Yuan et al., http://www.jcb.org/cgi/content/full/jcb.201205007/DC1

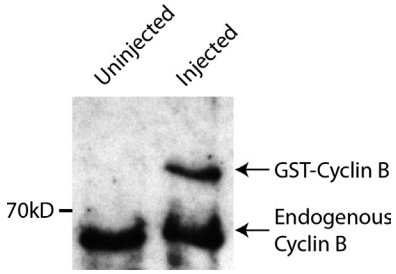


Figure S1. **Single-embryo Western blot of control and GST-CycB protein-injected embryos in prophase 13.** The amount of GST-CycB was approximately half of the total level of endogenous CycB. Because the injected GST-CycB only diffused to 20–30% of the entire embryo, we estimated that the GST-CycB injection increased the local CycB concentration by one- to threefold over endogenous CycB level.

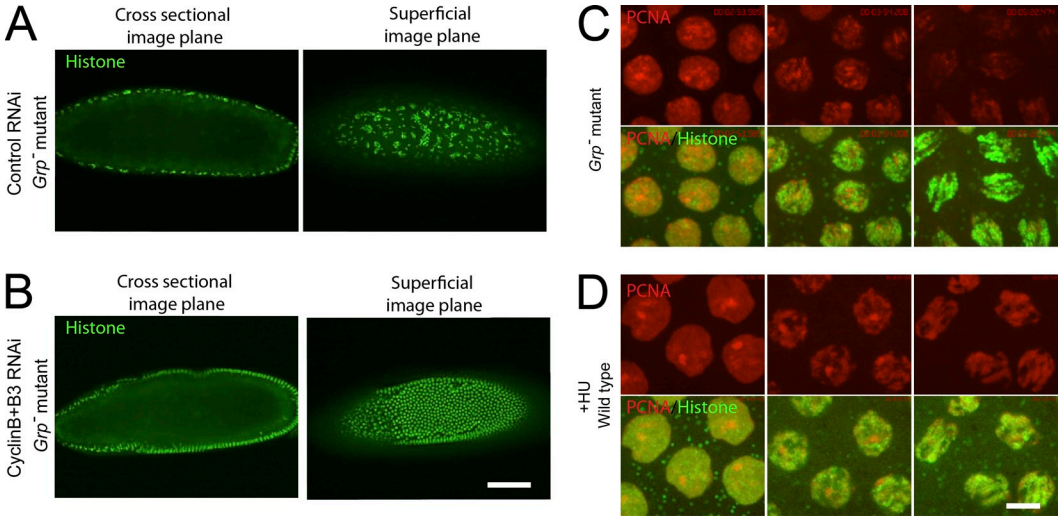


Figure S2. **Suppression of *grp* mutant phenotypes by cyclin knockdowns.** (A) After a failed mitosis 13, *grp* embryos continue to cycle catastrophically, producing grapes-like clusters of nuclei and fail to initiate MBT events. (B) Pairwise knockdown of cyclins largely suppresses these defects. (C) The gradual extension of early interphases is compromised in *grp* embryos, and after an inappropriately short interphase 13, mitosis 13 exhibits extensive anaphase bridging that characterizes the failed mitosis. Persistence of PCNA on mitotic chromosomes supports suggestions that this bridging results from entry into mitosis with incompletely replicated DNA. (D) A similar persistence of PCNA foci after hydroxyurea (HU) injection (an inhibitor of DNA synthesis) further supported this interpretation. Bars: (A and B) 100 µm; (C and D) 5 µm.

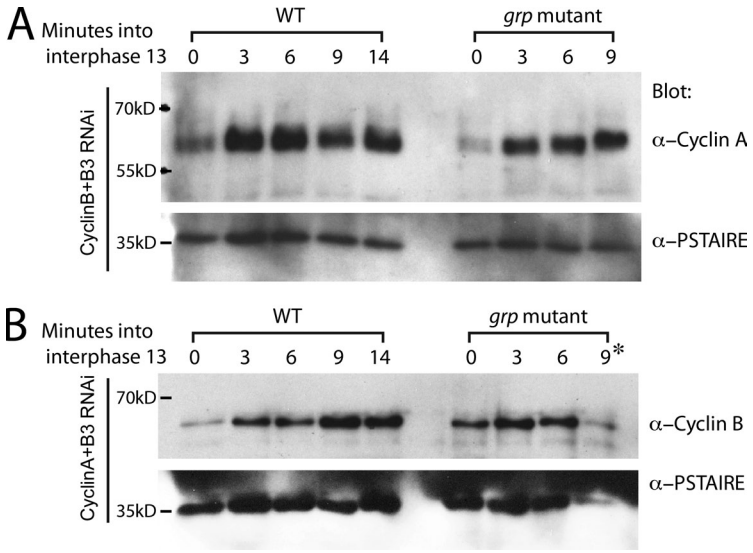
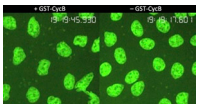


Figure S3. **Comparable cyclin accumulation in wild-type and *grp* mutant embryos.** (A and B) Immunoblots of single embryos show cyclin accumulation during S phase 13 in both wild-type and *grp* mutant embryos. Anti-PSTAIRE provides a loading control. CycA levels after knockdown of CycB and CycB3 (A) and CycB accumulation after knockdown of CycA and CycB3 (B). Asterisk shows a lane with low PSTAIRE signal and presumably poor sample recovery. WT, wild type.

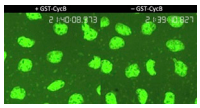
Table S1. **Average length of interphase 13 after different treatments**

Treatment	*n*	Genotype	Interphase 13 length
			min
Control RNAi	8	Wild type	12.02 ± 0.92
CycA+B RNAi	10	Wild type	19.19 ± 1.57
CycA+B3 RNAi	11	Wild type	16.76 ± 1.67
CycB+B3 RNAi	8	Wild type	24.81 ± 1.75
Control RNAi	6	*grp* mutant	7.00 ± 0.56
CycA+B RNAi	5	*grp* mutant	9.32 ± 1.03
CycA+B3 RNAi	5	*grp* mutant	11.00 ± 0.81
CycB+B3 RNAi	8	*grp* mutant	9.64 ± 0.84
Control RNAi + Geminin	3	Wild type	8.96 ± 0.50
CycA+B RNAi + Geminin	4	Wild type	12.38 ± 0.48
CycA+B3 RNAi + Geminin	7	Wild type	11.86 ± 0.90
CycB+B3 RNAi + Geminin	8	Wild type	13.44 ± 1.05

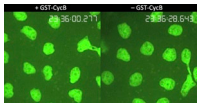
Corresponds to Fig. 4 A.



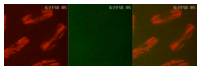
Video 1. **Injection of GST-CycB does not accelerate mitotic entry in CycA+B3 RNAi-treated embryos.** Embryos expressing histone-GFP (green) were first injected with dsRNA targeting CycA and CycB3 at both ends and then injected with recombinant rhodamine-labeled GST-CycB protein at one pole (see Fig. 2). Note that the GST–CycB protein, showing in red, was concentrated only around the injection end (the left side of the video). Both ends of the embryo entered mitosis 13 at the same time (19:37:48 on the left and 19:37:21 on the right). Images were analyzed by time-lapse confocal microscopy using a spinning-disk confocal microscope (PerkinElmer). Frames were taken every 30 s. This video corresponds to Fig. 2 B. The 00:00 frames in Fig. 2 B correspond to 19:19:45 and 19:19:17 in the video. Time is given in hours, minutes, and seconds.



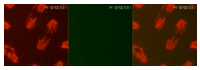
Video 2. **Injection of GST-CycB accelerates mitotic entry in CycB+B3 RNAi-treated embryos.** Embryos expressing histone-GFP (green) were first injected with dsRNA targeting CycB and CycB3 at both ends and then injected with recombinant rhodamine-labeled GST-CycB protein at one pole (see Fig. 2). Note that the GST-CycB protein, showing in red, was concentrated only around the injection end (the left side of the video). The GST-CycB–positive end of the embryo entered mitosis 13 earlier than the GST-CycB–negative end (21:55:48 on the left vs. 22:04:38 on the right). Images were analyzed by time-lapse confocal microscopy using a spinning-disk confocal microscope (PerkinElmer). Frames were taken every 30 s. This video corresponds to Fig. 2 C. The 00:00 frames in Fig. 2 C correspond to 21:40:08 and 21:39:10 in the video. Time is given in hours, minutes, and seconds.



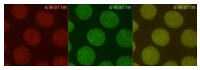
Video 3. **Injection of GST-CycB accelerates mitotic entry in CycA+B RNAi-treated embryos.** Embryos expressing histone-GFP (green) were first injected with dsRNA targeting CycA and CycB at both ends and then injected with recombinant rhodamine-labeled GST-CycB protein at one pole (see Fig. 2). Note that the GST-CycB protein, showing in red, was concentrated only around the injection end (the left section of the video). The GST-CycB–positive end of the embryo entered mitosis 13 earlier than the GST-CycB–negative end (23:50:45 on the left vs. 23:56:05 on the right). Images were analyzed by time-lapse confocal microscopy using a spinning-disk confocal microscope (PerkinElmer). Frames were taken every 30 s. Time is given in hours, minutes, and seconds.



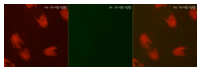
Video 4. **Interphase progression in control embryos.** Embryos expressing histone-RFP (red) were injected with control dsRNA targeting LacI and subsequently with recombinant GFP-PCNA protein (green). Nuclei near the injection site are shown as they progress through interphase 13. Images were analyzed by time-lapse confocal microscopy using a spinning-disk confocal microscope (PerkinElmer). Frames were taken every 30 s. This video corresponds to Fig. 3 A. The 00:00 frame in Fig. 3 A corresponds to 16:24:25 in the video. Time is given in hours, minutes, and seconds.



Video 5. **Interphase progression in CycA+B RNAi-treated embryos.** Embryos expressing histone-RFP (red) were injected with dsRNA targeting CycA and CycB and subsequently with recombinant GFP-PCNA protein (green). Nuclei near the injection site are shown as they progress through interphase 13. Images were analyzed by time-lapse confocal microscopy using a spinning-disk confocal microscope (PerkinElmer). Frames were taken every 30 s. This video corresponds to Fig. 3 B. The 00:00 frame in Fig. 3 B corresponds to 14:11:45 in the video. Note that GFP-PCNA foci disappeared around 14:28:11; however, the nuclei did not enter mitosis until 14:33:44. Time is given in hours, minutes, and seconds.



Video 6. **Interphase progression in CycA+B3 RNAi-treated embryos.** Embryos expressing histone-RFP (red) were injected with dsRNA targeting CycA and CycB3 and subsequently with recombinant GFP-PCNA protein (green). Nuclei near the injection site are shown as they progress through interphase 13. Images were analyzed by time-lapse confocal microscopy using a spinning-disk confocal microscope (PerkinElmer). Frames were taken every 30 s. This video corresponds to Fig. 3 C. The 00:00 frame in Fig. 3 C corresponds to 16:27:57 in the video. Note that GFP-PCNA foci disappeared around 16:42:40; however, the nuclei did not enter mitosis until 16:47:24. Time is given in hours, minutes, and seconds.



Video 7. **Interphase progression in CycB+B3 RNAi-treated embryos.** Embryos expressing histone-RFP (red) were injected with dsRNA targeting CycB and CycB3 and subsequently with recombinant GFP-PCNA protein (green). Nuclei near the injection site are shown as they progress through interphase 13. Images were analyzed by time-lapse confocal microscopy using a spinning-disk confocal microscope (PerkinElmer). Frames were taken every 30 s. This video corresponds to Fig. 3 D. The 00:00 frame in Fig. 3 D corresponds to 14:15:46 in the video. Note that GFP-PCNA foci disappeared around 14:28:51; however, the nuclei did not enter mitosis until 14:42:01. Time is given in hours, minutes, and seconds.

Video 8. **Cyclin knockdown rescues cellularization and gastrulation in the *grp* mutant.** *grp* mutant embryos expressing histone-GFP (green) were injected three times along the embryo with dsRNA targeting CycB and CycB3. A representative embryo is shown as it gastrulates in interphase 14. Images were analyzed by time-lapse confocal microscopy using a spinning-disk confocal microscope (PerkinElmer). Frames were taken every minute.