

Phosphatidylinositol 4,5-bisphosphate regulates cilium transition zone maturation in *Drosophila melanogaster*

Alind Gupta ^{*1,2}, Lacramioara Fabian ^{†2}, and Julie A. Brill ^{‡1,2,3}

¹Department of Molecular Genetics, University of Toronto, Toronto, Ontario, M5S 1A8, Canada

²Cell Biology Program, The Hospital for Sick Children, Toronto, Ontario, M5G 0A4, Canada

³Institute of Medical Science, University of Toronto, Toronto, Ontario, M5S 1A8, Canada

To whom correspondence should be addressed:

Julie A. Brill

Cell Biology Program

The Hospital for Sick Children

PGCRL Building, 15th Floor

686 Bay Street, Room 15.9716

Toronto, Ontario M5G 0A4

Canada

Phone: (416) 813-8863

Email: julie.brill@sickkids.ca

Short title: PIP₂ regulates the ciliary TZ

Keywords: PtdIns(4,5)P₂, Skittles, Cep290, cilia, transition zone, basal body

Brief summary statement: The authors show that the membrane phospholipid PIP₂, and the kinase that produces PIP₂ called Skittles, are needed for normal ciliary transition zone morphology and function in the *Drosophila* male germline.

*alind.gupta@mail.utoronto.ca

†lacramioara.fabian@sickkids.ca

‡julie.brill@sickkids.ca

1 **Abstract**

2 Cilia are cellular antennae that are essential for human development and physiology. A large
3 number of genetic disorders linked to cilium dysfunction are associated with proteins that
4 localize to the ciliary transition zone (TZ), a structure at the base of cilia that regulates
5 trafficking in and out of the cilium. Despite substantial effort to identify TZ proteins and
6 their roles in cilium assembly and function, processes underlying maturation of TZs are not
7 well understood. Here, we report a role for the membrane lipid phosphatidylinositol 4,5-
8 bisphosphate (PIP₂) in TZ maturation in the *Drosophila melanogaster* male germline. We show
9 that reduction of cellular PIP₂ levels by ectopic expression of a phosphoinositide phosphatase
10 or mutation of the type I phosphatidylinositol phosphate kinase Skittles induces formation of
11 longer than normal TZs. These hyperelongated TZs exhibit functional defects, including loss
12 of plasma membrane tethering. We also report that the *onion rings (onr)* allele of *Drosophila*
13 *exo84* decouples TZ hyperelongation from loss of cilium-plasma membrane tethering. Our
14 results reveal a requirement for PIP₂ in supporting ciliogenesis by promoting proper TZ
15 maturation.

16 Introduction

17 Cilia are sensory organelles that are important for signalling in response to extracellular cues,
18 and for cellular and extracellular fluid motility [1, 2, 3, 4]. Consistent with their importance,
19 defects in cilium formation (*i.e.* ciliogenesis) are associated with genetic disorders known
20 as ciliopathies, which can display neurological, skeletal and fertility defects, in addition to
21 other phenotypes [5, 6, 7, 8]. Many ciliopathies are associated with mutations in proteins that
22 localize to the transition zone (TZ), the proximal-most region of the cilium that functions as a
23 diffusion barrier and regulates the bidirectional transport of protein cargo at the cilium base
24 [9, 10]. For example, the conserved TZ protein CEP290 is mutated in at least six different
25 ciliopathies [11] and is important for cilium formation and function in humans [12, 13] and
26 *Drosophila* [14]. Although the protein composition of TZs has been investigated in various
27 studies [15], the process of TZ maturation, through which it is converted from an immature
28 form to one competent at supporting cilium assembly, is relatively understudied.

29 Ciliogenesis begins with assembly of a nascent TZ at the tip of the basal body (BB) [9]. During
30 TZ maturation, its structure and protein constituents change, allowing for establishment of a
31 compartmentalized space, bounded by the ciliary membrane and the TZ, where assembly of the
32 axoneme, a microtubule-based structure that forms the ciliary core, and signalling can occur. In
33 *Drosophila*, nascent TZs first assemble on BBs during early G2 phase in primary spermatocytes
34 [16]. This occurs concomitantly with anchoring of cilia to the plasma membrane (PM),
35 microtubule remodelling within the TZ [17, 18], and establishment of a ciliary membrane that
36 will persist through meiosis [16] (Figure 1A). TZ maturation has been described in *Paramecium*
37 [19], *Caenorhabditis elegans* [20] and *Drosophila* [18], and is most readily observed by an
38 increase in TZ length in the *Drosophila* male germline.

39 We previously showed that the membrane lipid phosphatidylinositol 4,5-bisphosphate (PIP₂) is
40 essential for formation of the axoneme in the *Drosophila* male germline [21, 22]. PIP₂, which
41 is one of seven different phosphoinositides (PIPs) present in eukaryotes, localizes primarily

42 to the PM, where it is required for vesicle trafficking, among other processes [23]. PIP₂ has
43 recently been linked to cilium function. Although the ciliary membrane contains very little
44 PIP₂ due to the action of the cilium resident PIP phosphatase INPP5E, the base of the cilium
45 is enriched in PIP₂ [24]. Inactivation of INPP5E causes a build up of intraciliary PIP₂, which
46 disrupts transport of Hedgehog signalling proteins in vertebrates [25, 26, 27] and ion channels
47 involved in mechanotransduction in *Drosophila* [28]. In light of the current understanding
48 of PIP₂ as a modulator of cilium function, we sought to investigate the cause of defects we
49 had observed in axoneme assembly in *Drosophila* male germ cells with reduced levels of PIP₂
50 [21, 22].

51 **Results**

52 *PIP₂ is essential for transition zone maturation*

53 To investigate how reduction of cellular PIP₂ affects ciliogenesis in the *Drosophila* male
54 germline, we used transgenic flies expressing the *Salmonella* PIP phosphatase SigD under
55 control of spermatocyte-specific β_2 -tubulin promoter (hereafter β_2 t-SigD) [21]. To examine
56 whether axoneme defects in β_2 t-SigD [21] were caused by aberrant TZ function, we examined
57 localization of fluorescently-tagged versions of the core centriolar/BB protein Ana1 (CEP295
58 homolog) [29, 30] and the conserved TZ protein Cep290 [14] during early steps of cilium
59 assembly. Cep290 distribution appeared similar in control and β_2 t-SigD in early G2 phase,
60 when TZs are still immature. In contrast, Cep290-labelled TZs were significantly longer
61 in β_2 t-SigD compared to controls by late G2 phase, following the period of TZ maturation
62 (Figure 1B and 1C). Unlike *Drosophila cep290* mutants, which contain longer than normal BBs
63 [14], Ana1 length was not affected in β_2 t-SigD, and we did not observe a strong correlation
64 between Cep290 and Ana1 lengths (Figure 1D). Consistent with this result, the ultrastructure
65 of BBs in β_2 t-SigD is normal, and localization of the centriolar marker GFP-PACT [31] is
66 similar, in controls and β_2 t-SigD [21]. In contrast, TZ proteins Chibby (Cby) [32] and Mks1

67 [33, 34] exhibited hyperelongation in β_2 t-SigD (Figure 1E), indicating that this phenotype is
68 not unique to Cep290. TZ hyperelongation was a highly penetrant phenotype (>70%) and
69 showed high correlation (>0.95) within syncytial germ cell cysts, suggestive of a dosage-based
70 response to a shared cellular factor, presumably SigD. Despite persistence of hyperelongated
71 TZs through meiosis, axonemes were able to elongate normally in post-meiotic cells (Figure
72 1F). Nonetheless, the ultrastructure of these axonemes is frequently aberrant, either lacking
73 nine-fold symmetry or containing triplet microtubules in addition to the usual doublets
74 [21].

75 *The type I PIP kinase Skittles regulates TZ length*

76 Although PIP₂ is its major substrate in eukaryotic cells *in vivo* [35, 36, 37], SigD can de-
77 phosphorylate multiple PIPs *in vitro* [38]. To address whether TZ hyperelongation observed
78 in β_2 t-SigD represented a physiologically relevant phenotype due to decreased PIP₂, we at-
79 tempted to rescue this phenotype by co-expressing β_2 t-SigD with fluorescently-tagged Skittles
80 (Sktl) under control of β_2 -tubulin promoter. We found that Sktl expression was able to suppress
81 TZ hyperelongation in a cilium-autonomous manner (Figure 2A and 2B). Furthermore, the
82 BB/TZ protein Unc-GFP [39, 21], exhibited TZ hyperelongation at a low penetrance in *skt1*^{2,3}
83 mutant clones (Figure 2C), indicating that Sktl is important for TZ maturation.

84 Vertebrate type I PIP kinase PIPKI γ has previously been shown to be important for cilium
85 formation in cultured cells [40]. The two *Drosophila* PIPKIs, Sktl and PIP5K59B, arose from
86 recent duplication of the ancestral PIPKI gene, and are not orthologous to specific vertebrate
87 PIPKI isoforms (Figure 2D). Sktl has diverged more than its paralog PIP5K59B and seems
88 to be functionally related to PIPKI γ and the *C. elegans* PPK-1 in having roles at cilia [41].
89 However, unlike the human PIPKI γ , which licenses TZ assembly by promoting CP110 removal
90 from BBs [40], our results suggest that Sktl functions in regulating TZ length but not TZ
91 assembly. Notably, neither inactivation nor overexpression of *cp110* affects cilium formation in
92 *Drosophila*, and Cp110 is removed from BBs in early primary spermatocytes [42].

93 ***Hyperelongated transition zones exhibit functional defects***

94 We next sought to examine whether TZ hyperelongation due to SigD expression affected TZ
95 function. Following meiosis in the *Drosophila* male germline, TZs detach from the BB and
96 migrate along the growing axoneme, maintaining a ciliary compartment at the distal-most
97 $\sim 5\mu\text{m}$ where tubulin is incorporated into the axoneme [14, 43]. As shown by Unc and Cep290
98 localization, TZs in $\beta_2\text{t-SigD}$ were frequently incapable of detaching from BBs and migrating
99 along axonemes despite axoneme and cell elongation (Figures 1E, 3A and 3B). Indeed, the
100 previously reported “comet-shaped” Unc-GFP localization in $\beta_2\text{t-SigD}$ [21] persists during cell
101 elongation after meiosis (Figure 3A, bottommost panel) despite elongation of the axoneme
102 (Figure 1E).

103 In *Drosophila* and humans, BBs consist of microtubule triplets [44, 45], whereas axonemes
104 contain microtubule doublets due to obstruction of C-tubules at the TZ [18]. Consistent
105 with a defect in this barrier and the presence of microtubule triplets in axonemes in $\beta_2\text{t-SigD}$
106 [21], a subset of cilia (<5%) in $\beta_2\text{t-SigD}$ contained puncta of Ana1 at the distal tips of TZs
107 (Figure 3C). Treatment of germ cells with the microtubule-stabilizing drug Taxol increased the
108 penetrance of this phenotype from <5% in untreated cells to >25% in cells treated with 4
109 μM Taxol (arrowheads in Figure 3D) without significantly affecting Cep290 length (Figure
110 3E). Taxol-treated controls did not exhibit TZ-distal Ana1 puncta ($p < 0.01$ at 5% penetrance).
111 Fluorescently-tagged Asterless (CEP152 homolog), a pericentriolar protein [46, 47], did not
112 localize to TZ-distal puncta in $\beta_2\text{t-SigD}$ ($p < 0.01$) suggesting that these TZ-distal sites are not
113 fully centriolar in protein composition. Taxol has been hypothesized to disrupt TZ maturation
114 by inhibiting microtubule remodelling in the *Drosophila* male germline [17]. Similar to
115 $\beta_2\text{t-SigD}$, Taxol-treated male germ cells assemble extremely long axonemes that contain triplet
116 microtubules [17], further supporting a functional relationship between PIP_2 and microtubule
117 reorganization in TZ maturation.

118 ***The onion rings (onr) mutant decouples defects found in cells with reduced levels of***
119 ***PIP₂***

120 Male flies homozygous for the *onion rings (onr)* mutant of *Drosophila* *exo84* are sterile and
121 exhibit defects in cell elongation and polarity similar to β_2t -SigD [21]. Exo84 is a component
122 of the octameric exocyst complex, which binds PIP₂ and regulates membrane trafficking at the
123 PM [48]. To investigate whether defects in TZ hyperelongation could be explained by defective
124 Exo84 function, we examined TZs in *onr* mutants. Unlike β_2t -SigD, *onr* did not display
125 hyperelongated TZs (Figure 4A), suggesting that Exo84 is dispensable for TZ maturation.

126 Due to involvement of the exocyst in trafficking at the PM, we examined whether cilium-
127 associated membranes were affected in β_2t -SigD or *onr* mutants in a manner similar to *dilatatory*;
128 *cby* mutants [33]. Dilatory (Dila), a conserved TZ protein, cooperates with Cby to assemble
129 TZs in the *Drosophila* male germline [33]. Whereas TZs in β_2t -SigD and *onr* cells were able
130 to dock at the PM initially, they were unable to maintain membrane connections, and were
131 rendered cytoplasmic (Figure 4B and C), similar to TZs in *dila*; *cby* mutants. We found that
132 fluorescently-tagged Exo70, a PIP₂-binding exocyst subunit, localized to BBs (Figure 4D). Our
133 results suggest that the exocyst, and Exo84 in particular, regulates cilium-PM associations,
134 similar to PIP₂, and that TZ hyperelongation and loss of cilium-PM association are genetically
135 separable phenotypes.

136 **Discussion**

137 The process of maturation of a TZ from a nascent form to a fully functional state, leading
138 ultimately to axoneme assembly and ciliary signalling, requires orchestration of various pro-
139 teins and cellular pathways [9, 15]. Our results indicate that normal execution of this process
140 requires PIP₂ and that depletion of PIP₂ induces TZs to grow longer than normal. Similar to β_2t -
141 SigD, *Drosophila* *dila*; *cby* and *cby* mutants display hyperelongated TZs [32, 33], whereas *mks1*
142 mutants have shorter TZs [34]. Because both Cby and Mks1 are hyperelongated in β_2t -SigD

143 cells, PIP₂ regulates TZ length independently of an effect on Cby or Mks1 recruitment.

144 We also show that hyperelongated TZs are dysfunctional. Similar to *dila*; *cby* [33] and *cep290*
145 [14] mutants, axonemes can assemble in β_2 t-SigD, albeit with aberrant ultrastructure [21],
146 despite the lack of functional TZs or membrane association. The presence of TZ-distal Ana1
147 puncta in β_2 t-SigD cells, without the increase in BB length seen in *cep290* mutants lacking
148 a functional TZ barrier, suggests that β_2 t-SigD expression selectively disrupts the ability of
149 TZs to restrict C-tubules and Ana1 without abolishing the TZ barrier entirely. CEP295, the
150 human Ana1 ortholog, regulates post-translational modification of centriolar microtubules
151 [49], which may explain the presence of Ana1 along with supernumerary microtubules in
152 β_2 t-SigD cells. Asterless (Asl), a pericentriolar protein important for centrosome formation
153 and centriole duplication [46, 47], did not exhibit this TZ-distal localization, possibly due to
154 differences dynamics of Ana1 and Asl loading onto centrioles [50, 51] or the more peripheral
155 nature of Asl within the centriole [46].

156 The majority of PIP₂ at the PM is produced by PIPKs [23, 52]. In this study, we showed
157 that mutation of the PIPKI Sktl induced hyperelongated TZs and that expression of Sktl
158 could suppress TZ hyperelongation in β_2 t-SigD, with some cells showing cilium-autonomous
159 suppression, suggesting Sktl might function *in situ* to regulate TZ length. In humans, *PIPKI γ* is
160 linked to lethal congenital contractural syndrome type 3 (LCCS3), which has been suggested
161 to represent a ciliopathy [40]. The recent discovery of a role for LCCS1-associated GLE1
162 protein in cilium function [53] corroborates this hypothesis. Our data support the idea that
163 PIPKs might represent ciliopathy-associated genes or genetic modifiers of disease.

164 Members of the exocyst complex such as Sec10 and Sec8 are important for cilium formation
165 in cultured cell lines and zebrafish [54, 55, 56], but their precise roles in ciliogenesis are
166 not well understood. The subunits Sec3 and Exo70 regulate exocyst targeting to the plasma
167 membrane through a direct interaction with PIP₂ [48, 57]. We previously showed that the
168 *onr* allele of *Drosophila* *exo84* phenocopies defects in cell polarity and elongation observed in
169 β_2 t-SigD [22]. Here, we show that the *onr* mutation phenocopies the loss of cilium-membrane

170 contacts in β_2t -SigD, similar to *dila*; *cby* mutants [33], but not TZ hyperelongation. Thus, TZ
171 hyperelongation is not a prerequisite for the failure of cilium-PM association in male germ
172 cells, and Exo84 uniquely regulates the latter process, potentially by supplying membrane
173 required to maintain cilium-PM association. This result is supported by the *Drosophila cep290*
174 mutant, which lacks a functional TZ but retains cilium-PM association [14]. Notably, *EXOC8*,
175 which encodes the human Exo84, has been linked to the ciliopathy Joubert syndrome [58],
176 and a similar process might underlie defects in humans with mutations in *EXOC8*.

177 **Methods**

178 *Transgenic flies*

179 *Drosophila* stocks were cultured on cornmeal molasses agar medium at 25°C and 50% humidity.
180 Stocks expressing $\beta_2t::Styp\SigD$ (chromosome 3) and $\beta_2t::YFP-Sktl$ (chromosome 2) were
181 described previously [21, 59]. GFP-Exo70 was cloned into the low-level expression vector *tv3*
182 [59] and transgenic flies were generated using standard *P* element-mediated transformation.
183 Ana1-tdTomato (chromosome 2) and Cep290-GFP (chromosome 3) were provided by T.
184 Avidor-Reiss [14]. *Sp/CyO*; Unc-GFP was originally provided by M. Kernan [39]. Stocks
185 expressing GFP-tagged Chibby and Mks1 were provided by B. Durand [32, 33]. The *onr*
186 mutant was described previously [60]. Stocks for generating *skt*^{2,3} clones were originally
187 provided by A. Guichet [61]. Clones were induced by heat shock for two hours on days 3, 4
188 and 5 after egg laying. *w*¹¹¹⁸ was used as the wild-type control.

189 *Antibodies*

190 The following primary antibodies were used for immunofluorescence at the indicated concen-
191 trations: chicken anti-GFP IgY (abcam), 1:1000; rat anti-RFP IgG (5F8, ChromoTek), 1:1000;
192 rabbit anti-Centrin (C7736, Sigma-Aldrich), 1:500; mouse anti-acetylated α -tubulin 6-11-B

193 (Sigma-Aldrich), 1:1000. Secondary antibodies were Alexa 488- and Alexa 568-conjugated
194 anti-mouse, anti-rabbit and anti-chicken IgG (Molecular Probes) at 1:1000. DAPI at 1:1000
195 was used to stain for DNA.

196 *Fluorescence microscopy*

197 For live imaging, testes were dissected in phosphate buffered saline (PBS). To stain for DNA,
198 intact testes were incubated in PBS with Hoechst 33342 (1:5000) for 5 minutes. Testes were
199 transferred to a polylysine-coated glass slide (Thermo Fisher Scientific) in a drop of PBS,
200 ruptured using a syringe needle and squashed under a glass coverslip using Kimwipes. The
201 edges of the coverslip were sealed with nail polish and the specimen was visualized using
202 an epifluorescence microscope (Zeiss Axioplan 2) with an AxioCam CCD camera. Cells were
203 examined live whenever possible to avoid artefacts from immunostaining.

204 For Taxol treatments, testes from larvae or pupae expressing Ana1-tdTomato; Cep290-GFP
205 were dissected into Shields and Sang M3 medium (Sigma-Aldrich) supplemented with a
206 predefined concentration of Taxol (Sigma-Aldrich) in DMSO and incubated overnight in a
207 humidified sterile chamber in the dark at room temperature. These were then squashed in
208 PBS and imaged live.

209 For CellMask staining, cells were spilled from testes in M3 medium onto a sterilized glass-
210 bottom dish pre-treated with sterile polylysine solution to enable cells to adhere. CellMask
211 Deep Red (Invitrogen) solution (20 $\mu\text{g}/\text{mL}$) was added to the medium dropwise immediately
212 before visualization under a confocal microscope.

213 For immunocytochemistry, testes were dissected in PBS, transferred to a polylysine-coated
214 glass slide in a drop of PBS, ruptured with a needle, squashed and frozen in liquid nitrogen
215 for 5 minutes. Slides were transferred to ice-cold methanol for 5-10 minutes for fixation.
216 Samples were then permeabilized and blocked in PBS with 0.1% Triton-X and 0.3% bovine
217 serum albumin, and incubated with primary antibodies overnight at 4°C, followed by three

218 5-minute washes with PBS, 1 hour incubation with secondary antibodies, and three 5-minute
219 washes with PBS. Samples were mounted in Dako (Agilent) and imaged with a Zeiss Axioplan
220 2 epifluorescence microscope or a Nikon A1R scanning confocal microscope (SickKids imaging
221 facility).

222 ***Statistical methods***

223 Statistical analysis and graphing was performed using R (version 3.4). A Gaussian jitter was
224 applied when plotting results in Figures 1 and 2 for clearer visualization of trends, but raw
225 data was used for all analyses. Statistical tests for “absence of phenotype” were computed
226 using a binomial test under the assumption that the probability of the phenotype occurring
227 was fixed. All *t*-tests were unpaired and two-sided with Welch’s correction for unequal
228 variances. *n* represents the pooled number of samples (individual cilia) from multiple flies.
229 A significance level of 0.01 was fixed *a priori* for all classical analyses. All raw data and
230 code for analysis and plotting can be found online at [http://www.github.com/alindgupta/
231 germline-paper/](http://www.github.com/alindgupta/germline-paper/).

232 ***Phylogenetic analysis***

233 Candidate orthologs of Skittles and PIP5K9B were queried from Inparanoid (version 8.0) and
234 FlyBase (version FB2017_05). Poorly annotated protein sequences were confirmed to encode
235 type I phosphatidylinositol phosphate kinases using reciprocal BLAST search. Phylogeny.fr
236 (<http://www.phylogeny.fr>) [62] was used for phylogenetic reconstruction with T-Coffee for
237 multiple alignment and MrBayes for tree construction. The output was converted to a vector
238 image in Illustrator and colours were added for the purpose of illustration.

239 **Acknowledgements**

240 We are grateful to Brian Ciruna for insightful discussions, to Bénédicte Durand, Tomer Avidor-
241 Reiss, Antoine Guichet and Maurice Kernan for providing fly stocks, and to Bénédicte Durand
242 and Bill Trimble for critical comments on the manuscript. This research was supported by
243 a grant from the Canadian Institutes for Health and Research to J.A.B (MOP #130437).
244 A.G. was supported by a University of Toronto Open Fellowship and an Ontario Graduate
245 Scholarship.

246 **Author contributions**

247 A.G. and J.A.B. conceived the project. A.G. performed all the experiments and analyses, and
248 wrote the manuscript. L.F. generated GFP-Exo70 flies. J.A.B. edited the manuscript and
249 supervised the project.

250 **Declaration of interests**

251 The authors declare no competing interests.

252 **Figure legends**

253 ***Figure 1. SigD expression induces transition zone hyperelongation.***

254 (A) Schematic diagram of the stages of ciliogenesis in the *Drosophila* male germline. Stages
255 in parentheses correspond to those from [63].

256 (B) β_2 t-SigD expression induces Cep290 hyperelongation in cilia at late G2 phase (white
257 arrowheads).

- 258 (C) Quantification of paired Ana1-Cep290 lengths in early and late G2 phase in spermatocytes
259 ($n > 30$ and > 65 respectively).
- 260 (D) Lengths of Ana1-tdTomato versus Cep290-GFP in control and β_2t -SigD cells at late G2
261 phase from (C) showing negligible correlation. Regression lines are plotted in red, and
262 the Pearson correlation coefficient (r) is indicated on the bottom-left.
- 263 (E) β_2t -SigD expression induces hyperelongation of the TZ proteins Chibby (Cby) and Mks1
264 in late G2 phase (white arrowheads).
- 265 (F) TZ hyperelongation persists through meiosis (white arrowhead) but does not affect
266 axoneme outgrowth. Acetylated tubulin (AcTub) labels the axoneme.

267 **Figure 2. *Sktl* is important for transition zone maturation.**

- 268 (A) Expression of full-length *Sktl* can suppress β_2t -SigD-induced TZ hyperelongation in a
269 cilium-autonomous manner. Images were chosen to demonstrate varying levels of rescue
270 of Cep290-GFP length in β_2t -YFP-*Sktl*; β_2t -SigD. White arrowheads mark rescued Cep290
271 distribution for comparison.
- 272 (B) Quantification of Cep290 and Ana1 lengths from control, β_2t -SigD from (A) and β_2t -YFP-
273 *Sktl*; β_2t -SigD ($n = 100$).
- 274 (C) *sktl*^{2,3} clones exhibit TZ hyperelongation (white arrowheads), as marked by Unc-GFP
275 (left). Quantification of Unc-GFP lengths in control ($n = 53$), *sktl*^{2,3} ($n = 31$) and
276 β_2t -SigD ($n = 51$) spermatocytes at late G2 phase (right).
- 277 (D) Phylogenetic tree of PIP5Ks showing evolutionary conservation of cilium-associated
278 functions. Scale bar (bottom) represents expected amino acid substitutions per site.
279 Branch support values are shown in red (a value of 1 indicates maximum support). Black
280 arrows represent previous evidence of involvement in cilium-associated functions (from
281 [40]). Black arrowhead indicates *Sktl*. Abbreviations: Cele (*Caenorhabditis elegans*),
282 Spur (*Strongylocentrotus purpuratus*), Amel (*Apis mellifera*), Aaeg (*Aedes aegypti*), Dana
283 (*Drosophila ananassae*), Dmel (*Drosophila melanogaster*), Hsap (*Homo sapiens*), Mmus
284 (*Mus musculus*), Xtro (*Xenopus tropicalis*), Cint (*Ciona intestinalis*), Scer (*Saccharomyces*

285 *cerevisiae*).

286 **Figure 3. Hyperelongated transition zones display functional defects.**

- 287 (A) Unc-GFP is unable to detach from the basal body and migrate in spermatids expressing
288 β_2 t-SigD (white arrowhead). Cell elongation in spermatids is concomitant with elon-
289 gation of the mitochondrial derivative (dark organelles in the phase-contrast images).
290 Insets show phase-contrast images corresponding to the region shown in the fluorescence
291 images.
- 292 (B) Cep290 is unable to detach and migrate from the basal body at the onset of axoneme
293 assembly in β_2 t-SigD spermatids (white arrowhead). Insets show phase contrast images
294 corresponding to the region shown in the fluorescence images, with the elongating
295 mitochondrial derivative delineated by a yellow dashed line.
- 296 (C) Structured illumination micrographs of β_2 t-SigD cells showing TZ-distal puncta of the
297 centriolar protein Ana1 (yellow arrowheads).
- 298 (D) Treatment of control and β_2 t-SigD cells with the microtubule stabilizing drug Taxol.
299 Images demonstrate the variety in Cep290 distribution. Yellow arrowheads mark TZ-
300 distal Ana1.
- 301 (E) Quantification of Cep290 lengths in Taxol-treated control and β_2 t-SigD cells from (D) (*n*
302 is between 30 and 40).

303 **Figure 4. The onion rings (*onr*) allele of *exo84* decouples TZ hyperelongation from loss of**
304 ***plasma membrane contacts.***

- 305 (A) *onr* mutant cells do not display hyperelongated acetylated tubulin signal at the cilium
306 (white arrowhead). Acetylated tubulin marks the axoneme, which colocalizes with the
307 TZ in spermatocytes [34].
- 308 (B) Cells expressing β_2 t-SigD fail to maintain cilium-PM tethering despite initially anchoring
309 to the PM. The PM is marked with CellMask, a cell impermeable dye.
- 310 (C) *onr* mutants do not maintain PM-cilium tethering.

311 (D) GFP-tagged Exo70 localizes to BBs in spermatocytes.

List of Abbreviations

β_2 t-SigD	SigD driven by the male germline-specific β_2 -tubulin promoter
PIP ₂	phosphatidylinositol 4,5-bisphosphate
PIP	phosphoinositide, also known as phosphatidylinositol phosphate
TZ	transition zone
BB	basal body
PM	plasma membrane
<i>onr</i>	<i>onion rings</i> (an allele of <i>exo84</i>)

References

1. Satir, P., Pedersen, L. B., and Christensen, S. T. (2010). The primary cilium at a glance. *J Cell Sci*, 123(4):499–503.
2. Marshall, W. F. and Nonaka, S. (2006). Cilia: tuning in to the cell's antenna. *Current Biology*, 16(15):R604–R614.
3. Eley, L., Yates, L. M., and Goodship, J. A. (2005). Cilia and disease. *Current Opinion in Genetics & Development*, 15(3):308–314.
4. Brooks, E. R. and Wallingford, J. B. (2014). Multiciliated cells. *Current Biology*, 24(19):R973–R982.
5. Waters, A. M. and Beales, P. L. (2011). Ciliopathies: an expanding disease spectrum. *Pediatric Nephrology*, 26(7):1039–1056.
6. Valente, E. M., Rosti, R. O., Gibbs, E., and Gleeson, J. G. (2014). Primary cilia in neurodevelopmental disorders. *Nature Reviews Neurology*, 10(1):27.
7. Hammarsjö, A., Wang, Z., Vaz, R., Taylan, F., Sedghi, M., Girisha, K., Chitayat, D., Neethukrishna, K., Shannon, P., Godoy, R., et al. (2017). Novel KIAA0753 mutations extend the phenotype of skeletal ciliopathies. *Scientific Reports*, 7(1):15585.
8. Inaba, K. and Mizuno, K. (2016). Sperm dysfunction and ciliopathy. *Reproductive Medicine and Biology*, 15(2):77–94.

9. Reiter, J. F., Blacque, O. E., and Leroux, M. R. (2012). The base of the cilium: roles for transition fibres and the transition zone in ciliary formation, maintenance and compartmentalization. *EMBO Reports*, 13(7):608–618.
10. Szymanska, K. and Johnson, C. A. (2012). The transition zone: an essential functional compartment of cilia. *Cilia*, 1(1):10.
11. Rachel, R. A., Li, T., and Swaroop, A. (2012). Photoreceptor sensory cilia and ciliopathies: focus on CEP290, RPGR and their interacting proteins. *Cilia*, 1(1):22.
12. Shimada, H., Lu, Q., Insinna-Kettenhofen, C., Nagashima, K., English, M. A., Semler, E. M., Mahgerefteh, J., Cideciyan, A. V., Li, T., Brooks, B. P., et al. (2017). In vitro modeling using ciliopathy-patient-derived cells reveals distinct cilia dysfunctions caused by CEP290 mutations. *Cell Reports*, 20(2):384–396.
13. Stowe, T. R., Wilkinson, C. J., Iqbal, A., and Stearns, T. (2012). The centriolar satellite proteins Cep72 and Cep290 interact and are required for recruitment of BBS proteins to the cilium. *Molecular Biology of the Cell*, 23(17):3322–3335.
14. Basiri, M. L., Ha, A., Chadha, A., Clark, N. M., Polyanovsky, A., Cook, B., and Avidor-Reiss, T. (2014). A migrating ciliary gate compartmentalizes the site of axoneme assembly in *Drosophila* spermatids. *Current Biology*, 24(22):2622–2631.
15. Gonçalves, J. and Pelletier, L. (2017). The ciliary transition zone: finding the pieces and assembling the gate. *Molecules and cells*, 40(4):243.
16. Riparbelli, M. G., Callaini, G., and Megraw, T. L. (2012). Assembly and persistence of primary cilia in dividing *Drosophila* spermatocytes. *Developmental Cell*, 23(2):425–432.
17. Riparbelli, M. G., Cabrera, O. A., Callaini, G., and Megraw, T. L. (2013). Unique properties of *Drosophila* spermatocyte primary cilia. *Biology Open*, 2(11):1137–1147.
18. Gottardo, M., Callaini, G., and Riparbelli, M. G. (2013). The cilium-like region of the *Drosophila* spermatocyte: an emerging flagellum? *J Cell Sci*, 126(23):5441–5452.
19. Aubusson-Fleury, A., Lemullois, M., Bengueddach, H., Abdallah, S., Shi, L., Cohen, J., Tassin, A., and Koll, F. (2015). Transition zone: the sequential assembly of its components parallels its dual role in basal body anchoring and ciliary function. *Cilia*, 4(1):(Supplement 1) P26.
20. Serwas, D., Su, T. Y., Roessler, M., Wang, S., and Dammermann, A. (2017). Centrioles initiate cilia assembly but are dispensable for maturation and maintenance in *C. elegans*. *J Cell Biol*, 216(6):1659–1671.
21. Wei, H.-C., Rollins, J., Fabian, L., Hayes, M., Polevoy, G., Bazinet, C., and Brill, J. A. (2008). Depletion of plasma membrane PtdIns (4, 5) P2 reveals essential roles for phosphoinositides in flagellar biogenesis. *Journal of Cell Science*, 121(7):1076–1084.
22. Fabian, L., Wei, H.-C., Rollins, J., Noguchi, T., Blankenship, J. T., Bellamkonda, K., Polevoy, G., Gervais, L., Guichet, A., Fuller, M. T., et al. (2010). Phosphatidylinositol 4, 5-bisphosphate directs spermatid cell polarity and exocyst localization in *Drosophila*. *Molecular Biology of the Cell*, 21(9):1546–1555.

23. Balla, T. (2013). Phosphoinositides: tiny lipids with giant impact on cell regulation. *Physiological Reviews*, 93(3):1019–1137.
24. Nakatsu, F. (2015). A phosphoinositide code for primary cilia. *Developmental Cell*, 34(4):379–380.
25. Chávez, M., Ena, S., Van Sande, J., de Kerchove d’Exaerde, A., Schurmans, S., and Schiffmann, S. N. (2015). Modulation of ciliary phosphoinositide content regulates trafficking and sonic hedgehog signaling output. *Developmental Cell*, 34(3):338–350.
26. Garcia-Gonzalo, F. R., Phua, S. C., Roberson, E. C., Garcia, G., Abedin, M., Schurmans, S., Inoue, T., and Reiter, J. F. (2015). Phosphoinositides regulate ciliary protein trafficking to modulate hedgehog signaling. *Developmental Cell*, 34(4):400–409.
27. Conduit, S., Ramaswamy, V., Remke, M., Watkins, D., Wainwright, B., Taylor, M., Mitchell, C., and Dyson, J. (2017). A compartmentalized phosphoinositide signaling axis at cilia is regulated by INPP5E to maintain cilia and promote Sonic Hedgehog medulloblastoma. *Oncogene*, 36(43):5969.
28. Park, J., Lee, N., Kavoussi, A., Seo, J. T., Kim, C. H., and Moon, S. J. (2015). Ciliary phosphoinositide regulates ciliary protein trafficking in *Drosophila*. *Cell Reports*, 13(12):2808–2816.
29. Goshima, G., Wollman, R., Goodwin, S. S., Zhang, N., Scholey, J. M., Vale, R. D., and Stuurman, N. (2007). Genes required for mitotic spindle assembly in *Drosophila* S2 cells. *Science*, 316(5823):417–421.
30. Blachon, S., Cai, X., Roberts, K. A., Yang, K., Polyanovsky, A., Church, A., and Avidor-Reiss, T. (2009). A proximal centriole-like structure is present in *Drosophila* spermatids and can serve as a model to study centriole duplication. *Genetics*, 182(1):133–144.
31. Basto, R., Lau, J., Vinogradova, T., Gardiol, A., Woods, C. G., Khodjakov, A., and Raff, J. W. (2006). Flies without centrioles. *Cell*, 125(7):1375–1386.
32. Enjolras, C., Thomas, J., Chhin, B., Cortier, E., Duteyrat, J.-L., Soulavie, F., Kernan, M. J., Laurençon, A., and Durand, B. (2012). *Drosophila* chibby is required for basal body formation and ciliogenesis but not for Wg signaling. *J Cell Biol*, 197(2):313–325.
33. Vieillard, J., Paschaki, M., Duteyrat, J.-L., Augière, C., Cortier, E., Lapart, J.-A., Thomas, J., and Durand, B. (2016). Transition zone assembly and its contribution to axoneme formation in *Drosophila* male germ cells. *J Cell Biol*, 214(7):875–889.
34. Pratt, M. B., Titlow, J. S., Davis, I., Barker, A. R., Dawe, H. R., Raff, J. W., and Roque, H. (2016). *Drosophila* sensory cilia lacking MKS proteins exhibit striking defects in development but only subtle defects in adults. *J Cell Sci*, 129(20):3732–3743.
35. Terebiznik, M. R., Vieira, O. V., Marcus, S. L., Slade, A., Yip, C. M., Trimble, W. S., Meyer, T., Finlay, B. B., and Grinstein, S. (2002). Elimination of host cell PtdIns (4, 5) P 2 by bacterial SigD promotes membrane fission during invasion by *Salmonella*. *Nature Cell Biology*, 4(10):766.

36. Zhou, D., Chen, L.-M., Hernandez, L., Shears, S. B., and Galán, J. E. (2001). A Salmonella inositol polyphosphatase acts in conjunction with other bacterial effectors to promote host cell actin cytoskeleton rearrangements and bacterial internalization. *Molecular Microbiology*, 39(2):248–260.
37. Sengupta, S., Barber, T. R., Xia, H., Ready, D. F., and Hardie, R. C. (2013). Depletion of PtdIns (4, 5) P₂ underlies retinal degeneration in *Drosophila* trp mutants. *J Cell Sci*, 126(5):1247–1259.
38. Norris, F. A., Wilson, M. P., Wallis, T. S., Galyov, E. E., and Majerus, P. W. (1998). SopB, a protein required for virulence of *Salmonella* dublin, is an inositol phosphate phosphatase. *Proceedings of the National Academy of Sciences*, 95(24):14057–14059.
39. Baker, J. D., Adhikarakunnathu, S., and Kernan, M. J. (2004). Mechanosensory-defective, male-sterile unc mutants identify a novel basal body protein required for ciliogenesis in *Drosophila*. *Development*, 131(14):3411–3422.
40. Xu, Q., Zhang, Y., Wei, Q., Huang, Y., Hu, J., and Ling, K. (2016). Phosphatidylinositol phosphate kinase PIPKI γ and phosphatase INPP5E coordinate initiation of ciliogenesis. *Nature communications*, 7:10777.
41. Xu, Q., Zhang, Y., Xiong, X., Huang, Y., Salisbury, J. L., Hu, J., and Ling, K. (2014). PIPKI γ targets to the centrosome and restrains centriole duplication. *J Cell Sci*, 127(6):1293–1305.
42. Franz, A., Roque, H., Saurya, S., Dobbelaere, J., and Raff, J. W. (2013). CP110 exhibits novel regulatory activities during centriole assembly in *Drosophila*. *J Cell Biol*, 203(5):785–799.
43. Fabian, L. and Brill, J. A. (2012). *Drosophila* spermiogenesis: big things come from little packages. *Spermatogenesis*, 2(3):197–212.
44. Jana, S. C., Bettencourt-Dias, M., Durand, B., and Megraw, T. L. (2016). *Drosophila melanogaster* as a model for basal body research. *Cilia*, 5(1):22.
45. Lattao, R., Kovács, L., and Glover, D. M. (2017). The Centrioles, Centrosomes, Basal Bodies, and Cilia of *Drosophila melanogaster*. *Genetics*, 206(1):33–53.
46. Blachon, S., Gopalakrishnan, J., Omori, Y., Polyanovsky, A., Church, A., Nicastro, D., Malicki, J., and Avidor-Reiss, T. (2008). *Drosophila* asterless and vertebrate Cep152 Are orthologs essential for centriole duplication. *Genetics*, 180(4):2081–2094.
47. Dzhindzhev, N. S., Quan, D. Y., Weiskopf, K., Tzolovsky, G., Cunha-Ferreira, I., Riparbelli, M., Rodrigues-Martins, A., Bettencourt-Dias, M., Callaini, G., and Glover, D. M. (2010). Asterless is a scaffold for the onset of centriole assembly. *Nature*, 467(7316):714.
48. He, B., Xi, F., Zhang, X., Zhang, J., and Guo, W. (2007). Exo70 interacts with phospholipids and mediates the targeting of the exocyst to the plasma membrane. *The EMBO journal*, 26(18):4053–4065.
49. Chang, C.-W., Hsu, W.-B., Tsai, J.-J., Tang, C.-J. C., and Tang, T. K. (2016). CEP295 interacts with microtubules and is required for centriole elongation. *J Cell Sci*, 129(13):2501–2513.

50. Fu, J., Lipinszki, Z., Rangone, H., Min, M., Mykura, C., Chao-Chu, J., Schneider, S., Dzhindzhev, N. S., Gottardo, M., Riparbelli, M. G., et al. (2016). Conserved molecular interactions in centriole-to-centrosome conversion. *Nature Cell Biology*, 18(1):87.
51. Saurya, S., Roque, H., Novak, Z. A., Wainman, A., Aydogan, M. G., Volanakis, A., Sieber, B., Pinto, D. M. S., and Raff, J. W. (2016). Drosophila Ana1 is required for centrosome assembly and centriole elongation. *J Cell Sci*, 129(13):2514–2525.
52. Hammond, G. R., Fischer, M. J., Anderson, K. E., Holdich, J., Koteci, A., Balla, T., and Irvine, R. F. (2012). PI4P and PI (4, 5) P2 are essential but independent lipid determinants of membrane identity. *Science*, 337(6095):727–730.
53. Jao, L.-E., Akef, A., and Wentz, S. R. (2017). A role for Gle1, a regulator of DEAD-box RNA helicases, at centrosomes and basal bodies. *Molecular biology of the cell*, 28(1):120–127.
54. Zuo, X., Guo, W., and Lipschutz, J. H. (2009). The exocyst protein Sec10 is necessary for primary ciliogenesis and cystogenesis in vitro. *Molecular Biology of the Cell*, 20(10):2522–2529.
55. Lobo, G. P., Fulmer, D., Guo, L., Zuo, X., Dang, Y., Kim, S.-H., Su, Y., George, K., Obert, E., Fogelgren, B., et al. (2017). The exocyst is required for photoreceptor ciliogenesis and retinal development. *Journal of Biological Chemistry*, 292(36):14814–14826.
56. Seixas, C., Choi, S. Y., Polgar, N., Umberger, N. L., East, M. P., Zuo, X., Moreiras, H., Ghossoub, R., Benmerah, A., Kahn, R. A., et al. (2016). Arl13b and the exocyst interact synergistically in ciliogenesis. *Molecular Biology of the Cell*, 27(2):308–320.
57. Zhang, X., Orlando, K., He, B., Xi, F., Zhang, J., Zajac, A., and Guo, W. (2008). Membrane association and functional regulation of Sec3 by phospholipids and Cdc42. *The Journal of Cell Biology*, 180(1):145–158.
58. Dixon-Salazar, T. J., Silhavy, J. L., Udpa, N., Schroth, J., Bielas, S., Schaffer, A. E., Olvera, J., Bafna, V., Zaki, M. S., Abdel-Salam, G. H., et al. (2012). Exome sequencing can improve diagnosis and alter patient management. *Science Translational Medicine*, 4(138):138ra78–138ra78.
59. Wong, R., Hadjiyanni, I., Wei, H.-C., Polevoy, G., McBride, R., Sem, K.-P., and Brill, J. A. (2005). PIP2 hydrolysis and calcium release are required for cytokinesis in Drosophila spermatocytes. *Current Biology*, 15(15):1401–1406.
60. Giansanti, M. G., Vanderleest, T. E., Jewett, C. E., Sechi, S., Frappaolo, A., Fabian, L., Robinett, C. C., Brill, J. A., Loerke, D., Fuller, M. T., et al. (2015). Exocyst-dependent membrane addition is required for anaphase cell elongation and cytokinesis in Drosophila. *PLoS Genetics*, 11(11):e1005632.
61. Gervais, L., Claret, S., Januschke, J., Roth, S., and Guichet, A. (2008). PIP5K-dependent production of PIP2 sustains microtubule organization to establish polarized transport in the Drosophila oocyte. *Development*, 135(23):3829–3838.
62. Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., Dufayard, J.-F., Guindon, S., Lefort, V., Lescot, M., et al. (2008). Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic acids research*, 36(suppl_2):W465–W469.

63. Cenci, G., Bonaccorsi, S., Pisano, C., Verni, F., and Gatti, M. (1994). Chromatin and microtubule organization during premeiotic, meiotic and early postmeiotic stages of *Drosophila melanogaster* spermatogenesis. *Journal of Cell Science*, 107(12):3521–3534.

Figures

Figure 1

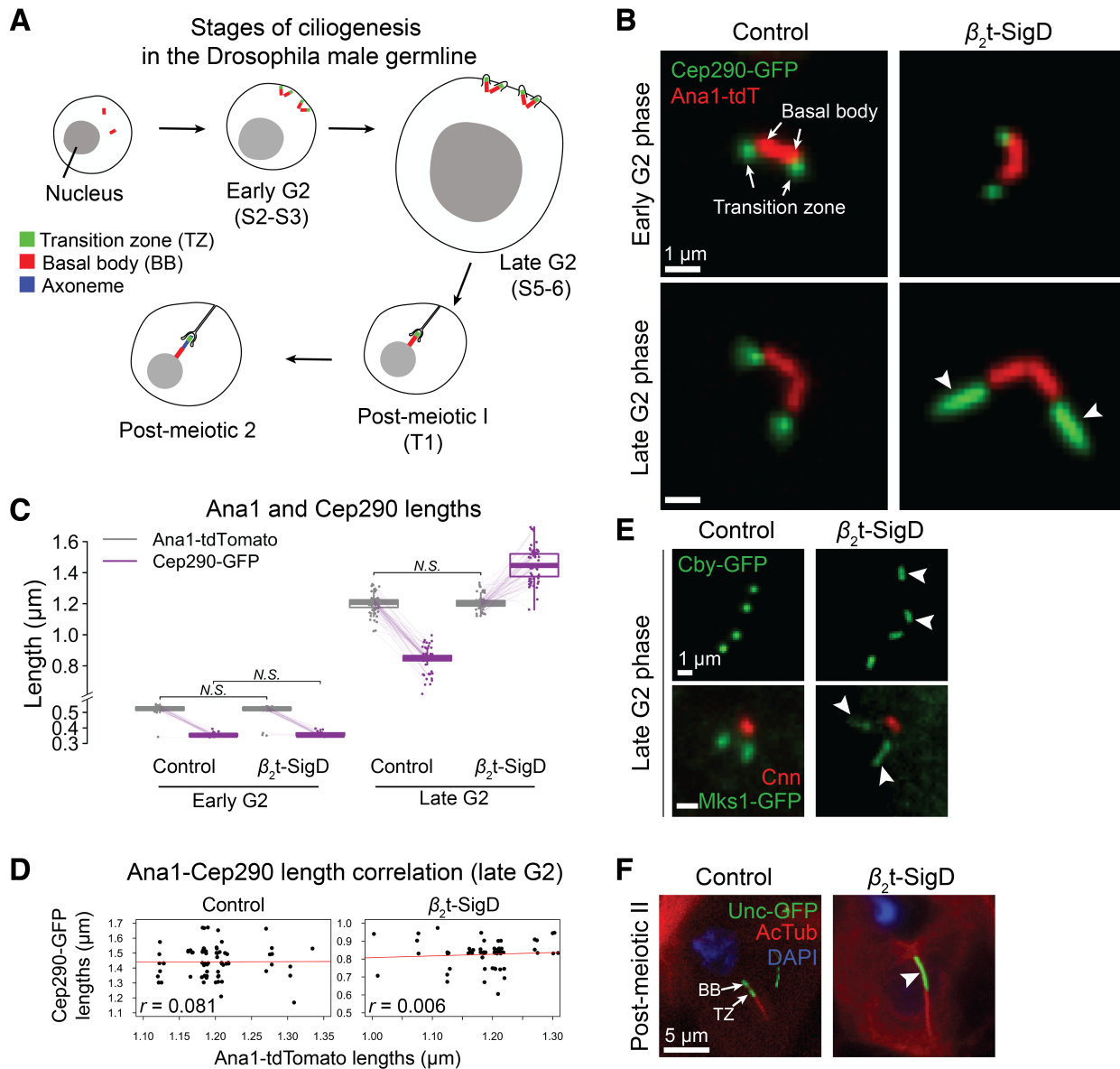


Figure 2

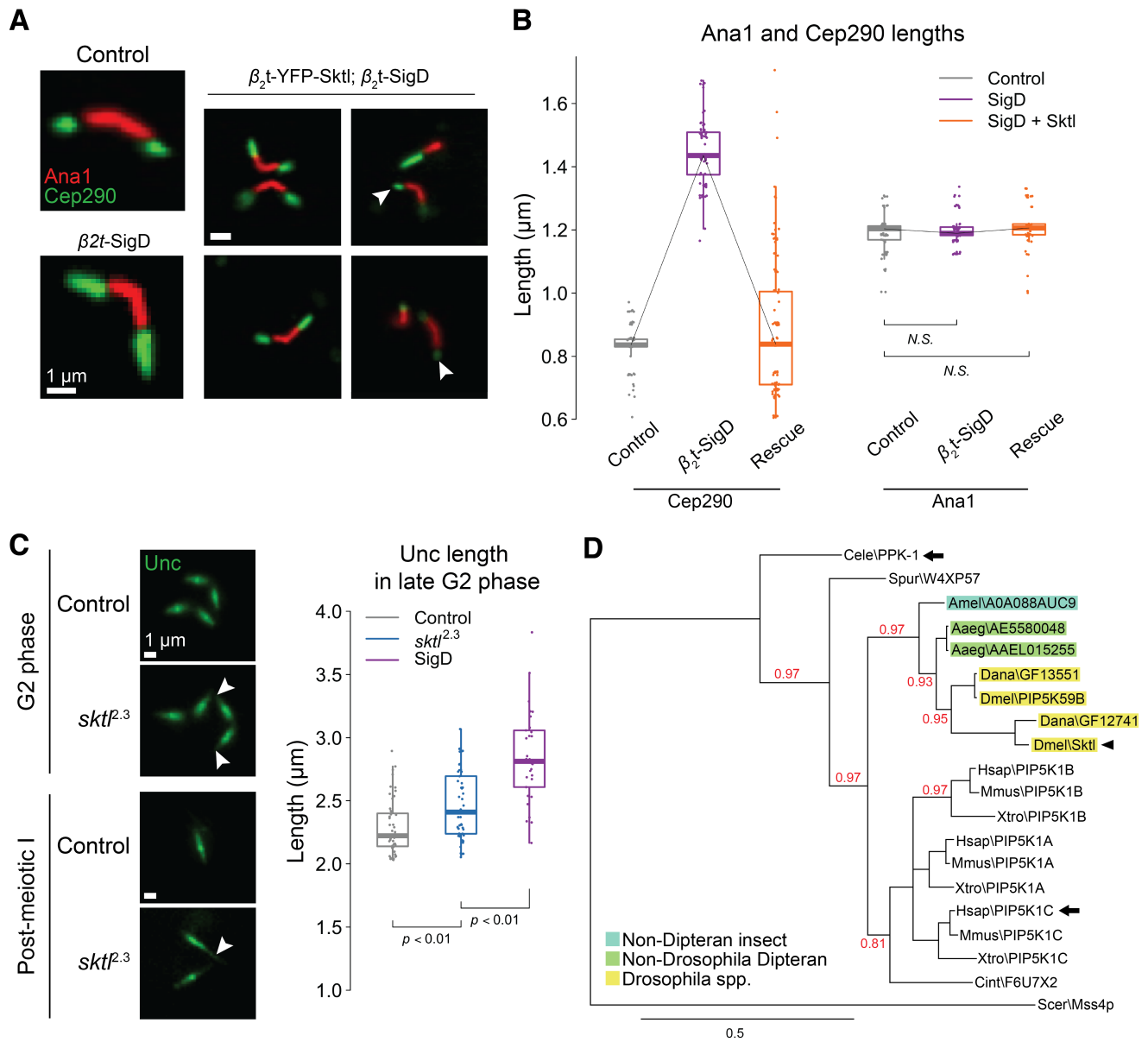


Figure 3

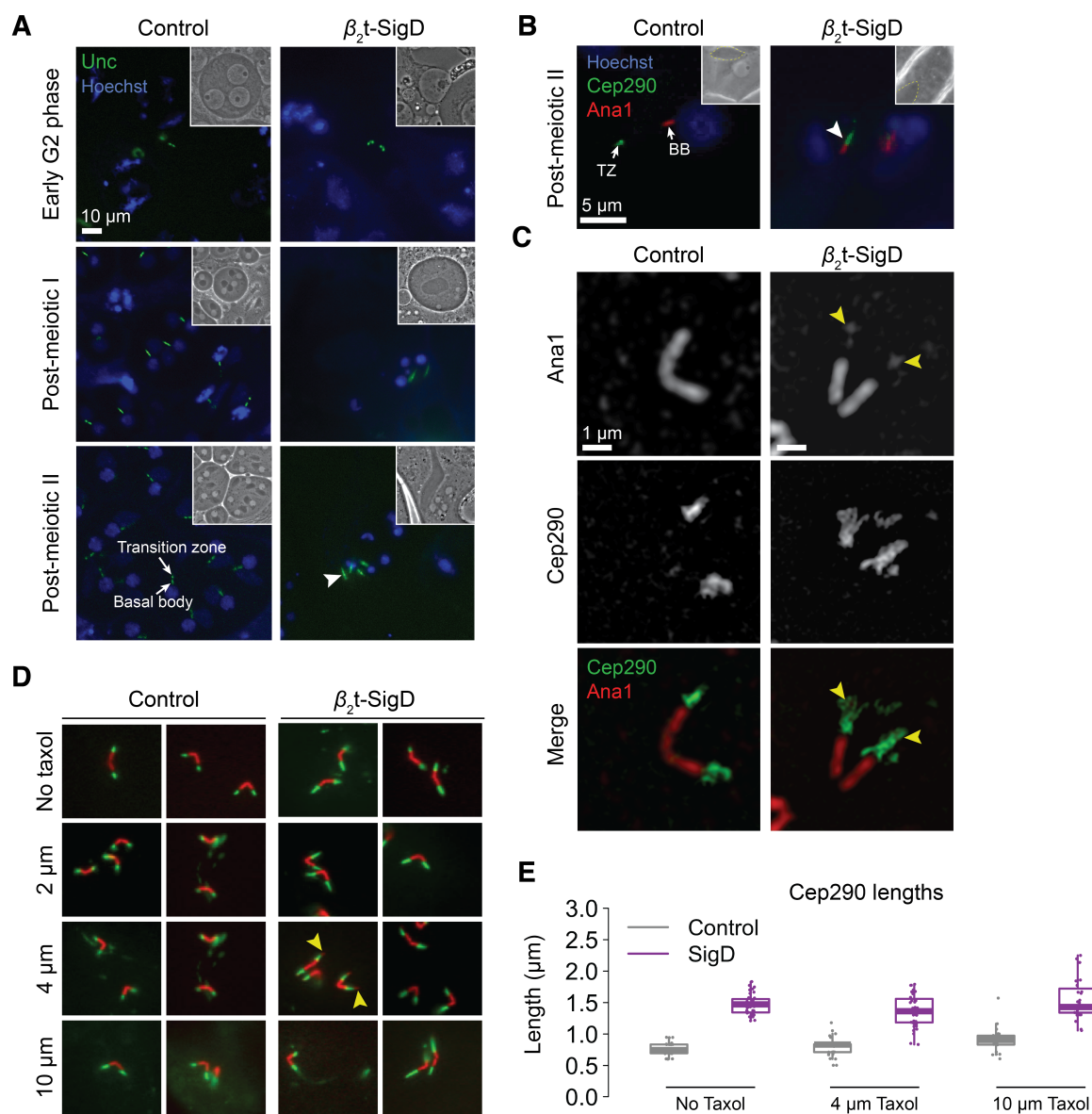


Figure 4

