File S1

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],y^{1} w^{1118};;Dr^{Mio}/TM3,ry* Sb) and 2149 (w*;;P[neoFRT]82B P[$ovoD^{1\text{-}18}$,W^{+}]3R/TM3) were used to generate asl^{mecD} mutant embryos. Bloomington stock 5748 (P[neoFRT]82B cu^{1} sr^{1} e^{s} ca^{1}) 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- -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.

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