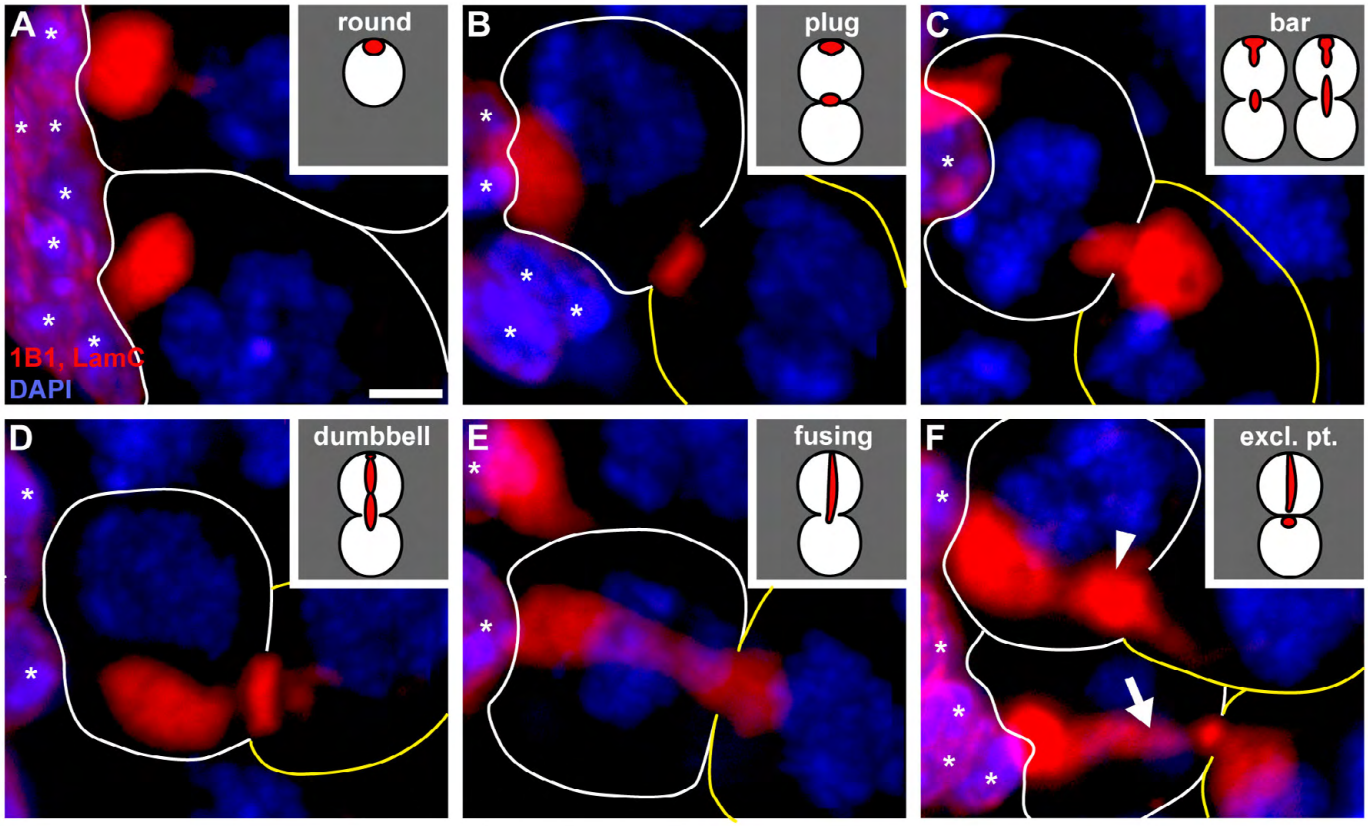

Fig. S1. Summary of fusome morphologies according to the cell cycle in GSCs. (**A-F**) Representative maximum intensity projection (5-7 μm thickness) images of GSCs displaying different forms of the fusome. Cap cells are indicated by asterisks; GSCs are outlined in white; cystoblasts are outlined in yellow. 1B1 labels fusomes (red); LamC labels cap cell nuclear envelopes (red); DAPI labels nuclei (blue). As previously described, the majority of GSCs (see Table S1) have round fusomes (juxtaposed to the GSC-cap cell interface) (A), indicative of G2 and M (de Cuevas and Spradling, 1998; Hsu et al., 2008). As M phase is completed, a small plug of fusome material (B) appears at the junction between the GSC and the nascent cystoblast. Subsequently, the fusome extends posteriorly from the vicinity of the GSC-cap cell interface, and accumulates at the plug and extends anteriorly, acquiring a 'bar' morphology that spans a transient ring canal (de Cuevas and Spradling, 1998) (C). Upon close inspection of fusome morphology in combination with EdU incorporation (not shown), we noticed a close correlation between this S-phase marker and a specific GSC fusome stage that we termed 'dumbbell' (D). The majority (82%) of GSCs with 'dumbbell' fusomes are EdU positive; however, not all GSCs in S phase display this fusome morphology (see Table S1). The 'dumbbell' fusome appears to be an intermediate stage, appearing just before the extending fusome pieces become connected to form the previously described 'fusing' fusome (E) (de Cuevas and Spradling, 1998). The GSC-cystoblast transient ring canal persists until early G2, when cytokinesis completes and pinches the fusome, giving rise to the 'exclamation point' morphology (F, arrow) (de Cuevas and Spradling, 1998). Arrowhead indicates a neighboring GSC with a 'dumbbell' fusome. Scale bar: 2 μm.

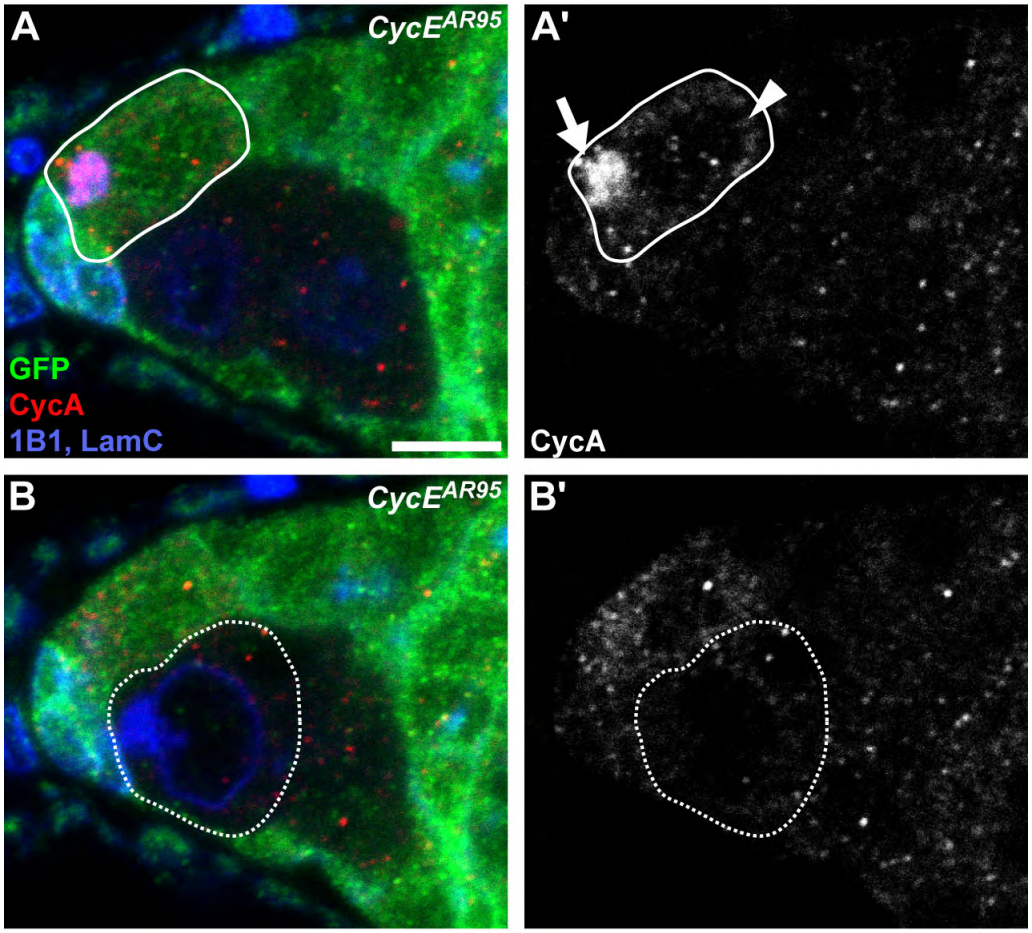

Fig. S2. Null *CycE*^{AR95} GSCs do not express CycA. (**A-B′**) Two optical sections of a mosaic germarium showing a GFP-positive wild-type GSC (A; solid outline) and a GFP-negative *CycE*^{AR95} GSC (B; dashed outline). GFP (green), wild-type control cells; CycA (red; grayscale image in A′); 1B1 (blue), fusomes; LamC (blue), cap cell nuclear envelopes. LamC is also prominent in *CycE* mutant germ cell nuclear envelopes. Arrow and arrowhead in A′ indicate CycA at the fusome and in the cytoplasm, respectively. Scale bar: 5 μm.

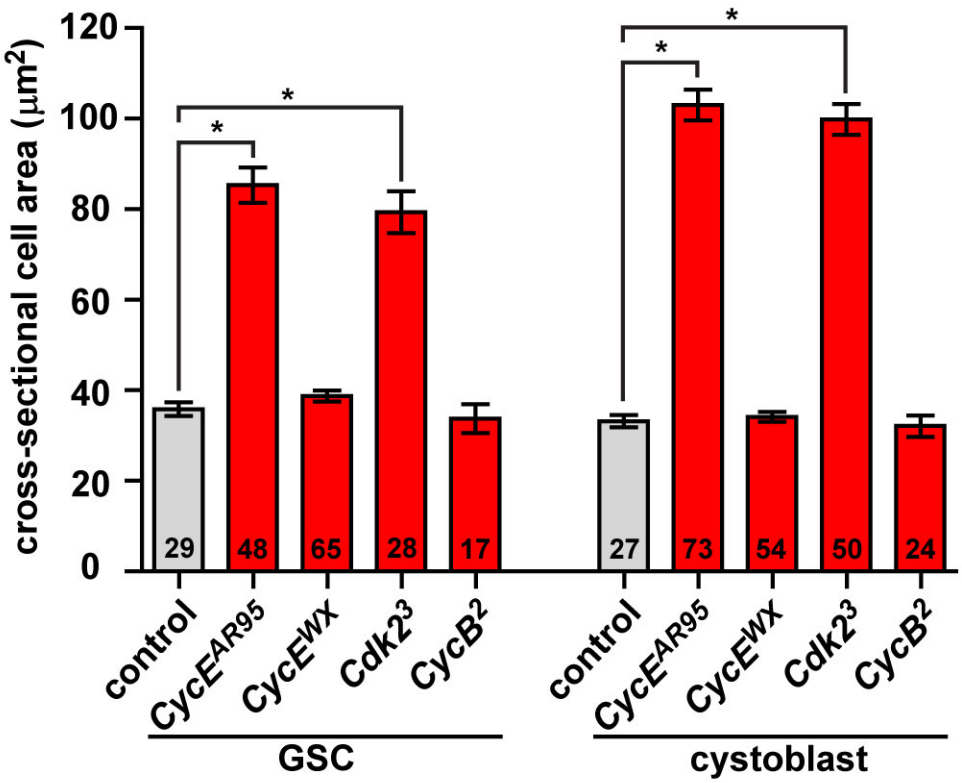

Fig. S3. Null *CycE*^{AR95} and *Cdk2*^{3} GSCs are larger than wild-type GSCs. Average cross-sectional area of GSCs in control or mutant mosaic germaria. See Materials and methods for experimental details. Numbers in bars represent number of GFP-negative GSCs analyzed. Standard bars represent s.e.m. *P<0.0001.

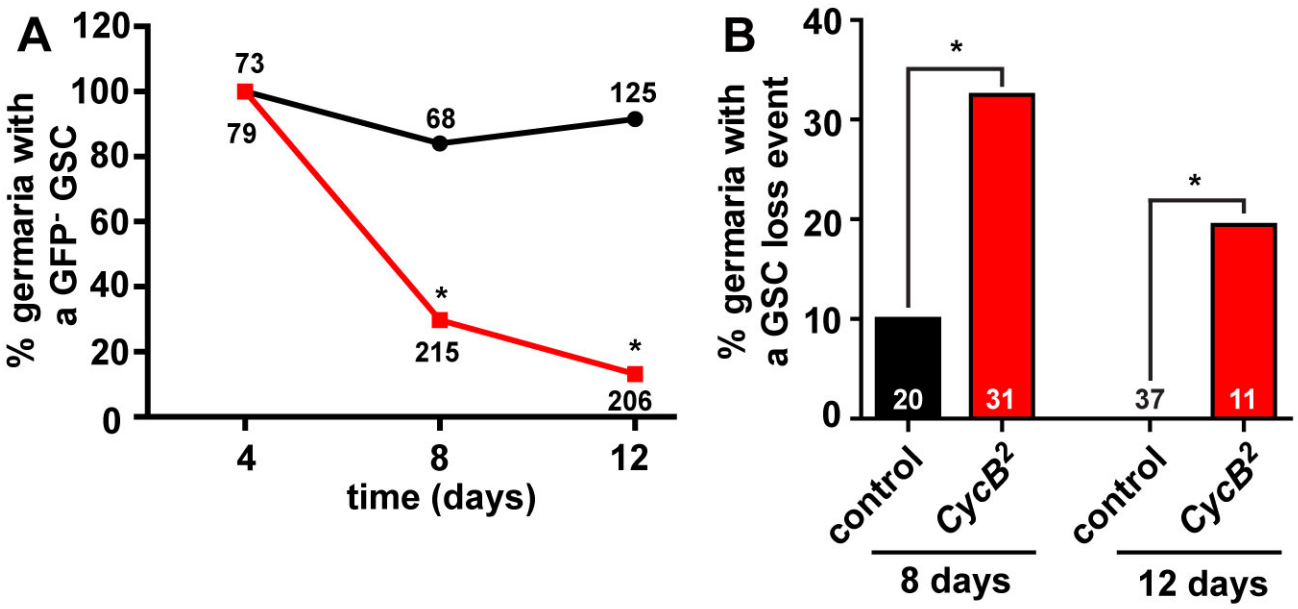

Fig. S4. Quantification of *CycB* requirement for GSC maintenance. (**A**) Percentage of germaria from control (black line) or *CycB*^{2} (red line) mosaic females with at least one GFP-negative GSC 4, 8 and 12 days after clone induction. (**B**) Percentage of germline-mosaic germaria with a GSC loss event 8 or 12 days after clone induction. See Materials and methods for experimental details. Numbers above or below datapoints (A) or in the bars (B) represent the sample size (total number of germaria or of germline-mosaic germaria analyzed, respectively). *$P \leq 0.001$.

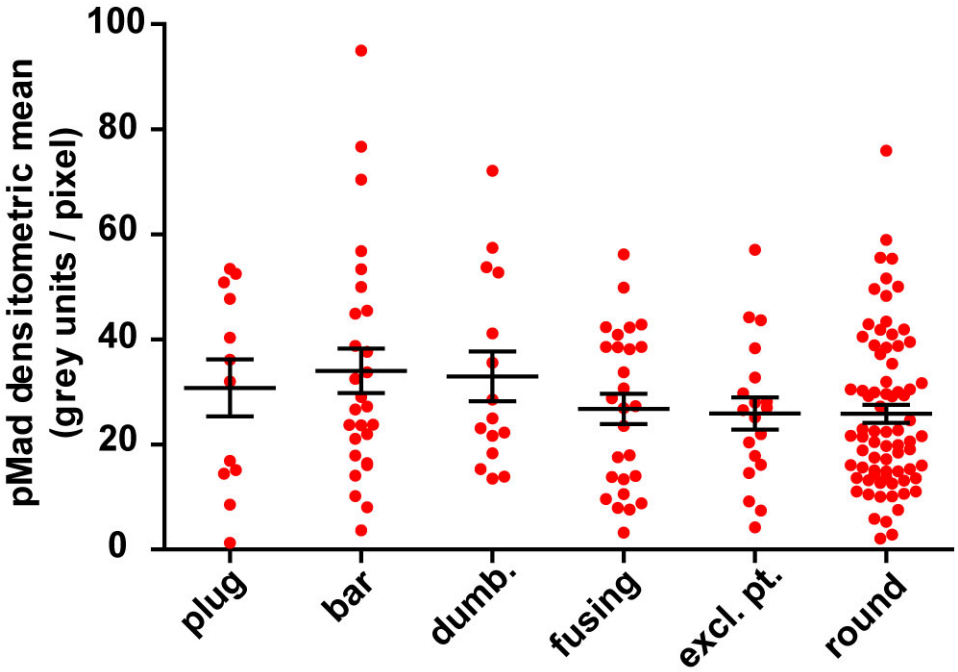

Fig. S5. pMad levels are not cell cycle regulated in GSCs. Quantification of average pMad fluorescence intensity in GSCs according to fusome morphology (see Materials and methods). *n*=175 GSCs. Bars represent s.e.m.

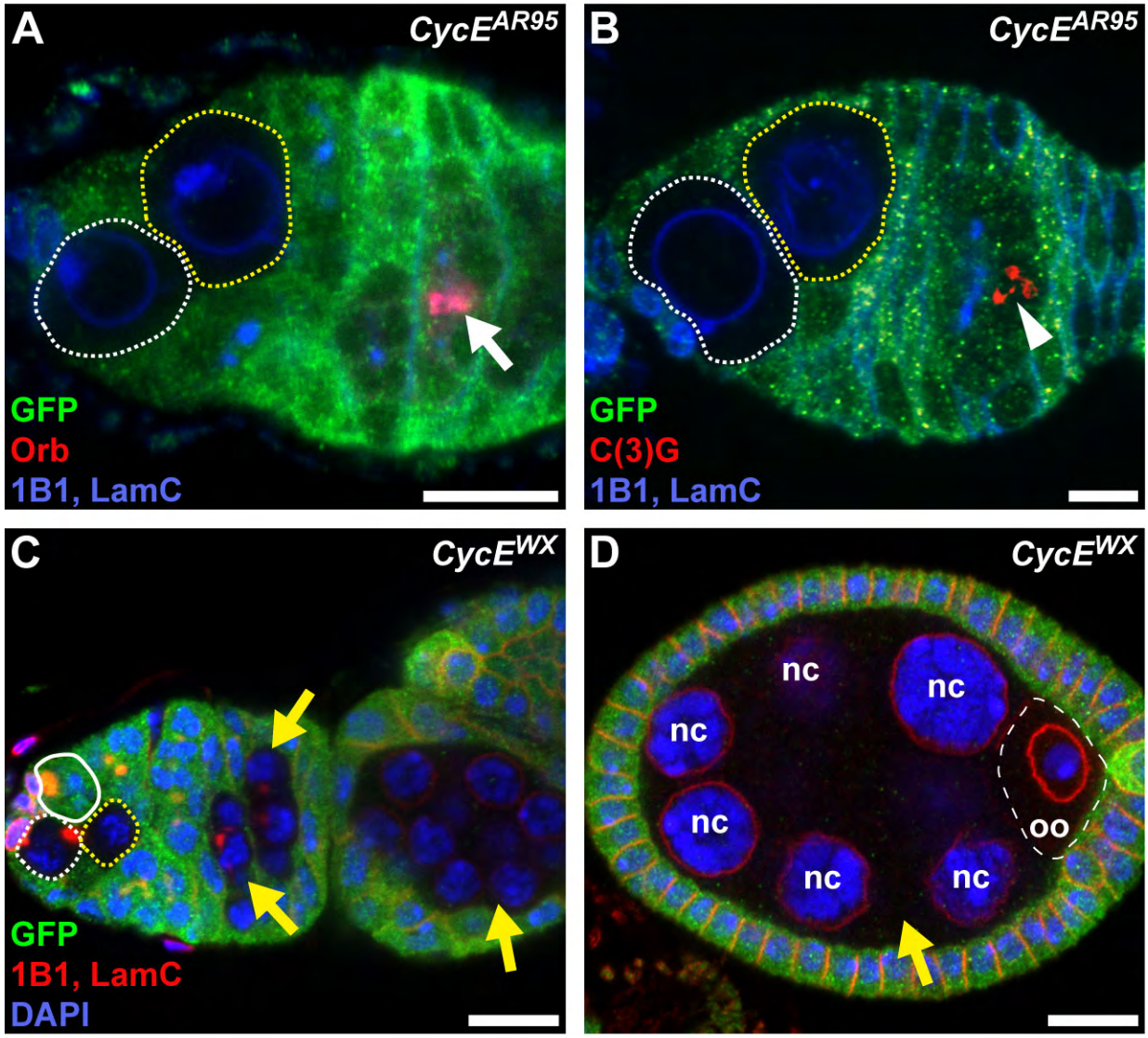

Fig. S6. *CycE*^{AR95} germ cells do not express oocyte markers; this is probably secondary to the failure of these cells to proliferate. (**A**,**B**) Mosaic germaria showing GFP-negative *CycE*^{AR95} germ cells that do not express the oocytes markers Orb (A) or C(3) G (B). GFP (green), wild-type control cells; Orb (red in A), *Drosophila* CPEB homolog (Lantz et al., 1994); C(3)G (red in B), a synaptonemal complex protein (Hong et al., 2003). *CycE*^{AR95} GSCs are outlined in white; *CycE*^{AR95} cystoblast-like cells are outlined in yellow. Arrow and arrowhead indicate oocytes within wild-type 16-cell cysts that express Orb or C(3)G, respectively. (**C**,**D**) Mosaic germaria showing differentiated GFP-negative *CycE*^{WX} germ cells in the germarium (C) and in previtellogenic egg chambers (C,D). DAPI (blue), nuclei. *CycE*^{WX} GSCs are outlined in white; *CycE*^{WX} cystoblast-like cells are outlined in yellow. Yellow arrows indicate *CycE*^{WX} germline cysts. oo, oocyte; nc, nurse cells. 1B1 (blue), fusomes and follicle cell membranes; LamC (blue) cap cell nuclear envelopes. LamC is also prominent in *CycE* mutant germ cell nuclear envelopes. Scale bars: 10 μm.

Table S1. Quantification of GSCs with different fusome morphologies relative to S and M phases of the GSC division cycle

Fusome morphology*	Experiment 1			Experiment 2		Total	
	% of Total GSCs‡	% GSCs labeled with EdU§	% GSCs labeled with pHH3¶	% of Total GSCs‡	% GSCs labeled with EdU§	% of Total GSCs‡	% GSCs labeled with EdU§
Plug**	2.9 (4)	0	0	7.9 (26)	46 (12)	6.4 (30)	40 (12)
Bar**	11 (15)	27 (4)	0	7.0 (23)	26 (6)	8.1 (38)	26 (10)
Dumbbell	7.3 (10)	70 (7)	0	7.0 (23)	87 (20)	7.1 (33)	82 (27)
Fusing	20 (27)	7.4 (2)	0	13 (42)	0	15 (69)	2.9 (2)
Exclamation point	7.3 (10)	0	0	12 (40)	0	11 (50)	0
Round	52 (72)	0	4.2 (3)	53 (175)	0	53 (247)	0
Total	*138*	*12 (8.7%)‡‡*	*3 (2.2%)‡‡*	*329*	*38 (12%)‡‡*	*467*	*50 (11%)‡‡*

*Newly eclosed *y w* wild-type females were maintained at 25°C for 2 days on standard media supplemented with yeast paste.

‡Total number of GSCs with the indicated fusome morphology (in parentheses) is shown as a percentage of the total number of GSCs examined.

§Total number of GSCs labeled with EdU (an S phase marker; in parentheses) is shown as a percentage of the total number of GSCs with the indicated fusome morphology.

¶Total number of GSCs labeled with antibodies against phosphorylated histone H3 (pHH3; an M phase marker; in parentheses) is shown as a percentage of the total number of GSCs with the indicated fusome morphology.

**A small fraction of GSCs displaying either plug or bar morphology are EdU positive; this is probably due to a very short G1 and the imperfect synchrony between fusome dynamics and the G1-to-S transition.

‡‡The percentages of total GSCs analyzed (shown in parentheses) that are EdU or pHH3 positive are consistent with previous reports (Ables and Drummond-Barbosa, 2010; Hsu et al., 2008; LaFever et al., 2010).

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Table S2. Quantification of GSC loss in *CycE* and *Cdk2* mutant mosaic germaria

Mosaic genotype	Days*	Method I‡		Method II‡	
		Number of germline-mosaic germaria analyzed	% Germaria with a GSC loss event§	Number of germaria analyzed	% Germaria with a GFP$^-$ GSC¶
FRT40A					
Control	4	--	--	282	35 (100%)
	8	90	3.2	241	36 (103%)
	12	74	1.8	208	35 (100%)
CycE^{AR95}	4	--	--	130	42 (100%)
	8	104	29**	162	46 (108%)
	12	77	56**	164	21 (49%)
CycE^{KG00239}	4	--	--	128	44 (100%)
	8	54	28**	103	38 (87%)
	12	88	46**	202	24 (54%)
CycE^{WX}	4	--	--	137	40 (100%)
	8	67	18**	169	33 (83%)
	12	81	19**	209	31 (78%)
CycE^{1F36}	4	--	--	131	44 (100%)
	8	75	5.7	164	43 (98%)
	12	93	9.7**	234	36 (81%)
CycE^{JP}	4	--	--	132	36 (100%)
	8	47	4.5	170	27 (73%)
	12	89	4.5	208	41 (112%)

FRT82B					
Control	4	--	--	n. d.	n. d.
	8	107	1.9	173	61
	12	102	4.9	190	51
***Cdk2*^{3}**	4	--	--	n. d.	n. d.
	8	144	41**	263	32
	12	116	67**	266	14

*Days after clone induction. One- to two-day-old females were heat shocked twice daily for 3 days, maintained at 25°C on standard media supplemented with yeast, and dissected 4, 8 or 12 days after the last heat shock.

‡See Materials and methods for detailed explanation of Methods I and II for quantification of GSC loss.

§Number of germline-mosaic germaria displaying a GSC loss event divided by the total number of germline mosaic germaria analyzed, represented as a percentage. GSC loss events were only quantified at 8- or 12-day timepoints to ensure that all germline clones within germaria descended from a GFP-negative GSC.

¶Number of germaria with at least one GFP-negative GSC divided by the total number of germaria analyzed, represented as a percentage.

***P*<0.0001 relative to corresponding *FRT* control, as determined by χ^2 analysis.