Zhou et al., http://www.jcb.org/cgi/content/full/jcb.200902101/DC1

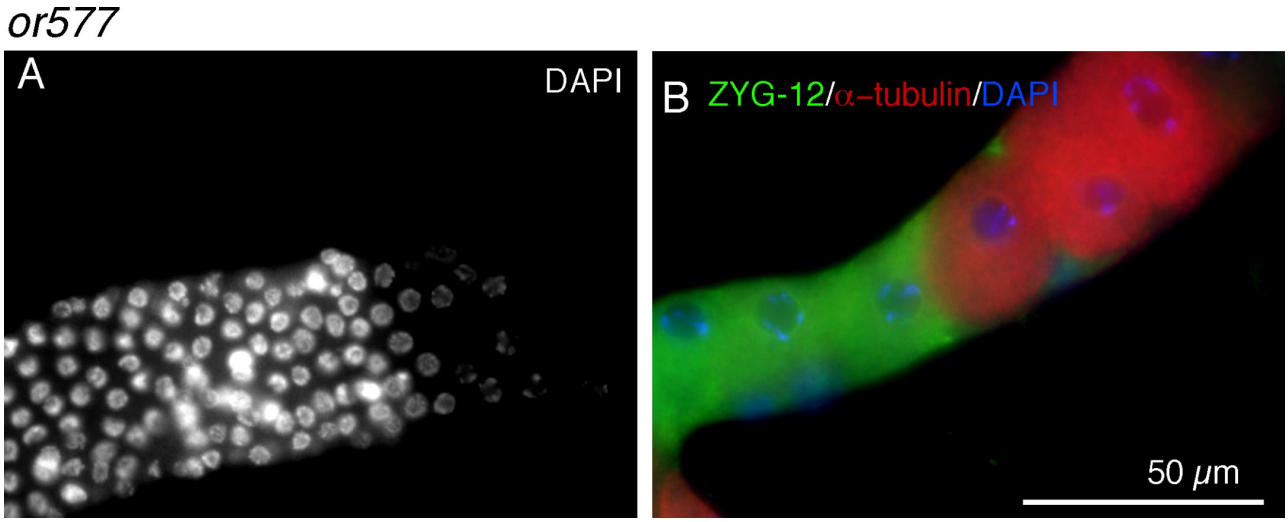

Figure S1. ***zyg-12(or577)* has normal germline nuclear positioning when raised at the nonpermissive temperature from the L1 stage.** (A) The nuclei (DAPI) are regularly arrayed in the distal gonad of an adult *zyg-12(or577)* animal. (B) The proximal gonad of *zyg-12(or577)* animals have evenly spaced oocytes with one nucleus each.

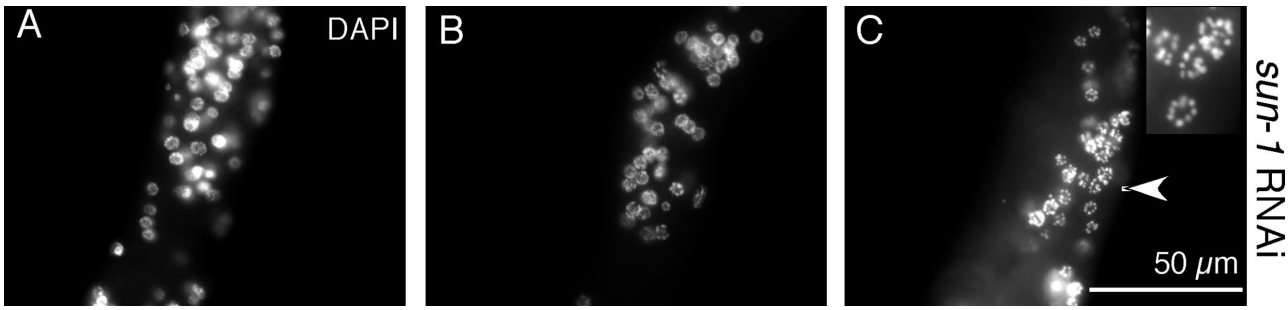

Figure S2. ***sun-1* RNAi disrupts gonad nuclear arrangement.** (A) DAPI-stained distal gonads show disorganized nuclei. (B and C) Proximal nuclei are disorganized and abnormally small, likely reflecting a defect in meiotic progression. (inset) A higher magnification view of the region near the arrowhead, showing nuclei with additional DNA fragments during diakinesis.

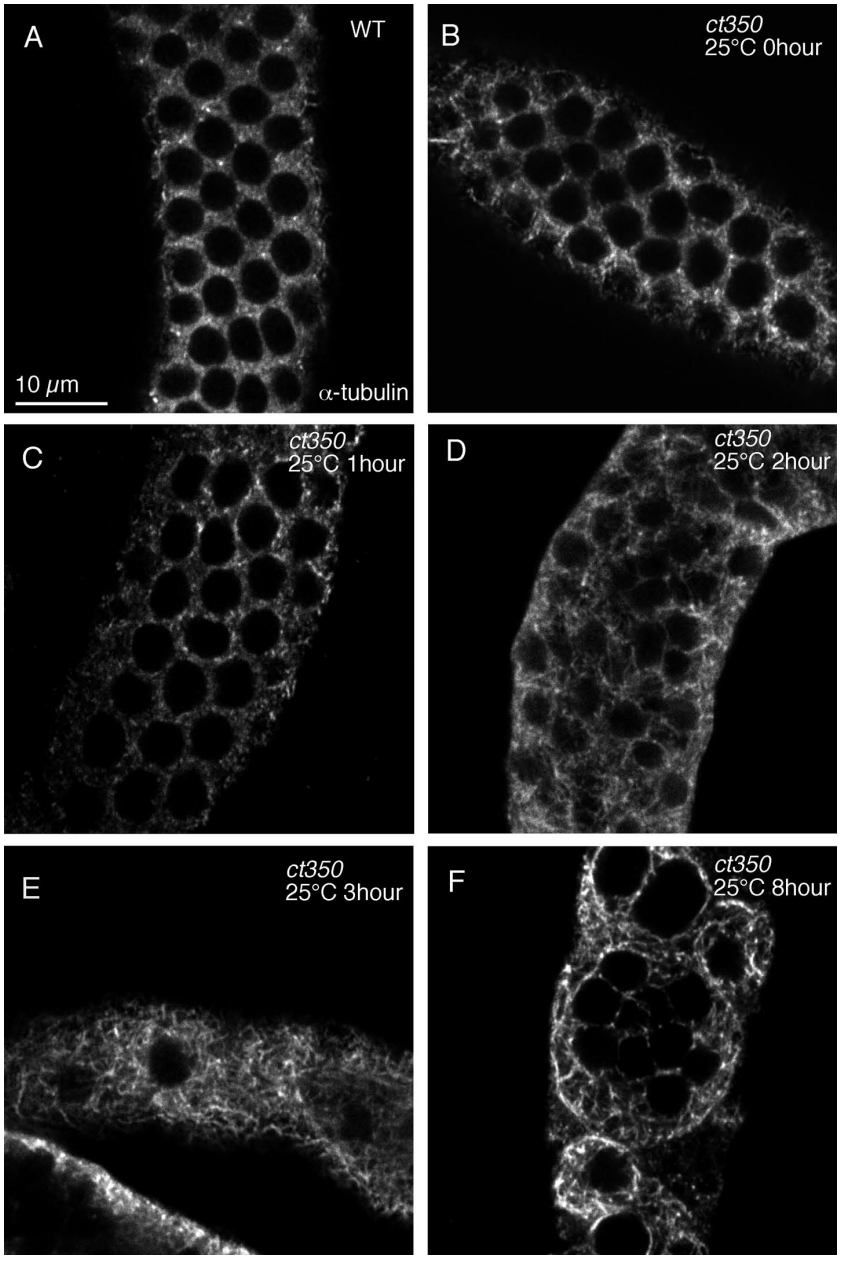

Figure S3. **Microtubules in the gonad of *zyg-12(ct350)* rearrange after shift to the nonpermissive temperature.** (A) Immunofluorescent image stained for α-tubulin shows the microtubule structure of a wild-type (WT) gonad. Microtubules appear in a punctate pattern surrounding the nucleus in this single frame of a confocal z series. In Fig. S4 A′, the 3D projection of the same z series reveals a meshwork-like structure of microtubules surrounding the nucleus. (B) When raised at the permissive temperature, *zyg-12 (ct350)* has the same microtubule structure as wild type. (C) 1 h after the shift to the restrictive temperature, microtubule structure remains intact. (D) After 2 h at restrictive temperature, microtubule arrangement is partially disrupted and is accompanied by the disruption of nuclear position. (E) We observed dramatic microtubule rearrangement as early as 3 h at the restrictive temperature. (F) After 8 h at the restrictive temperature, clusters of multiple nuclei were wrapped in a reorganized microtubule network. All panels are a single frame of a confocal z series.

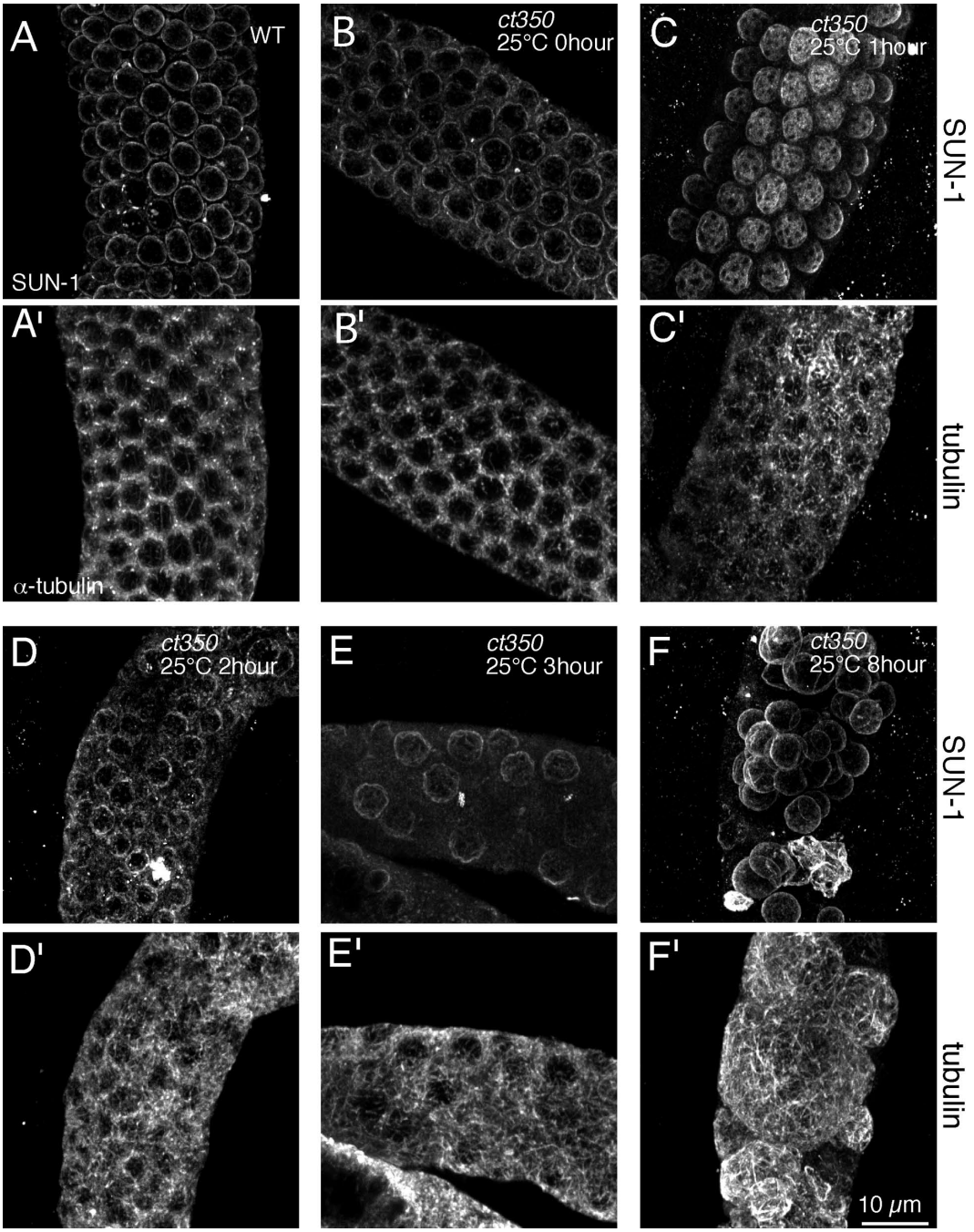

Figure S4. **3D projections of confocal z series show the nuclear position and microtubule structure of animals.** (A–F′) A, B, C, D, E, and F are stained with SUN-1 antibody to indicate the nuclear position. A′, B′, C′, D′, E′, and F′ show corresponding animals stained with α-tubulin antibody. Wild type (WT; A and A′) and *zyg-12 (ct350)* (B–F′) after the indicated times at the restrictive temperature. 3D projections correspond to those in Fig. S3.

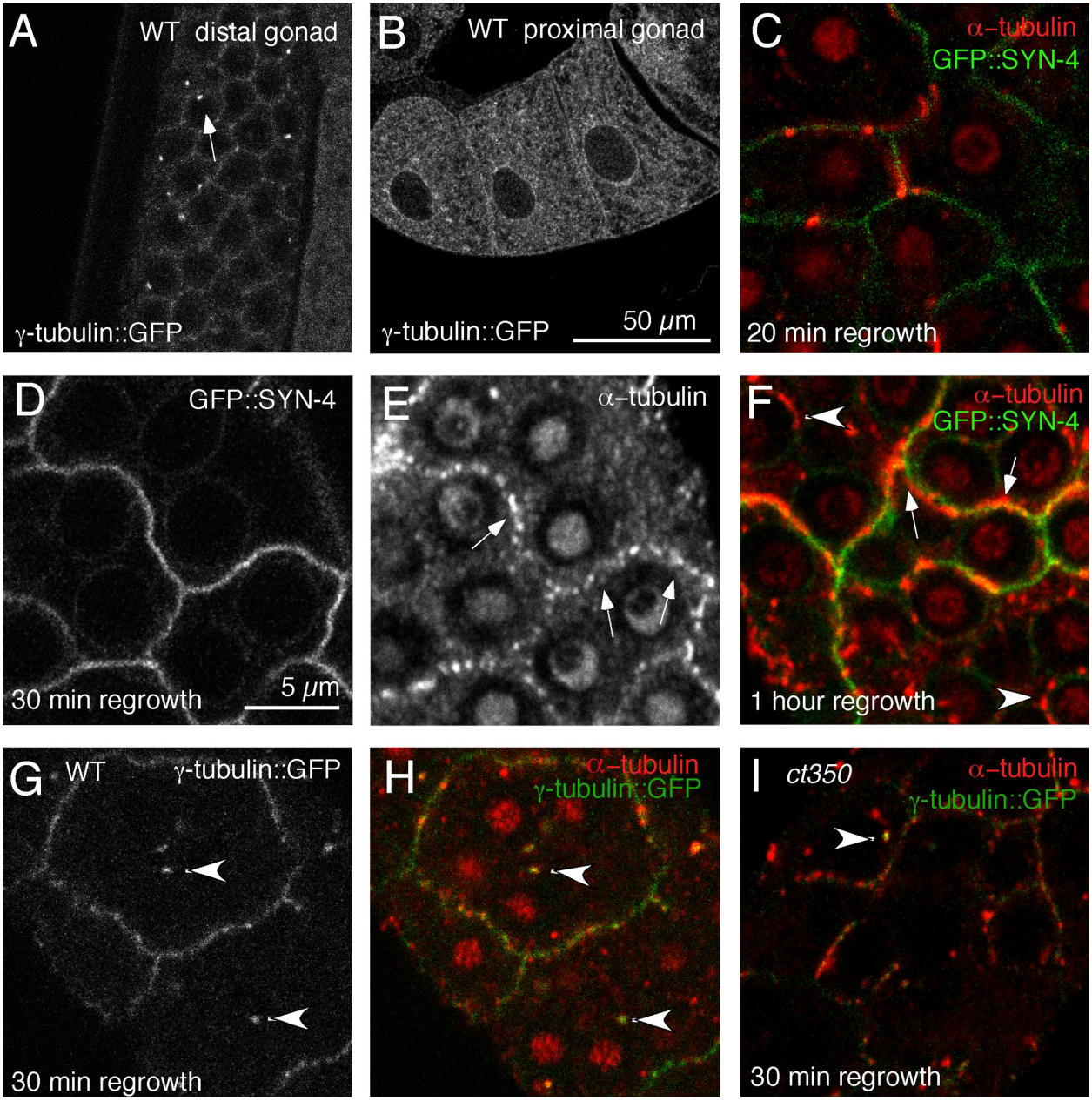

Figure S5. **Nucleation of microtubules from germ cell membranes.** (A) Scanning confocal microscopy of the gonad of a live animal expressing γ-tubulin::GFP. GFP signal is detected on cell membrane and centrosome (white arrow). (B) GFP antibody stain of the proximal gonad of animal expressing γ-tubulin::GFP. Accumulation of GFP signal on nuclear envelope can be seen, but the centrosomal GFP signal shown in distal gonad is not detected in the oocyte. (C–F) Wild-type animals expressing GFP::SYN-4 subjected to microtubules regrowth experiment. (C) 20 min after nocodazole removal, only a few punctate microtubules appear on plasma membrane. (D and E) 30 min after nocodazole removal, punctate microtubule signals (stained with α-tubulin antibody; E, white arrows) align with GFP::SYN-4–marked plasma membrane (D). (F) 1 h after the removal of nocodazole, membrane-associated microtubules grow into filaments (white arrows). Microtubules are also detected on the nuclear envelope (arrowheads). (G and H) Wild-type (WT) animal expressing γ-tubulin::GFP. 30 min after nocodazole removal, microtubule puncta (H, red) associate with γ-tubulin::GFP–marked membrane and centrosomes (arrowheads). (I) *ct350* expressing γ-tubulin::GFP. Microtubules regrow from centrosome (arrowhead) at restrictive temperature.