Sperm Head-Tail Linkage Requires Restriction of Pericentriolar Material to the Proximal Centriole End

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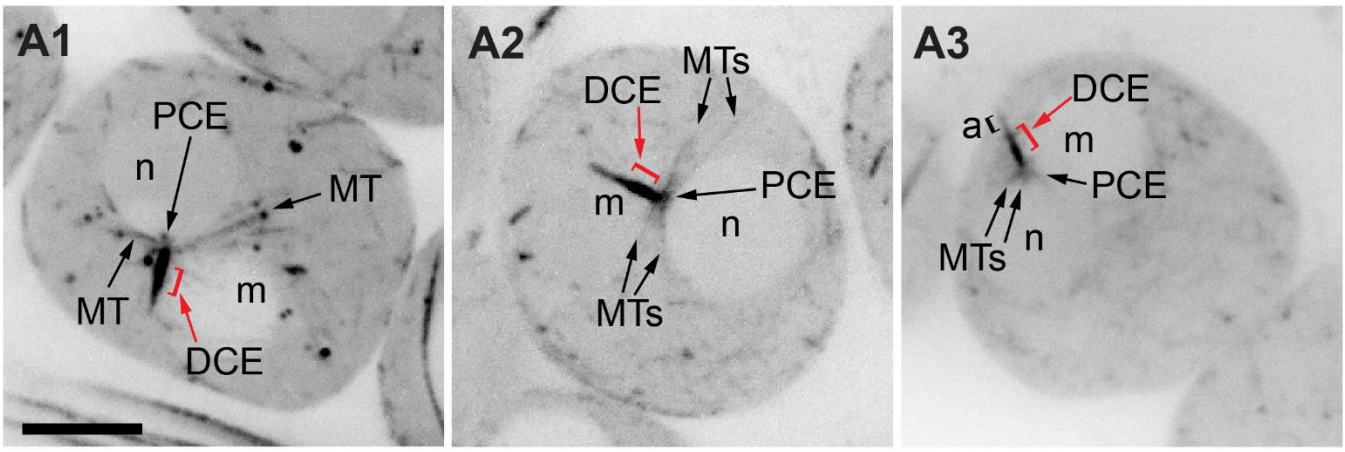
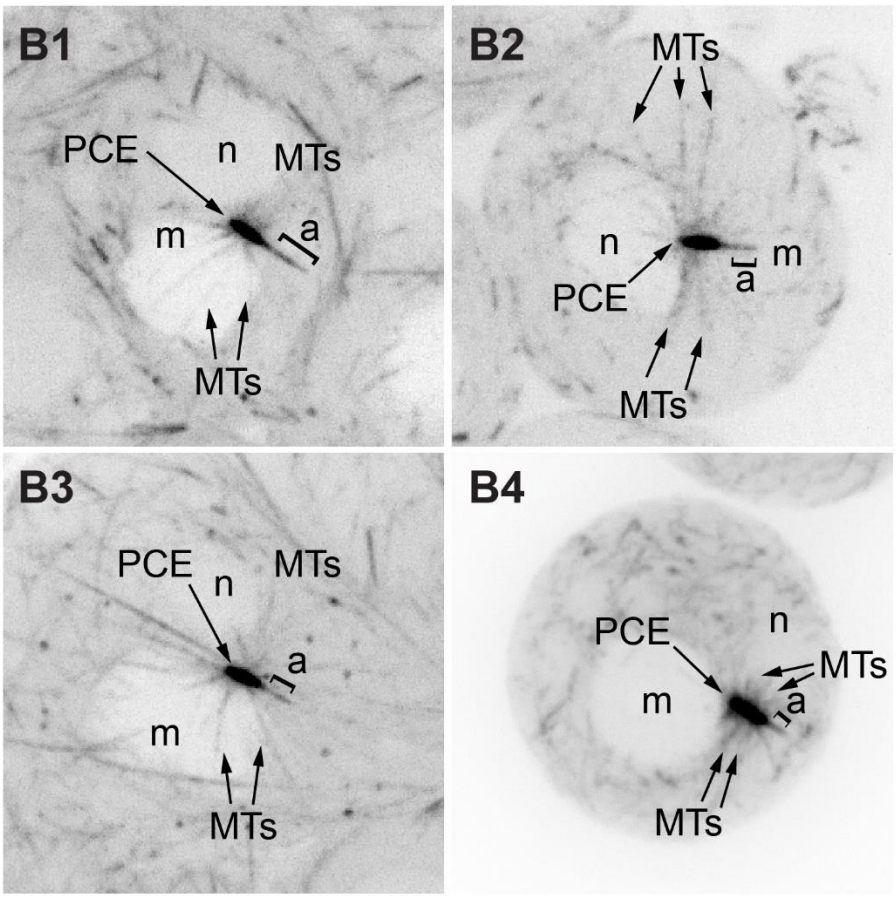
Supplemental information

ubi-Tubulin::GFP



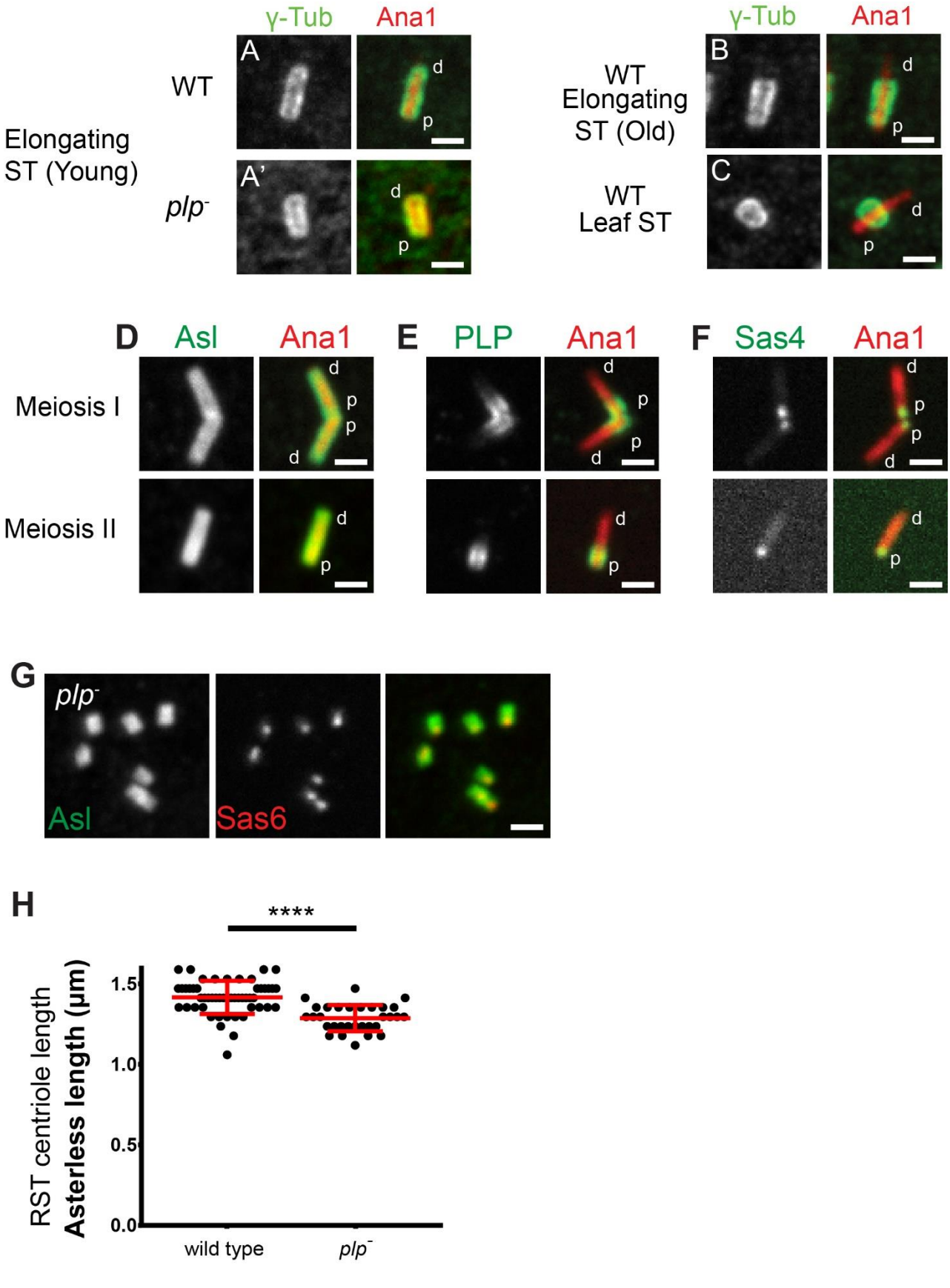
ubi-PLP::GFP / ubi-tubulin::GFP



Galletta et al., Figure S1

Figure S1. Additional examples of microtubules in RSTs (related to Figures 1,5)

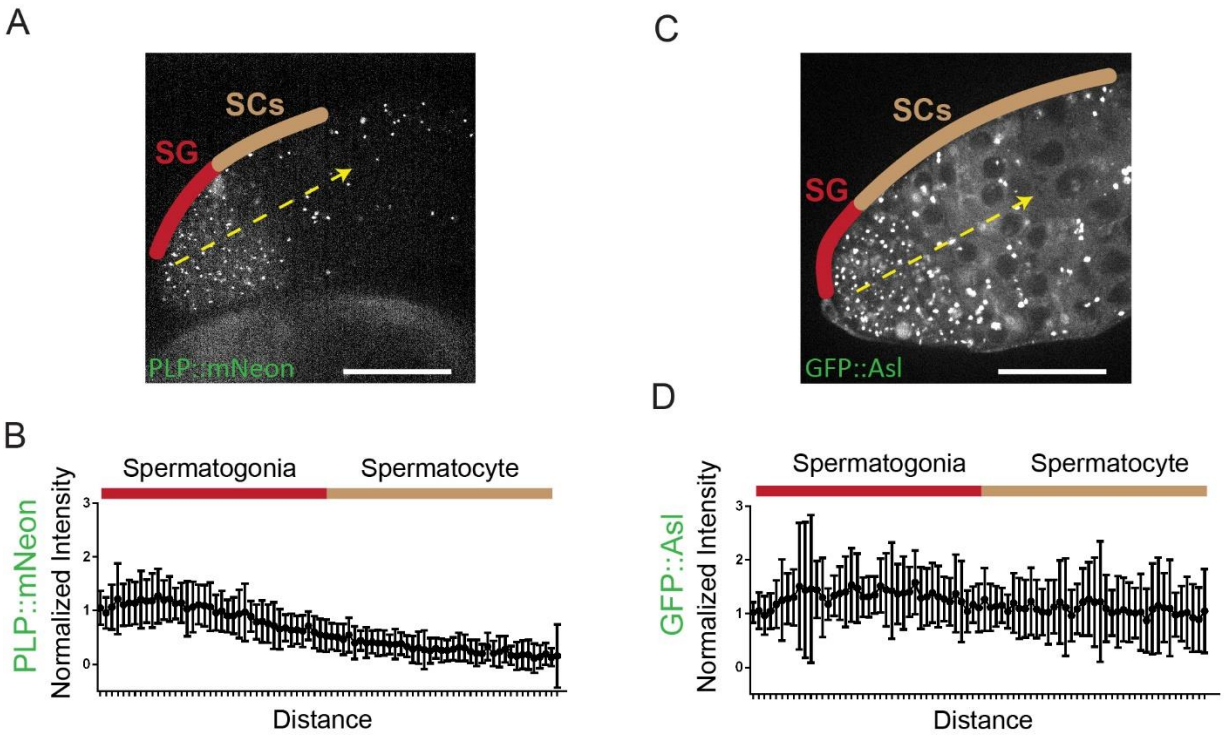
Additional examples supporting Figures 1E, 5N and 5O. Max intensity projection of confocal slices through live RSTs expressing GFP::tubulin. Labels - n = nucleus, m = mitochondrial derivative, a = axoneme, PCE = proximal centriole end, DCE = distal centriole end (red bracket), MTs = microtubules. **A)** wildtype - MTs emanate from the PCE, which is against the nucleus. **B)** ubi-PLP::GFP – MTs emanate from along the length of the centriole.



Galletta et al., Figure S2

Figure S2. γ-tub in older spermatids, bridge protein position on meiotic centrioles and additional phenotypes in *plp* mutant testes (related to Figure 2)

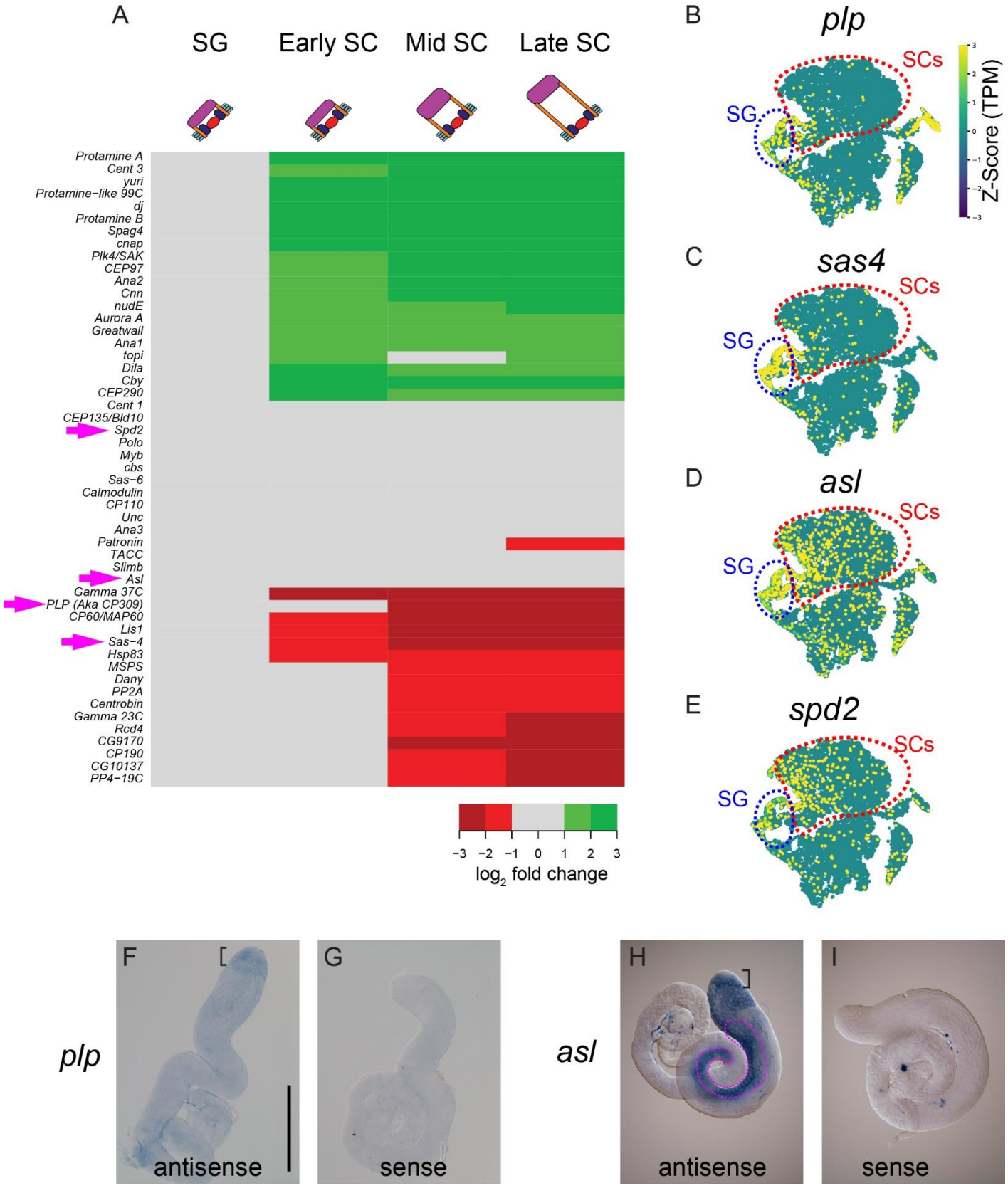
A) Localization of γ-tub (green) along centrioles (Ana1, red) in young elongating STs in wildtype (**A**) and *plp* mutants (**A'**). Unlike in RSTs, γ-tub is localized along the length of the centriole in these older STs in wildtype (52/52) and in *plp* mutants (23/23). **B-C)** Localization of γ-tub (green) along centrioles (Ana1, red) in old elongating STs (**B**) and leaf stage STs (**C**). γ-tub is reorganized around the centriole at these stages. **D-F)** The position of the bridge proteins (green) along centrioles (Ana1, red) in wildtype SCs during meiosis. Asl (**D**) is localized along the whole centriole length, while PLP (**E**) and Sas4 (**F**) are localized proximally. **G)** Examples of disorganized centrioles (Asl, green) in *plp* mutant young SCs. Every centriole contains a tagRFP::Sas6 (Red) focus. The presence of Sas6 on all centrioles (N = 714) suggests these centrioles all originated via canonical centriole duplication mechanisms and none are the result of centriole fragmentation. It therefore remains an open question as to why an unusual clustered arrangement of centrioles is present in *plp*- SCs. **H)** Length of the centriole as determined from Asl immunofluorescence. *plp* mutant RSTs (N = 31) have slightly shorter (1.3 ± 0.1 µm) centrioles than wildtype (1.4 ± 0.1 µm; N = 52). No evidence of major fragmentation. **** = $p \leq 0.0001$.



Galletta et al., Figure S3

Figure S3. Cytoplasmic PLP levels drop along the length of the testis (related to Figure 4)

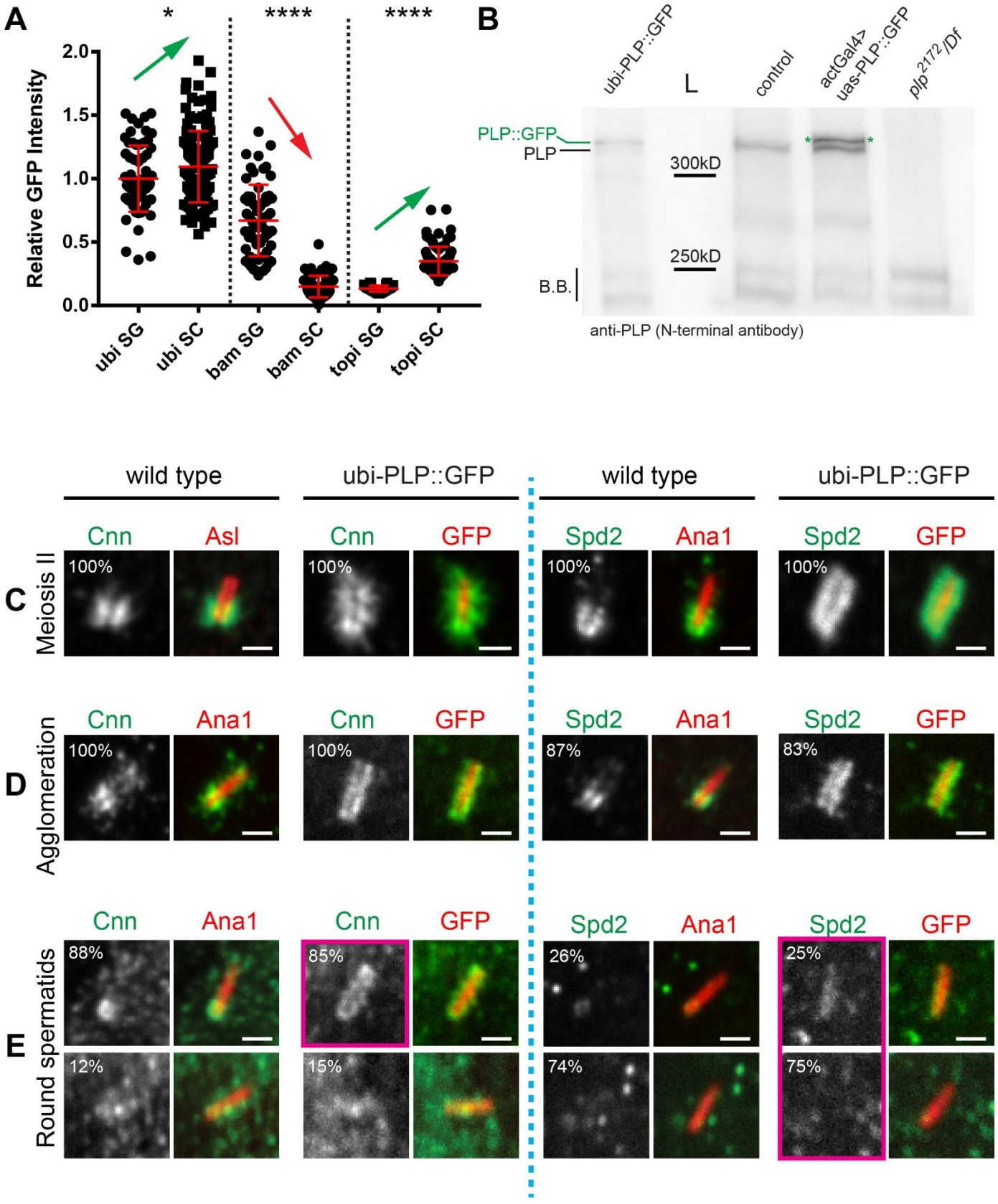
Confocal images of live testes from flies with PLP tagged with mNeon (PLP::mNeon) at the endogenous locus (**A**) or with a transgene containing the genomic region surrounding Asl with the sequence of GFP inserted at the start codon of Asl (GFP::Asl; **C**). The approximate positions of SG and SCs are indicated. Note the higher intensity of cytoplasmic PLP signal near the tip of the testis where the SG reside. In contrast, cytoplasmic Asl signal does not decrease. The arrow is an example of the direction of the line scans taken to generate the intensity vs. position profiles in **B** and **D**. The PLP::mNeon and GFP::Asl signals have been heavily contrast adjusted to allow the cytoplasmic staining to be visualized for presentation purposes only. Scale bar = 50 µm **B-D)** Average of line scans along live testes from flies expressing PLP::mNeon (N = 20) from the endogenous locus **(B)** or GFP::Asl (N=15) under the control of its endogenous promoter **(D)**. Value at each point is the mean ± SD.



Galletta et al., Figure S4

Figure S4. **Additional information about mRNA levels in testes (related to Figure 4)**

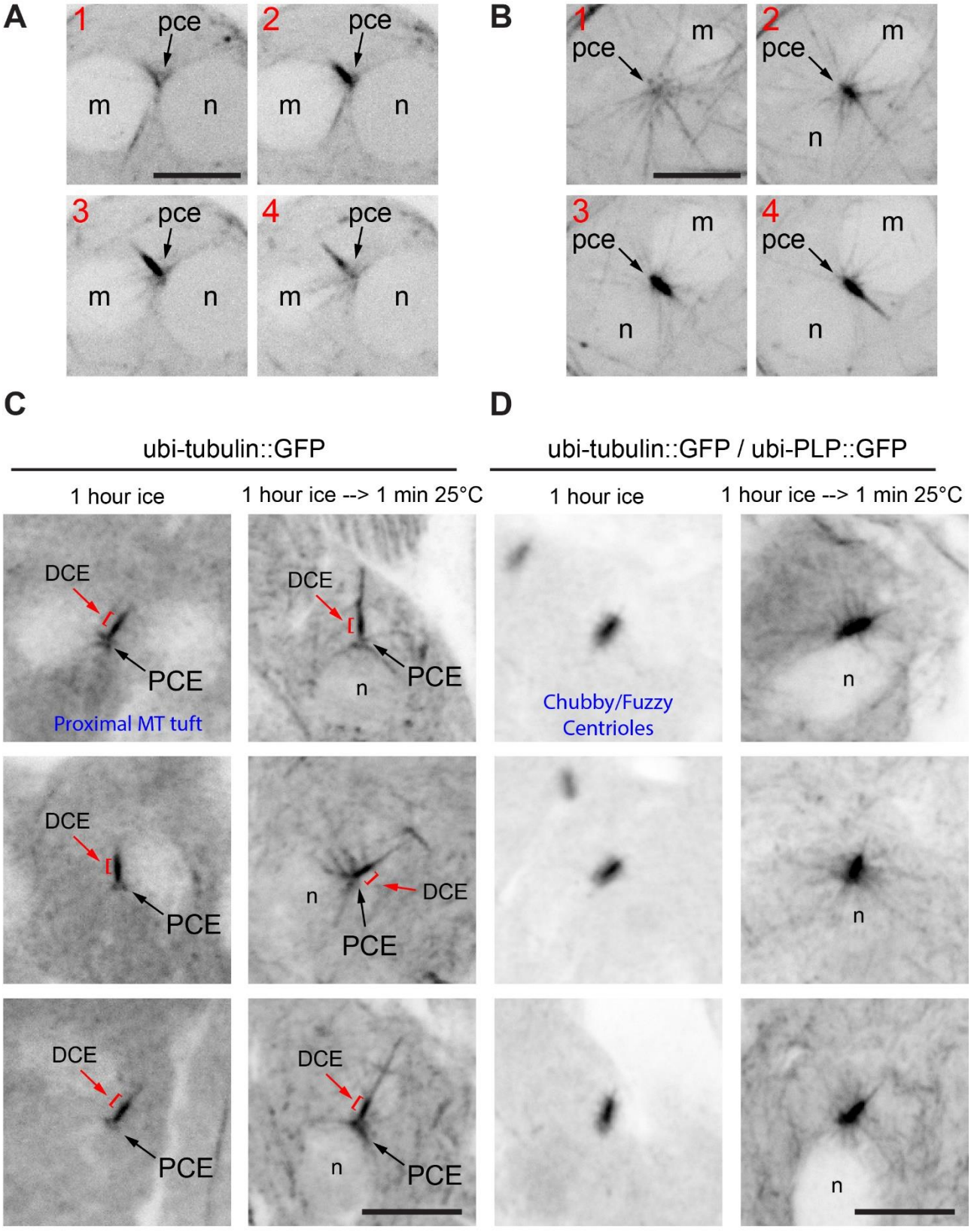
A) Heat map of the changes in selected centriole, cilia and spermiogenesis genes throughout germline development found using scRNAseq. Each row represents a single gene. Each column represents a single developmental stage. The RPKM for each gene at each stage was determined from all the cells assigned to a cluster. The log2 fold change, relative to SG was calculated for each stage. Genes with less than a two-fold change up or down at a given stage are presented in grey. Genes more than two-fold increased at a given stage are in shades of green and genes more than two-fold decreased are in shades of red. Bridge proteins are indicated with arrows. **B-E)** tSNE projections of the cells from single cell RNAseq, as in Figure 4A. Each spot represents a single cell and is color coded to show the Z-score (TPM, legend in B) for the indicated gene relative to that transcript's representation across all cells. Yellow spots indicate cells with an enrichment of the indicated transcript. **F-I)** Whole mount *in situs* of adult testes using the indicated probes. **F)** *plp* antisense – *plp* transcript is enriched at the tip of the testis where the youngest germline cells are located (bracket). **G)** *plp* sense – background control. **H)** *asl* antisense – *asl* transcript is strongly enriched throughout all spermatogenesis stages, young (bracket) and old (pink outline). The area lacking signal is where packed sperm tails reside. **I)** *asl* sense – background control. Bar F-I = 250 µm



Galletta et al., Figure S5

Figure S5. PLP::GFP levels at centriole and PCM position during "Nuclear Search" and "Nuclear Attachment" (related to Figures 4,5)

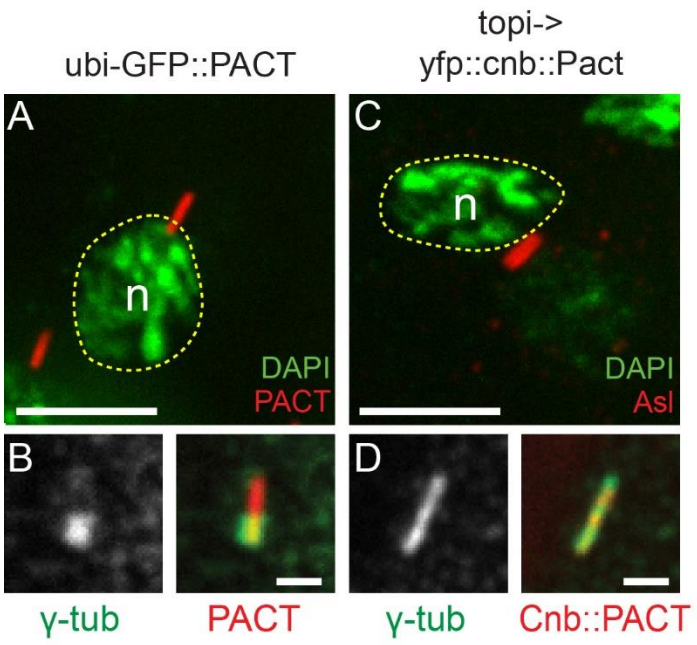
A) Measurements of cytoplasmic fluorescence in live SG and SC of the indicated genotypes. All data is normalized to mean cytoplasmic florescence measured in late SG of testes expressing PLP::GFP under control of the ubiquitin (ubi) promoter for a given imaging session. Arrows indicate the change in levels from SG to SC. ubi SG (N = 68), ubi SC (N = 146), bam SG (N = 62), bam SC (N = 64), topi SG (N = 39), topi SC (N =82), * - p = 0.021, **** - p ≤ 0.0001. **B)** Western blot (anti-PLP, n-terminal antibody) of brain extracts showing that UAS-PLP::GFP expresses a protein of the appropriate length (asterisks). Ubi-PLP:GFP was used a known control to show the GFP shift (Galletta, 2014). plp- brains show no PLP protein. Background bands (B.B.) indicate nearly identical protein loading. **C-E)** Position of Cnn (green, left) and Spd2 (green, right) in wildtype and ubi-PLP::GFP cells in Meiosis II (**C**), Agglomeration STs (**D**) and RSTs (**E**). The percentage of centrioles with a similar distribution of the given PCM protein are indicated and were determined as follows: Cnn, wildtype - Meiosis II – 3/3 proximal or proximally enriched (previously observed(Fu and Glover, 2012; Galletta et al., 2016b), Agglomeration – 19/19 proximal or proximally enriched, RSTs (53/60) Proximal or proximally enriched; Cnn, ubi-PLP::GFP - Meiosis II (17/17) along centriole length, Agglomeration (4/4) along length, RSTs (40/47) along length; Spd2, wildtype - Meiosis II (11/11) proximal or proximally enriched (previously observed; (Galletta et al., 2016b; Giansanti et al., 2008), Agglomeration (27/31) proximal or proximally enriched, RSTs (11/43) proximal or proximally enriched, (32/43) little or no Spd2; Spd2, ubi-PLP::GFP - Meiosis II (22/22) along length, Agglomeration (7/12) along whole length, RSTs (16/63) had patchy or faint Spd2 on centriole, (47/63) little or no Spd2. Bar = 1 µm

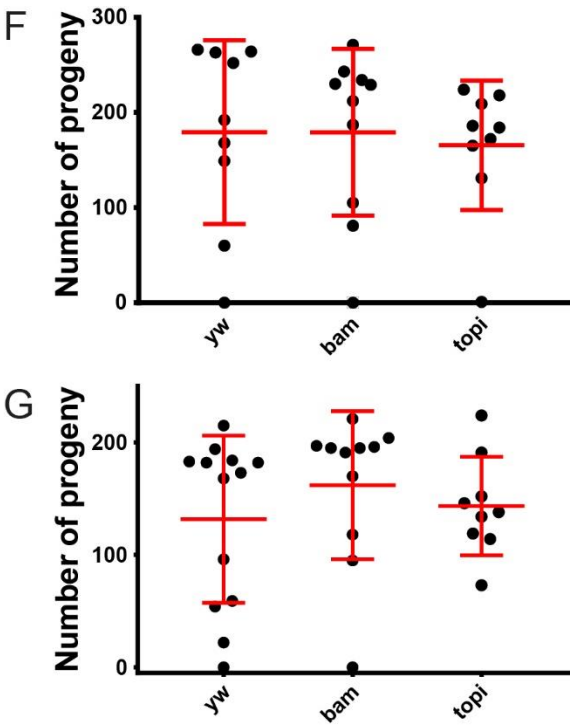


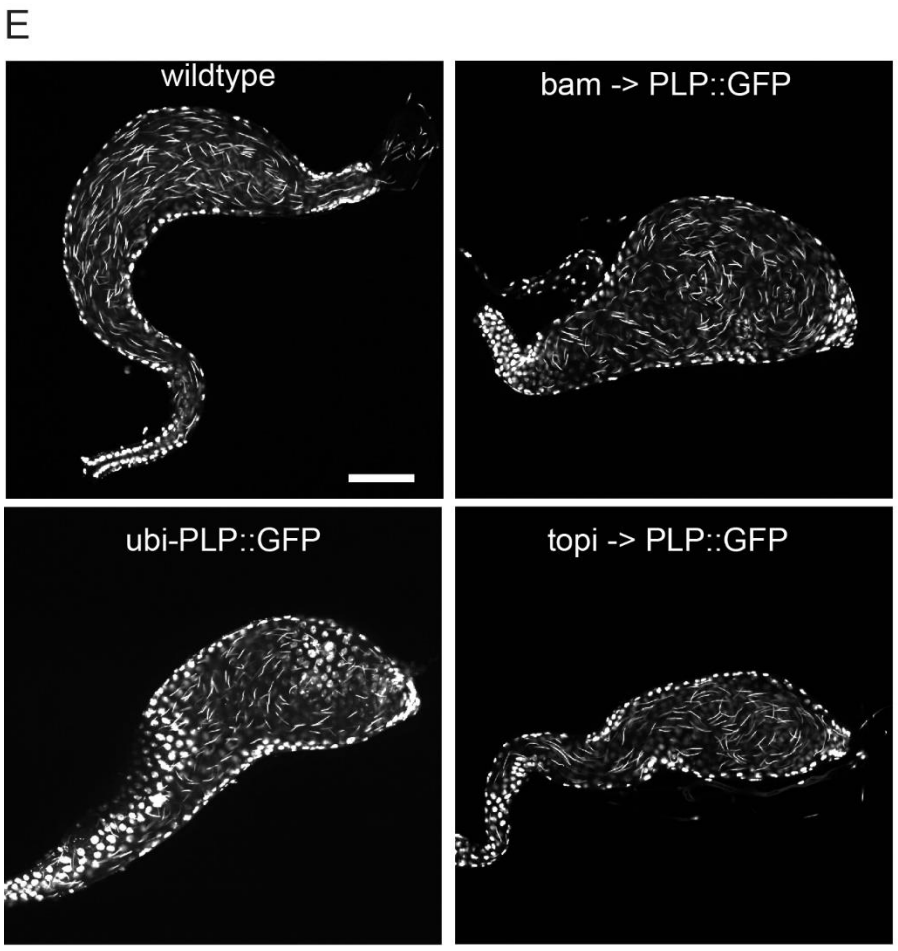
Galletta et al., Figure S6

Figure S6. Additional support for the location of MTs along centrioles (related to Figure 5)

A, B) Serial confocal slices through centrioles in RSTs expressing tubulin:GFP from wildtype (**A**) and ubi-PLP::GFP (**B**) testes. Slices are numbered to indicate order and are separated by 500 µm in Z. Microtubules are predominantly coming from the proximal end of the centriole in wildtype and along the length of the centriole in ubi-PLP::GFP. **C, D)** Microtubule (tubulin::GFP) regrowth assays in RSTs from wildtype or ubi-PLP::GFP expressing testes. Left – treated for 1 hour on ice. Right – treated for 1 hour on ice followed by 1 minute regrowth at 25°C. In wildtype after ice treatment (**C**, left) the centriole has a tuft of microtubules at the proximal end (24/43) or no obvious microtubules emanating from it (19/43). Upon regrowth in wildtype (**C**, right) we predominantly observed microtubule signal at the proximal end either as MTs emanating from the proximal end (26/36) or a tuft a microtubules at the proximal end (8/36). In ubi-PLP::GFP testes after ice treatment (**D**, left) 9/18 centrioles had chubby or fuzzy tubulin around centrioles, 6/18 had no tubulin, 3/18 had a tuft at the proximal centriole end. After regrowth in ubi-PLP::GFP testes (**D**, right), 20/29 centrioles had MTs emanating from the whole length, 7/29 were chubby or fuzzy, 1/29 had MTs from only one end, and 1/29 had no obvious MTs. PCE = proximal centriole end, DCE = distal centriole end, m = mitochondrial derivative, n = nucleus Bars = 5 µm.







Galletta et al., Figure S7

Figure S7. Controls for PLP mispositioning and fertility assays (related to Figure 5)

A-D) Controls for the effect of PLP mispositioning on nuclear search and attachment. Position of centriole (red) relative to the nucleus (DAPI, green) and the position of γ-tub along the centriole in the following genotypes: **A, B)** ubi-GFP::PACT. Centriole marked with GFP::PACT. **C, D)** topi-GAL4 driving UAS-YFP::Cnb::PACT. Centriole marked with Cnb::PACT. The expression of GFP::PACT along the centriole does not affect the position of PCM nor the docking to the nucleus. The expression of Cnb::PACT mispositions PCM along the centriole length and interferes with docking. Quantification of these phenotypes in Figure 5M. **E**) Seminal vesicles from 6 – 24 hour old naïve males stained with DAPI. Sperm nuclei are needle shaped structures. Sperm were found in all seminal vesicles. wt = 9/9, bam = 5/5, ubi = 5/5, topi 8/8. **F, G)** Two independent fertility assays on wildtype (*yw*), bam->PLP::GFP (bam) or topi->PLP::GFP (topi) males. **E**) 16 -24 hour old naïve males were crossed with 2 virgin *yw* females for 24 hours. The male was removed after this period and the females were allowed to lay in the vial for 1 week. All adult progeny were counted. Bars are mean ± standard deviation. Differences were not statistically significant. yw – N = 9, bam – N = 10, topi – N = 9. **F**) 16 -24 hour old naïve males were crossed with 1 virgin *yw* female for 48 hours. The male was removed after this period. The female was moved to a second vial and allowed to lay for 48 hours. The female was then moved to a third vial and allowed to lay for 48 hours. The females was then sacrificed. All adult progeny from all vials were counted. Bars are mean ± standard deviation. Differences were not statistically significant. yw – N = 13, bam – N = 11, topi – N = 9. Bars A,C = 5 µm, B, D = 1 µm, E = 50 µm