

**Table S1.** The rescue assay of *cycB3* mutants by the transgenic line of P{attB-*cycB3*-gDNA}

Genotype	Age <sup>1</sup>	Germaria				Total
		Empty	0 GSC (cysts only)	1 GSC	2-3 GSCs	
<i>cycB3<sup>2</sup>/cycB3<sup>2</sup></i>	day1	3.0%	2.0%	5.6%	89.4%	198
	day14	41.8%	11.6%	23.3%	23.3%	146
<i>attB-cycB3-gDNA</i> ; <i>cycB3<sup>2</sup>/cycB3<sup>2</sup></i>	day1	2.1%	0	1.6%	96.3%	190
	day14	0	0.9%	0.9%	98.2%	108*
<i>cycB3<sup>2</sup>/cycB3<sup>EY08012</sup></i>	day1	2.5%	1.7%	2.5%	93.3%	120
	day14	24.0%	5.3%	24.0%	46.7%	167
<i>attB-cycB3-gDNA</i> ; <i>cycB3<sup>2</sup>/cycB3<sup>EY08012</sup></i>	day1	0	2.5%	1.0%	96.4%	196
	day14	0	0	2.6%	97.4%	193*

<sup>1</sup>the days after eclosion. \* $P < 0.001$  ( $\chi^2$  test) when the total percentages of abnormal germaria (containing 1 GSC, 0 GSC and empty) from different *cycB3*-rescuing ovaries were compared with their corresponding mutants. 14-day-old flies were selectively analyzed.

**Table S2.** *cycB3* is intrinsically required for GSC maintenance in *Drosophila* ovary

Genotype	Age <sup>1</sup>	Germaria				Total
		Empty	0 GSC (cysts only)	1 GSC	2-3 GSCs	
<i>cycB3<sup>2</sup>/cycB3<sup>2</sup></i>	day1	2.4%	2.4%	7.0%	88.2%	212
	day14	40.3%	12.9%	23.7%	23.1%	186
<i>UASp-cycB3;nosP-gvp</i> ; <i>cycB3<sup>2</sup>/cycB3<sup>2</sup></i>	day1	1.0%	0	3.3%	95.7%	210
	day14	0	0.7%	3.0%	96.3%	134*
<i>nosP-cycB3</i> ; <i>cycB3<sup>2</sup>/cycB3<sup>2</sup></i>	day1	0	0	2.2%	97.8%	312
	day14	1.2%	0.5%	1.2%	97.1%	347*
<i>cycB3<sup>2</sup>/cycB3<sup>EY08012</sup></i>	day1	2.0%	0.5%	5.0%	92.5%	200
	day14	34.7%	13.5%	26.5%	25.2%	230
<i>UASp-cycB3/nosP-gvp</i> ; <i>cycB3<sup>2</sup>/cycB3<sup>EY08012</sup></i>	day1	0	0.4%	2.1%	97.5%	237
	day14	0	0	4.0%	96.0%	125*
<i>nosP-cycB3</i> ; <i>cycB3<sup>2</sup>/cycB3<sup>EY08012</sup></i>	day1	0	0	2.7%	97.3%	339
	day14	0	0	1.9%	98.1%	159*
<i>c587-gal4;UASp-cycB3</i> ; <i>cycB3<sup>2</sup>/cycB3<sup>2</sup></i>	day1	4.5%	0.8%	9.1%	85.6%	132
	day14	32.9%	5.1%	32.9%	29.1%	237*
<i>c587-gal4;UASp-cycB3</i> ; <i>cycB3<sup>2</sup>/cycB3<sup>EY08012</sup></i>	day1	2.3%	0	8.7%	89.0%	219
	day14	44.8%	10.5%	17.5%	27.2%	257*

<sup>1</sup>the days after eclosion. \* $P < 0.001$  ( $\chi^2$  test) when the total percentages of abnormal germaria (containing 1 GSC, 0

GSC and empty) from different *cycB3*-rescuing ovaries were compared with their corresponding mutants.  $^*P>0.05$  ( $\chi^2$  test) when the total percentages of abnormal germaria from *cycB3*-rescuing ovaries were compared with their corresponding mutants. 14-day-old flies were selectively analyzed.

**Table S3.** The analysis of the cell death in *cycB3* mutant ovaries.

Genotype	The percentages of apoptotic GSCs between wild type and <i>cycB3</i> mutant fly ovaries
<i>Oregon-R</i>	1.4% (N=210)
<i>cycB3<sup>2</sup>/cycB3<sup>2</sup></i>	1.8% (N=223)
<i>FRT control</i>	1.5% (N=201)
<i>FRT cycB3<sup>2</sup></i>	1.0% (N=197)

N, the total number of GSCs examined.

**Table S4.** Primers used for generating *cycB3* transgenic vectors.

No.	Primer Sequences	Restriction enzymes (underlined)
P1	5'-AAAC <u>CCTGCAGG</u> CCAATGTTGCCCTAAGAAATCATAATGGG- 3'(forward)	<i>SbfI</i>
P2	5'-TTT <u>GGTACCT</u> TAAACCTTTGACATCTAGGAGTTTATGCTC- 3'(reverse)	<i>KpnI</i>
P3	5'-TTT <u>GGCGCGCC</u> ATGGCGCCCACAAAAGCAACAAC -3' (forward)	<i>AscI</i>
P4	5'-TTT <u>GGTACC</u> CTACGACAGATTGCTTTTCGTTTC-3' (reverse)	<i>KpnI</i>
P5	5'-TTT <u>GGCGCGCC</u> CGACAGATTGCTTTTCGTTTCAGGTCAAG- 3'(reverse)	<i>AscI</i>
P6	5'-AA <u>AGGTACC</u> GCCGCTGTGGGCAAAGGA-3'(forward)	<i>KpnI</i>
P7	5'-TTT <u>GGTACC</u> TACTTGTACAGCTCGTCCATGCCG-3'(reverse)	<i>KpnI</i>