Supplemental Materials

Molecular Biology of the Cell

Pierron et al.

Supplementary Figure legends

Table S1. Summary of results.

Figure S1. hsPlk1 localizes at centrioles during meiosis in *P. miniata* and *A. forbesi,* schematic of mild centrifugation procedure as well as centriole tracking in centrifuged oocytes.

(A) Still images from dual color time-lapse confocal microscopy of *P. miniata* oocyte expressing hsEB3::mCherry3 and hsPlk1::mEGFP (green), which localizes at centrioles (insets) and kinetochores (arrows point to three of them). Note also hsPlk1::mEGFP signal in polar bodies, perhaps reflecting both kinetochore and centriolar localizations; this is also visible in *A. forbesi* (see Fig. S1B) and, to a lesser extent, with PmPlk1::mEGFP in *P. miniata* (see Fig. 1C). Here, as well as in panel B, time is indicated in minutes:seconds starting from meiosis II onset and scale bars are 5 µm. (B) Still images from dual color time-lapse confocal microscopy of *A. forbesi* oocyte expressing hsEB3:mCherry3 and hsPLk1::mEGFP. hsPlk1 localizes at centrioles (see insets 5-7 in particular) and kinetochores (-10:00, arrows point to three of them). At time 00:00, the outer pole of the meiosis II spindle shown in inset 3 is hidden below labeled chromosomes in the merged overview image. Note that hsPlk1::mEGFP is particularly bright at kinetochores in this particular oocyte. (C) Schematic representation of a microinjection chamber containing mature oocytes held between 2 coverslips assembled and spaced using double-sided tape. (D) Schematic representation of microinjection chamber placed into a plastic holder in a 50ml Falcon tube filled with seawater for centrifugation. (E) Upon mild centrifugation, the nucleus, which is less dense than the cytoplasm, moves away from the animal pole, whereas centrioles remain attached to the cell cortex below the plasma membrane. The maximal distance between centrioles and nucleus is obtained when the animal pole is opposite from the centrifugation force, i.e. close to the double-sided tape. Only oocytes with this orientation in the chamber were microinjected prior to centrifugation. (F) Sequence of events during meiosis I and II following mild centrifugation and 1-MA addition. Note that all centrioles are retained in the oocyte proper. The same color code is used as in Figure 1. See text for details. (G, H) Time-resolved images of centrioles and microtubules in centrifuged *P. miniata* oocytes expressing pmCentrin2::mEGFP and hsEB3::mCh3,

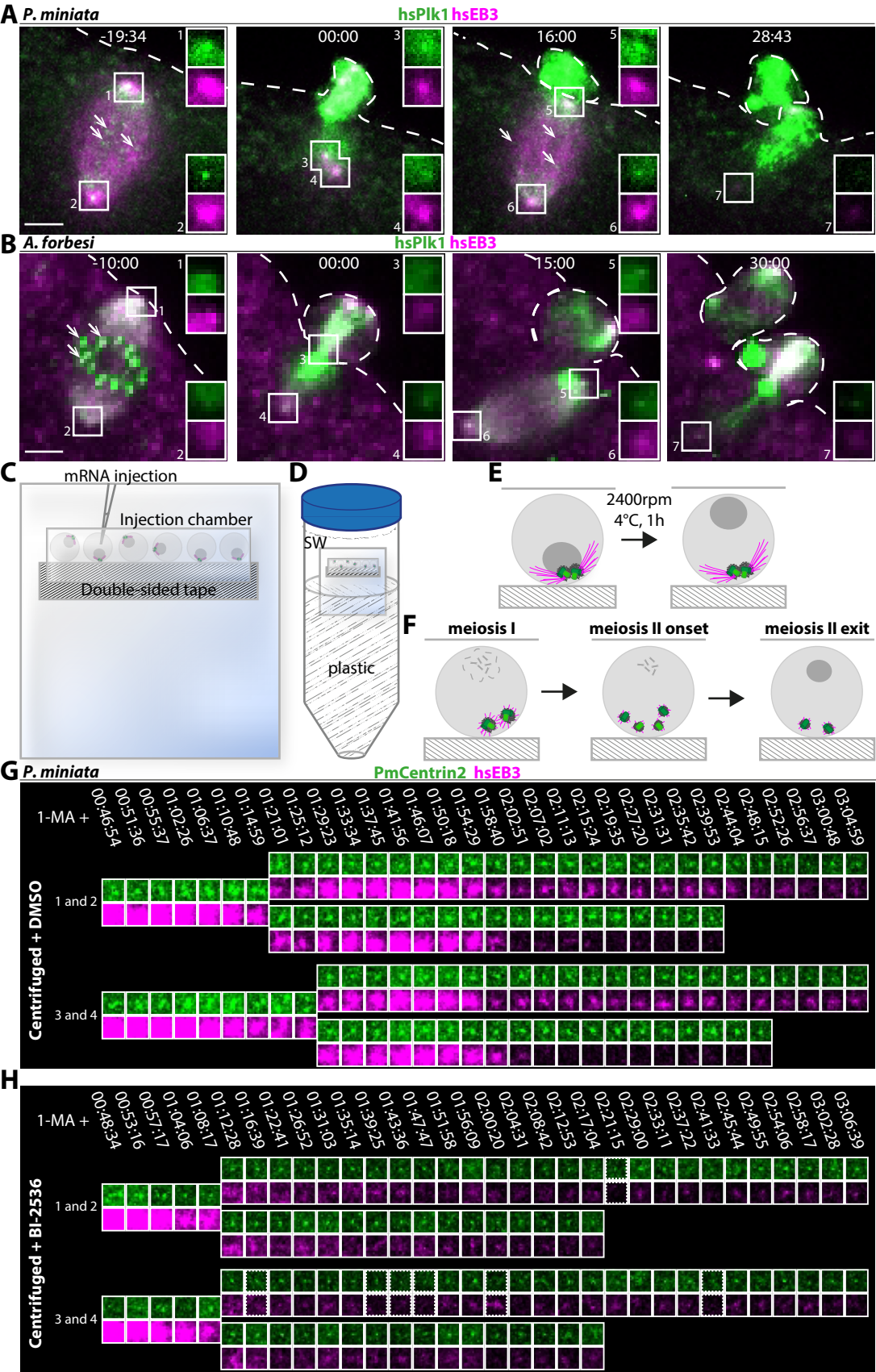
and treated with DMSO (G) or BI-2536 (H). Images boxed by a dashed line indicate time frames when the centriolar focus could not be found with certainty in the oocyte cytoplasm due to movement, loss of focus or dim signal. Time is indicated in hours:minutes:seconds after 1-MA addition. Each image is 3.73x3.73 microns.

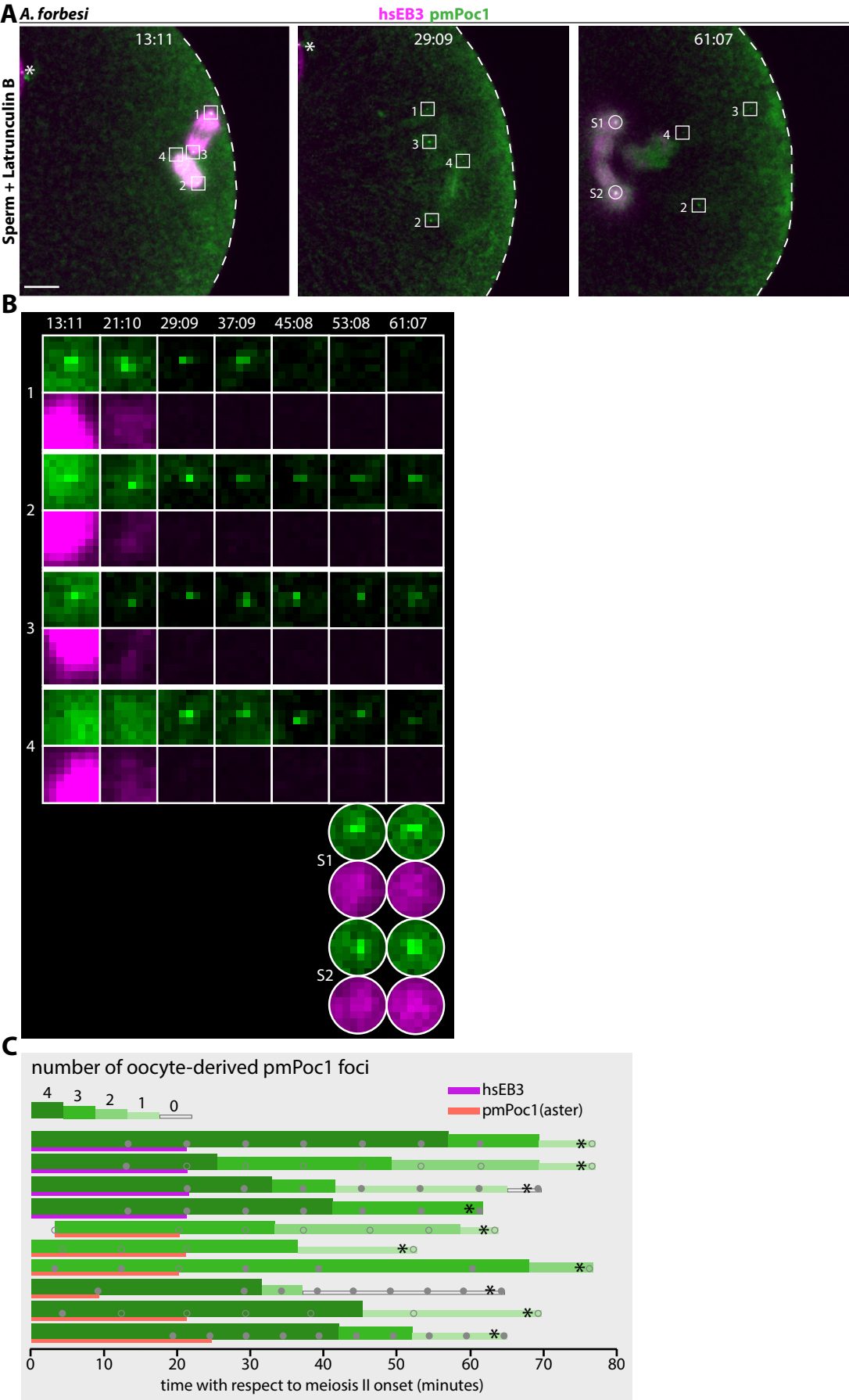
Figure S2. Retained foci of pmPoc1::mEGFP persist until mitosis in *A. forbesi*.
(A) Still images from dual color time-lapse confocal microscopy of Latrunculin B-treated *A. forbesi* zygote from meiosis II onset (time 00:00) until mitosis, with microtubules labeled with hsEB3::mCherry3 and centrioles marked with pmPoc1::mEGFP. Oocyte-derived centrioles are bounded by squares, sperm-derived centrioles by circles. Asterisks indicate sperm-derived centrioles at the edge of the image. The sequence of events for pmPoc1::mEGFP was more variable than for PACT::mEGFP (see Table S1). Scale bar: 10 µm. (B) Single confocal z-planes showing differential persistence of MTOC activity and presence of pmPoc1::mEGFP foci in individual oocyte-derived versus sperm-derived centrioles from meiosis II until mitosis. Each image is 3.98x3.98 µm. (C) Oocyte-derived centriole number over time as monitored by pmPoc1::mEGFP foci (green) and MTOC activity as monitored by hsEB3::mCherry3 (magenta) or astral pmPoc1::mEGFP (orange). Each line corresponds to one oocyte, with asterisks indicating mitosis onset. Foci number is indicated with different shades of green and line thicknesses. Grey filled discs: data points; grey circles: ambiguous data points due to foci being out-of-focus or to the presence of multiple, probably spurious, foci.

Movie S1. Two retained foci of PACT::mEGFP persist in *A. forbesi.* Movie from dual color time-lapse confocal microscopy from meiosis II onset until mitosis of *A. forbesi* zygote treated with Latrunculin B and expressing hsEB3::mCherry3 (magenta) to label microtubules as well as PACT::mEGFP (green) to mark centrioles (related to Figure 5A and 5B). Sperm asters are on the left and maternal bipolar MII spindles are on the right. Time is indicated in hours:minutes:seconds. Scale bar: 10 µm.

Experiment	Figures	# oocytes	Results in brief	Comments
mEGFP::pmPlk1 localization in *P. miniata*	Fig. 1C	17	17/17: enrichment at MI and MII spindle poles and kinetochores. 13/17: brighter at mother than at daughter during MII. 2/17: similarly bright at mother and daughter during MII. 2/17: brightness difference between mother and daughter could not be determined with certainty due to imaging issues.	1 oocyte in which Plk1 localization was observed at kinetochores and spindle poles during MI but not at the poles of the MII spindle was not considered, because the signal was generally very weak.
hsPlk1::mEGFP localization in *P. miniata*	Fig. S1A	14	14/14: enrichment at MI and MII spindle poles and kinetochores. 9/14: brighter at mother than at daughter during MII. 5/14: brightness difference between mother and daughter could not be determined with certainty due to imaging issues.	4/14: also expressed hsEB3::mCh3.
hsPlk1::mEGFP localization in *A. forbesi*	Fig. S1B	3	3/3: enrichment at MI and MII poles, as well as kinetochores.	3/3: also expressed hsEB3::mCh3. 2/3: fertilized.
Fate of centrioles marked by mEGFP::pmCentrin2 upon Plk1 inhibition by BI-2536 in *P. miniata* (not centrifuged)	Fig. 2A, 2B	DMSO: 4	4/4 proper meiotic progression.	-
		BI-2536: 4	4/4 failure of bipolar spindle formation. 2/4: persistence of 2 centrioles. 1/4: persistence of at least 1 centriole. 1/4: not analyzed for centriole fate because centrioles moved deeply into the oocyte proper.	4/4: observed for only 143 min post 1-MA.
Fate of centrioles marked by mEGFP::pmCentrin2 upon Plk1 inhibition by BI-2536 in *P. miniata* (centrifuged)	Fig. 2C, 2D, 2E	DMSO: 11	11/11: persistence of 2 centrioles.	2 oocytes were not considered because of the appearance of extra foci (n=1) or because foci were very dim to start with (n=1).
		BI-2536: 10	5/10: persistence of 2 centrioles. 5/10: persistence of 1 centriole.	6 further oocytes were excluded because foci were dim to start with (n=3) or because they went out-of-focus (n=3).

Experiment	Figures	# oocytes	Results in brief	Comments
Fate of mother centrioles marked by pmOdf2::mEGFP in *A. forbesi*	Fig. 3	14	14/14: bright foci at each pole of MI spindle and solely at outer pole of MII spindle.	-
MTOC activity of retained oocyte-derived centrioles in *A. forbesi*	Fig. 4	29	28/29: no remaining MTOC activity. 1/29: extrusion of first polar body, one oocyte-derived MTOC active following retention of the second polar body.	18/29: MTOC marked by hEB3::mCh3. 11/29: MTOC marked by pmPoc1::mEGFP (also analyzed for Fig S2, see below). In a few cases, more than one sperm fertilized the oocyte. 6 oocytes were not considered as they exhibited unusual cytoplasmic texture and/or extensive polyspermy, so that MTOC activity of oocyte-derived centrioles could not be determined with certainty.
Fate of oocyte-derived centrioles as marked by mEGFP::PACT in *A. forbesi*	Fig. 5	5	4/5: persistence of 2 foci until mitosis onset. 1/5: persistence of 2 foci until mitosis onset, plus transient appearance of 1 focus during mitosis.	2/5: fertilized by 2 sperm. 2 oocytes from one animal were not considered as they exhibited unusual cytoplasmic texture and/or potential polyspermy, so that the MTOC capability of oocyte-derived centrioles could not be determined with certainty.
Fate of oocyte-derived centrioles as marked by pmPoc1::mEGFP in *A. forbesi*	Fig. S2	10	6/10: persistence of 1 focus until mitosis onset. 1/10: persistence of 2 foci until mitosis onset. 1/10: persistence of 3 foci until mitosis onset. 2/10: no oocyte-derived pmPoc1::mEGFP focus at mitosis onset.	4/10: also expressed hsEB3::mCh3. 2/10: fertilized by more than one sperm. 2 further oocytes could not be analyzed because tracking of centriolar pmPoc1::mEGFP was impossible due to spurious dots (n=1) or because sperm-derived centrioles were in the vicinity of oocyte-derived centrioles (n=1).


A
P. miniata
hsPlk1 hsEB3
-19:34
00:00
16:00
28:43
B
A. forbesi
hsPlk1 hsEB3
-10:00
00:00
15:00
30:00
C
mRNA injection
Injection chamber
Double sided tape
D
SW
plastic
E
2400rpm
4°C, 1h
F
meiosis I
meiosis II onset
meiosis II exit
G
P. miniata
PmCentrin2 hsEB3
1-MA +
00:46:54
00:51:36
00:55:37
01:02:26
01:06:37
01:10:48
01:14:59
01:21:01
01:25:12
01:29:23
01:33:34
01:37:45
01:41:56
01:46:07
01:50:18
01:54:29
01:58:40
02:02:51
02:07:02
02:11:13
02:15:24
02:19:35
02:27:20
02:31:31
02:35:42
02:39:53
02:44:04
02:48:15
02:52:26
02:56:37
03:00:48
03:04:59
Centrifuged + DMSO
1 and 2
3 and 4
H
1-MA +
00:48:34
00:53:16
00:57:17
01:04:06
01:08:17
01:12:28
01:16:39
01:22:41
01:26:52
01:31:03
01:35:14
01:39:25
01:43:36
01:47:47
01:51:58
01:56:09
02:00:20
02:04:31
02:08:42
02:12:53
02:17:04
02:21:15
02:29:00
02:33:11
02:37:22
02:41:33
02:45:44
02:49:55
02:54:06
02:58:17
03:02:28
03:06:39
Centrifuged + BI-2536
1 and 2
3 and 4


A
A. forbesi
hsEB3 pmPoc1
Sperm + Latrunculin B
13:11
29:09
61:07
1
2
3
4
S1
S2
B
13:11
21:10
29:09
37:09
45:08
53:08
61:07
C
number of oocyte-derived pmPoc1 foci
4
3
2
1
0
hsEB3
pmPoc1(aster)
0
10
20
30
40
50
60
70
80
time with respect to meiosis II onset (minutes)