Figure S1  Klp10A localization under different fixation conditions. Spindles from late-stage oocytes were examined for the localization of endogenous KLP10A (A) and HA-tagged KLP10A (B, C, D). The HA-tagged KLP10A (B, C, D) was expressed in a wild-type background. (A) In oocytes fixed with formaldehyde/heptane, microtubules were well preserved and endogenous KLP10A localized throughout the meiotic spindle. (B) A similar result was observed in oocytes expressing HA-tagged KLP10A fixed with methanol. (C, D) In oocytes fixed with formaldehyde/cacodylate solution, KLP10A is much more difficult to detect, but the microtubules are better preserved and the “curly pole” phenotype is observable. The “curly pole” phenotype was not observed using a methanol fixation because the spindle microtubules are preserved poorly. In all images, DNA is shown in blue and microtubules are shown in green. KLP10A is in red in merged images and in white in single channel images. Scale bars are 5 μm. Methanol fixation was performed essentially as described (Tavosanis et al. 1997). Briefly, 20 to 30 females were fattened on yeast for three to five days. Oocytes were dissected directly into methanol, then the chorion and vitelline membranes were removed by repeated pulses of low-strength sonication. Oocytes were transferred to fresh methanol for two hours, followed by stepwise rehydration into PBS. Oocytes were then immunostained as described (McKim et al. 2009).