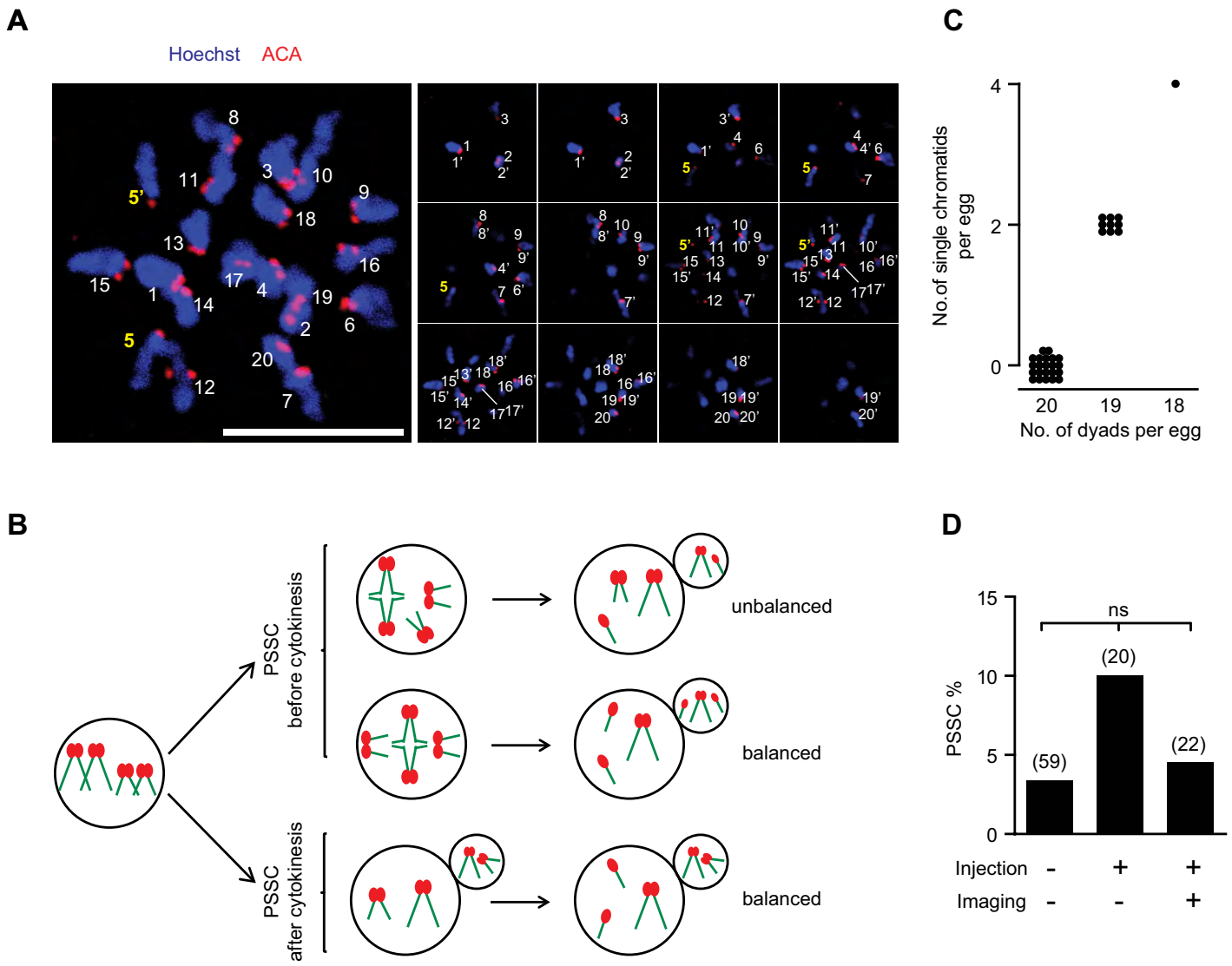


Fig. S1. PSSC in aged oocytes. (A) The left image is a maximal intensity z-projection of chromosomes (blue; Hoechst) and their kinetochores (red; anti-ACA); as shown in Fig 1A. Sister chromatid pairs are labeled in white and two single chromatids are labeled in yellow. Right images are sister chromatid pairs/single chromatids taken from individual z-sections labeled with corresponding numbers. Note that the kinetochores of chromosomes 2 and 19 overlap in the maximum intensity projection, but are readily resolved in the individual z-sections. Scale bar represents 10 µm. (B) Schematic showing balanced and unbalanced pre-division. If sister chromatid separation happens before cytokinesis this can result in either balanced or unbalanced division, but only balanced pre-division can ever be observed if premature separation happens after cytokinesis. (C) The number of single chromatids and dyads observed per egg, showing only balanced pre-division from aged mice. (D) PSSC rate in metII eggs associated with injection and imaging during in vitro maturation. Neither causes a significant increase in PSSC.

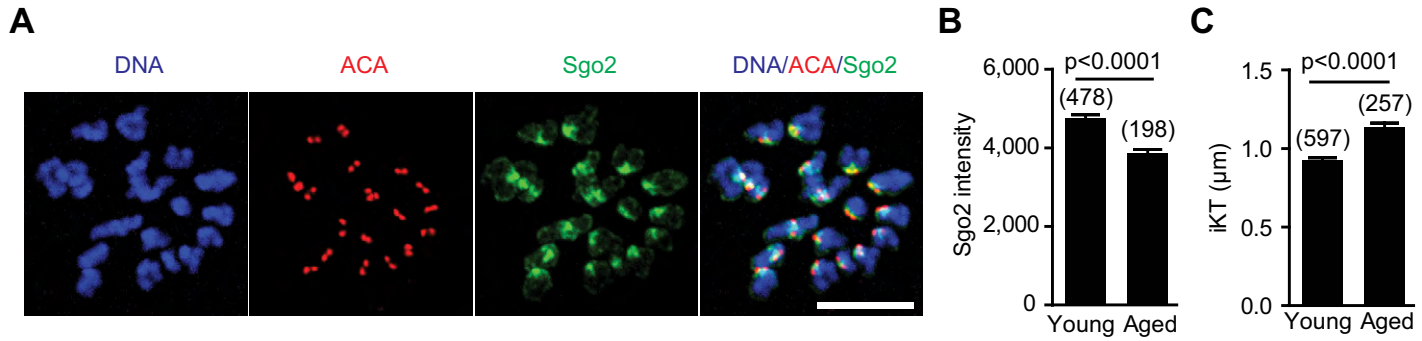


Fig. S2. SGO2 intensity and iKT distance measurements in young and aged metII eggs. (A) Chromosomes from a metII egg with SGO2 and kinetochore (ACA) immunostaining. Scale bar represents 10 μm. (B) Comparison of centromeric SGO2 levels between young and aged metII eggs, showing significantly less SGO2 with age. (C) iKT distance measurements, made between the kinetochores of the sister chromatid pair, in young and aged eggs, showing greater distance with age. (B,C) shown are mean and s.e.m.

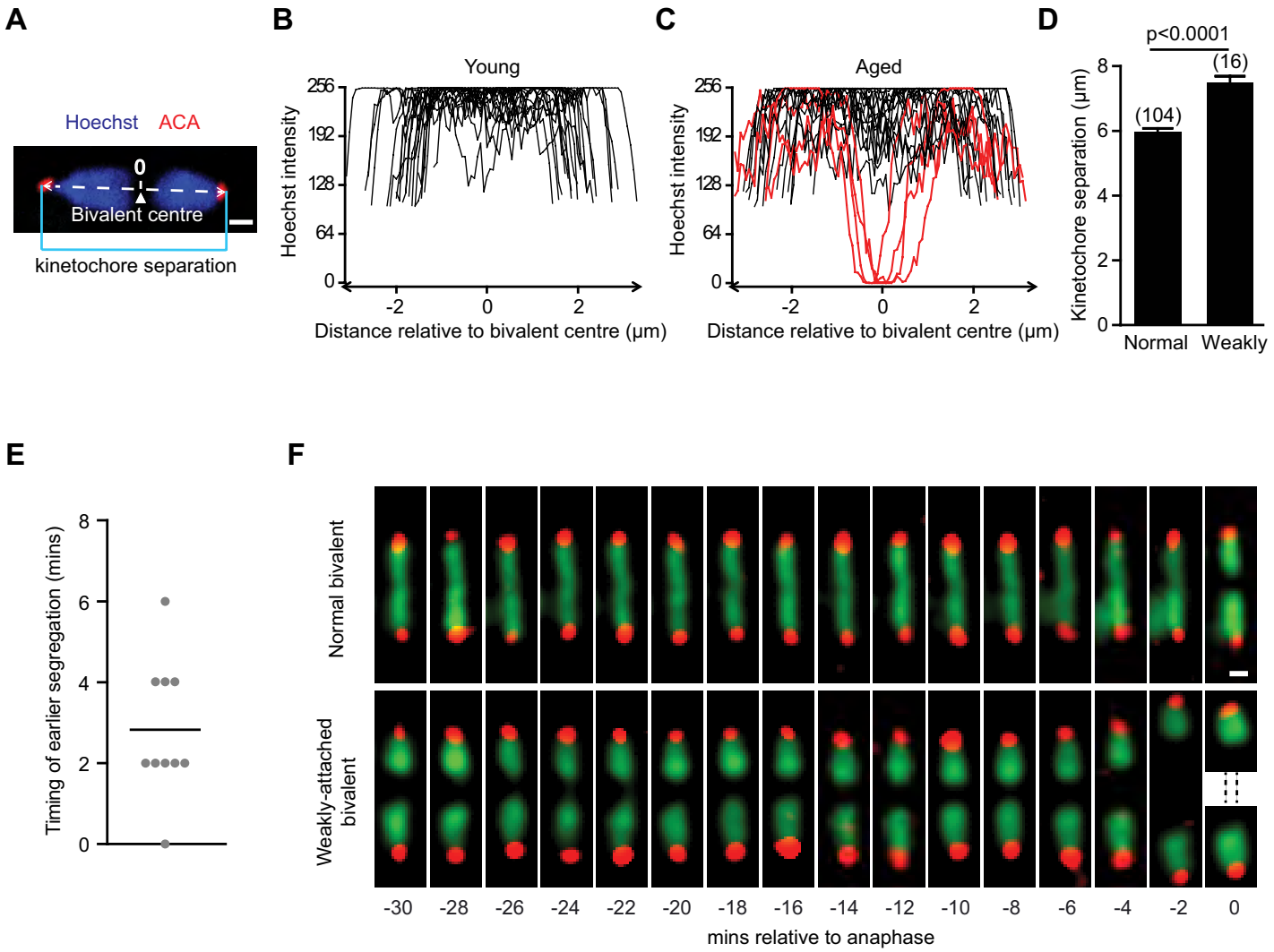


Fig. S3. Weakly-attached bivalent measurement and premature segregation. (A) Measurement of Hoechst intensity and kinetochore separation for individual bivalents from fixed oocytes. Intensity score was calculated along a line joining the two sister kinetochore pairs within the bivalent. Kinetochore separation is the distance between the two pairs of sister kinetochores. (B, C) Measurement score of Hoechst intensity from 20 bivalents of a young (B) and aged (C) oocyte. Red lines in (C) indicate four weakly-attached bivalents, as shown in Fig 3A. (D) Kinetochore separation in weakly-attached bivalents is significantly increased (mean and s.e.m.). Number of bivalents indicated in parenthesis. (E) Segregation timing of weakly-attached bivalents (10 bivalents from 5 aged oocytes) relative to their normally-attached siblings from the same oocyte. Horizontal line represents the mean. (F) Representative images of a normal and weakly-attached bivalent tracked during the last 30 minutes before anaphase onset. The weakly-attached bivalent showed earlier (4 minutes) segregation. (A, F) Scale bar represents 1 μm.

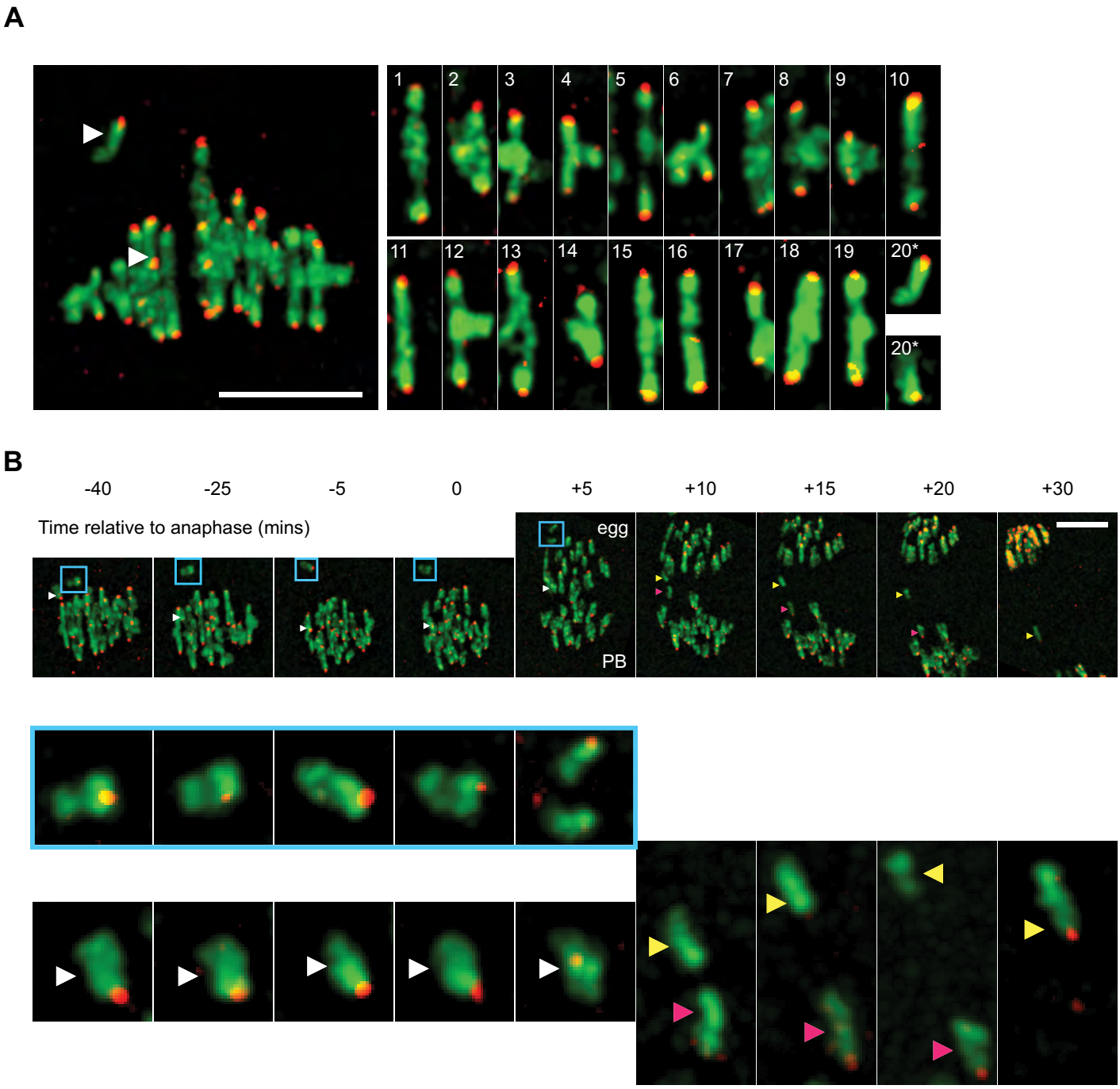
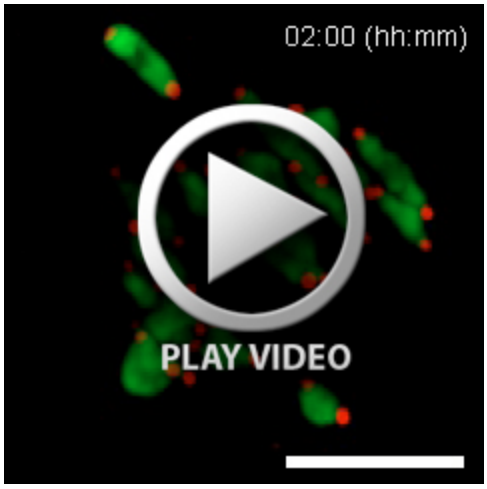
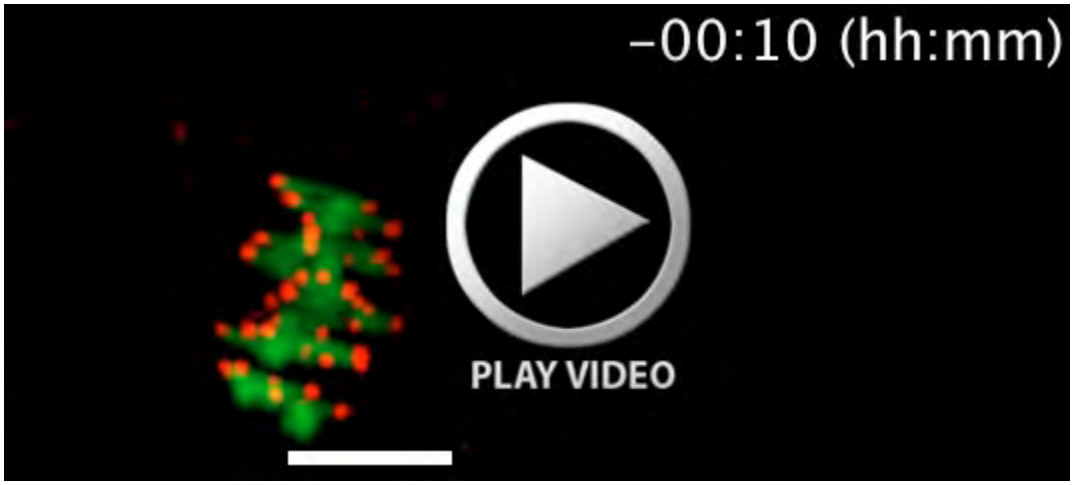


Fig. S4. The fate of univalents at anaphase I in an aged oocyte. (A) Left image shows a maximum intensity z-projection of an aged oocyte containing two univalents (arrowheads) 3 hours before anaphase onset. Right images show individual z-sections of the bivalents (1-19) and two univalents (20*). (B) The fate of the two univalents at anaphase shown in a maximum intensity z-projection (top row) and in enlarged, single z-sections (bottom two rows) at the times indicated, relative to anaphase onset. One univalent (blue box) separates into two single chromatids, which both partition into the oocyte. The other univalent (unboxed, white arrowhead), separates into two single chromatids (pink and yellow arrowheads), one of which (yellow) lags at the spindle equator leading to co-segregation into the polar body. (A,B) Scale bar represents 5 µm.



Movie 1. Oocyte with chromosome and kinetochore labeling undergoing faithful segregation at anaphase. Elapsed time given from GVB. Kinetochores (EGFP-CenpC) in red, chromosomes (H2B-mCherry) in green. Scale bar represents 10 μm.



Movie 2. Generation of single chromatids following chromosome recondensation during Movie 2. Elapsed time given from anaphase-onset. Kinetochores in red, chromatin in green. Some of the images from this movie are displayed in Fig. 7. Following anaphase onset, chromatin is observed to decondense (0:08-1:06). Chromatin in the first polar body (PB1) is not tracked and so is lost from the field of view at 0:50. Chromosome recondensation (1:06-1:38) is followed by separation of a dyad (arrowheads) at 2:04, and the resulting single chromatids oscillate about the spindle equator. *White box*, showing the separation event, is enlarged on right hand side of video (2x magnification). Scale bar represents 10 μm.