Supporting Information

Kolano et al. 10.1073/pnas.1204686109

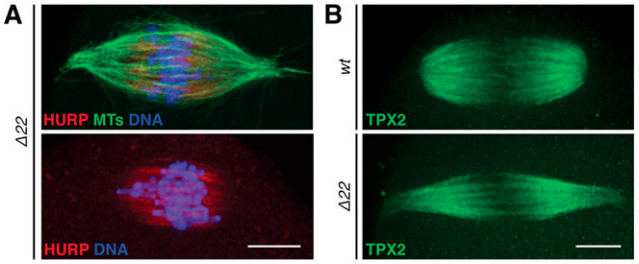


Fig. S1. Functional NuMA is required for polar but not central spindle organization. (*A*) HURP is localized properly in the central spindle of Δ*22* oocytes. MI oocytes were labeled with HURP (red), tubulin (green), and DNA (blue). HURP has the expected localization in the central spindle region. (*B*) TPX2 labeling extends to spindle extremities. MII oocytes from *wt* (*Upper*) or Δ*22* (*Lower*) oocytes were labeled for TPX2.

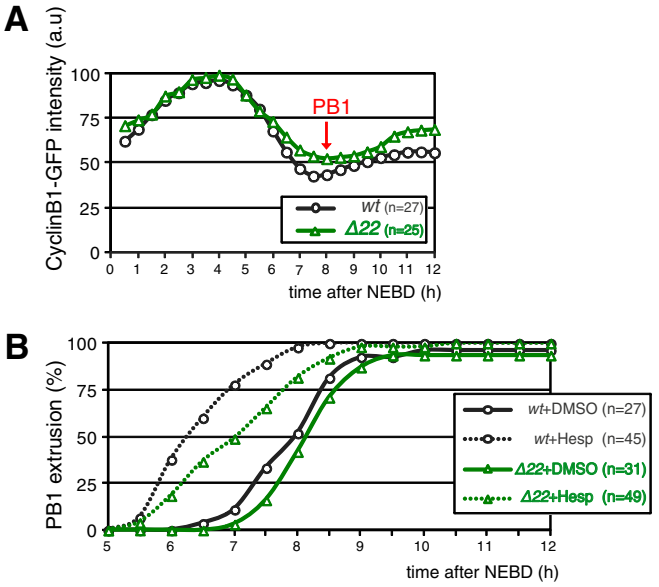


Fig. S2. Cyclin B1-GFP is degraded at anaphase I with identical kinetics in control vs. Δ*22* oocytes. (*A*) Graph shows the kinetics of cyclin B1-GFP accumulation during meiotic maturation in control (gray) vs. Δ*22* (green) oocytes. The fluorescence intensity of cyclin B1-GFP in each condition was normalized to the maximum intensity reached at NEBD + 4 h. The red arrow indicates the time of PB1. (*B*) Treatment with hesperadin, an Aurora B/C inhibitor, accelerates the extrusion of PB1 more efficiently in controls (gray) compared to Δ*22* oocytes (green). Hesperadin (100 nM) was added at NEBD + 4 h, and oocytes were scored for PB1. Mutant oocytes are advanced after hesperadin treatment: The mean time of PB1 is 6 h and 40 min ± 40 min for *wt* oocytes (gray dotted line) and 7 h and 20 min ± 1 h for Δ*22* oocytes (green dotted line) treated with hesperadin compared to 8 h and 20 min ± 1 h and 50 min for *wt* oocytes and 8 h and 40 min ± 1 h and 20 min for Δ*22* oocytes treated with DMSO. The difference in mean PB1 timing was extremely significant ($P < 0.0001$) between controls and hesperadin-treated oocytes, and the difference between *wt* and Δ*22* oocytes treated with hesperadin was very significant ($P = 0.0013$).

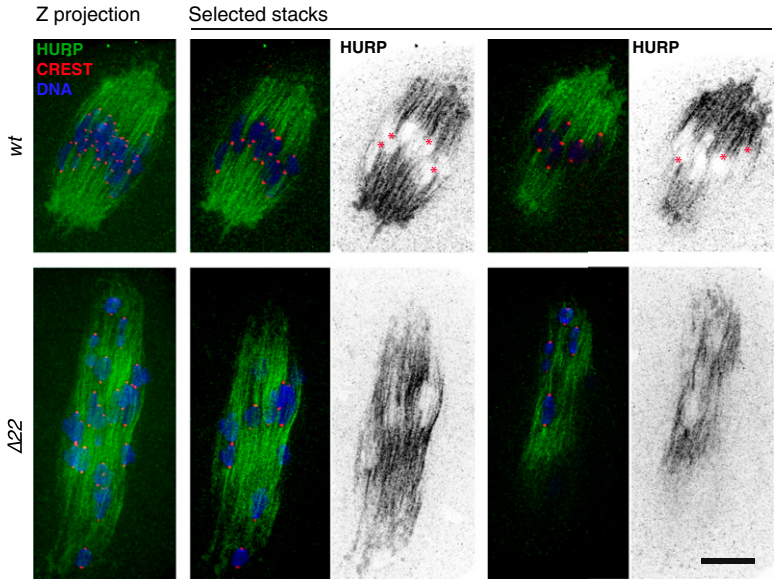
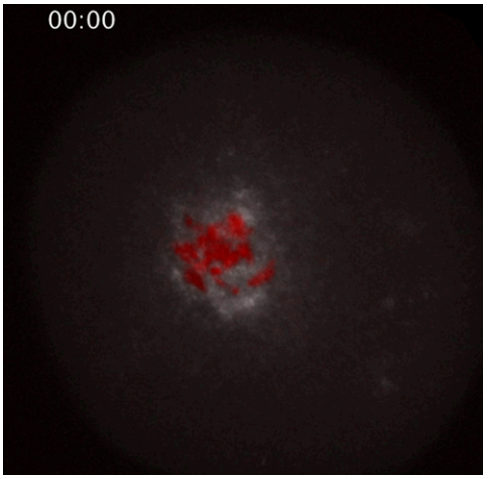
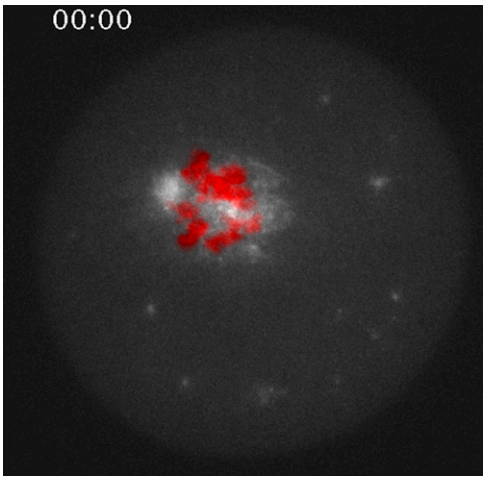


Fig. S3. NuMA Δ*22* oocytes do not exhibit K-fibers defects at the end of MI. Controls (*n* = 14) and NuMA Δ*22* oocytes (*n* = 13) were fixed and stained at NEBD + 7 h with Hoechst for chromosomes (blue), CREST for kinetochores (red), and HURP (green) for interzonal and kinetochore microtubules (MTs). Maximum projections and selected stacks are presented. Asterisks highlight kinetochore MTs. (Scale bar: 10 μm)



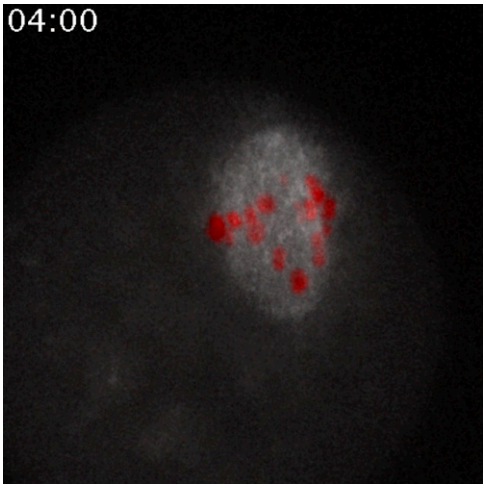
Movie S1. Early steps of meiotic spindle formation in a *wt* oocyte. Time-lapse microscopic analysis shows a live *wt* oocyte expressing EB3-GFP (gray) and H2B-RFP (red). The movie starts at NEBD. Maximal Z-projections of six confocal sections are shown (Z-step = 3 μm). Time in hours and minutes (h:min) after NEBD is shown. Acquisitions were taken every 20 min.

Movie S1



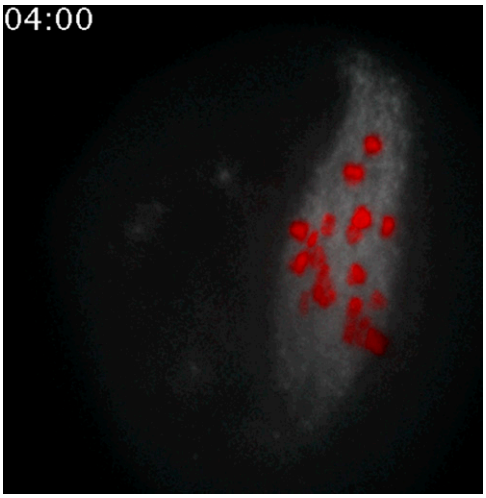
Movie S2. Chaotic spindle assembly in a Δ*22* oocyte. Time-lapse microscopic analysis shows a live Δ*22* oocyte that expresses EB3-GFP (gray) and H2B-RFP (red) starting from NEBD. Maximal Z-projections of six confocal sections are shown (Z-step = 3 μm). Time in hours and minutes (h:min) after NEBD is shown. Acquisitions were taken every 30 min.

Movie S2



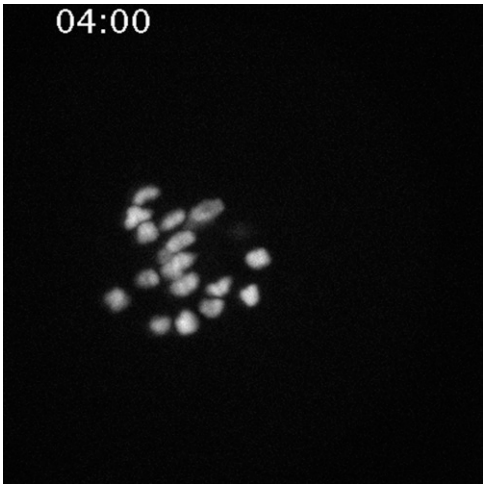
Movie S3. Mid-MI and extrusion of PB1 in a *wt* oocyte. Time-lapse microscopic analysis shows a live *wt* oocyte that expresses EB3-GFP (gray) and H2B-RFP (red). The movie starts from NEBD + 4 h. Maximal Z-projections of seven confocal sections taken every 3 μm are shown. Time in hours and minutes (h:min) after NEBD is shown. Acquisitions were taken every 20 min.

Movie S3



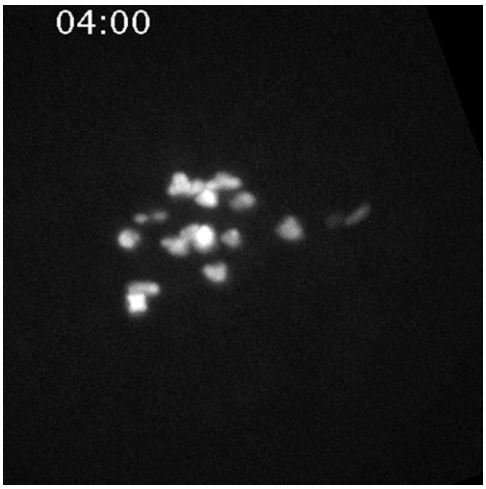
Movie S4. Rescue of spindle morphology defects and extrusion of PB1 in a Δ*22* oocyte. Time-lapse microscopic analysis shows a live Δ*22* oocyte that is expressing EB3-GFP (gray) and H2B-RFP (red). The movie starts from NEBD + 4 h. Maximal Z-projections of seven confocal sections taken every 3 μm are shown. Time in hours and minutes (h:min) after NEBD is shown. Acquisitions were taken every 20 min.

Movie S4



Movie S5. Chromosome segregation in a *wt* oocyte. Time-lapse microscopic analysis shows a live control oocyte expressing H2B-RFP (gray) from mid-MI (NEBD + 4 h) until PB1 extrusion. Maximal Z-projections of seven confocal sections are shown (Z-step = 3 μm). Time in hours and minutes (h:min) after NEBD is shown. Acquisitions were taken every 20 min.

Movie S5



Movie S6. Lagging chromosomes in a Δ*22* oocyte. Time-lapse microscopic analysis shows a live Δ*22* oocyte expressing H2B-RFP (gray) from mid-MI (NEBD + 4 h) until PB1 extrusion. Maximal Z-projections of seven confocal sections are shown (Z-step = 3 μm). Time in hours and minutes (h:min) after NEBD is shown. Acquisitions were taken every 20 min.

Movie S6