

Figure S1. **MKS components are present at the TZ of sensory cilia, and B9d2 and Tctn are required to recruit other MKS components.** (A) Confocal imaging of *Drosophila* antennae stained for Futsch (22c10 antibody) and different MKS proteins or Cep290 in wild-type or *B9d2, tctn*-deleted flies. All MKS components are present at the base of sensory cilia. MKS components are absent from the TZ in *B9d2, tctn*-deleted antennae. Cep290 is maintained. The apparent slight reduction in Cep290 staining was quantified but is not significant. (B) B9d2 is localized at the dendritic ending in chordotonal neurons of wild-type antennae. (C) Tctn::GFP expression is only detectable by rescuing the *B9d2, tctn* mutant strain, indicating a competition with the endogenous protein. (D) 3D-SIM imaging of the chordotonal neurons showing Cep290 and B9d2 domain above the distal BB labeled by PLP antibody. Note that B9d2 domain diameter is wider than Cep290. DBB, distal basal body; PBB, proximal basal body. Bars: (A–C) 10 μm; (D) 1 μm.

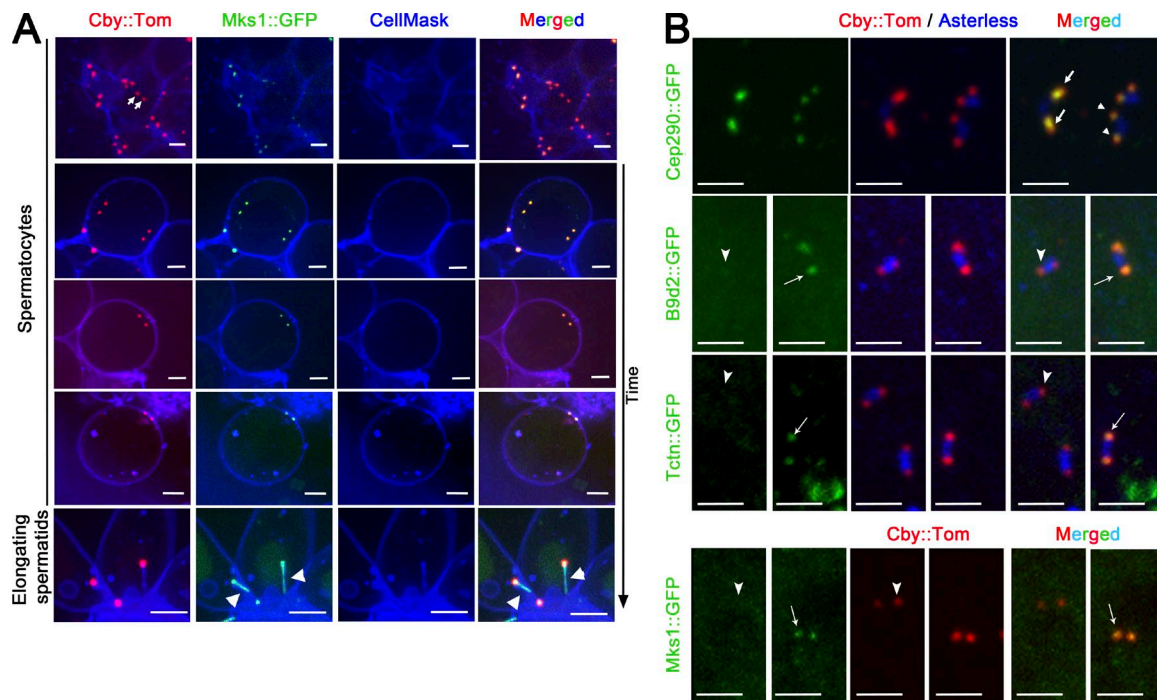


Figure S2. **Time course of TZ assembly in spermatogenesis.** (A) Time-lapse imaging of cultured *Drosophila* male germ cells showing TZ dynamics from spermatocytes to spermatids elongation. Cby is recruited first during centriole maturation (arrows point to centrioles with Cby, but not Mks1, labeling). MKS components are next recruited before docking of the centriole to the plasma membrane (second to fourth rows of images). The ciliary cap is next elongated at the onset of spermatid lengthening (fifth row of images, arrowheads). CellMask labels cell membranes. (B) Magnification of fixed, whole-mount testes showing the distribution of Cby relative to Cep290 or MKS components in spermatocytes of different stages. Cby and Cep290 are recruited concomitantly in early spermatocytes (arrowheads), and the amount of both proteins increases in late spermatocytes (arrows). MKS components are recruited during spermatocytes maturation, as centrioles showing Cby labeling with no MKS staining are observed in young spermatocytes (arrowheads), whereas both proteins are observed on centrioles in older spermatocytes (arrows). Centrioles are labeled by Asterless antibody (blue) for the three top rows. Bars: (A) 5 μ m; (B) 2 μ m.

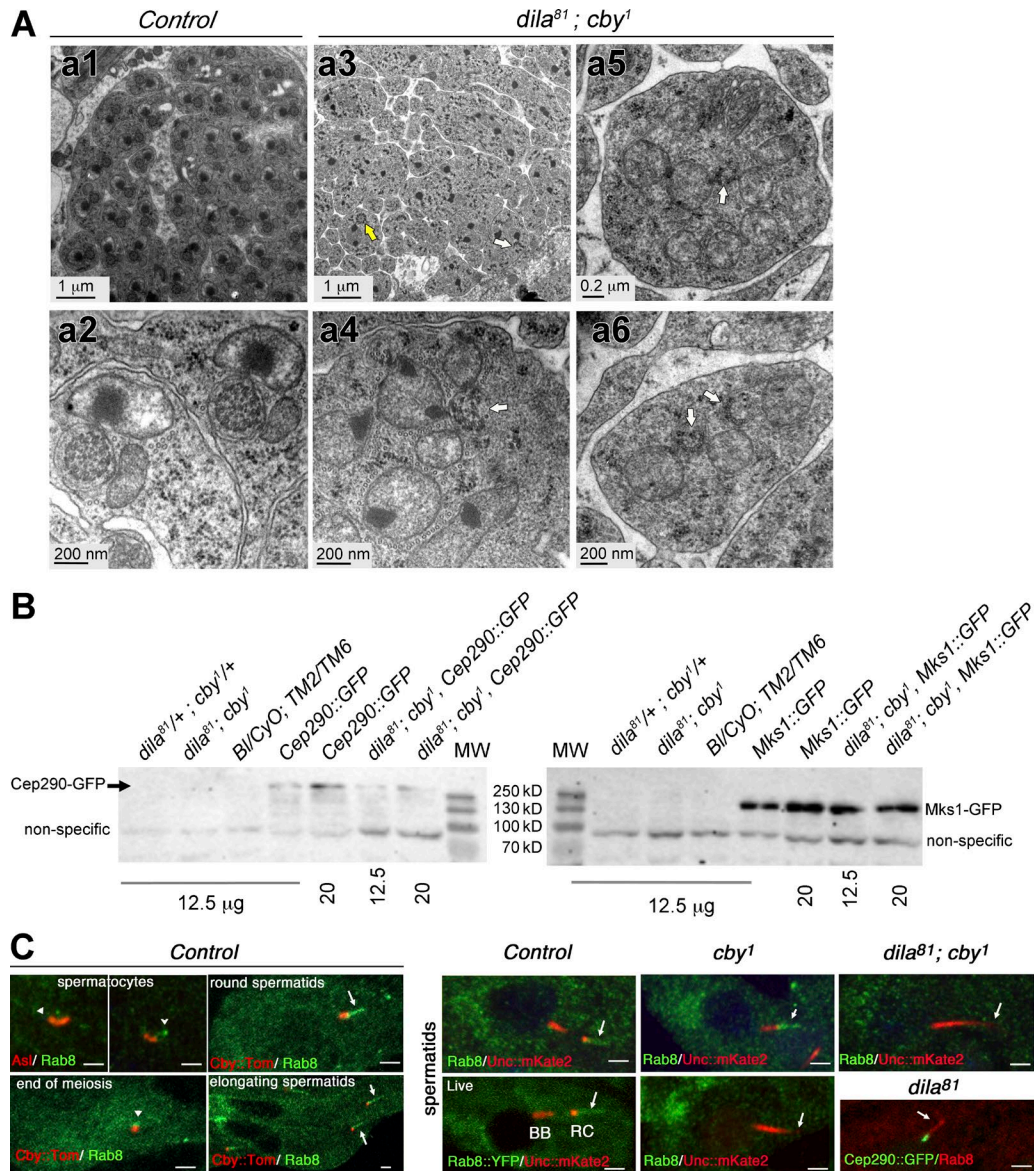


Figure S3. **Phenotypic analysis of testes from *dila⁸¹; cby¹* double mutants.** (A) EM structural analysis. (a1) Each mitochondria is associated with one axoneme in control testes. (a2) Each axoneme shows a typical 9 + 2 architecture in control testes. (a3–a6) *dila⁸¹; cby¹* mutant cysts are severely disorganized. Almost no intact axonemes (yellow arrow) can be observed. Remaining axonemes are broken (white arrows) and only axonemal remnants can be observed. (B) Western blot of protein extracts from whole *Drosophila* testes detected with an anti-GFP antibody. The amount of proteins loaded in each lane is indicated. No difference in Cep290-GFP or Mks1-GFP intensity can be observed in control or *dila⁸¹; cby¹* testes. (C) Fluorescence imaging of squashed or live testes with the different markers as indicated on each panel. In control testes, Rab8 clearly labels the ciliary cap (arrows) from round to late elongating spermatids. Rab8 is usually difficult to observe in spermatocytes (arrowhead, top left panel) but in rare occasions can be observed at the distal tip of spermatocyte centrioles (arrowhead) or during meiosis. Rab8 always labels the ciliary cap in *dila⁸¹* spermatids but never does so in *dila⁸¹; cby¹* spermatids, and Unc signal does not split between basal body (BB) and ring centriole (RC). In *cby¹* testes, Rab8 labels the ciliary cap in ~35% of spermatids, but Rab8 staining is missing in the remaining spermatids. Note that Rab8 antibodies or Rab8-YFP tagged proteins show an identical localization profile. Bars, 2 µm.

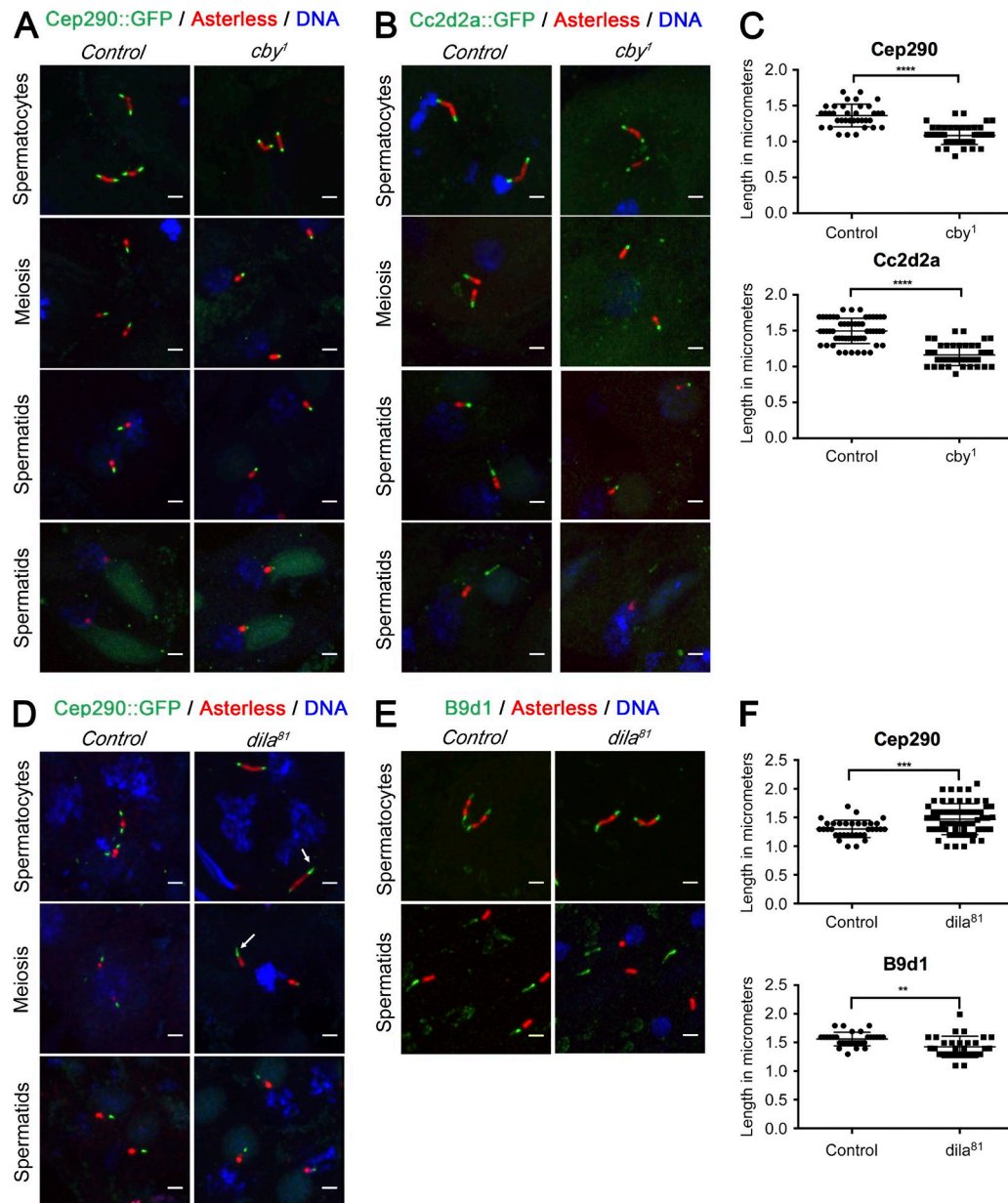


Figure S4. **Organization of the TZ in *dila⁸¹* or *cby¹* single-mutant flies.** (A) Cep290::GFP and (B) Cc2d2a::GFP costaining with Asterless (red) in squashed control or *cby¹* mutant testes. Both Cep290 and Cc2d2a are present at all stages, but their expression domain is reduced. (C) Scattered plots with mean and SD of Cep290 (control $n = 36$ and *cby¹* $n = 51$) and Cc2d2a (control $n = 54$ and *cby¹* $n = 44$) length at the TZ during meiosis indicate a reduction of the expression domain of both proteins. (D) Cep290::GFP and Asterless (red) observation in squashed control or *dila⁸¹* mutant testes showing that Cep290 is still present. Note that the Cep290 domain is sometimes extended (arrows). (E) B9d1 (green) and Asterless (red) observation in squashed control or *dila⁸¹* mutant testes showing that B9d1 is still present. (F) Scattered plots with mean and SD of Cep290 (control $n = 34$ and *dila⁸¹* $n = 63$) and B9d1 (control and *dila⁸¹* $n = 30$) length at the TZ during meiosis showing a significant extension of Cep290 in *dila⁸¹* mutant context and a significant decrease of B9d1. Bars, 2 μ m. **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

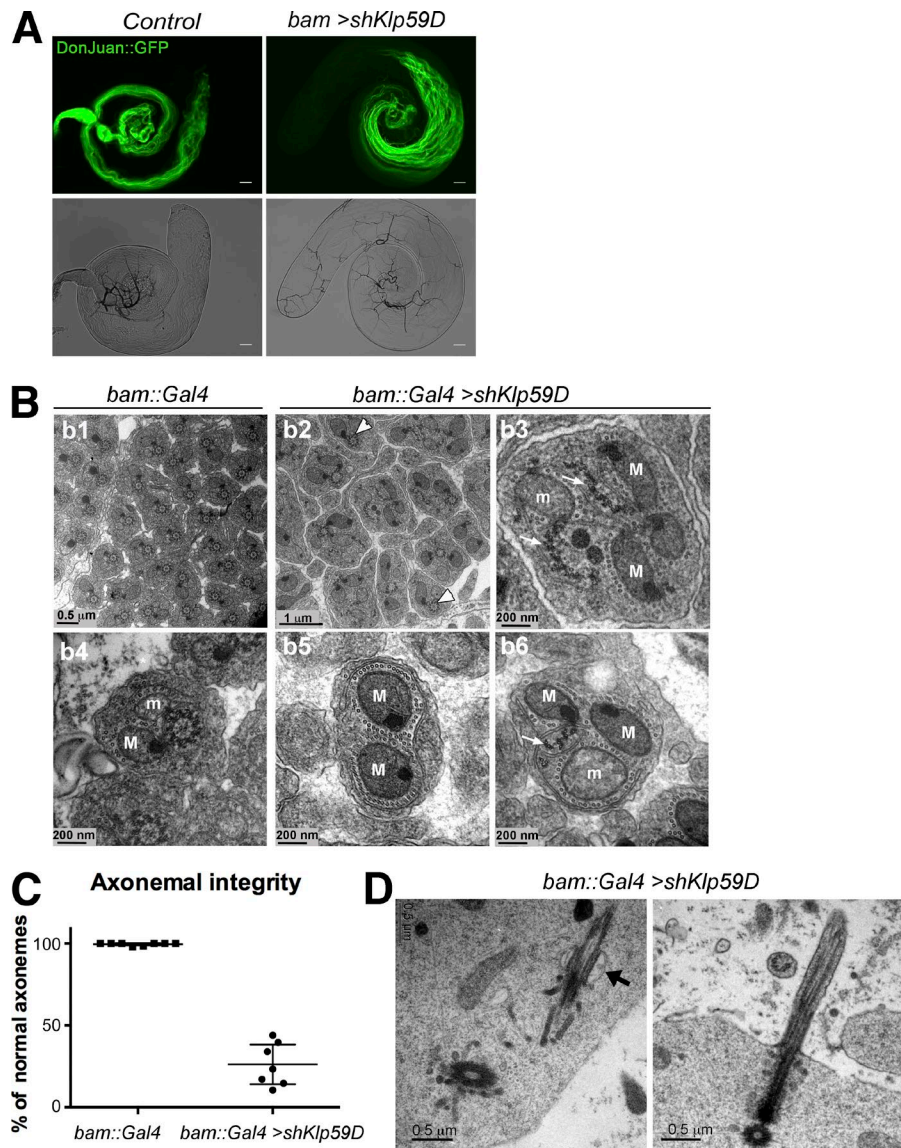


Figure S5. **Phenotypes of *Klp59D* KD testes.** (A) Whole-mount control or *Klp59D* KD *Drosophila* testis expressing the tagged mitochondria protein DonJuan::GFP observed by brightfield and confocal microscopy. Bars, 50 μ m. (B) Transmission EM analysis of testes. Severe axonemal defects are observed in *Klp59D* KD testes (b2, b3, b5, and b6) compared with controls (b1 and b4). Microtubule doublets are sometimes assembled but break apart compared with controls (arrows in b3). Only a few axonemes are complete (arrowheads in b2). Many axonemes are missing; two major mitochondria derivatives (M) are observed in b5 and b6, whereas no or only one broken axoneme is seen (arrow). Numerous microtubules surround the mitochondria in *Klp59D* KD testes (b5 and b6). m, minor mitochondria derivative. (C) Quantification of the percentage of axonemal defects per cysts (control $n = 8$ and *Klp59D* KD $n = 7$); results are presented as a scattered plot with mean and SD. $P < 0.0001$. (D) Structure of spermatocytes cilia showing the defective ciliary cap (left, arrow) or huge cilia extension (right).