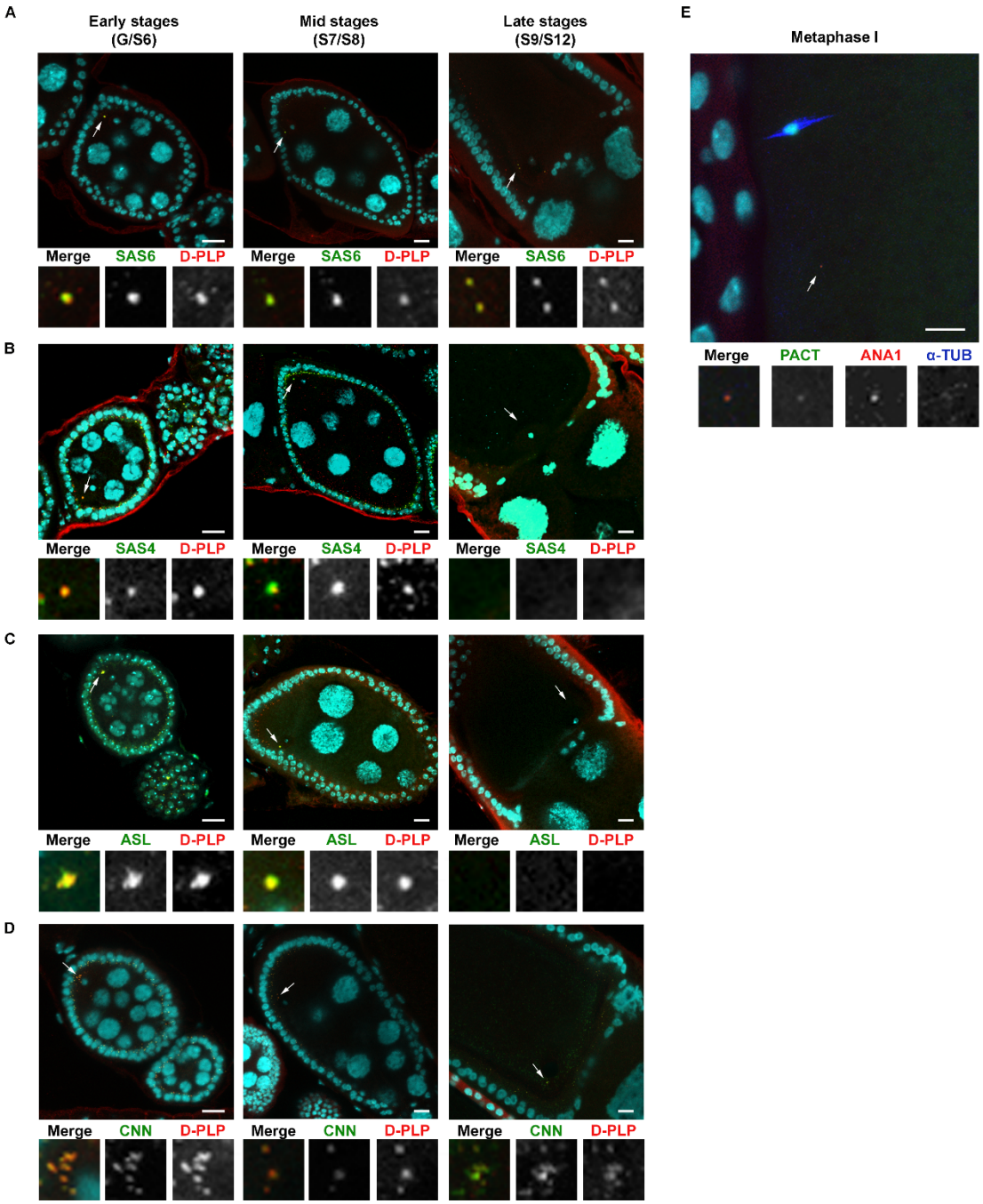
Supplementary Material

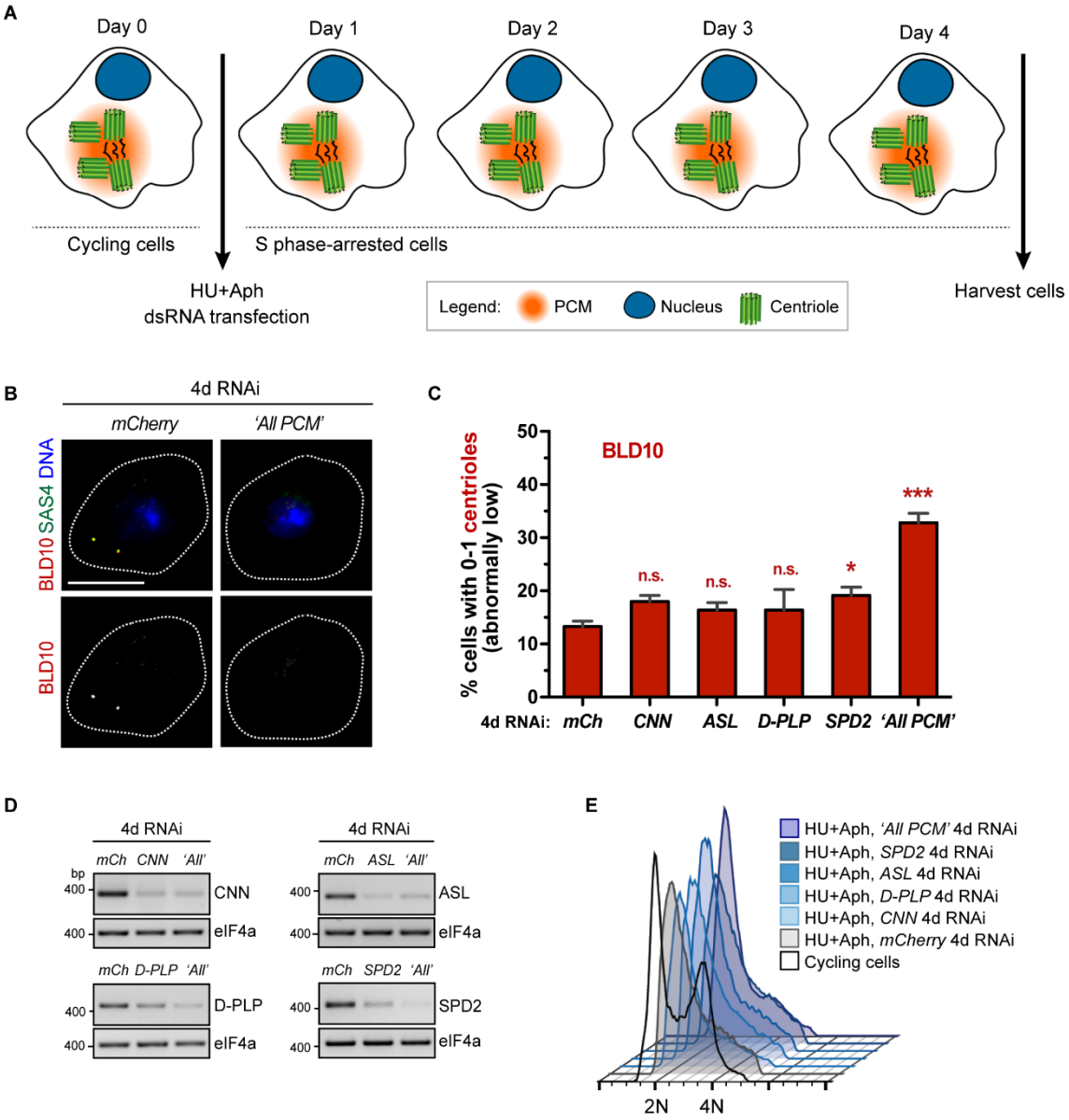
Marques & Bento *et al*

Supplementary Figures



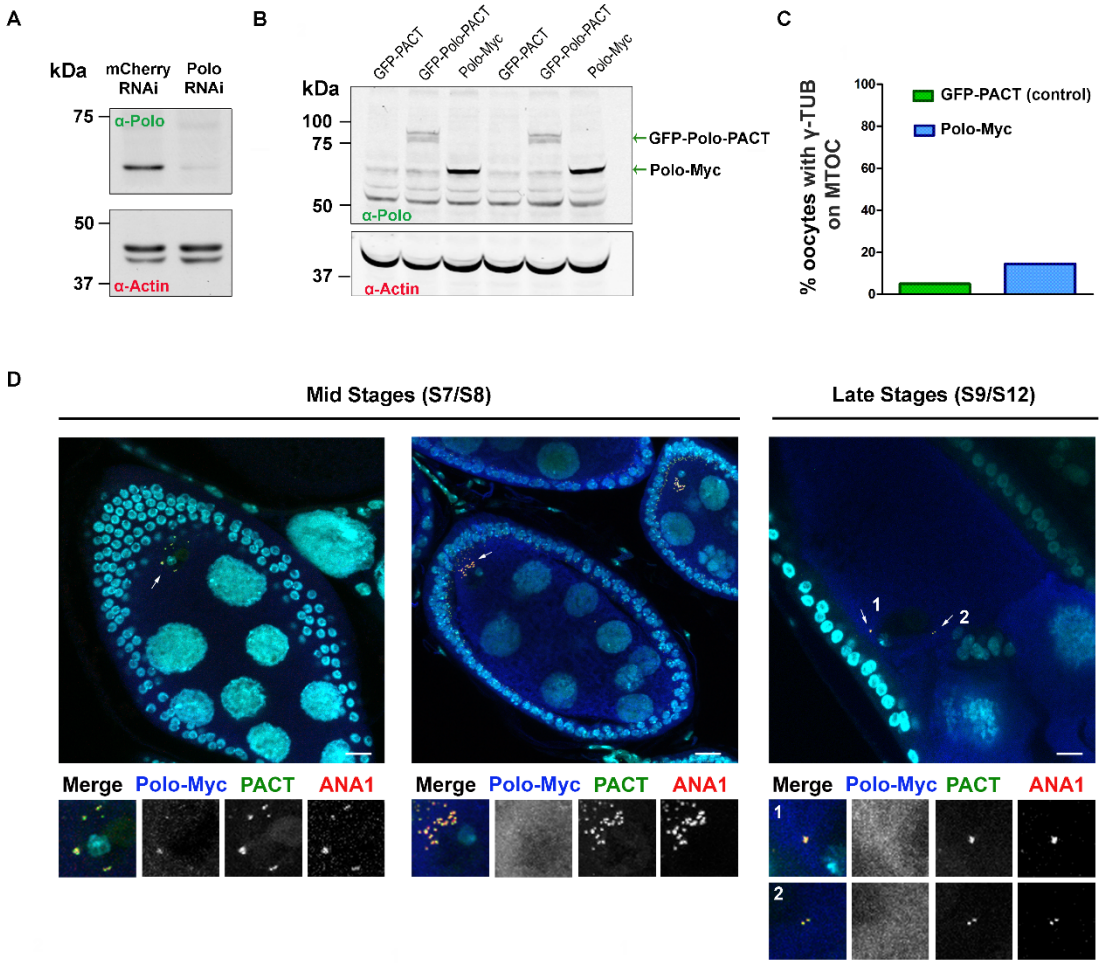
Supp. Fig. 1. Related to Fig 1. (A-D) Localization of the core centriolar protein SAS6 (A) and PCM-associated components SAS4 (B), ASL (C) and CNN (D) in the oocyte. Co-staining with D-PLP as a marker for the MTOC. SAS6 is detected close to the oocyte's nucleus, from early to late stages of oogenesis. PCM proteins are also detected close to the oocyte's nucleus from germarium to mid stages of oogenesis. In late oogenesis PCM components tend to disappear.

Enlargements of the indicated areas (arrows) are shown (5.3x and 7.5x magnification in early stages and mid/late stages, respectively, so that the images are set to comparable scales). Scale bars, 10 µm. **(E) Rare presence of inactive centrioles in metaphase I.** Two (out of twenty) metaphase I eggs showed centrioles. These were not associated with the spindle. Centriole identification was done by co-localization of ANA1 (centriolar marker) and PACT, the PLP centriole-targeting domain (GFP-PACT). Note that these centrioles were unable to nucleate microtubules. Enlargement (1.7x) of the indicated area (arrow) is shown. Scale bar, 10 µm.



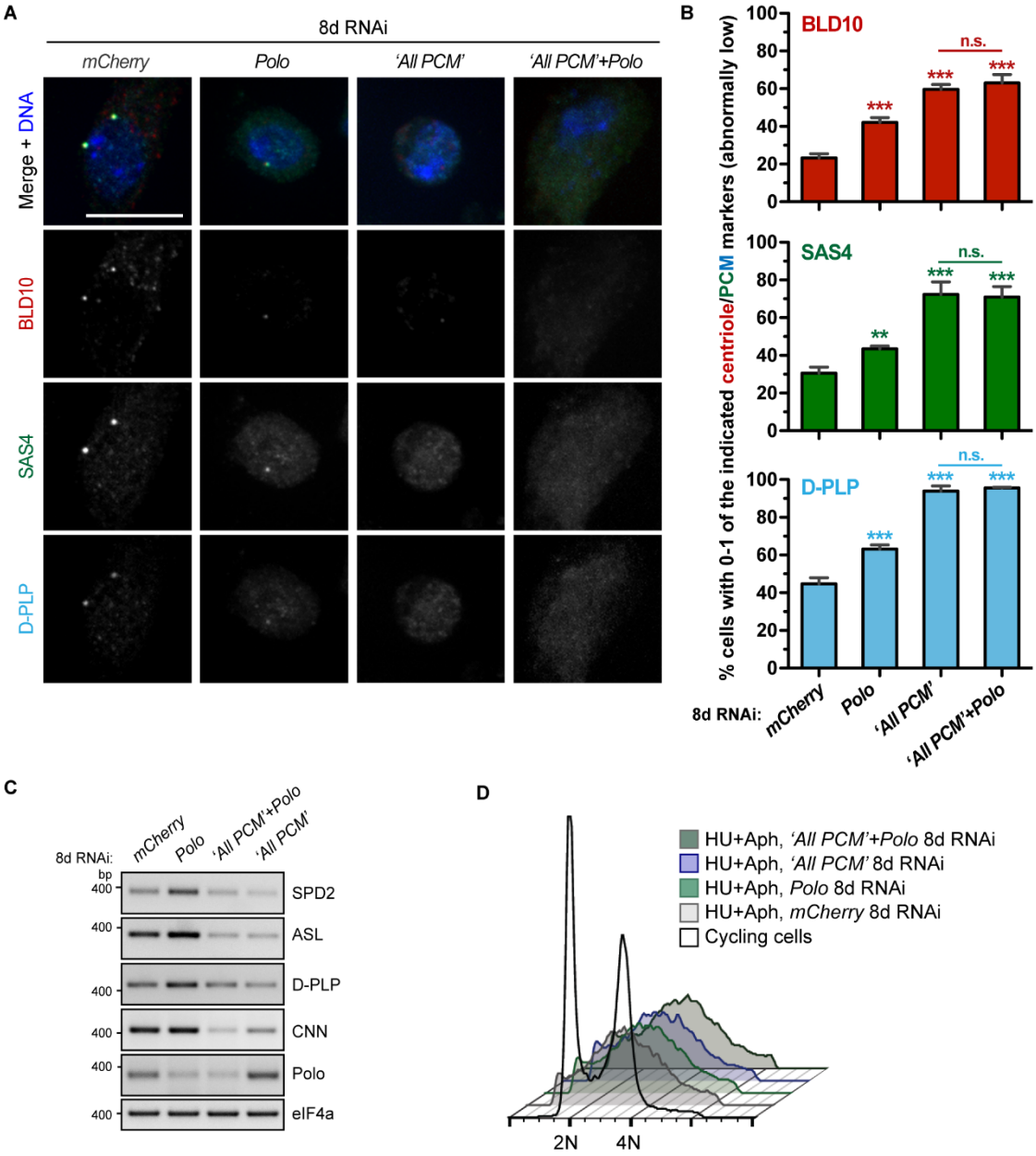
Supp. Fig. 2. Related to Fig 1. Depletion of four major PCM components induces centriole loss in *Drosophila* culture cells. (A) 'Centriole Stability Assay'. *Drosophila melanogaster* cultured cells (DMEL) were simultaneously transfected with dsRNA and treated with hydroxyurea (HU) and aphidicolin (Aph) for 4 days. Note that with this assay all cells are arrested in S phase when the RNAi starts to have an effect (day 2). Arrested cells will have duplicated their centrioles (should have a total of 4) and cannot re-duplicate them (*1*), hence

uncoupling an effect of depletion on centriole stability vs. centriole replication. **(B-E)** 'Centriole Stability Assay' in DMEL cells depleted of individual PCM components (*CNN*, *ASL*, *D-PLP* or *SPD2*) or where all four PCM components were simultaneously removed (*CNN+ASL+D-PLP+SPD2*, i.e. *'All PCM'*). *mCherry* RNAi was used as a control. After 4 days, cells were collected and assayed for centriole numbers by immunofluorescence (B-C), efficient depletion by RT-PCR (D) and cell cycle profile by flow-cytometry (E). **(B)** Cells were stained for BLD10 (best centriole marker in these cells; red), SAS4 (green) and DNA (blue). Representative images are shown. Scale bar, 10 µm. **(C)** Quantification of centriole numbers using BLD10 as a marker. As this assay is focused on centriole stability, we counted the percentage of cells with abnormally low numbers (i.e. 0-1). Note that simultaneous removal of *SPD2*, *D-PLP*, *ASL* and *CNN*, i.e. '*All PCM'* leads to centriole elimination. Data shown are the average of 3 independent experiments (n±100 cells in each experiment). The statistical difference between samples was evaluated with a Pearson's X^2 test (***, $p<0.001$; **, $p<0.01$; *, $p<0.05$; n.s., not statistically significant). **(D)** RNAi efficiency was confirmed by RT-PCR. elF4a elongation factor was used as a loading control. **(E)** Histograms of DNA content show the overlay of cycling and S-phase-arrested cells. Note that arrested cells show "S phase" DNA content.



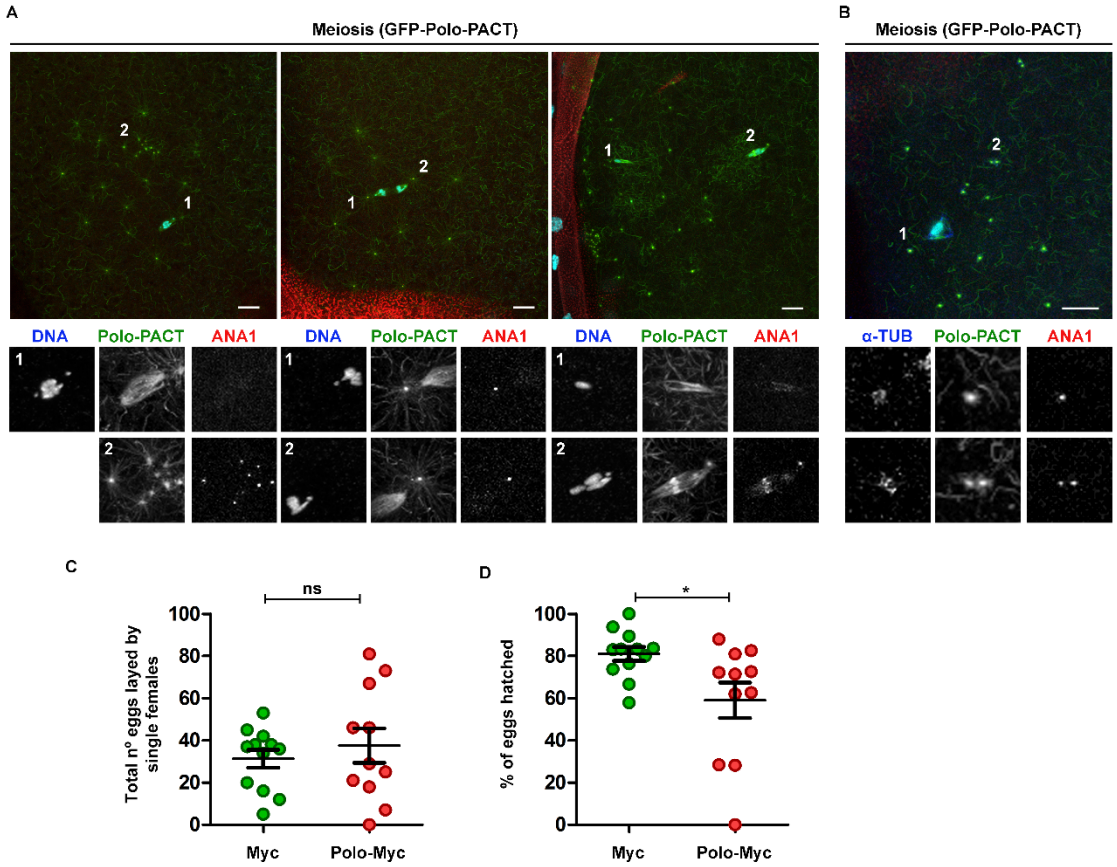
Supp. Fig. 3. Related to Figs. 2 and 3. *Polo* depletion and expression of GFP-PACT, GFP-Polo-PACT, Polo-Myc and Myc in oogenesis. (A) Western blot showing that Polo protein expression is reduced by RNAi treatment in ovaries. Egg chambers expressing *mCherry* (control) or *Polo* RNAi were prepared and analyzed by Western blot for Polo antibody. Actin was used as a loading control. Note that *Polo* shRNA expression was driven after stages 3/4 of oogenesis, which is posterior to the initial 4 successive mitosis that occur in the germarium and oocyte specification. This allowed us to circumvent the problems described for early depletion of *Polo* (*2*). **(B) Western blot showing levels of expression of different Polo constructs in female germline**. Egg chambers expressing GFP-PACT (control), GFP-Polo-PACT and Polo-Myc were prepared and analyzed by Western blot for Polo antibody (to detect GFP-Polo-PACT and Polo-Myc). Actin was used as a loading control. Note that Polo-Myc is more expressed then GFP-Polo-PACT. **(C) Overexpression of Polo-Myc is not sufficient to prevent the normally observed γ-tubulin loss.** Presence of γ-tubulin (γ-TUB) on centrioles in oocytes expressing GFP-PACT (control) or Polo-Myc in late stages of oogenesis (S12/13). Note a very small increase in presence of γ-TUB upon expression of Polo-Myc. This is in contrast to what is seen using GFP-Polo-PACT (Fig. 3). **(D) Overexpression of Polo-Myc is not sufficient to sustain its localization at the MTOC.** Note that in mid stages Polo-Myc does not always co-localize with the centrioles (left panel shows co-localization; right panel does not show co-localization). Late stage egg chambers rarely show the presence of Polo-Myc at the centrioles. Immunostaining for Myc

(Polo-Myc); expression of GFP-PACT and ANA1-tdTomato under the control of the endogenous promoter. Enlargements (2.3x) of the indicated areas (arrows) are shown. Scale bars, 10 µm.



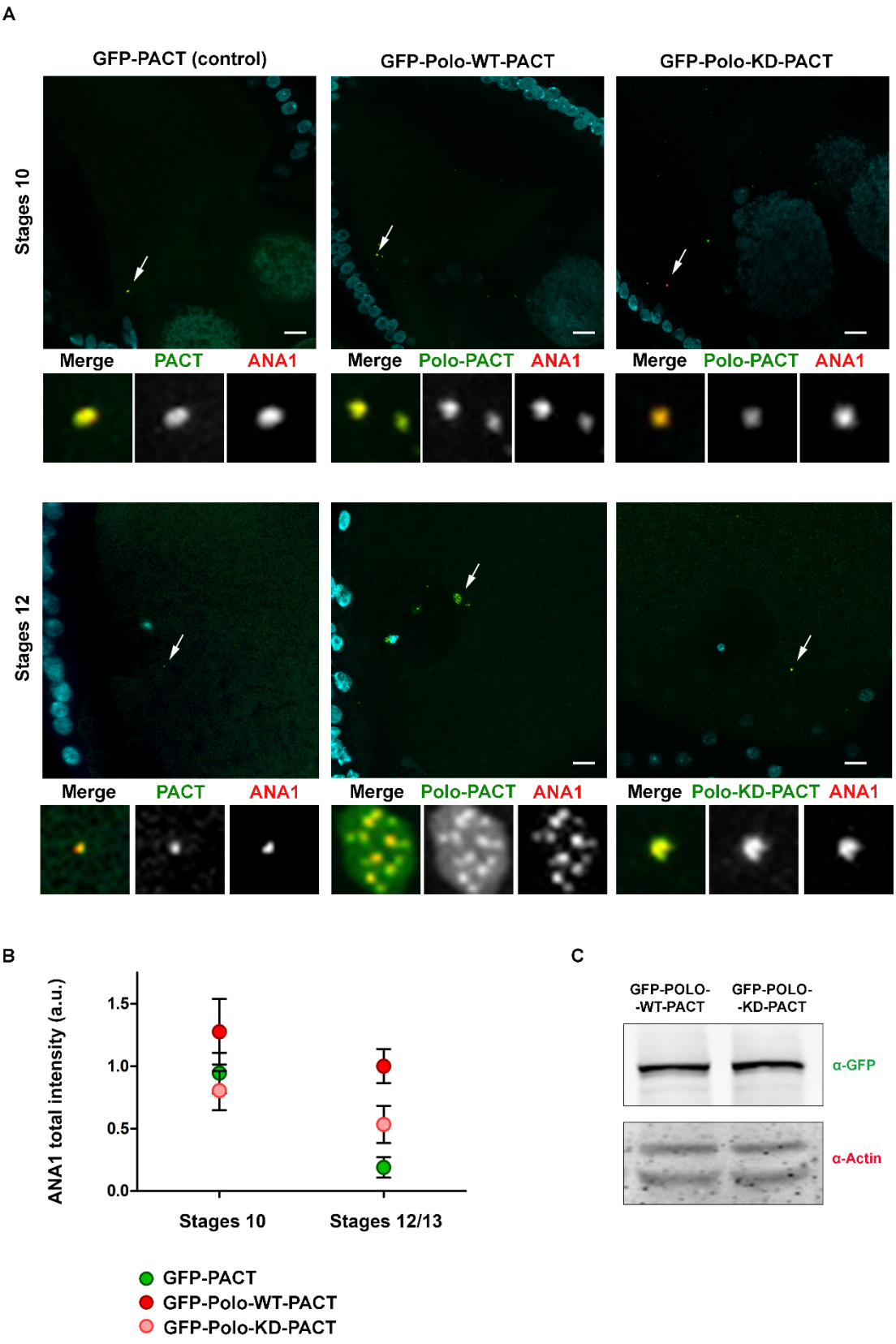
Supp. Fig. 4. Related to Fig. 2. Depletion of *Polo* induces centriole loss in *Drosophila* culture cells. 'Centriole Stability Assay' in DMEL cells depleted of *Polo*, *'All PCM'* or *'All PCM'* and *Polo ('All PCM'+Polo)*. *mCherry* RNAi was used as a control. This assay is an optimization of the assay shown in fig. S2, as it runs for longer (8 vs. 4 days) and it achieves more centriole elimination. After the initial 4-day assay, cells were subject to a second round of dsRNA transfection and treatment with HU and APH for 4 more days. After a total of 8 days of RNAi and HU+Aph, cells were harvested and assayed for centriole numbers by immunofluorescence (A-B), efficient depletion by RT-PCR (C) and cell cycle profile by flow-cytometry (D). **(A)** Cells were stained for BLD10 (red), SAS4 (green), D-PLP (Cyan) and DNA (blue). Representative images are shown. Scale bar, 10 µm. **(B)** Histograms show the percentage of cells with abnormally low numbers (i.e. 0-1) of the indicated centriole (BLD10) and PCM (SAS4 and D-PLP) markers. Data shown

are the average of 3 independent experiments (n±100 cells in each experiment). The statistical difference between samples was evaluated with a Pearson's X^2 test as compared to controls, unless otherwise stated (***, p<0.001; **, p<0.01; n.s., not statistically significant). Note that depletion of *Polo* alone has an effect on PCM loss (similar results were observed for the PCM proteins SPD2 and γ-tubulin; not shown), similar to its effect on centriole loss; moreover Polo and the PCM are likely to be acting on the same "centriole maintenance pathway" as the effect of co-depletion is similar to depletion of *'All PCM'*. Note that the effect of the loss of the PCM on centriole loss was increased as compared to Fig. S2, where a 4-day assay was used. **(C)** RNAi efficiency was confirmed by RT-PCR. eIF4a elongation factor was used as a loading control. **(D)** Histograms of DNA content show the overlay of cycling and S-phase-arrested cells.

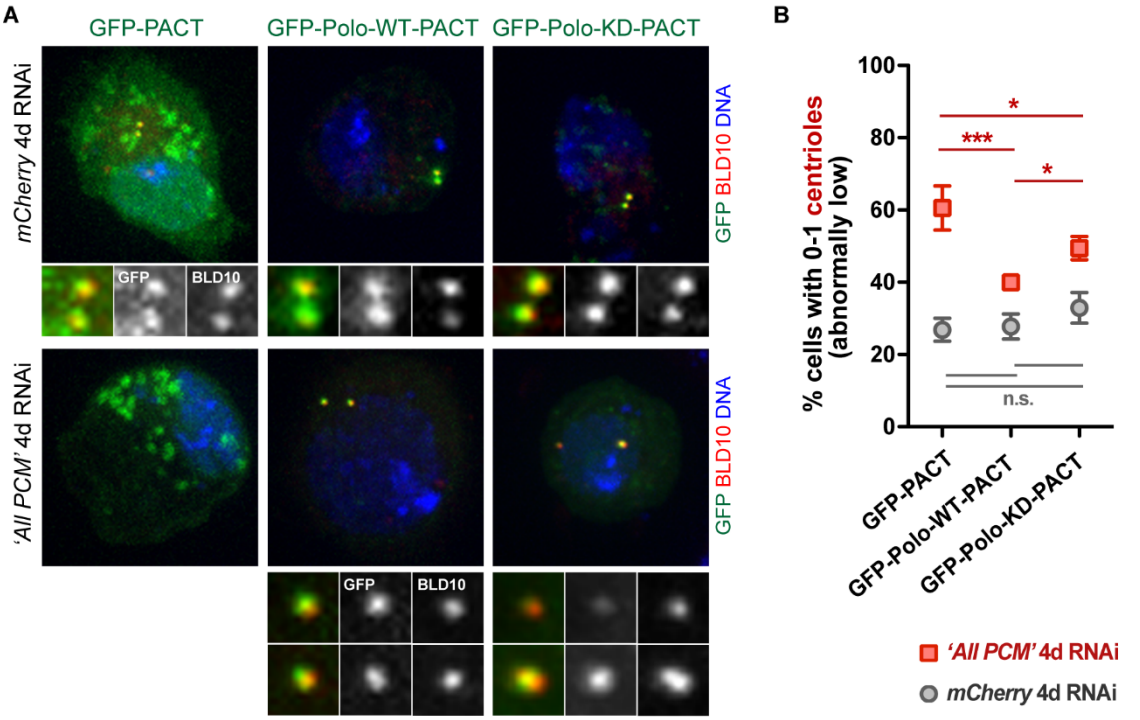


Supp. Fig. 5. Related to Fig. 3. Expression of centriole tethered Polo leads to meiotic defects and aborted embryonic development. (A) Stage 14 eggs expressing GFP-Polo-PACT show several randomly distributed centrioles in the anterior pole and abnormal meiosis (see control in Fig. 3). GFP-Polo-PACT, ANA1 (centriolar marker) and DNA. Note that at this stage GFP-Polo-PACT localizes to both Polo and PACT localization places, i.e. centrioles, microtubules and kinetochores. Observe scattered DNA in right panel (1 and 2). Scale bars, 10 µm. **(B) Upon expression of GFP-Polo-PACT, centrioles present in meiosis have microtubule nucleating capacity**. Immunostaining of egg chambers with α-tubulin (α-TUB). Expression of GFP-PACT and ANA1-tdTomato (centriolar marker) under the control of the endogenous promoter. Scale bar, 10 µm. **(C-D) Expression of Polo-Myc does not affect egg laying and only slightly perturbs egg viability as compared with GFP-Polo-PACT (Fig. 4). (C)** Quantification of the number of eggs laid by Myc (control) and Polo-Myc females (11 females per condition). **(D)** Quantification

of eggs that hatched from the total number of eggs laid by Myc or Polo-Myc expressing females. The statistical difference between samples was evaluated with a t-test (*, p<0.05; ns, not statistically significant).

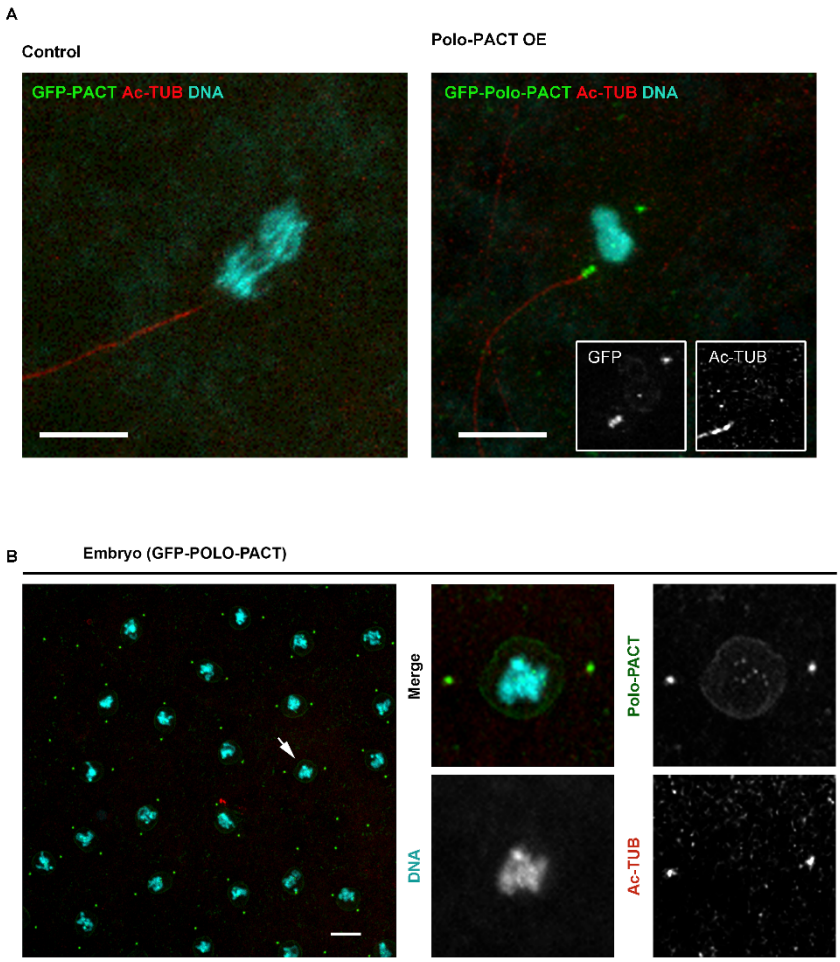


Supp. Fig. 6. Related to Fig. 3. Maintenance of centrioles in oogenesis is Polo kinase activity-dependent. (A) Targeting Polo and Polo kinase dead (catalytically inactive) to centrioles. GFP-PACT (control), GFP-Polo-WT-PACT and GFP-Polo-KD-PACT were expressed in the germline (PACT is the centriolar targeting domain of PLP (*3*)) from stage 3/4 onwards. ANA1-tdTomato expression under endogenous promoter was used as a robust centriolar marker. Enlargements (8.5x) of the indicated areas (arrows) are shown. All images were acquired with same exposure. We noted that centrioles in Polo-KD-PACT have a similar behavior to control ones as they are more clumped in early and mid stages of oogenesis (in contrast to the overexpression of catalytically active Polo, where centrioles are more dispersed (see stage 10 and 12 images); this supports Polo-KD-PACT is catalytically dead). Scale bars, 10 µm. **(B) Quantification of total ANA1-tdTomato levels (endogenous promoter) in stages 10 and stages 12 (late stages) of oogenesis in GFP-PACT, GFP-Polo-WT-PACT and GFP-Polo-KD-PACT expressing oocytes**. Note that GFP-Polo-KD-PACT is not able to fully rescue centriole loss as compared with GFP-Polo-PACT; however, we systematically observed a stronger centriole signal in GFP-Polo-KD-PACT as compared to GFP-PACT controls. **(C) Western blot showing levels of expression of GFP-Polo-WT-PACT and GFP-Polo-KD-PACT constructs in female germline**. Protein extracts were prepared from ovaries expressing GFP-Polo-WT-PACT and GFP-Polo-KD-PACT and analyzed by western blot for GFP antibody. Note that these constructs are expressed at equal levels (GFP-PACT is also expressed at similar levels, not shown).



Supp. Fig. 7. Related to Fig. 3. Ectopic tethering of Polo to the centrioles prevents centriole loss in *Drosophila* culture cells in the context of PCM depletion. After transfection with dsRNA against either *mCherry* (control) or *'All PCM'* and concomitant treatment with hydroxyurea (HU) and aphidicolin (Aph) (centriole stability assay, 4 days), DMEL cells were transiently transfected with GFP-PACT, GFP-Polo-WT-PACT and GFP-Polo-KD-PACT constructs. After 4 days, cells were harvested and assayed for centriole numbers by immunofluorescence. **(A)** Cells were stained for BLD10 (red) and DNA (blue). Representative images of transfected cells

are shown. Scale bar, 10 µm. Enlargements of the centrioles are shown. **(B)** Histogram shows the percentage of cells with abnormally low numbers (i.e. 0-1) of centrioles (BLD10). Data shown are the average of 3 independent experiments (n±100 cells in each experiment). The statistical difference between samples was evaluated with a Pearson's X^2 test (***, p<0.001; *, p<0.05; n.s., not statistically significant). Note that catalytically active Polo is more efficient at rescuing centriole loss in the context of PCM depletion as compared to GFP-Polo-KD-PACT. However, we systematically observed more centrioles in GFP-Polo-KD-GFP as compared to GFP-PACT controls.



Supp. Fig. 8. Related to Fig. 4. (A) Eggs from females expressing GFP-Polo-PACT can be fertilized. Eggs from females expressing either GFP-PACT (control) or GFP-Polo-PACT were stained for acetylated tubulin (Ac-TUB) to visualize the sperm tail. Left panel shows the first mitosis (anaphase) of a control egg (GFP-PACT) where the aster from the sperm tail is still visible by staining of acetylated tubulin. The right panel shows an egg from a female expressing GFP-Polo-PACT (condensing DNA), also fertilized. Insets show enlargement of the poles. Note the presence of supernumerary centrosomes (see 3 at lower pole; at least 5 in total in the inset). The signal for GFP-PACT (left) is very weak in the first mitosis. Scale bars, 10µm. **(B) Presence of GFP-Polo-PACT in syncytial embryos.** GFP-Polo-PACT expression did not lead to severe defects in syncytial nuclear divisions in the very few escaper embryos observed upon maternal expression of GFP-Polo-PACT. Note that GFP-Polo-PACT localizes to both Polo and PACT targeting sites, i.e. centrosomes and kinetochores. While some supernumerary

centrosomes are still detected, no major mitotic defects are observed suggesting the possibility that the presence of GFP-Polo-PACT *per se* in the embryo is not detrimental. Enlargement (3.5x) of the indicated area (arrow) is shown. Scale bar, 10 µm.

Materials and Methods

Fly Stocks

For SAS6, BLD10 and ANA1 analyses we used SAS6-GFP/Cyo, BLD10-GFP/Cyo and W; ANA1-tdTomato lines, where transgene expression was under the control of the respective endogenous promoters. Heterozygous flies were used for these experiments. These fly stocks were kindly provided by Tomer Avidor-Reiss (Harvard Medical School, USA; (*4, 5*)). CNN and SAS4 analyses were done by using pUbq-GFP-CNN/Cyo and pUbq-SAS4-GFP/Cyo lines. In these cases, transgene expression was under the control of a polyubiquitin promoter that allows basal level expression along the entire organism. Homozygous flies were used for these analyses. These lines were kindly provided by Jordan Raff (University of Oxford, UK; (*6, 7*)). W; β-Tubulin-GFP line was used to identify the metaphase or anaphase spindle of meiosis I (*8*). For the expression of non tethered Polo in the germline we used UASp-Polo-WT-Myc (*9*). For GFP-Polo-PACT overexpression experiments we generate transgenes, GFP-PACT (control), GFP-Polo-WT-PACT and GFP-Polo-kinase-dead-KD-PACT, which were then cloned into the UASp vector. The kinase dead version of GFP-Polo-PACT was generated by substituting the conserved amino acid aspartate in position 176 to aspargine in the catalytic domain, as described before for PLK1 and several other kinases (*10*). Transgenic flies were generated via plasmid injection (BestGene Inc.). Knockdown for *polo* with UAS-RNAi was carried out with the line *Phf7-P{TRiP.GL00014}attP2* (BDSC # 35146) from the *Drosophila* transgene RNAi Project (TriP; (*11*)). This is a GL RNAi line which is constructed in the VALIUM22 vector that has expression in the female germline. A UAS-RNAi line for *mCherry* was used as control (BDSC #35785). We generated fly lines in which both *Polo*- and *mCherry*-RNAi were combined with genotypes expressing Ana1-tdTomato (under the control of the endogenous promoter) and GFP-PACT (under the control of a Poliubiquitin promoter). The maternal germline-specific overexpression involved crossing of the above lines with the maternal germline-specific driver V32 and G302-Gal4/Cyo (Daniel St. Johnston; Gurdon Institute, UK). All flies were raised at 25°C unless otherwise indicated, using standard techniques.

Egg laying and egg hatch rate assays

Well-fed virgin females 2 days old were mated with single W^{1118} males. Single female and male crosses were performed in cages with agar plates supplemented with apple juice. The number of eggs laid was counted during 5 days. Each plate was kept at 25°C for 3 extra days and examined for the number of larvae hatched. Egg hatching rates were calculated as the percentage of larvae that ecloded from the total number of eggs laid by each single female. Each experiment was done in 10 or more independent replicates.

Ovaries immunostaining

For ovary stainings we anesthetized 3-4 day-old well fed females with ether. Females were transferred to pre-warmed (25°C) BRB80 buffer (80 mM Pipes pH 6.8, 1 mM $MgCl_2$, 1 mM EGTA) supplemented with 1X protease inhibitors (Roche), and their ovaries were extracted with pre-cleaned forceps. Individualized ovaries were then incubated for 1 hour (h) at 25°C in BRB80 with 1% Triton X-100 without agitation, followed by a 15 minutes (min) fixation step at -20°C in chilled methanol. 3 wash steps of 15 min each and overnight permeabilization were done in PBST (1X PBS with 0.1% Tween). Blocking for 1 h was done in PBST with 2% BSA (Gibco). Primary antibodies were incubated overnight at 4°C in PBS with 1% BSA (PBSB) followed by 3 wash steps. Secondary antibodies were diluted in PBSB and incubated for 2 h at room temperature (RT). Ovaries were washed in PBS and DNA was counterstained with Toto-3-Iodide (Molecular Probes) or by using Vectashield mounting medium with DAPI. Ovaries were mounted onto coverslides in Vectashield mounting media (Vector Laboratories). Unless stated otherwise, chemicals were purchased from Sigma.

Stage 14 oocyte immunostaining

Ovaries were dissected in BRB80 buffer supplemented with 1X protease inhibitors (Roche) and transferred to a 1.5 ml plastic tube containing ~1 ml of fresh methanol. About 10-20 single ovaries prepared in this way were sonicated three times for ~1 second (sec) each. Sonicated ovaries were prepared as described for ovaries immunostaining. Once the immunostaining was concluded, stage 14 dechorionated

oocytes were selected and mounted as previously described. (Protocol kindly provided by Hiroyuki Ohkura, University of Edinburgh, UK).

Embryo immunostaining

For embryo analysis, embryos were collected 1 h after egg laying and fixed (after dechorionation in 50% bleach for 5 min) by gentle shaking for 1 hr in 4 mL heptane, 0.125 mL 37% formaldehyde and 0.875 mL PBS. Fixation was followed of devitellinization by addition of 4 mL methanol and shaking vigorously during 1 min. Following rehydration, embryos were blocked in PBSTB (PBS containing 0.1% triton X-100 and 1% BSA) at 4°C overnight. Primary antibody incubations were carried in PBSTB at 4°C overnight. Embryos were washed extensively in PBSTB and secondaries were incubated in this same solution for 2 h at RT. After extensive wash in PBS with 0.1% triton and PBS, embryos were mounted onto coverslips in Vectashield mounting media with DAPI (Vector Laboratories).

Imaging, analysis and quantification

Ovary and embryo preparations were imaged as Z-series (0.5 µm apart) on a Leica SP5 high-speed and high-resolution spectra confocal microscope and Leica TCS SP5 upright confocal laser-scanning microscope. For comparisons across oogenesis, images were acquired with same exposure for each protein at different stages of oogenesis. Images were processed as maximum-intensity projections and assembled into panels using Image J (NIH) and Adobe Photoshop CS (Adobe). Intensity measurements were performed using ImageJ software (NIH). The centrosomal region was determined by the co-localization of the GFP signal from either the GFP-PACT or the GFP-Polo-PACT with the ANA1-tdTomato signal. The intensity of centriolar γ-tubulin and ANA1, was analyzed by measuring the intensity in these co-localizing dots. To assess the cytoplasmic staining (background), the intensity of 3 different regions of 10 px diameters was measured from the maximum projection. Afterwards, that intensity was reduced from the original image stacks and the centriolar signal (for either γ-tubulin or ANA1) was measured from the sum projections of those stacks. In experiments were PCM and centriolar proteins presence or absence was valuated, absence of signal was defined as failure to detect significant signal above oocyte background signal.

Super-Resolution Structured Illumination Microscopy (SIM)

To study the centrioles using SIM, ovaries from 3 day-old females expressing either GFP-PACT (control) or GFP-Polo-WT-PACT were dissected in BRB80 buffer supplemented with 1X protease inhibitors (Roche) and transferred to a 1.5 ml plastic tube containing ~1 ml of fresh cold methanol. About 10-20 single ovaries prepared in this way were sonicated three times for ~1 sec each to dechorionate the non-transparent chorion from the late stage oocytes. They were further washed and stained with DAPI. All imaged ovaries were expressing the centriolar protein ANA1 tagged with tdTomato and under the control of the endogenous promoter. Samples were mounted on the poly-L-Lysine coated coverslips in Vectashield mounting media (Vector Laboratories) so that the samples remain very close to the cover glass during imaging. The images were then collected using Elyra Zeiss microscope (Zeiss, Germany). 3D-Structuted illumination micrographs were generated in ZEN Blue software (Zeiss, Germany). Finally, all images are processed in ImageJ (USA) and Adobe Photoshop (Adobe Systems, USA).

Western blotting

For western blotting, 10 ovaries of each sample were dissected in PBS containing protease inhibitors (Roche). For each sample, ovaries were squashed in SDS-PAGE sample buffer and boiled for 5 min before loading. For *polo*-RNAi experiment in the germline, ovary protein extracts were prepared as previously described (*12*). Standard western blotting procedures involved blocking in TBS-T (0.1% Triton X-100 in TBS) supplemented with 2% BSA powder, and 1% BSA powder in TBS-T for antibody incubations and washes. The antibodies used for western blotting were the following: mouse anti-Polo (1:50 dilution; kindly provided by David Glover, University of Cambridge, UK), rabbit anti-Actin (1:2000 dilution, Sigma, USA) and IRDye secondary antibodies (1:5.000 dilution; Odyssey, LI-COR Biosciences).

Protein depletion in DMEL cells

D. melanogaster culture cells (DMEL) were maintained in Express5 SFM medium (Gibco,USA) supplemented with 1x L-Glutamine-Penicilin-Streptomycin according to

standard tissue culture techniques. Synthesis of *mCherry*, *d-plp*, *cnn*, *asl*, *spd2* and *polo* dsRNAs were performed as previously described (*13*). For dsRNA transient transfection we used 12 million cells. The experiment involved using different dsRNA amounts and combinations: 80 µg *mCherry* alone; 20 µg individual PCM components or *polo* combined with 60 µg *mCherry*; 20 µg each *cnn*, *asl*, *d-plp*, and *spd2* for *'All PCM'*; or 20 µg each *cnn*, *asl*, *d-plp*, *spd2* and *polo* for *'All PCM'+Polo*. Primers used for dsRNA production are listed in Supplementary Table 1.

DMEL cells 'Centriole Stability Assay'

DMEL cells were S-phase-arrested using a protocol described elsewhere (*1, 14*). Briefly, 1 h after dsRNA transfection we co-treated cells with 10 µM aphidicolin (Aph), a specific Eukaryotic DNA polymerase inhibitor, and 1.5 mM hydroxyurea (HU), a drug which reduces deoxyribonucleotide production. For the 4-days 'Centriole Stability Assay', cells were collected after 4 days of dsRNA and Aph+HU treatment (fig. S2 and S7). For the 8-days 'Centriole Stability Assay' (fig. S7), cells were subject to a second round of dsRNA transfection and Aph+HU treatment after the initial 4-day assay and collected on the 8th day of the assay. Collected cells were used for immunostaining, RT-PCR and/or flow-cytometry.

Plasmid transfections for rescue experiments in DMEL 'Centriole Stability Assay'

After transfection with dsRNA against either *mCherry* (control) or *'All PCM'* and concomitant treatment with hydroxyurea (HU) and aphidicolin (Aph) ('Centriole Stability Assay'), DMEL cells were transiently transfected with GFP-PACT, GFP-Polo-WT-PACT and GFP-Polo-KD-PACT constructs. Since these constructs contain an UASp promoter (*15*), each of these constructs were simultaneously co-transfected with an Actin5C-Gal4 plasmid (*16*). Transfection was performed with Effectene reagent (Qiagen, USA) according to the manual recommendations. Briefly, 200 ng of each plasmid DNA were mixed with 3.5 µl Enhancer reagent and incubated for 5 min at RT; 10 µl Effectene transfection reagent were added to the previous solution, mixed and incubated at RT for 10 min; 1.5 ml medium with 1.5 mM HU and 10 µM Aph were

added to the final mix and the solution was added to the cells in a drop wise manner. Transfections proceeded for the remainder of the 'Centriole Stability Assay'.

Immunostaining and imaging of *D. melanogaster* culture cells

DMEL cells were plated onto glass coverslips and allowed to adhere for 1h. After medium removal, cells were fixed at -20ºC for 10 minutes in chilled methanol. Cells were then permeabilized and washed in PBSTB (PBS containing 0.1% Triton X-100 and 1% BSA). Immunostainings were performed as previously described (*17*). Cells were mounted with a mounting medium containing DAPI to stain DNA (Vector Laboratories, USA). Cell imaging and centriole scoring were performed on a Leica DMRA2 microscope with a Cool SNAP HQ camera (Photometrics), or with a Nikon Eclipse Ti-E (Nikon) microscope with a Evolve EMCCD camera (Photometrics) and controlled by Metamorph 7.5 software (Molecular Devices). Images were acquired as Z-series (0.3 µm z-interval) and are presented as maximal intensity projections. All images were prepared with Adobe Photoshop and Illustrator (Adobe Systems, USA) and ImageJ (NIH, USA). Centrioles were scored blindly in a total of 100 cells per sample per slide. Statistical analysis was performed with a Pearson's X^2 test.

Flow Cytometry

Cells collected 4 days after transfection were fixed in 70% ice-cold ethanol. Fixed cells were incubated at 37ºC for 30 min in PBS supplemented with 100 µg/ml RNase and 100 µg/ml propidium iodide. DNA content analysis of 30,000 cells per sample was performed in a Becton Dickison FACScan and Becton Dickinson LSR. Results were processed with FlowJo software.

RT-PCR

Total mRNA was extracted using the RNeasy Mini Kit (Qiagen) according to manufacturer instructions. cDNA was synthesized with the Transcriptor First Strand cDNA Synthesis Kit (Roche). PCR of the gene-of-interest was carried out with the primers used for dsRNA synthesis (see supplementary table 1). eIF4a was used as a loading control.

Antibodies

Primary antibodies and dilutions used for immunostaining were as follows: chicken anti-D-PLP (1:500 dilution; (*18*)), rabbit anti-Asterless (1:500 dilution) and mouse anti-Polo (1:50 dilution) (both kindly provided by David Glover, University of Cambridge, UK), rabbit anti-SPD2 (1:500 dilution; (*17*)), mouse anti-γ-tubulin (1:50 dilution; clone GTU88, Sigma), rat anti-α-tubulin (1:50 dilution; clone YL1/2, Oxford Biosciences), mouse anti-acetylated tubulin (1:50 dilution; clone 6-11B-1, Sigma), mouse anti-Myc (1:350 dilution; Santa Cruz Biotechnologies), rabbit and rat anti-SAS4 (1:500; (*6*)), rat anti-SAS4 (1:500; *(1)*) and rabbit anti-BLD10 (1:500; Tim Megraw; The Florida State University, USA (*19*)). Secondary antibodies (Jackson Immunoresearch Laboratories) were used at 1:250 dilution for ovaries immunostaining and 1:100 dilution for DMEL cells immunostaining.

Supplementary Table 1.

Table 1. List of primers used for dsRNA synthesis and RT-PCR		
Name	**CG No.**	**Sequence (5'-3')**
***mCherry* dsRNA**	-	**Fw:** TAATACGACTCACTATAGGGATGGTGAGCAAGGG **Rev:** TAATACGACTCACTATAGGGGTTGACGTTGTAGG
***asl* dsRNA and RT-PCR**	2919	**Fw:** TAATACGACTCACTATAGGGAGATTATGGTGAATGCCTTCGAC **Rev:** TAATACGACTCACTATAGGGAGACTAGCTCAGCCTGCATGATG
***spd2* dsRNA and RT-PCR**	17286	**Fw:** TAATACGACTCACTATAGGGAGAGTCGCGTTCCAGCCAAGCAAAGA **Rev:** TAATACGACTCACTATAGGGAGAAATCCCCCACCTCCGTTAAGACTCAG
***d-plp* dsRNA and RT-PCR**	33957	**Fw:** TAATACGACTCACTATAGGGAGAGGAGCGCCTAAAGAACAGTG **Rev:** TAATACGACTCACTATAGGGAGACTGATCGAGCTGTTTGTGGA
***cnn* dsRNA and RT-PCR**	4832	**Fw:** TAATACGACTCACTATAGGGAGAACCTCCAGGCGGCGGCAACT **Rev:** TAATACGACTCACTATAGGGAGATGGCTCGAGCGGCATCCTT
***Polo* dsRNA and RT-PCR**	12306	**Fw:** TAATACGACTCACTATAGGGAGACGTTCTCCGCTTTGTGCTTGGTTTTCGTG **Rev:** TAATACGACTCACTATAGGGAGACGCTTGTAGGTTTTCCGCTGGTTGATGTCG
***elF4a* RT-PCR**		**Fw:** TAATACGACTCACTATAGGGAGAGAAATGAGATACCTCAGGATGGCCC **Rev:** TAATACGACTCACTATAGGGAGAACGTTAGTGCCGCCAATGCA

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