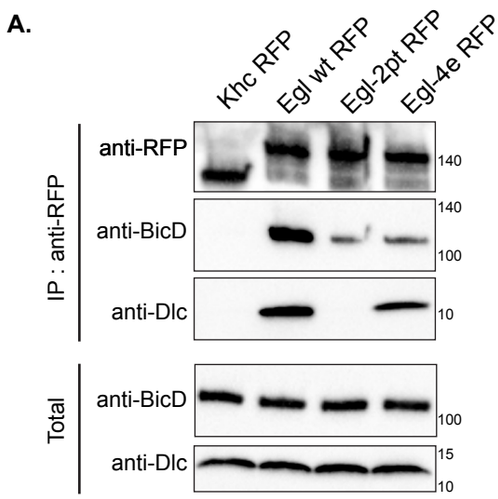
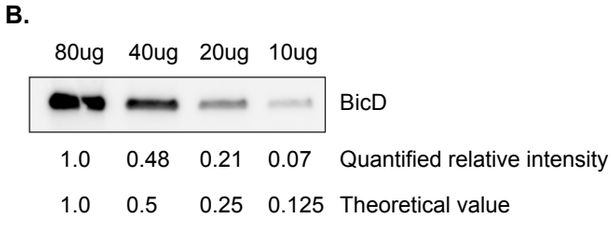
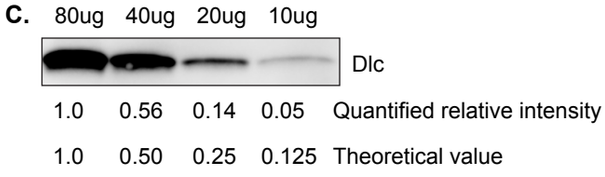
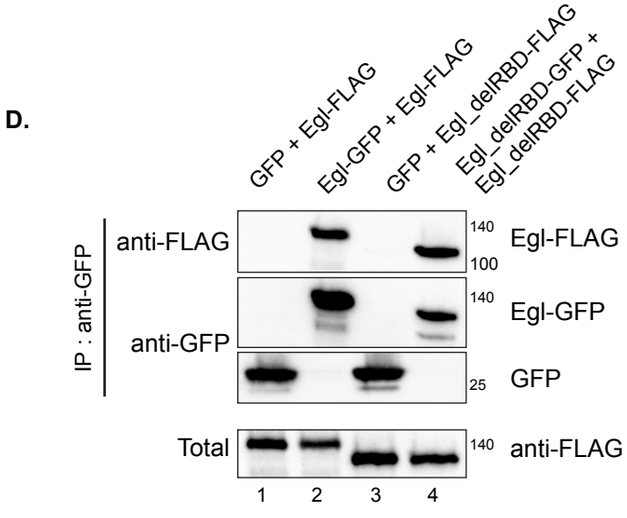
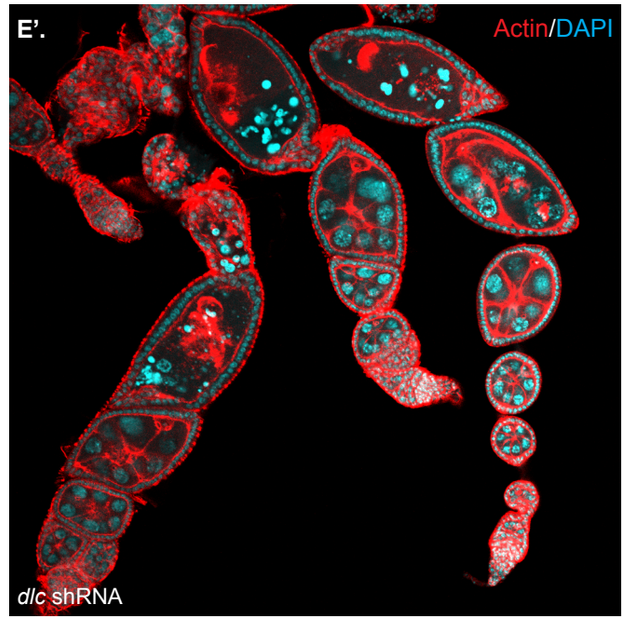
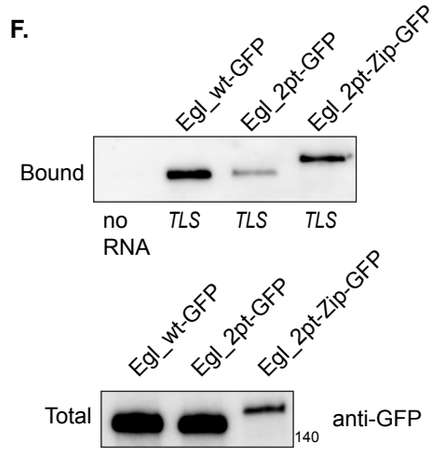








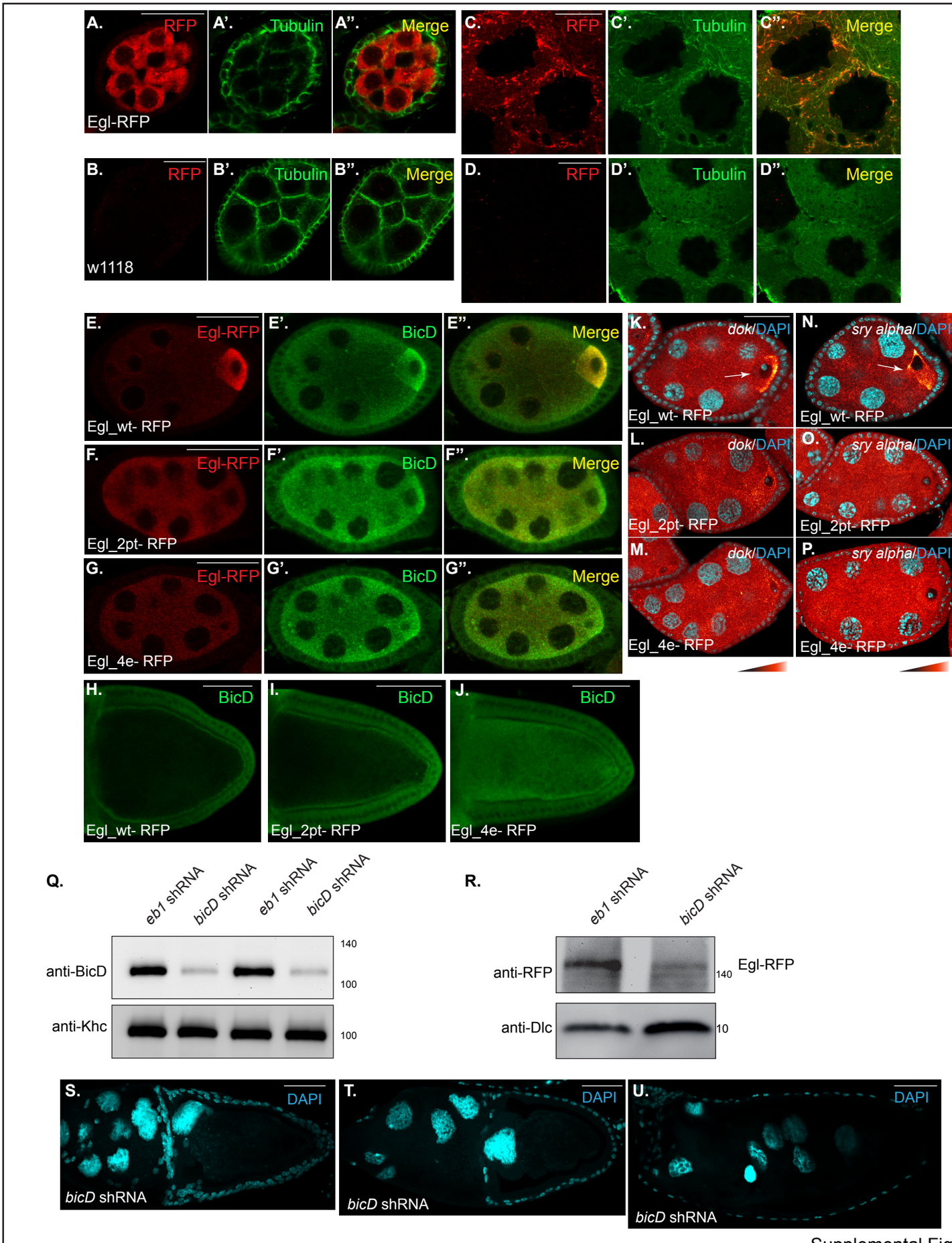




Supplemental Fig.1

Fig. S1

(A) A co-immunoprecipitation was set up using S2 cell lysates from strains expressing the indicated constructs. The lysates were incubated with RFP-Trap beads. After binding and wash steps, the bound proteins were eluted and analyzed by western blotting using the indicated antibodies. Wild-type Egl was able to co-precipitate BicD and Dlc. Egl_4e-RFP was able to co-precipitate Dlc but not BicD. Egl_2pt-RFP was deficient for binding both Dlc and BicD. **(B, C)** Ovarian lysates from strains expressing Egl_wt-RFP and depleted of endogenous Egl were analyzed by western blotting using an antibody against BicD (B) or Dlc (C). The indicated concentration of lysates were used. Band intensities were quantified using the Biorad Chemidoc MP imaging system and Image lab software. Band intensities were normalized to the respective 80ug sample. Quantified intensity values and theoretical values are shown. **(D)** A co-immunoprecipitation experiment was set up using S2 cells expressing the indicated constructs. The lysates were incubated with GFP -trap beads. The bound proteins were analyzed using the indicated antibodies. An Egl construct lacking the RNA binding domain is still able to dimerize. **(E)** Ovaries from flies expressing either a control shRNA (*eb1* shRNA, E) or shRNA against *dlc* (E') were fixed and processed using TRITC-Phalloidin and DAPI. The driver used for this experiment is restricted to the germline and is turned on in early-stage egg chambers (Sanghavi et al., 2016). Depletion of Dlc using this driver results in oogenesis arrest. **(F)** The *TLS* localization element was bound to beads and incubated with S2 cell lysates expressing the indicated constructs. Bound proteins were analyzed by blotting using a GFP antibody. Although Egl_2pt is compromised for RNA binding, artificial dimerization (Egl_2pt-Zip) restores RNA binding.



Supplemental Fig.2

Fig. S2

(A-D) Ovaries from flies expressing Egl_wt-RFP (A, C) or from a control strain (w1118, B, D) were fixed and processed using the home-made polyclonal RFP antibody (A, B, C, D, red). The egg chambers were co-stained with alpha-Tubulin directly conjugated to FITC (A', B', C', D', green). A merged image is also shown (A'', B'', C'', D''). Robust RFP signal is seen in the strain expressing Egl_wt-RFP but not in the control strain. **(E-J)** Ovaries from strains expressing Egl_wt-RFP, Egl_2pt-RFP, or Egl_4e-RFP and depleted of endogenous Egl were fixed and processed for immunofluorescence using an antibody against RFP (red) and BicD (green). Representative stage 5 (E-G) and stage 10 (H-J) egg chambers are shown. **(K-P)** The same strains were fixed and processed for smFISH using probes against *dok* (K-M) or *sry alpha* (N-P). The in situ signal is depicted using a red to white LUT; red pixels represent low-intensity signal and white pixels represent high-intensity signal. Arrows indicate the oocyte enrichment of *dok* (K) or *sry alpha* (N). **(Q, R)** Ovarian **l**ysates from flies expressing the control *eb1* shRNA or *bicD* shRNA were analyzed by western blotting using the indicated antibodies. BicD is depleted using this shRNA and driver (Q). However, the expression of Egl_wt-RFP in these same lysates is also greatly reduced (R). The Khc and Dlc blots serve as loading controls. **(S-U)** The strain expressing *bicD* shRNA was fixed and stained with DAPI. Depletion of BicD results in disorganized (S, T) and atrophying (U) egg chambers. The scale bar in H-J and S-U represent 50 microns; the scale bar in the remaining images represent 25 microns.