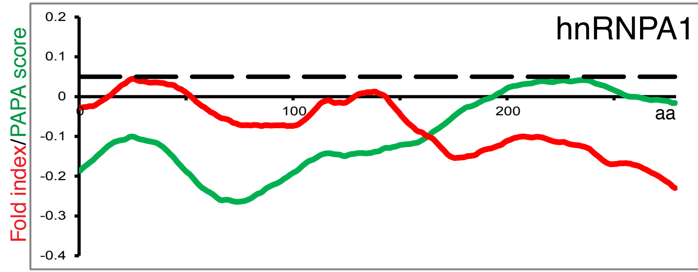
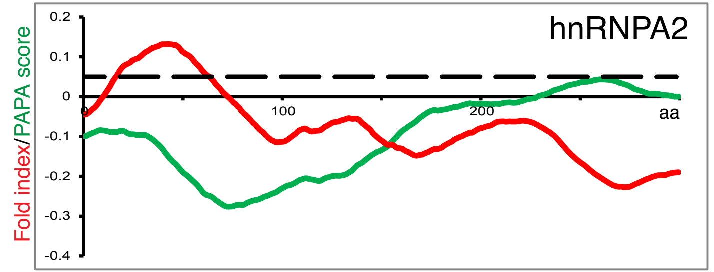
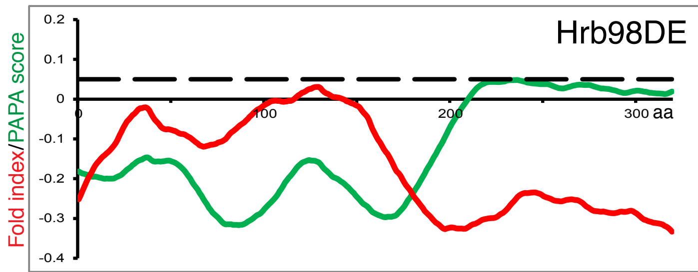
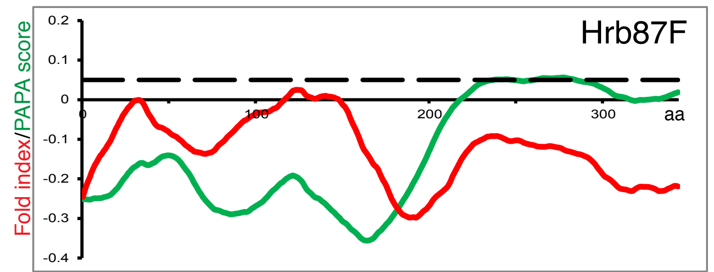
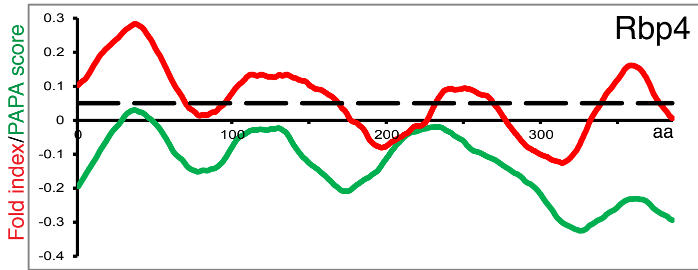










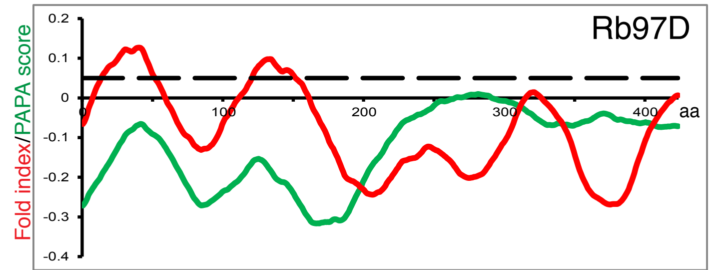


Figure S1. PAPA scores and fold indexes of human and *Drosophila* proteins that are homologous to hnRNPA2B1 and A1.

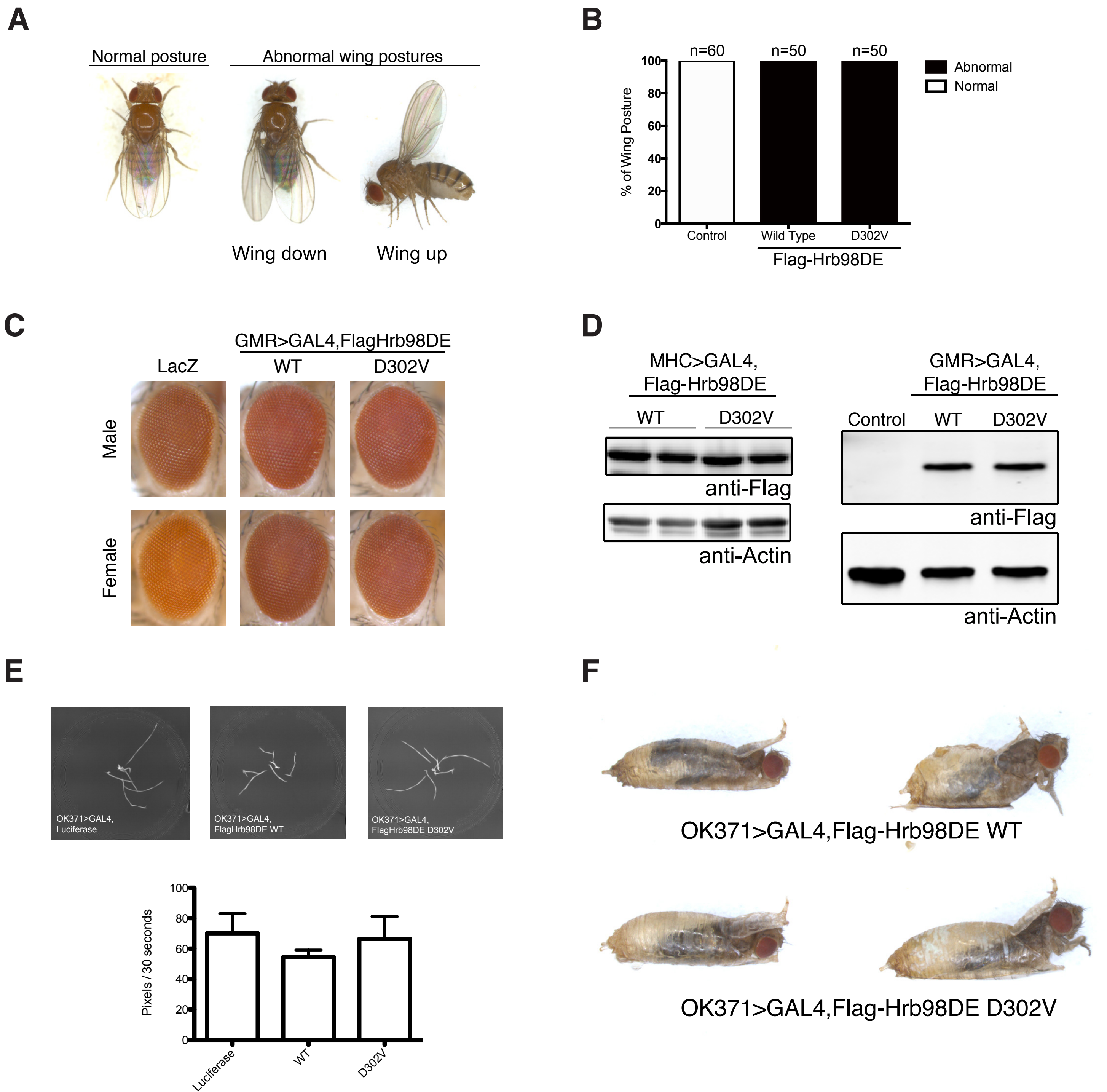


Figure S2. Characterization of Hrb98DE WT and D302V phenotypes in *Drosophila* muscle, eye and motor neuron. (A) Examples of abnormal wing postures caused by Hrb98DE expression in *Drosophila* under control of the MHC-GAL4. (B) Quantification of (A). Ectopic expression of Flag-Hrb98DE in *Drosophila* indirect flight muscle caused 100% abnormal wing-up or wing-down posture phenotype. (C) Flag-Hrb98DE WT or D302V was expressed in adult *Drosophila* eyes under control of the GMR-GAL4 promoter. The eyes did not show abnormal phenotype. (D) Western blotting assays show protein expression levels of Flag-Hrb98DE WT or D302V in *Drosophila* muscle and eye. (E) Locomotor (Crawling assay) was evaluated in 3rd instar larvae expressing Flag-Hrb98DE WT and D302V under the control of the OK371-GAL4 promoter. No significant difference was detected between the control and experiment groups. (F) Late pupal lethality was detected in *Drosophila* expressing Flag-Hrb98DE WT or D302V under control of the OK371-GAL4 promoter. Almost 100% of flies died when trying to leave the pupal case.

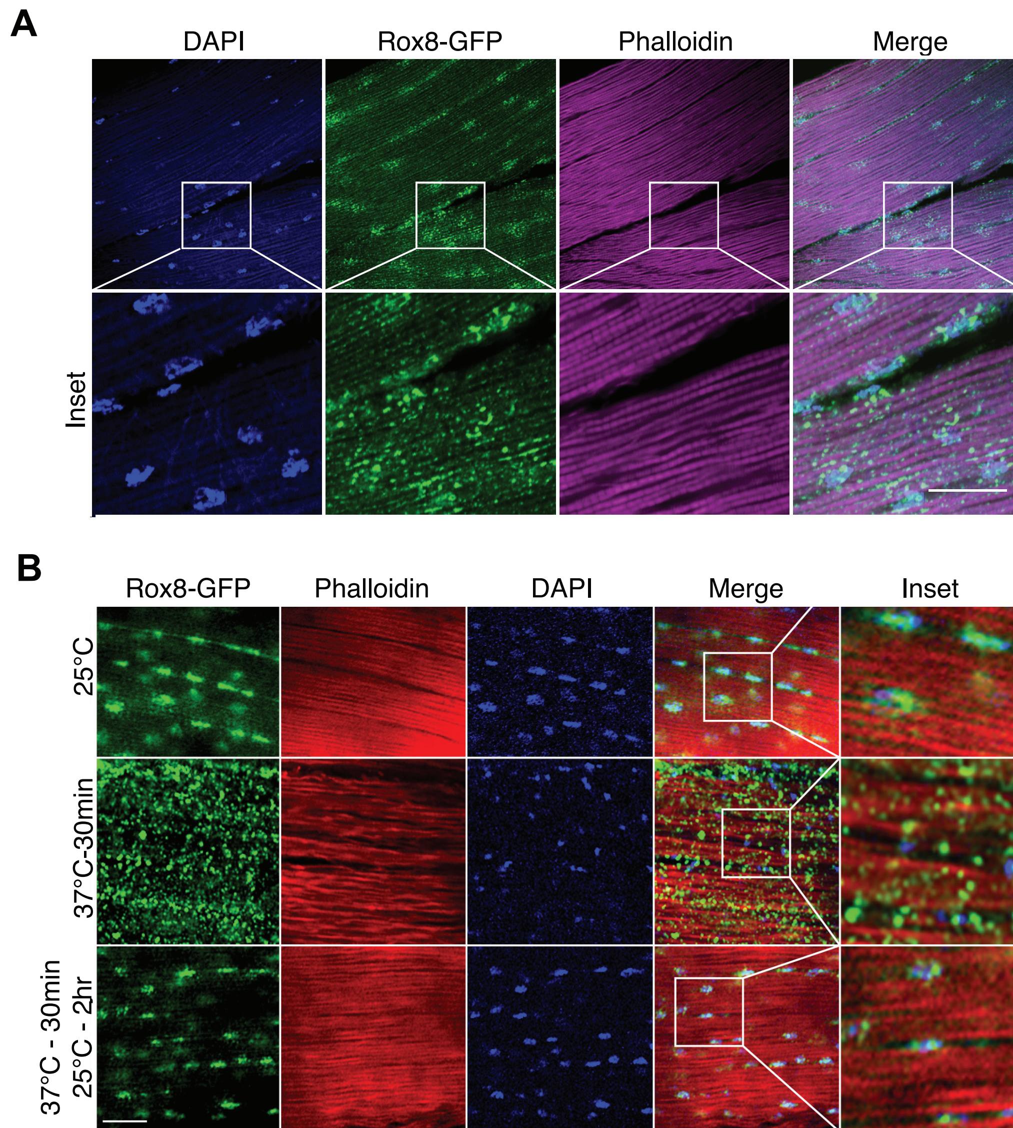


Figure S3. Generation and characterization of flies expressing Rox8-GFP in adult indirect flight muscle. (A) Ectopic expression of ROX8-GFP under the control of the Mhc-GAL4 promoter showed a granulated pattern that was mostly located in the cytoplasm, with enrichment in the para-nuclear area. (B) Formation of the heat shock-induced ROX8 aggregates in Mhc-GAL4> ROX8-GFP *Drosophila* showed stress granule–like behavior in the indirect flight muscle. Scale bars in A and B, 20 μm.

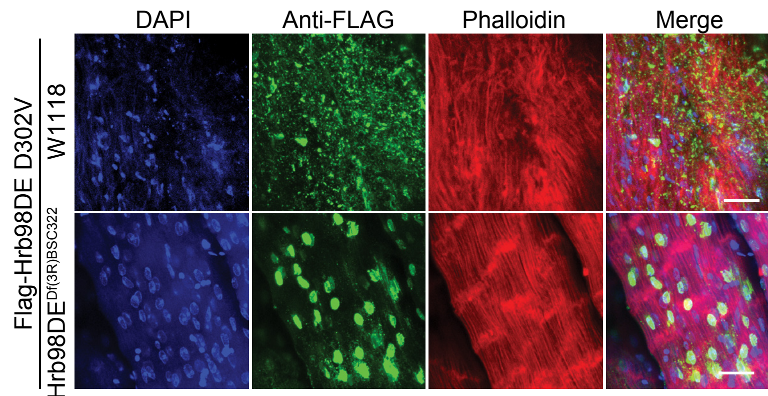
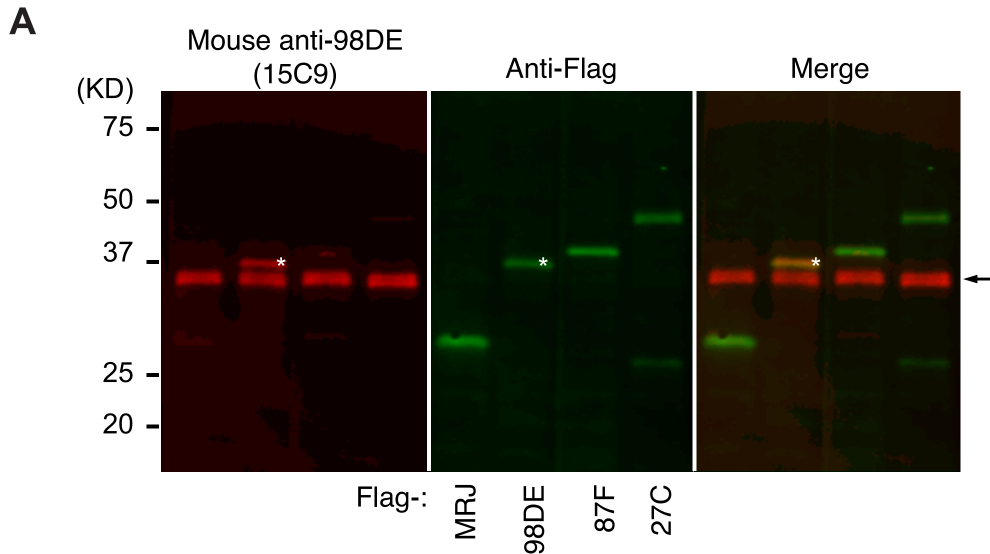


Figure S4. Knockdown of endogenous Hrb98DE partially rescues cytoplasmic inclusions generated by Hrb98DE D302V. Muscle from day 5 adult *Drosophila* expressing FLAG-Hrb98DE D302V was dissected to examine the cellular localization of the mutant protein. FLAG-Hrb98DE D302V was localized primarily in cytoplasmic inclusions (top). When endogenous Hrb98DE protein is knocked down using the classical allele Df(3R)BSC322, this partially rescues the localization of FLAG-Hrb98DE D302V into cytoplasmic inclusions (bottom). FLAG-Hrb98DE D302V was visualized with anti-FLAG antibody (green), DAPI was used to visualize the nucleus (blue) and a phalloidin stain was used to mark the muscle morphology (red). Size bars, 20 μm.



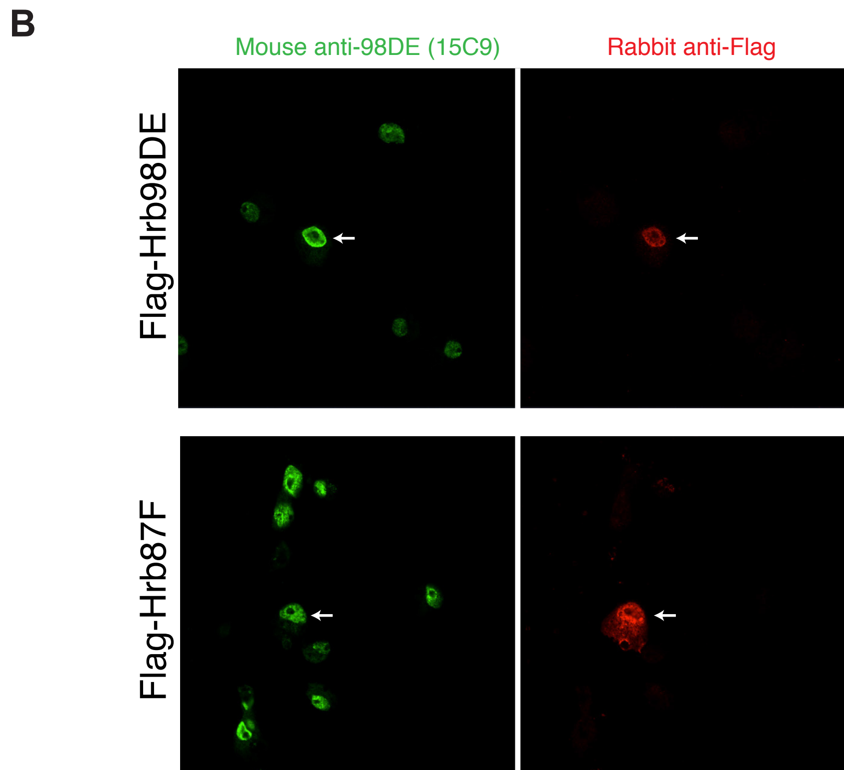


Figure S5. Generation and characterization of anti-Hrb98DE monoclonal antibody (15C9).
(A) Western blot assays showing the specificity of 15C9. S2 cell lysates prepared from cells transfected with Flag-MRJ, Flag-Hrb98DE, Flag-Hrb87F, or Hrb27C were used to perform immunofluorescence-based western blot assays. Mouse 15C9 (red) and rabbit anti-Flag (green) antibodies were used to detect corresponding protein on the same membrane.
(B) Indirect immunofluorescence (IIF) showing the specificity of 15C9. S2 cells transfected with either Flag-Hrb98DE (upper panels) or Flag-Hrb87F (lower panels) were attached to slides by cytospin and then IIF was performed. Mouse 15C9 (green) and rabbit anti-Flag (red) were used to detect the corresponding proteins in cells.

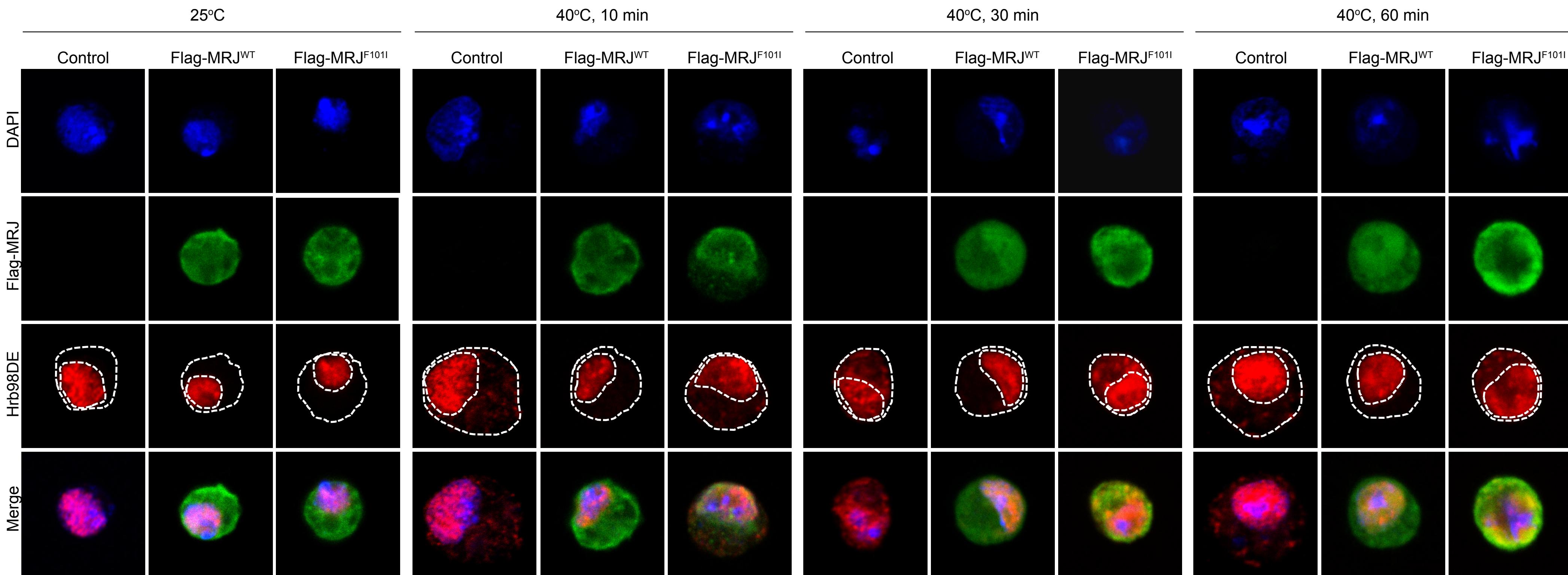


Figure S6. Endogenous Hrb98DE is re-localized to cytoplasmic punta after heat shock and MRJ WT, not LGMD1E-associated mutant F101I, expression inhibits re-localization of Hrb98DE. S2 cells transfected with either Flag-MRJ WT or F101I mutant were fixed before or after 40°C heat shock and stained with anti-Flag (green) and anti-Hrb98DE (red) to determine the cellular localization of these proteins.

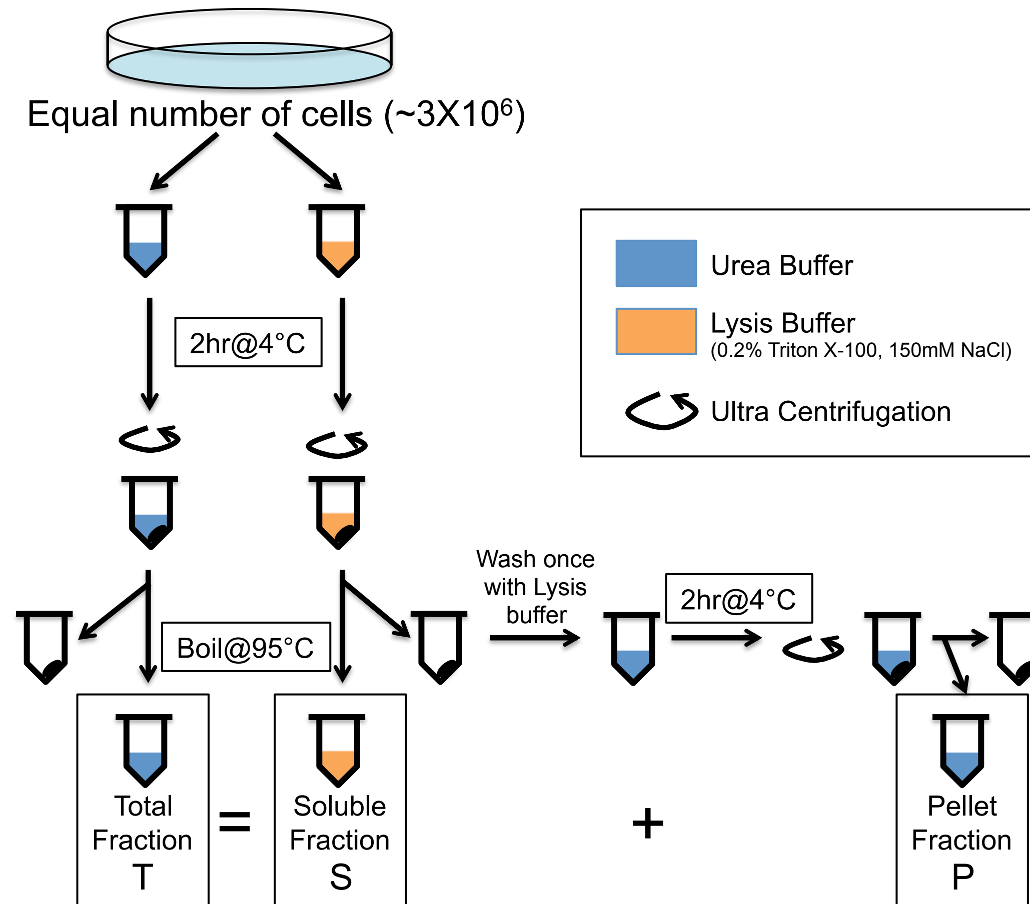


Figure S7. Schematics for Protein Fractionation Assay. Twenty-four hours post transfection, the total cell harvest was split 50:50 and the first fraction was lysed in Urea buffer and defined as total protein (T). The second fraction underwent sequential solubilization with 0.2% Triton X-100 followed by urea, and these were defined a soluble (S) and insoluble pellet (P).

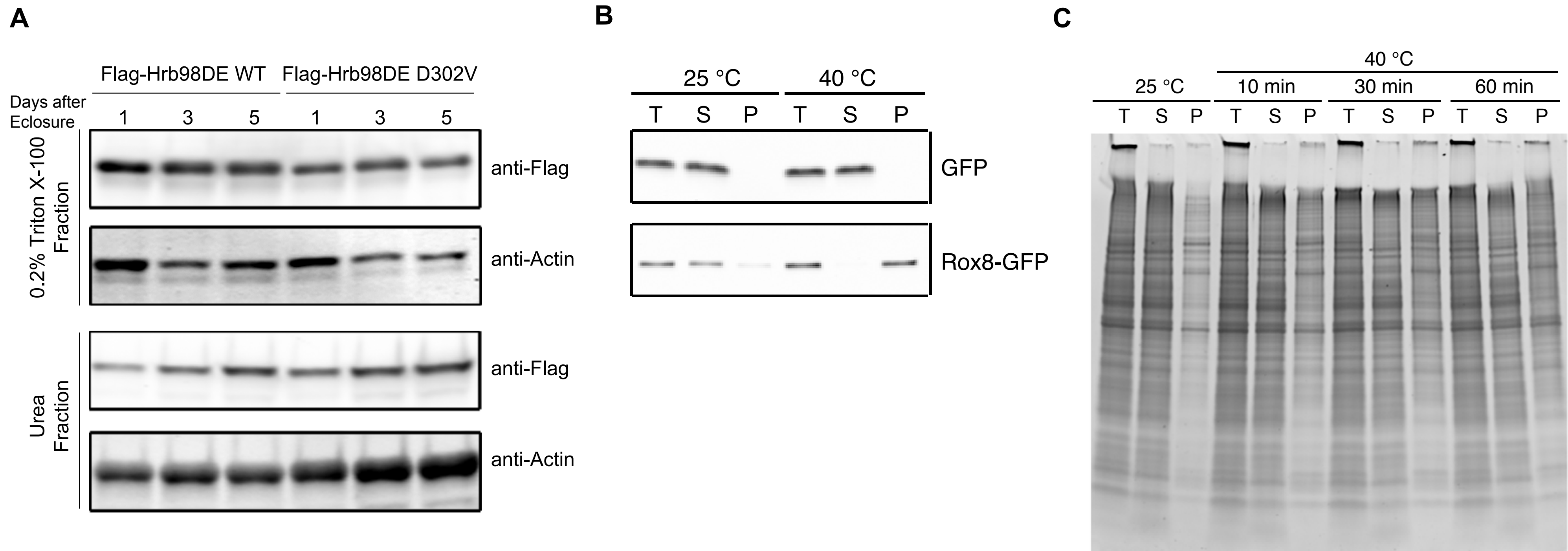


Figure S8. Protein solubiity after fractionation assay. (A) Thoraces of adult flies were dissected and sequential extractions were performed to examine the solubility profile of Flag-Hrb98DE WT and D302V. Result shows that mutant Hrb98DE is less soluble than WT protein and greater amounts of mutant Hrb98DE are recovered in the Triton X-100 insoluble fraction. (B) S2 cells were transfected with either GFP or Rox8-GFP and cell lysates were fractionaed before and after heat shock at 40 °C. Total (T), detergent-soluble (S), and insoluble pellet (P) fractions were blotted with anti-GFP antibody. (C) After a fractionation assay as described in Figure 7D, cell lysates were loaded onto the SDS-PAGE gel and stained with Sypro Ruby to vidualize the total proteins loaded.