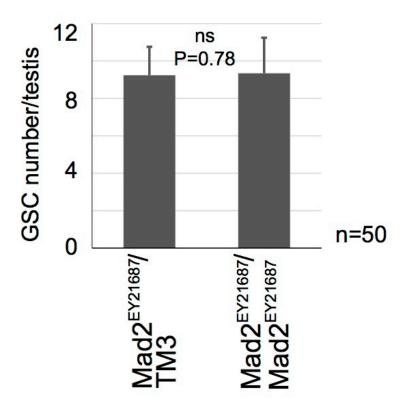


**Fig. S1. Effect of** *ex vivo* **colcemid treatment on adult testis.** Apical tip region of mock-(A) and colcemid-(B) treated testes after 1-hour incubation with DMSO or colcemid. (A', B') Images in (A) and (B), respectively, merged with images of Vasa and DAPI staining. Red: Vasa. Green: α-tubulin. Blue: DAPI. Asterisks (\*) indicate the hub. Scale bars:  $10 \, \mu m$ . (C) GSC number per testis after *ex vivo* treatment with DMSO or colcemid for 6 hours. Error bar indicates s.d. P values were determined by two-tailed two-sample t-test..



**Fig. S2. GSC number in** *mad2* **mutant adult testis.** GSC number per testis from *mad2* heterozygous and homozygous mutants. Error bar represents s.d.. P values were determined by two-tailed two-sample t-test.

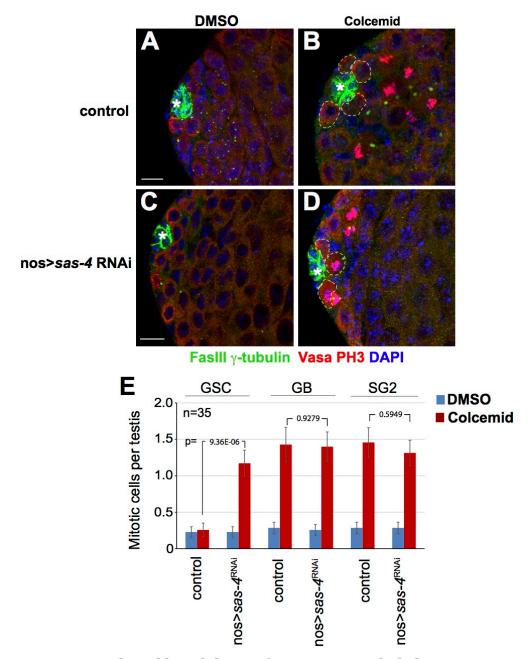


Fig. S3. RNAi-mediated knockdown of sas-4 in GSCs abolishes G2 arrest upon microtubule (MT) depolymerization. (A-D) Examples of apical tip in mock-treated control (A), colcemid-treated control (B), mock-treated nos-gal4>sas-4<sup>RNAi</sup> (TRiP.HMS01463) (C), and colcemid-treated nos-gal4>sas-4<sup>RNAi</sup> testes after 4.5 hours. Red: Vasa, PH3. Green: FasIII,  $\gamma$ -tubulin. Blue: DAPI. Asterisks (\*) indicate the hub. Scale bars: 10  $\mu$ m. (E) Mitotic index of germline cells from control and sas-4<sup>RNAi</sup> adult testes after 4.5 hours of mock (DMSO) or colcemid treatment. Error bars indicate s.e.m. P values were determined by two-tailed two-sample t-test.