

Expanded View Figures

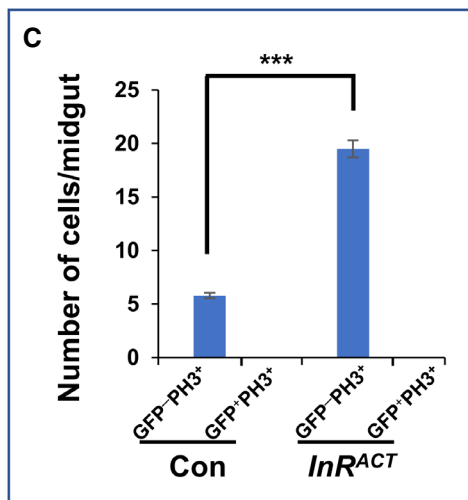
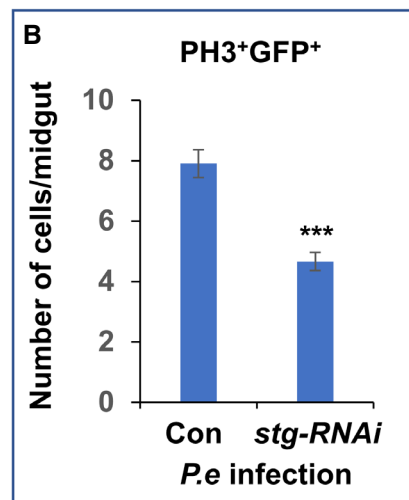
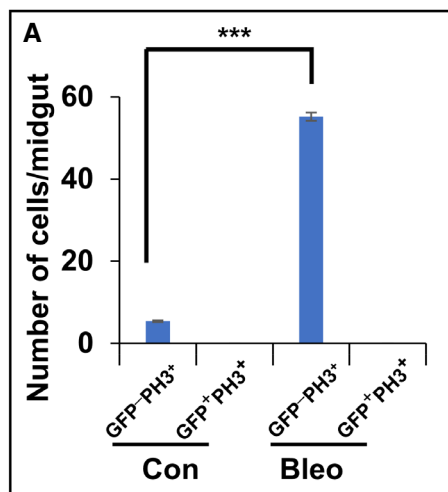


Figure EV1. Mitosis in the midguts in response to different treatments.

- A** Feeding with Bleo can induce mitosis in ISCs, but not in EBs, and quantification of $PH3^+GFP^-$ ISCs and $PH3^+GFP^+$ EBs with given treatments. $n = 21$ guts for each treatment. Three independent experiments were performed, and error bars are $\pm$ SEM. *** $p < 0.001$ (Student's t-test).
- B** Knockdown of *stg* in EBs can reduce EB mitosis upon *P.e.* infection, and quantification of $PH3^+$ in EBs (GFP^+) in the *Drosophila* midguts expressing *Su(H)^{ES}>CD8:GFP* with or without *UAS-stg-RNAi* upon *P.e.* infection. $n = 10$ guts for each genotype. Three independent experiments were performed, and error bars $\pm$ SEM. *** $p < 0.001$ (Student's t-test).
- C** Activation of Insulin signaling with *InR^{ACT}* in EBs noncell-autonomously promotes ISC proliferation, and quantification of $PH3^+$ in ISCs (GFP^-) and EBs (GFP^+) in the *Drosophila* midguts expressing *Su(H)^{ES}>GFP* with or without *InR^{ACT}* for 4 days at 29°C. $n = 12$ guts for each genotype. Three independent experiments were performed, and error bars $\pm$ SEM. *** $p < 0.001$ (Student's t-test).

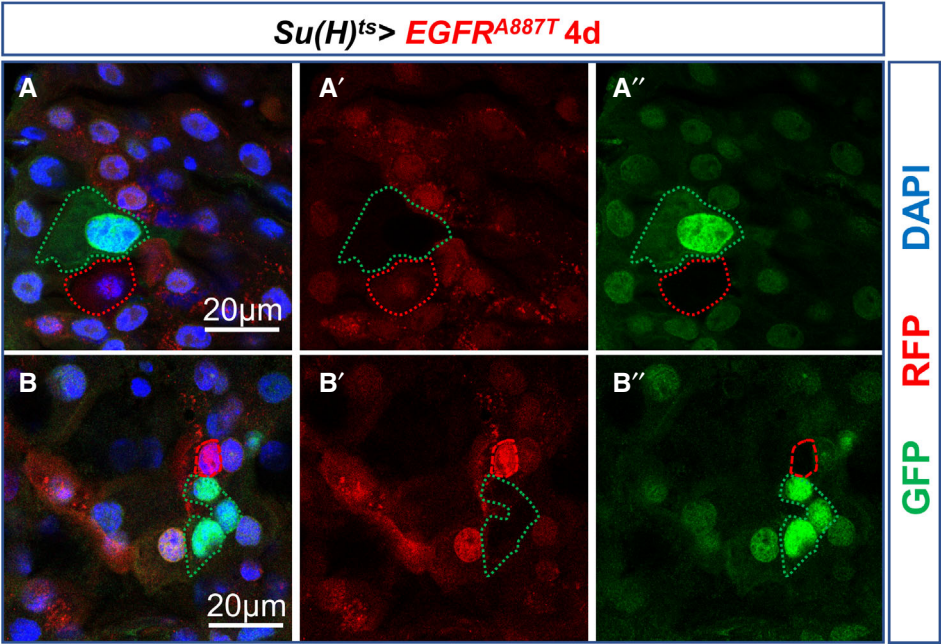


Figure EV2. Activation of EGFR-Ras signaling in EBs induces an EB to generate one ISCs or non-ISCs.

A–B'' Representative twin clones from midguts show symmetric division in EBs to produce two non-ISCs (A–A'') or asymmetric division in EB to produce one ISC with multiple cells (green) and one non-ISC (red) (B–B'').

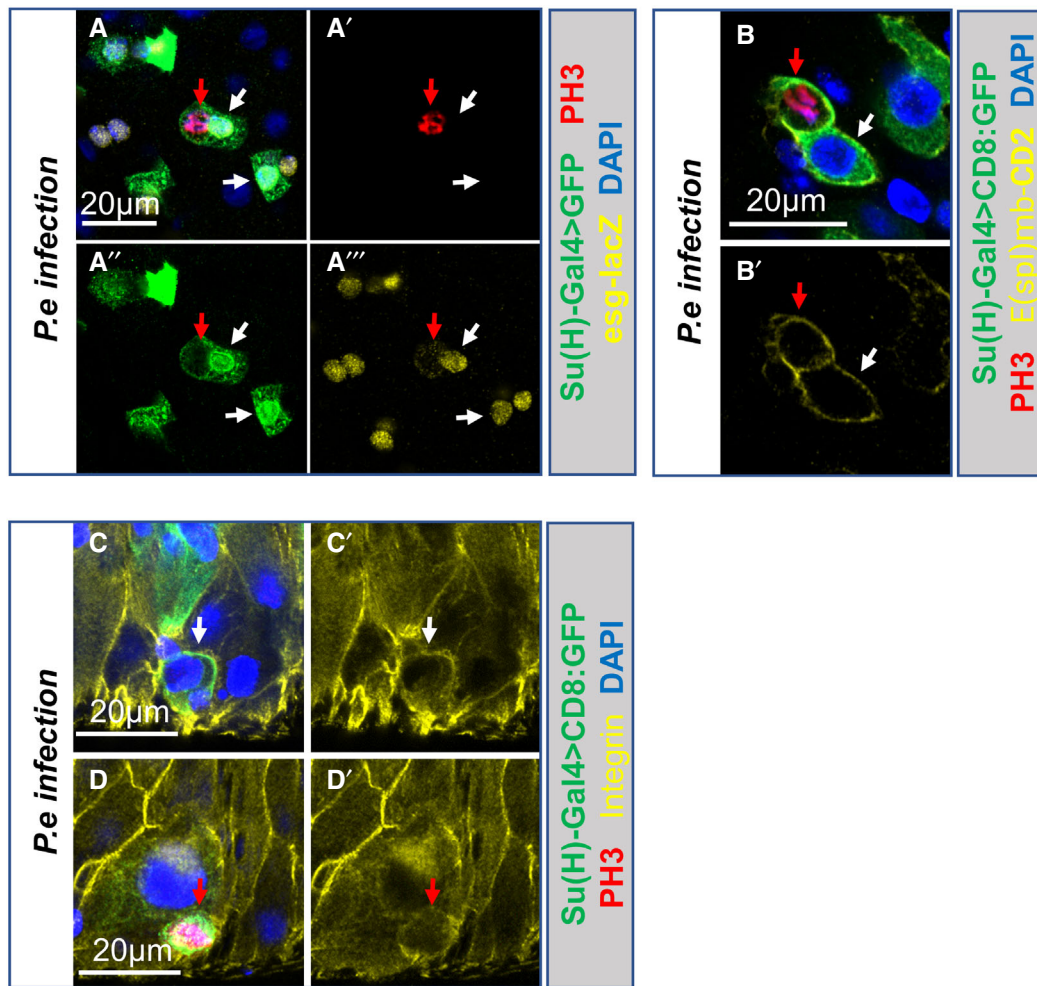


Figure EV3. Expression of reporters in the dividing EBs.

A–A''' A representative image showed that *esg* expression with a reporter *esg-lacZ* was down-regulated in mitotic EBs upon *P.e.* infection (red arrows). White arrows indicate GFP⁺ EBs without PH3 expression.
 B–B' The Notch reporter *E(spl)mb-CD2* was normally expressed in dividing EBs (red arrows). White arrows indicate GFP⁺ EBs without PH3 expression.
 C–D' The integrin was normally expressed and localized in dividing EBs (D, D', red arrows) compared with nondividing EBs (C, C', white arrows).

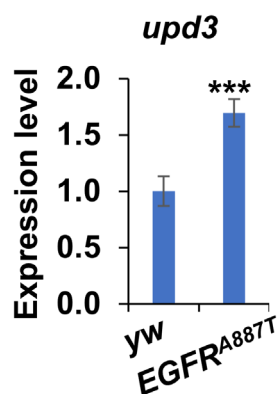


Figure EV4. Activation of EGFR-Ras signaling in EBs up-regulates *up3* expression.

Activation of EGFR-Ras signaling in EBs with *Su(H)^{ts}>UAS-EGFR^{A887T}* up-regulates the expression of *up3*. Three independent experiments were performed, and error bars are $\pm$ SEM. *** $p < 0.001$ (Student's *t*-test).

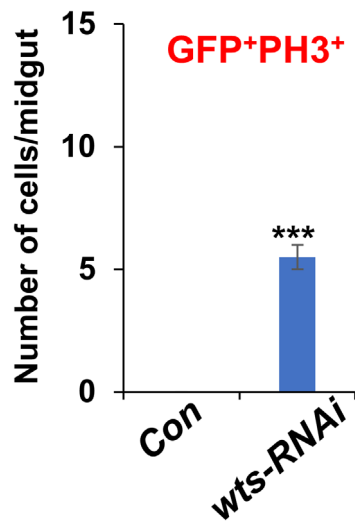


Figure EV5. Knockdown of *wts* in EBs promotes EB mitosis.

Quantification of PH3⁺ in EBs (GFP⁺) in the midguts expressing *Su(H)^{ts}>CD8:GFP* with or without *UAS-wts-RNAi* for 5 days at 29°C. *n* = 10 guts for each genotype. Three independent experiments were performed, and error bars $\pm$ SEM.

****p* < 0.001 (Student's *t*-test).