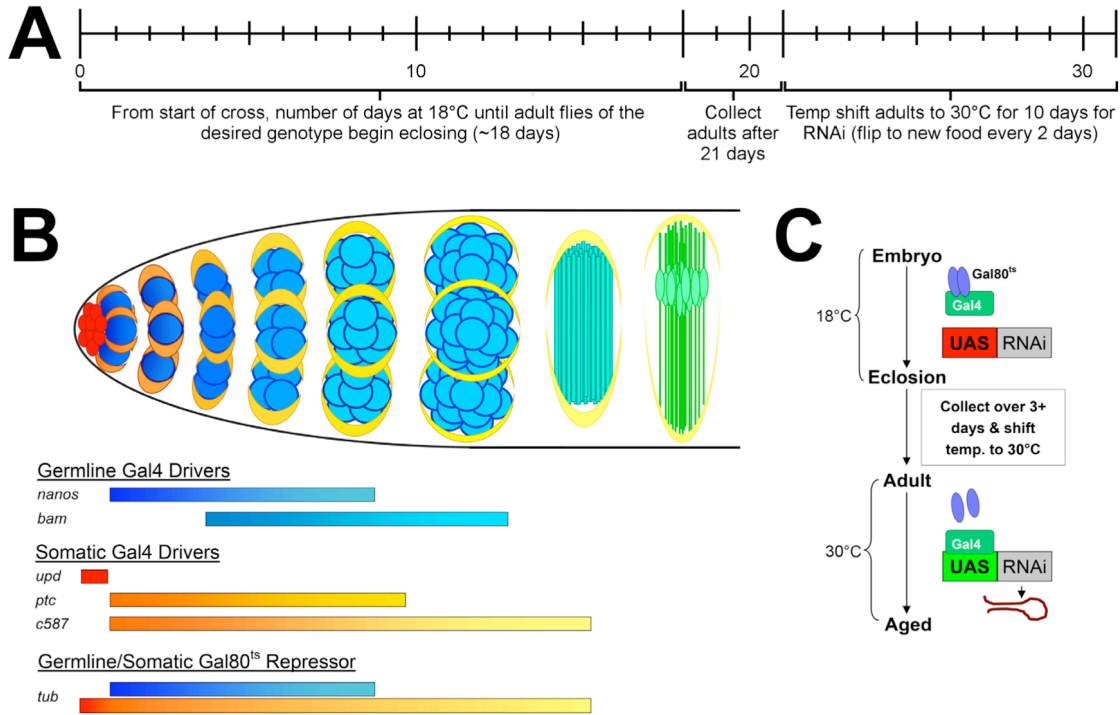


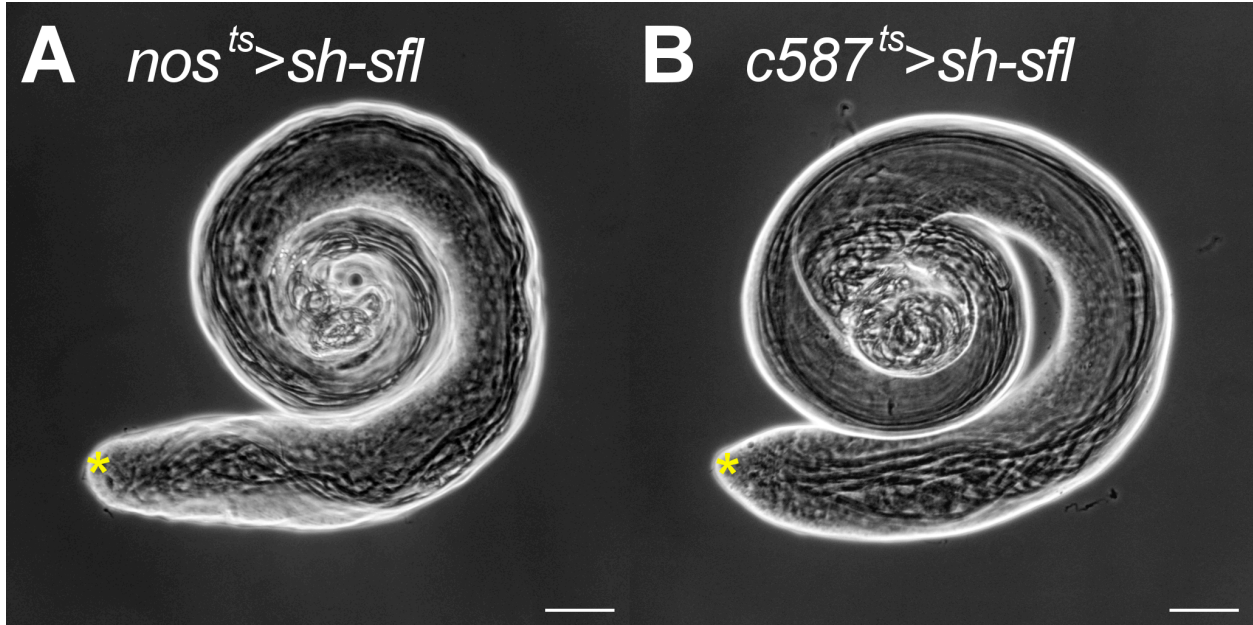
Supplemental Materials



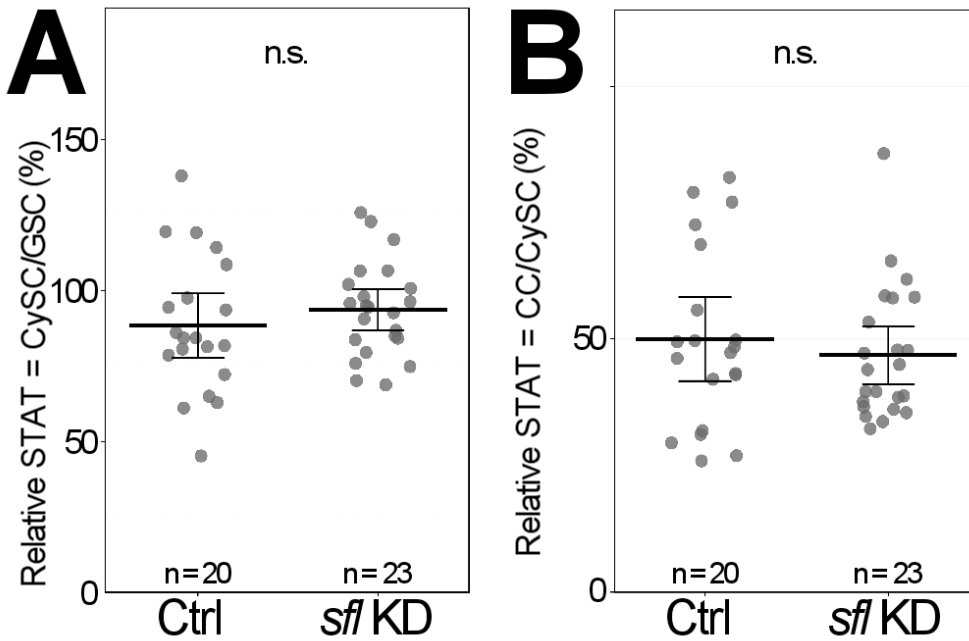
Supplemental Figure 1. Gal4-Gal80^{ts} (TARGET) in the *Drosophila* testis

(A) Experimental timeline for TARGET in testis. Flies are crossed in a bottle kept at 18°C and allowed to lay for 10-16 days. Adults are removed and flipped to a new bottle at 18°C. At approximately 18 days (between 18-20 days, depending on the strain) adults of the desired TARGET genotype began eclosing. Adults are collected 3 days from the start of eclosion (so the collected adults will be from 0-3 days old). These adults are cultured in vials with no more than 20 males and 20 females, and then cultured at 30°C for 10 days (or indicated time) before the testes are dissected. (B) Gal4 driver expression profiles are shown for the testis. Cell types found in the testis are indicated by different colors (top). Different Gal4 lines can drive expression in specific cell types, including the hub (red), germline cells (blue), and CySCs/CCs (orange to yellow). Some lines, such as *arm-Gal4*, drive expression more ubiquitously in the soma. The

Gal80^{ts} repressor is expressed ubiquitously in the germline and soma. (C) Schematic of experimental protocol for use of TARGET in the testis. In short, flies carrying a cell type-specific Gal4 and ubiquitous Gal80^{ts} are raised at 18°C, the temperature at which Gal80^{ts} effectively represses Gal4 activity. Adult flies are collected over several days after eclosion, and then shifted to 30°C, which is the Gal80^{ts} restrictive temperature. This temperature increase represses Gal80^{ts}, thus allowing Gal4 activity, leading to expression of a UAS-linked transgene such as an RNAi construct.



Supplemental Figure 2. Loss of HS in germline and somatic cells does not result in tumors. Phase contrast images of testes from *nos^{ts}>sh-sfl* (A) and *c587^{ts}>sh-sfl* (B). Both genotypes show normal testis morphology and progressive organization of spermatogenic cells. No tumorous testes were ever observed. Yellow asterisks indicate hub location. Bars: 100 μ m.



Supplemental Figure 3. Non-eCC somatic cells show similar relative Stat levels between control and hub *sfl* RNAi.

Quantification of relative Jak/Stat signaling between different cell types. (A) When CySC Stat signal was divided by GSC's in each testis, no difference in Stat signal was detected between control and hub *sfl* RNAi. As previous studies have reported, GSCs showed higher Stat signal than CySCs on average, in both genotypes. (B) When the CC Stat signal was divided by CySC signal in each testis, no difference in Stat signal was detected between control and hub *sfl* RNAi. Both genotypes showed a significant reduction in Stat levels in CCs, compared to CySCs, as expected. Numerical figures depict the mean \pm SE. n.s.: not significant. n: number of testes assayed.