

Figure S3: Lid acts in germ cells to maintain proper hub size. (A) Quantification of average hub area in wt ($113.9 \pm 28.2 \mu\text{m}^2$) versus *lid* ($231.5 \pm 84.2 \mu\text{m}^2$) testes. (B) Quantification of average hub area in testes from males of the following genotypes: *upd-Gal4* control ($134 \pm 14 \mu\text{m}^2$); *upd-Gal4;; UAS-lid shmiRNA* ($123 \pm 17 \mu\text{m}^2$); *c587-Gal4* control ($125 \pm 34 \mu\text{m}^2$); *c587-Gal4;; UAS-lid shmiRNA* ($137 \pm 24 \mu\text{m}^2$); *nos-Gal4* control ($120 \pm 25 \mu\text{m}^2$); *nos-Gal4/ UAS-lid shmiRNA* ($208 \pm 50 \mu\text{m}^2$). *P*-values calculated using Student's *t*-test. Error bars represent s.d.

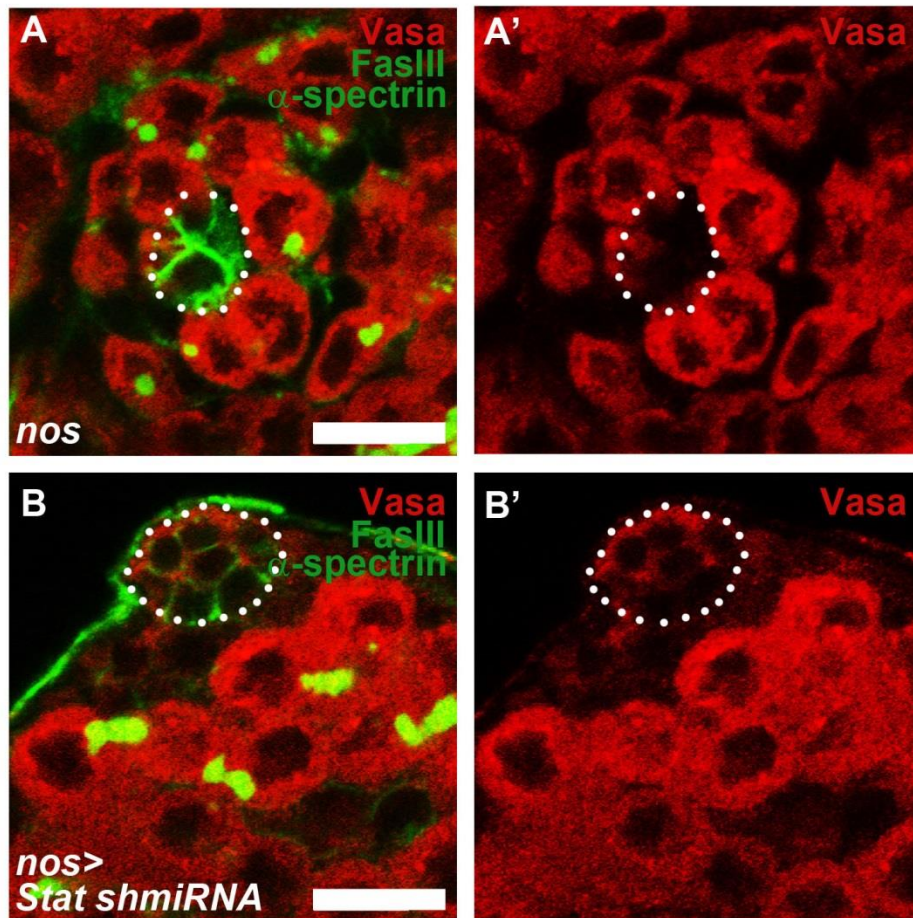


Figure S4: Knockdown of germline *Stat92E* results in GSC displacement. (A-B')

Immunostaining using antibodies against Vasa (red), FasIII (green), and α -spectrin (green) in *nos-Gal4* (A-A') and *nos-Gal4; UAS-Stat shmiRNA* (B-B') testes. Note detachment of GSCs from the hub in (B-B'). Hub area is outlined (white dotted line). Scale bars: 10 μ m.

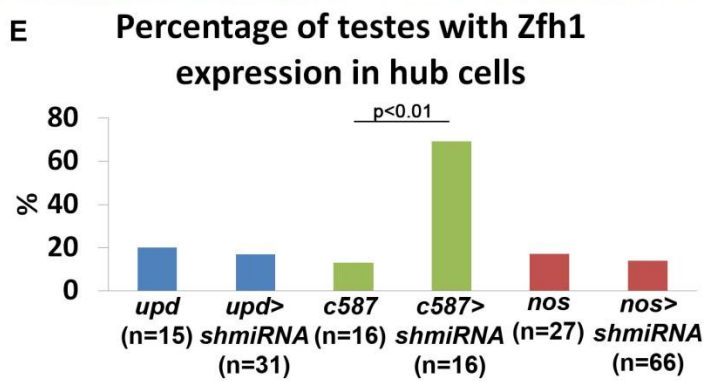
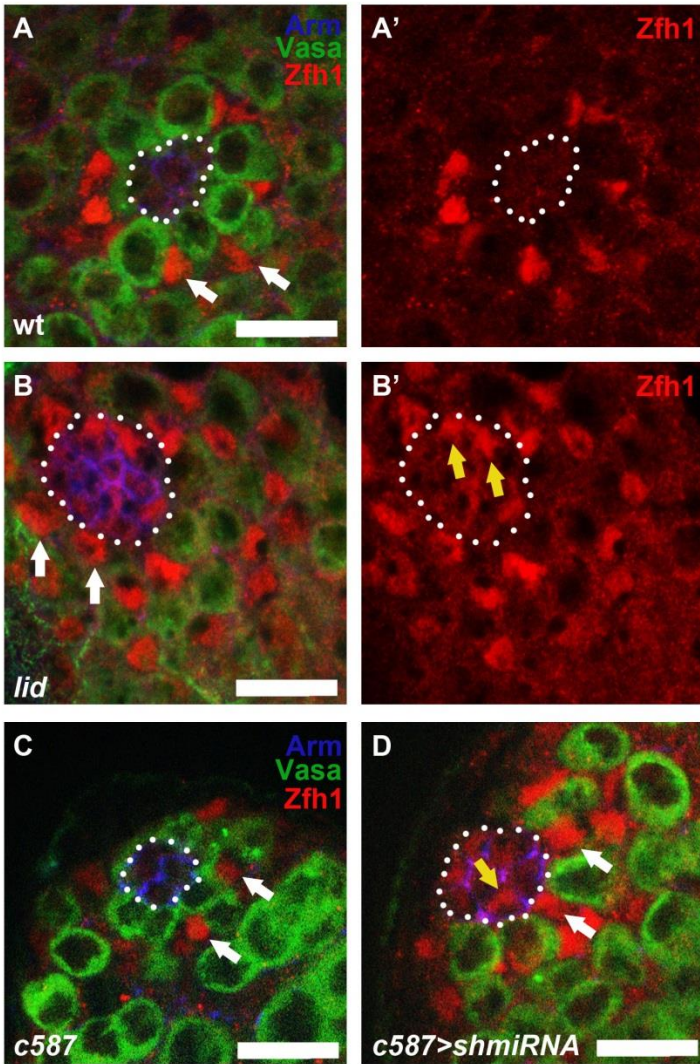


Figure S5: Lid acts in CySCs and/or early cyst cells to repress overpopulation of Zfh1-expressing cells around the hub and ectopic Zfh1 expression in hub cells. (A-D)

Immunostaining using antibodies against Arm (blue), Vasa (green), and Zfh1 (red). Hub area is

outlined (white dotted line). **(A-A')** wt testis. White arrows point to CySCs with normal distribution around the GSCs with thin extensions towards the hub. **(B-B')** *lid* testis. White arrows point to overpopulation of Zfh1-expressing cells with nuclei that directly contact the hub. Yellow arrows point to Zfh1-expressing cells in the hub. **(C)** *c587-Gal4* testis. White arrows point to CySCs with normal distribution around the GSCs with thin extensions towards the hub. **(D)** *c587-Gal4;; UAS-lid shmiRNA* testis. White arrows point to overpopulation of Zfh1-expressing with nuclei that directly contact the hub. Yellow arrows point to Zfh1-expressing cells in the hub. **(E)** Percentage of testes with ectopic Zfh1 expression in hub cells. *P*-values calculated using Fisher's test.