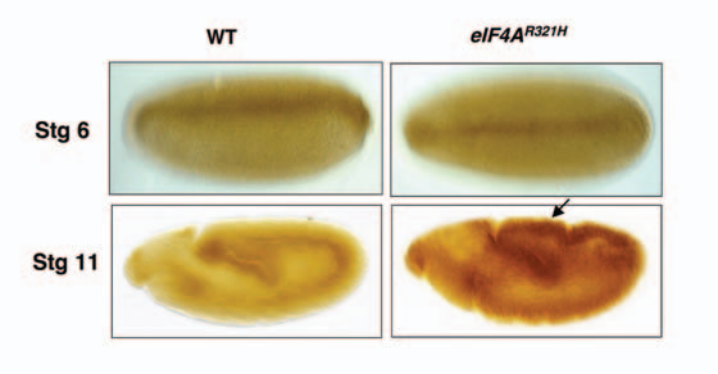
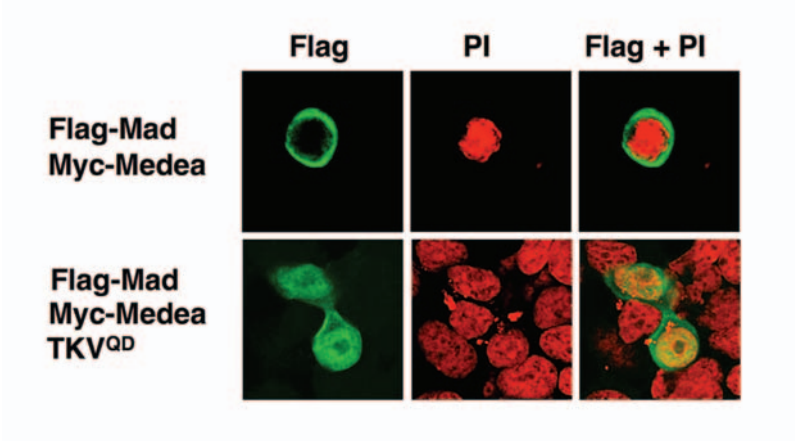
Supplementary Figure 1. Mad activation in early eIF4A mutant embryos



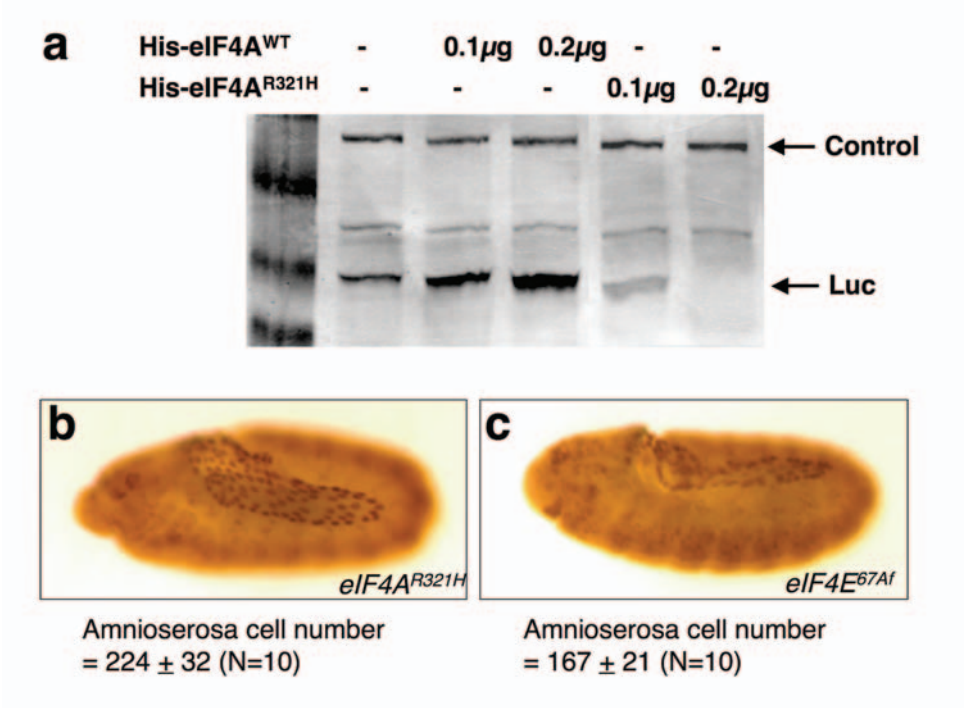
Mad activation is detected by anti-pMad. Wild-type control (left) and *eIF4A*^{R321H} mutant embryos (right) are shown anterior left and dorsal up. At stage 6, no obvious change in pMad signals were detected in *eIF4A*^{R321H} mutant as compared with wild type, although we cannot rule out that there might be small differences. In contrast, in later stages, significant increases in spatial domains of pMad signals were detected *eIF4A*^{R321H} embryos as compared with wild type. Stage 11 embryos are shown for comparison.

Supplementary Figure 2. Signal-dependent Mad nuclear translocation



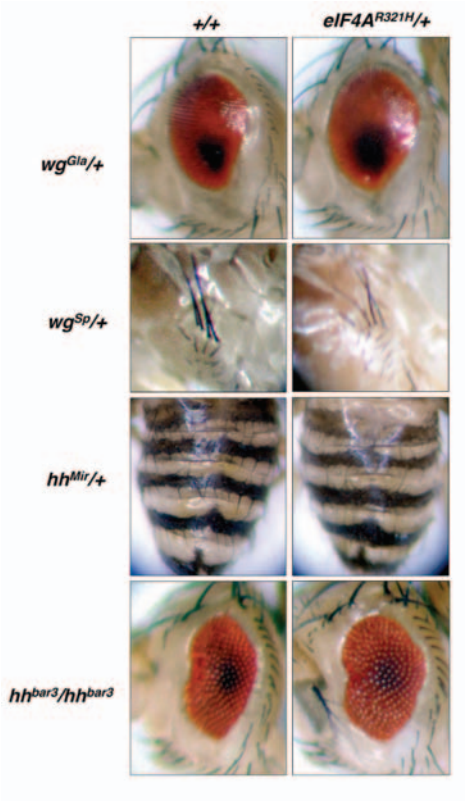
293T cells were transfected as in Fig. 2a. In the absence of cotransfected eIF4A, transfected Mad resides in the nucleus in the presence of cotransfected Tkv^{QD}.

Supplementary Figure 3. The function of eIF4A in modulating Dpp signaling is independent of its role in protein translation



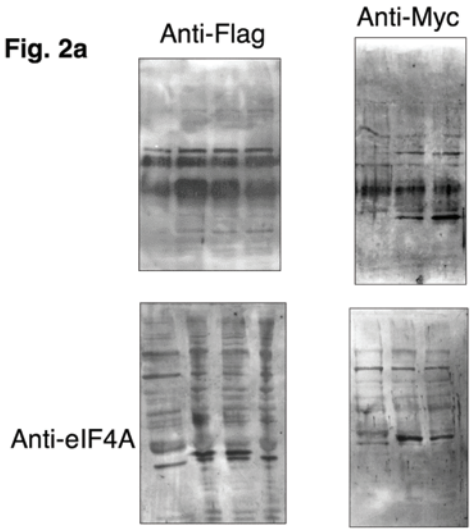
(a) Dose-dependent effects of *Drosophila* eIF4A^{WT} and eIF4A^{R321H} on in vitro protein translation. Bacterially expressed eIF4A^{WT} and eIF4A^{R321H} was added to a reticulocyte lysate system in indicated quantities. Translation rate is measured by the amount of luciferase (luc) synthesized compared with an internal control (upper band). Note eIF4A^{WT} enhanced and eIF4A^{R321H} inhibited luciferase translation, respectively. (b, c) Differential effects of eIF4A and eIF4E mutations on amnioserosa cell number. Amnioserosa cells are shown as large Kr-positive cells (brown staining) in the central region of stage 12 embryos. Representative pictures are shown. (b) *eIF4A^{R321H}* homozygous embryos had significantly more amnioserosa cells. (c) The number of amnioserosa cells in *eIF4E^{67Af}* (a null allele) homozygous embryos was indistinguishable from that of wild-type embryos (not shown).

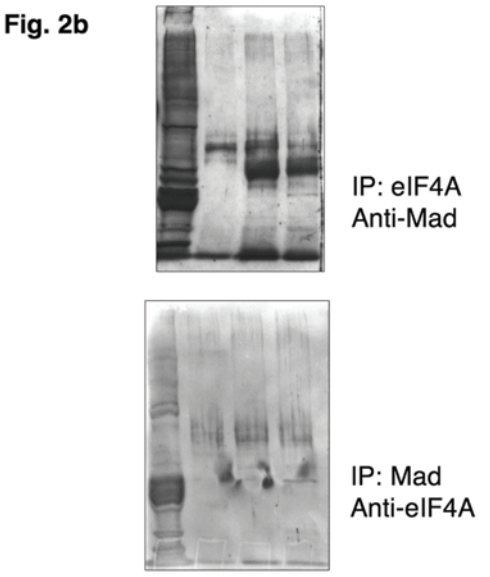
Supplementary Fig. 4. eIF4A does not appear to be involved in Wingless or Hedgehog signaling

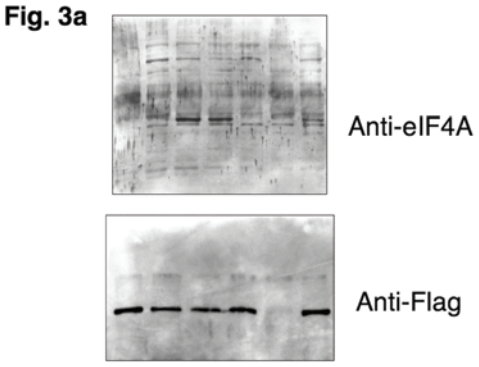


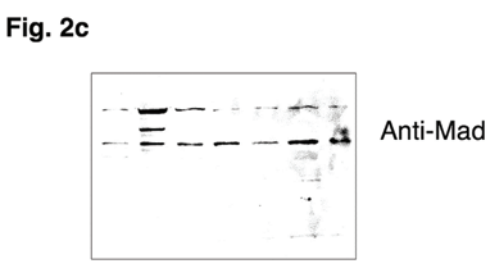
The dominant-negative allele *eIF4A*^{R321H} does not modify visible adult phenotypes of gain- or loss-of-function alleles *wg* or *hh* under the conditions tested. *wg*^{Gla} is a gain-of-function allele of *wg*, which is associated with coalesced ommatidia due to *wg* ectopic expression[1]. This phenotype was not modified in *eIF4A*^{R321H} heterozygotes. *wg*^{Sp} is a regulatory mutant *wg* allele associated with increased sternopleural bristle numbers[2]. This phenotype was not modified by *eIF4A*^{R321H}. *hh*^{Mir} is a gain-of-function allele of *hh*. It causes transformation of anterior structures toward posterior in the dorsal abdomen, a phenotype not sensitive to dosages of *dpp* or *wg* (ref 3). In *eIF4A*^{R321H} heterozygous background, the mirror-image duplication of tergites remained indistinguishable from those of otherwise wild-type background. Finally, *eIF4A*^{R321H} did not appear to significantly modify a *hh* hypomorphic allele, *hh*^{bar3} (ref 4), although there was a slight increase in the eye size of *eIF4A*^{R321H}/+; *hh*^{bar3} flies, possibly due to an increase in Dpp signaling.

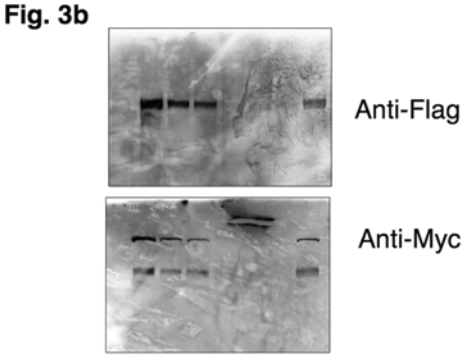
Supplementary Fig. 5. Full-length gels of key Western data











Reference:

1. Brunner, E., Brunner, D., Fu, W., Hafen, E. & Basler, K. The dominant mutation Glazed is a gain-of-function allele of wingless that, similar to loss of APC, interferes with normal eye development. Dev Biol 206, 178-88 (1999).
2. Neumann, C. J. & Cohen, S. M. Sternopleural is a regulatory mutation of wingless with both dominant and recessive effects on larval development of Drosophila melanogaster. Genetics 142, 1147-55 (1996).
3. Kopp, A., Muskavitch, M. A. & Duncan, I. The roles of hedgehog and engrailed in patterning adult abdominal segments of Drosophila. Development 124, 3703-14 (1997).
4. Lee, J. J., von Kessler, D. P., Parks, S. & Beachy, P. A. Secretion and localized transcription suggest a role in positional signaling for products of the segmentation gene hedgehog. Cell 71, 33-50 (1992).