

Expanded View Figures

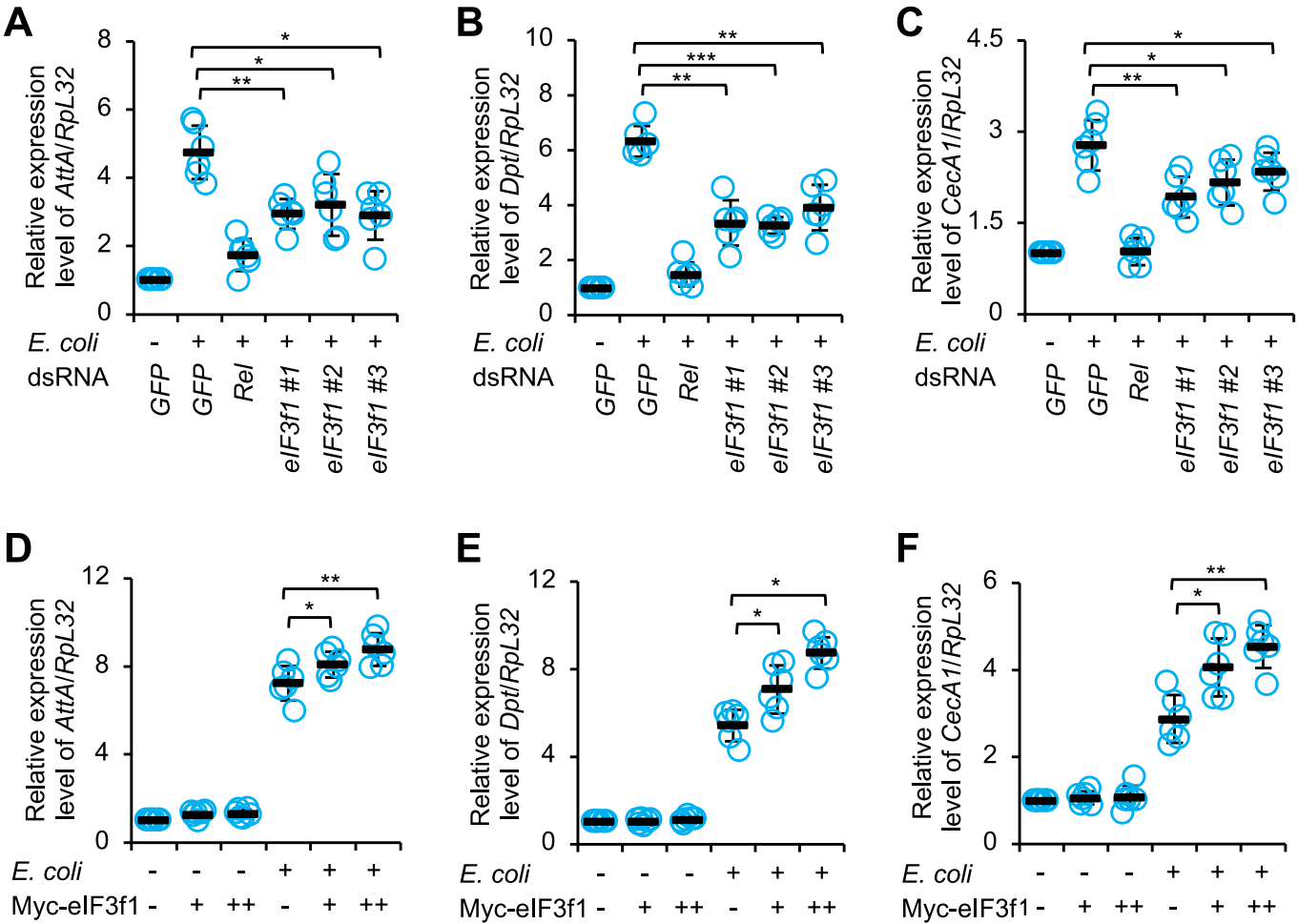


Figure EV1. eIF3f1 modulates IMD signaling in *Drosophila*.

(A–C) S2 cells were treated with indicated dsRNAs for 48 h and then treated with heat-killed *E. coli* (MOI is around 5) for 6 h. Samples were subjected to RT-qPCR experiments to monitor the mRNA levels of *Att* (A), *Dpt* (B), or *CecA1* (C). (D–F) S2 cells were transfected with indicated expressing plasmids for 36 h, followed by treatment of heat-killed *E. coli* for 6 h. Samples were then subjected to RT-qPCR assays. Data Information: (A–F) Each dot represents one biological biological replicate and data are shown as mean ± SD. The nonparametric Kruskal-Wallis test was used for statistical analyses. **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

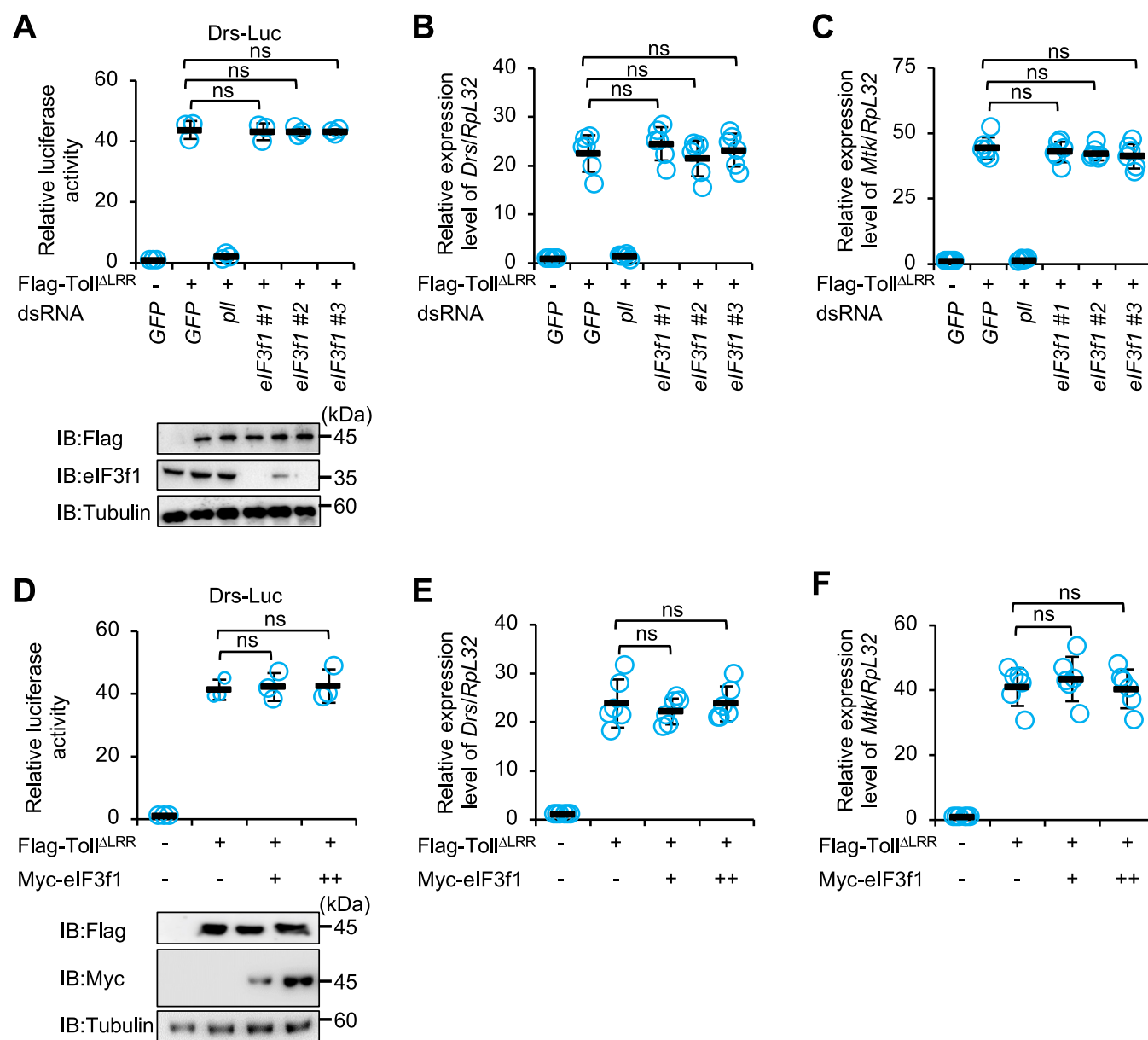


Figure EV2. eIF3f1 does not affect Toll signaling in S2 cells.

(A–C) S2 cells were treated with indicated dsRNAs for 48 h. Cells were then transfected with various combinations of expressing plasmids for 36 h, followed by dual-luciferase assays (A) or RT-qPCR experiments to monitor the mRNA levels of *Drs* (B) or *Mtk* (C). (D–F) S2 cells were transfected with indicated expressing plasmids. 36 h post transfection, cells were harvested for dual-luciferase (D) or RT-qPCR (E, F) assays. Data Information: (A–F) Each dot represents one biological replicate and data are shown as mean ± SD. The nonparametric Kruskal-Wallis test was used for statistical analyses. ns, not significant, $P > 0.05$.

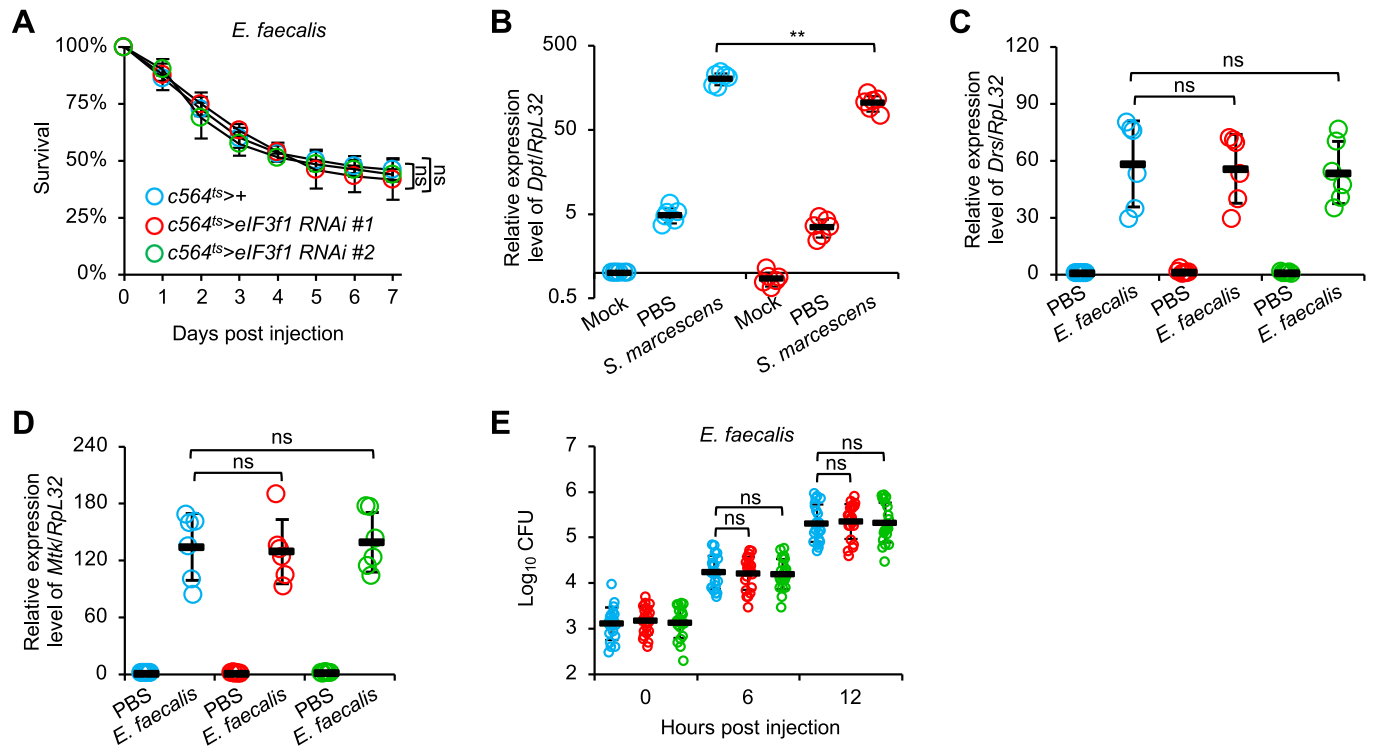


Figure EV3. *eIF3f1* is dispensable for the fly immune defense upon *E. faecalis* infection.

(A–E) Adult flies including $c564^{ts}>+$, $c564^{ts}>eIF3f1$ RNAi #1, and $c564^{ts}>eIF3f1$ RNAi #2 were infected with *E. faecalis* (A, C–E), *S. marcescens* (B), PBS (B–D), or without treatment (mock in B). Flies were subjected to survival analysis (A), or RT-qPCR assays (6 h post-infection, B–D), or bacterial burden assay (E). Data Information: (A) Data are shown as mean $\pm$ SD and the Log-Rank test was used for statistical analyses. The numbers of flies are as follows. $c564^{ts}>+$: 89, 85, 89; $c564^{ts}>eIF3f1$ RNAi #1: 84, 87, 85; $c564^{ts}>eIF3f1$ RNAi #2: 90, 92, 84. (B–E) Each dot represents one biological replicate and data are shown as mean $\pm$ SD. The nonparametric Kruskal–Wallis test was used for statistical analyses. ** $P < 0.01$; ns, not significant, $P > 0.05$.

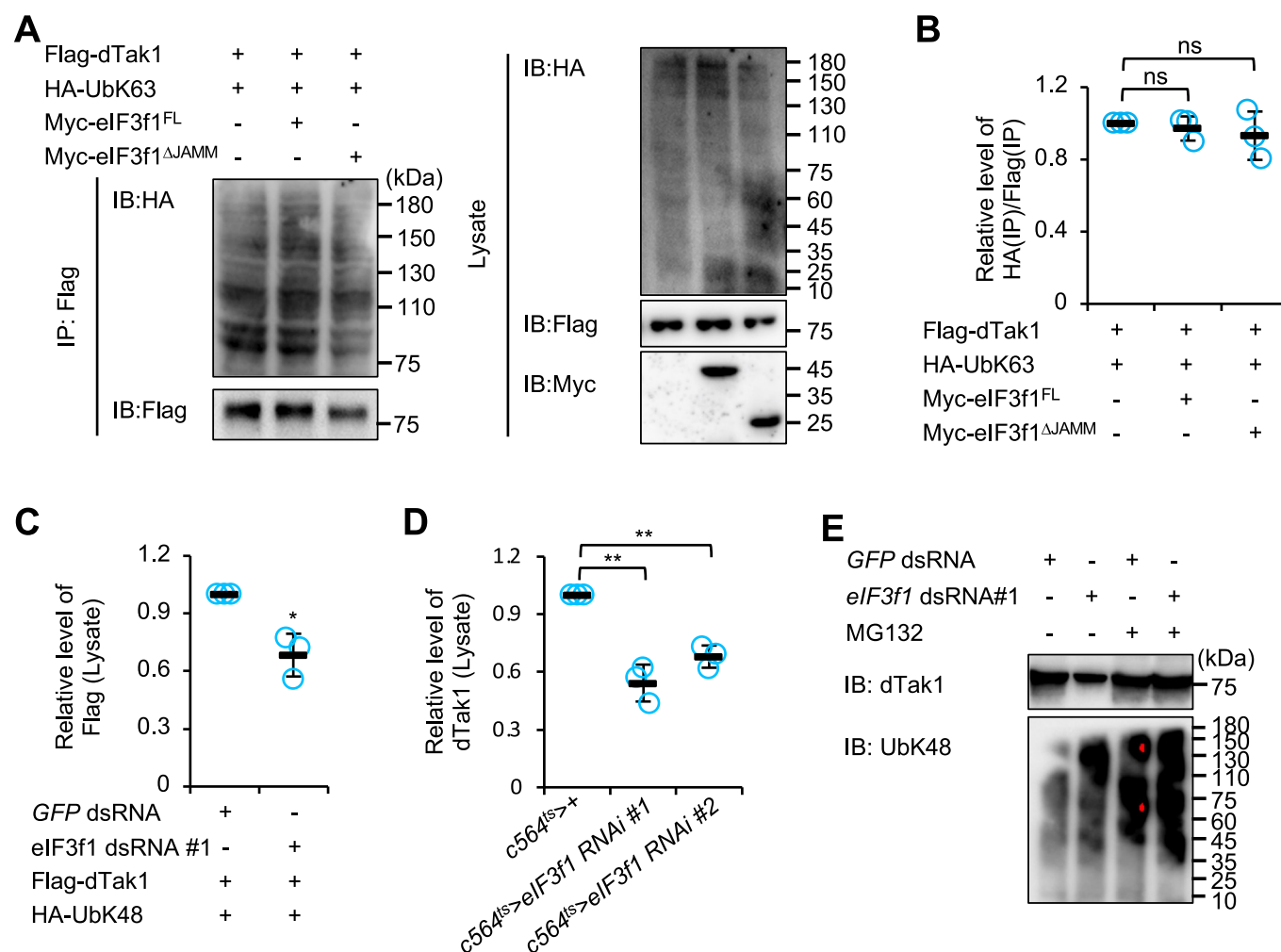


Figure EV4. eIF3f1 is not involved in regulating the K63-linked ubiquitination of dTak1.

(A, B) S2 cells were transfected with indicated combinations of expressing plasmids. 48 h post transfection, cells were harvested for ubiquitination assay (A). Densitometry analysis to quantify the K63-linked ubiquitination levels of dTak1 is shown in (B). (C) Densitometry analysis to quantify Flag-dTak1 expression level in Fig. 5J. (D) Densitometry analysis to quantify dTak1 expression level in Fig. 5L. (E) S2 cells were treated with GFP or eIF3f1 dsRNAs for 48 h, followed by MG132 treatment for 6 h as indicated. Cells were lysed for western blot assays using anti-dTak1 and anti-UbK48 antibodies. Data Information: (B, D) Each dot represents one biological replicate and data are shown as mean $\pm$ SD. The nonparametric Kruskal-Wallis test was used for statistical analyses. (C) The Tukey's test was used for statistical analysis. * $P < 0.05$; ** $P < 0.01$; ns, not significant, $P > 0.05$.

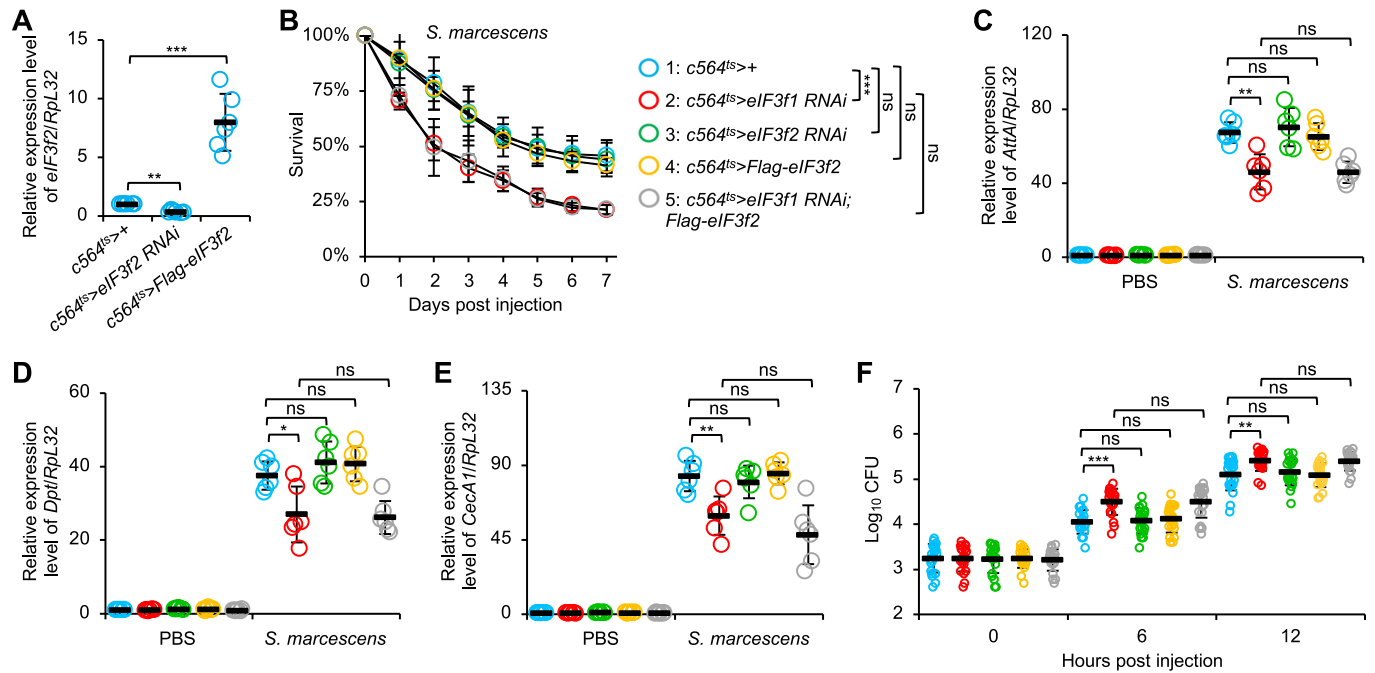


Figure EV5. *eIF3f2* is not involved in mediating the fly immune defense upon microbial infection.

(A) RT-qPCR assays examining the knockdown or overexpression effect of *eIF3f2*. (B–F) Adult flies including *c564^{ts}>+*, *c564^{ts}>eIF3f1 RNAi*, *c564^{ts}>eIF3f2 RNAi*, *c564^{ts}>Flag-eIF3f2*, and *c564^{ts}>eIF3f1 RNAi;Flag-eIF3f2* were infected with *S. marcescens* or PBS (control). Flies were subjected to survival analysis (B), or RT-qPCR assays (6 h post-infection, C–E), or bacterial burden assay (F). Data Information: (A, C–F) Each dot represents one biological replicate. Data are shown as mean $\pm$ SD. The nonparametric Kruskal-Wallis test was used for statistical analyses. (B) Data are shown as mean $\pm$ SD and the Log-Rank test was used for statistical analyses. The numbers of flies are as follows. *c564^{ts}>+*: 89, 88, 94; *c564^{ts}>eIF3f1 RNAi*: 91, 89, 84; *c564^{ts}>eIF3f2 RNAi*: 96, 87, 89; *c564^{ts}>Flag-eIF3f2*: 88, 89, 89; *c564^{ts}>eIF3f1 RNAi;Flag-eIF3f2*: 89, 93, 94. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, not significant, $P > 0.05$.