Supplemental Figures

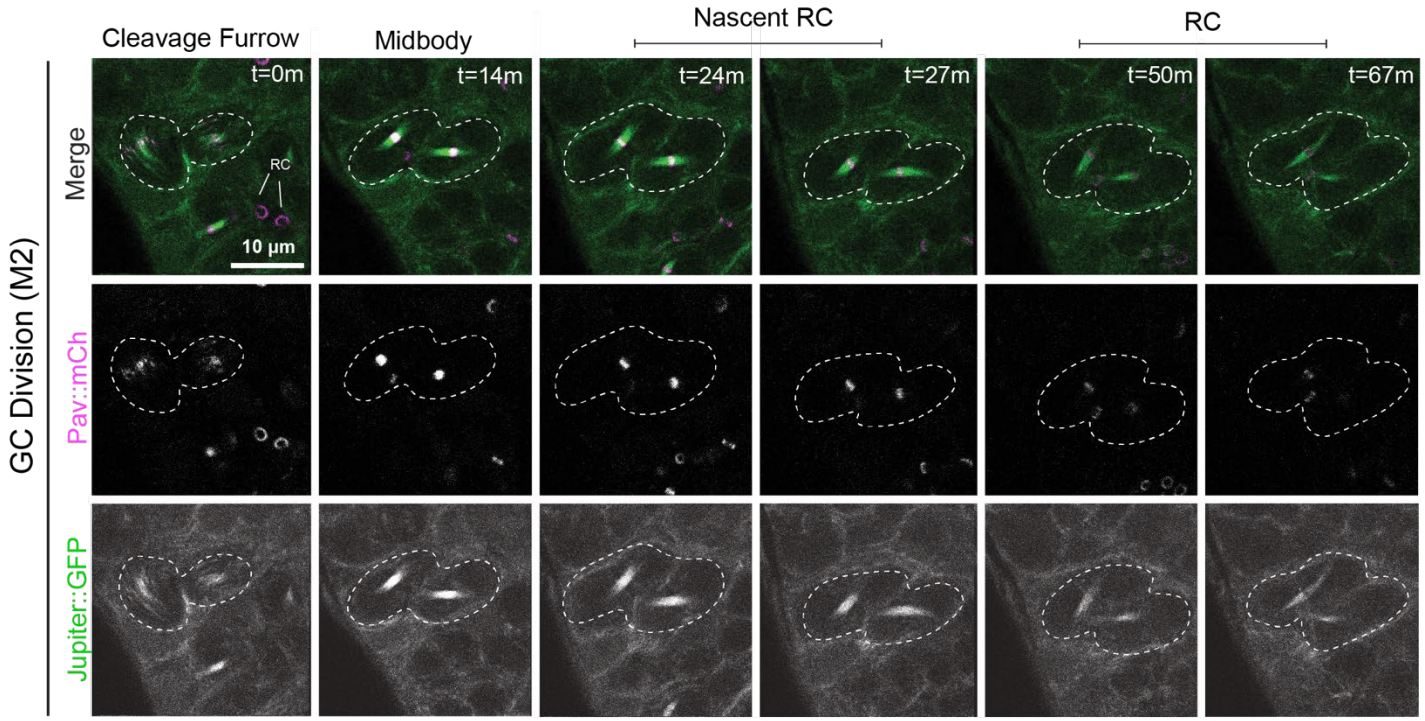


Figure S1. Stable intercellular bridge microtubules are maintained during ring canal formation. Related to Figure 1.

Pav::mCherry was imaged with Jupiter::GFP to visualize microtubules. M, minutes. An actively dividing two-cell cyst (outlined) with single, mature ring canal (arrowhead in t=14m frame) will generate two new ring canals (labeled “RC” at t=50m) that maintain Jupiter::GFP-labeled microtubules in the lumen. In contrast, two mature ring canals (arrowheads in t=0m frame) do not contain luminal Jupiter::GFP signal revealing the microtubules, while present during the midbody-to-ring canal transition, are eventually removed in the mature ring canal.

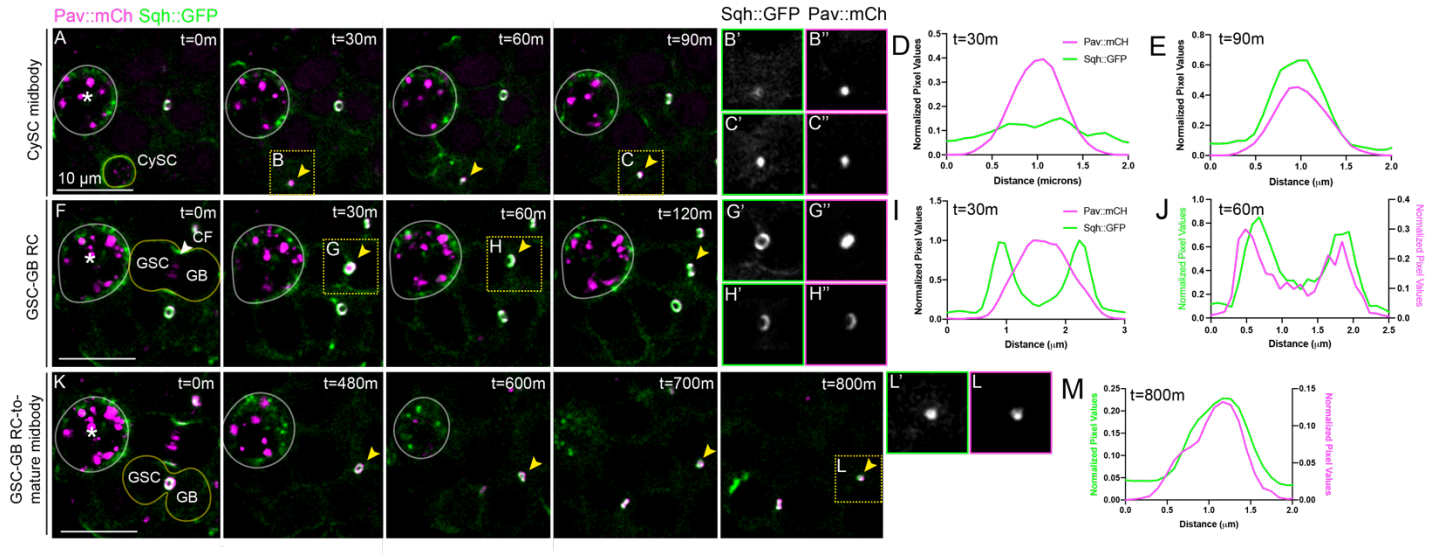


Figure S2. Germ cell midbody reorganization is a feature of germline stem cell division. Related to Figure 1.

Live imaging of Pav::mCherry and Sqh::GFP. (A) Cyst stem cell (CySC) division. Asterisk marks the stem cell niche. (B) Midbody that forms during CySC division. (B'-B'') Enlarged image of (B) revealing the formation of a midbody ring (Sqh::GFP, green) and midbody focus (Pav::mCherry, magenta) and accompanying quantification in (D). (C"-C") Enlarged image of (C) revealing the mature midbody of the CySC division where the midbody ring and midbody core distinctions are no longer present. (E) Quantification of Pav::mCherry and Sqh::GFP from the mature midbody of the CySC in (C). (F) Dividing germline stem cell (GSC). CF, cleavage furrow. (G-G") Pav::mCherry midbody focus and accompanying Sqh::GFP-labeled midbody ring. (H-H") Transient ring canal during the GSC-GB division. (I) Quantification of Pav::mCherry and Sqh::GFP from the GSC midbody canal shown in (G). (J) Quantification of Pav::mCherry and Sqh::GFP in the transient ring canal shown in (H). (K) The transient ring canal that connects the germline stem cell and gonialblast undergoes apparent collapse to form a mature midbody in preparation for abscission. (L'-L") Enlarged image of (L) showing the mature midbody focus. (M) Quantification of Pav::mCherry and Sqh::GFP in the mature midbody of the GSC-GB division. All quantifications are normalized to the pixel intensities of Pav::mCherry and Sqh::GFP in the midbody/midbody ring during formation of the transient ring canal in the GSC-GB division (G).

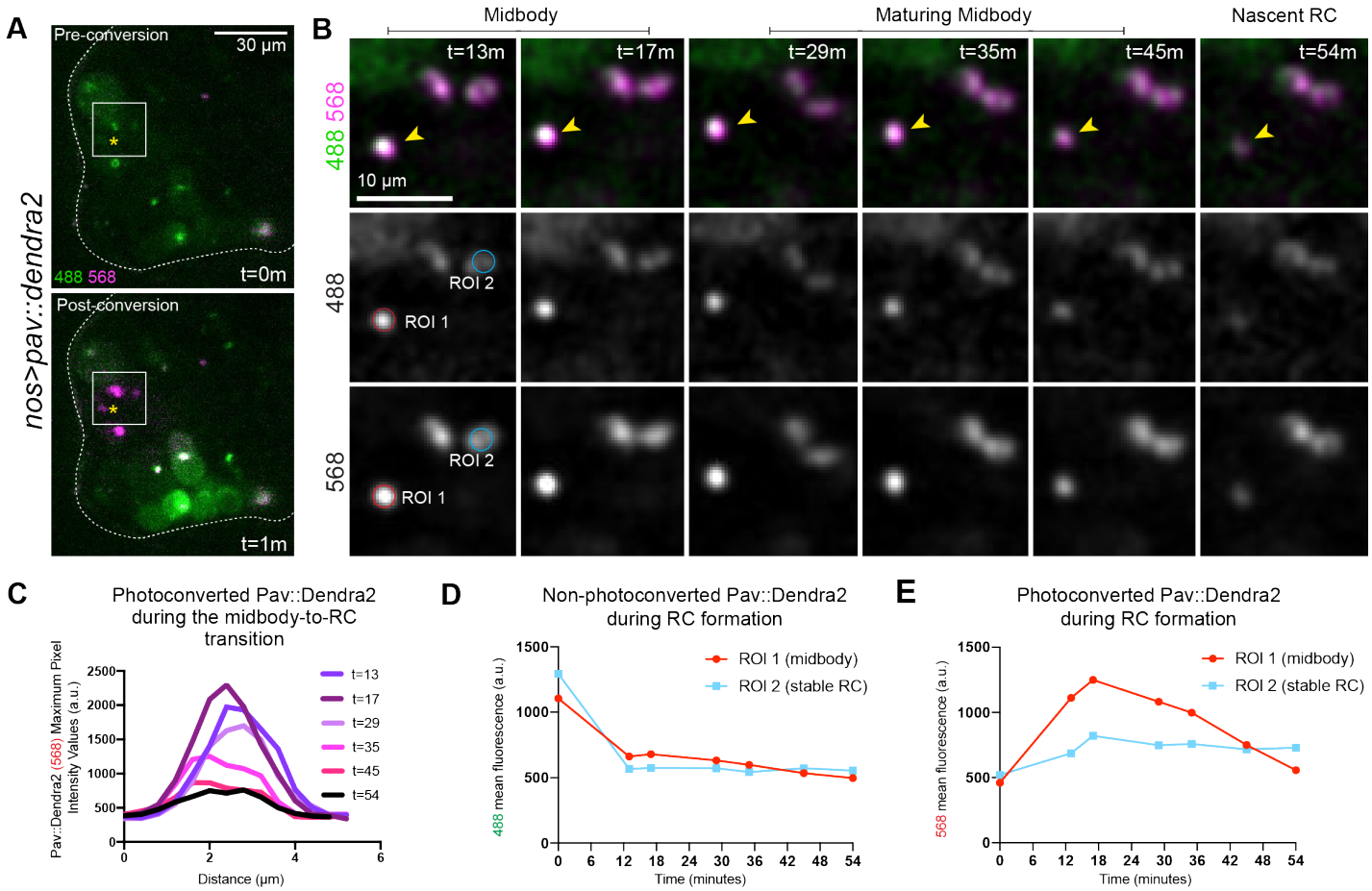


Figure S3. Imaging of Pav::Dendra2 reveals that ring canals are derived from germ cell midbodies. Related to Figure 2.

(A) *nos>pav::dendra2* testes before photoconversion (top, green) and after photoconversion (bottom, magenta). The photoconverted region of interest is outlined in the white box; the yellow asterisk marks the cleavage furrow of a dividing spermatogonial cell. Photoconversion occurred during cleavage furrow formation, prior to accumulation of Pav::Dendra2 in the germ cell midbody. (B) Stills from a time-lapse movie of the testis in (A) following photoconversion. (C) Fluorescence intensity across the center of Pav::Dendra2 in the midbody and ring canal of the representative sample in (A-B) over the course of time-lapse imaging. Photoconverted Pav::Dendra2 undergoes the same changes in fluorescence intensity as endogenous Pav and a ring canal localization of Pav::Dendra2 is marked at t=54 minutes. (D) Mean fluorescence intensities of a representative non-photoconverted midbody (ROI 1) and ring canal (ROI 2) imaged with 488 nm wavelength demonstrates that photoconversion of GFP-to-RFP is concomitant with a decrease in GFP signal. Following conversion, the fluorescence of GFP remains mostly stable demonstrating that no new Pav protein is added to the midbody or ring canal over the course of imaging. (E) Mean fluorescence intensities of the same representative midbody (ROI 1) and ring canal (ROI 2) reveal that the levels of photoconverted Pav::Dendra2 increase upon photoconversion and formation of the midbody and then decrease during ring canal formation whereas the levels of photoconverted Pav::Dendra2 in the ring canal are unchanged over the course of imaging.

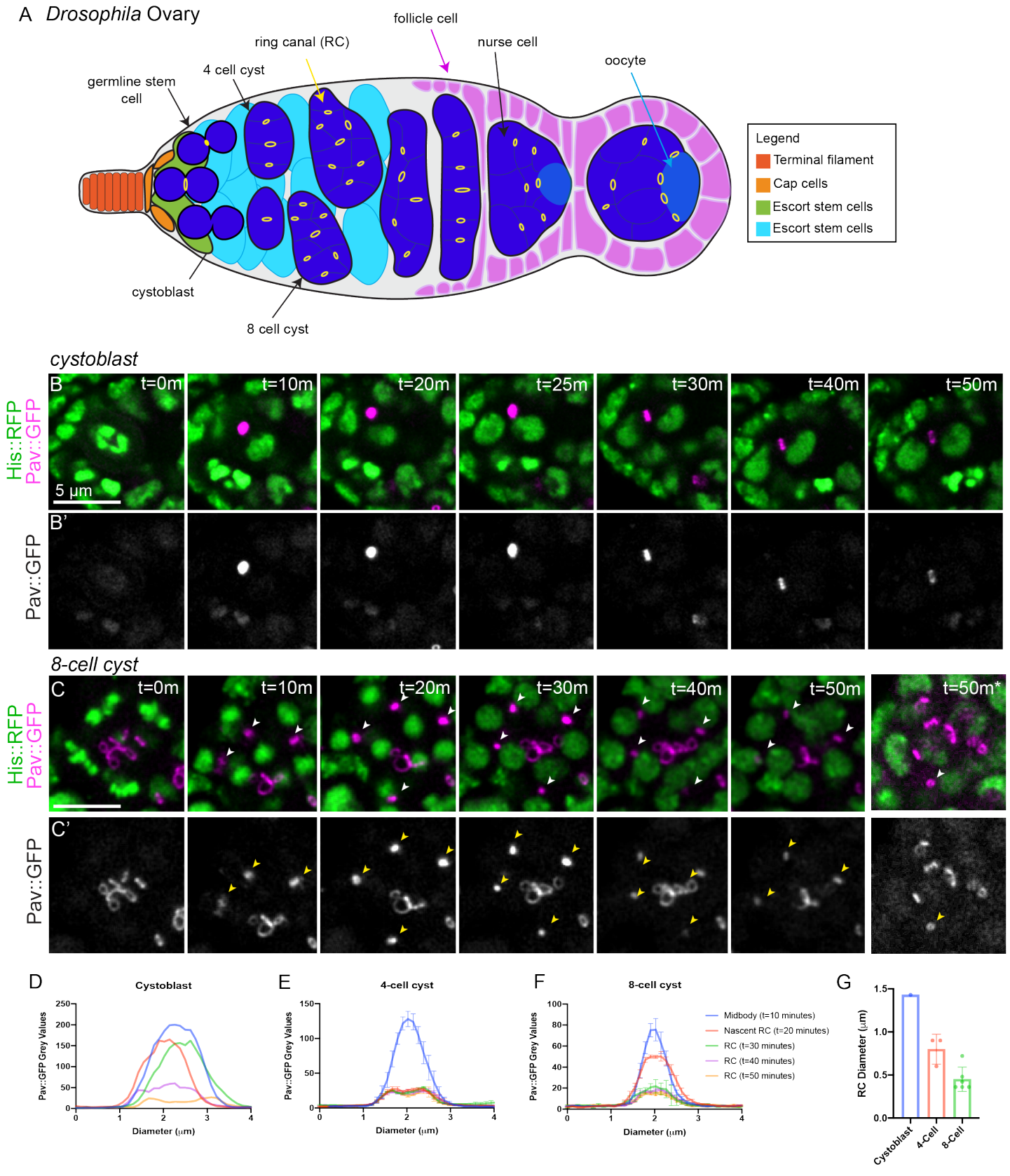


Figure S4. Ring canal formation in the *Drosophila* ovary occurs via a midbody-like intermediate. Related to Figure 3.

(A) Schematic of the *Drosophila* germarium, where four incomplete mitotic cell divisions result in a 16-cell cyst connected by 15 ring canals. (B) Live imaging of a cystoblast division. M, minutes. (C) A dividing 8-cell cyst, four

dividing cells are in the plane of focus as marked by four metaphase plates (t=0m). The midbodies that result are marked with arrowheads and tracked over the course of the movie; at t=40m, one midbody has started to move out of the plane of focus, and by t=50m signal from three ring canals are in visible. t=50m* is a different Z plane to better visualize the ring canal that has formed in the new 16-cell cyst. (D-F) Line scans of Pav::GFP pixel intensity in the cystoblast (D), dividing 4-cell cyst from Figure 3 (E), and dividing 8-cell cyst (F). For all quantifications, line scans were taken from images that were imaged and brightness and contrast scaled identically to allow comparison of pixel intensity values. (G) Ring canal diameters, average diameter ± SEM is plotted.

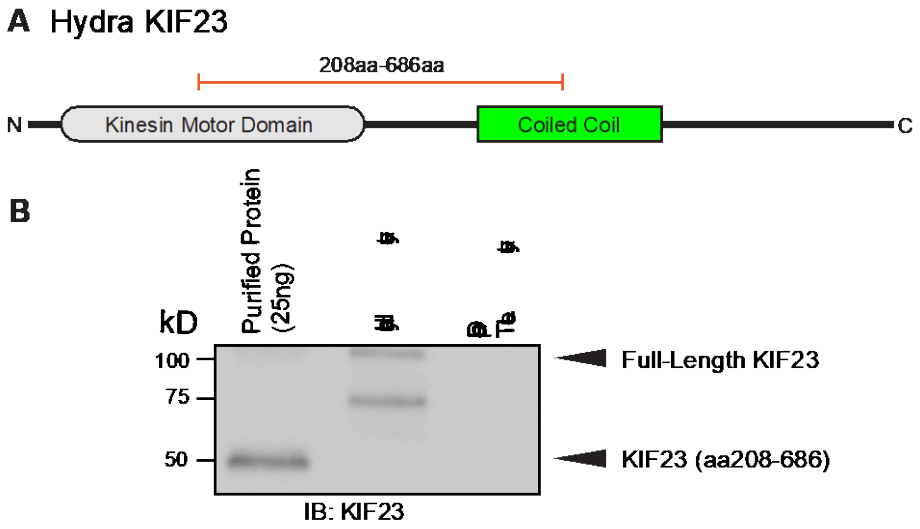


Figure S5. Validation of *Hydra* KIF23 polyclonal antibody. Related to Figure 3.

(A) Schematic of *Hydra* KIF23 protein (105 kD, predicted). Amino acids 208-686 (orange bar) were used as immunogen to generate a polyclonal antibody. This strategy was similar to the strategy used to make an antibody to *Drosophila* Pav (Adams et al., 1998). (B) Western blot of the purified fragment (208-686aa), *Hydra* whole cell lysate, and *Drosophila* testis whole cell lysate probed with *Hydra* anti-KIF23 antibody (n=2 biological replicates).

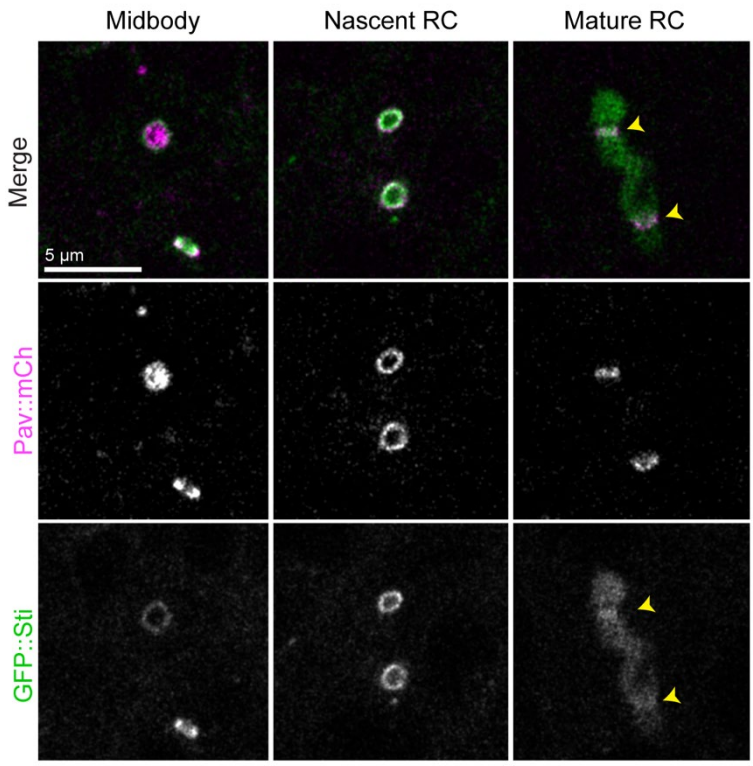
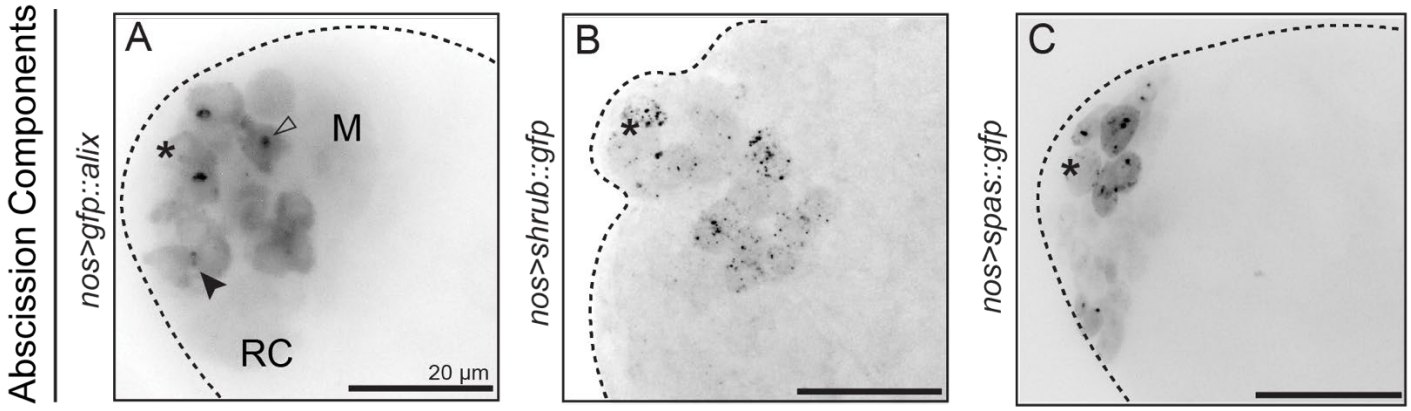


Figure S6. Transgenic GFP::Sticky localization. Related to Figure 5.

GFP::Sticky localizes to the midbody ring and nascent ring canal in spermatogonia. In cysts with mature ring canals, like those in primary spermatocytes (depicted), GFP::Sticky localizes to the fusome and very weakly to the mature ring canal.



Extended Data Figure 7. Examination of abscission components. Related to Figure 7.

Images of fixed tissue expressing transgenic GFP::Alix (n=29) (A), Shrub::GFP (n=53) (B), or Spastin::GFP (n=38) (C). Only GFP::Alix localizes to the germ cell midbody (M) and nascent ring canal (RC). Asterisk, stem cell niche.