Supplementary figures

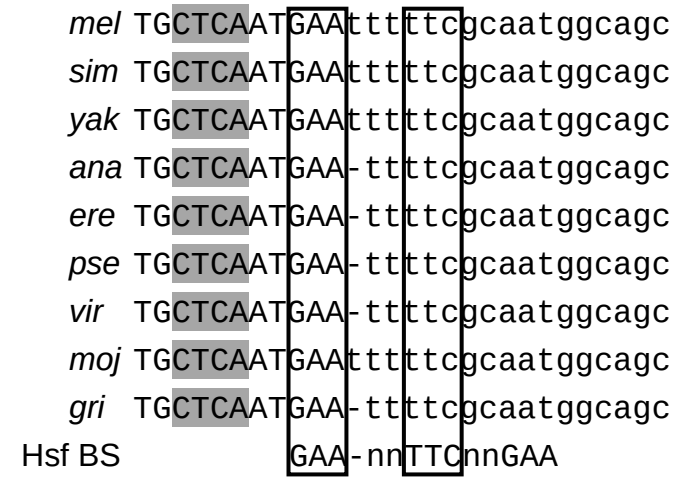

Figure S1. DNA sequences in the *tor*-RE and downstream region are highly conserved among nine *Drosophila* species.

DNA sequences of the *tor*-RE (upper case) and downstream region (lower case) in *Drosophila melanogaster* (*mel*), *simulans* (*sim*), *yakuba* (*yak*), *ananassae* (*ana*), *erecta* (*ere*), *pseudoobscura* (*pse*), *virilis* (*vir*), *mojavensis* (*moj*) and *grimshawi* (*gri*) were retrieved from the UCSC Genome Bioinformatics (http://genome.ucsc.edu/). The consensus sequence bound by Hsf trimer (Hsf BS) is shown below the multiple-aligned sequences. The TGAG sequences in the reverse strand of the *tor*-RE are shaded. Two GAAs in an inverted-repeat fashion at the 3' end and flanking region of the *tor*-RE are boxed. The spacing between the GAA repeats is 3 bp in *D. mel*, *sim*, *yak* and *moj*, and is 2 bp in the remaining five species.

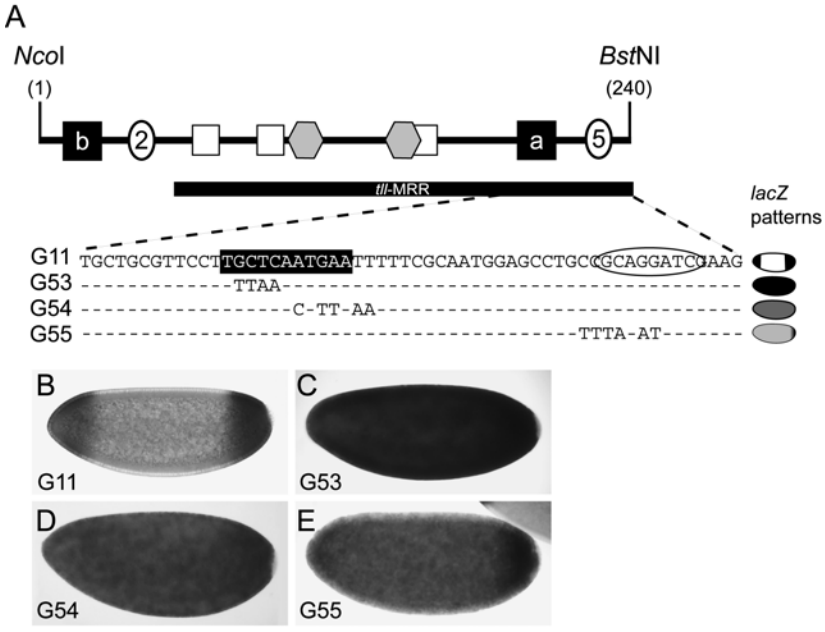

Figure S2. Two different factors may bind to the *tor*-RE.

(A) The diagram shows the locations of the *tor*-REs (solid squares), GAF (open squares) and Ttk69 (open ovals) binding sites in a 240-bp *tll* cis-regulatory region from *Nco*I to *Bst*NI. The DNA sequence of the 3' end of *tll* minimal regulatory region (*tll*-MRR; 186 bp in length) is shown (1). Shaded hexagons represent two putative Hsf binding sites in addition to the *tor*-RE. DNA sequence in 3' portion of the *tll*-MRR is shown below the diagram. Closed rectangle and open oval delimitate sequences for the *tor*-RE and the Ttk69 binding site, TC5, respectively. DNA sequences with or without base substitutions are indicated by letters and "-", respectively. *lacZ* expression patterns were determined by in situ hybridization with a digoxigenin-labeled RNA as a probe. The cartoons at the right summarize the patterns driven by wild-type (G11, B) or three different mutated *tll*-MRRs, G53 (C), G54 (D) or G55 (E). Except for the pattern of G54, the other expression patterns have been published (2).

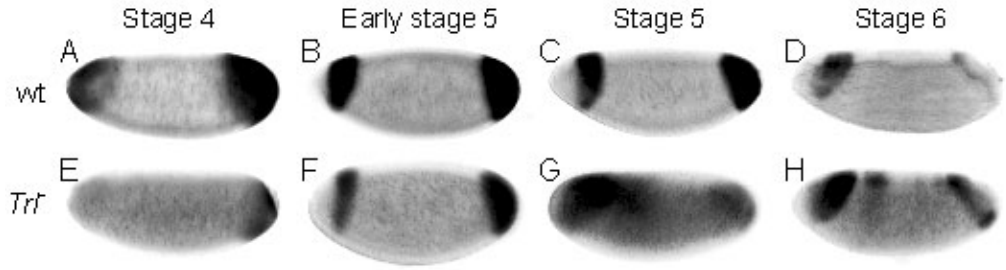

Figure S3. The anterior stripe of *tll* expression in embryos with reduced *Trl* activity shifts posteriorly at late stage 5.

Embryos from females obtained from GLC with Trl^{R85} (E-H) were used to determine *tll* expression using in situ hybridization. *tll* expression patterns in embryos from GLC with wild type serve as controls (A-D). The embryonic stages are late stage 4 (stage 4; A and E), early (B and F) and late stage 5 (C and G), and stage 6 (D and H). Embryos are arranged in a sagittal view, with the anterior towards the left.

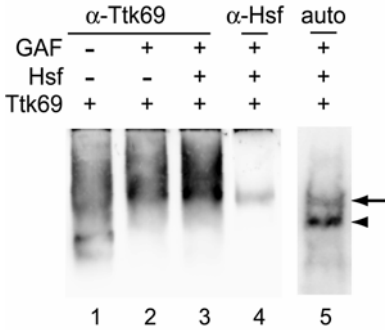

Figure S4. Hsf and Ttk69 exist in the *tor*-RE-protein complex.

Shift-western blotting of the complexes formed with GAF (2 μl in lanes 2-5), Hsf (3 μl in lanes 3-5), Ttk69 (4 μl in lanes 1-5) and [^{32}P]-labeled *tor*-RE, which are the same as those used in EMSA (Figure 4A). The binding reaction was carried out at room temperature. The DNA-protein complexes were subjected to be separated in a 6% of native polyacrylamide gel and transferred onto stacked nitrocellulose and nylon membranes. Hsf and Ttk69 on nitrocellulose membrane were detected by anti-Hsf and Ttk69 antibodies, respectively. "auto" represents an autoradiogram that shows position of the radiolabeled probe on nylon membrane.

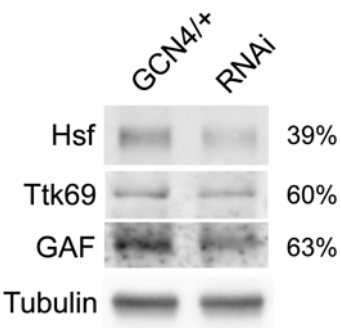

Figure S5. Reduced levels of GAF, Hsf and Ttk69 proteins in embryos with simultaneous knock-down of the three genes.

Proteins extracted from embryos laid by females of *GCN4>hsf*, *Trl* and *ttk69* RNAi were separated in an 8% SDS polyacrylamide gel and detected by western blotting with anti-GAF, Hsf or Ttk69 antibodies and a chemilluminescent assay kit. Tubulin served as a loading control to normalize the percentage of protein reduction. The percentages of the remaining proteins in the embryos are shown at the right.

References

1. Liaw, G.J., Rudolph, K.M., Huang, J.D., Dubnicoff, T., Courey, A.J. and Lengyel, J.A. (1995) The *torso* response element binds GAGA and NTF-1/Elf-1, and regulates *tailless* by relief of repression. *Genes Dev*, **9**, 3163-3176.
2. Chen, Y.J., Chiang, C.S., Weng, L.C., Lengyel, J.A. and Liaw, G.J. (2002) Tramtrack69 is required for the early repression of *tailless* expression. *Mech Dev*, **116**, 75-83.