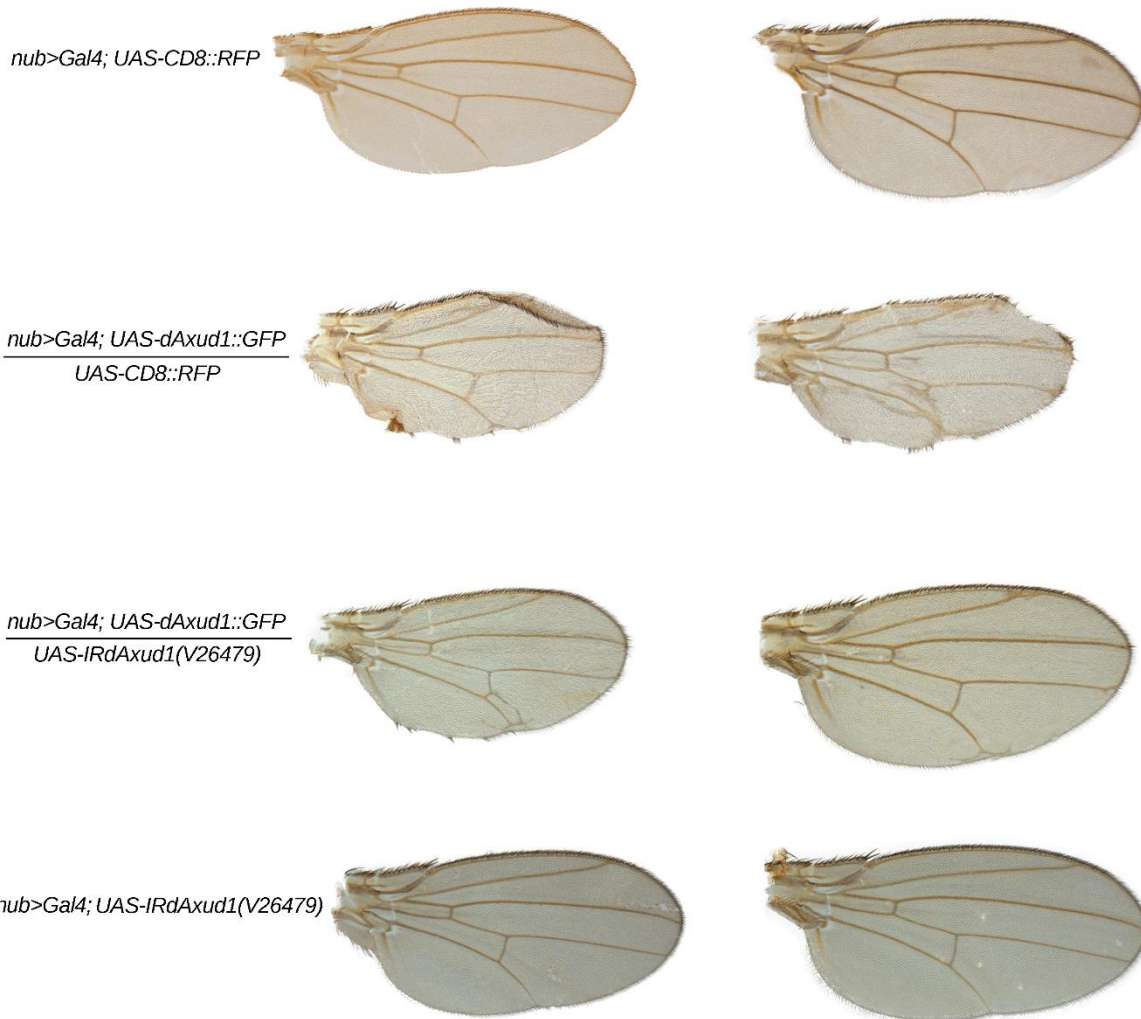
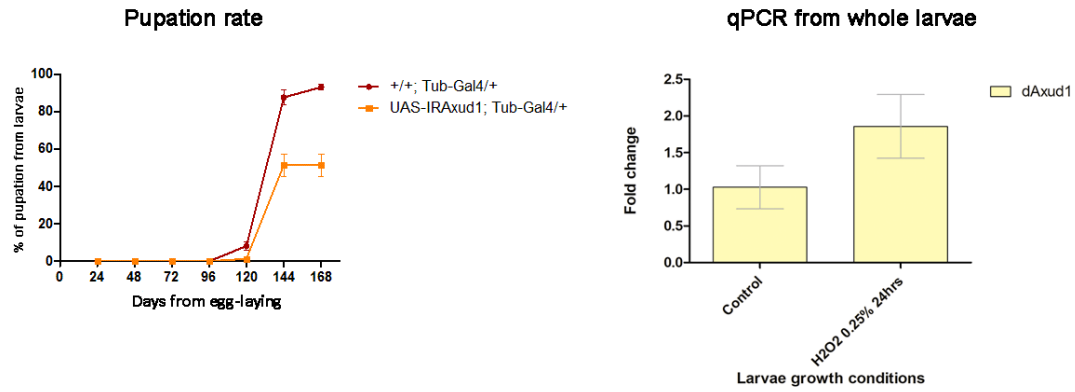


Male

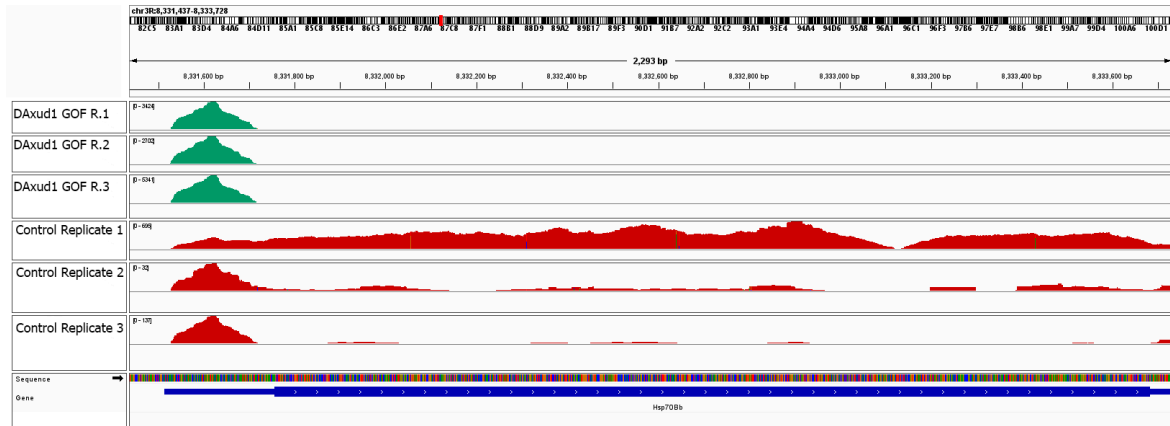
Female



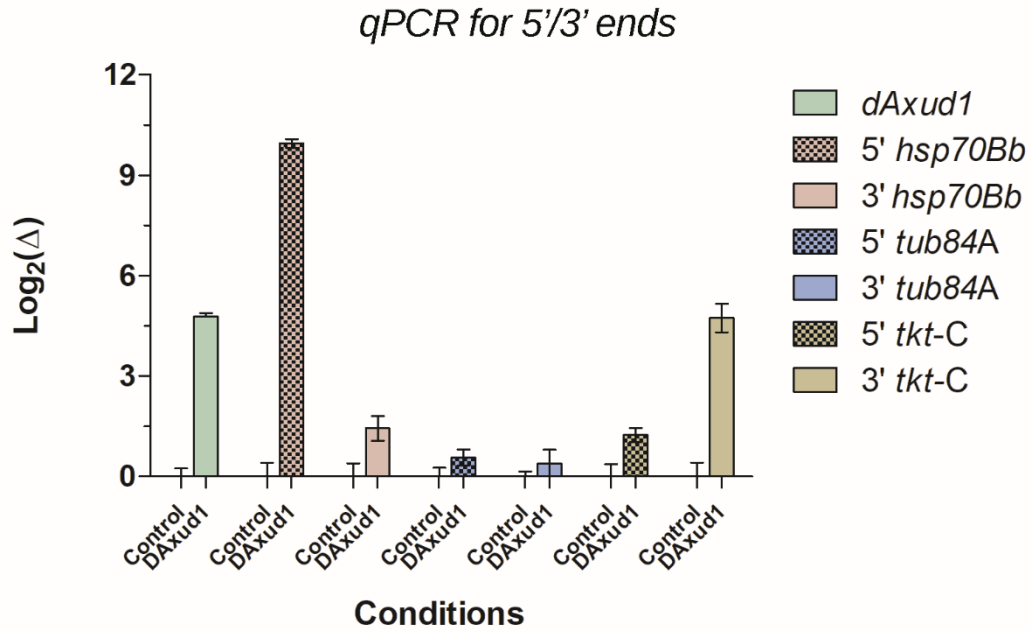
Additional Figure S1. Phenotype analysis of knockdown of *dAxud1* RNAi. Co-expression of *IR-dAxud1* can reverse the phenotype of overexpression of *dAxud1* and there is no evident phenotype on *dAxud1* RNA knockdown. Flies were grown at 29°C.



Additional Figure S2. *dAxud1* RNA knockdown has a peroxide-sensitive phenotype in pupation rate, and *dAxud1* rises its mRNA levels at 24 hours on larvae feed with peroxide food 0.5% supplement. The RNA was extracted from larvae on 72-96 hours after egg-laying. P-value = 0.02 from t-test. qPCR was normalized with *actin42C*.

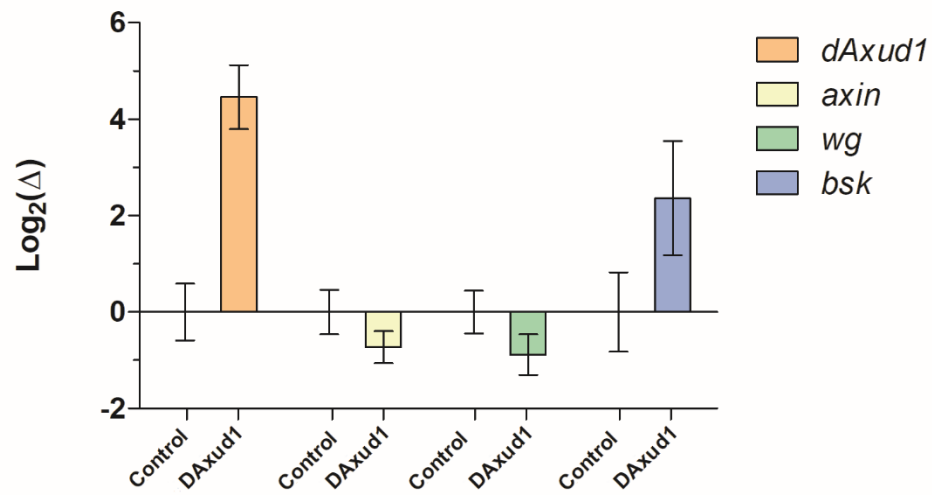


Additional Figure S3: Imaginal wing discs RNA-seq alignment from count profiles *hsp70B* locus. Three *hsp70* paralogous are shows in coordinates in red and the abundance of alignment counts are in blue in sequence positions. There is a high abundance of count on 5' side of *hsp70* genes only in GOF of *dAxud1* records.



Additional Figure S4: qPCR of 5' and 3' sides of genes in *dAxud1* overexpression (DAXud1) with Gal4/UAS (*nub>Gal4*; *UAS-dAxud1::GFP*) in imaginal wing discs compared with control genetic background (*nub>Gal4*). Primers were design to span 5' and 3' ends on *hsp70* gene. The profiles are compared with *tubulin84 A* and *tkt-C*, this last gene rises its expression in *dAxud1* overexpression. Ct data were normalized with *actin42C*.

qPCR GOF dAxud1 Imaginal wing disc



Additional Figure S5: *dAxud1* GOF qPCR for readout genes from pathways related with *dAxud1* function. Actin42C was used as normalizer gene and RNA was extracted from third instar imaginal wing disc from larvae growth at 25°C.



nub>Gal4; UAS-dAxud1



nub>Gal4; UAS-dAxud1/spt5

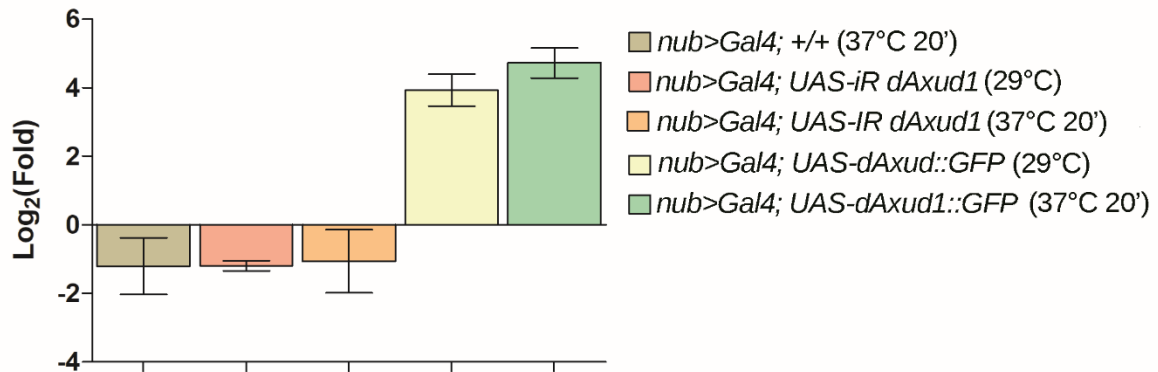
Additional Figure S6: *spt5* deficiency rescues DAxud1 overexpression phenotype.



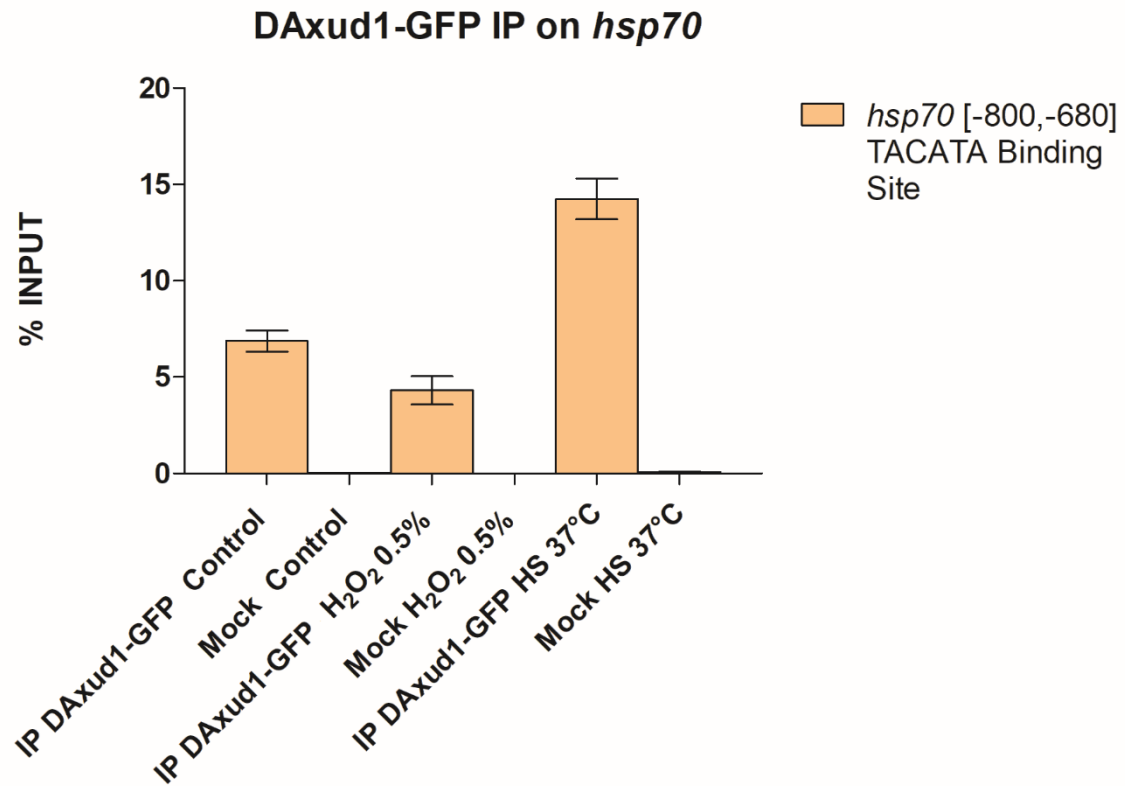
Additional Figure S7: genetic *dAxud1* interaction with Nelf-B gain and loss of function. Overexpression condition was achieved expressing NELF-B-HA (FlyORF F003904), and knockdown was induced expressing RNAi (TRiP collection Bloomington 34847).

qPCR for dAxud1 levels under differentes conditions

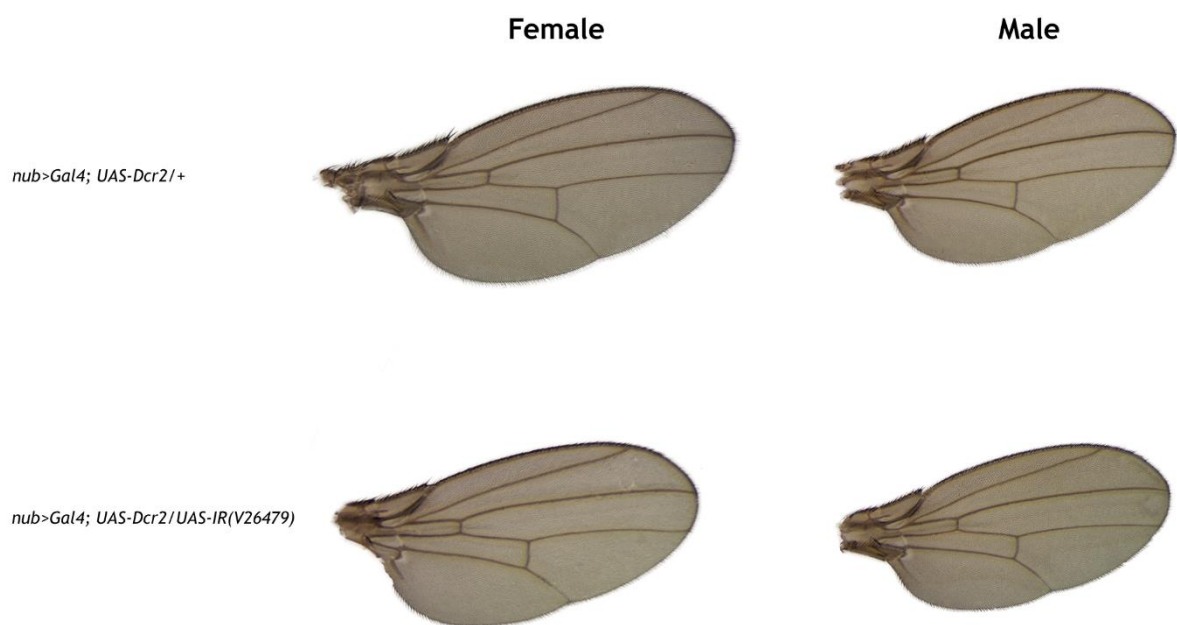
$\log_2(\text{Fold})$ Fold = Conditions vs. Control 29°C *nub>Gal4*



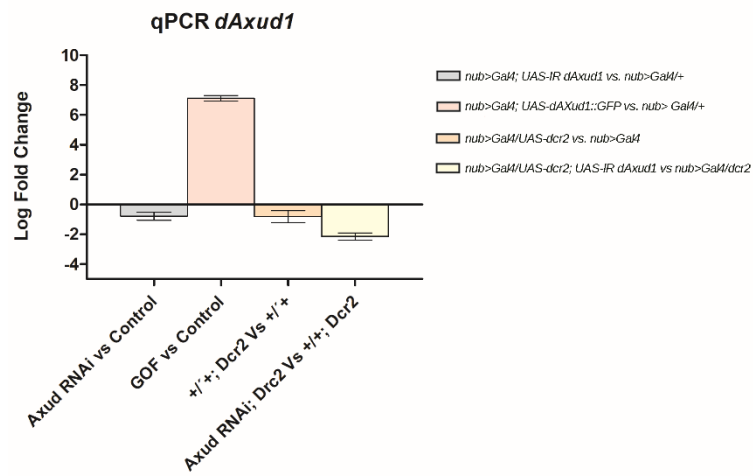
Additional Figure S8: qPCR of *dAxud1* levels in different conditions. Actin42A was used as normalizer. Heat shock was performed for 20'.



Additional Figure S9: CHIP qPCR for *hsp70* promoter zone with TACATA DamID consensus motif.



Additional Figure S10: Wing phenotypes from RNAi knockdown enhanced by co-expression with Dicer 2.



Additional Figure S10: qPCR for *dAxud1* in different genotypes including expression of Dicer 2 to enhance the effect of the RNAi to manipulate *dAxud1* mRNA levels.