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Supplemental Information

Germ Cell-less Promotes Centrosome Segregation to Induce Germ Cell Formation

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Supplemental Figures

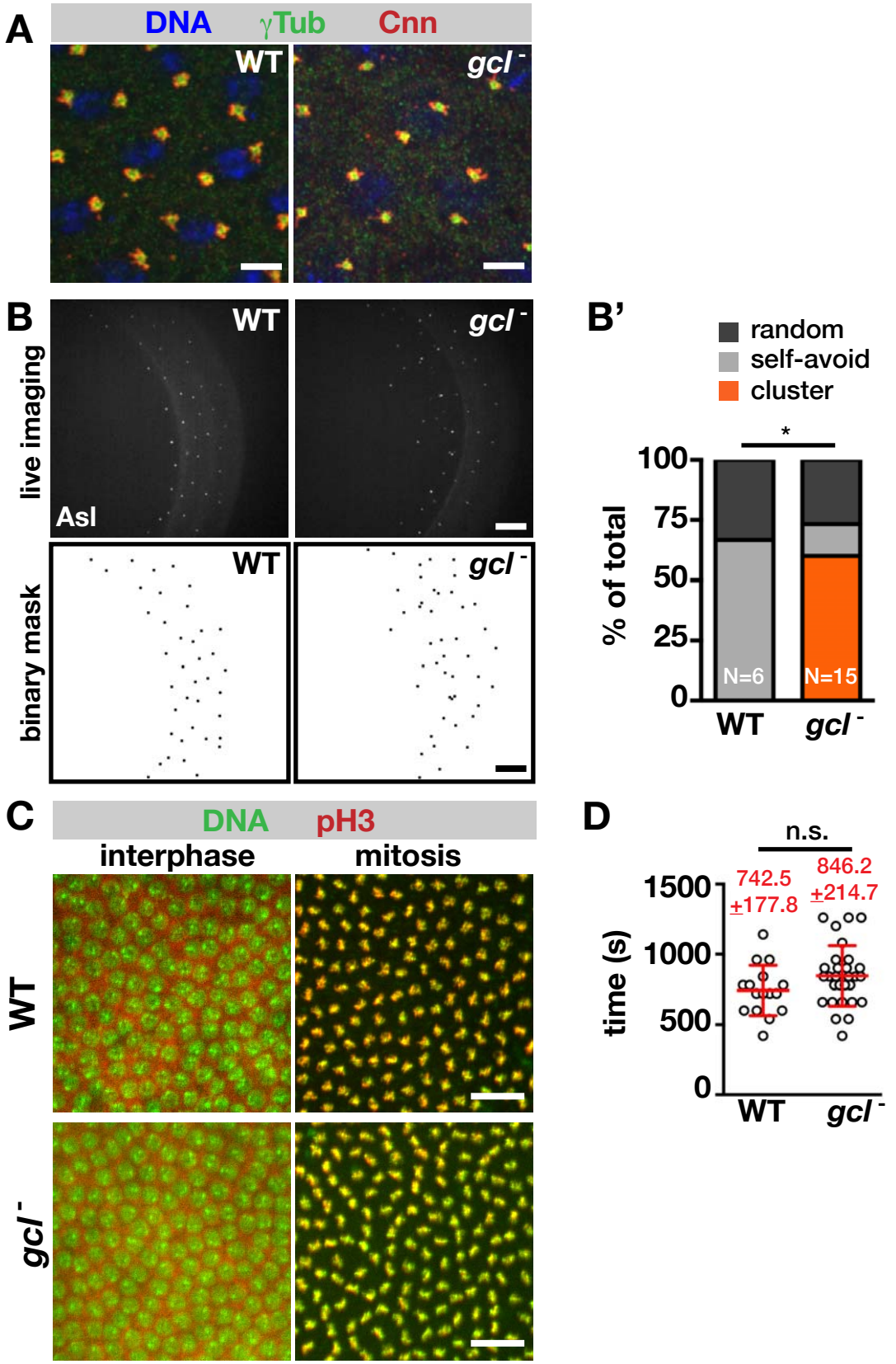

Figure S1. Gcl regulates centrosome separation locally at PB nuclei, Related to Figure 2.
(A) Immunofluorescence shows the PCM proteins γTub (green) and Cnn (red) at centrosomes from the somatic mid-region of 1-2 hr embryos. (B) Stills from live imaging of centrosome separation in 1-2 hr WT and *gcl* mutant prophase-stage embryos expressing Asl-YFP (top) were used to generate binary masks (below) using FIJI software. For this image, objects (single pixel black dots) were dilated several times to facilitate visualization. (B') Frequency plot from centrosome nearest-neighbor analysis (FIJI, NIH) taken from WT N=6 embryos and *gcl* N=15 embryos. Statistical significance was determined by chi-square test; * p=0.0189. (C) Maximum intensity projections show the somatic mid-region of 1-2 hr embryos stained for pH3 (red) to show mitotic nuclei and DAPI (green) to label all nuclei. Mitotic synchrony is not lost in *gcl* mutants. (D) Quantification of cell cycle duration derived from live imaging where each data point indicates the timing for a single NC (WT N=8 embryos; *gcl* N=17 embryos). Significance determined by unpaired t-test; n.s., not significant. Bars: (A) 2.5 µm; (B) 5 µm; (C) 20 µm.

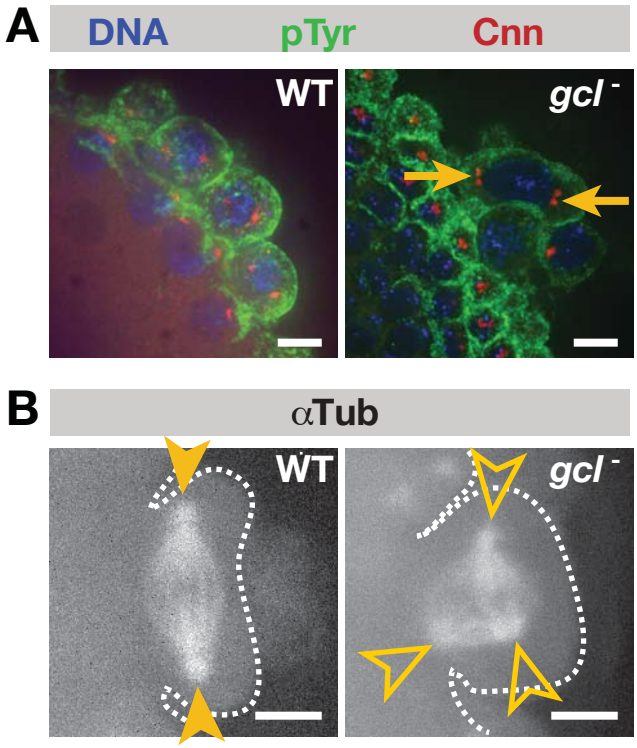

Figure S2. Aberrant centrosome numbers in *gcl* PGCs, Related to Figure 2.
(A) Immunofluorescence for pTyr (green) and Cnn (red) to show centrosomes within PGCs. In the *gcl* mutant, supernumerary centrosomes are highlighted with arrows (2 centrosomes/arrow). (B) Stills from live imaging of PB spindles within 1-2 hr embryos expressing γTub-GFP. WT spindles have 2 poles (closed arrowheads), while multipolar spindles (open arrowheads) are observed in N=2/9 *gcl* mutants. Dashed lines mark the posterior cortex. Bars: (A) 2.5 μm; (B) 5 μm.

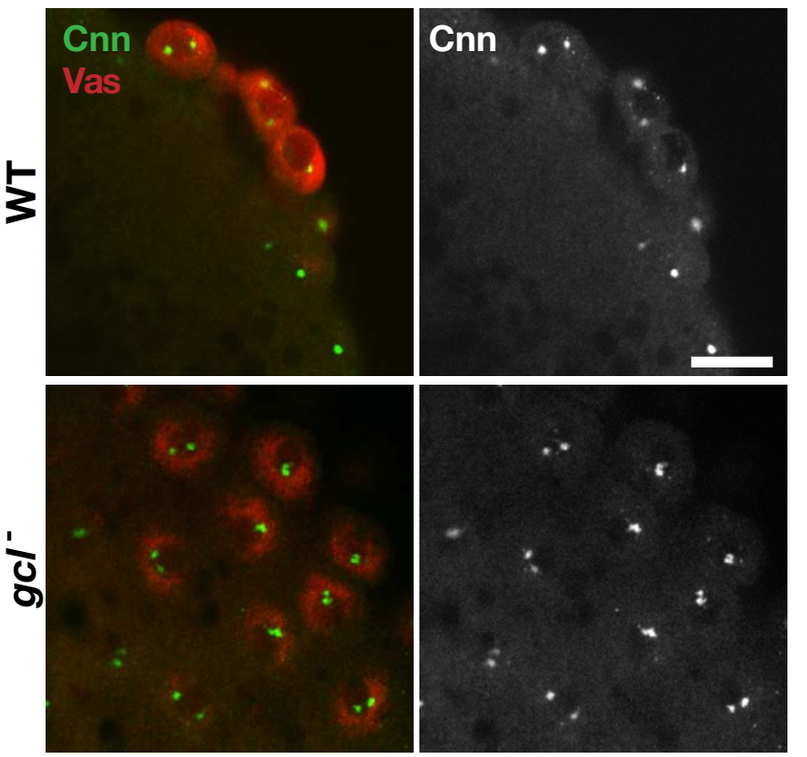

Figure S3. Gcl is required to partition germ plasm to the PGCs, Related to Figure 3.
Immunofluorescence shows Vas (red) and Cnn (green) in 1-2 hr embryos. Unevenly spaced centrosomes correlate with asymmetric accumulation of Vas at posterior nuclei in the *gcl* mutant. Bar: 20 µm.

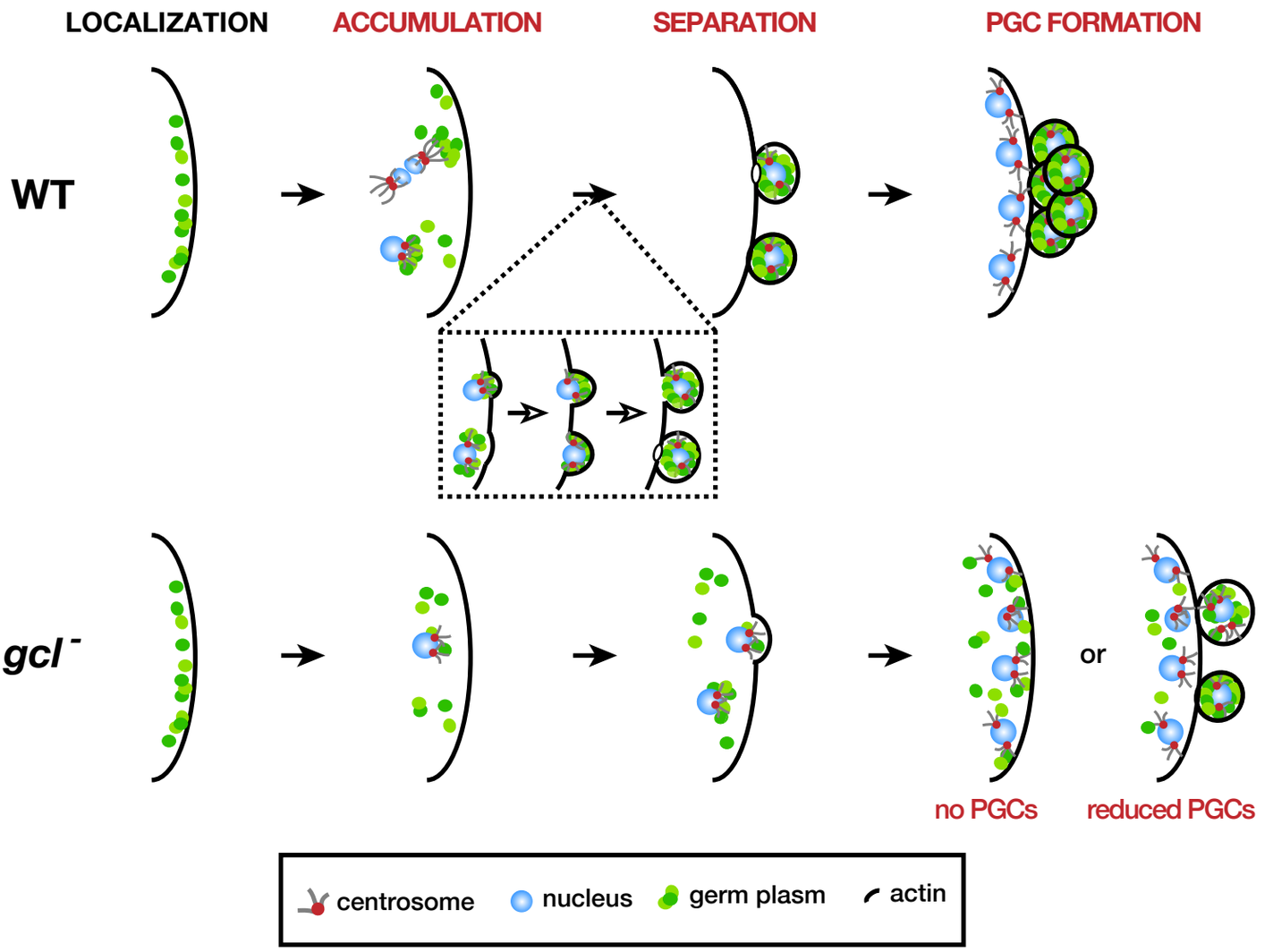

Figure S4. Model of Gcl-mediated regulation of centrosome separation during PB formation, Related to Figure 4.
Cartoon comparing the progression of PGC development in WT and *gcl* mutants. Normally, proximity of the centrosomes/astral MTs induces release of the localized germ plasm from the posterior cortex and permits its symmetric accumulation at PB nuclei. Inset region (dashed box) shows how centrosome separation drives PB growth and protrusion until the PB furrow forms and completes PGC cellularization. Steps where evidence supports a role for Gcl function are highlighted in red and include the symmetric accumulation of germ plasm around PB nuclei, uniform centrosome separation, and PB/PGC formation. We propose Gcl facilitates proper PGC development by promoting centrosome segregation.

Supplemental Videos

Video 1. Symmetric accumulation of *nosGFP during PB formation in a WT embryo (Related to Fig. 1B)**
High resolution confocal time-lapse movie of a 1-2 hr WT embryo expressing *nos**GFP. Posterior is down. Images were captured at 3 frames/s, and each second represents 10 s of elapsed time.

Video 2. Asymmetric accumulation of *nosGFP during PB formation in a *gcl* embryo (Related to Fig. 1B)**
High resolution confocal time-lapse movie of a 1-2 hr *gcl* embryo expressing *nos**GFP. Posterior is down. Images were captured at 3 frames/s, and each second represents 10s of elapsed time.

Video 3. Accumulation of *nosGFP around PB nuclei (Related to Figs. 1D and 1E)**
High resolution confocal time-lapse movie of 1-2 hr WT and *gcl* embryos expressing *nos**GFP. Posterior is to the right. Images were captured at 3 frames/s, and each second represents 10 s of elapsed time. N marks PB nuclei.

Supplemental Experimental Procedures

Imaging

Embryos were prepared for live imaging as described in (Lerit and Gavis, 2011). Briefly, dechorionated embryos were adhered to a sticky 22x30 mm #1.5 glass coverslip and covered with a thin layer of halocarbon oil. Imaging of *nos**GFP was captured at 300 ms intervals and performed on a Leica TCS SP5 confocal microscope with a 100x, 1.49 NA oil immersion objective. Live images of *nos**GFP were post-processed with a 3x3 median filter and normalized for photobleaching using LAS AF (Leica) and ImageJ (NIH) software. Imaging of Asl-YFP and αTub-GFP was collected at 0.5 µm intervals over a 15-20 µm volume at 60 s time intervals and performed on a Nikon Eclipse Ti microscope with 40x, 1.3 NA or 100x, 1.49 NA oil immersion objectives using a spinning disk confocal head (CSU-22, Yokagawa) and an EMCCD camera (Clara; Andor).

Immunofluorescence

Embryos were fixed in a 1:4 solution of 4% paraformaldehyde:heptane for 20 min and devitellinized in methanol. For visualization of MTs, embryos were fixed according to the method of Theurkauf (Theurkauf, 1994). Briefly, embryos were fixed in 1:1 37% paraformaldehyde:heptane for 3 min, rinsed in PBS, and manually devitellinized using 30G PrecisionGlide Needles (BD). For all experiments, embryos were blocked in BBT (PBS supplemented with 0.1% Tween-20 and 0.1% BSA), incubated overnight at 4 ^{o}C with primary antibody in BBT, further blocked in BBT supplemented with 2% normal goat serum, and incubated for 2 hours at room temperature with secondary antibody with DAPI. Embryos were mounted in Aqua-Poly/Mount (Polysciences, Inc.).

The following primary antibodies were used: rabbit anti-Vasa (1:10,000, gift from Ruth Lehmann), rabbit anti-Vasa (1:2,000, gift from Paul Lasko), rabbit anti-Cnn (1:3,000, gift from Tim Megraw), guinea pig anti-Asl (1:3000, gift from Greg Rogers), rabbit anti-Anillin (1:1000, gift from Christine Field), mouse anti-γ-Tubulin ascites GTU88 (1:50, Sigma-Aldrich), mouse anti-α-Tubulin DM1α (1:500, Sigma-Aldrich), rabbit anti-phospho-Histone H3 Ser10 (pH3; 1:1000, Millipore), mouse anti-phospho-Tyrosine 4G10 (1:1000, Millipore). Secondary antibodies: Alexa Fluor 488, 568, or 627 (1:500, Molecular Probes), and DAPI (4',6-diamidino-2-phenylindole, dihydrochloride; 10 ng/mL, ThermoFisher).

Supplemental References

THEURKAUF, W. E. 1994. Immunofluorescence analysis of the cytoskeleton during oogenesis and early embryogenesis. *Methods Cell Biol.,* 44**,** 489-505.