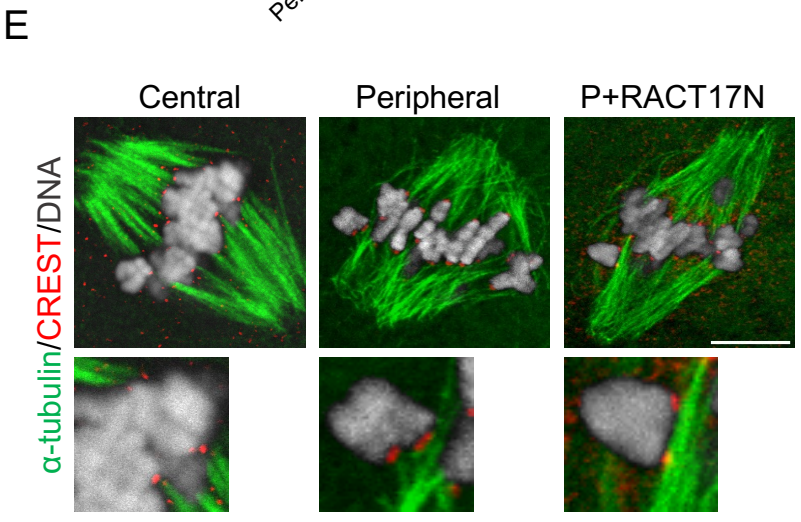
fig. S4

Fig. S4: Full-grown prophase I-arrested oocytes were collected and sorted into central, intermediate, and peripheral groups based on the positioning of their nucleus and imaged live using time-lapse microscopy during *in vitro* maturation. (A) Quantification of the change in the distance of the nucleus to the nearest cortex from the initial position of the nucleus at the prophase I-arrested stage to the final destination of the nucleus immediately prior to NEBD. (B) Representative fluorescence and bright-field image sequence depicting the timing of chromosome alignment (labeled by SiR-DNA, red) in oocytes with a central or peripheral nucleus. Images were taken every 45 minutes using a Z-interval of 7 μm. White arrows represent the timing of chromosome alignment. Scale bars represent 50 μm. (C) Quantification of the distance of chromosomes to the nearest cortex at the time of alignment. (D) Quantification of the time of chromosome alignment. One-way ANOVA and Tukey's post hoc test were performed to determine statistical significance. Data are displayed as mean ± SEM. Values with asterisks vary significantly, ** $P < 0.01$, *** $P < 0.001$. The total number of analyzed oocytes (from 3 independent replicates) is specified in each graph.


A
PAK-YFP
DNA/Merge
PAK-YFP (enhanced)
Central
Peripheral
1
2
B
1 (nearest cortex)
2 (opposite)
PAK-YFP fluorescence intensity
Distance


C
(25)
(29)
****
Nearest : Opposite PAK-YFP cortical ratio
Central
Peripheral


D
(22)
(12)
(15)
(19)
% Unattached kinetochores Per oocyte
Central
C+ML141
Peripheral
P+ML141


E
Central
Peripheral
P+RACT17N
α-tubulin/CREST/DNA

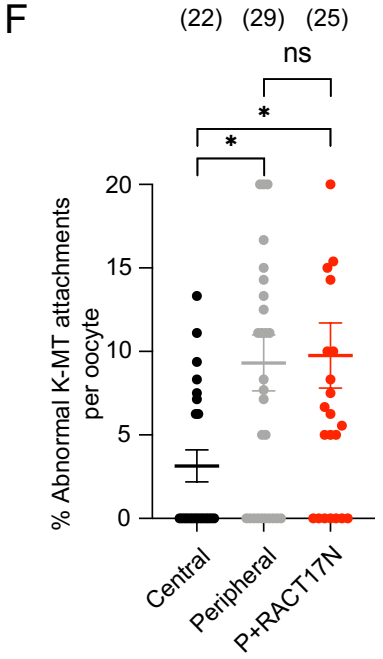

F
(22)
(29)
(25)
ns
*
*
% Abnormal K-MT attachments per oocyte
Central
Peripheral
P+RACT17N

fig S5

Fig. S5: (A) Representative confocal (single slice) images of central and peripheral prometaphase I oocytes expressing RAC biosensor (PAK-YFP) to assess RAC-GTP cortical enrichment. The DNA was stained with DAPI. (B) Examples of fluorescence intensity of PAK-YFP in A, using the 'plot profiles' function in ImageJ. (C) RAC-GTP cortical enrichment was quantified as the ratio of PAK-YFP fluorescence intensity at the cortical side closest to the chromosomes to PAK-YFP intensity at the cortical side opposite to chromosome localization. (D) Quantification of the percentage of unattached kinetochores in oocytes treated with ML141 from Fig. 3E. (E) Representative images of K-MT attachments in central (as a control) and peripheral oocytes, as well as peripheral oocytes expressing RAC dominant-negative mutant (P+RACT17N). The oocytes were matured to metaphase I and immunostained with α-tubulin (to label MTs), CREST (to label kinetochores), stained with DAPI (to label DNA), and imaged using confocal microscopy to assess K-MT attachments. (F) Quantification of the average incidence of abnormal K-MT attachments for the oocyte populations illustrated in E. Scale bars represent 10 µm. Student t-test and one-way ANOVA followed by Tukey's post hoc tests were performed to determine statistical significance among groups. Data are displayed as mean ± SEM. Values with asterisks vary significantly, * $P < 0.05$, **** $P < 0.0001$. The total number of analyzed oocytes (from at least 3 independent replicates) is specified above each graph.

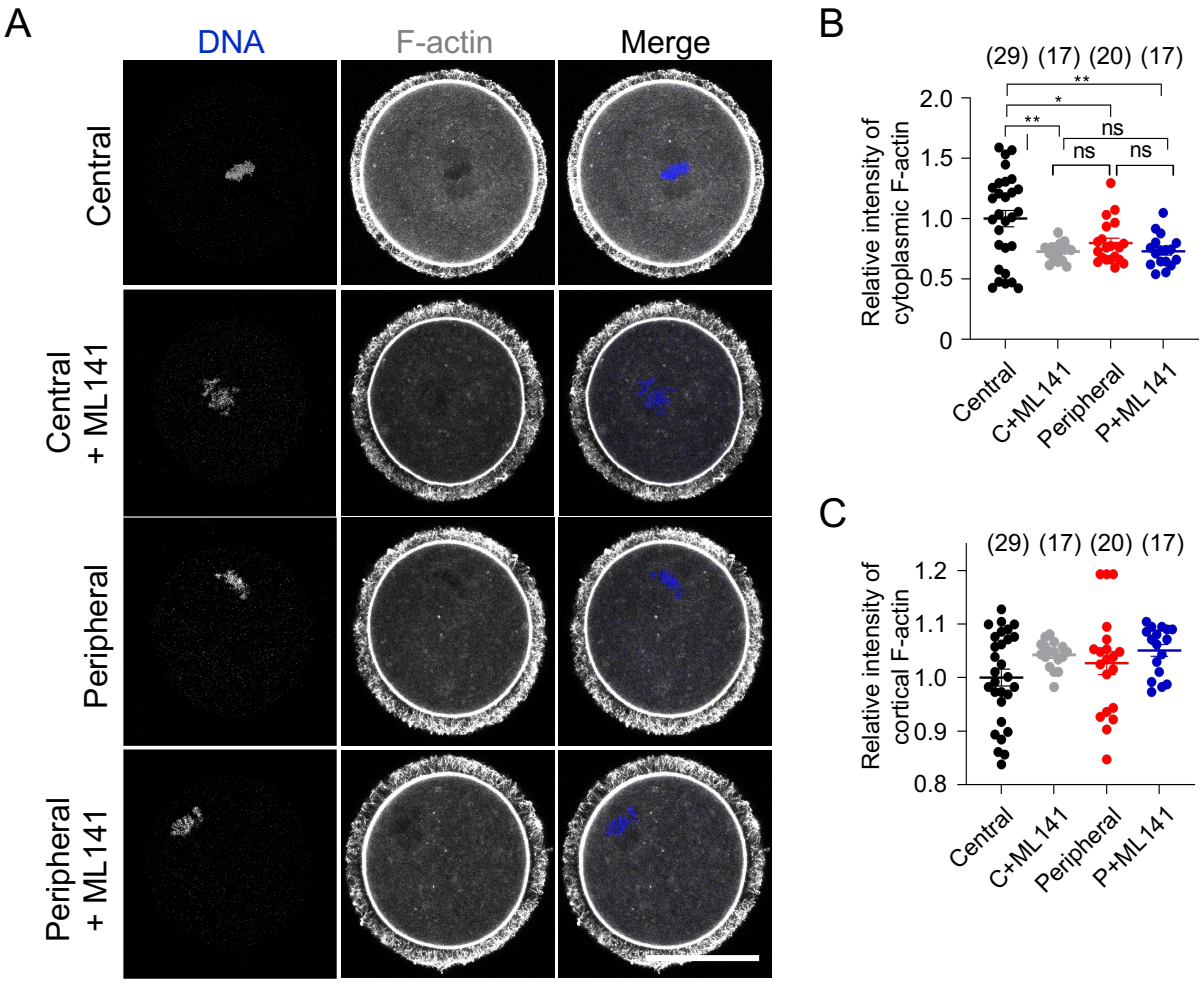
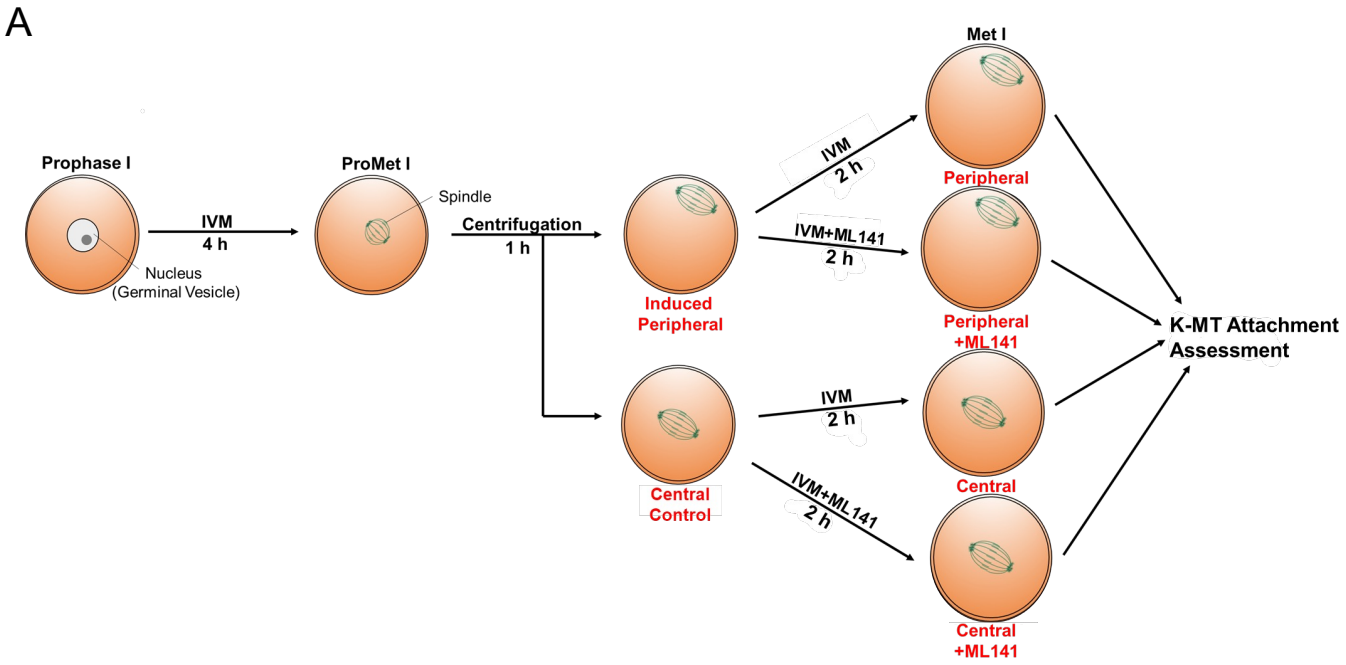
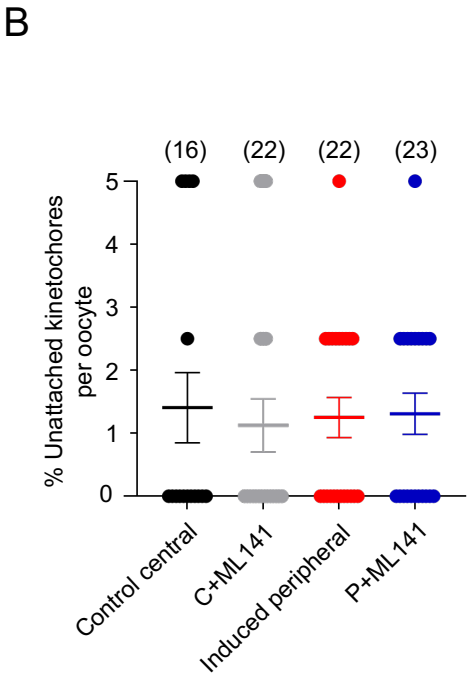

A
DNA
F-actin
Merge
Central
Central + ML141
Peripheral
Peripheral + ML141
B
(29) (17) (20) (17)
Relative intensity of cytoplasmic F-actin
2.0
1.5
1.0
0.5
0
**
*
**
ns
ns
ns
Central
C+ML141
Peripheral
P+ML141
C
(29) (17) (20) (17)
Relative intensity of cortical F-actin
1.2
1.1
1.0
0.9
0.8
Central
C+ML141
Peripheral
P+ML141

fig. S6

Fig. S6: (A) Representative images of central and peripheral oocytes treated with or without ML141. The scale bar represents 50 μm. (B,C) Quantification of the relative intensity of F-actin in the cytoplasm and cortex of oocytes, respectively. DNA was stained with DAPI (blue in merge). One-way ANOVA and Tukey's post hoc test were performed to determine statistical significance. Data are displayed as mean ± SEM. Values with asterisks vary significantly, * $P<0.05$, ** $P<0.01$. No significant difference among the groups presented in C. The total number of analyzed oocytes (from 4 independent replicates) is specified above each graph.


A
Prophase I
IVM
4 h
Nucleus
(Germinal Vesicle)
ProMet I
Spindle
Centrifugation
1 h
Induced
Peripheral
Central
Control
IVM
2 h
IVM+ML141
2 h
Met I
Peripheral
Peripheral
+ML141
Central
Central
+ML141
K-MT Attachment
Assessment


B
(16)
(22)
(22)
(23)
% Unattached kinetochores
per oocyte
0
1
2
3
4
5
Control central
C+ML141
Induced peripheral
P+ML141

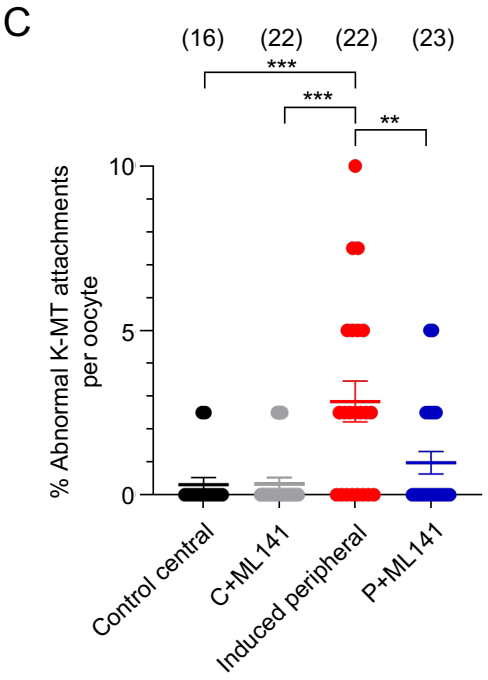
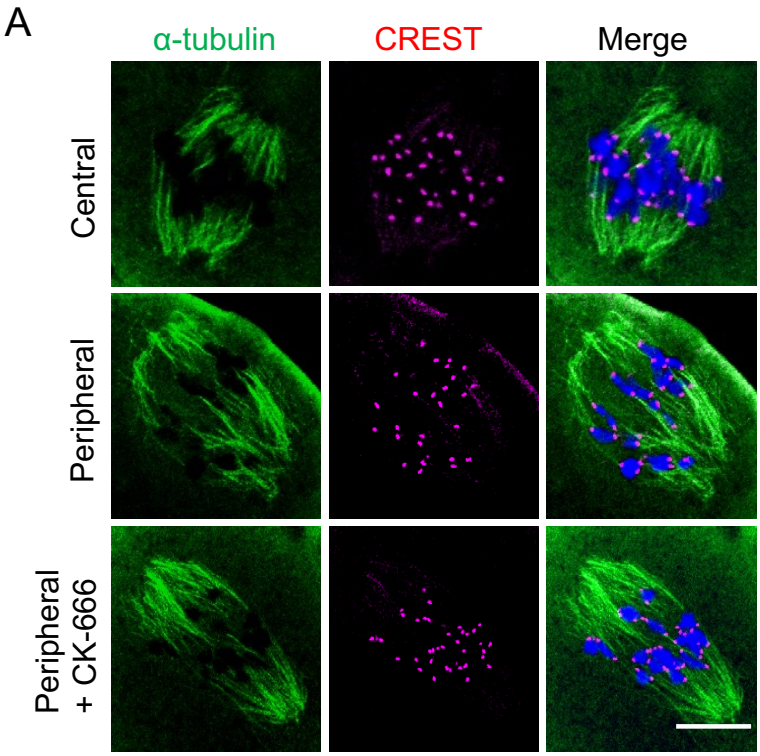
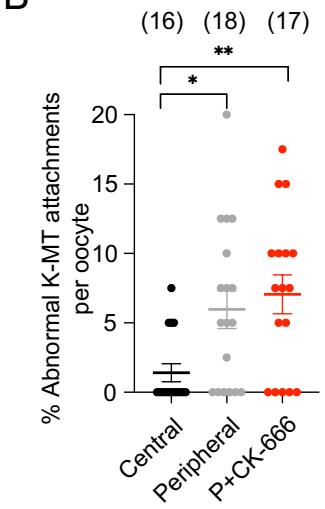

C
(16)
(22)
(22)
(23)
***
***
**
% Abnormal K-MT attachments
per oocyte
0
5
10
Control central
C+ML141
Induced peripheral
P+ML141

fig. S7

Fig. S7: (A) Schematic depicting the induction of peripheral spindle positioning and treatment with ML141 in oocytes with a centrally positioned nucleus. (B) Quantification of the percentage of unattached kinetochores for the oocyte populations illustrated in A. (C) Quantification of the average incidence of abnormal K-MT attachment for the oocyte populations illustrated in A. One-way ANOVA and Tukey's post hoc test were performed to determine statistical significance. Data are displayed as mean ± SEM. Values with asterisks vary significantly, ** $P < 0.01$, *** $P < 0.001$. The total number of analyzed oocytes (from 3 independent replicates) is specified above each graph.


A
α-tubulin
CREST
Merge
Central
Peripheral
Peripheral
+ CK-666


B
(16)
(18)
(17)
**
*
% Abnormal K-MT attachments per oocyte
20
15
10
5
0
Central
Peripheral
P+CK-666

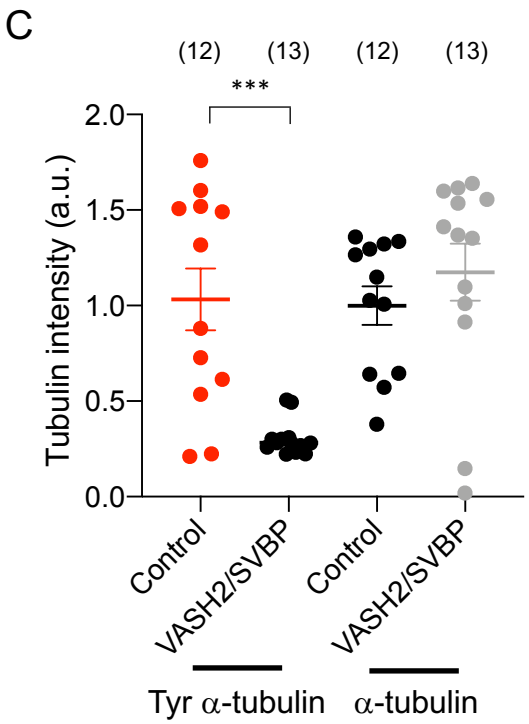

C
(12)
(13)
(12)
(13)
***
Tubulin intensity (a.u.)
2.0
1.5
1.0
0.5
0.0
Control
VASH2/SVBP
Control
VASH2/SVBP
Tyr α-tubulin
α-tubulin

fig. S8

Fig. S8: (A) Representative images of K-MT attachment in metaphase I oocytes treated with or without ARP2/3 inhibitor, CK-666 at 3 h post-NEBD. The scale bar represents 10 μm. (B) Quantification of the average incidence of abnormal K-MT attachments in central, peripheral and CK-666-tretaed peripheral oocytes. The total number of analyzed oocytes (from 3 independent replicates) is specified above the graph. (C) Quantification of tyrosinated α-tubulin and α-tubulin intensities in control oocytes expressing GFP and oocytes expressing VASH2/SVBP. Full-grown GV oocytes were collected and immediately microinjected with Vash2/SVBP cRNAs in milrinone-free MEM medium and then matured in milrinone-free CZB medium to metaphase I stage. The total number of analyzed oocytes (from 2 independent replicates) is specified above the graph. One-way ANOVA and Tukey's post hoc test were performed to determine statistical significance. Data are displayed as mean ± SEM. Values with asterisks vary significantly, * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Movie S1: Time-lapse microscopy of a germinal vesicle (GV) oocyte cultured in milrinone-containing medium to maintain prophase I arrest. DIC images were captured every 10 minutes at Z-intervals of 5 μm. The scale bar represents 50 μm.

Movie S2: Time-lapse microscopy of an oocyte with a central nucleus matured in the presence of SiR-DNA to label the DNA (red). Fluorescence and bright-field images were captured every 45 minutes at Z-intervals of 7 μm. The scale bar represents 50 μm.

Movie S3: Time-lapse microscopy of an oocyte with a peripheral nucleus matured in the presence of SiR-DNA to label the DNA (red). Fluorescence and bright-field images were captured every 45 minutes at Z-intervals of 7 μm. The scale bar represents 50 μm.