## Supplemental material

Borrego-Pinto et al., http://www.jcb.org/cgi/content/full/jcb.201510083/DC1



Figure S1. Automated counting of centriole configuration. (A and B) The general marker pmPoc1-mCherry and the mother centriole marker pmOdf2-mEGFP were expressed in starfish embryos. (A) Example of raw images used for automated quantification. Arrows show examples of pmPoc1-mCherry pairs that cannot be resolved by light microscopy. Maximum-intensity projections. Bar,  $5 \mu$ m. (B) 81% of the pmPoc1-mCherry—labeled centriole pairs have one of the centrioles labeled by pmOdf2-mEGFP, following the expected pattern. We considered only the Poc1-mCherry pairs, which can be resolved by light microscopy. For details, see Materials and methods.



Figure S2. **mEGFP-pmCentrin-2 has the same localization as pmPoc1-mEGFP, and pmChibby-mEGFP same as pmOdf2-mEGFP.** (A) An oocyte expressing mEGFP-pmCentrin-2 was imaged starting at metaphase I and then throughout meiosis by 3D confocal microscopy. The images show maximum-intensity projection of the z-stacks; the insets are single confocal sections. Insets are shown for individual centrioles as marked on the overview images. z-Stacks were acquired every 21–24 s. Time is shown in mm:ss. Bars: (overview) 5 µm; (insets) 1 µm. (B and C) The two mother centrioles are extruded into the two polar bodies. Examples of a pmPoc1-mCherry (magenta) expressing oocyte coinjected with the mother centriole markers pmOdf2-mEGFP (B) or pmChibby-mEGFP (C) are shown. The images show maximum-intensity projections of z-stacks. Bar, 5 µm.



Figure S3. The mother centriole is transported to the plasma membrane shortly after PBI extrusion. Plots of the centriole-plasma membrane distance over time for the full dataset (12 oocytes). The mother centriole is shown in green, the daughter in pink, along with individual transport velocities (v) fitted onto the 3D trajectory of the fast transport phase as shown on Fig. 3 D.



Figure S4. The mother centriole remains anchored to the cell cortex after latB treatment. (A) An ocyte expressing pmPoc1-mEGFP arrested by MG-132 and treated with latB, imaged and visualized as described for Fig. 5 B. (B) Distance measurements of mother and daughter centrioles to the plasma membrane for the ocyte shown in A. t = 0 s corresponds to the time of drug addition. z-Stacks were acquired every 37 s. Time is shown in mm:ss. Bar, 5 µm.



Figure S5. **Persistence of mothers is independent of fertilization.** Oocytes expressing pmOdf2-mEGFP and hsEB3-mCherry were imaged ~2 h after completion of meiosis. Oocytes were treated with equal amount of DMSO or latB to prevent extrusion of PBII or of both polar bodies, respectively. White dashed lines delineate the pronucleus. Bar, 10 µm.



Video 1. Centrioles organize the meiotic spindles in the starfish oocyte. An oocyte injected with pmPoc1-mEGFP, which labels the four centrioles and the spindle microtubules. Maximum intensity projections of z-stacks acquired every 30 s for a total time of 48 min.



Video 2. Mother centrioles are extruded into the polar bodies. Mother centrioles are labeled in green by pmOdf2-mEGFP; the meiotic spindle is labeled in magenta by Cy3-tubulin. Maximum-intensity projections of z-stacks acquired every 38 s for a total time of 42 min.



Video 3. A single daughter centriole remains in the mature egg. Daughter centrioles are labeled in green by hsCentrobinmEGFP; the meiotic spindle is labeled in magenta by pmPoc1-mCherry. Maximum-intensity projections of z-stacks acquired every 35 s for a total time of 47 min.



Video 4. The mother centriole is transported and anchored to the plasma membrane shortly after MI. Centrioles after tracking (mother and daughter centrioles are represented by the green and pink sphere, respectively). Recording was started shortly after PBI extrusion. Video shows a 3D volume rendering of the data overlaid with an isosurface reconstruction of the cell outline (gray). z-Stacks were acquired every 12 s for a total time of 7 min.



Video 5. Mother centriole transport does not depend on PBI cytokinesis. Tracked positions of mother and daughter centrioles are marked with green and pink spheres, respectively. See Fig. 3 (F and G) for details. t = 0 is shortly after NEBD. z-Stacks were acquired every 50 s for a total time of 62 min.



Video 6. Mother centriole transport is dynein-dependent. Centriole positions were tracked in an oocyte expressing hsEB3mCherry3 and treated with ciliobrevin D at PBI extrusion. Centrioles are marked by green and pink spheres. See Fig. 3 (H and I) for details. *t* = 0 is shortly after anaphase I. z-Stacks were acquired every 57 s for ~30 min.



Video 7. **Dynamics of microtubule nucleating activities of centrioles during meiosis.** Maximum-intensity projections of z-stacks of oocytes expressing pmPoc1-mEGFP (green) and hsEB3-mCherry3 (magenta) acquired every 36 s for a total time of  $\sim$ 1 h. Oocyte was centrifuged to spatially separate spindle and centrioles.



Video 8. The first zygotic spindle is organized by the sperm centrioles. Maximum intensity projections of z-stacks of oocytes expressing hsEB3-mCherry3 and pmOdf2-mEGFP (not depicted) acquired every 79 s for a total time of 59 min. Oocyte was fertilized in MI and DMSO was added at MII as control.



Video 9. When one mother centriole is artificially retained in the egg, it remains active and participates in the formation of a tripolar spindle. Maximum-intensity projections of z-stacks of oocytes expressing hsEB3-mCherry3 and pmOdf2-mEGFP (not depicted) acquired every 47–51 s for a total time of 52 min. Oocyte was fertilized in MI and treated with latB at MII.



Video 10. When two mother centrioles are artificially retained in the egg, they remain active and participate in formation of a tetrapolar spindle. Maximum-intensity projections of z-stacks of occytes expressing hsEB3-mCherry3 and pmOdf2-mEGFP (not depicted) acquired every 101 s for a total time of 76 min. Occyte was fertilized in MI and treated with latB at MI.

## Table S1. Centriolar composition in Patiria miniata

Name	Starfish (P. miniata) gene models	Starfish ( <i>P. miniata</i> ) transcriptome	Sea urchin ( <i>S. purpuratus</i> )	Human (H. sapiens)
EB1	PMI_010172	KU512182	SPU_027631	AAC09471.1
α-Tubulin	PMI_000200	KU512183	SPU_012679	AAA91576.1
β-Tubulin	PMI_017127	KU512184	SPU_000062	AAB59507.1
δ-Tubulin	PMI_019313	KU512185	SPU_004266	AAF09584.1
ε-Tubulin	PMI_003328	KU512186	SPU_002663	AAF09585.1
γ-Tubulin	PMI_005801	KU512187	SPU_020943	AAF34188.1
PLK1/Polo/Plk1/2	PMI_005755	KU512188	SPU_017949	AAA56634.1
PLK4/Sak/Zyg-1	PMI_018726	KU512189	SPU_016352	NP_055079.3
SAS-6	PMI_018471	KU512190	SPU_026405	NP_919268.1
CPAP/SAS-4	PMI_023921+ PMI_026370	KU512191 KU512192 KU512193	SPU_002588	NP_060921.3
STIL/Sas-5/Ana2	PMI_005076	KU512194	SPU_006333	NM_001048166.1
Cep135/Bld10	PMI_010556+ PMI_010557+ PMI_010558	KU512195	SPU_018300	NP_079285.2
CP110	PMI_000096	KU512196	SPU_023395.1	NP_001185951.1
Cep192/Spd2	Not found	KU512197 KU512198	SPU_017452	NP_115518.3
CEP152/ Asterless	PMI_011887+ PMI_013017	KU512199	SPU_021838	NP_001181927.1
Pericentrin	PMI_001302	KU512200	SPU_015653	NP_006022
Poc 1	PMI_023696+ PMI_023695	KU512201	SPU_023342	NP_758440.1
Centrin-2	PMI_011671	KU512202	SPU_028660	NP_004335.1
Poc5	PMI_029127	KU512203	SPU_008198	NP_001092741.1
Ninein	PMI_024194+ PMI_018774	KU512204	SPU_001232	AAF23015.2
Cep164	PMI_007847	KU512205	SPU_021618	NP_055771.4
Odf2/Cenexin	PMI_017972	KU512206	SPU_025208	AAH91500.1
Cep170	PMI_002626+ PMI_002624	not found	SPU_008958	NP_055627.2
Chibby	PMI_002897	KU512207	SPU_005105	AAH16139.1
Centrobin	not found	KU512208	SPU_007275	NP_001032221.1

IDs of centriolar proteins listed in Fig. 1 A. Listed are, for human proteins, the GenBank ID; for sea urchin proteins, the SPBase ID (http://www.echinobase.org/); for starfish, the IDs of the proteins predicted from the genome assembly V1.0. GenBank IDs of the submitted transcriptome sequences are listed.