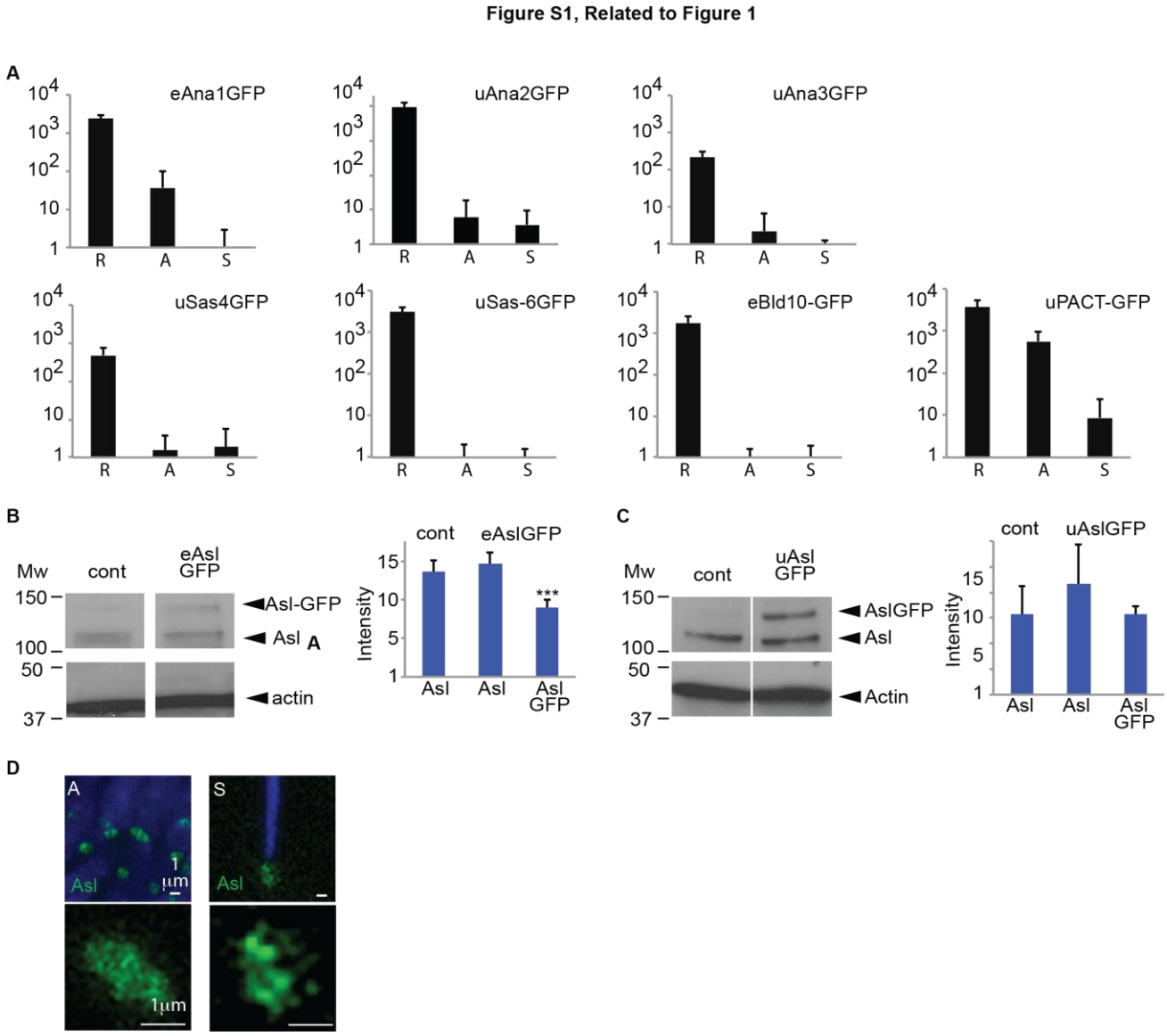
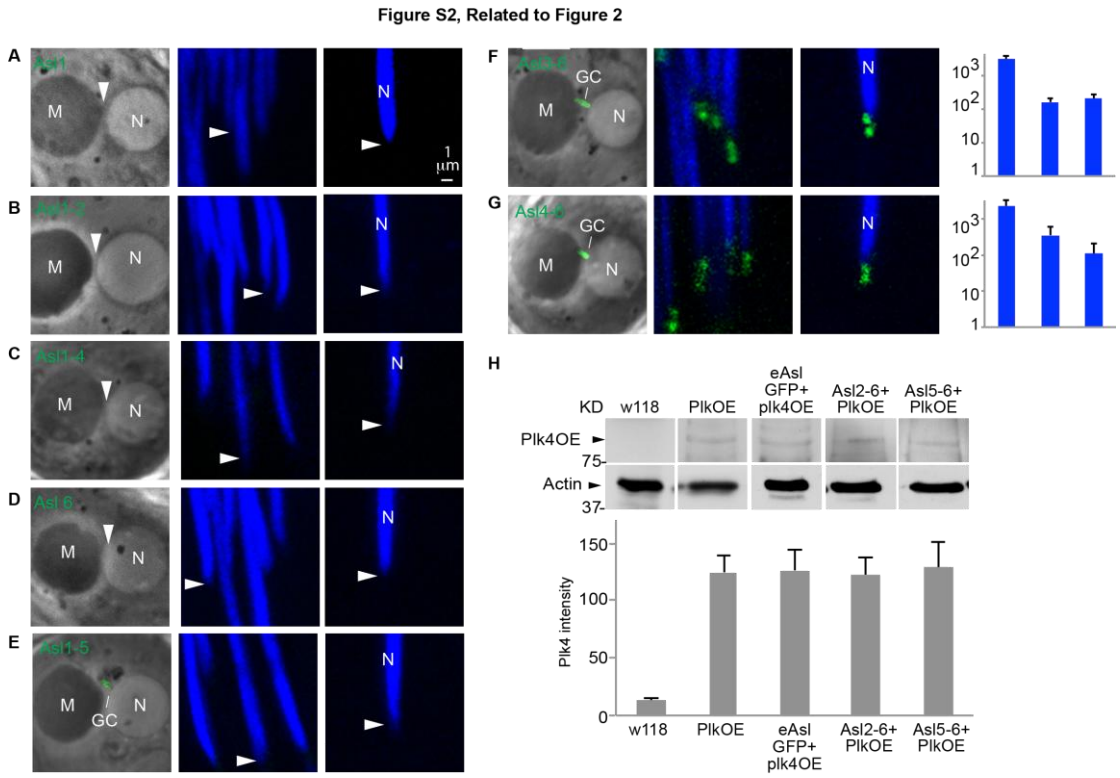


Supplemental Fig 1 (supplement to Fig 1).Asl is overexpressed in the testes of uAslGFP and eAslGFP.

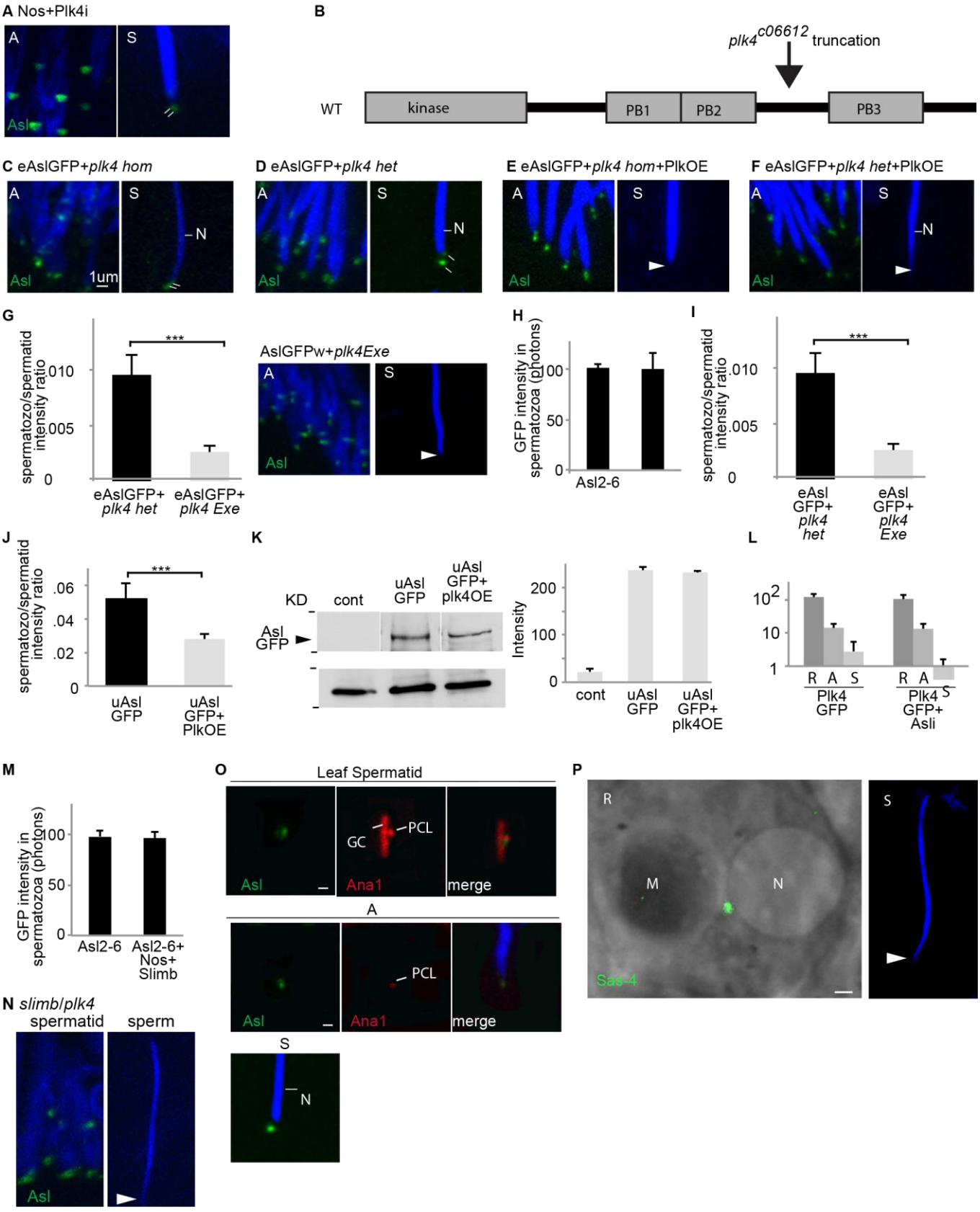
(A) Quantification of centrosomal protein reduction in *Drosophila* at three stages (R: round spermatid, A: almost-needle spermatid, or S: spermatozoa). Ana1 was expressed under the control of the endogenous Ana1 promoter. All other proteins were expressed under the control of the exogenous ubiquitin promoter [S1]. **(B-C**) Testes of control (cont), uAslGFP, and eAslGFP were analyzed by western blot using anti-Asl and anti-actin (loading control). Quantification of Asl intensity *N*=3, Mean±s.d.,two-sided *t*-test, ***$P<0.0001$.**(D**) Attenuated Asl reduction in uAslGFP and AslGFP is consistently observed in almost-needle spermatid (left two panels) and spermatozoa (right two panels). The size and location of Asl In the spermatozoa suggest that uAslGFP label both the GC and the PCL.



Supplemental Fig 2 (supplement to Fig 2). Asl truncation proteins.

(A–E) Representative figures of Asl truncation proteins (Asl1, Asl1-2, Asl1-4, Asl1-5, and Asl6) that do not appear to express during spermiogenesis.**(F-G**) Representative figures of Asl truncation proteins Asl3-6 and Asl4-6 that express during spermiogenesis and quantification of their reduction. N, nucleus; M, mitochondrion; an arrowhead points to where Asl would be expected to be in spermatids or spermatozoa lacking Asl. Scale bar, 5μm (round spermatids) and 1μm (almost-needle and spermatozoa).*N*≥5;Mean±s.d.Note that the fusion protein having only coiled-coil domain 6 lacked GFP localization even in the round spermatid stage (**Fig 2A**), suggesting that domain 5 is essential for centriole localization. Also note that of the fusion proteins having C-terminal deletions, only the fusion protein having Asl's coiled-coil domains1 to 5 (Asl1–5) retained GFP localization by the round spermatid stage; when compared to wild-type Asl, Asl1-5's GFP localization was prematurely lost by the almost-needle spermatid stage. Because this fusion protein's signal in round spermatid is weak, when compared to others, its absence at later stages likely represents the outcome of centrosome reduction that beginning at a lower signal intensity. **(H**)Plk4GFP level is not affected by expression of Asl deletion construct Asl2-6, and Asl5-6.Western blot using anti-GFP and anti-actin (loading control).Asl, were analyzed by Quantification of GFP intensity *N*=3, Mean±s.d., two-sided *t*-test, ***P<0.0001.cont, control,

Figure S3, Related to Figure 3

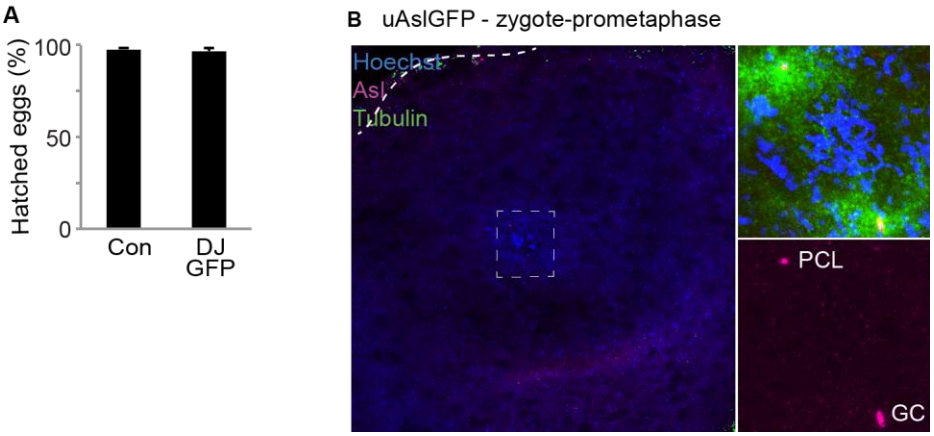


Supplemental Fig 3 (supplement to Fig 3).

Plk4 regulates Asl reduction.

(A)Plk4 knockdown by Nos-GAL4/UAS-Plk4 RNAi results in attenuated Asl reduction (Nos+Plk4i). Two lines point to the two Asl foci observed in the spermatozoa. **(B**) Plk4 domain organization and the *plkc*^{066122}mutation. An arrow points to the insertion site of the transposon in the *plk4*^{c06612} mutation. PB, polo-binding domain.(**C-F**) Spermatozoa homozygous(**C**) or heterozygous (**D**) for *plk4*^{c06612}have attenuated Asl reduction. However, overexpression of Plk4 (Plk4OE) in *plk4*^{c06612}homozygotes (hom) (**E**) or heterozygotes (het) (**F**) rescues the *plk4*^{c06612}–dependent attenuated Asl reduction. Two lines point to the two Asl foci observed in the spermatozoa. In some cases as in "C" one of the foci is longer and likely represents a GC labeled with Asl along its length. In other cases, both foci are small and may be a PCL and a partial labeling of the GC. (**G**)Excising the *plk4*^{c06612}transposon restores Asl reduction in eAslGFP flies. Two-sided *t*-test, ***P<0.001; *N*≥5;Mean±s.d.(**H**) Plk4OE does not affect the level of Asl2-6 in spermatozoon centrioles.(**I-K**) Plk4 overexpression enhances eAslGFP (**I**) and uAslGFP reduction (**J**).Two-sided *t*-test, ***P<0.001; *N*≥5;Mean±s.d.Plk4 overexpression does not affect expression of uAslGFP in the testes (**K**).**L**) Overexpression of Slimb using Nos-GAL4 does not affect the level of Asl2-6 in spermatozoa. (**M**) Asl RNAi (Asl) does not affect Plk4GFP reduction. (**N**) Flies heterozygous for both *slimb* and *plk4* mutations have undetectable uAslGFP in spermatozoa. **O-P**) Heterozygous mutants of *plk4* have attenuated Asl reduction (eAslGFP),but not attenuatedAna1 reduction **(O**) or Sas-4 reduction **(P**).N, nucleus; M, mitochondrion, A,almost-needle spermatids; S, spermatozoa. Scale bar, 5μm (round spermatids) and 1μm (leaf and almost-needle spermatids as well as spermatozoa).

Figure S4, Related to Figure 4



Supplemental Fig 4 (supplement to Fig 4).

Asl reduction is essential for embryo development

(A) Embryos fathered by control flies (w^{1118}) and Don Juan (DJGFP) expressing flies, produced embryos that hatched.

(B) During mitosis, zygotes fathered by spermatozoa with increased Asl (uAslGFP) have a bipolar spindle. Because of the delayed development in uAslGFP-fathered zygotes, embryos were collected every 4 minutes.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Transgenic Flies.

All *Drosophila* stocks were cultured on standard media at 25°C. For *in vivo* expression of Asl deletions, the *asl* promoter and cDNA were subcloned into a pUAS vector with an in-frame C-terminal GFP tag (for N-terminal deletions) or N-terminal GFP (for C-terminal deletions) tag such that Asl deletions were expressed using the Asl promoter in the absence of GAL4. Germline transformations were performed by BestGene Inc. (Chino Hills, CA), using w^{1118} flies. Plk4^{KD}GFP flies (D156N point mutation in Plk4-wt)were generated in pUAS vector using a pMT/V5-HisCplasmid obtained from Dr. Gregory Rogers [S2].

eAna1GFP, Ana1-td-tomato, Sas-4GFP and eAslGFP, were previously described and are expressed by their own promoters [S3, S4]. uAslGFP, Plk4GFP, uAna2GFP, uSas-4GFP, uSas 6GFP and uPACTGFP were expressed using a (strong) ubiquitin promoter and were provided by Jordan Raff [S5, S6]. Bld10 was expressed using own promoters promoter and was provided [S4]. Asl-RNAi (Bloomington stock# 38220) was expressed using Bam-Gal4 promoter. Bam-Gal4 fly line was provided by Yokiko Yamashita. SlimbGFP was expressed using a UAS promoter and were provided by Dr. Daniel St Johnston [S7].Piggybac Transposase(stock #32070), Nos-GAL4 (stock #7303), $slimb^{00295}$(stock #11493), and $plk4^{c06612}$ (stock #7774) were obtained from the Bloomington Stock Center.

Antibodies.

The following antibodies were used for immunofluorescence and western blots at the indicated concentrations.

Primary antibodies for immunofluorescence: E7-mouse anti-β-tubulin,1:200 (AB_2315513, DSHB at The University of Iowa); rabbit anti-Asl [S3].

Secondary antibodies for immunofluorescence:Cy™5 AffiniPure Goat Anti-Rabbit IgG (H+L), 1:200; Alexa Fluor® 488-conjugated donkey anti-rabbit IgG, 1:200 (Jackson ImmunoResearch).

Primary antibodies for western blot: anti-βactin (Abcam-ab8227), 1: 10000, anti-Asl,1:5000, and anti-GFP (Thermo Scientific, MA5-15256), 1: 2000.

Primary antibodies for immunoprecipitation: Ascites Mouse Anti-Ubiquitin (BD-Pharmingen-550944),1:25, and

Mouse p-Threonine(42H4) (Cell Signaling-9386S),1:50.

Fluorescence Microscopy.

Images were taken by Leica SP8 scanning confocal microscope as Z stacks. Maximal projection images were then modified using Adobe Photoshop and annotated using Adobe Illustrator.

Testes Fluorescence Microscopy.

Testes imaging was performed as described in Basiri *et al*. (2013) [S8]. Testes of pharate adult pupae were dissected in PBS, fixed in 3.7% formaldehyde in PBS for 5 min at room temperature, squashed with a coverslip, frozen in liquid nitrogen for 5 min, and washed with PBST(PBS+ 0.1% Triton).Antibodies were incubated in PBST-B(PBS + 0.1%Triton+ 1% BSA) for 1h at room temperature followed by three washes in PBS For photon counting, at least 5 testes were analyzed. In each testes, photons were counted from 5 centrioles at each stage. Pictures were taken in the photon counting mode using 63X objective with a zoom of 6, pin holeof 1.2, resolution of 512×512 pixel, 488 nm laser power of 1% of 20% and using measuring box of 1μm by 2.5μm that was placed on top of the centriole.

Embryo Fluorescence Microscopy.

Fifty male and fifty virgin female flies less than 5 days old were placed in an egg collection chamber with a grape agar plate supplemented with yeast paste. Chambers were used for 3 days and embryos collected every 4 min. Immediately after collection, the embryos were placed in a mac-tech dish and washed with 100μl of distilled water, and then washed in a wash buffer (0.7% NaCl+0.05% Triton 100-X). Afterwards, 50% bleach solution was added to the embryos until the appendages of the embryos disassociated. The embryos were rinsed twice with the wash buffer, fixed in a 1:1 solution of heptane and methanol, and shaken vigorously by vortex, until the embryos settled into the methanol layer. This was followed by removal of the fixative, and suspending the embryos in acetone. At this stage, the embryos were usually stored at -20°C. Prior to staining, embryos were rehydrated sequentially in 70%, 50%, 30%, and 10% methanol in PBS, and then in PBS alone. Embryos were then incubated in PBT (PBS+1% Triton) for 30 min, and blocked in PBST (PBT+3%BSA) for 1 h. Then the embryos were incubated with primary antibodies in PBST for 1 h at room temperature. After three 5-min washes in PBT, the embryos were incubated with secondary antibodies for 1 h at room temperature. The embryos were washed three times: first with PBT for 5 min, next with PBT Hoechst (1μg/ml) for 20 min, and

finally in PBS for 5 min. The embryos were mounted on a slide using a mounting medium (PBS, 50% glycerol, 0.5%N-propyl-gallate) and imaged.

Western blot

Testes were collected in PBS buffer and boiled for 5 min in 95 °C with 1X Laemmli buffer samples. Twelve testes were run per lane in 10% polyacrylamide gels and transferred into nitrocellulose membranes. The blots were incubated with primary antibodies overnight at 4°C followed by peroxidase-conjugated secondary antibodies at RT for 1h. Super Signal West Pico (for tubulin staining) or Femto (for Asl and GFP staining) Chemilluminescent substrate (Pierce) was used to detect peroxidase activity. Molecular masses were determined by comparison to Precision Plus Protein Standard (Bio-Rad). Western blots were analyzed with ImageJ (National Institute of Mental Health, Bethesda, Maryland, USA) and intensity of total area was calculated using an identical rectangular box.

Ubiquitination IP

Twenty testes were lysed in immunoprecipitation (IP) lysis buffer (50mM Tris-HCl [pH 7.5], 1% Triton X-100, 150mM NaCl, 0.1% 2-mercaptoethanol, 1mM Na_3VO_4, 1µM leupeptin, 1µM aprotinin, 1 mM PMSF) supplemented by Phosphatase inhibitor cocktail 3 (Sigma) diluted 1000 fold and *N*-Ethylmaleimide at a final concentration of 10 mM. Immunoprecipitation was carried out by adding to the lysed Protein-G Sepharose Beads (GE Healthcare) and the Anti-ubiquitin antibody. Anti-Asl antibody was used in the immunoblot.

Phosphorylation IP:

Fifty testes were lysed in Immunoprecipitation (IP) lysis buffer supplemented by Phosphatase inhibitor cocktail 3 (Sigma) diluted 1000 fold. Immunoprecipitation was carried out by adding to the lysed Protein-G Sepharose Beads (GE Healthcare) and the Anti-Phospho-Threonine antibody. Anti-Asl antibody was used in the immunoblot.

Larval hatching.

Five males and five females were placed in humidified mating chamber at 25°C to lay eggs for 24h. The parents were removed and embryos counted. Seventy-two hours later hatched eggs were counted and the

percent of hatched eggs was calculated. Experiments were repeated at least 5 times. Each experiment typically resulted in 80 or more embryos.

Embryo development.

Twenty-five males and twenty-five females were placed in humidified mating chamber at 25°C to lay eggs for 5 min. The parents were removed and embryos were placed for 1 h in a MatTek dish containing wash buffer (0.7% NaCl+0.05% Triton 100-X).The embryos were processed for embryo fluorescence microscopy staining.

Statistical Methods.

Statistical analyses were done with Excel and Graph Pad Prism 5. A two-tailed, unpaired Student's *t*-test (with samples that do not have equal variances) was used.

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