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A liquid-like spindle domain promotes acentrosomal spindle assembly in mammalian oocytes

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Abstract

Mammalian oocytes segregate chromosomes with a microtubule spindle that lacks centrosomes. How acentrosomal spindles are organized and function is largely unclear. Here, we identify a conserved subcellular structure in mammalian oocytes that forms by phase separation. This structure, which we term the liquid-like meiotic spindle domain (LISD), permeates the spindle poles and forms dynamic protrusions that extend well beyond the spindle. The LISD selectively concentrates multiple microtubule regulatory factors, and allows them to diffuse rapidly within the spindle volume. Disruption of the LISD via different means disperses these factors and leads to severe spindle assembly defects. Our data suggest a model whereby the LISD promotes meiotic spindle assembly by serving as a reservoir that sequesters and mobilizes microtubule regulatory factors in proximity of spindle microtubules.

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Once every menstrual cycle, an oocyte progresses through the first meiotic division to mature into a fertilizable egg. To this end, the oocyte eliminates half of its chromosomes in a small cell, called a polar body. The remaining chromosomes become aligned in the second metaphase spindle, and the egg is released into the fallopian tube, where it can be fertilized. Upon fertilization, the egg completes the second meiotic division, during which it eliminates half of the remaining sister chromatids into the second polar body. Subsequently, the male and female pronuclei form, progress towards each other, and the mitotic divisions of the embryo begin.

Mammalian embryos frequently develop abnormally though, resulting in miscarriages and genetic disorders such as Down syndrome. The major cause for aberrant embryonic development is aneuploidy in the egg, which results from chromosome segregation errors during oocyte meiosis. Unlike somatic cells and male germ cells, oocytes segregate chromosomes with a specialized microtubule spindle that lacks centrosomes (1). Canonical centrosomes consist of a pair of centrioles surrounded by pericentriolar material, and are the main microtubule organizing centers in centrosomal spindles. They serve as the major sites of microtubule nucleation and form the two poles of mitotic spindles. Oocytes have developed mechanisms to nucleate microtubules independently of centrosomes. For instance, in *Drosophila* oocytes, the augmin complex and Subito (kinesin-6) mediate microtubule nucleation from the spindle poles and spindle equator respectively, by recruiting the γ -tubulin complex (2–4). The γ -tubulin complex resides at the minus ends of microtubules and serves as a template for microtubule nucleation (5). Chromosomes can also serve as sites of microtubule nucleation, as studied most comprehensively in *Xenopus* egg extracts: they locally activate the small GTPase Ran, which releases spindle assembly factors from inhibitory binding to importins to promote local microtubule assembly (6).

How acentrosomal spindles are organized in mammalian oocytes is still incompletely understood. Despite the absence of centrosomes, mammalian oocytes express many centrosomal proteins (7). Some of these proteins have been mapped to the acentriolar microtubule organizing centers (aMTOCs) (table S1), which functionally replace centrosomes in mouse oocytes (8, 9). However, a comprehensive map of centrosomal protein localization in oocytes is lacking. Such a map would not only shed light on the roles of centrosomal proteins during oocyte meiosis, but might also reveal novel functions and subdomains of centrosomes in other cell types.

Results

Identification of the LISD – a liquid-like meiotic spindle domain

We analysed the localization of 70 centrosomal and spindle-related proteins in mouse metaphase I oocytes (Fig. 1A, fig. S1, A and B, and table S1). We identified several new aMTOC components, including CEP120, CP110, DISC1, KIF2B, MCRC1 and TOP2A. Of the 17 centriolar proteins that we examined, only CNTROB, CNAP1 and CP110 localized to aMTOCs, consistent with the absence of centrioles in oocytes (1). Proteins that constitute the pericentriolar material of centrosomes mostly localized to aMTOCs. Several centrosome-associated regulatory kinases and their substrates also localized to aMTOCs, and many of the mapped proteins showed enrichment to the overall spindle region.

Unexpectedly, we observed a previously undescribed domain that contains 19 proteins, including centrosomal proteins (AKAP450, CEP170 and KIZ), centriolar satellite proteins (CEP72, PCM1 and LRRC36), minus-end binding proteins (CAMSAP3 and KANSL3), dynein-related proteins (HOOK3, NDE1, NDEL1 and SPDL1) and proteins that control microtubule nucleation and stability (CHC17, chTOG, GTSE1, HAUS6, MCAK, MYO10 and TACC3) (fig. S1, A and B, and table S1). This domain permeated a large region of the spindle and formed prominent spherical protrusions that extended well beyond the spindle poles during late meiosis I as well as in the metaphase II spindle (Fig. 1, A to C), and was conserved in oocytes from other mammalian species (Fig. 1C, and fig. S2).

Many of the proteins observed within this domain have been studied extensively in mitosis, yet similar structures have not been reported in somatic cells. To investigate whether the domain is a specific feature of acentrosomal spindles in mammalian oocytes, we depleted centrosomes in somatic cells by centrinone treatment (10) (fig. S3, A and B). Intriguingly, mitotic spindles in centrosome-depleted mouse embryonic fibroblasts did not possess a related domain (fig. S3, B to D). This result suggests that the domain is not a general feature of acentrosomal spindles, but likely unique to female meiotic spindles. As we outline in detail below, this domain forms by phase separation and behaves similar to a liquid. We will therefore refer to it as the liquid-like meiotic spindle domain (LISD).

AURA, TACC3 and CHC17 are essential for LISD assembly

To investigate LISD assembly, we first examined the role of the regulatory kinases Aurora A (AURA), Polo-like kinase 1 (PLK1) and Polo-like kinase 4 (PLK4), which control spindle assembly in multiple systems, including oocytes (11). Whereas pharmacological inhibition of PLK1 and PLK4 did not affect LISD assembly, inhibition of AURA led to disruption of the LISD (fig. S4, A to E). Of the LISD-associated proteins that we identified, only TACC3 and GTSE1 are known mitotic substrates of AURA (12–15). Specific depletion of endogenous TACC3 by Trim-Away fully disassembled the domain (fig. S5, A to D), leading to complete dispersion of multiple LISD-associated proteins, including CAMSAP3 (minus-end stability) (16), chTOG (microtubule nucleation and stability) (17, 18) and GTSE1 (microtubule stability) (19, 20) (fig. S6). Some LISD-associated proteins, such as CHC17 (microtubule stability) (21), HAUS6 (microtubule nucleation) (22, 23) and KANSL3 (minus-end stability) (24) were no longer detected as prominent protrusions and strongly depleted from the spindle, but still showed some residual association with microtubules upon depletion of TACC3 (fig. S6). In contrast, depletion of GTSE1 only had a minor effect on LISD assembly (fig. S7, A and B). LISD assembly was also disrupted upon depletion of CHC17 (fig. S8, A and B), which binds to microtubules together with TACC3 (25, 26). TACC3 truncations (26) that are unable to interact with CHC17 (fig. S8C) or bind microtubules (fig. S8D) were not incorporated into the LISD, consistent with the co-dependence of LISD assembly on TACC3 and CHC17. Thus, AURA, TACC3 and CHC17 are essential for LISD assembly.

The LISD is a liquid-like assembly

To further analyse LISD assembly, we performed live imaging and immunofluorescence on mouse oocytes at different maturation stages. We utilized TACC3 as the reporter, as it is a

core component of the LISD. The LISD accumulated within the center of the microtubule mass after nuclear envelope breakdown (NEBD), permeated the entire microtubule ball as the spindle grew and the aMTOCs fragmented, and translocated towards the two spindle poles together with the aMTOCs during spindle bipolarization (Fig. 2, A and B). As the bipolar spindle increased in size from early to late metaphase I, the LISD grew and extended beyond the spindle poles (Fig. 2, A and B). The LISD was not only enriched in the pole region, but also formed projections towards the midzone in the metaphase I spindle (Fig. 1B, and movie S1). Protein-retention expansion microscopy and short-term treatment with EG5 inhibitor monastrol further demonstrated that the LISD was present throughout the spindle body (Fig. 2C, and fig. S9), with enrichments on kinetochore fibers (K-fibers) confirmed by cold-stable assays and immunoelectron microscopy (Fig. 2C, and fig. S10, A to D). Also the LISD components CHC17 and PCM1 showed similar localization patterns as TACC3 throughout oocyte maturation (fig. S11, A to C).

High-resolution time-lapse microscopy of fluorescently-labelled TACC3 further revealed that the spindle pole-associated protrusions of the LISD were dynamic and fused with each other, generating larger protrusions (Fig. 3A). Given that the protrusions of the LISD existed in a region that is largely devoid of microtubules, we asked if the LISD can be maintained independently of microtubules. Indeed, mouse oocytes acutely treated with the microtubule-depolymerizing drug nocodazole transiently maintained the LISD, even after the bulk of microtubules had disappeared (Fig. 3B, and movie S2). The LISD eventually disappeared, and then reassembled into new AURA-dependent spherical condensates after a few minutes (Fig. 3B, fig. S12, and movie S2). Endogenous TACC3 and PCM1 also formed these spherical condensates in oocytes fixed after nocodazole addition (Fig. 3C), demonstrating that they were not an artifact of ectopically expressed, fluorescently-labelled TACC3.

Interestingly, even in the absence of microtubules, the spherical condensates fused with each other and formed larger foci (Fig. 3D, and movie S3). This droplet-like behavior was reminiscent of properties of phase-separated structures reported in recent studies (27). We hence asked if the LISD might form by phase separation. Phase separation is characterized by the absence of membrane surrounding the phase-separated structure. To test for the absence of membrane, we established a workflow for focused ion beam-scanning electron microscopy (FIB-SEM) of mouse oocytes and imaged the meiotic spindle pole at isotropic resolution in 3D (fig. S13A). This analysis confirmed that the LISD was not enclosed by membranes (fig. S13, B and C).

Phase-separated structures are further characterized by their ability to rearrange internally. To test for rearrangement, we performed fluorescence recovery after photobleaching (FRAP) on half of a TACC3-labelled spherical condensate in nocodazole-treated oocytes. The TACC3 signal recovered most prominently directly adjacent to the bleached region, indicating diffusion of fluorescent TACC3 from within the non-bleached region into the bleached region. While the signal recovered in the bleached region, the non-bleached region became dimmer over time, consistent with internal rearrangement within the spherical condensate (Fig. 3E). Consistent with these results, photoactivated bars of TACC3 and PCM1 in intact spindles rapidly spread out bidirectionally within the LISD. In contrast, the photoactivated bar of the microtubule-associated protein TPX2, which is not a LISD

component, moved slowly and unidirectionally towards the spindle pole (Fig. 3F). In addition to droplet-like behavior and internal rearrangement, proteins in the LISD and spherical condensates recovered after bleaching (fig. S14, A and B). Remarkably, proteins in the LISD recovered with similar, rapid kinetics (fig. S14B). In contrast, core aMTOC proteins such as CDK5RAP2, CEP192 and PCNT showed no prominent turnover on aMTOCs in metaphase I spindles (fig. S14C).

Depending on protein dynamics and reversibility, phase-separated structures can be further classified into liquid-like (dynamic and reversible), gel-like (arrested and reversible) and solid-like (arrested and irreversible) (28). As proteins in the LISD were highly dynamic (fig. S14B) and the LISD reversibly disassembled and reassembled upon nocodazole addition and washout (Fig. 2B, and fig. S15A), we hypothesized that the LISD is liquid-like. To test this hypothesis further, we discriminated liquid-like from solid-like condensates in oocytes by treating them with 1,6-hexanediol, which dissolves liquid-like condensates by disrupting weak hydrophobic interactions (29). The spherical condensates in nocodazole-treated oocytes dispersed within minutes of 1,6-hexanediol addition (Fig. 3G). 1,6-hexanediol treatment also strongly depleted the LISD on intact spindles, though not completely (fig. S15, B and C). Finally, thioflavin T, which stains the amyloid-like interactions that are characteristic of solid-like condensates, did not stain the LISD (fig. S15D). Together, these data strongly suggest that the LISD is a liquid-like condensate.

TACC3 phase-separates via its N-terminus

We discovered that the spherical condensates in nocodazole-treated oocytes were dependent on TACC3, but only mildly affected by CHC17 depletion (fig. S16, A and B). We thus tested whether TACC3 can phase-separate on its own *in vitro*. In the presence of the macromolecular crowding agent polyethylene glycol (PEG), recombinant TACC3 self-assembled into micron-sized spherical droplets over a range of pH values, ionic strengths, PEG concentrations and protein concentrations (fig. S17, A to C). TACC3 droplets were able to wet glass surfaces, fused into larger droplets and excluded fluorescently-labelled 70 kDa dextran (Fig. 4, A to C), consistent with characteristics of phase separation (27). In further support of liquid-liquid phase separation, TACC3 droplets rapidly recovered after bleaching (Fig. 4D, and fig. S17D).

In silico predictions suggested that both human and mouse TACC3 are bipartite, with a disordered N-terminus and a structured, coiled-coil-containing C-terminus (Fig. 4E). Both disordered and coiled-coil domains have been implicated in driving phase separation (30). To address which region of TACC3 mediates phase separation, we purified TACC3 fragments that contained only the coiled-coil TACC domain (aa 594-838) or the rest of the protein TACC3(TACC) (aa 1-593). TACC3(TACC) retained the ability to form droplets, similar to full-length TACC3 (Fig. 4F). By contrast, the TACC domain self-organized into network-like structures (Fig. 4F). These results reveal that the previously uncharacterized, disordered N-terminus of TACC3 is necessary and sufficient for phase separation *in vitro*.

Although the N-terminus of TACC3 is required for its phase separation *in vitro*, it is dispensable for TACC3 localization in *Xenopus* mitotic extracts (15) and in mitotic cells (26). To determine if the TACC3 N-terminus is essential for LISD assembly, we expressed

TACC3(NT) (aa 522-838) in TACC3-depleted oocytes. TACC3(NT) lacks the disordered N-terminus but still contains the regions required to bind both CHC17 and microtubules. Although TACC3(NT) localized properly to the spindle and associated with K-fibers, as confirmed by cold treatment, it failed to restore LISD assembly (Fig. 4, G and H). Instead, consistent with the behavior of the purified TACC domain in vitro (Fig. 4F), TACC3(NT) and CHC17 assembled network-like structures at the spindle poles in vivo (Fig. 4G). These data indicate that the N-terminus of TACC3 is essential for phase separation in vivo. Notably though, the N-terminus of TACC3 is not sufficient for TACC3 recruitment to the LISD in vivo, as TACC3(TACC) was not incorporated into the LISD (fig. S8D). Microtubule binding via the TACC domain is apparently required for LISD formation and may help to stabilize the condensates in vivo, as also suggested by the higher resistance of TACC3 condensates to 1,6-hexanediol treatment on intact spindles than in nocodazole-treated oocytes (Fig. 3G and fig. S15, B and C).

The LISD promotes acentrosomal spindle formation by sequestering microtubule regulatory factors

Phase separation can selectively enrich factors to promote reactions or storage (27). Recent studies proposed that the phase-separated *Xenopus* microtubule-binding protein BuGZ, regulatory kinase PLK4 and the *Caenorhabditis elegans* centrosomal protein SPD-5 promote microtubule nucleation in centrosomal spindles by locally enriching tubulin dimers by a factor of 4 to 10 (31–33). In contrast, LISD protrusions and spherical condensates did not significantly concentrate tubulin dimers (fig. S18, A to E). Interestingly, however, microtubule dynamics were altered in TACC3-depleted oocytes: microtubule growth rates were significantly decreased, and their overall turnover was significantly increased (fig. S19, A to D). We thus hypothesized that the LISD locally concentrates microtubule regulatory factors to promote spindle assembly. To test this hypothesis, we examined acentrosomal spindle assembly upon ablation of the LISD by three different means: inhibition of AURA, depletion of TACC3 and depletion of CHC17, which are all essential for LISD assembly (see above). Under all conditions, the total microtubule intensity and spindle volume were severely reduced, to about half of the values in control oocytes (Fig. 5, A to C, fig. S20, A to E, fig. S21, A to I, and movie S4 and S5). Moreover, aMTOCs were no longer scattered around the spindle poles, but coalesced into two single foci (fig. S22, A to K, and S23, A to I). Similarly, AURA inhibition and TACC3 depletion in bovine oocytes caused a severe reduction in microtubules as well as spindle assembly defects (Fig. 5, D to F, fig. S24), in line with the abnormal spindles observed in a previous RNA interference (RNAi) study (34).

In mitotic cells, depletion of TACC3 causes a minor loss of spindle microtubules (17, 23), but results in misaligned chromosomes and metaphase arrest (25, 35–38). To uncover LISD-specific functions, we took advantage of TACC3(NT), which can neither phase-separate in vitro nor restore LISD assembly in vivo (Fig. 4G). We do not exclude the possibility that there are additional, phase separation-independent functions associated with the N-terminus of TACC3. However, TACC3(NT) has been shown to rescue the phenotypes induced by TACC3 depletion in *Xenopus* mitotic extracts and in mitotic cells, and the N-terminus of TACC3 has hence been suggested to be dispensable for TACC3 function in these systems (15, 26). Strikingly, expression of TACC3(NT) in TACC3-depleted mouse oocytes did not

rescue the reduction in total microtubule intensity, and the spindle volume was still significantly reduced compared to wild-type oocytes, albeit not as strongly as in TACC3-depleted oocytes, as microtubules in the spindle appeared less densely packed and more spread out (Fig. 6, A to C, and movie S6). Moreover, spindle bipolarization was severely delayed in TACC3(NT)-TACC3-depleted oocytes relative to wild-type oocytes, and spindles progressed through a prolonged phase of spindle instability and fragmentation before becoming bipolar (Fig. 6A and fig. S25, A and B). Also the association between aMTOCs and spindles in TACC3(NT)-TACC3-depleted oocytes was abnormal (fig. S25C). In contrast, TACC3(NT) rescued progression into anaphase and markedly reduced lagging chromosomes in TACC3-depleted oocytes, demonstrating that the construct is functional (fig. S25, D to G). Together, these data establish that the LISD is required for efficient microtubule assembly and to form stable acentrosomal spindles.

The LISD is required to form K-fibers and interpolar microtubules

To further investigate the role of the LISD in spindle assembly, we examined K-fibers and interpolar microtubules. To assess K-fibers, we briefly placed oocytes on ice, which depolymerizes dynamic microtubules within the spindle while preserving the more stable, kinetochore-bound microtubules. K-fibers were significantly diminished in TACC3-depleted oocytes (Fig. 7, A to C). To assess interpolar microtubules, we treated oocytes with calcium, which preserves both K-fibers and stable interpolar microtubules in mouse oocytes (39). Stable interpolar microtubules were strongly depleted in TACC3-depleted oocytes (Fig. 7D, and movie S7 and S8). Quantification revealed a more severe microtubule loss than in the cold-stable assay (Fig. 7, E and F). We also labelled spindles with the microtubule cross-linker PRC1, which associates with interpolar microtubules (40). Consistent with the calcium-stable assay, PRC1-marked microtubules were drastically reduced in TACC3-depleted oocytes (Fig. 7, G and H, and movie S9 and S10). In addition, oocytes in anaphase displayed a significant reduction in the total intensity and volume of the central spindle (Fig. S26, A to C), which consists of interpolar microtubules and is largely devoid of K-fibers (41). Thus, the LISD is essential for the correct assembly of both K-fibers and interpolar microtubules in mammalian oocytes.

Discussion

In summary, our data uncover a new principle of acentrosomal spindle assembly in mammalian oocytes: meiotic spindle assembly is facilitated by a prominent liquid-like domain that contains multiple microtubule regulatory factors, and enriches them in a dynamic manner in proximity of spindle microtubules. The domain permeates the spindle poles and forms dynamic protrusions extending outward well beyond the spindle region in mouse, bovine, ovine and porcine oocytes. Ablating the domain results in microtubule loss and defective spindle assembly.

Enriching microtubule regulatory factors in local proximity of the spindle may be particularly important for spindle assembly in large cells such as oocytes, where they would otherwise be dispersed throughout the large cytoplasmic volume. Liquid-liquid phase separation may be an ideal principle for this purpose: it sequesters factors within proximity

of microtubules, but still allows them to diffuse dynamically throughout the spindle region. This could also help to control the local concentration of certain spindle-related factors and prevent them from accumulating within specific spindle regions such as the spindle poles or kinetochores, but instead promote their even distribution throughout the entire spindle volume.

Does the LISD play a role in spindle assembly beyond mammalian oocytes? Structures that resemble the LISD have not yet been reported in other cell types (42), although many LISD proteins have been extensively studied in mitotic cells. Given the absence of LISD-related structures in centrosome-depleted mitotic cells, it appears likely that the LISD is an oocyte-specific structure. Intriguingly though, when TACC3 is highly overexpressed in somatic cells, it forms large structures that can associate with the mitotic spindle (43). Whether phase separation also plays a role in the formation of these structures is unclear.

In centrosomal spindles, phase separation has been proposed to enrich tubulin dimers to facilitate microtubule nucleation (31–33). However, the LISD does not concentrate tubulin dimers, but enriches different microtubule regulatory factors in acentrosomal spindles. One could speculate that an enrichment of tubulin dimers is less important in large oocytes, as tubulin is not limiting in large cytoplasmic volumes (44, 45). Indeed, recent studies imply microtubule dynamics as a key factor in determining spindle size when tubulin is not limiting (46). Mammalian oocytes may use phase separation of microtubule regulatory factors as a mechanism to modulate microtubule dynamics and to thereby promote the assembly of large meiotic spindles. The LISD differs from other phase-separated structures in terms of morphology, composition and mechanism of action, underscoring that phase separation is a widely used principle that can promote spindle assembly via distinct mechanisms in different species and cell types.

Materials and Methods

Preparation and culture of mouse oocytes and follicles

All mice were maintained in a specific pathogen-free environment according to animal ethics guidelines of the Animal Facility of Max Planck Institute for Biophysical Chemistry and UK Home Office regulations.

Oocytes were isolated from ovaries of 8 - 12-week-old FVB/N or CD1 female mice. For some experiments that required large numbers of germinal vesicle (GV) oocytes, the mice were primed with 7.5 IU of pregnant mare serum gonadotropin 48 h before isolation. Fully grown oocytes of ~75 μm in diameter with a centered GV were maintained at prophase arrest in homemade phenol red-free M2 medium supplemented with 250 μM dibutyryl cyclic AMP (dbcAMP) (Sigma-Aldrich) under paraffin oil (ACROS Organics) at 37°C.

Follicles were isolated from 12-day-old (C57BL $\times$ CBA) F1 female mice as previously described (49) with some minor modifications. Briefly, compact follicles of ~100 μm in diameter with a centered oocyte were cultured in MEM-alpha with GlutaMax (Gibco) supplemented with 5% fetal bovine serum (FBS) (Gibco), 0.01 $\mu\text{g/ml}$ ovine follicle stimulating hormone (National Hormone and Peptide Program), 1 $\times$ insulin/transferrin/

sodium selenite (Sigma-Aldrich) and 0.1× penicillin G/streptomycin (Gibco) on collagen-coated inserts (Corning) at 37°C/5% CO₂. Medium surrounding the insert was replaced every 3 d. After 8 d of culture, in vitro grown oocytes were denuded and matured in modified M2 medium with 10% FBS instead of 4 mg/ml bovine serum albumin (BSA).

Preparation and culture of bovine, ovine and porcine oocytes

All ovaries were obtained from local slaughterhouses. Bovine ovaries were transported to the laboratory within 2 h of retrieval in a thermo-flask. Ovine and porcine ovaries were transported to the laboratory within 1 h of retrieval in a portable 37°C incubator in M2 medium supplemented with 1 mM dbcAMP. Oocytes were recovered by aspiration of antral follicles with an 18-gauge needle affixed to a 1 ml syringe. For bovine oocytes, 140 µl of 5000 IU/ml heparin (Merck Millipore) was added to every 20 ml of aspirates. Cumulus-oocyte complexes (COCs) were allowed to sediment and then washed extensively with HEPES-buffered medium 199 (for bovine oocytes) or M2 medium (for ovine and porcine oocytes) supplemented with 1 mM dbcAMP. Only fully grown oocytes with a homogeneous cytoplasm and several layers of compact cumulus cells were selected for experiments. Bovine and ovine/porcine oocytes were maintained in prophase arrest in dbcAMP-containing medium at 39°C and 37°C, respectively. Surrounding cumulus cells of bovine and ovine/porcine oocytes were removed by vortexing and with 30 nM hyaluronidase (Sigma-Aldrich), respectively. Ovine and porcine oocytes were released into dbcAMP-free M2 medium to resume meiosis.

Cell culture

NIH3T3 cells (ATCC) were cultured in high glucose DMEM supplemented with GlutaMAX, pyruvate and 10% calf serum (Gibco). To deplete centrosomes, cells were treated with 300 nM centrinone (Tocris Bioscience) for 12 days. To obtain mitotic spindles, cells were synchronized with 10 µM RO-3306 (Tocris Bioscience) for 1 day and then released into RO-3306-free medium for 45 min.

Immunofluorescence

To obtain mouse and ovine/porcine metaphase I spindles, oocytes were incubated at 37°C for around 7 and 12 h, respectively, upon release into dbcAMP-free medium. To obtain bovine metaphase I spindles, oocytes were incubated at 39°C/5% CO₂ for around 12 h upon release into dbcAMP-free medium. To obtain mouse, bovine, ovine and porcine metaphase II spindles, oocytes were incubated for around 16 h upon release into dbcAMP-free medium. None of the oocytes used for immunofluorescence analyses was subjected to live imaging before fixation.

Oocytes and cells were fixed in 100 mM HEPES (pH 7.0, titrated with KOH), 50 mM EGTA (pH 7.0, titrated with KOH), 10 mM MgSO₄, 2% methanol-free formaldehyde and 0.5% triton X-100 at 37°C for 15 - 60 min. Fixed oocytes and cells were extracted in phosphate-buffered saline (PBS) with 0.5% triton X-100 (PBT) overnight at 4°C and blocked in PBT with 5% BSA (PBT-BSA) overnight at 4°C. All antibody incubations were performed in PBT-BSA at 10 µg/ml overnight at 4°C (for primary antibodies) or at 20 µg/ml for 1 h at room temperature (for secondary antibodies). Primary antibodies used were human anti-

centromere antibody (ACA) (FZ90C-CS1058; Europa Bioproducts), rabbit anti-AKAP450 (NBP1-89167; Novus Biological), rabbit anti-ASPM (NB100-2278; Novus Biological), rat anti- α -tubulin (MCA78G; Bio-Rad), mouse anti-AURA (NBP2-50041; Novus Biological), rabbit anti-pT288-AURA (NB100-2371; Novus Biological), rabbit anti-CAMSAP3 (50), rabbit anti-CDK5RAP2 (ABE236; Merck Millipore), mouse anti-pan centrin (04-1624; Merck Millipore), rabbit anti-CEP63 (06-1292; Merck Millipore), rabbit anti-CEP120 (PA5-55985; Thermo Fisher Scientific), rabbit anti-CEP135 (ab75005; Abcam), rabbit anti-CEP152 (ab183911; Abcam), rabbit anti-CEP164 (HPA037606; Sigma-Aldrich), rabbit anti-CEP170 (27325-1-AP; Proteintech), rabbit anti-CEP192 (18832-1-AP; Proteintech), mouse anti-CHC17 (610500; BD Biosciences), rabbit anti-CHC17 (ab21679; Abcam), rabbit anti-chTOG (PA5-59150 and PA5-58763; Thermo Fisher Scientific), rabbit anti-CNAP1 (14498-1-AP; Proteintech), rabbit anti-CP110 (12780-1-AP; Proteintech), rabbit anti-DHC (12345-1-AP; Proteintech), rabbit anti-DISC1 (NB110-40773; Novus Biological), goat anti-GFP (600-101-215; Rockland Immunochemicals), mouse anti-GM130 (610822; BD Biosciences), mouse anti-GMAP210 (611712; BD Biosciences), rabbit anti-GTSE1 (20), rabbit anti-GTSE1 (A302-425A; Bethyl Laboratories), mouse anti- γ -tubulin (11-465-C025; Exbio), rabbit anti-HAUS6 (51), rabbit anti-HOOK3 (NBP2-44279; Novus Biological), rabbit anti-KANSL3 (HPA035018; Sigma-Aldrich), rabbit anti-KIF2A (NB500-180; Novus Biologicals), rabbit anti-KIZ (21177-1-AP; Proteintech), mouse anti-LIS1 (H00005048-M03; Abnova), rabbit anti-MCRS1 (HPA039057; Sigma-Aldrich), sheep anti-MCAK (52), rabbit anti-MKLP2 (53), goat anti-MYO10 (sc-23137; Santa Cruz Biotechnology), rabbit anti-NDE1 (10233-1-AP; Proteintech), rabbit anti-NDEL1 (H00081565-D01P; Abnova), mouse anti-NEDD1 (H00121441-M05; Abnova), rabbit anti-ninein (ABN1720; Merck Millipore), mouse anti-NUMA (610561; BD Biosciences), rabbit anti-pS395-NUMA (3429; Cell Signaling Technology), rabbit anti-NUMA (14951; Cell Signaling Technology), rabbit anti-ODF2 (12058-1-AP; Proteintech), mouse anti-P50 (611002; BD Biosciences), mouse anti-P150 (612708; BD Biosciences), rabbit anti-PCM1 (54), rabbit anti-PCM1 (HPA023374; Sigma-Aldrich), mouse anti-Pericentrin (611814; BD Biosciences), rabbit anti-pericentrin (ab4448; Abcam), mouse anti-PLK1 (ab17056; Abcam), mouse anti-pT210-PLK1 (558400; BD Biosciences), goat anti-PLK4 (NB100-894; Novus Biological), rabbit anti-SSX2IP (HPA027306; Sigma-Aldrich), rabbit anti-STIL (ab89314; Abcam), rabbit anti-SPDL1 (55), goat anti-TACC3 (AF5720-SP; R&D Systems), mouse anti-TACC3 (H00010460-M02; Abnova), rabbit anti-TACC3 (ab134154; Abcam), rabbit anti-TGN46 (ABT95; Merck Millipore), mouse anti-TOP2A (MAB4197; Merck Millipore) and rabbit anti-TPX2 (NB500-179; Novus Biological). Secondary antibodies used were Alexa Fluor 405-, 488-, 568- or 647-conjugated anti-human IgG, goat IgG, mouse IgG, mouse IgM, rabbit IgG, rat IgG or sheep IgG (Molecular Probes). DNA was stained with Hoechst 33342 (Molecular Probes).

Optical clearing of bovine, ovine and porcine oocytes

Oocytes were fixed, extracted and blocked as for routine immunofluorescence. Prior incubation with primary antibodies, lipid droplets in oocytes were cleared with 4000 U/ml lipase from *Candida rugosa* (Sigma-Aldrich) in 400 mM NaCl, 50 mM tris (pH 7.2), 5 mM CaCl₂ and 0.2% sodium taurocholate supplemented with cOmplete, EDTA-free Protease Inhibitor Cocktail (Roche) at room temperature for 20-40 min.

Protein-retention expansion microscopy

Expansion microscopy was performed as previously described for cell and tissue samples (56) using the Expansion Microscopy Kit (Expansion Technologies).

Expression constructs, messenger RNA (mRNA) synthesis, protein expression and purification

To generate constructs for mRNA synthesis, we fused previously published coding sequences with meGFP (Clontech), mClover3 or 3×mClover3 (57), mPA-GFP (58), 3×CyOFP (59), mCherry (60), mScarlet (61) or mRFP (62), and sub-cloned them into pGEMHE (63) to obtain meGFP-AKAP450 (64), AURA-meGFP (65), Bbs4-meGFP (66), meGFP-BBS14 (67), TurboGFP-BUGZ (68), mCherry-CDK5RAP2 (69), CEP72-meGFP (70), mScarlet-CEP192 (71), CETN2-meGFP (72), meGFP-centrobin (73), mClover3-CHC17 (21), chTOG-mScarlet (74), mEmerald-CLIP170 (M. Davidson, unpublished), meGFP-CPAP (75), mClover3-GTSE1 (74), H2B-mRFP (4), meGFP-HOOK3 (76), meGFP-KIF2B (77), LRRC36-meGFP (70), LRRC45-meGFP (78), mClover3-MAP4-MTBD (79), 3×CyOFP-MAP4-MTBD (79), NEDD1-mCherry (80), meGFP-NEK2A (81), meGFP-P50 (82), meGFP-P150 (83), meGFP-PAR6a (84), PCM1-mClover3 (Source BioScience), PCM1-mPA-GFP (Source Bioscience), meGFP-Pericentrin (85), PRC1-3×mClover3 (74), meGFP-rootletin (86), meGFP-SAS6 (87), TACC3-mClover3 (74), TACC3-mPA-GFP (74), mPA-GFP-TPX2 (88), (bovine) TRIM21 (Thermo Fisher Scientific), (mouse) TRIM21 (89) and γ -tubulin-meGFP (90). EB3-3×mCherry was sub-cloned from pEB3-mCherry (J. Ellenberg, unpublished) into pGEMHE. pGEMHE-TACC3(CID)-mClover3, pGEMHE-TACC3(TACC)-mClover3 and pGEMHE-TACC3(NT) were constructed from pGEMHE-TACC3-mClover3 using QuikChange Lightning Multi Site-Directed Mutagenesis Kit (Agilent) or In-Fusion HD Cloning Plus (Clontech) or Q5 Site-Directed Mutagenesis Kit (NEB). pGEMHE-mCherry-MAP4-MTBD (79), pGEMHE-mPA- α -tubulin (79), pGEMHE-CEP250-meGFP (91) and pGEMHE-mCherry-PLK1 (91) were also used. All mRNAs were synthesized and quantified as previously described (92).

To generate constructs for protein expression, TACC3(TACC) (aa 594-838)-His6 and TACC (aa594-838)-His were inserted into pGEX-6p-1 (GE Healthcare), and His6-TACC3-His6 was inserted into pET-28c(+) (Novagen). GST-TACC3-His6, GST-TACC3(TACC)-His6, GST-TACC-His6 were expressed in and purified from BL21(DE3)pLysS Competent Cells (Promega) as previously described (26) with some modifications. Briefly, recombinant proteins were purified first using Ni-NTA Agarose (Qiagen), followed by Q Sepharose Fast Flow (GE Healthcare). His6-TACC3-His was expressed in NiCo21(DE3) (NEB) and purified first using Ni-NTA Agarose, followed by Chitin Resin (NEB).

Short-interfering RNAs (siRNAs)

All siRNAs were purchased from Qiagen. For knockdown of TACC3 using RNAi, a mix of the following siRNAs were used: 5'-AAGTCCTAACATGACCAATAA-3', 5'-CTGCATGTCTTAAATGACGAA-3', 5'-AAGACTAAAGTTAATCTCAA-3' and 5'-AAGGAAATAGCTGAAGACAAA-3'. AllStars Negative Control (Qiagen) was used as a control.

Microinjection of mouse oocytes, follicles and bovine oocytes

Mouse oocytes were microinjected with 3.5 pl of mRNAs as previously described (4). mClover3-MAP4-MTBD mRNA was microinjected at a needle concentration of 83.5 ng/μl, PCM1-mPA-GFP mRNA at 165.8 ng/μl, PRC1-3xmClover3 mRNA at 97 ng/μl, TACC3-mClover3 mRNA at 208.1 ng/μl, TACC3-mPA-GFP at 277.4 ng/μl, mPA-GFP-α-tubulin mRNA at 250.5 ng/μl, 3xCyOFP-MAP4-MTBD mRNA at 166.4 ng/μl, CEP192-mScarlet mRNA at 165.8 ng/μl, EB3-3xmCherry mRNA at 138.8 ng/μl, H2B-miRFP mRNA at 28.4 ng/μl, and Trim21 mRNA at 421 ng/μl. For other mRNAs, they were individually titrated and care was taken that they did not induce phenotypes due to overexpression. Oocytes were allowed to express mRNAs for 3-4 h before released into dbcAMP-free medium for imaging.

Mouse follicles were microinjected with 6 pl of siRNAs at a needle concentration of 2 μM as previously described (49).

Bovine oocytes were microinjected with 7 pl of TACC3-mClover3 mRNA and bovine TRIM21 mRNA at a needle concentration of 208.1 and 421 ng/μl, respectively. Oocytes were allowed to express mRNAs for 3-9 h before released into dbcAMP-free, bicarbonate-buffered BO-IVM (IVF Bioscience).

Peptide pre-incubation assay

Oocytes were fixed, extracted and blocked as for routine immunofluorescence. Rabbit polyclonal anti-CHC17, polyclonal anti-GTSE1 and monoclonal anti-TACC3 were pre-incubated with peptide PQAPFGYGYTAPPYGPQPGFGYSM (GenScript), recombinant GTSE1 (OriGene) and recombinant GST-TACC3-His6 (homemade), respectively, before applied to oocytes as previously described (92).

Trim-Away in mouse and bovine oocytes

Only affinity purified antibodies were used in this study for Trim-Away-mediated protein depletion. Rabbit polyclonal anti-CHC17, polyclonal anti-GTSE1 and monoclonal anti-TACC3 were purified as previously described (92). The control IgG used was normal rabbit IgG (12-370; Millipore).

For constitutive Trim-Away in mouse GV oocytes, 3.5 pl of mRNAs and 3.5 pl of antibodies were microinjected as previously described (92). All antibodies were microinjected at a needle concentration of 1 mg/ml with 0.1% NP-40. Target proteins were allowed to be depleted for 3-4 h before the oocytes were released into dbcAMP-free medium for imaging. For acute Trim-Away in mouse metaphase I oocytes, oocytes were first microinjected with 3.5 pl of mRNAs and then released into dbcAMP-free medium after 3 h. At around 6 h upon release, oocytes were further microinjected with 3.5 pl of antibodies immediately before imaging.

For constitutive Trim-Away in bovine GV oocytes, 7 pl of bovine TRIM21 mRNA and 7 pl of anti-TACC3 at a needle concentration of 2 mg/ml with 0.1% NP-40 were microinjected. TACC3 was allowed to be depleted for 3 – 9 h before the oocytes were released into dbcAMP-free, bicarbonate-buffered BO-IVM.

Immunoblotting

30 mouse metaphase I oocytes per lane were washed in BSA-free M2 medium and re-suspended in 4 μ l of BSA-free M2 medium. 12 μ l of 1.333 $\times$ NuPAGE LDS sample buffer (Thermo Fisher Scientific) with 100 mM DTT was then added, and the mixture was immediately snap-frozen in liquid nitrogen for 5 min. Samples were thawed and frozen twice more before heated at 100°C for 5 min. Samples were resolved on a 15-well NuPAGE 4 - 12% bis-tris protein gel of 1.0 mm thickness (Thermo Fisher Scientific) with NuPAGE MOPS SDS Running Buffer (Thermo Fisher Scientific). Proteins were transferred onto a 0.45 μ m PVDF membrane with SDS-free Towbin buffer at 200 mA for 2 h on ice. Blocking and antibody incubations were performed in tris-buffered saline (TBS) with 5% skim milk and 0.1% tween-20. Primary antibodies used were mouse anti-AURA (610938; BD Biosciences), rabbit anti-chTOG, mouse anti-CHC17 rabbit anti-TACC3 and rat- α -tubulin. Secondary antibodies used were HRP-conjugated anti-mouse (P0447; Dako), anti-rabbit (31462; Invitrogen) and anti-rat (sc-2032; Santa Cruz Biotechnology). Blots were developed with SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientific) and documented with Amersham Imager 600 (GE Healthcare). Care was taken that the exposure time did not cause saturation.

Confocal and super-resolution microscopy

For confocal imaging, oocytes were imaged in 1 - 2 μ l of M2 medium (for live mouse oocytes) or PBS with 1% polyvinylpyrrolidone (PVP) and 0.5 mg/ml BSA (for fixed oocytes) under paraffin oil in a 35 mm dish with a #1.0 coverslip. Images were acquired with LSM 880 confocal laser scanning microscopes (Zeiss) equipped with an environmental incubator box and a 40 $\times$ C-Apochromat 1.2 NA water-immersion objective. A volume of 50 μ m $\times$ 50 μ m $\times$ 37.5 μ m or 35 μ m $\times$ 35 μ m $\times$ 37.5 μ m centered around the chromosomes was typically recorded. Automatic 3D tracking was implemented for time-lapse imaging with a temporal resolution of 5 - 15 min using AutofocusScreen (93) or MyPiC (94). mClover3 and mGFP were excited with a 488 nm laser line and detected at 493 - 571 nm. CyOFP was excited with a 488 nm laser line and detected at 571 - 638 nm. mScarlet and mCherry were excited with a 561 nm laser line and detected at 571 - 638 nm. mRFP was excited with a 633 nm laser line and detected at 638 - 700 nm. Images of the control and experimental groups were acquired under identical imaging conditions on the same microscope. For some images, shot noise was reduced with a Gaussian filter. Airyscan images were acquired using the Airyscan module on LSM800 and LSM880 confocal laser scanning microscopes (Zeiss) and processed in ZEN (Zeiss) after acquisition. Care was taken that the imaging conditions (laser power, pixel-dwell time and detector gain) did not cause phototoxicity (for live imaging), photobleaching or saturation.

Drug addition and washout

All drugs, except 1,6-hexanediol were prepared in DMSO (Sigma-Aldrich) as 1000 $\times$ stocks. To depolymerize microtubules, metaphase I oocytes were treated with 10 μ M nocodazole (Sigma-Aldrich). To regrow microtubules, metaphase I oocytes were first washed into 10 μ M nocodazole for 45–60 min and then washed out into medium without drug. To inhibit EG5, metaphase I oocytes were treated with 100 μ M monastrol (Sigma-Aldrich) for 30–90 min.

To inhibit AURA, PLK1 and PLK4 in metaphase I oocytes, MLN8237 (Selleckchem), BI2536 (Selleckchem) and centrinone were used at 500 nM, 100 nM and 5 μ M, respectively. Previous studies have shown that these drugs do not exhibit non-specific inhibition on other kinases at the indicated concentrations (5, 91, 95). 1,6-hexanediol (Sigma-Aldrich) was prepared as a 10 $\times$ stock in medium and used at a final concentration of 3.5%.

Electron microscopy staining, immuno-electron microscopy and focused ion beam-scanning electron microscopy (FIB-SEM)

For electron microscopy staining, mouse metaphase I oocytes were fixed as previously described for mitotic cells (96) with some minor modifications. Briefly, oocytes were fixed in 100 mM HEPES (pH 7.0, titrated with KOH), 50 mM EGTA (pH 7.0, titrated with KOH), 10 mM MgSO₄, 3% sucrose, 3% EM-grade glutaraldehyde, 0.5% methanol-free formaldehyde and 0.1% tannic acid at 37°C for 1 h. Fixed oocytes were first stained with 2% osmium tetroxide (Electron Microscopy Sciences)-1.5% potassium ferrocyanide (Electron Microscopy Sciences) in 0.1 M phosphate buffer (pH 7.4) for 1 h. Oocytes were then incubated with 0.1% tannic acid (Sigma-Aldrich) for 30 min and stained with 2% osmium tetroxide in water for 40 min. After overnight staining with 1% uranyl acetate (SPI-Chem) in water at 4°C, oocytes were further stained with 0.02 M lead nitrate (Merck Millipore)-0.03 M aspartic acid (Sigma-Aldrich) (pH 5.5) for 30 min. Oocytes were washed three times with water for 5 min in between every staining step.

For immuno-electron microscopy of TACC3, mouse metaphase I oocytes were microinjected with 7 μ l of 0.3 mg/ml US Immunogold-conjugated rabbit Fab anti-TACC3 (Aurion) with 0.1% NP-40. After fixation as described above, silver enhancement was performed with R-Gent SE-EM (Aurion). All following processing steps were performed in a microwave (Ted Pella) and oocytes were washed three times with water for 40 s at 250 W in between every staining step. Fixed oocytes were first stained with 2% osmium tetroxide-1.5% potassium ferrocyanide in 0.1 M phosphate buffer (pH 7.4) for 8 min at 100 W (microwave cycling between on-off every 2 min). Oocytes were then incubated with 1% thiocarbohydrazide (Sigma-Aldrich) for 12 min at 100 W (microwave cycling between on-off every 2 min) and stained with 2% osmium tetroxide in water for 12 min at 100 W (microwave cycling between on-off every 2 min). After overnight staining with 1% uranyl acetate in water at 4°C, oocytes were further stained with 0.02 M lead nitrate-0.03 M aspartic acid (pH 5.5) for 12 min at 100 W (microwave cycling between on-off every 2 min).

Oocytes were subsequently dehydrated in a graded ethanol series (10, 30, 50, 75, 90, 100 and 100%) for 40 s at 250 W and infiltrated in a graded series (25, 50, 75, 90, 100 and 100%) of Durcupan resin (Sigma-Aldrich) in ethanol for 3 min at 250 W. Infiltrated oocytes were embedded with minimal amount of resin on top of an Aclar film as previously described (97). Embedded oocytes were cut out and attached to a SEM stub (Science Services) using silver-filled EPO-TEK EE129-4 adhesive (Electron Microscopy Sciences), and were cured overnight at 60°C. Samples were coated with an 8 nm platinum layer using the high vacuum sputter coater EM ACE600 (Leica) at 35 mA current. Afterwards, samples were placed in the Crossbeam 540 FIB-SEM (Zeiss). To ensure even milling and to protect the surface, a 400 nm platinum layer was deposited on top of the region of interest at 3 nA

current. Atlas 3D (Fibics) was used to collect the 3D datasets. Samples were exposed with a 15 nA current and a 7 nA current was used to polish the cross-section surface. Images were acquired at 1.5 kV with the ESB detector at a grid voltage of 1100 V (5 nm pixel size in x-y) in a continuous mill and acquire mode using 700 pA for the milling aperture (5 nm z-step).

After acquisition, images were first aligned using Linear Stack Alignment with SIFT in Fiji (NIH) (98). Datasets were then cropped, inverted and smoothed in Fiji. For better visualization of microtubules, datasets were further subjected to two rounds of Local contrast enhancement (CLAHE) in Fiji. Specific parameters used for the first round were: 127 for blocksize, 100 for histogram bins and 1.5 for maximum slope. Specific parameters used for the second round were 50 for blocksize, 256 for histogram bins and 2.0 for maximum slope. To obtain a metaphase I spindle parallel to the imaging plane, the resulting image stacks were rotated and resliced with Interactive Stack Rotation in Fiji. 3D segmentation and surface rendering were performed with Microscopy Image Browser (99) and Imaris, respectively.

Fluorescence recovery after photobleaching (FRAP)

For analyses of protein dynamics, oocytes co-expressing fluorescent reporter(s) of interest with H2B-miRFP were rotated with an unbroken microinjection needle on stage to obtain meiotic spindles parallel to the imaging plane. Rectangular or circular regions of interest (ROIs) were marked and photobleached using the corresponding excitation laser line (405, 488, 561 and/or 633 nm) at the maximum power after the 3rd or 5th time point.

Photoactivation

For analyses of dissipation, oocytes co-expressing mPA- α -tubulin, PCM1-mPA-GFP, TACC3-mPA-GFP or mPA-GFP-TPX2 with H2B-miRFP were rotated with an unbroken microinjection needle on stage to obtain meiotic spindles parallel to the imaging plane. Rectangular ROIs were marked and photoactivated using the 405 nm laser line at the maximum power after the 3rd or 5th time point.

In vitro droplet assembly and dextran exclusion assay

TACC3 droplets were assembled in an imaging chamber as previously described for SPD-5 (32). For most experiments, 20 μ M purified His-tagged proteins or recombinant GST (GenScript) in 50 mM tris (pH 7.4), 500 mM NaCl, 0.5 mM DTT, 1% glycerol and 0.1% CHAPS (all from Sigma-Aldrich) was added to 25 mM HEPES (pH 6.4), 150 mM KCl, 0.5 mM DTT and 12% PEG-3350 with 0.1 mg/ml NTA-Atto 647 N (all from Sigma-Aldrich). For dextran exclusion assay, 2.5 mg/ml Oregon Green 488-conjugated dextran of 70,000 M.W. (Molecular Probes) was included during the assembly of the reaction.

Thioflavin T staining

Oocytes were fixed, extracted and blocked as for routine immunofluorescence. 10 μ M thioflavin T was applied to oocytes for 10 min as previously described (100).

Cold-stable assay

Mouse metaphase I oocytes were incubated on ice for 15 min and immediately fixed as for routine immunofluorescence.

Calcium-stable assay

Mouse metaphase I oocytes were incubated in 100 mM PIPES (pH 7.0, titrated with KOH), 1 mM MgCl₂, 0.1 mM CaCl₂ and 0.1% triton X-100 at 37°C for 15 min and immediately fixed as for routine immunofluorescence.

General quantification

Time-lapse movies of live mouse oocytes were analyzed using Imaris (Bitplane). To determine the stages of meiosis and to score for chromosome segregation defects, the timing of meiotic progression was quantified relative to the time of NEBD. NEBD was defined as the time point when the sharp boundary between the nucleus and cytoplasm disappeared. Chromosome(s) that failed to congress to the metaphase plate before anaphase onset or the end of the video were scored as misaligned chromosomes. Anaphase onset was defined as the last time point of metaphase (5 - 6 min) before chromosome separation was first observed. Chromosome(s) that failed to clear the central spindle region within 10-12 and 20-24 min of anaphase onset were scored as mildly and severely lagging chromosomes, respectively. The presence of misaligned or lagging chromosome(s) were further confirmed by 3D reconstruction of chromosomes in Imaris as previously described (79).

Quantification of FRAP experiments

Mean intensities of photobleached areas over time were exported from ZEN into Excel (Microsoft) for further processing. The intensity of an unbleached area in the cytoplasm of the same oocyte was subtracted to correct for cytoplasmic background. Background-corrected data were then normalized to the mean intensity of pre-bleach time points (F_0). As the post-bleach intensity could not be normalized between individual plots due to variable photobleaching efficiency, plots of intensity (F) against time were fitted to single exponential functions ($F(t) = c - F_{\infty}e^{-t/\tau}$ where c is the offset, F_{∞} is the amplitude of maximum intensity recovered after equilibrium and τ is the time constant) in OriginPro (OriginLab). Half-times of maximum recovery ($t_{1/2}$) and mobile fractions were determined by $\tau \times \ln(2)$ and $F_{\infty}/(F_0 - F')$ (where F' is the minimum intensity measured immediately after photobleaching), respectively.

Manual and automatic quantification of plus-end growth velocity

To determine the growth rate of plus-ends of astral microtubules, comet velocities were determined using the Kymograph function in Fiji. Tracks were analyzed in Excel.

For plus-ends of microtubules within the spindle, comets were automatically tracked using u-track 2.2 (101) in MATLAB R2017b (MathWorks). For comet detection, low-pass and high-pass Gaussian standard deviations were set to 2 and 6 pixels, respectively. For comet tracking, maximum gap to close was set to 0 frame to eliminate discontinuous tracks. As astral microtubules were protruding from the spindle in all directions and their comets

should be out of focus before comets of spindle microtubules, tracks longer than 5 frames were used for further processing. This resulted in on average >500 (for control group) and >300 (for experimental group) tracks per oocyte within 2.5 min.

Quantification of photoactivation experiments

To determine dissipation rates of α -tubulin, mean intensities of photoactivated areas over time were exported from ZEN into Excel for further processing. Data were first corrected for cytoplasmic background by subtracting the intensity of the photoactivated areas before photoactivation. Background-corrected data were then normalized to the intensity of the first post-activation time point (F_0). Plots of intensity (F) against time were fitted to one-component exponential functions ($y = Ae^{(c-x)/\tau}$, where c is the offset, A is the fraction of the component and τ is the time constant) in OriginPro. Half-times of decay ($t_{1/2}$) were calculated by $\tau \times \ln(2)$. Single-component exponential fitting was used because double-component exponential fitting was unable to yield two distinct τ , consistent with our previous studies on metaphase II spindles (79). This was likely due to the fact that the fraction of dynamic non-kinetochore-bound microtubules is much more abundant than that of stable kinetochore microtubules in mouse meiotic spindles.

Manual quantification of fluorescence intensities and volume by 3D reconstruction of spindles and aMTOCs

Spindles and aMTOCs were labelled (with MAP4-MTBD and CEP192, respectively) in live and (with α -tubulin and Pericentrin, respectively) fixed oocytes. They were segmented by applying a threshold in the corresponding channel using the Surface function of Imaris. Cytoplasmic aMTOCs that were not part of the spindle were manually removed. Depending on the signal-to-noise ratio in each oocyte, a suitable threshold value was selected and maintained for the entire time series. The volume of spindles and aMTOCs were exported into Excel. The mean and total intensities for spindle proteins (α -tubulin, MAP4-MTBD, PRC1 or TACC3) and aMTOCs proteins (CEP192 or Pericentrin) were exported into Excel.

For live imaging, data from individual oocytes were aligned to the time of NEBD or the onset of microtubule nucleation. To normalize individual dataset, all (mean or sum) intensities for a channel from both the control and experimental groups were divided by the average (mean or total) intensity for that channel from either the control or experimental group at steady-state. For some experiments, normalized data from different datasets were interpolated to identical time intervals in OriginPro.

For immunofluorescence, all (mean or sum) intensities for a channel from both the control and experimental groups were normalized to the average (mean or total) intensity for that channel from the control group.

Automatic quantification of fluorescence intensities and volume by 3D reconstruction of spindles

To consistently reconstruct the spindles from different cold-stable assays, an in-house-developed MATLAB script was used for automatic surface creation in Imaris.

Individual sections of a dataset were background-subtracted by a Gaussian smoothed image with a sigma equal to 80% of the background scale given and smoothed with a median filter. The size of the filter was determined by the nearest odd integer to twice the object detail given.

To automatically determine a threshold for the Surface function in Imaris, Otsu's method was implemented via the "multithresh.m" function in MATLAB. This function allows the computation of multiple thresholds for multilevel images. A single threshold was first computed and the volume of the surface was approximated by multiplying the number of voxels above the threshold with the volume of a voxel. When the volume was not within the range of possible volumes, more thresholds were computed. This was repeated until an appropriate threshold was found or a number of 5 thresholds was reached. The unaltered values of object detail, background scale and a filter based on the intensity mean of the second channel were then used for surface creation in Imaris. For spindles, the common centre of homogeneous mass of all surfaces was computed, and all surfaces with a centre of mass >10 μm from the common one were removed.

Specific parameters used for spindles were: 0.15 μm for object detail, 0.6 μm for background scale and 50-1000 μm^3 for volume range.

Statistical analysis

Average (mean) and standard deviation (S.D.) were calculated in Excel. Statistical significance based on unpaired, two-tailed Student's t-test (for absolute values) and two-tailed Fisher's exact test (for categorical values) were calculated in OriginPro or Prism (GraphPad). All box plots show median (horizontal black line), mean (small black squares), 25th and 75th percentiles (boxes), 5th and 95th percentiles (whiskers) and 1st and 99th percentiles (crosses). All data are from at least two independent experiments. *P* values are designated as **P* < 0.05, ***P* < 0.01, ****P* < 0.001 and *****P* < 0.0001. Nonsignificant values are indicated as N.S.

Supplementary Material

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One Sentence Summary

Phase separation of microtubule regulatory factors promotes acentrosomal spindle assembly in mammalian oocytes.

Fig. 1. Identification of a novel spindle domain in mammalian oocytes.

(A) Schematic representation of the mouse metaphase I spindle (scheme; LISD in green, aMTOCs in magenta, kinetochores in blue, Golgi in purple and spindle microtubules in grey). (B) Immunofluorescence images of a mouse metaphase I spindle. Green, LISD (TACC3). Magenta, aMTOCs (Pericentrin). Gray, microtubules (α -tubulin). (C) Immunofluorescence images of metaphase I and II spindles in mouse, bovine, ovine and porcine oocytes. Green, LISD (TACC3). Magenta, microtubules (α -tubulin). Scale bars, 5 μ m.

Fig. 2. Dynamics of LISD assembly during meiosis I in mouse oocytes.

(A) Immunofluorescence images of mouse oocytes fixed at different times after nuclear envelope breakdown (NEBD). Gray, microtubules (α -tubulin). Green, LISD (TACC3). Magenta, aMTOCs (Pericentrin). Insets are magnifications of regions marked by dashed line boxes. (B) Stills from time-lapse movies of meiotic maturation of mouse oocytes. Green, LISD (TACC3-mClover3). Magenta, aMTOCs (CEP192-mScarlet). Time, hours:minutes after NEBD. (C) Single sections of expansion microscopy of a mouse early metaphase I spindle. Gray, LISD (TACC3). Yellow arrowheads highlight spherical protrusions at the spindle poles. White arrowheads highlight the K-fibers. Chr. denotes chromosome. Scale bars, 5 μm .

Fig. 3. The LISD forms by phase separation.

(A) Stills from time-lapse movies of mouse late metaphase I oocytes. Gray, LISD (TACC3-mClover3). Yellow lines mark the position of xz planes on the corresponding xy planes. Arrowheads highlight fusing LISD protrusions. (B) Stills from time-lapse movies of acutely nocodazole-treated mouse metaphase I oocytes. Green, LISD (TACC3-mClover3). Magenta, microtubules (EB3-3×mCherry). Time, minutes after 10 μM nocodazole addition. (C) Immunofluorescence images of spherical condensates in acutely 10 μM nocodazole-treated mouse metaphase I oocytes. Gray, microtubules (α-tubulin). Green, spherical condensates

(TACC3 or PCM1). Magenta, aMTOCs (Pericentrin). **(D)** Stills from time-lapse movies of acutely 10 μ M nocodazole-treated mouse metaphase I oocytes. Gray, spherical condensates (TACC3-mClover3). Arrowheads highlight fusing spherical condensates. **(E)** Partial bleaching of TACC3-mClover3 in a spherical condensate in acutely 10 μ M nocodazole-treated mouse metaphase I oocytes. Bleached area is marked by the dashed line box. Scale bar, 1 μ m. **(F)** Photoactivation (PA) of different proteins on mouse metaphase I spindles. Spindle poles are outlined with white lines. Photoactivated bars are marked with yellow lines. Time, minutes after photoactivation with 405 nm laser. **(G)** Stills from time-lapse movies of acutely 1,6-hexanediol-treated mouse metaphase I oocytes pre-treated with 10 μ M nocodazole. Time, minutes after 3.5% 1,6-hexanediol addition. Scale bars, 5 μ m unless otherwise specified.

Fig. 4. The N-terminus of TACC3 is necessary for phase separation in vitro and in vivo. (A) Bright-field and fluorescence images of GST-TACC3 droplets in vitro. Inset is the magnification of the region marked by the dashed line box. (B) Stills from time-lapse movies of fusing GST-TACC3 droplets in vitro. Scale bar, 1 μ m. Arrowheads highlight fusing GST-TACC3 droplets. (C) Fluorescence images of GST-TACC3 droplets in the presence of 70 kDa dextran in vitro. Green, GST-TACC3. Magenta, 70 kDa dextran. Insets are magnifications of regions marked by dashed line boxes. Scale bar, 2.5 μ m. (D) FRAP of GST-TACC3 droplets in vitro. Gray, GST-TACC3. The number of analyzed droplets is

specified in italics. M.F. denotes mobile fraction. Scale bar, 1 μm . **(E)** Domain organization of human and mouse TACC3 showing the disordered region [shown in purple, analysis with DisEMBL (47)] and the coiled-coil domain [shown in yellow, analysis with MARCOIL (48)]. **(F)** Fluorescence images of GST, GST-TACC3, GST-TACC3(TACC) and GST-TACC in vitro. **(G and H)** Immunofluorescence images of the metaphase I spindle in control, TACC3-depleted and TACC3(NT)-TACC3-depleted mouse oocytes with and without cold treatment. Insets are magnifications of regions marked by dashed line boxes. All in vitro assays were performed in pH 6.4 buffer with 150 mM KCl and 12% PEG. Scale bars, 5 μm unless otherwise specified.

Fig. 5. Microtubule loss and defective spindle assembly in TACC3-depleted oocytes.

(A) Stills from time-lapse movies of control and TACC3-depleted mouse oocytes. Green, microtubules (mClover3-MAP4-MTBD). Magenta, aMTOCs (CEP192-mScarlet). Blue, chromosomes (H2B-miRFP). Time, hours:minutes after NEBD. (B and C) Quantification of total fluorescence intensity of microtubules and spindle volume in control and Tacc3-depleted mouse oocytes. (D) Immunofluorescence images of the metaphase I spindle in control and TACC3-depleted bovine oocytes. Gray, LISD (TACC3). Green, microtubules (α -tubulin). Magenta, chromosomes (Hoechst). (E and F) Quantification of total fluorescence

intensity of microtubules and spindle volume in control and TACC3-depleted bovine metaphase I oocytes. The number of analyzed oocytes is specified in italics. Error bars (shaded areas) represent S.D. Scale bars, 5 μ m.

Fig. 6. The N-terminus of TACC3 is required for spindle assembly in mouse oocytes. (A) Stills from time-lapse movies of control, TACC3-depleted and TACC3(NT)-TACC3-depleted mouse oocytes. Green, microtubules (mClover3-MAP4-MTBD). Magenta, aMTOCs (CEP192-mScarlet). Blue, chromosomes (H2B-miRFP). Time, hours:minutes after NEBD. Yellow asterisks highlight the unfocused spindle intermediate. (B and C) Quantification of total fluorescence intensity of microtubules and spindle volume in control, TACC3-depleted and TACC3(NT)-TACC3-depleted mouse oocytes. The number of analyzed oocytes is specified in italics. Error bars (shaded areas) represent S.D. Scale bars, 5 μm .

Fig. 7. Loss of K-fibers and interpolar microtubules in TACC3-depleted oocytes.

(A) Immunofluorescence images of the metaphase I spindle in cold-treated control and TACC3-depleted mouse oocytes. Gray, LISD (TACC3). Green, microtubules (α -tubulin). Magenta, kinetochores (ACA). Blue, chromosomes (Hoechst). (B and C) Quantification of total fluorescence intensity of cold-stable microtubules and spindle volume in cold-treated control and TACC3-depleted mouse metaphase I oocytes. (D) Immunofluorescence images of the metaphase I spindle in calcium-treated control and TACC3-depleted mouse oocytes. Green arrowheads highlight stable interpolar microtubules. Magenta arrowheads highlight

K-fibers. **(E and F)** Quantification of total fluorescence intensity of calcium-stable microtubules and spindle volume in calcium-treated control and TACC3-depleted mouse metaphase I oocytes. **(G)** Immunofluorescence images of PRC1-3×mClover3 in control and TACC3-depleted mouse metaphase I oocytes. Green, PRC1 (GFP). Magenta, TPX2. **(H)** Quantification of total fluorescence intensity of PRC1-marked microtubules in control and TACC3-depleted mouse oocytes. The number of analyzed oocytes is specified in italics. Error bars represent S.D. Scale bars, 5 μ m.

Acentrosomal spindle in a mouse egg.

A liquid-like meiotic spindle domain (LISD, cyan) forms prominent spherical protrusions at acentrosomal spindle poles and extends into the spindle region (magenta, right) toward chromosomes (magenta, left). The LISD forms by phase separation and is required for spindle assembly, serving as a reservoir that locally sequesters and mobilizes microtubule regulatory factors within the large egg cytoplasm. Scale bar, 5 μm .