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Supplemental Information

Klp10A, a Microtubule-Depolymerizing Kinesin-13, Cooperates with CP110 to Control *Drosophila* Centriole Length

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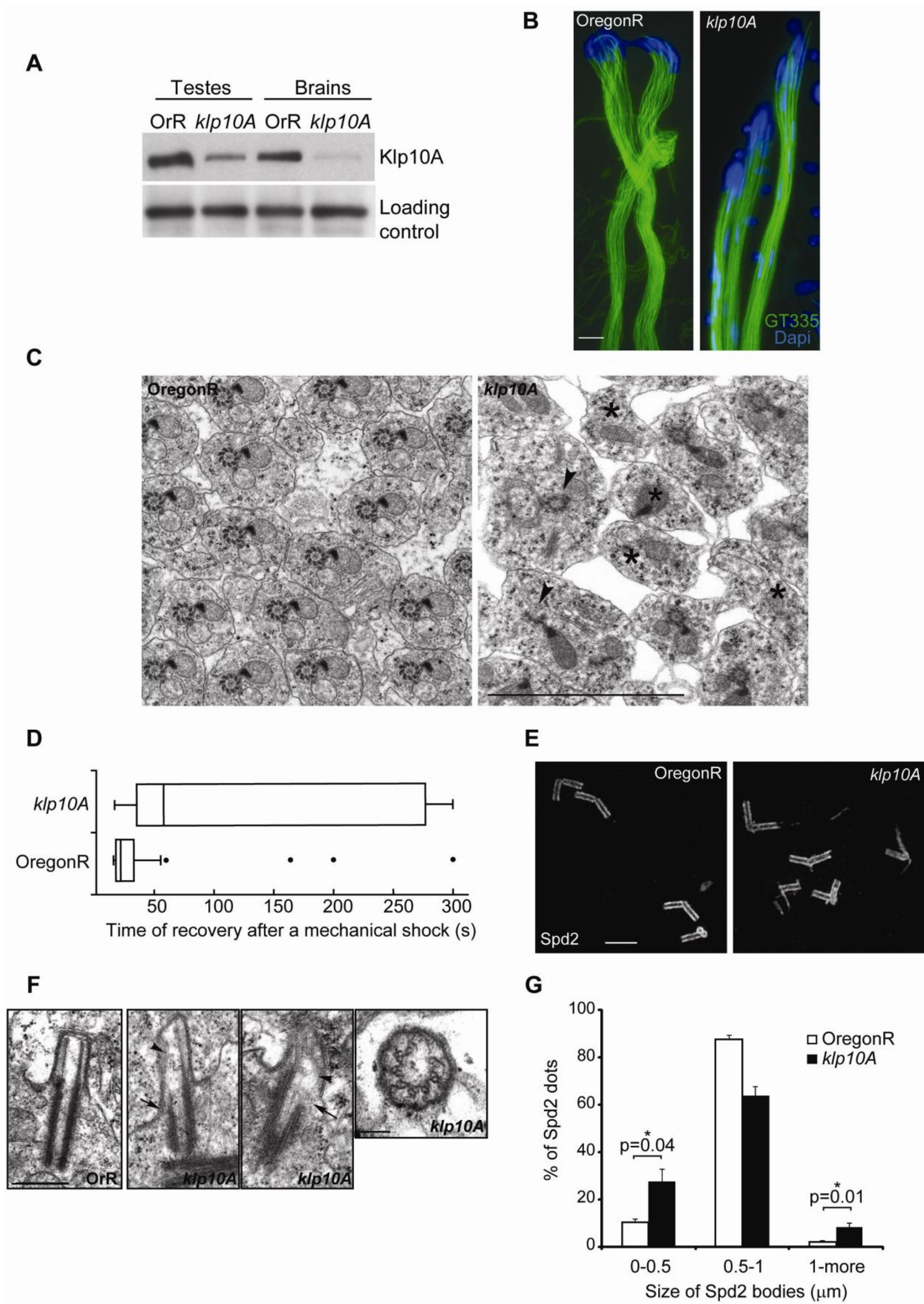
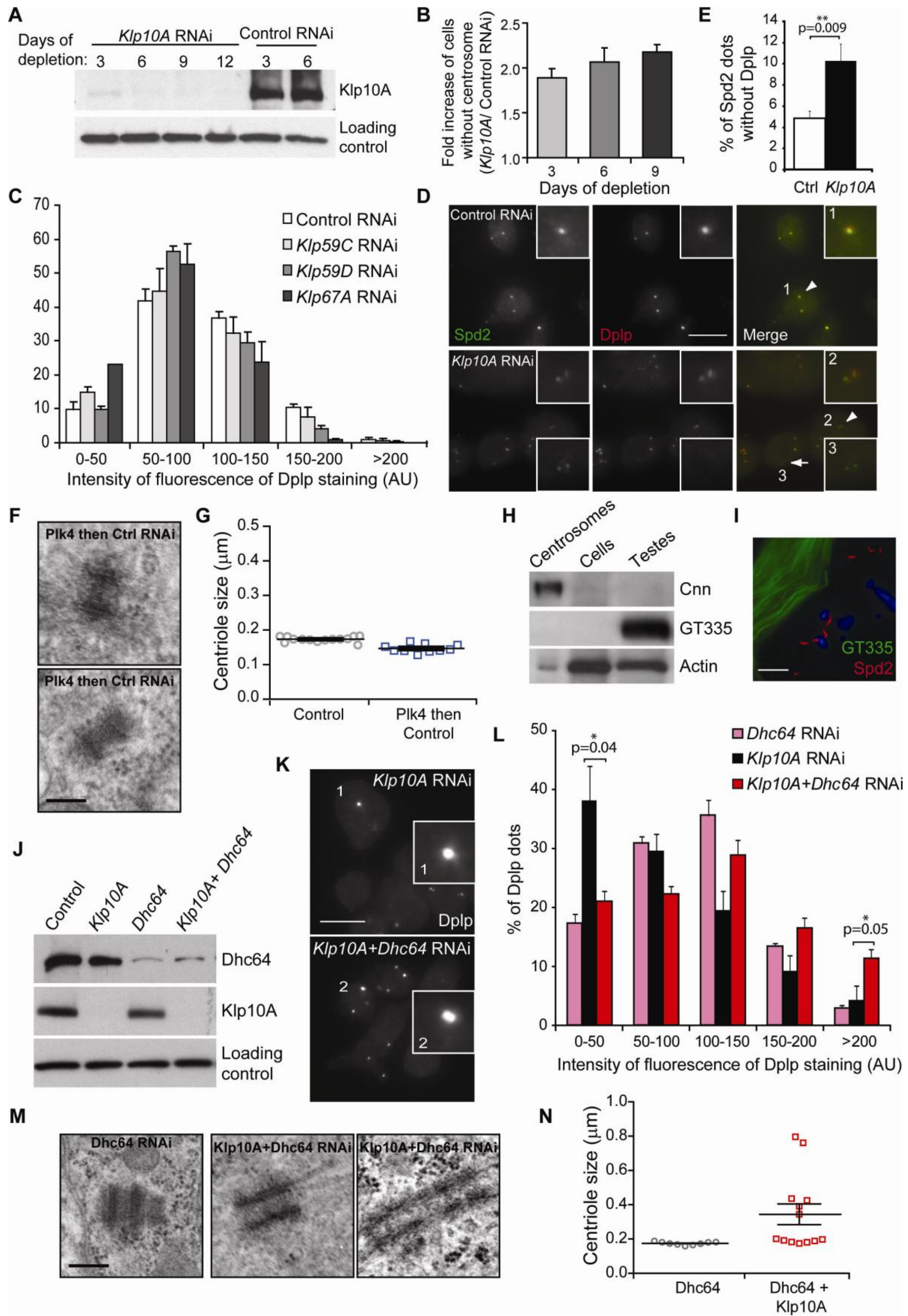


Figure S1.



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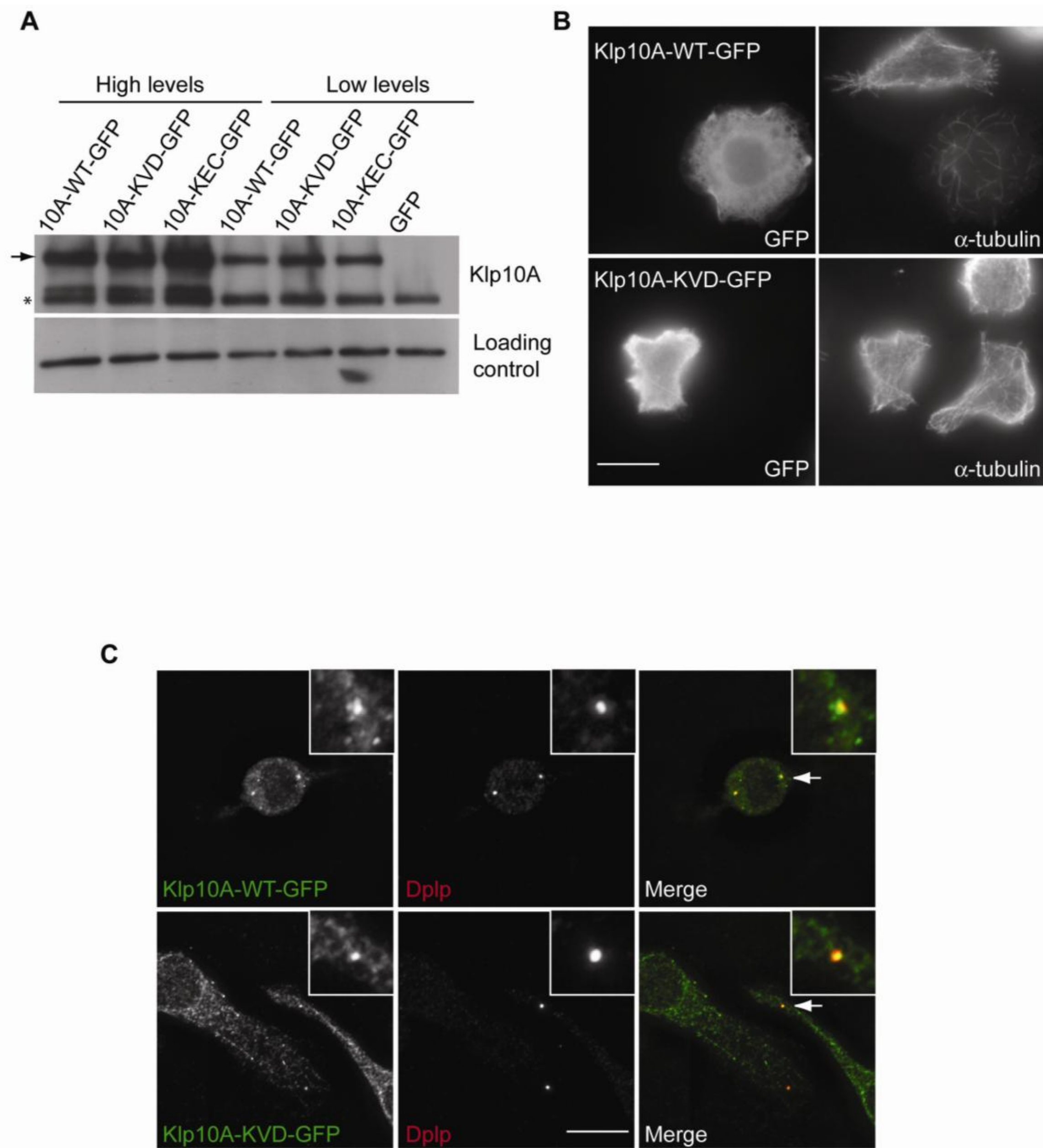

Figure S3.

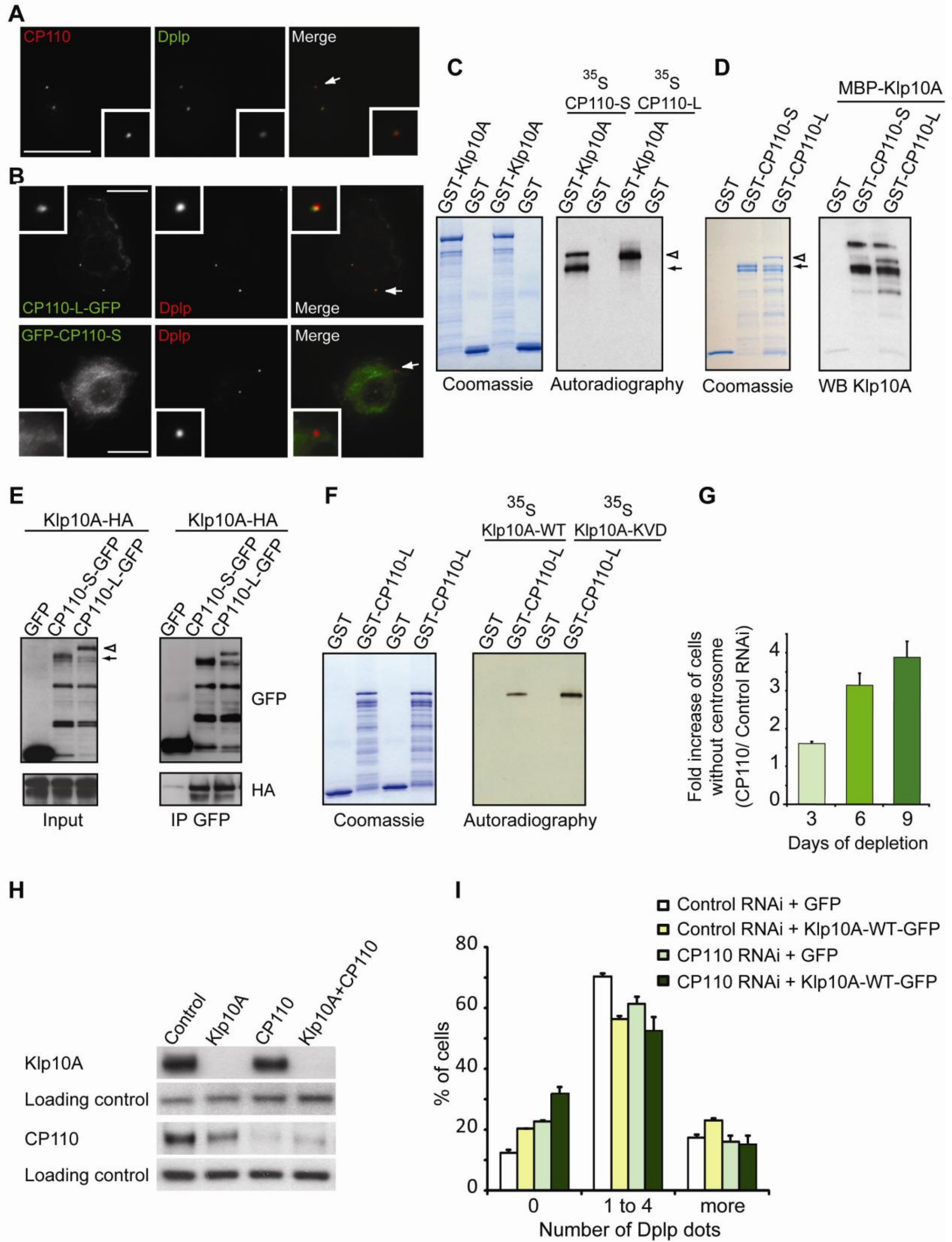

Figure S4.

Supplemental Figure Legends

Figure S1, related to Figure 1. Klp10A Levels, Spermiogenesis, and Coordination Defects in *Klp10A* Mutant Flies

A. Western blot analysis for Klp10A and α-tubulin (loading control) of wild-type (OregonR) and *Klp10A* mutant testis and brain extracts showing diminution of Klp10A levels in both mutant tissues.

B. Elongating spermatids of wild-type (OregonR) or *Klp10A* mutant flies stained for polyglutamylated tubulin (GT335, green) and Dapi (blue) showing thinner spermatid bundles and nuclei scattered along the length of cyst in the mutant testes. Flagella appear shorter in *Klp10A* mutant than in control flies : 1.76±0.009mm compared to 1.91±0.007mm. (Scale bar represents 10μm).

C. Electron microscopy showing cross-sections of elongating spermatids in wild-type (Oregon R) or *Klp10A* mutant flies. *Klp10A* mutant flies exhibit abnormal incomplete axonemes (arrowheads, 9%) or are completely missing axonemes (asterisks, 15%). (Scale bar represents 2μm).

D. Box-plot of the time required for the wild-type (OregonR) and *Klp10A* mutant flies to recover from a mechanical shock (the band inside the box represents the median, the box represents 50% of the data, with the whiskers it represents 75% of the data, the dots represents the outliers) indicating that *Klp10A* mutant flies are uncoordinated compared to the OregonR flies.

E. Structured-illumination microscopy of spermatocytes from wild-type (OregonR) or *Klp10A* mutant flies showing Spd2 stained centriolar structures. In *Klp10A* mutants centrioles appear frayed all along their lengths or at their extremity only. Some fragments remain attached to centriolar barrels while some others are fully dissociated. (Scale bar represents 2μm).

F. Electron micrograph of longitudinal sections of primary cilia showing incomplete basal bodies (arrows) and axonemes (arrowheads) in *Klp10A* mutant spermatocytes compared to wild-type (OrR). (Scale bar represents 0.5μm). Cross-section of a mutant primary cilium showing incomplete number of doublet. (Scale bar represents 0.1μm).

G. Size distribution of Spd2 bodies in spermatogonia of *Klp10A* and wild-type (OregonR) flies (error bars = SEM of 3 independent experiments; p values from Student's t-test; >700 Dplp bodies scored).

Figure S2, related to Figure 2. Centrosome Fragmentation in Cells Depleted of Klp10A Is Dynein Dependent, and Centriole Elongation in Cells Depleted of Klp10A Is Not a Consequence of De Novo Centriole Formation

A. Western blot of Klp10A and actin (loading control) in Dmel cell extracts 3, 6, 9 or 12 days after transfection with *Klp10A* or control RNAi.

B. Ratio of the number of cells without centrosomes between *Klp10A* RNAi and Control RNAi treatments after 3, 6 or 9 days showing that the number of cells without centrosomes stays constant with time (error bars = SEM of 3 independent experiments; >600 cells counted).

C. Distribution of the intensity of fluorescence of Dplp centrosomal dots in Dmel cells depleted for the kinesins Klp59C, Klp59D and Klp67A. None of the kinesin leads to the formation of bright Dplp-bodies (error bars=SEM of 2 independent experiments, >300 centrioles counted).

D. Representative images of cells immunostained to reveal Spd2 (green) and Dplp (red,) 6 days after transfection with control or *Klp10A* RNAi. After Klp10A depletion some dots were labeled with both centrosomal markers (arrowhead) whereas others were labeled with only one of them (arrow) (Scale bar represents 10µm). Numbers indicate the centrosomes in inset (magnification=3x).

E. Quantification of the number of centrosomal dots labeled with Spd2 but not Dplp as in D (error bars = SEM of 4 independent experiments; p value from Student's t-test; >250 dots quantified).

F and G. Electron micrograph of centrioles allowed to regrow in cells after transient depletion of Plk4. These centrioles formed *de novo* are not longer than the control population of centrioles. (Scale bar represents 0.25µm).

H. Western blot of centrosomes enriched fraction (Centrosome), Dmel cell protein extract (Cells) and testes protein extract (Testes) for CNN (centrosome marker), GT335 (polyglutamylated tubulin) and actin, reveals that *Drosophila* centrioles are not polyglutamylated in contrast to the sperm flagella.

I. Immunostaining of testis from wild-type flies showing polyglutamylated flagellar microtubules (detected with GT335 antibody, green) by contrast to centriolar microtubules (detected with Spd2, red but not GT335) (Dapi in blue, Scale bar represents 10µm).

J. Western blot analysis of cells treated with control, *Klp10A*, *Dynein* (Dhc64) or both *Klp10A* and *Dynein* RNAi revealing an almost complete depletion of Klp10A and partial depletion of Dynein.

K. Representative images of Dmel cells immunostained to reveal Dplp after treatment with *Dynein* (Dhc64) RNAi, with *Klp10A* RNAi or with both *Klp10A* and *Dhc64* RNAi. Numbers indicate centrosomes magnified in the inset (magnification=3x). (Scale bar represents 10µm).

L. Distribution of the intensity of fluorescence of dots labeled by Dplp after treatment with *Dhc64* or *Klp10A* or both *Klp10A* and *Dhc64* RNAi as above. Note the decrease in weakly stained Dplp dots and the increase of brightly stained Dplp dots when both Klp10A and Dynein are depleted compared to Klp10A only (error bars = SEM of 3 independent experiments; p values from Student's t-test, KS test p=0.0004, Chi^2 test $p=2.874\times10^{-7}$; >400 dots quantified).

M and N. Electron micrograph of cells depleted for Dhc64 or both Dhc64 and Klp10A showing very long centrioles in the co-depleted cells. (Scale bar represents 0.25µm). Size measurement of these centrioles on longitudinal sections reveals that Dhc64 depletion does not affect the length of centrioles whereas co-depletion of Dhc64 and Klp10A leads to the formation of longer centrioles than after depletion of Klp10A only (to compare with quantification in Fig. 4H).

Figure S3, related to Figure 3. Activity and Localization of Klp10A Wild-Type or Mutant Forms

A. Western blot of protein extracts from Dmel cells transiently transfected (over 24h) with Klp10A-GFP wild-type, or mutant (Klp10A-KVD-GFP and Klp10A-KEC-GFP) forms driven by Klp10A endogenous promoter at concentrations leading to elevated levels of expression (high levels) (arrow=GFP-tagged proteins, asterisk=endogenous Klp10A). Stable cell lines expressing lower levels of the same wild-type and mutant proteins were generated (low levels).

B. Representative images of cells transiently over-expressing wild-type (Klp10A-WT-GFP) or mutant (Klp10A-KVD-GFP) Klp10A immunostained for GFP (right panels) and microtubules

(α-tubulin, left panels). Over-expression of wild type but not mutant Klp10A leads to generalized depolymerization of interphase microtubules (Scale bar represents 10μm).

C. Immunostaining of Dmel cells stably expressing low levels of wild-type (Klp10A-WT-GFP) or mutant (Klp10A-KVD-GFP) Klp10A to reveal GFP (green) and Dplp (red) showing that both forms localise to the centrosome in interphase cells.

Figure S4, related to Figure 4. CP110 Has a Role in the Control of Centriole Size and Localizes to the Centrosome

A. Dmel cells were stained for CP110 (red) and Dplp (green) showing that endogenous CP110 localises to centrosome (Scale bar represents 10μm).

B. Dmel cells were transiently transfected with either the CP110 short (CP110-S) or long (CP110-L) isoform tagged with GFP. Representative images of cells 1 day after transfection immunostained to reveal Dplp (red) and GFP (green), showing that only the long isoform localises to the centrosome. (Scale bar represents 10μm).

C. Assay for binding of GST-Klp10A or GST, affinity purified from *E. coli* extracts (Coomassie stain) to ^{35}S-Methionine labeled CP110 isoforms (short (S) and long (L)) synthesized by coupled *in vitro* transcription-translation (Autoradiography). An additional band of higher apparent molecular weight is observed with time after *in vitro* translation of CP110 short isoform that we interpret to be a post-translational modification occurring in the reticulocyte lysate (arrow is short isoform; arrowhead, long isoform).

D. Assay for direct binding of GST, GST-CP110-S (arrow) or GST-CP110-L (arrowhead) affinity purified from *E.coli* on sepharose beads (Coomassie stain) with MBP-Klp10A purified from *E.coli* and eluted from amylose beads (Western Blots with anti-Klp10A antibody).

E. Western blots of extracts of Dmel cells transiently co-transfected with each of the GFP-tagged-CP110 isoforms (arrowhead=long isoform, arrow=short isoform) and Klp10A-HA and probed with anti-GFP or anti-HA antibodies. Input= left panels; anti-GFP immunoprecipitation=right panels, shows CP110 interacts with Klp10A.

F. *In vitro* binding assay between GST or GST CP110 long isoform (GST-CP110 L) affinity purified from *E.coli* (Coomassie stain) and ^{35}S-Methionine labeled Klp10A wild type (^{35}S Klp10A-WT) or motor mutant (^{35}S Klp10A-KVD) synthesized by coupled transcription-translation *in vitro* (Autoradiography). CP110 and Klp10A are interacting independently of Klp10A microtubule depolymerase activity.

G. Ratio of the number of cells without centrosomes between *CP110* RNAi and Control RNAi treatments after 3, 6 or 9 days showing that the number of cells without centrosomes increases with time (error bars = SEM of 3 independent experiments; >600 cells counted).

H. Western blot analysis of cells treated with control, *Klp10A*, *CP110* or both combined *Klp10A* and *CP110* RNAi showing the expression levels of the proteins after depletion.

I. Distribution of Dplp centrosomal dots in Dmel cells expressing Klp10A-GFP (under an inducible promoter) and then depleted for CP110. The number of centrosomes stained by Dplp was assessed 3 days after depletion and show an increased population of cells lacking centrosomes when Klp10A is overexpressed and CP110 depleted compared to single treatment (error bars=SEM of 2 independent experiments, >600 centrioles counted).

Table S1. Primers List

Gateway primers	
Klp10A for C-terminal fusion entry clone : HR16_AttB-KLP10A_FL_Cter_for	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGACATGATTACGGTGGGGCAGAGC
Klp10A for C-terminal fusion entry clone : HR17_AttB_KLP10A_FL_Cter_rev	GGGGACCACTTTGTACAAGAAAGCTGGGTCACGCTTGCCATTCGGCGAATTGAAG
Klp10A for N-terminal fusion entry clone : HR18_AttB_KLP10A_FL_Nter_for	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGACATGATTACGGTGGGGCAGA
Klp10A for N-terminal fusion entry clone : HR19_AttB_KLP10A_FL_Nter_rev	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAACGCTTGCCATTCGGCGAATTGAAG
Spd2 for N-terminal fusion entry clone: CG17286entryF	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGACAGTAGCAGTGGAAGCCAAG
Spd2 for N-terminal fusion entry clone: CG17286entryRstop	GGGGACCACTTTGTACAAGAAAGCTGGGTCTTAAAATTTAAAACTAATCGGGACACT
CP110 for C and N-terminal fusion entry clone: HR101_CP110_RA+RBFL_for	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCaccatgGATGCGACGTGGGC
CP110 short for C-terminal fusion entry clone : HR102_CP110_RAFL_Cter_rev	GGGGACCACTTTGTACAAGAAAGCTGGGTCGGGCTTCTTGCGATTGCC
CP110 short for N-terminal fusion entry clone : HR103_CP110_RAFL_Nter_rev	GGGGACCACTTTGTACAAGAAAGCTGGGTCctaGGGCTTCTTGCGATTGCC
CP110 long for C-terminal fusion entry clone : HR105_ CP110_RBFL_Cter _rev	GGGGACCACTTTGTACAAGAAAGCTGGGTCATCCAATCGGCGATGTTTGA
CP110 long for N-terminal fusion entry clone : HR104_ CP110_RBFL_Nter _rev	GGGGACCACTTTGTACAAGAAAGCTGGGTCctaATCCAATCGGCGATGTTTG
Cloning endogenous promoter-Klp10A-GFP	
HR90_KLP10A_KpnIFullEndogSeq_for	GGTGGTACCGCTTTCCCTTCAACGCACAAAC
HR93_ KLP10A_ Exon1BamHI_rev	CCGGATCCTGGTTCATCAG
HR100_KLP10A_Exon1_for	GCCGGAAACATGCTGAACAA
XhoI Klp10A (no stop, 2415) Rev	GCTTCTCGAGACGCTTGCCATTCGGCG
XhoI 4G GFP (1) Fw	GCTTCTCGAGGGAGGAGGAGGAATGGTGAGCAAGGGC
HIII 3UTR_SV40(1596)R	GCAAAGCTTGATCCAGACATGATAAGATACATTG
dsRNA Primers	
dsRNA Klp10A ORF: T7 Klp10A (1) Fw	TAATACGACTCACTATAGGGAGAATGGACATGATTACGGTG
dsRNA Klp10A ORF: T7 Klp10A (898 ex1) Rev	TAATACGACTCACTATAGGGAGACATCGATCTCCTTGCGATT
dsRNA 3'UTR Klp10A: T7 Klp10A (2427;3'U) Fw	TAATACGACTCACTATAGGGAGAGCATTCGACCCGTTC
dsRNA 3'UTR Klp10A: T7 Klp10A (2854; 3'U) Rev	TAATACGACTCACTATAGGGAGAACAATTAGAGTATCGACTGCG
dsRNA Dhc64: Dhc64C.f1	TAATACGACTCACTATAGGGAGAGCCTGGCCGATTTGCTTGGAAAGAT
dsRNA Dhc64: Dhc64C.r1	TAATACGACTCACTATAGGGAGAGTTGTTCTCAGAAGCCTGCTGAAGG
dsRNA Plk4: sak-7186 Fw	TAATACGACTCACTATAGGGAGAATACGGGAGGAATTTAAGCAAGTC
dsRNA Plk4: sak-7186 Rev	TAATACGACTCACTATAGGGAGATTATAACGCGTCGGAAGCAGTCT
dsRNA Control: GST-RNAi-F	TAATACGACTCACTATAGGGAGATTTGTATGAGCGCGATGAAG
dsRNA Control: GST-RNAi-R	TAATACGACTCACTATAGGGAGAATCCGATTTTGGAGGATGGT

dsRNA Control: GFPT7F	TAATACGACTCACTATAGGGAGACTTCAGCCGCTACCCC
dsRNA Control: GFPT7R	TAATACGACTCACTATAGGGAGATGTCGGGCAGCACG
dsRNA CP110: T7 CP110 (195ex3) Fw	TAATACGACTCACTATAGGGAGAATGACGAGCGCCAAG
dsRNA CP110: T7 CP110 (619ex3) Rev	TAATACGACTCACTATAGGGAGACCGTTGTGTCCTTTGT ATCC
dsRNA Klp59C Fw	TAATACGACTCACTATAGGGATGGATAAGTTGTCGATCG AA
dsRNA Klp59C rev	TAATACGACTCACTATAGGGTCCAGTTTGTCCGTAGGCG
dsRNA Klp59D Fw	TAATACGACTCACTATAGGGGGATCGCATCAAAATTGG
dsRNA Klp59D Rev	TAATACGACTCACTATAGGGCGTAGACCAGCGCATTG
dsRNA Klp67A Fw	TAATACGACTCACTATAGGGTGAAGTAACAACTATTCCGC
dsRNA Klp67A FRev	TAATACGACTCACTATAGGGTGCTTGGATGAGGGCAAGG
dsRNA Sas6 Fw	TAATACGACTCACTATAGGGAGATGTAGTGCGCCATGCT GAAGGAC
dsRNA Sas6 Rev	TAATACGACTCACTATAGGGAGAGCTGCGCTGCTCGTTT ATTTTG
Directed mutagnesis primers	
Klp10A_KVD317AAA_F	CACGAGCCGCGCAGCGCGGCCGCCCTCACCAAGTTCCT
Klp10A_KVD317AAA_R	AGGAACTTGGTGAGGGCGGCCGCGCTGCGCGGCTCGTG
Klp10A_KEC546AAA_F	CAAATCGCTGCTGGCCCTCGCGGCCGCCATTCGTGCGTT GGGCAAA
Klp10A_KEC546AAA_R	TTTGCCCAACGCACGAATGGCGGCCGCGAGGGCCAGCA GCGATTTG
T7 promoter primers	
CP110 flanked with T7 sequences : HR107_CP110_RA+RBFL_T7_for	TAATACGACTCACTATAGGGAGAATGGATGCGACGTGG GC
CP110 short flanked with T7 sequences : HR108_ CP110_RAFL_stop _rev	CTAGGGCTTCTTGCGATTGCC
CP110 long flanked with T7 sequences : HR109_ CP110_RB+RDFL_stop _rev	CTAATCCAATCGGCGATGTTTG
Klp10A flanked with T7 sequences : HR117_Klp10A_ FL_T7_for	TAATACGACTCACTATAGGGAGAATGGACATGATTACG GTGGGGC
Klp10A flanked with T7 sequences :HR118_ Klp10A_FL_stop _rev	CTAACGCTTGCCATTCGGCG

Supplemental Experimental Procedures

Fly Stocks

OregonR flies were used as wild type. The *Klp10A* mutant line, carrying the *ThbA* allele, was obtained from the study described in [9].

Mobilization of the P-element with a P-element transposase restored the male fertility exhibited by *Klp10A* mutant flies. The long centrioles frequently observed in the spermatogonia of *Klp10A* mutant flies were never observed in the lines obtained after remobilization.

Cell Culture

Dmel cells were grown at 25°C in Express Five SFM Drosophila media (Invitrogen) complemented with L-glutamine (2mM, Gibco) and penicillin-streptomycin (50000units/L-50000µg/L, Gibco).

dsRNA Experiments

dsRNA against Klp10A, Dhc64, Plk4, Klp59C, Klp59D, Klp67A, Sas6 and GFP or GST (as control) were made from oligodT cDNA generated from Dmel cells or plasmid DNA. A list of primer pairs is given in the primers list (Table S1). Cells were plated at $1.5x10^6$ cells in 6-well plates a few hours before transfection with 25µg of dsRNA in 10µl of H_2O. For co-depletions, 25µg of each dsRNA were used and single dsRNAs were supplemented with 25µg of control dsRNA. dsRNAs were incubated with 20µl of Transfast (Promega) and 970µl of media for 15min before transfection [36]. The dsRNA solution (1ml mix) was then incubated on the cells for 1h prior to the addition of 3ml of media. Cells were harvested after 3 days and re-submitted to the same transfection protocol where indicated.

Constructs

The Klp10A entry clone, the CP110 entry clone and MTp-GFP-Spd2 constructs were generated according to the instructions for the Gateway system (see also primers list in Table S1). Klp10A (CG1453) and Spd2 (CG17286) cDNAs were obtained from DRGC clone LD29208 and LD24702 respectively (http://dgrc.cgb.indiana.edu/), whereas, CP110 (CG14617) long and short isoforms were generated from oligo dT cDNA generated from Dmel cells. Expression clones were obtained by LR reaction into pDEST15 (for GST N-terminal tag, Invitrogen), pKM596 (for MBP N-terminal tag, Addgene), pAHW (for C-terminal HA tag driven by the Actin5 promoter, DGRC), pAGW (for N-terminal GFP tag driven by the Actin5 promoter, DGRC), the vector for C-terminal GFP tag driven by the Actin5 promoter described in D'Avino et al [37], and pMTBlast-Dest13-CtermEGFP (for C-terminal GFP tag driven by metallothionein promoter) generated by Joao Rocha. Cloning of Klp10A driven by its own promoter was performed in two steps: 1) a PCR product, flanked with KpnI and BamHI for Klp10A promoter and beginning of exon1, was amplified from *Drosophila*'s genomic DNA. In parallel a PCR product flanked with BamHI and XhoI for Klp10A ORF was amplified from Klp10A entry clone. The fragments were cloned together into a pBS-SK+ vector between KpnI and XhoI (see primers list in Table S1). 2) For this last construct, a PCR product for GFP and the 3'UTR of SV40 from pHWG (DRGC) was cloned between XhoI and HindIII.

Mutations in Klp10A were made in the endogenous Klp10A promoter-Klp10A-GFP constructs with the quick-change XL Site-Directed Mutagenesis Kit (Stratagene, see primers list in Table S1).

Stable Cell Lines and Transient Transfections

Stable cell lines expressing MTp-GFP-Spd2 or endogenous promoter-Klp10A wt or mutant-GFP, were obtained as follow: $3x10^6$ cells were plated on a 6-well plate; a few hours later either 3µg of GFP-Spd2 or 0.5µg of endogenous promoter-Klp10A constructs were mixed with 0.5µg of picoblast vector (carrying the blasticydin resistance gene (Invitrogen)) in 100µl of H_2O. 15µl of Fugen-HD (Roche) was mixed with the DNA and incubated at room temperature for 15min. The mix was then added drop by drop to the cells. 48h later, the cells were harvested re-inoculated in a 6-well plate in media containing 20µg/ml of blasticydin. Cells were maintained with this concentration of antibiotic for 10 days.

Transient transfections were performed to assess the effect of over-expressing endogenous promoter-Klp10A wt or mutants-GFP using 5µg of the plasmid-DNA of interest. The same protocol was used as with the generation of stable cell lines but without co-transfection with the picoblast. Cells were harvested 48h after transfection and used for immunofluorescence analysis.

Expression of Klp10A-GFP from the metallothionein promoter was induced with 75µM $CuS0_4$ for 3 or 6 days.

Antibodies

The following antibodies were used: Chicken anti-Dplp [20], immunofluorescence (IF) 1/2000; rabbit anti-Dplp kindly provided by Jordan Raff [10], IF 1/300; chicken anti-Sas6 [38], IF 1/1000; rabbit anti-Spd2 [38], IF 1/2000; rabbit anti-Klp10A (rabbit 57) [13] , IF 1/400, WB 1/4000; mouse anti-alpha-tubulin (DM1A, affinity purified, Sigma), IF 1/1000, WB 1/10000; mouse anti-Dhc64 kindly provided by Tom Hays [39], WB 1/2000; mouse anti-GFP (Roche), IF 1/700, WB 1/1000; rabbit anti-centrosomin (Cnn, rabbit 7647) [16], WB 1/500; mouse anti-polyglutamylated tubulin antibody (GT335) kindly provided by Carsten Jenke [40], IF 1/2500, WB 1/500; rabbit anti-actin (A2066 Sigma), WB 1/2000; GFP rabbit A6455 Molecular Probes, IP 1/72; mouse HA-11 Covance Ab (clone 16B12), WB 1/1000; rabbit anti-CP110 antibody produced by Guojie Mao and affinity purified on a membrane carrying the antigen, IF 1/600, WB 1/1000.

The secondary antibodies used were conjugated with Rhodamine Redex (Jackson Immunochemicals), Alexa 488 or Alexa 350 (Molecular Probes, 1/700) and peroxidase (Jackson Immunochemicals, 1/10000).

Immunofluorescence and Fluorescence Microscopy

Testes from young pupae were dissected in PBS, transferred in PBS-5% glycerol before being squashed between slide and coverslip. After snap freezing in liquid nitrogen, testes on slides were fixed in methanol, quickly rehydrated in PBS-0.5% Triton-X100, rinse for 10min in PBS and used immediately for staining.

Dmel cells were harvested and plated on 13mm diameter glass coverslips in a 24-well plate either at $3x10^5$ cells per well 3h before fixation or at $1.5x10^5$ cells per well the day before. Generally, cells were then pre-extracted 10s in 0.1% NP40 in BRB80 buffer (80mM K-Pipes pH6.8, 1mM $MgCl_2$, 1mM Na-EDTA pH8) and immediately fixed in BRB80-4% formaldehyde for 20min. They were then permeabilized in BRB80-0.1% Triton-X100 for 10min.

Staining of cells on coverslips or testes on slides was performed as follows: cells or testis were incubated for 1h at room temperature or overnight at 4°C respectively, in primary antibodies solution and 45min or 3h respectively, in secondary antibodies solution at room

temperature. Antibodies were diluted in PBS containing 0.1% Tween 20-3% BSA. Samples were washed after each incubation in PBS-0.1% Tween 20. Finally, cells were rinsed in water and mounted on slides in Vectashield (with or without DAPI) (Vector Laboratories).

Images were acquired using a Zeiss Axiovert 200M microscope using a 100x1.4 NA objective and a Coolsnap HQ2 camera controlled by Metamorph software (Universal Imaging). Figures shown are the maximum-intensity projections of optical sections acquired at 0.25µm z steps.

Centriole size was assessed on spermatogonia stained for Dplp or Spd2 by measuring the length of the dot using Metamorph software.

Staining was used to score the number of Dplp dots as 0, 1 to 4 or more than 4 Dplp dots per cells. In cells, the categories 1 to 4 dots were plotted together as a centrosome (and not a centriole) marker was used.

Quantification of Fluorescence Intensity on Digital Images

Fluorescence intensity of the centrosome of cells stained for Dplp or Spd2 was determined on digital images. Dplp and Spd2 were used since interphase Drosophila cells have very limited amount of PCM material and all other antibodies tested either did not recognise the interphase centriole or gave a high background. Average fluorescence-intensity values were measured on maximum intensity projections (image depth:16bits) in a constant region (305 pixels) centred on the centrosome using Metamorph software. Images were acquired under conditions that ensured that saturation was not reached. Only the centrosomes in focus after maximum intensity projection of 21 Z planes acquired at 0.25µm z steps were measured. An average fluorescence-intensity value for a background area of the same size near each Dplp dot was subtracted for each dot measured. In each experiment, the intensity of fluorescence of each Dplp dot is normalized to the mean value of fluorescence intensity of the control set to 100 (arbitrary units). The intensities of fluorescence of the centrosomes in the different samples are analyzed through their distribution from low to high intensity. The binning interval was chosen to have <50 that is half the size of the average centriole in the control (duplicating centrioles, PCM satellites would be likely to be included in this category) and >200 as centriole just having duplicated by not yet disengaged are likely to be twice as bright than the average control centriole. Cells in mitosis (recognized by a Phospho-histone H3 staining) were not included in the quantification since PCM is likely to accumulate at mitosis onset.

Structured-Illumination Microscopy

Images were acquired using DeltaVision OMX 3D-SIM System V3 (Applied Precision) with a 100x1.4 NA objective. Sections were aquired at 0.125µm z steps and their number was adjusted to accommodate the volume of the centrioles. Raw 3-phase images were rendered and reconstructed in 3D by softWoRx 5.0.0 (Applied Precision) software. Images were then processed in ImageJ software for maximum-intensity projections. To generate longitudinal view of centrioles, 3D images generated by softWoRx software were rotated 90°C around the Y axis with the VolumeViewer function. Centriole size was assessed on the longitudinal views of centrioles by measuring the length of the barrel using ImageJ software.

Time-Lapse Analysis

Time lapse recordings were carried out using a Zeiss Axiovert 200M microscope using a 100x1.4 NA objective and a Coolsnap HQ2 camera controlled by Metamorph software

(Universal Imaging). 11 fluorescence images were acquired at a *z*-distance of 0.4µm between planes using 2x2 binning along with a single differential interference contrast image in the middle focal plane, every 20min. Cells stably expressing MTp-GFP-SPD2 and showing 2 centrosomes only for at least 40min were analyzed.

Preparation of Extracts and Western Blot

Protein extracts from tissue cultured cells were prepared after homogenization in 1D buffer (50mM Tris pH8, 150mM NaCl and 1%NP40) and SDS-PAGE sample buffer, boiled 5min and incubated with benzonase (Novagen) on ice for 20min to remove DNA. Extracts equivalent to $1.5x10^5$ cells were processed for Western-Blot analysis.

Brains from third instar larvae and testis from pharate adult were dissected in PBS and kept at -80°C. Tissues were pestle homogenized in 1D lysis buffer, incubated on ice for 20min and centrifuged at 13000rpm for 10min. Soluble proteins fractions were quantified by Bradford assay (BIO-RAD Protein Assay, Biorad) and 10µg of soluble proteins extracts were processed for Western-Blot analysis.

Centrosomes enriched fractions were obtained from 0-2h collections of syncytial embryos according to the protocol described in [41]. Briefly, embryos were dechorionated and homogenized with a Dounce homogenizer in homogenization buffer (400mM K-Pipes pH6.8, 5mM $MgCl_2$, 5mM Na_2EGTA pH8, 100mM KCl, 14% sucrose, 1mM PMSF and protease inhibitors). Homogenate was centrifuged twice at 3000g for 10min and the supernatant equilibrated at 50% sucrose with 0.5% Triton-X100. Extract was then loaded onto a discontinuous 70%-55% sucrose gradient (containing 1mM GTP) and submitted to ultracentrifugation during 1h30 at 27000rpm. Fractions were collected from the bottom of the gradient and centrosomes enriched fractions were identified by Western-Blot with centrosomal markers such as centrosomin.

Cell or fly extracts were loaded onto SDS-PAGE (ProGel Tris Glycin 8-16%, Anamed) and transferred onto nitrocellulose membranes (Hybond ECL, Amersham Biosciences). Membranes were blocked with TBS-0.2% Tween20-3% BSA for 30min at room temperature. Incubation with primary or secondary antibodies diluted into TBS-0.2% Tween20-3% BSA were performed at 4°C overnight or 1h at room temperature respectively. Peroxidase activity was detected with Amersham ECL plus western blotting detection system (GE healthcare).

In Vitro Binding Assay

PCR products flanked with T7 RNA polymerase promoter sequences were generated from cDNA for CP110 isoforms and Klp10A. These PCR products were then gel extracted and purified (Quiagen) before use in *in vitro* transcription/translation assay based on reticulocyte lysate (TNT-T7 Quick coupled Transcription/Translation System, Promega). The incubation time was 1h for CP110 translation and 1h30 for Klp10A with the mix provided by the manufacturer and supplemented with ^{35}S methionine (Perkin Elmer).

In vitro translated CP110 and Klp10A proteins were incubated with either GST-Klp10A or GST-CP110 long isoform respectively (or GST for control) in 300µl binding buffer (20mM Hepes pH7.5, 150mM NaCl, 1mM EDTA, 1mM DTT, 0.1% triton, proteases inhibitors) for 1h on ice with shaking every 10-15min. GST beads were then centrifuged for 10s at 5000g and washed 5 times in binding buffer. Final bead pellets were resuspended in SDS-PAGE buffer, boiled for 5min and loaded on SDS-PAGE (ProGel Tris Glycin 8-16%, Anamed). Gels were stained with Coomassie blue (Biorad) and exposed at -80°C for autoradiography.

GST-Klp10A and GST-CP110 long isoform were produced in BL21 AI *E.coli* (Invitrogen). Bacteria were lysed by sonication in sarkosyl containing buffer (20mM Hepes, 500mM NaCl, 1mM EDTA, 1mM DTT, 2% sarkosyl, protease inhibitors) to solubilise the proteins and extracts were incubated 20min on ice before addition of Triton-X100 to 4% and centrifugation at 10000g for 20min at 4°C. Soluble proteins were then purified with GS beads (Amersham).

MBP-Klp10A was produced in BL21 star *E.coli* (Invitrogen). Bacteria were lysed by sonication in PBS buffer with protease inhibitors and extracts were incubated 20min on ice before centrifugation at 10000g for 20min at 4°C. Soluble proteins were then purified with amylose beads (New England Biolabs).

For the direct binding assay equivalent amounts (as detected on Coomassie stained gel) of GST-CP110 purified on sepharose beads and of MBP-Klp10A eluted from the amylase resin were incubated for 1h30 on ice with shaking every 10-15min in binding buffer (20mM NaHepes pH7.5, 150mM NaCl, 1mM Na-EDTA, 1mM DTT, 0.1% Triton-X100, protease inhibitors). The beads were spun and rinsed 5 times in binding buffer before being resuspended in SDS-PAGE buffer, boiled for 5min and loaded on SDS-PAGE (ProGel Tris Glycin 8-16%, Anamed).

Immunoprecipitation Assay

Before immunoprecipitation GFP-CP110 (or GFP only for control) and Klp10A-HA proteins were expressed in cultured cells by transient co-transfection.

24h after transfection cell pellets were collected and homogenized in 100µl of 2D lysis buffer (50mM Tris pH8, 150mM NaCl, 1%NP40 and 0.5% deoxycholic acid, sodium salt). Cells were lysed by 3 freeze/thaw cycles on dry ice and incubated for 15min on ice before centrifugation at 13000rpm for 6min at 4°C. 500µg of soluble proteins were then incubated overnight at 4°C with GFP antibody bound to Dynabeads Protein G in a total of 500µl 2D lysis buffer. Dynabeads Protein G (Invitrogen, 35µl per condition) were prepared the day before and incubated overnight at 4°C with the GFP antibody (7µl of rabbit polyclonal antibody). Proteins immunoprecipitated on beads were then washed 4 times in 2D lysis buffer and resuspended in 40µl of SDS-PAGE sample buffer before processing for western-blot analysis.

Uncoordination Assay

Klp10A mutant males or control males, aged 1 or 4 days old, were distributed between individual vials. Each vial was vortexed at maximum speed for 15s and time of recovery from the mechanical shock was measured as the time needed by the fly to right itself and go up. When flies were still at the bottom of the vial, time measurement was stopped at 300s and time of recovery for the fly was considered as 300s. When death occurred, flies were excluded from the analysis.

Electron Microscopy

Cultured cells on coverslips or testes dissected in phosphate buffer, fixed in 4% paraformaldehyde (EM grade) in PBS for 15min were immersed in 2.5% glutaraldehyde in 0.1M phosphate buffer (pH7.2) for 2h at 4ºC. They were then washed three times for 30min in phosphate buffer, postfixed with 1% OsO4 for 1h at 4ºC, washed once in phosphate buffer, and then in distilled water. Samples were stained for 1h in uranyl acetate. They were washed again and then dehydrated in a graded series of ethanols and flat-embedded in a mixture of Epon and Araldite. After polymerization for 2 days at 60ºC, the coverslips were removed from the resin

after a short immersion in liquid nitrogen. Ultrathin serial sections were obtained with a LKB ultratome, stained with uranyl acetate and lead citrate, and observed and photographed with a Philips CM10 electron microscope at 80kV.

Supplemental References

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