

Figure S1. Starvation has no significant effect on hub size, Upd expression, or Stat92E levels in GSCs. (A) Quantification of hub cell number on day 9 of adulthood in fed or starved conditions. Data presented as mean \pm s.d. p -value > 0.05 . (B) Expression of Upd-YFP protein under endogenous promoter (green) in the hub (dotted outline) was unchanged in fed vs. starved conditions. (C) Stat92E-GFP (green) expression in GSCs (dotted outline) attached to hub (asterisk) was unchanged in fed vs. starved conditions. Scale bars = 10 μ m.

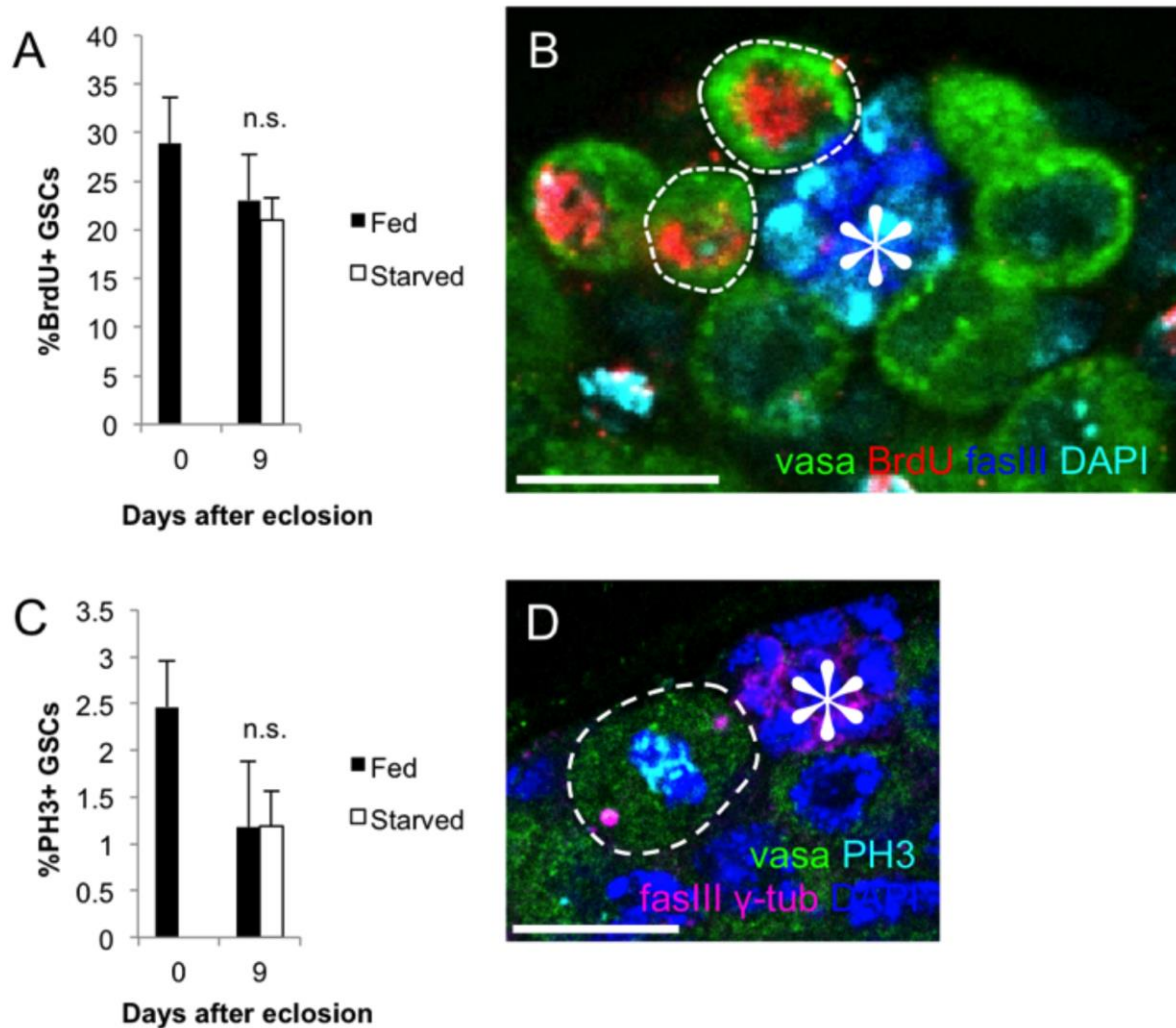


Figure S2. S-phase index and mitotic index decrease with age but are not significantly affected by protein starvation. (A) Frequency of GSCs in S-phase at 0 and 9 days of protein starvation. GSCs in S phase were detected by soaking dissected testes in BrdU-containing media for 45 minutes. $N > 100 \times 3$ GSCs per data point. Data presented as mean \pm s.d. (B) Representative image of GSCs in S-phase (dotted outline) with BrdU+ nuclei (red). (C) Frequency of GSCs in mitosis at 0 and 9 days of starvation. Mitotic GSCs were detected by staining with anti-phospho-histone H3 (PH3-phospho-Thr3) antibody. $N > 500 \times 3$ GSCs per data point. Data presented as mean \pm s.d. (D) Representative image of a GSC in mitosis (dotted outline). Hub is denoted by asterisk. GSCs are indicated by broken lines. Scale bars = 10 μ m.

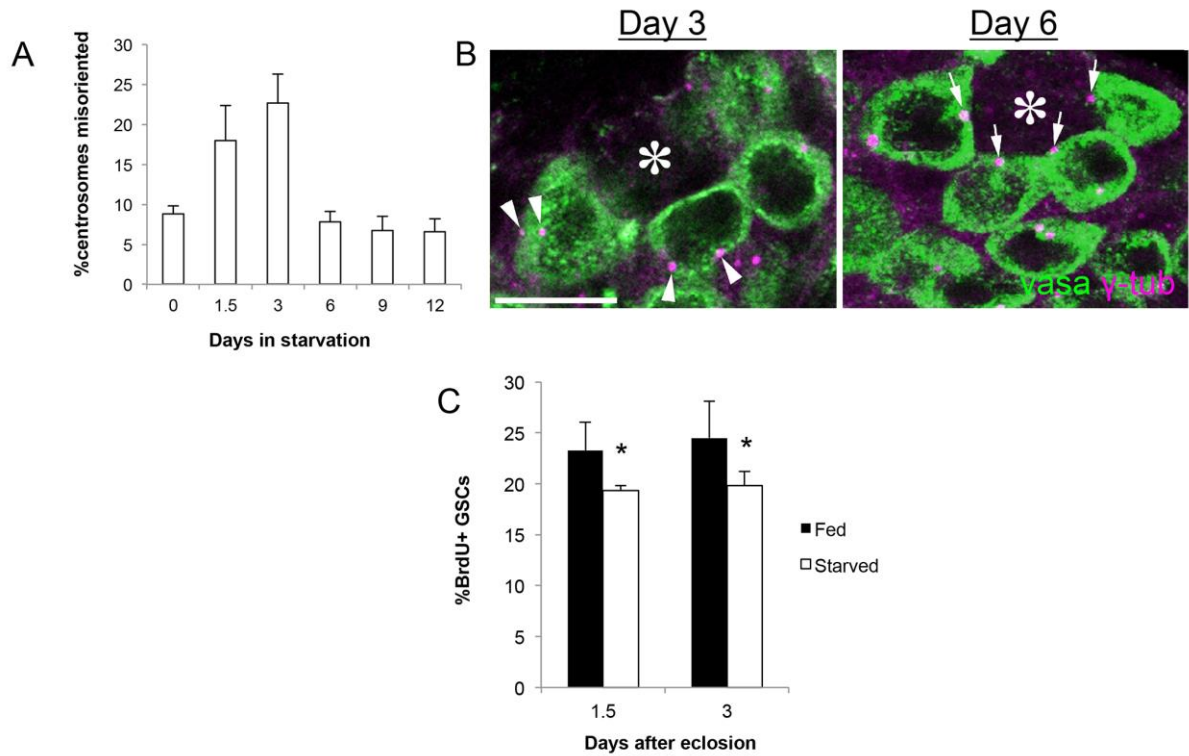


Figure S3. Transient centrosome misorientation and reduced cell cycle progression in GSCs upon protein starvation. (A) Percent of GSCs with misoriented centrosomes over 12 days of protein starvation. $N > 100 \times 3$ GSCs per data point. Data presented as mean \pm s.d. (B) Representative images of GSCs with misoriented centrosomes (arrowheads) on day 3 of starvation and oriented centrosomes (arrows) on day 6 of starvation. The hub is denoted by an asterisk (*). Scale bar = 10 μ m. (C) Percent of GSCs in S-phase after 1.5 and 3 days of starvation, demonstrating a transient slowdown of cell cycle during the initial stage of protein starvation. $N > 100 \times 3$ GSCs per data point. Data presented as mean \pm s.d. asterisk (*) indicates $p < 0.05$.

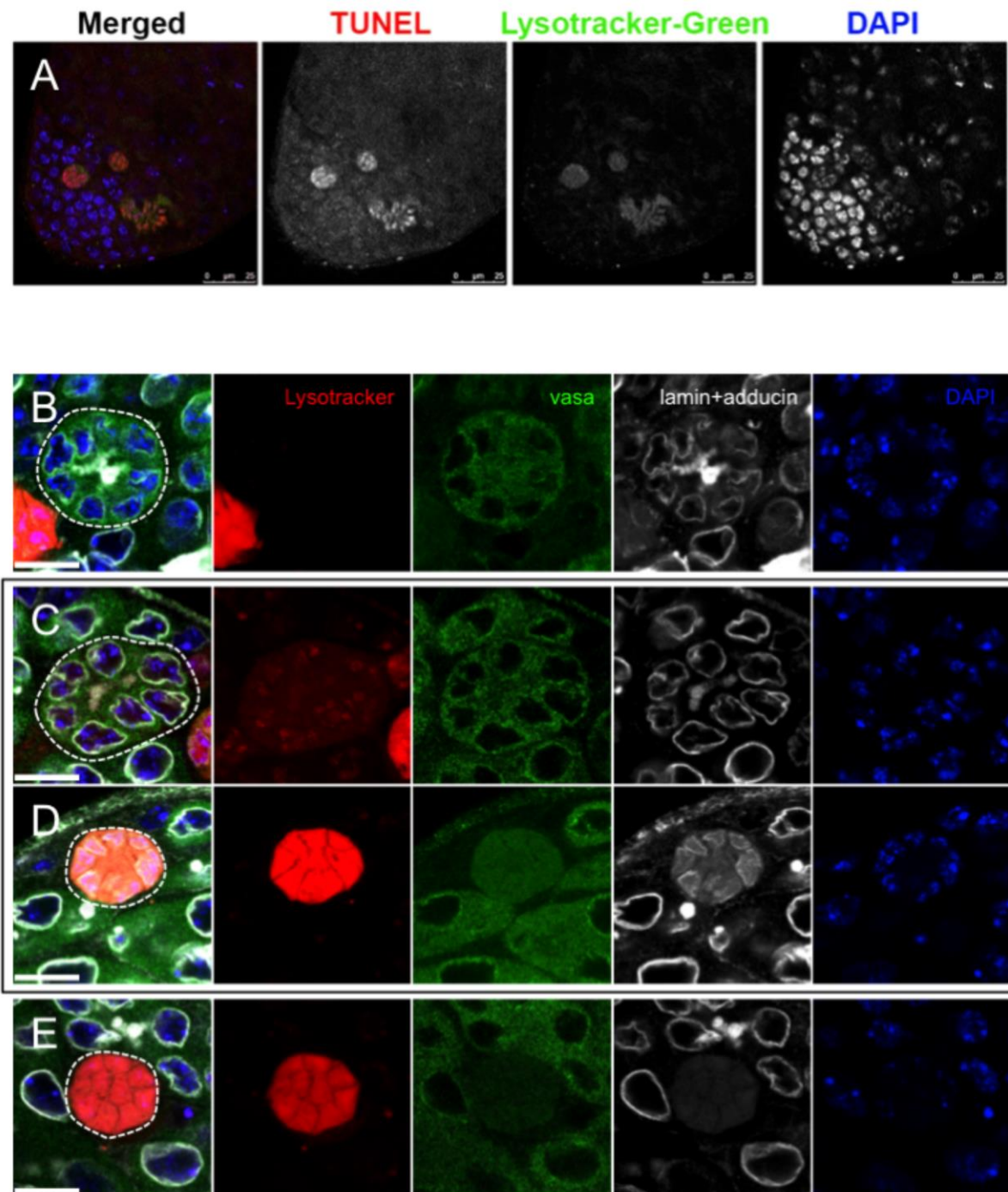


Figure S4. Dying SGs can be quantified according to SG stage using Lysotracker and Lamin staining. (A) TUNEL and Lysotracker completely colocalize in testes that were starved for 3 days. SGs die synchronously by (B) rounding up, (C) staining positive for Lysotracker, (D) losing cytoplasmic vasa staining, and (E) losing all identifying markers except for Lysotracker. To accurately quantify SG death by stage, only (C) and (D), which are positive for Lysotracker and Lamin, were scored. (Note: panel D is same as “16-cell stage” shown in Fig. 2B). Scale bar = 10 μm.

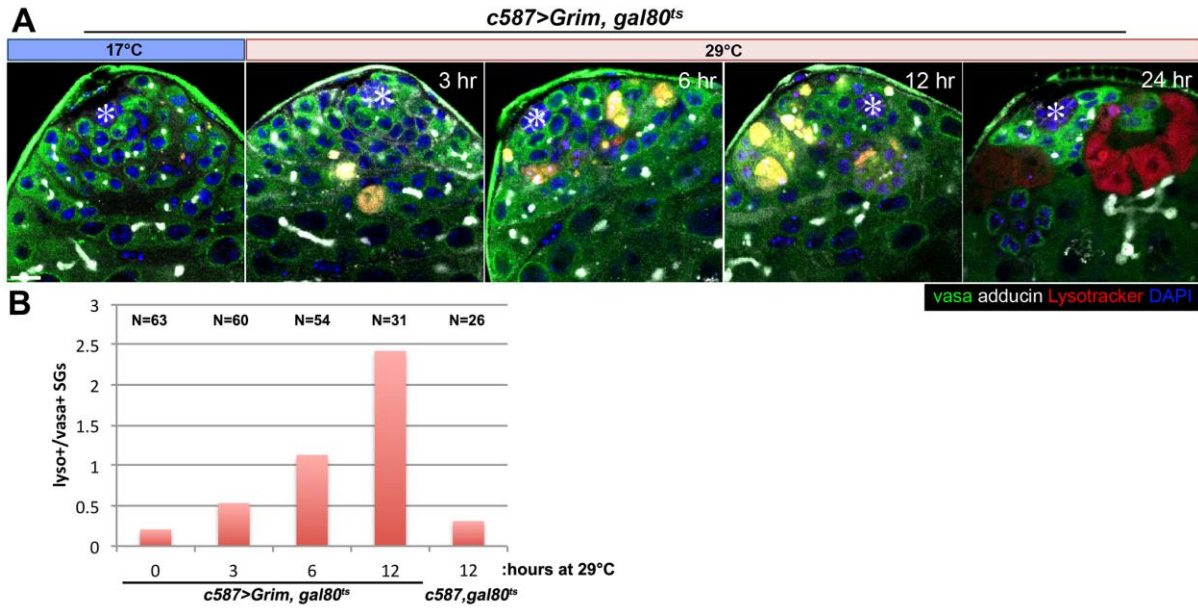


Figure S5. CC apoptosis is sufficient to initiate SG death. (A) SG death (red, Lysotracker) was dramatically increased upon expression of a proapoptotic gene, Grim, in CySC/CC lineage (*c587-gal4, gal80^{ts}, UAS-Grim*). Hub is denoted by asterisk (*). Scale bars = 10 μ m. (B) Quantification of dying SGs (Lysotracker+, Vasa+) upon induction of Grim expression. N; the number of testes scored.

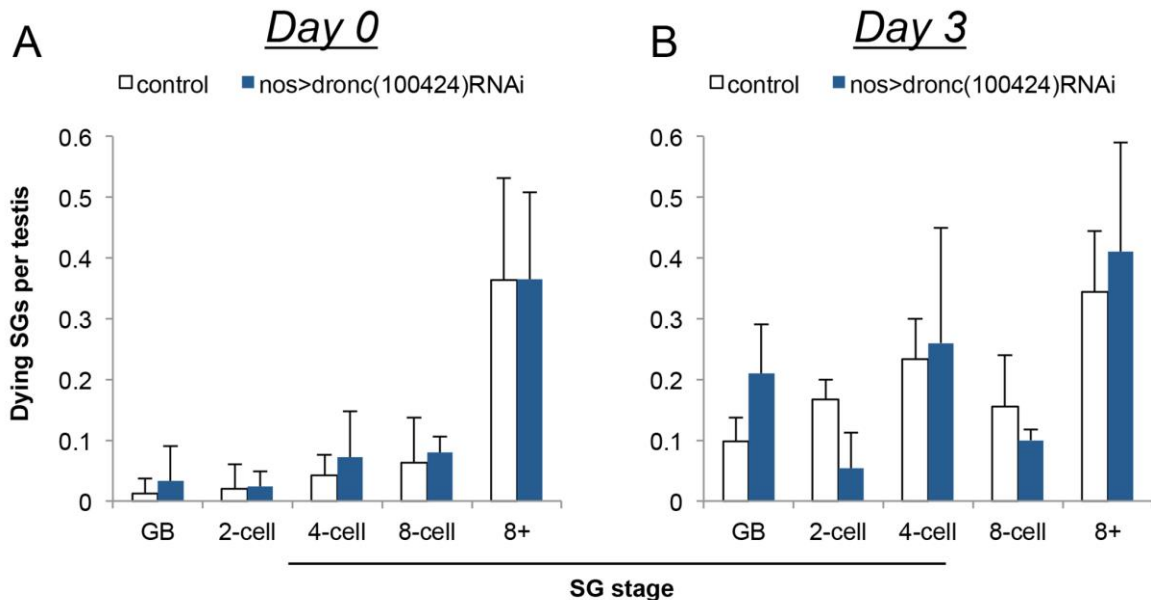


Figure S6. SG death is not suppressed by RNAi knockdown of Dronc in the germline. Quantification of germ cell death in control and *nos>Dronc-RNAi* testes (A) upon eclosion and (B) after 3 days of starvation. Data is presented as mean \pm s.e. N > 30 x 3 testes in triplicate per data point.

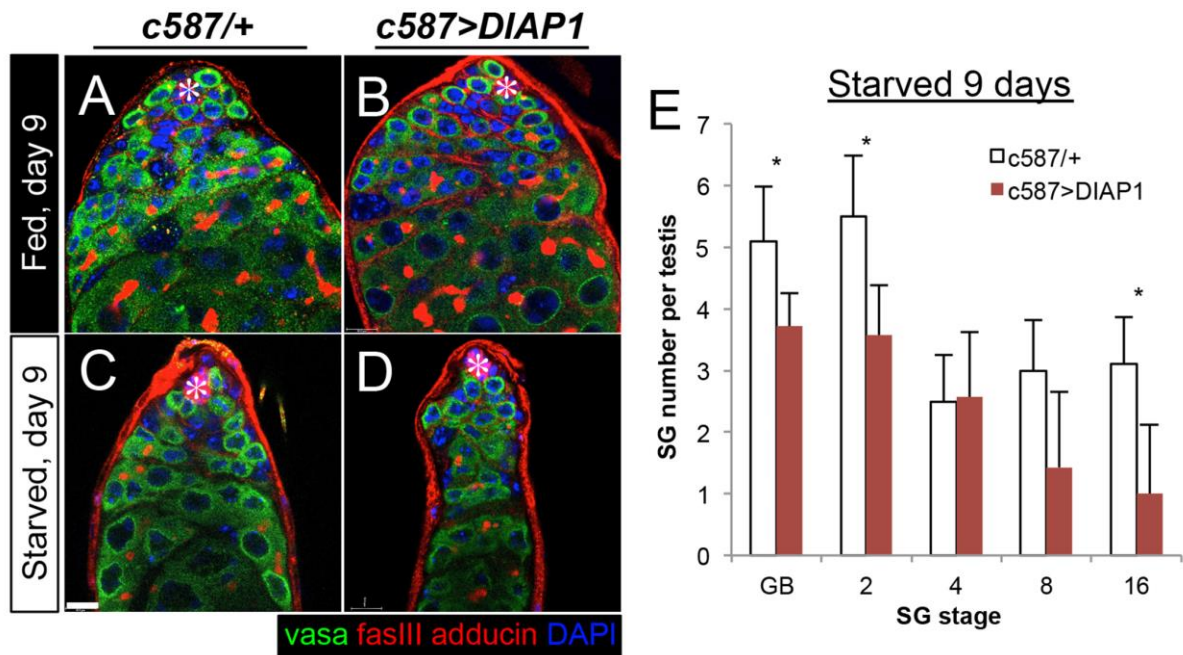


Figure S7. Expression of DIAP1 in CCs leads to tissue involution upon protein starvation. (A-D) representative images of testis apical tip in control vs. DIAP1-expressing testes in fed/starved conditions. Hub is denoted by asterisk. (E) SG number upon expression of DIAP1 under starved conditions. $N > 10 \times 3$ testes per condition. Data presented as mean \pm s.d. Asterisk (*) indicates $p < 0.05$.

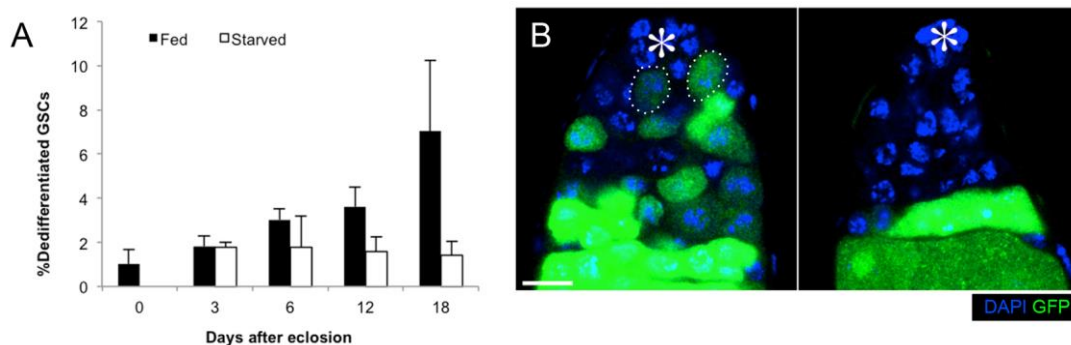


Figure S8. Dedifferentiation decreases upon protein starvation. (A) The percent of GSCs that are dedifferentiated over 18 days in fed vs. starved conditions. Data is shown as mean \pm s.e. $N > 50 \times 3$ testes in triplicate per data point. (B) Dedifferentiated GSCs (dotted outline) are labeled by permanent marking of SGs at the Bam+ stage with GFP (Bam-gal4, UAS-FLP, nos-FRT-stop-FRT-gal4, UAS-GFP). Asterisk (*) denotes hub. Scale bars = 10 μ m.