



Figure S1 Generating an embryo from *asl^{mecd}* homozygote oocytes (related to Figure 4). We recently showed that *asl^{mecd}*, unlike any other *Drosophila* mutant, completely blocks centriole duplication (BLACHON *et al.* 2008). Since the *asl^{mecd}* flies die shortly after emerging from pupa, these flies are not suitable for mating. This lethality can be avoided by using heterozygous *asl^{mecd}* females. However, these females provide wild type *asl* mRNA to their oocytes via the maternal contribution; thus, centriole duplication will not be completely blocked in these oocytes. To address this, we generated oocytes that completely lack the Asl protein except in heterozygous *asl^{mecd}* females. For this, we created germline *asl* mutant fly lines that lacked any *asl* maternal contribution. This was done using a FLP-FRT recombination technique and *ovo^{D1}* mutation that blocks the formation of oocytes that are not *asl^{mecd}* homozygotes (CHOU and PERRIMON 1996). Recombination was induced in larvae via a heat shock-inducible Flippase enzyme. Thus, as adults, they produced homozygous *asl^{mecd}* oocytes that lacked any Asl protein. Adult females were then mated to wild type males and their zygotes were analyzed.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Transgenic Flies

All *Drosophila* stocks were cultured on standard media at 25°C. Ana1-GFP, Ana1-tdtomato, Bld10-GFP, Asl-GFP, Sas-6-GFP, and Sas-4-GFP are expressed to near physiological levels using their corresponding promoter, and were previously described (BLACHON *et al.* 2009; BLACHON *et al.* 2008). Ana2-GFP is expressed under the strong ubiquitin promoter (STEVENS *et al.* 2010). Bloomington stock number 7 (P [hsFLP],y¹w¹¹¹⁸;;Dr^{Mio}/TM3,ry^{*}Sb) and 2149 (w^{*};;P[neoFRT]82B P[ovoD¹⁻¹⁸,W⁺]3R/TM3) were used to generate *asl^{mecD}* mutant embryos. Bloomington stock 5748 (P[neoFRT]82B *cu¹ sr¹ e^s ca¹*) and meiotic recombination was used to make the fly containing FRT82B and *asl^{mecD}*. It was selected for the presence of neomycin resistance and lack of *cu*, and was confirmed by failure to complement *asl^{mecD}*.

Antibodies

The following primary antibodies were used for immunofluorescence at the indicated concentrations: Guinea-pig anti-Cnn, 1:200 (a kind gift from Thomas C. Kaufman); mouse anti-β-tubulin, 1:50 (Developmental Studies Hybridoma Bank); Rabbit Anti-Asl, 1:200 (Ap1193. (BLACHON *et al.* 2008)); mouse anti-α-tubulin, 1:200 (Sigma); Rabbit anti-alpha-tubulin, rabbit anti-DSpd-2 1:200 (a kind gift from Maurizio Gatti); Rabbit anti-GFP (1:200; Fitzgerald Industries). The following secondary antibodies were used: Alexa Fluor® 488-conjugated goat anti-mouse IgG, 1:800; Alexa Fluor® 647-conjugated goat anti-guinea pig IgG, 1:800; Cyanine Cy3-conjugated goat anti-rabbit IgG, 1:800 ; Rhodamine goat anti-mouse 1:200; Rhodamine goat anti-rabbit 1:200 (Jackson ImmunoResearch). DAPI was used at final concentration of 1 µg/ml (Sigma).

Generating homozygote *asl^{mecD}* embryos

Three crosses were performed. In the first cross, 50 males having the FRT (Flippase recognition target) were crossed with the 50 females having Flippase enzyme coding gene (FLP) on the first chromosome. The resultant F1 generation males were selected which had FLP gene on the first chromosome and FRT site on the third chromosome along with TM3 balancer. In the second cross, 50 males from the F1 generation were then crossed with 50 females, which were heterozygous for *asl^{mecD}* having a FRT site along with *asl^{mecD}* on the third chromosome. After 3 days, the flies were moved to a new vial and the vial with embryos was subjected to heat shock at 37°C for 1 hour. The heat shock was performed each day for the next 3 days, thus activating the Flippase enzyme and carrying out the recombination reaction at the FRT site and making the resultant F2 homozygous for *asl^{mecD}* mutation. The females lacking TM6B (*Humeral⁺*) were selected from this F2 generation, and then were finally crossed with the wild type males and *asl^{mecD}* mutant embryos were collected.

SUPPLEMENTAL BIBLIOGRAPHY

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