SUPPLEMENTAL FIGURE TITLES AND LEGENDS

Supplementary Figure 1: Mouse oocytes contain two different sets of MTOCs. Related to Figure 1. (A) Quantifications of average mcMTOC numbers at metaphase I per oocyte. (B-E) Full-grown prophase-I oocytes were injected with *Aurka-Gfp*, incubated in milrinone-containing CZB medium for 3 h, followed by *in vitro* maturation. Metaphase I oocytes were fixed and immunostained using pericentrin antibody to label MTOCs (red, B) or using pericentrin (green) and α-tubulin (magenta) antibodies (C). DAPI was used to detect DNA (blue). (D) Quantifications of average mcMTOC numbers in "C". (D) Quantifications of average mcMTOC volume in "C". The data are expressed as mean ± SEM. Student t-test was used to analyze the data. (F) MTOCs in the cytoplasm and at the spindle poles of meiotic oocytes collected from C57BL/6 mice. Full-grown prophase-I oocytes collected from C57BL/6 mice were *in vitro* matured for 7 h. Metaphase I oocytes were fixed and immunostained using γ-tubulin and α-tubulin antibodies to label MTOCs (red) and microtubules (pseudo grey). DAPI was used to detect DNA (blue). White arrows represent mcMTOCs. Shown are representative Z-projection of confocal images. Scale bars represent 10 μm (zoomed panels, 2 μm).

Supplementary Figure 2: mcMTOC movement does not follow the movement of the cytoplasm. Related to Figure 2. Representative images (Z-projection of 16 sections every 3 μm) of time-lapse confocal microscopy of live oocytes expressing AURKA-GFP (MTOCs, green) and H2B-mCherry (chromosomes) from a time course. Fluorescence and bright-field images were captured every 15 min (time, h:min). The white arrow represents a cytoplasmic droplet. Lower panels show the tracking of both the cytoplasmic droplet (green) and an mcMTOC (blue). The scale bar represents 10 μm.

Supplementary Figure 3: Microtubules but not F-actin anchor the spindle to the oocyte cortex. Related to Figure 4. (A) F-actin localization in mouse oocytes. Full-grown prophase-I oocytes were *in vitro* matured for 7 h. Metaphase I oocytes were fixed and immunostained using γ-tubulin to label MTOCs (magenta) and α-tubulin to label the spindle (pseudo blue). Hoechst stain was used to detect DNA (pseudo white) and phalloidin stain was used to detect F-actin (pseudo green). Fluorescence signals were detected under a 100 X objective using STED super-resolution system. Shown are representative Z-projection. The scale bar represents 25 μm. (B) Fully grown prophase-I-arrested oocytes were *in vitro* matured for 6 h (metaphase I) prior to fixation and immunocytochemistry using pericentrin and α-tubulin antibodies to label MTOCs (pseudo grey) and microtubules (magenta). Fluorescence signals were detected under a 100 X objective using confocal microscopy (left panels) and STED super-resolution system (right zoomed enhanced panel). Shown is a representative image (Z-projection of 42 sections every 0.5 μm). The scale bar represents 25 μm (zoomed panel, 10 μm). (C,D) Inhibition of MTs accelerates chromosome migration towards the cortex in meiotic oocytes. Full-grown prophase-I oocytes were divided into two groups and treated with DMSO or nocodazole (added at 0 h after collection) followed by *in vitro* maturation and time-lapse imaging. Images were captured every 15 min. (B) Quantification of the average time spent by chromosomes until reaching the cortex. (C) Quantification of average chromosome speed during migration. The data are expressed as mean ± SEM. Student-t test was used to analyze the data. Values with asterisks vary significantly, ***$P < 0.001$, ****$P < 0.0001$. The total number of analyzed oocytes (from two independent replicates) is specified above each graph.

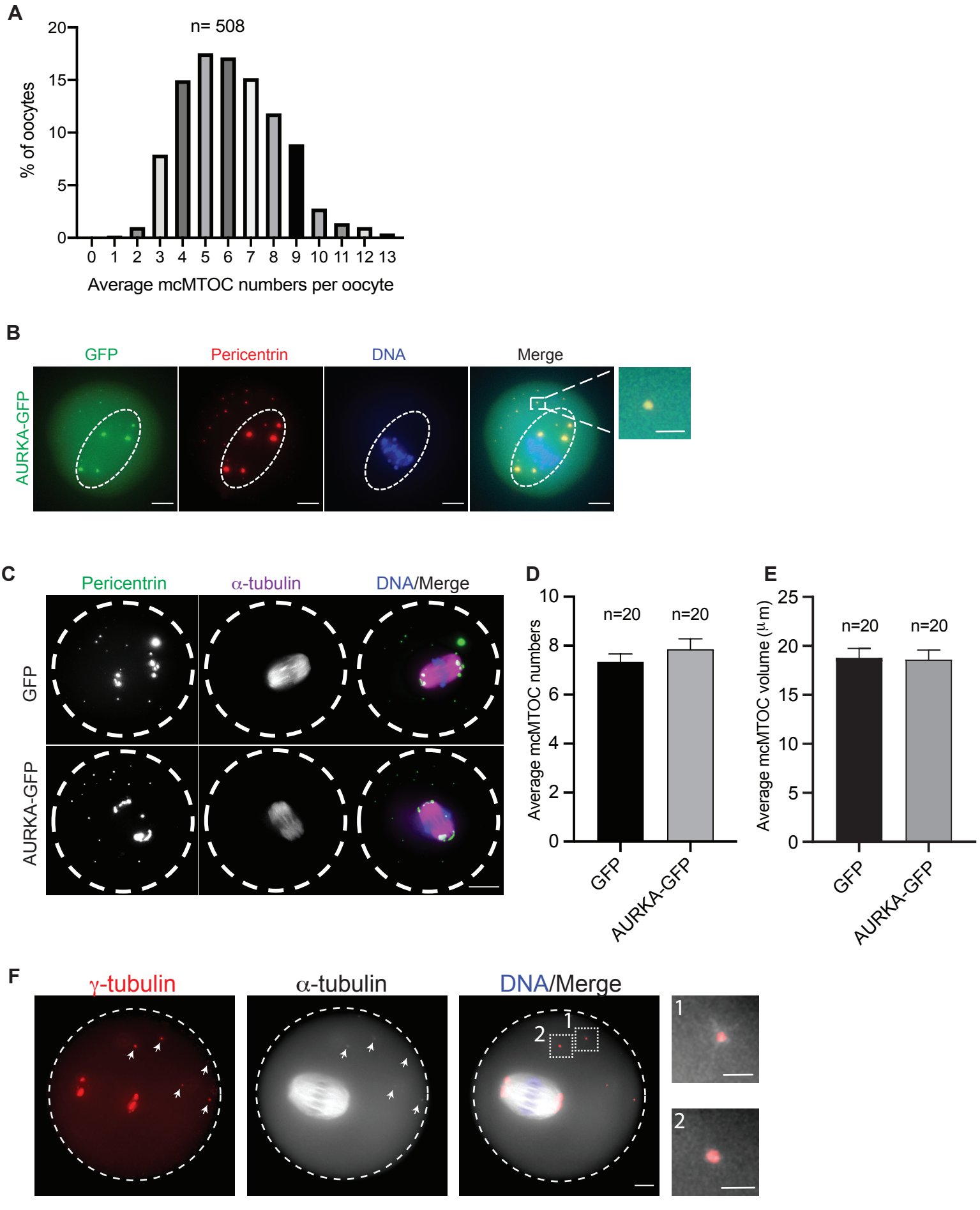
Supplementary Figure 4: mcMTOC depletion by two-photon laser ablation perturbs spindle positioning in Met I arrested oocytes. Related to Figure 5. Oocytes expressing AURKA-GFP and eGFP-EB3 were *in vitro* maturated for 6 h (metaphase I, Met I), transferred to CZB medium with MG-132, followed by mcMTOC depletion using two-photon laser ablation. Small square area(s) surrounding mcMTOCs were marked and then exposed to a laser with 925 nm wavelength. Control oocytes were exposed to the same parameters except ablating random areas of the cytoplasm equal to the same size and number of mcMTOCs. (A) Control and mcMTOC-ablated oocytes were fixed and immunostained using γ-tubulin antibody to label MTOCs. DAPI was used to detect DNA (blue). Arrowheads represent mcMTOCs. Shown are representative Z-projection of confocal images. The scale bar represents 10 μm. (B) Z-projection (16 sections every 3 μm) of 3D time-lapse microscopy of control-ablated and mcMTOC-depleted (see Video S7) Met I oocytes while cultured in MG-132-containing medium for additional 9 h to track the spindle. Fluorescence images were captured every 3 min (time, h:min). White arrowheads represent the tracking path of all time periods. Black arrows represent examples of mcMTOCs on different focal planes. The scale bar represents 10 μm. (C) Quantification of total distance traveled by the spindle. (D) Quantification of average spindle velocity. The data are expressed as mean ± SEM. Student t-test was used to analyze the data. Values with asterisks vary significantly, ****$P < 0.0001$. The total number of analyzed oocytes in each group (from two independent replicates) is specified above each condition within each graph.

Supplementary Figure 5: 3-MA treatment increases mcMTOC numbers and mcMTOC-nucleated MTs. Related to Figure 6. (A) Full-grown prophase-I oocytes were divided into two groups and treated with DMSO or 3-MA (added at NEBD) followed by *in vitro* maturation until metaphase I (Met I, 7h). Met I oocytes were fixed and immunostained using CEP192 and α-tubulin antibodies to label MTOCs (red) and microtubules (pseudo grey). DAPI was used to detect DNA (blue). Arrowheads represent mcMTOCs. Scale bars represent 10 μm. (B) Quantification of average mcMTOC numbers in "A". (C) Quantification of average pMTOC numbers in "A".

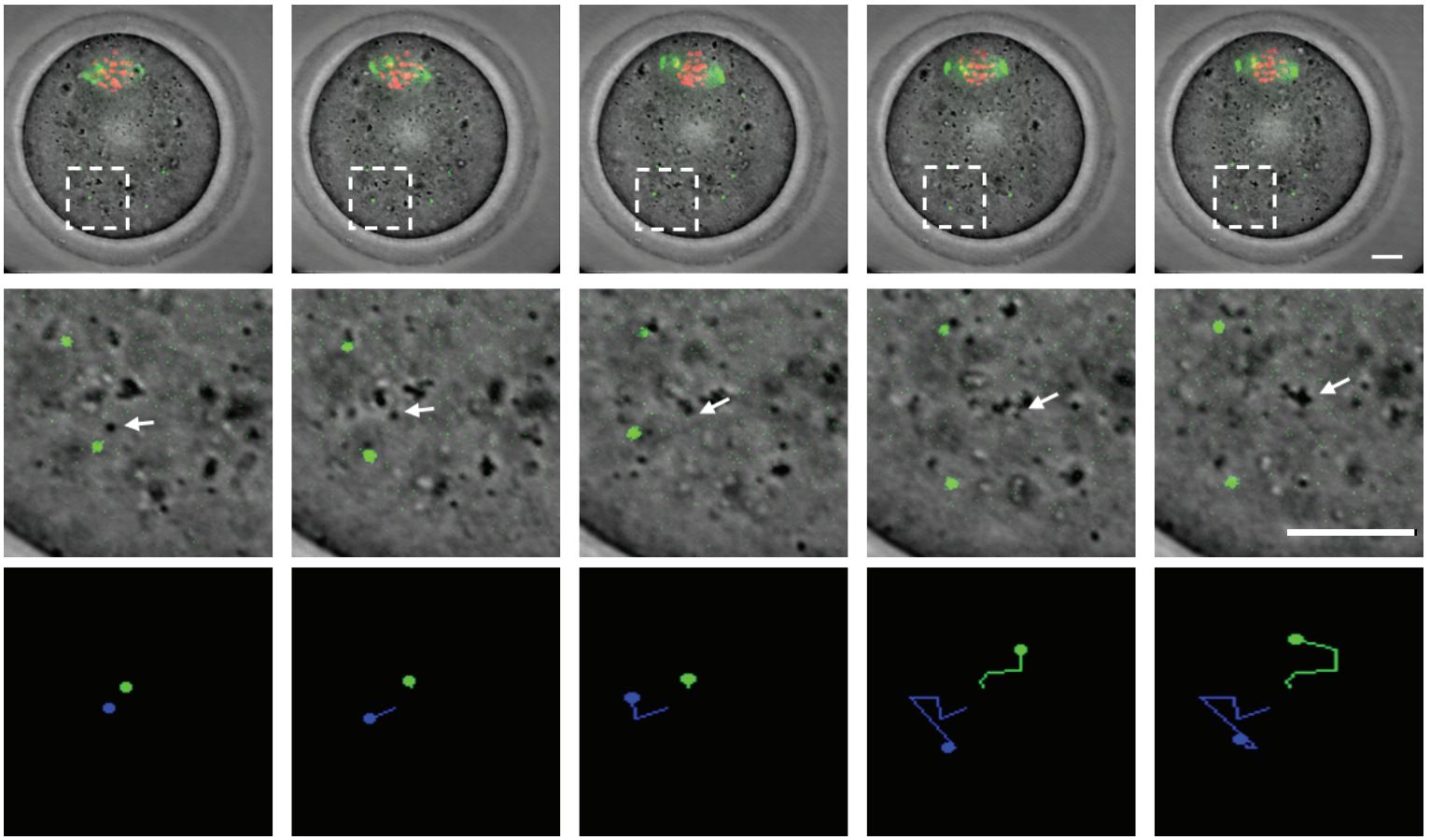
(D) Control and 3-MA-treated oocytes were *in vitro* matured for 6 h (metaphase I) prior to fixation and immunocytochemistry using pericentrin and α-tubulin antibodies to label MTOCs (pseudo grey) and microtubules (magenta). Fluorescence signals were detected under a 100 X objective using confocal microscopy (left panels) and STED super-resolution system (right panel). Shown is a representative image. The scale bar represents 20 μm. (E) Quantifications of mcMTOC-nucleated microtubule intensity in "D". (F) Control, mcMTOC-ablated and 3-MA-treated oocytes were *in vitro* matured for 7 h (metaphase I) prior to fixation and immunocytochemistry using pericentrin (pseudo grey). Phalloidin was used to label F-actin (pseudo green). Fluorescence signals were detected under a 100 X objective using STED super-resolution system. Shown are representative images (Z-projection of 42 sections every 0.5 μm). The scale bar represents 20 μm. (G) Quantifications of cytoplasmic F-actin intensity in "F". The data are expressed as mean ± SEM. Student t-test (B,C,E) or one-way ANOVA (G) was used to analyze the data. Values with asterisks vary significantly, ****$P < 0.0001$. The total number of analyzed oocytes (from three independent replicates) is specified above each graph.

Supplementary Figure 6: 3D time-lapse imaging of a 3-MA-treated oocyte during mcMTOC ablation. Related to Figure 6. Full-grown prophase I-arrested oocytes were collected from *Cep192-eGfp* reporter mice (MTOCs are labeled green) and *in vitro* matured in CZB medium containing SiR-tubulin (to label microtubules, magenta) for 2 h. 3-MA was added to the maturation medium at NEBD. At prometaphase I, 6 out of 14 mcMTOCs were depleted using laser ablation. Small square area(s) surrounding mcMTOCs were marked and then exposed to a laser with 820 nm wavelength. White arrowheads represent laser beam targets. Shown are the two Z-sections in which 6 mcMTOCs were ablated. Same oocyte as shown in Fig. 6A (lower panels). The scale bar represents 10 μm.

Supp. Fig. 1

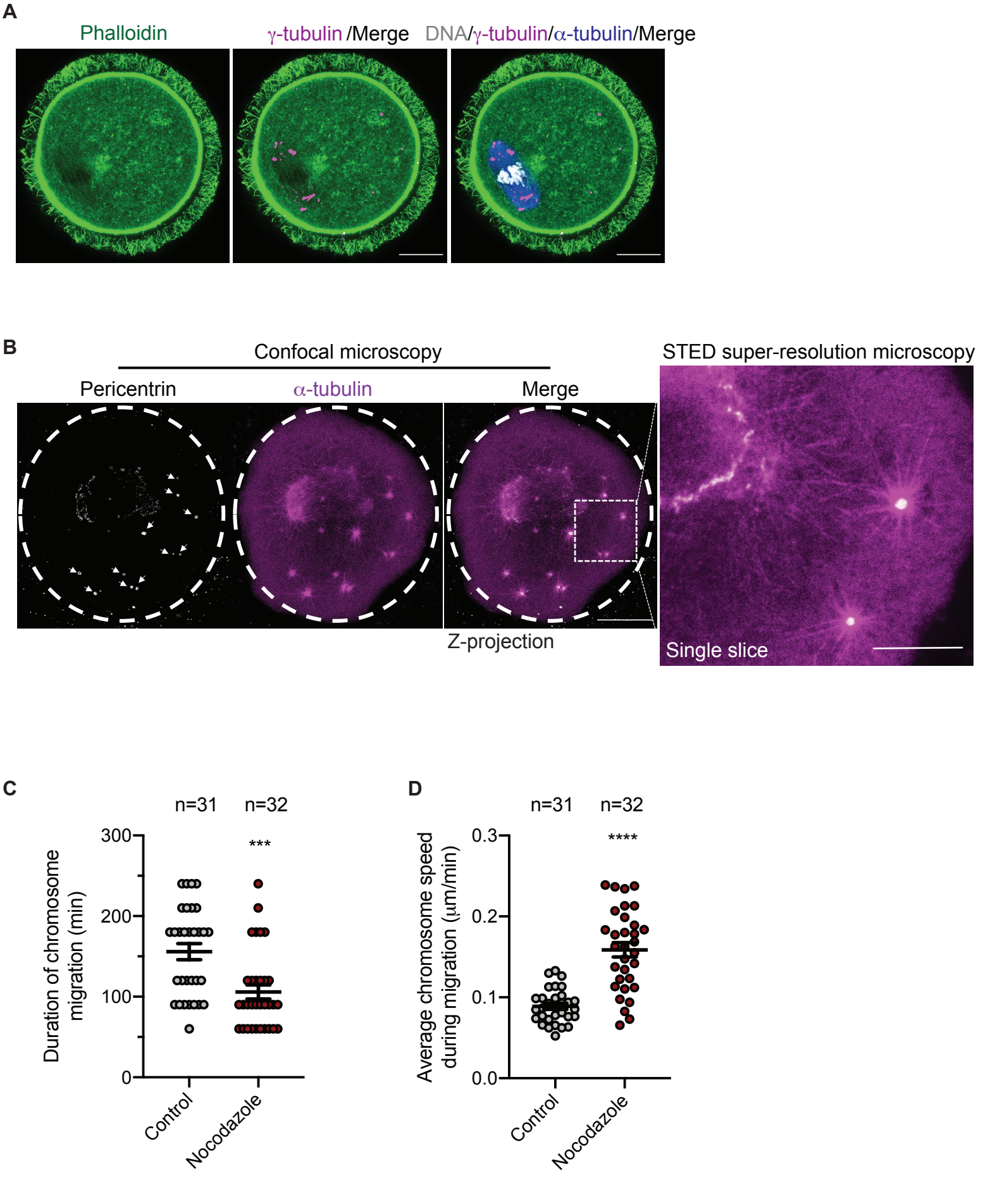

A
n= 508
% of oocytes
Average mcMTOC numbers per oocyte
B
GFP
Pericentrin
DNA
Merge
AURKA-GFP
C
Pericentrin
α-tubulin
DNA/Merge
GFP
AURKA-GFP
D
Average mcMTOC numbers
n=20
n=20
GFP
AURKA-GFP
E
Average mcMTOC volume (μm)
n=20
n=20
GFP
AURKA-GFP
F
γ-tubulin
α-tubulin
DNA/Merge
1
2

Supp. Fig. 2

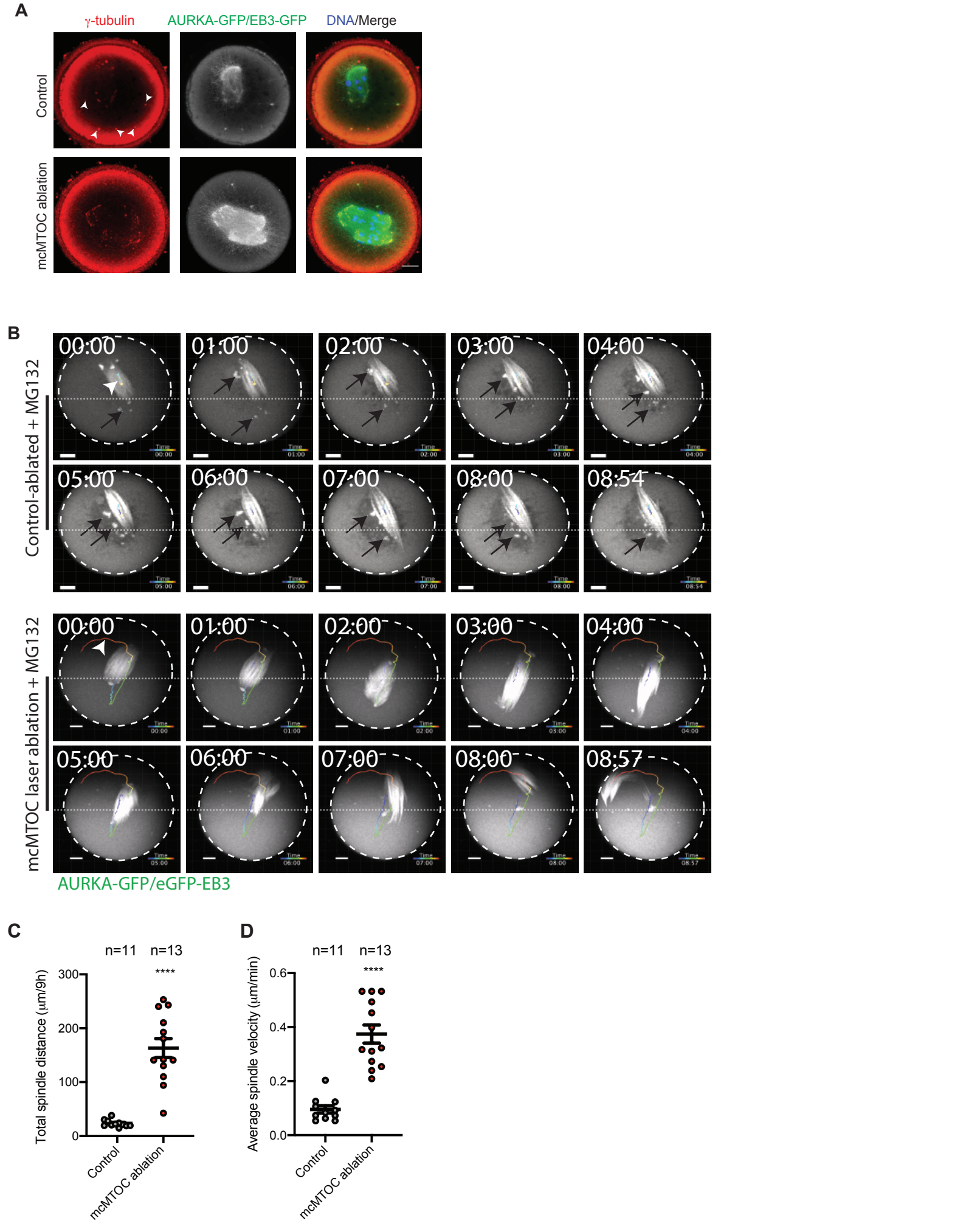


AURKA-eGFP/Bright-field/H2B-mCherry

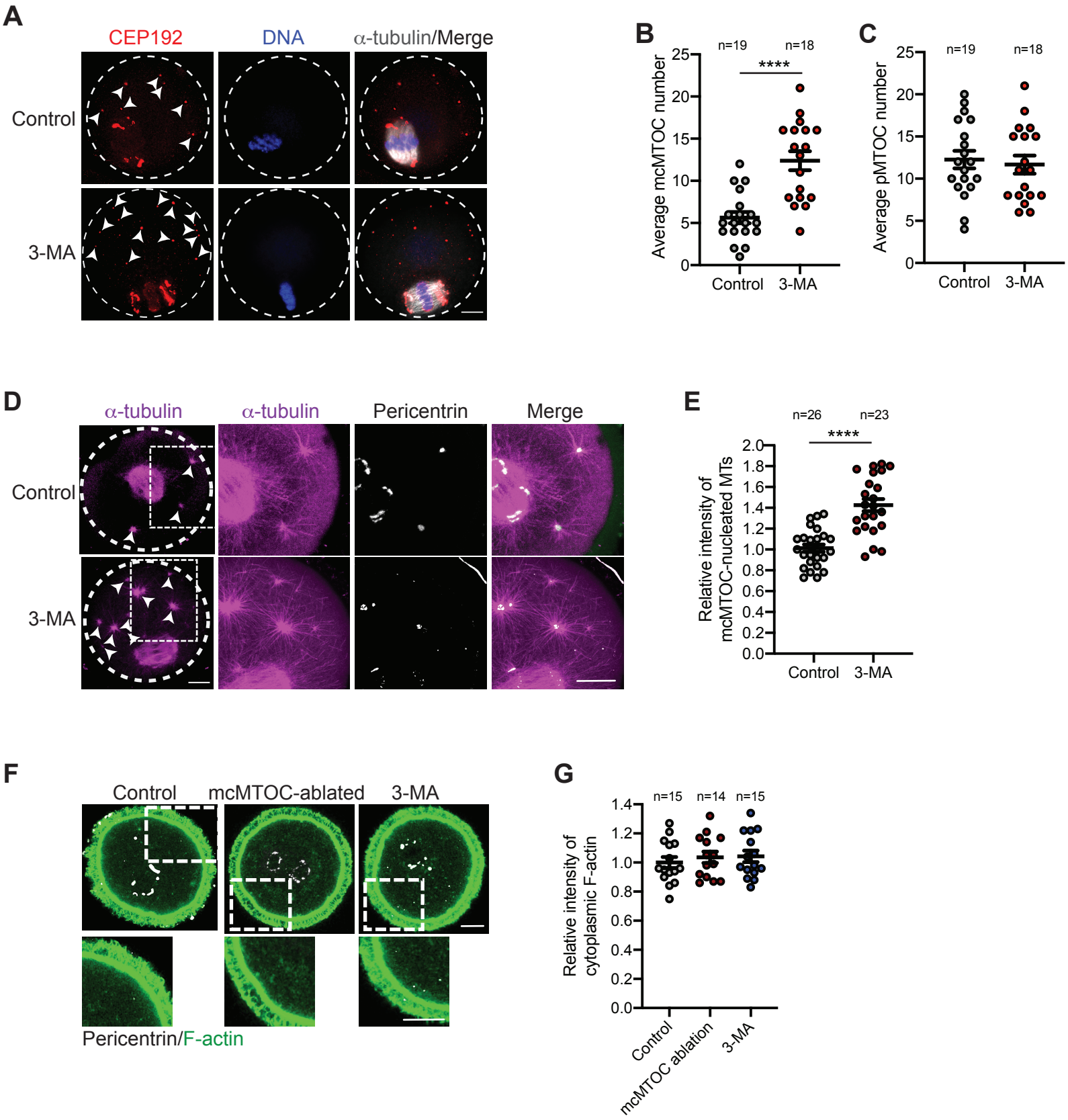
Supp. Fig. 3


A
Phalloidin
γ-tubulin /Merge
DNA/γ-tubulin/α-tubulin/Merge
B
Confocal microscopy
STED super-resolution microscopy
Pericentrin
α-tubulin
Merge
Z-projection
Single slice
C
n=31
n=32
***
Duration of chromosome migration (min)
300
200
100
0
Control
Nocodazole
D
n=31
n=32
****
Average chromosome speed during migration (μm/min)
0.3
0.2
0.1
0.0
Control
Nocodazole

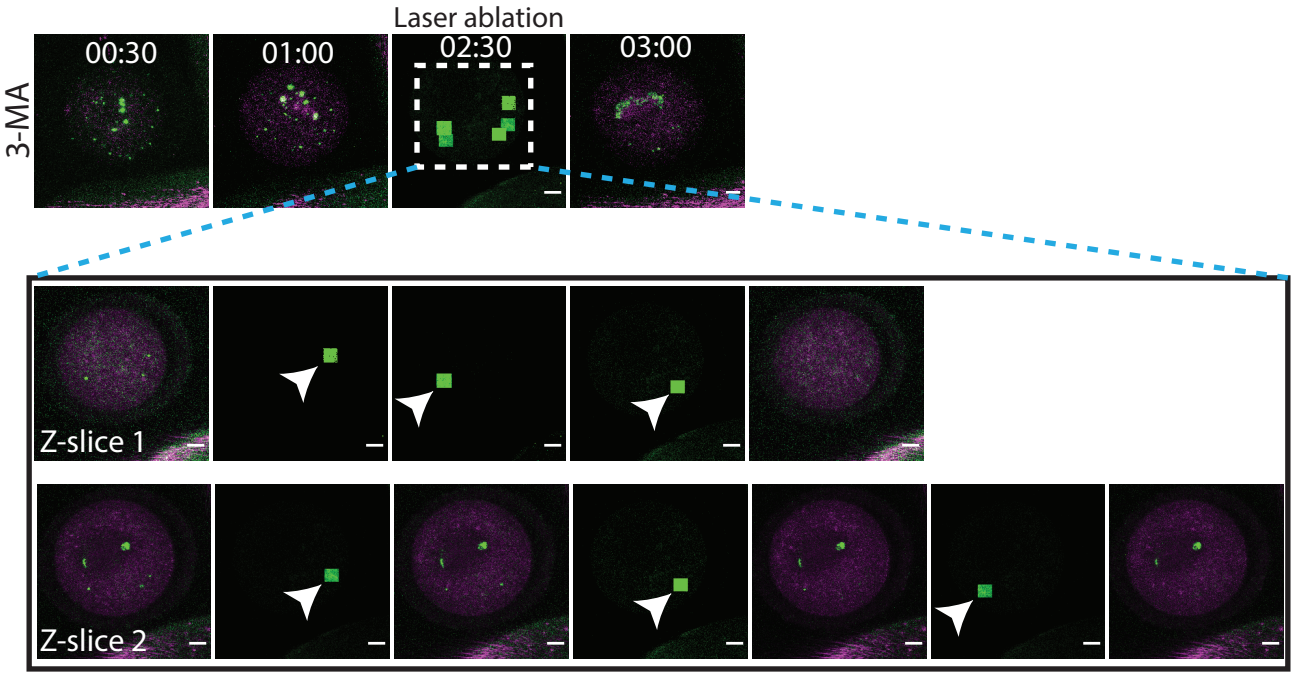
Supp. Fig. 4


A
γ-tubulin
AURKA-GFP/EB3-GFP
DNA/Merge
Control
mcMTOC ablation
B
Control-ablated + MG132
mcMTOC laser ablation + MG132
00:00
01:00
02:00
03:00
04:00
05:00
06:00
07:00
08:00
08:54
08:57
AURKA-GFP/eGFP-EB3
C
n=11
n=13
****
Total spindle distance (μm/9h)
300
200
100
0
Control
mcMTOC ablation
D
n=11
n=13
****
Average spindle velocity (μm/min)
0.6
0.4
0.2
0.0
Control
mcMTOC ablation

Supp. Fig. 5


A
CEP192
DNA
α-tubulin/Merge
Control
3-MA
B
n=19
n=18
****
Average mcMTOC number
Control
3-MA
C
n=19
n=18
Average pMTOC number
Control
3-MA
D
α-tubulin
α-tubulin
Pericentrin
Merge
Control
3-MA
E
n=26
n=23
****
Relative intensity of mcMTOC-nucleated MTs
Control
3-MA
F
Control
mcMTOC-ablated
3-MA
Pericentrin/F-actin
G
n=15
n=14
n=15
Relative intensity of cytoplasmic F-actin
Control
mcMTOC ablation
3-MA

Supp. Fig. 6



CEP192-EGFP/SiR-tubulin