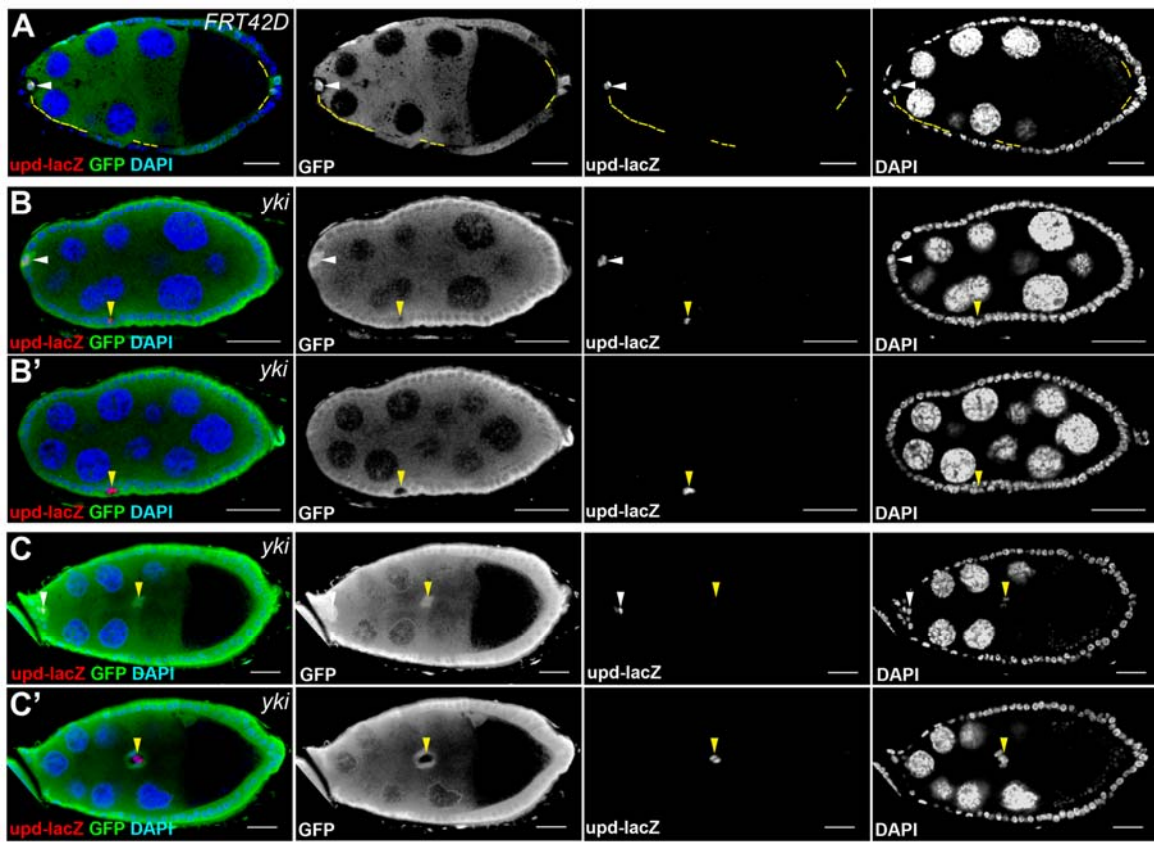


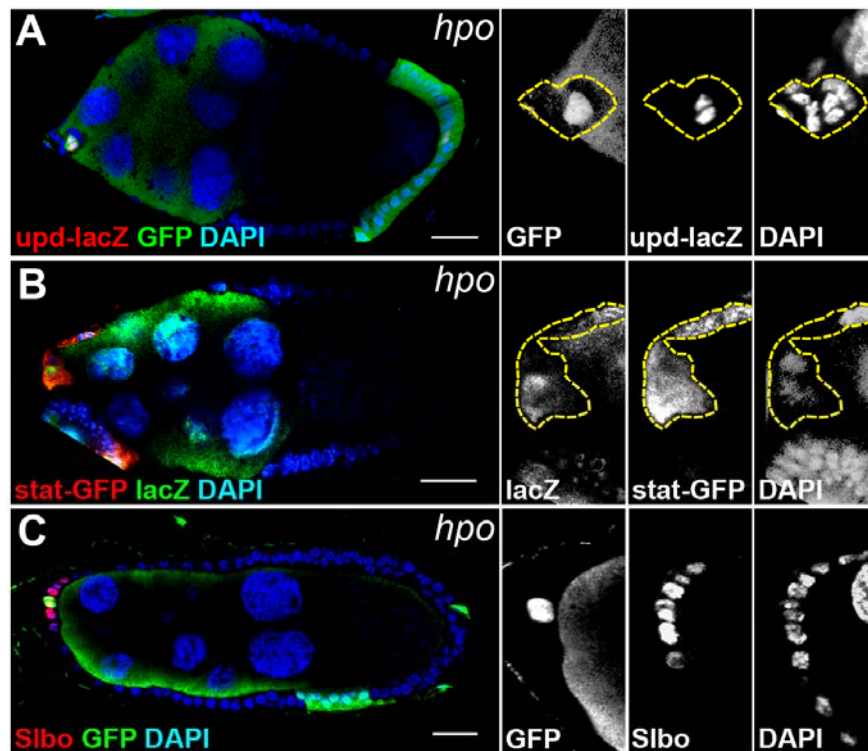
**Figure S1 The Hippo pathway regulates border cell migration after polar cell specification.**

*upd-Gal4* was used to knockdown or over-express genes in polar cells. The temperature-sensitive Gal80 system was adapted to control knockdown or expression of genes temporally. *UAS-GFP* was used as a control. (A) Quantification and percentage distribution of border cell migration after adult flies were incubated at 29°C for 40 hours. Knockdown of *wts* in polar cells attenuated border cell migration. (B) Quantification and percentage distribution of border cell migration after adult flies were incubated at 29°C for 54 hours. Knockdown of *wts* or over-expression of *yki-3SA* in polar cells severely attenuated border cell migration. Wilcoxon rank-sum test, \*:  $p < 0.05$ , \*\*:  $p < 0.01$ .



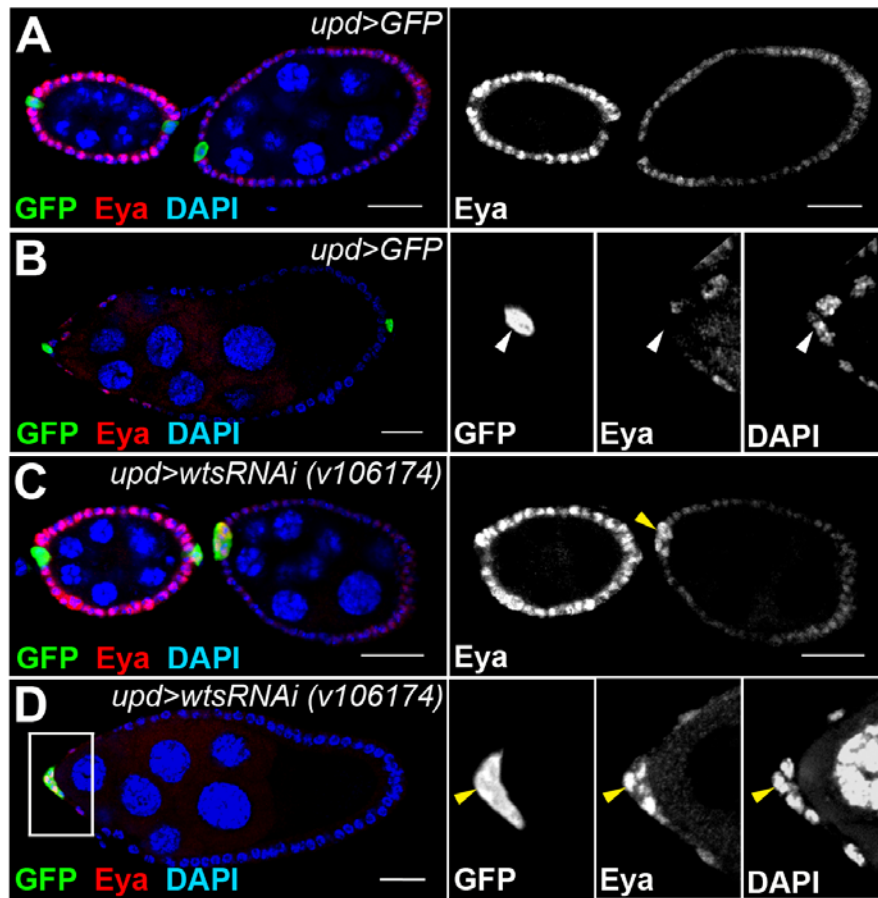
**Figure S2 Yki suppresses *upd* expression and controls border cell induction and migration.**

Mitotic clones of *FRT42D* (A) or *FRT42D yki<sup>B5</sup>* (B, C) were generated in an *upd-lacZ* background and examined six days after clone induction. The ovaries were immunostained with anti- $\beta$ -Gal and anti-GFP. (A) *upd-lacZ* was expressed in polar cells (white arrowheads) but not GFP-negative *FRT42D* cells (marked by yellow dashed lines). (B, B') Two focal planes of an egg chamber. A border cell cluster with GFP-positive polar cells (white arrowheads) expressed *upd-lacZ*. *yki* mutant cells (yellow arrowheads) also expressed *upd-lacZ* ectopically. (C, C') Two border cell clusters on different focal planes of an egg chamber. One cluster with wild-type GFP-positive polar cells expressing *upd-lacZ* remained at the anterior end (white arrowheads). The other cluster with GFP-negative *yki* mutant polar cells expressing *upd-lacZ* was migrating toward the oocyte (yellow arrowheads). Length of scale bar is 20  $\mu$ m for all panels.



**Figure S3 The Hippo pathway in outer border cells does not control JAK/STAT signaling in border cell clusters.**

Mitotic clones of *FRT42D hpo<sup>42-47</sup>* were generated and examined six days after clone induction. Egg chambers at stages 9 or 10 were selected and oriented as anterior towards the left. High magnification views of the border cell cluster are shown in the panels on the right. The ovaries were immunostained with anti-GFP, anti- $\beta$ -Gal (A, B), and anti-Slbo (C). (A) *hpo* mutant clones labeled by the absence of GFP were generated in an *upd-lacZ* background. A border cell cluster with *hpo* mutant outer border cells and GFP-positive control polar cells showed normal *upd-lacZ* expression in polar cells. (B) *hpo* mutant clones labeled by the absence of  $\beta$ -Gal were generated in a *stat-GFP* background. A border cell cluster with *hpo* mutant outer border cells and  $\beta$ -Gal-positive control polar cells showed normal *stat-GFP* expression in outer polar cells. (C) *hpo* mutant clone labeled by the absence of GFP were generated. A border cell cluster with *hpo* mutant outer border cells and GFP-positive control polar cells were positive for Slbo. Length of scale bar is 20  $\mu$ m for all panels.



**Figure S4 The Hippo pathway decreases the level of Eya in polar cells.**

*wts* was knocked down in polar cells by using *upd-Gal4*. *UAS-GFP* was used as a control (A, C). The ovaries were immunostained with anti-Fas3 and anti-Eya. Egg chambers at stages 5, 7 (A, C), and stage 9 (B, D) were selected and oriented as anterior towards the left. High magnification views of border cell clusters at stage 9 are shown in the panels on the right. (A) In the control, Eya was detected in main-body follicle cells but not polar cells. The level of Eya was reduced when main-body follicle cells entered endoreplication after stage 6. (B) In the control, low level of Eya was detected in outer border cells and stretch cells at stage 9. No Eya was detected in polar cells (white arrowheads). (C) Eya was detected in *wts*-knockdown cells at stage 7 (yellow arrowheads) but not stage 5 (yellow arrows). (D) Eya was detected in *wts*-knockdown cells at stage 9 (yellow arrowheads). Length of scale bar is 20  $\mu$ m for all panels.