Supporting Online Material

Materials and methods

Fly stocks and culture

Flies were raised on standard yeast/molasses medium at 25°C unless otherwise stated. The following stocks were used: *P[neoFRT]82B stat92E^{06346}/TM3 Sb* (*S1*), *P[neoFRT]82B stat92E^{j6C8}/TM3 Sb* (*S2*) (both from D. Harrison), *y w, P[hs-FLP], P[UAS-CD8-GFP]; P[tub-Gal4] P[neoFRT]82B P[tub-Gal80]/TM6B Tb* (*S3*) (from B. Ohlstein), *stat92E^{Frankenstein}* (*S4*) (from C. Dearolf), *hsp70-outstretched* (*S5*) and *UAS-outstretched* (*S6*) (from D. Harrison), *nanos-Gal4* (*S7*) (from M. van Doren), *c587-Gal4* (*S8*) (from A. Spradling), *socs36E^{PZ1647}* (a gift from A. Spradling), *UAS-socs36E-45* (*S9*) (from B. Callus), and *rhea$^{6-66}$* (*S10*) (from A. Ghabrial). Other stocks were from the Bloomington Stock Center.

Inducing or removing JAK-STAT signaling

Ectopic JAK-STAT signaling was induced via Outstretched overexpression by heat shocking male flies carrying the Outstretched cDNA under the control of the *hsp70* promoter (*hsp70-outstretched*) for 30 minutes at 37°C. JAK-STAT signaling was abolished through conditional removal of *stat92E* function by shifting *stat92E* temperature-sensitive flies (*stat92E^{Frankenstein}/stat92E^{06346}*) from the permissive temperature of 18°C to the restrictive temperature of 29°C for 1 day. These testes still contain hubs and somatic stem cells (*S11*).

Mosaic analysis

Stat92E: Positively marked clones homozygous for *stat92E*^{06346} or *stat92E*^{j6C8} were induced using MARCM (*S12*) in flies of the genotype *y w, P[hs-FLP], P[UAS-CD8-GFP]/Y; P[tub-Gal4] P[neoFRT]82B P[tub-Gal80]/P[neoFRT]82B stat92E*^{06346} or *y w, P[hs-FLP], P[UAS-CD8-GFP]/Y; P[tub-Gal4] P[neoFRT]82B P[tub-Gal80]/P[neoFRT]82B stat92E*^{j6C8}. Control clones were induced in *y w, P[hs-FLP], P[UAS-CD8-GFP]/Y; P[tub-Gal4] P[neoFRT]82B P[tub-Gal80]/P[neoFRT]82B ry*^{506}. Adult male flies were collected 0-5 days after eclosion and heat shocked for 1 hour in a 37˚C water bath; after heat shock, they were returned to 25˚C.

Socs36E: The Flipase (FLP)-mediated mitotic recombination technique (*S13*) was used to induce negatively marked *socs36E*^{PZ1647} homozygous mutant clones in the germline and/or soma in flies of the genotype *+/Y; socs36E*^{PZ1647} *P[neoFRT]40A/Ubi-GFP.nls P[neoFRT]40A; MKRS, P[hsFLP]*/+; control (wild-type) clones were induced in flies of the genotype *w/Y; P[neoFRT]40A/Ubi-GFP.nls P[neoFRT]40A; MKRS, P[hsFLP]*/+. Adult male flies were heat shocked three times at 37°C for 30 min. After heat shocking, flies were maintained at 25°C and subsequently dissected and stained 2, 6, 10, 14, 18, and 22 days after clone induction (ACI). *socs36E* mutant stem cells were identified by their absence of GFP and presence of the germline marker Vasa (for GSCs contacting the hub) or ZFH-1 (for CPCs and their daughters).

Immunofluorescence microscopy

Testes were dissected from newly eclosed flies (0-3 days old unless otherwise stated) and then fixed and immunostained as previously described (*S14*) using the following antibodies: rabbit anti-Vasa (d-260) and goat anti-Vasa (dN-13) (Santa Cruz Biotechnology; 1:200 dilution), rabbit anti-GFP (Torrey Pines Biolabs; 1:10,000 dilution), affinity-purified rabbit anti-STAT (from E. Bach; 1:400 dilution), guinea pig anti-ZFH-1 (from J. Skeath; 1:1000 dilution), mouse monoclonal antibody 1B1 and mouse anti-Fasciclin III (Developmental Studies Hybridoma Bank; 1:50 dilution), mouse anti-ßPS-integrin (Developmental Studies Hybridoma Bank; 1:20 dilution), and rabbit anti-phospho-Histone H3 (Upstate/Millipore; 1:200 dilution). Alexa 488- and Alexa 568-conjugated secondary antibodies (Molecular Probes/Invitrogen) were used at a 1:400 dilution. DNA was stained with 4,6-diamidino-2-phenylindole (DAPI; Sigma) at a concentration of 1 μg/ml. For Stat92E immunostains, testes were incubated sequentially with anti-STAT, then anti-Vasa antisera at 4°C overnight. Confocal images were obtained with a Leica TCS NT or Zeiss LSM 5 Pascal microscope. Figures were assembled using Adobe Photoshop 7.0, Adobe Illustrator CS, and Adobe InDesign.

Quantifying GSCs and CPCs

The number of GSCs/testis and CPCs/testis was determined using serial confocal reconstructions of the entire testis apex, which includes the stem cells, the spermatogonia, and some early spermatocytes. GSCs were identified as Vasa-positive cells containing a spherical fusome and contacting the hub. CPCs and their immediate daughters were identified as ZFH-1-positive cells with strong to medium staining as determined by the

rainbow indicator palette in the Zeiss Pascal software. An approximate number of CPCs was determined by dividing the number of ZFH-1-positive cells (CPCs and their respective daughter cyst cells) by 2. Mitotic ZFH-1 positive cells were identified by double staining with ZFH-1 and the mitotic marker, phospho-Histone H3. Statistical analysis of stem cell number (reported as mean ± standard error of the mean, SEM) was performed using the GraphPad Prism program. P-values were obtained between two groups by Student's t test or between more than two groups by analysis of variance (ANOVA).

In situ hybridization

In situ mRNA hybridizations of *Drosophila* testes were performed as previously described (*S15*). Digoxigenin-labeled anti-sense RNA probes (Roche) were synthesized according to the manufacturer's instructions. An antisense probe to *socs36E* was synthesized from a KpnI-digested pBS-*socs36E*-probe (plasmid from B. Callus) with T7 RNA polymerase. A sense probe to *socs36E* was synthesized from an XbaI-digested pBS-*socs36E*-probe with T3 RNA polymerase and used to assess background. CPCs were identified based on morphology and their proximity to the hub. *socs36E* expression pattern was recapitulated in the PZ1647 enhancer trap line by immunostaining with anti-ß-Galactosidase antisera.

Northern blot analysis

RNA samples *Drosophila* embryos were isolated using Trizol (Invitrogen) according to the manufacturer's instructions. Poly(A) RNA was separated from the total RNA

fraction using the QIAgen poly(A) RNA purification kit. The purified RNA was fractionated on formaldehyde/agarose gels, transferred to a nitrocellulose membrane, hybridized with a ^{32}P-radiolabeled *socs36E* probe and autoradiographed.

PCR

Inverse PCR and sequencing of the P-element line to determine the site of P-element insertion in the genome was performed according to the Berkeley Drosophila Genome Project. BLASTn results revealed the PZ-element was inserted into the *socs36E* gene.

Supporting Online Text

***socs36E* is a target and inhibitor of JAK-STAT signaling in the testis niche.** Ectopic JAK-STAT signaling expands the expression of *socs36E*, while removing JAK-STAT signaling abolishes *socs36E* expression (Fig. S1C-D), indicating that *socs36E* is a JAK-STAT target in the testis. To determine if *socs36E* can attenuate JAK-STAT signaling in this tissue, we ectopically expressed *socs36E* in the germline. The number of GSCs/testis decreased significantly from 9.9 ± 0.4 (wild-type, n = 14) to 1.0 ± 0.4 (genotype: *nanos-GAL4/UAS-Socs36E*, n = 15), P<0.0001(Student's t test), which is consistent with the requirement for JAK-STAT signaling in GSC maintenance.

***socs36E*^{PZ1647} interacts with JAK-STAT pathway members in a manner expected of a pathway inhibitor.** Reducing JAK-STAT signaling in the *socs36E*^{PZ1647} testes by adding a hypomorphic allele of the ligand outstretched (genotype: *os*^{s}*; socs36E*^{PZ1647}*)* restores the number of GSCs to near wild-type (7.9 ± 0.7). Conversely, although the

constitutively active JAK kinase allele $Hop^{Tum\text{-}L}$ is viable at 18°C, combining it with $socs36E^{PZ1647}$ results in lethality, likely from inappropriately high levels of JAK-STAT activation.

Competition for niche occupancy by CPCs requires ßPS-integrin. To investigate whether ßPS-integrin plays a role in stem cell competition, we could not reduce levels of ßPS-integrin since the gene encoding ßPS-integrin, *myospheroid*, is located on the X chromosome and hemizygous males are embryonic lethal. Instead, we reduced levels of *Drosophila* talin (*rhea*), which links integrins to the cytoskeleton and is required for integrin-mediated adhesion.

Supporting Online Figures

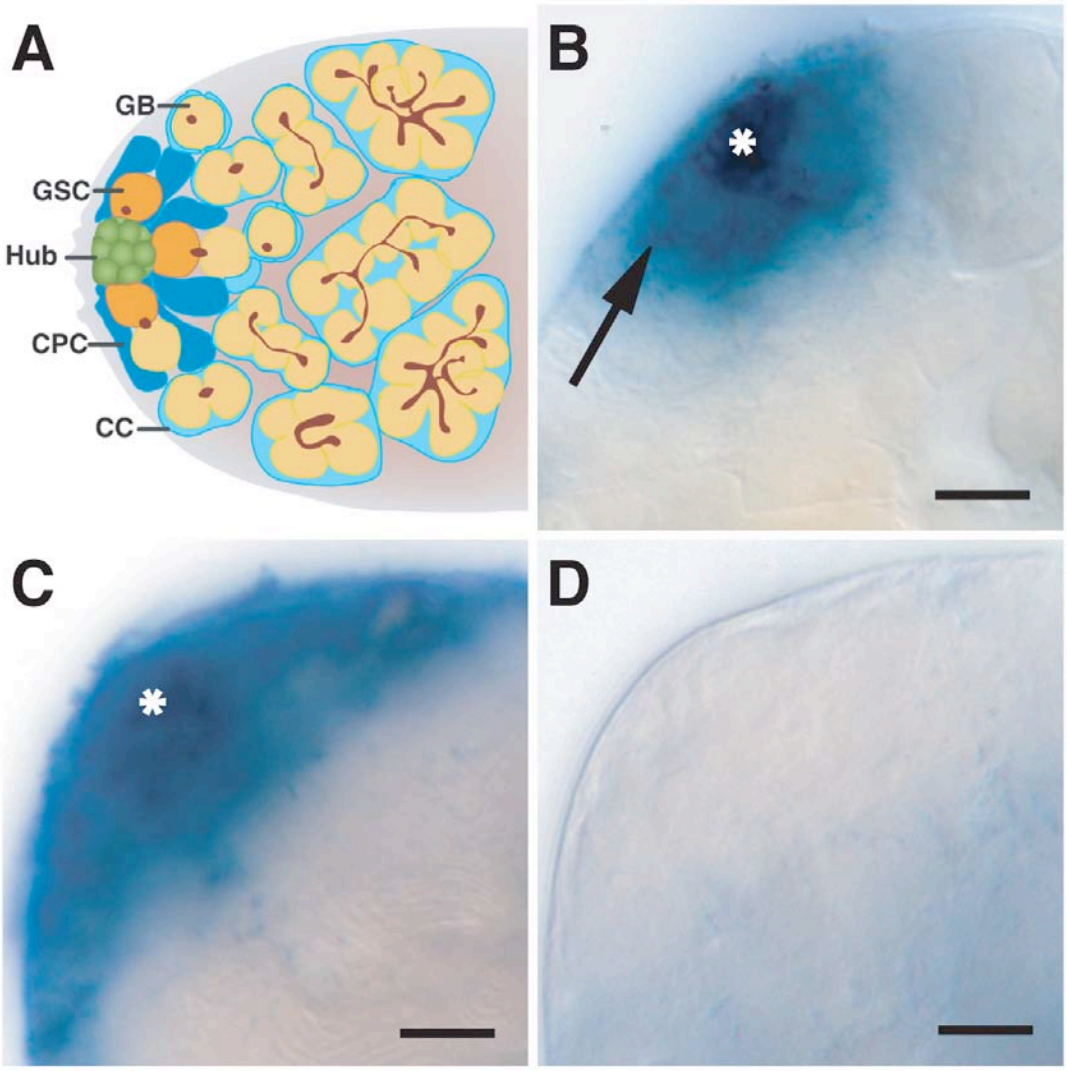

Figure S1. The JAK-STAT target *socs36E* is expressed in the *Drosophila* testis niche. **(A)** The *Drosophila* testis apex. Approximately 10 GSCs (3 shown, orange) attach to the hub (green). GSCs divide asymmetrically to produce daughters (gonialblasts, yellow) that are displaced from the hub and out of the niche. Gonialblasts undergo four rounds of mitosis, becoming 2-, 4-, 8- and 16-cell spermatogonial cysts (yellow). Fusomes (brown) distinguish germ cell stages; fusomes are spherical in GSCs, branched in differentiating spermatogonia. Approximately 2 CPCs (dark blue) flank each GSC and contact the hub with cytoplasmic extensions. CPCs divide to produce cyst cell daughters (light blue); two envelop each gonialblast and its descendants. **(B-D)** In situ hybridization with *socs36E* antisense probe on whole testes. **(B)** In wild-type, *socs36E* is expressed in the hub (asterisk) and CPCs (one indicated, arrow). **(C)** *socs36E* expression expands throughout the testis apex following ectopic JAK-STAT pathway activation in *hsp70-outstretched* flies. **(D)** *socs36E* expression is abolished in flies lacking JAK-STAT signaling (genotype: $stat92E^{Frankenstein}/stat92E^{06346}$). Bars, 10 microns.

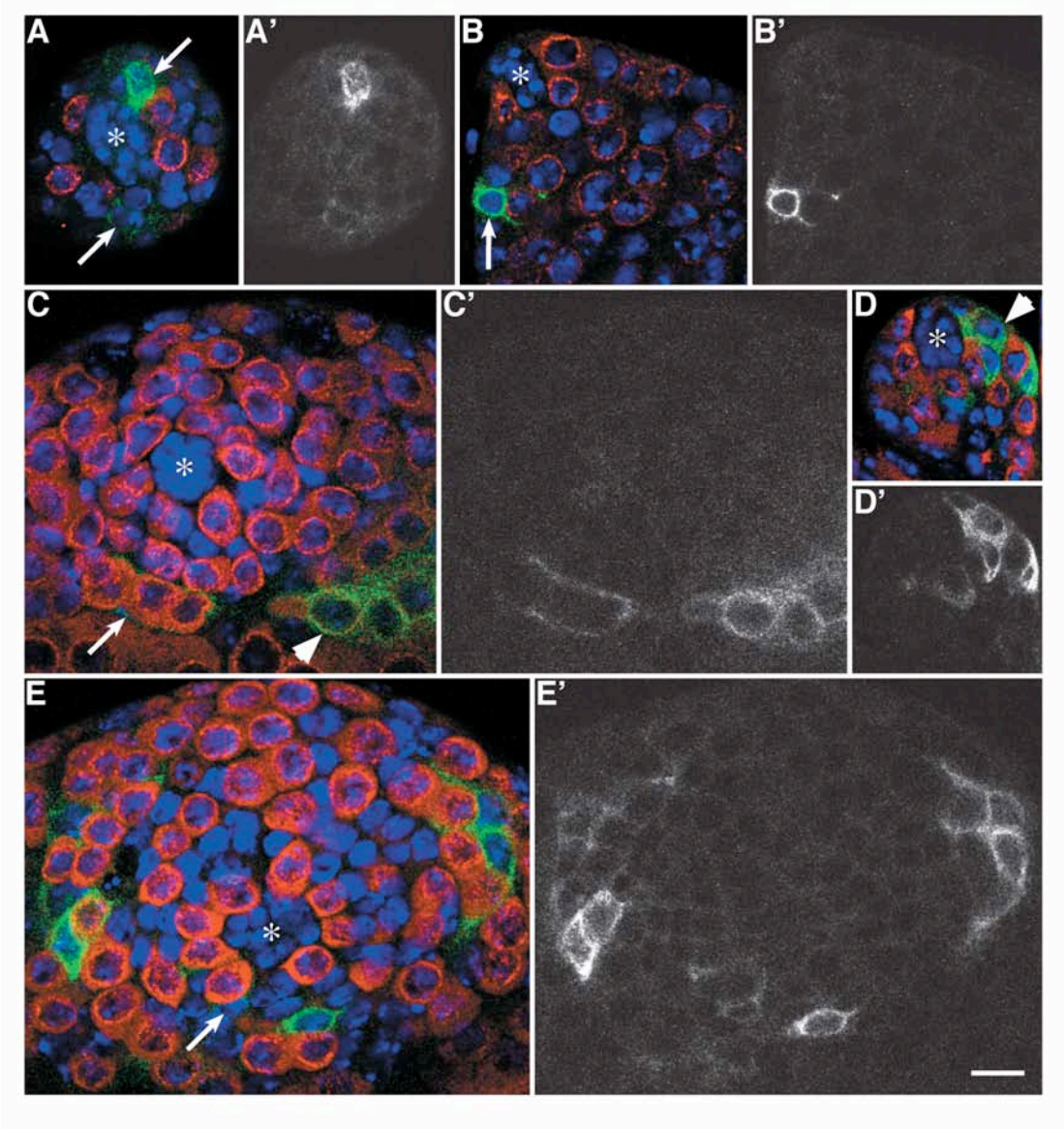

Figure S2. JAK-STAT signaling is required in CPCs for their self-renewal. All panels are confocal sections through the apex of testes containing *stat92E* **(A-C)** or wild-type **(D-E)** clones that are positively marked with GFP (MARCM technique). Testes are stained for DNA (DAPI; blue), germline (anti-Vasa; red) and GFP (anti-GFP; green). Black-and-white panels show the green channel only. In all panels, the hub is marked with an asterisk. Scale bar is 10 μm. **(A-C)** At 30 and 36 hr ACI, *stat92E* CPCs and cyst cells were frequently found; however, at 60 hr ACI, few *stat92E* CPCs remained. **(A, A')** 30 hr ACI; two $stat92E^{j6C8}$ CPCs are visible (arrows). **(B, B')** 36 hr ACI; one $stat92E^{j6C8}$ cyst cell is visible (arrow). **(C, C')** 60 hr ACI; one $stat92E^{06346}$ cyst cell is visible (arrow). Arrowhead points to marked germ cells. **(D-E)** In control testes, at 60 hr ACI, marked wild-type CPCs and cyst cells were both frequently found. Arrowhead in **(D)** and arrow in **(E)** point to marked CPCs. Additional marked CPCs and cyst cells are visible. All panels are single optical sections except for A and A', which are projections of several sections.

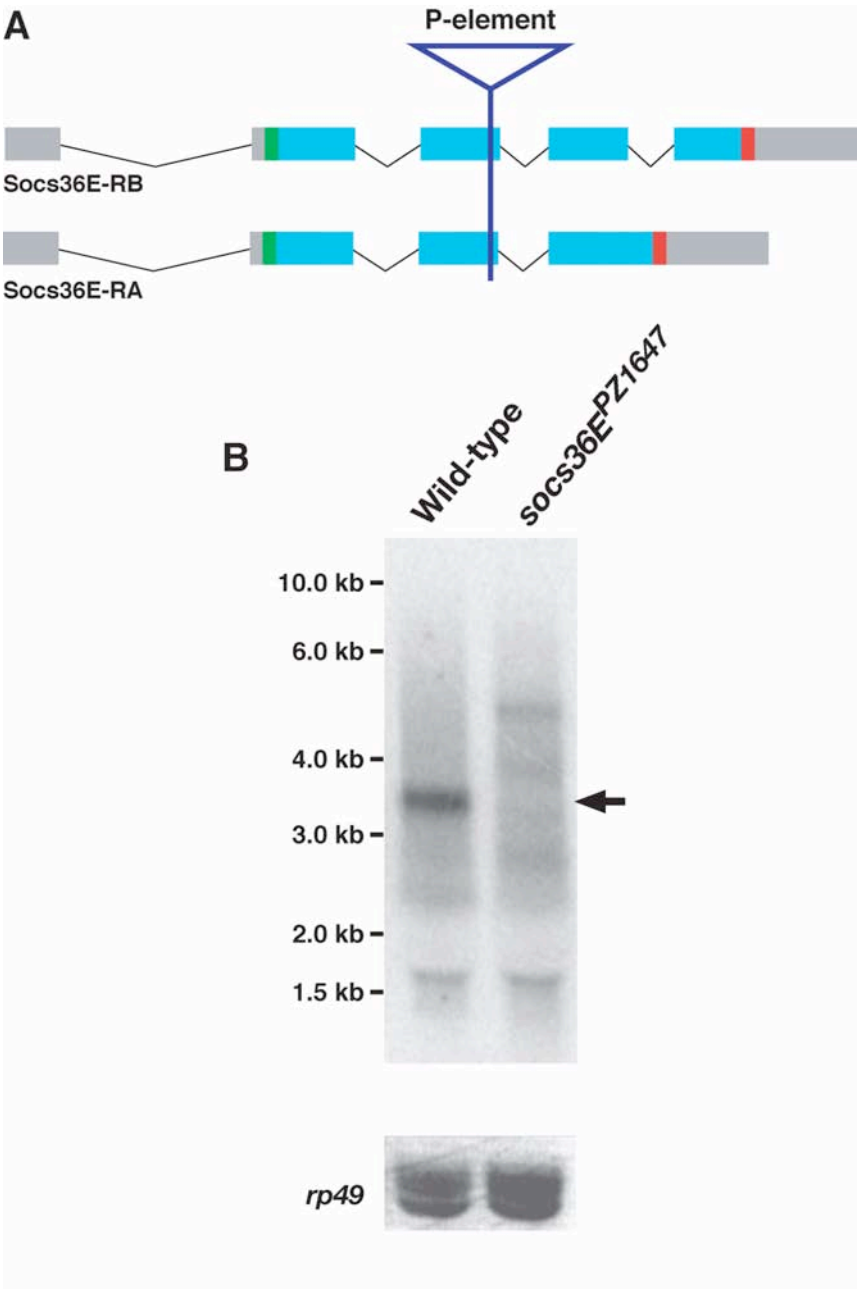

Figure S3. A P-element insertion in *socs36E* creates a strong loss-of-function allele, *socs36E*^{PZ1647}. **(A)** Schematic representation of the *socs36E* mRNA transcripts (RA and RB are alternate splice variants of *socs36E*) showing the relative arrangement of exons and introns and the coding regions (blue), untranslated regions (grey), initiation site (green) and termination site (red). A PZ-element is inserted in the 2nd exon of the *socs36E* gene after nucleotide 10663. **(B)** *socs36E* mRNA was detected after Northern blot hybridization with a ^{32}P-radiolabeled *socs36E* DNA probe in wild-type embryos (lane 1) but not *socs36E*^{PZ1647} embryos (lane 2). *Ribosomal protein 49* (*rp49*) was used as a loading control. The 3.5 kb *socs36E* transcript is indicated (arrow).

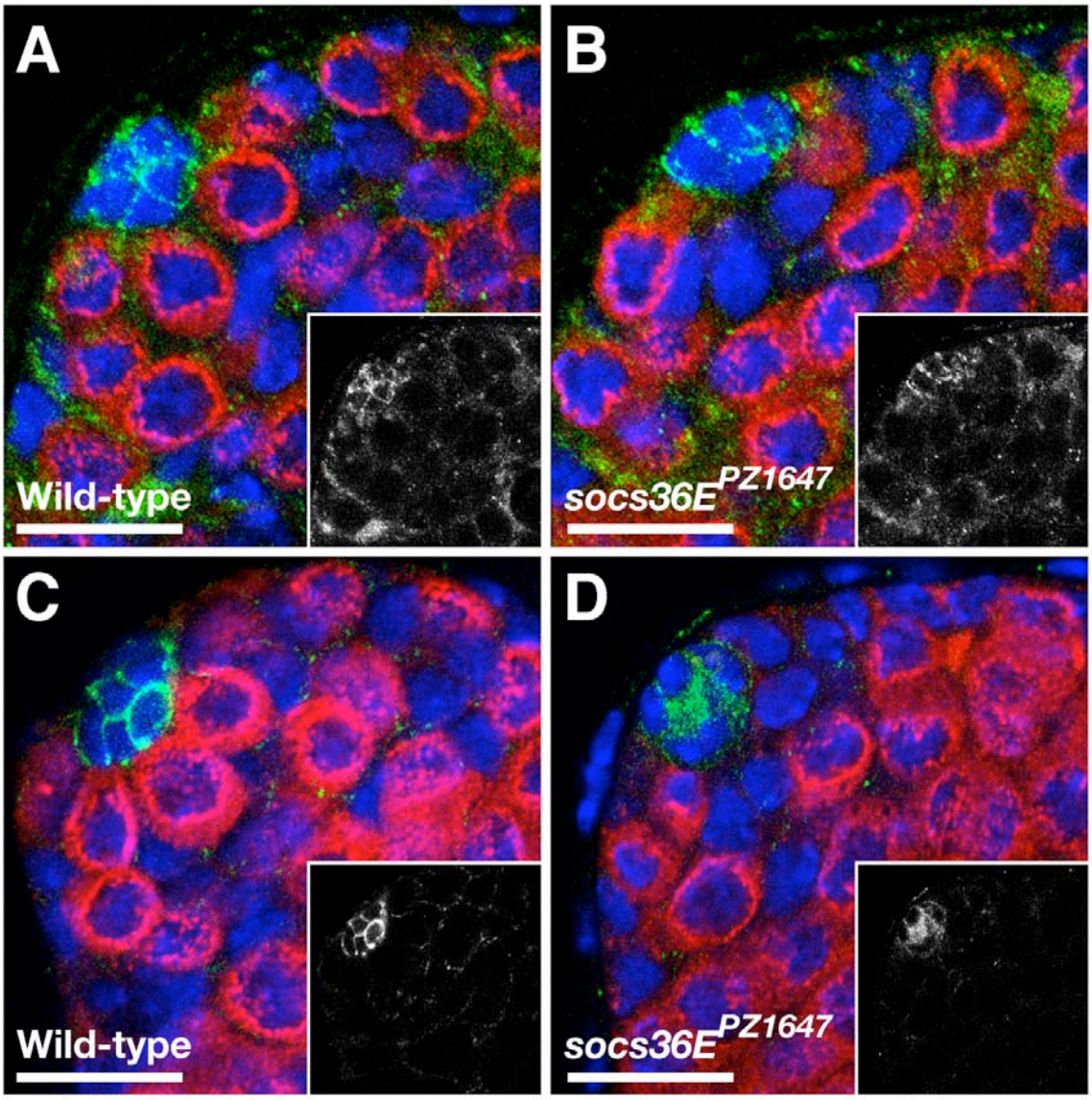

Figure S4. *socs36E* mutant testes do not have altered DE- or N-cadherin expression patterns. Confocal section through the testis apex in **(A, C)** wild-type and **(B, D)** *socs36E*^{PZ1647} flies immunostained with (**A, B**) anti-Vasa (red), anti-DE-Cadherin (green, also in insets), and DAPI (blue), or with **(C, D)** anti-Vasa (red), anti-N-Cadherin (green, also in insets), and DAPI (blue). The expression patterns of DE- and N-cadherin appear similar between wild-type and *socs36E*^{PZ1647} mutant testes.

Supporting Online Tables

Table S1. *stat92E*^{null} CPCs are not maintained. Marked *stat92E*^{null} CPCs were present initially but were rare by 60 hours after clone induction (ACI), whereas marked wild-type CPCs remained. Since the sensitivity of the MARCM system is time dependent and cells may take up to 2 days to express the marker, clones were analyzed beginning at 36 hours ACI (*S12*). The numbers of marked CPCs and daughter cyst cells are shown ± SEM.

Hours ACI	*stat92E* allele	Testes with marked CPCs or cyst cells/testes scored	Marked CPCs/testis	Marked cyst cells/testis
36	wild-type	30/99 (30%)	0.25 ± 0.06	0.73 ± 0.15
36	06346	25/45 (56%)	0.22 ± 0.10	1.20 ± 0.24
36	j6C8	52/89 (58%)	0.33 ± 0.07	1.36 ± 0.23
60	wild-type	44/76 (58%)	1.12 ± 0.16	3.13 ± 0.40
60	06346	25/68 (37%)	0.07 ± 0.03	0.93 ± 0.19
60	j6C8	26/56 (46%)	0.07 ± 0.03	1.05 ± 0.21
120	wild-type	29/71 (41%)	1.41 ± 0.30	2.86 ± 0.58
120	06346	1/45 (2%)	0.07 ± 0.07	0.11 ± 0.11
120	j6C8	4/62 (6%)	0.08 ± 0.05	0.15 ± 0.13
no h.s. control	wild-type	6/314 (2%)	0.06 ± 0.03	0.20 ± 0.10
no h.s. control	06346	0/68 (0%)	0	0
no h.s. control	j6C8	3/133 (2%)	0.02 ± 0.01	0

Table S2. Socs36E prevents CPCs from out-competing neighboring GSCs.

ACI (d)	*socs36E* allele	GSC clones/ testis		CPC clones/ testis		GSCs/testis		CPCs/testis	
2	Wild-type	0.8 ± 0.2 (45)	n.s.	1.6 ± 0.3 (36)	n.s.	8.4 ± 0.3 (45)	n.s.	18.4 ± 0.6 (36)	n.s.
6		0.8 ± 0.2 (41)		2.2 ± 1.4 (18)		8.4 ± 0.3 (41)		18.5 ± 1.1 (18)	
10		1.2 ± 0.4 (34)		1.7 ± 1.2 (18)		7.9 ± 0.3 (34)		18.4 ± 1.0 (18)	
14		0.7 ± 0.5 (12)		1.6 ± 0.9 (24)		7.5 ± 0.4 (12)		19.1 ± 0.9 (24)	
18		0.9 ± 0.4 (18)		1.9 ± 1.4 (18)		7.4 ± 0.5 (18)		18.1 ± 1.0 (18)	
22		0.8 ± 0.5 (20)		1.2 ± 0.8 (22)		7.2 ± 0.3 (20)		18.5 ± 1.1 (22)	
2	PZ1647	0.6 ± 0.1 (48)	n.s.	4.7 ± 0.8 (32)	*	7.9 ± 0.2 (48)	*	17.6 ± 0.4 (32)	***
6		0.6 ± 0.2 (49)		10.7 ± 2.3 (18)	***	6.9 ± 0.2 (49)	*	18.3 ± 0.8 (18)	
10		1.1 ± 0.3 (44)		8.7 ± 2.2 (18)		6.9 ± 0.2 (44)	***	18.4 ± 0.7 (18)	***
14		0.5 ± 0.3 (26)		8.6 ± 1.5 (36)		5.8 ± 0.4 (26)		18.1 ± 0.4 (36)	
18		0.3 ± 0.1 (24)		9.2 ± 1.9 (24)		5.6 ± 0.3 (24)		17.2 ± 0.5 (24)	
22		0.7 ± 0.3 (31)		12.2 ± 2.1 (18)		5.5 ± 0.3 (31)		16.9 ± 0.9 (18)	

*P < 0.05. ***P < 0.0001. n.s. not significant, P > 0.05. Data reported as mean ± SEM (n)

References for Supporting Online Material

S1. X. S. Hou, M. B. Melnick, N. Perrimon, *Cell* **84**, 411 (Feb 9, 1996).

S2. A. C. Spradling *et al*., *Genetics* **153**, 135 (Sep, 1999).

S3. J. D. Lee, J. E. Treisman, *Development* **128**, 1519 (May, 2001).

S4. K. Baksa, T. Parke, L. L. Dobens, C. R. Dearolf, *Dev Biol* **243**, 166 (Mar 1, 2002).

S5. J. R. McGregor, R. Xi, D. A. Harrison, *Development* **129**, 705 (Feb, 2002).

S6. D. A. Harrison, P. E. McCoon, R. Binari, M. Gilman, N. Perrimon, *Genes Dev* **12**, 3252 (Oct 15, 1998).

S7. M. Van Doren, A. L. Williamson, R. Lehmann, *Curr Biol* **8**, 243 (Feb 12, 1998).

S8. L. Manseau *et al*., *Dev Dyn* **209**, 310 (Jul, 1997).

S9. B. A. Callus, B. Mathey-Prevot, *Oncogene* **21**, 4812 (Jul 18, 2002).

S10. B. P. Levi, A. S. Ghabrial, M. A. Krasnow, *Development* **133**, 2383 (Jun, 2006).

S11. C. Brawley, E. Matunis, *Science* **304**, 1331 (May 28, 2004).

S12. T. Lee, L. Luo, *Neuron* **22**, 451 (Mar, 1999).

S13. T. Xu, G. M. Rubin, *Development* **117**, 1223 (Apr, 1993).

S14. E. Matunis, J. Tran, P. Gonczy, K. Caldwell, S. DiNardo, *Development* **124**, 4383 (Nov, 1997).

S15. R. Lehmann, D. Tautz, *Methods Cell Biol* **44**, 575 (1994).