McCleland et al., http://www.jcb.org/cgi/content/full/jcb.200909161/DC1

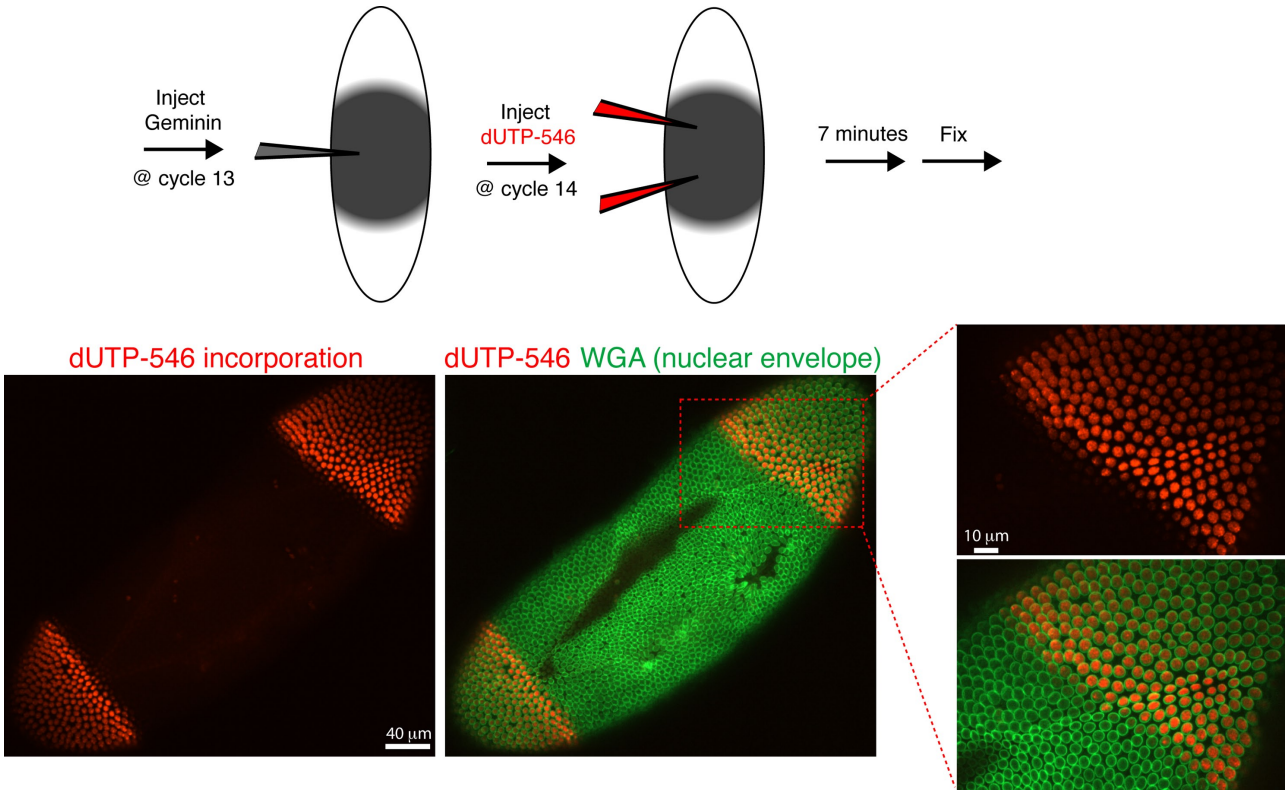


Figure S1. **Embryos injected with Geminin fail to initiate DNA replication.** Wild-type embryos were injected in the center lengthwise with Geminin during cycle 13 and incubated for 10 min. Fluorescent dUTP-546 nucleotide was subsequently injected throughout the embryos (note: embryos would be in early interphase 14). Embryos were fixed 7 min after introduction of nucleotide. Note that dUTP-546 is not incorporated in the region where Geminin was injected (middle of embryo), but that the embryo poles exhibit robust dUTP-546 incorporation. Red labeling corresponds to incorporation of dUTP-546 and green labeling highlights intact nuclear envelopes (stained with wheat germ agglutinin).

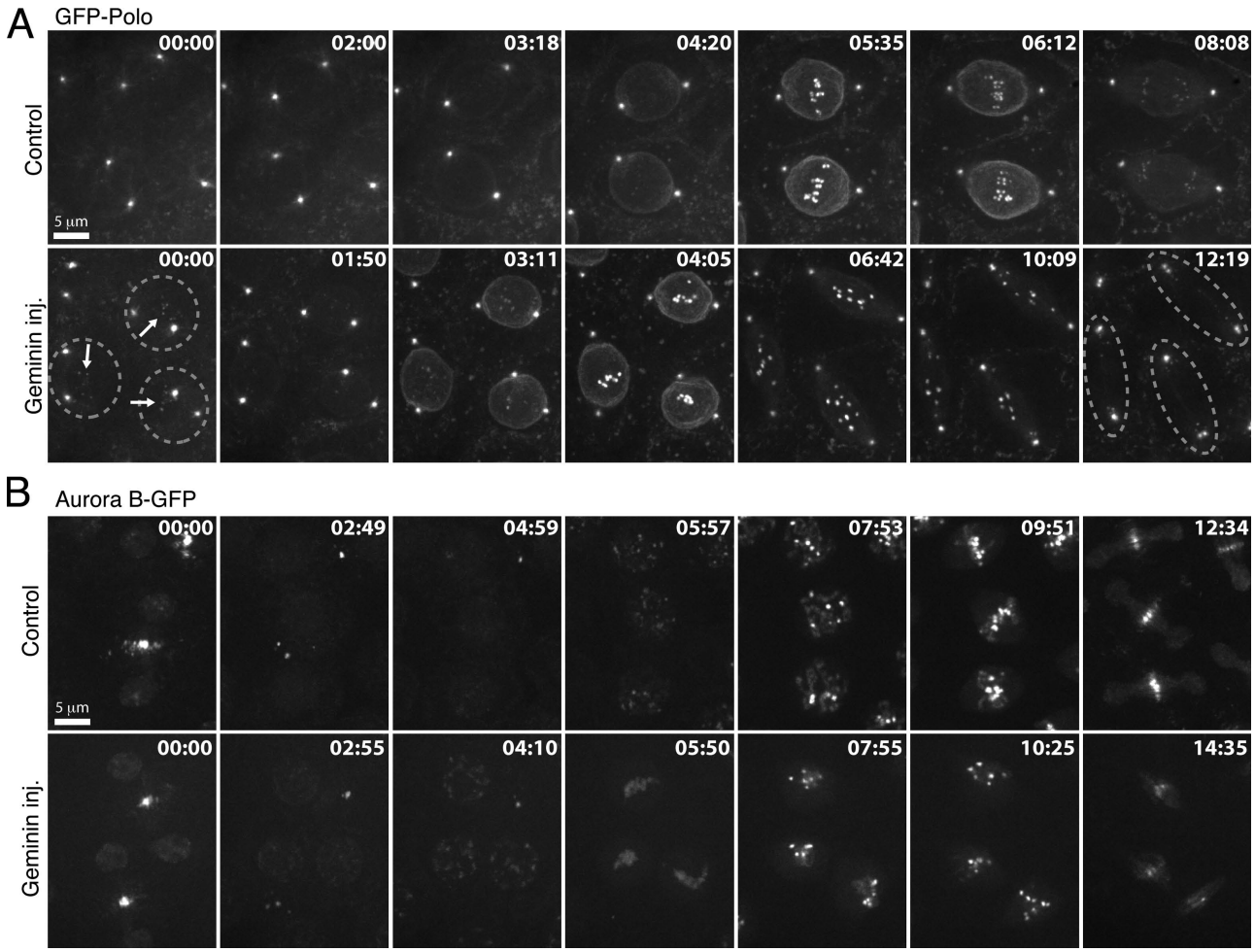


Figure S2. **Characterization of mitotic biomarkers after Geminin injection.** (A) GFP-Polo–expressing embryos were injected with control buffer or Geminin and followed during the subsequent cell cycle. Note that GFP-Polo enters the nucleus 3 min earlier than uninjected embryos, that GFP-Polo faintly decorates kinetochores throughout interphase (arrows highlight GFP-Polo–labeled kinetochores), and that the uninemic chromosomes fail to bi-orient. (B) Aurora B-GFP–expressing embryos were injected with control buffer or Geminin and followed during the subsequent cell cycle. Note that Aurora B-GFP, which normally binds between sister chromatids in mitosis, associated with the prematurely condensing chromatin in interphase and with the centromere in mitosis, despite the absence of a partner sister chromatid.

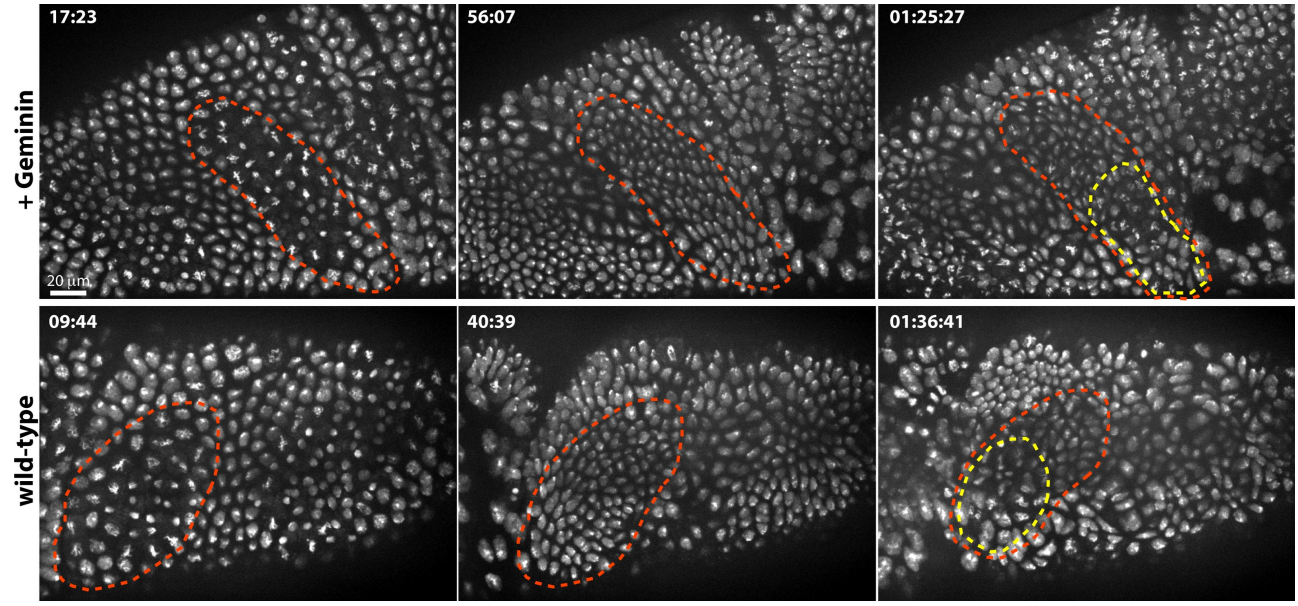
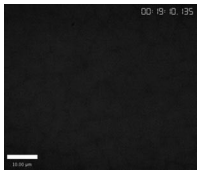
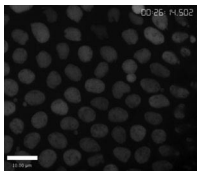


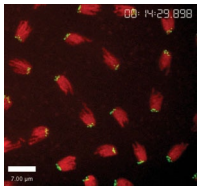
Figure S3. **DNA replication does not time mitotic entry in the post-MBT divisions.** Embryos expressing Histone-GFP were injected with Geminin during interphase of cycle 14 (before cellularization). Mitotic domain 5 of cycle 14, outlined by the red dashed line, was followed during cycle 15 to quantify the duration of interphase 15 in the absence of S phase. The yellow dashed line highlights nuclei that entered mitosis 15 with unreplicated chromatids in domain 5. Note that not all cells of domain 5 enter mitosis synchronously in cycle 15 (only those nuclei inside the yellow line). This Geminin-injected embryo corresponds to frames from Video 9 and can be used as a template to follow nuclei in domain 5 during cycle 14 and 15. A wild-type control is included to compare the mitotic domains during cycle 14 and 15. Note that although anterior is to the left in the Geminin-injected embryos, it is to the right in the wild-type. This wild-type embryo corresponds to Video 10 and can be used as a template to follow domain 5.



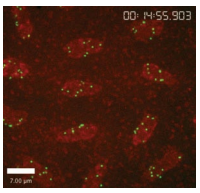
Video 1. ***Drosophila* embryos expressing YFP-PCNA were injected with control buffer and followed in real time.** Shown is an embryo progressing from mitosis of cycle 12 though mitosis of cycle 13. Note that YFP-PCNA is disperse in the cytoplasm during mitosis (beginning and end of movie). Also note that PCNA localizes to distinct foci in the nucleus during interphase and strongly stains the apical region of the nucleus at the end of interphase of cycle 13. This video corresponds to Fig. 1 C (video is a projection of five confocal sections 1.5 µm apart).



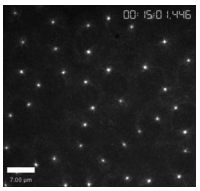
Video 2. ***Drosophila* embryos expressing YFP-PCNA were injected during interphase of cycle 12 with Geminin protein and followed in real time.** Shown is an embryo progressing from the end of S phase of cycle 12 until the beginning of cycle 14. Note that PCNA foci are not disturbed in interphase of cycle 12, but that PCNA foci are absent during interphase of cycle 13. This video corresponds to Fig. 1 C (video is a projection of five confocal sections 1.5 µm apart).



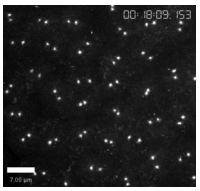
Video 3. ***Drosophila* embryos expressing Cid-GFP (green) and Histone-RFP (red) were injected with control buffer and followed in real time.** Shown is an embryo progressing from anaphase of cycle 11 until anaphase of cycle 12. This video corresponds to Fig. 2 A (video is a projection of five confocal sections 1.5 µm apart).



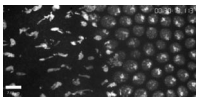
Video 4. ***Drosophila*** **embryos expressing Cid-GFP (green) and Histone-RFP (red) were injected during interphase of cycle 11 with Geminin protein and followed in real time.** Shown is an embryo progressing from anaphase of cycle 11 until interphase of cycle 13. Note that chromatin prematurely condenses into a mass in the middle of nuclei during interphase of cycle 12. Also note that Cid foci are unpaired throughout mitosis and never align at the metaphase plate. This video corresponds to Fig. 2 B (video is a projection of five confocal sections 1.5 µm apart).



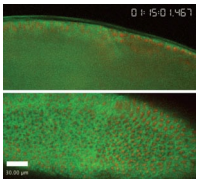
Video 5. ***Drosophila*** **embryos expressing GFP-Polo were injected with control buffer and followed in real time.** Shown is an embryo progressing from the beginning of interphase of cycle 12 through interphase of cycle 13. This video corresponds to Fig. S2 A (video is a projection of five confocal sections 1.5 µm apart).



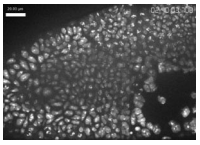
Video 6. ***Drosophila*** **embryos expressing GFP-Polo were injected during interphase of cycle 11 with Geminin protein and followed in real time.** Shown is an embryo progressing from anaphase of cycle 11 through the beginning of cycle 13. Note the faint labeling of kinetochores with GFP-Polo during interphase of cycle 12, premature entry of GFP-Polo into the nucleus before mitosis, and unpaired kinetochores throughout mitosis that oscillate between centrosomes. This video corresponds to Fig. S2 A (video is a projection of five confocal sections 1.5 µm apart).



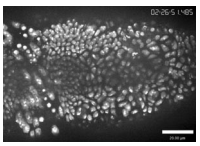
Video 7. ***Drosophila*** **embryos expressing Histone-GFP were injected at one pole (left) during interphase of cycle 13 with Geminin protein and followed in real time.** Shown is an embryo progressing from metaphase of cycle 13 through cycle 14. Note the extra syncytial division that occurs in the injected pole while nuclei toward the uninjected end of the embryo remain in interphase. This video corresponds to Fig. 4 A (video is a projection of five confocal sections 1.5 µm apart).



Video 8. ***Drosophila*** **embryos expressing Histone-RFP (red) and Sqh-GFP (green) were injected with Geminin at one pole (left) during cycle 13 and followed in real time to monitor cellularization at the MBT.** Sqh, the regulatory light chain for myosin, marks the leading edge of ingressing membrane. Images were acquired at the embryo surface to examine nuclear behavior (bottom) and in a sagittal view (top) to follow cellularization. Note that the pole in which Geminin was injected undergoes an extra syncytial division (4:08–18:59) and then subsequently cellularized (40:45–01:11:00). Also note the uninjected pole remains in interphase 14 and cellularizes ahead of the injected end (each video is a projection of five confocal sections 1.5 µm apart).



Video 9. ***Drosophila*** **embryos expressing Histone-GFP were injected with Geminin protein early during interphase of cycle 14 (before cellularization).** Shown is an embryo progressing from G2 of cycle 14 through cycle 15. Multiple mitotic domains are evident during cycle 14. Refer to Fig. S3 to locate mitotic domain 5. Note that mitosis of cycle 14 is normal, interphase of cycle 15 is not shortened, and mitosis of cycle 15 occurs with unreplicated sister chromatids. This video corresponds to Fig. S3 (video is a projection of five confocal sections 1.5 µm apart).



Video 10. ***Drosophila*** **embryos expressing Histone-GFP were filmed from G2 of cycle 14 through cycle 15.** Multiple mitotic domains are evident during cycle 14. Refer to Fig. S3 to locate mitotic domain 5 and note that the nuclei of domain 5 enter mitosis more asynchronously during cycle 15. This video corresponds to Fig. S3 (video is a projection of five confocal sections 1.5 µm apart).