Current Biology, Volume *29*

Supplemental Information

Meiotic Kinetochores Fragment into Multiple Lobes upon Cohesin Loss in Aging Eggs

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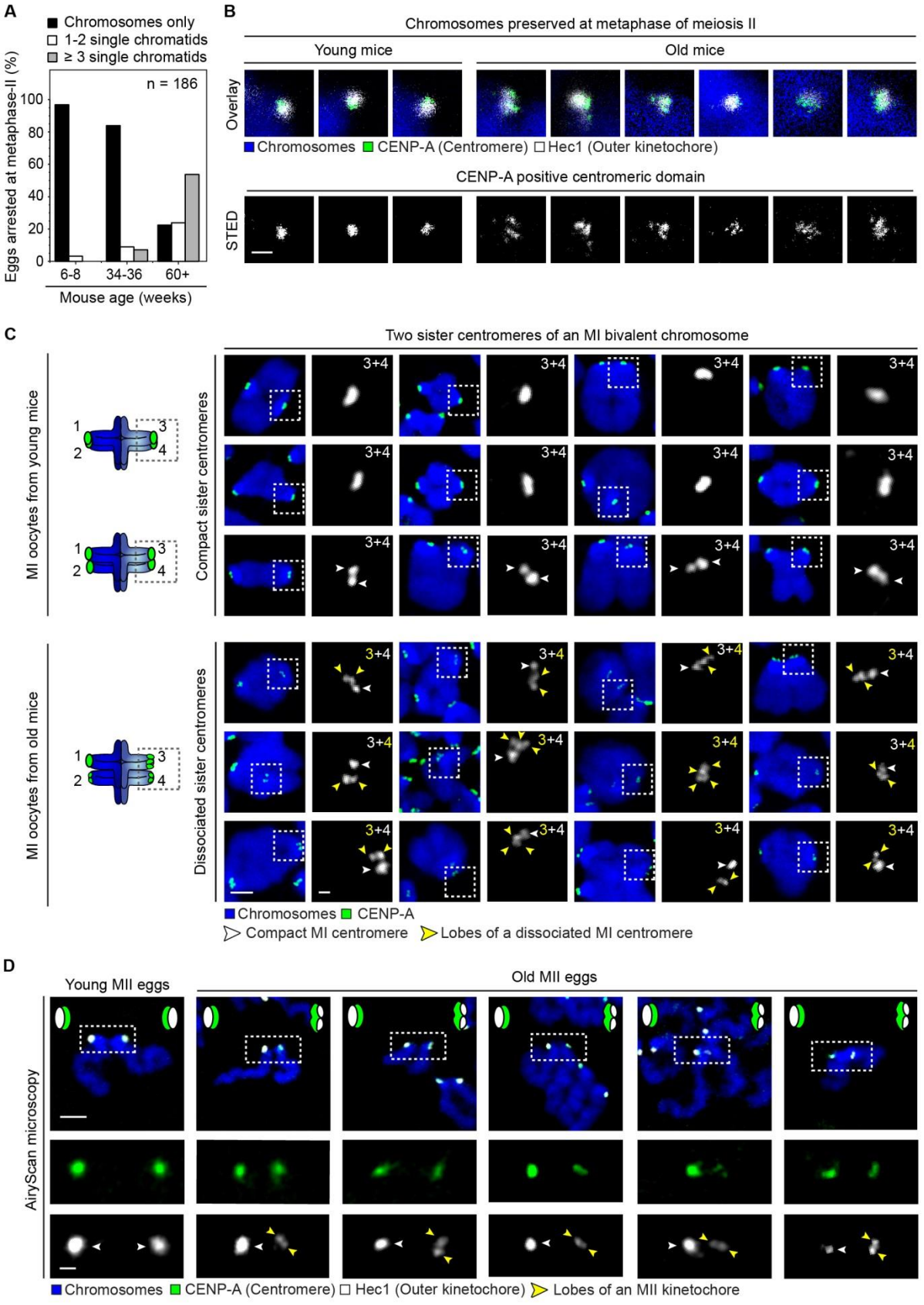

Figure S1| The centromeric CENP-A domain decompacts in aged MI oocytes and MII eggs, related to Figure 1

(A) Fraction of metaphase-II eggs from mice of different ages that contained single chromatids, indicative of pronounced weakening of centromeric cohesion. Data from 186 MII eggs (8 experiments).

(B) Representative images of CENP-A positive centromeric domains in metaphase-II eggs from mice aged either 8 weeks (young) or 60-64 weeks (old). Top panel shows an overlay of DNA signal (blue, Picogreen, confocal mode), outer kinetochore region (white, Hec1, confocal mode) and the centromeric signal (green, CENP-A, STED mode). Below each panel, the corresponding centromeric CENP-A signal is additionally shown in greyscale (STED mode). Scale bar: 0.5 µm.

(C) Representative images of the centromeric CENP-A domain in young (8 week old females) and old (65-67 week old females) oocytes preserved at metaphase of meiosis I and visualized with AiryScan microscopy. Numbers in insets refer to sister kinetochores shown (as in the schematic on the left).

(D) Representative metaphase-II chromosome spreads in eggs from aged mice (>60 weeks old) visualized with AiryScan microscopy. Centromeres are labelled in green (top, CENP-A), outer kinetochores in white (bottom, Hec1) and chromosomes in blue (Hoechst).

(**C** and **D**) Scale bars represent 2 µm in overviews and 0.5 µm in insets. White arrows point to compact kinetochores/centromeres and yellow arrows point to lobes within fragmented kinetochores/centromeres.

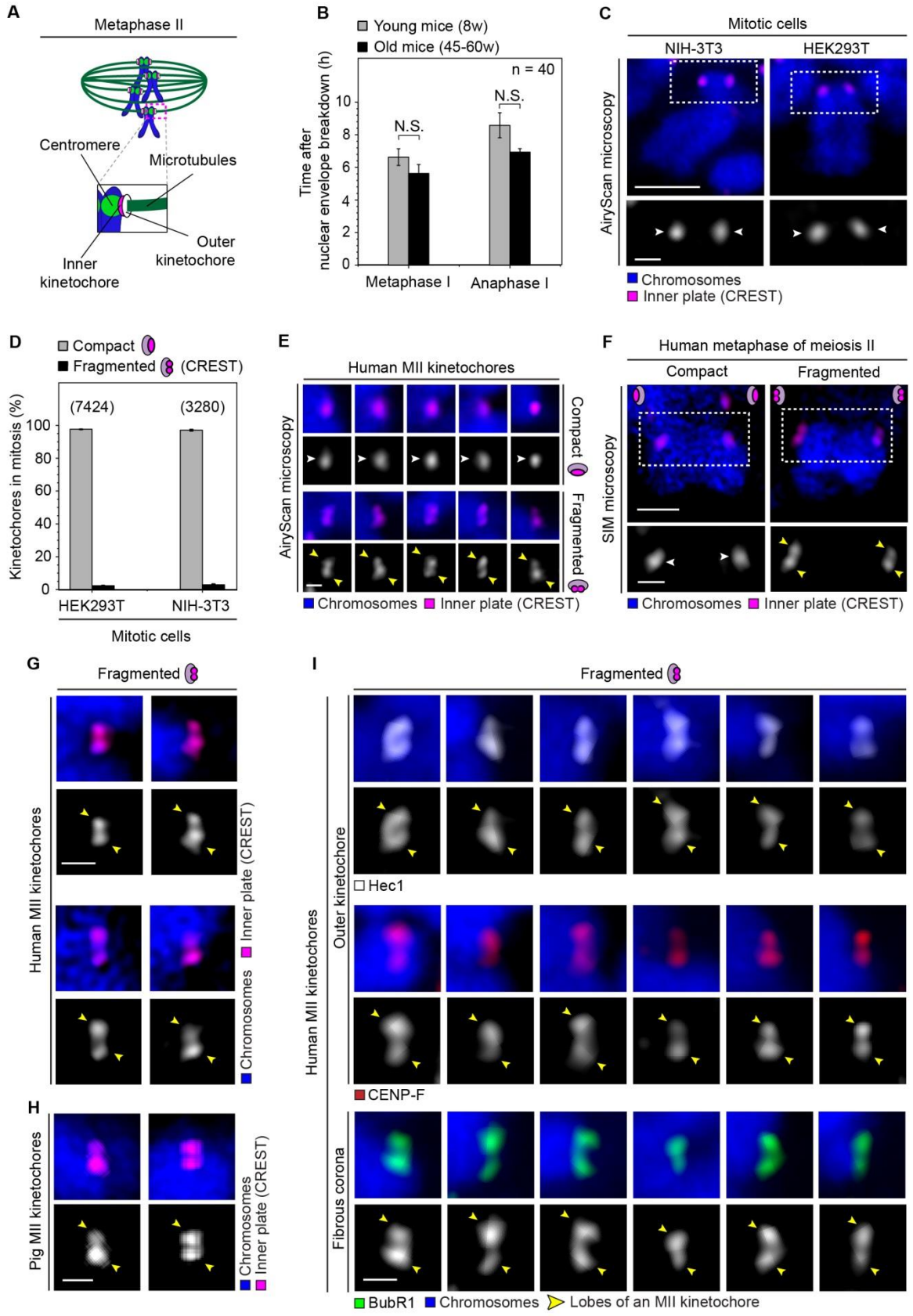

Figure S2| Meiosis II kinetochores in humans, pigs and mice frequently reorganize into two distinct lobes, related to Figure 2

(A) Schematic diagram showing the spatial arrangement of the centromere (green), the inner kinetochore (magenta) and the microtubule-interacting outer kinetochore (white) in a metaphase-II chromosome.

(B) Time required for oocytes from young mice (8 weeks) and old mice (45-62 weeks, 15 females) to complete the key meiotic events. Metaphase-I timepoint refers to full chromosome alignment on the MI spindle. Chromosome (DNA 5-TMR-Hoechst) and spindle (SiR-tubulin) dynamics were followed live using fluorescent dyes (4 experimental repetitions). Timings were compared using Student's t-test (p = 0.085, N.S.). Error bars show SEM.

(C) Representative immunofluorescence images of kinetochores in mouse and human mitotic cells imaged with AiryScan microscopy.

(D) Quantifications of kinetochore configurations in mitotic cells as in **(C)**. Data from 67 NIH-3T3 and 68 HEK293T cells (3 experiments each). Error bars show SEM.

(E) Representative images of compact (top panel) and fragmented (bottom panel) kinetochores in human MII eggs imaged with AiryScan microscopy.

(F) Representative SIM microscopy images of human MII chromosomes with both kinetochores compact (left) or both fragmented (right). Both chromosomes were captured on the same metaphase-II spindle. Scale bars: 1 µm in overviews and 0.5 µm in insets.

(G) Representative SIM microscopy examples of fragmented MII kinetochores in human eggs.

(H) Representative images of fragmented MII kinetochores in pigs, imaged with AiryScan.

(I) Representative images of fragmented human MII kinetochores labelled with outer kinetochore markers (Hec1, white and CENP-F, red), and stained for the fibrous corona component BubR1 (green). DNA is labelled with Hoechst (blue).

(C, E-H) Kinetochores are labelled with CREST (magenta) and the DNA is labelled with Hoechst (blue).

(C, F) Scale bars: 2 µm in overviews and 0.5 µm in insets.

(E, G, H and I) Scale bars: 0.5 µm.

White arrows point to compact kinetochores and yellow arrows point to lobes within a fragmented MII kinetochore.

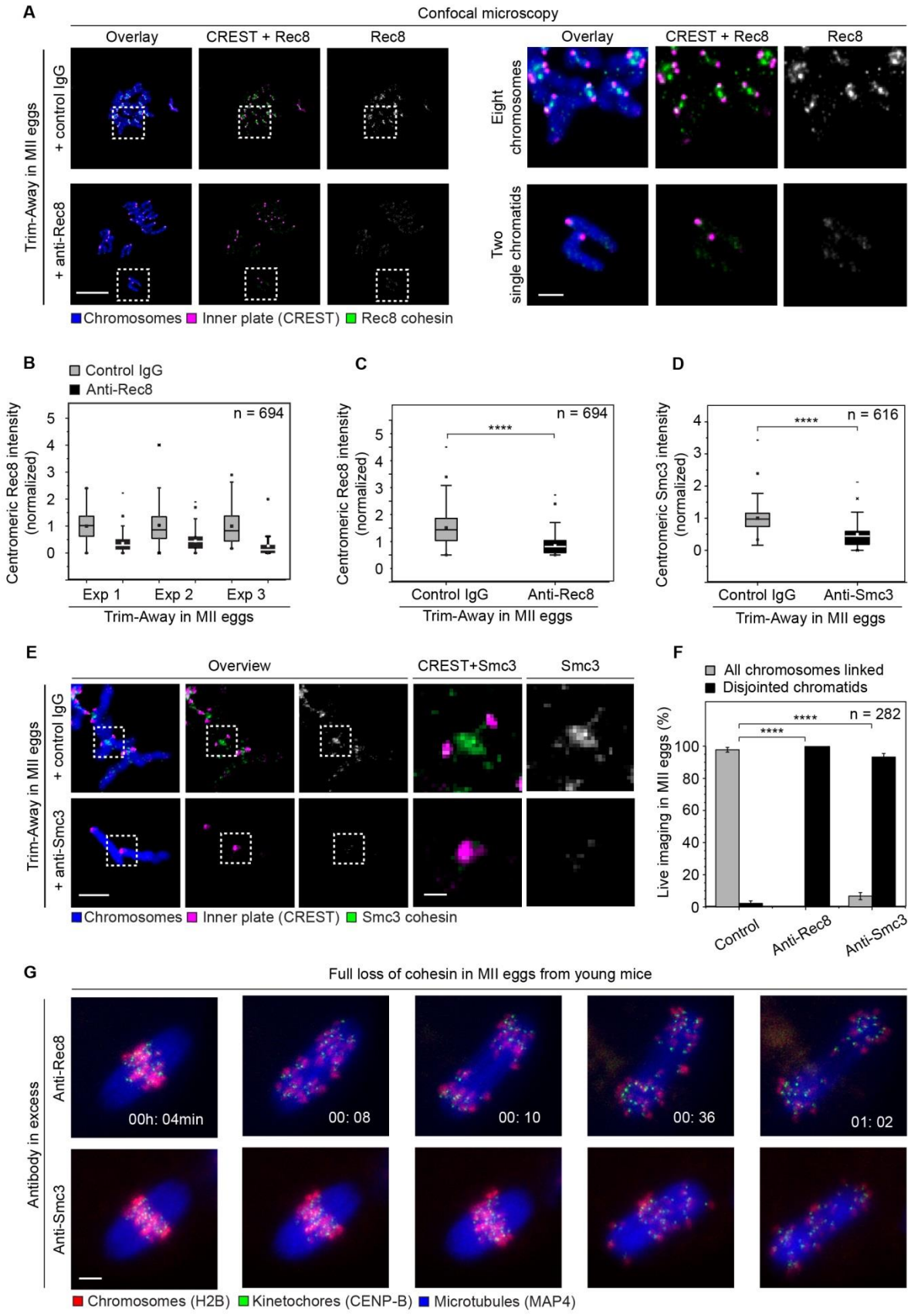

Figure S3| Trim-away efficiently depletes the centromeric cohesin pool in young eggs arrested at metaphase II, related to Figure 3

(A) Representative confocal images of metaphase-II chromosome spreads from young mice following the full Trim-Away assay as in **Figure 3A**, where TRIM21 overexpressing MII eggs were microinjected with either a control IgG antibody (top) or an anti-Rec8 antibody provided in excess (bottom). Images are maximum intensity z-projections of 19 z-sections acquired every 0.45 µm. The anti-Rec8 signal in both the control and the experimental groups is shown using identical brightness and contrast settings. Scale bars: 10 µm in overview and 2 µm in insets.

(B) Quantification of the centromeric Rec8 signal in MII eggs treated as in **(A)**. Measurements obtained for each experimental repetition are shown and these were normalized to the mean intensity of the young group. 29 MII eggs from 3 independent experimental repetitions were analyzed.

(C) Summary of all measurements obtained as in **(B)**

(D) Quantification of the centromeric Smc3 signal in MII eggs treated as in **(E)**. The measurements were normalized to the mean intensity of the young group. 22 MII eggs from 2 independent experimental repetitions were analysed.

(E) Representative confocal images of metaphase-II chromosome spreads from young mice following the full Trim-Away assay (as in **Figure 3A**), where TRIM21 overexpressing MII eggs were microinjected with either a control IgG antibody (top) or an anti-Smc3 antibody provided in excess (bottom). Images are maximum intensity z-projections of 12 z-sections acquired every 0.36 µm. The anti-Smc3 signal in both the control and the experimental groups is shown using identical brightness and contrast settings. Scale bars: 5 µm in overview and 1 µm in insets.

(F) Evaluation of the efficiency of the Trim-Away approach to induce loss of chromosome integrity, as in **(G)**. Data from 282 live MII eggs (young mice) from 19 experiments.

(G) Frames from time-lapse movies of live TRIM21 overexpressing MII eggs from young mice, microinjected with either an excess of anti-Rec8 or anti-Smc3. Chromosomes are labelled in red (H2B-mRFP), kinetochores in green (CENP-B-mEmerald) and microtubules in blue (MAP4-MTBD-Snap647). Time shows minutes (min) from antibody microinjection. Scale bar: 5 μm.

(A and **E)** Kinetochores are labelled with CREST (magenta), DNA is labelled with Hoechst (blue) and cohesins (green) are labelled in **(A)** with anti-Rec8 and in **(E)** with anti-Smc3.

P values are designated as *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001. *P* values in **(F)** were calculated with Student's t-test and in (**B-D**) the two groups were compared by one-way ANOVA followed by Turkey's test. Error bars show SEM. Box plots show median (horizontal lines), mean (small squares), 25th and 75th percentiles (boxes) and 5th and 95th percentiles (whiskers).

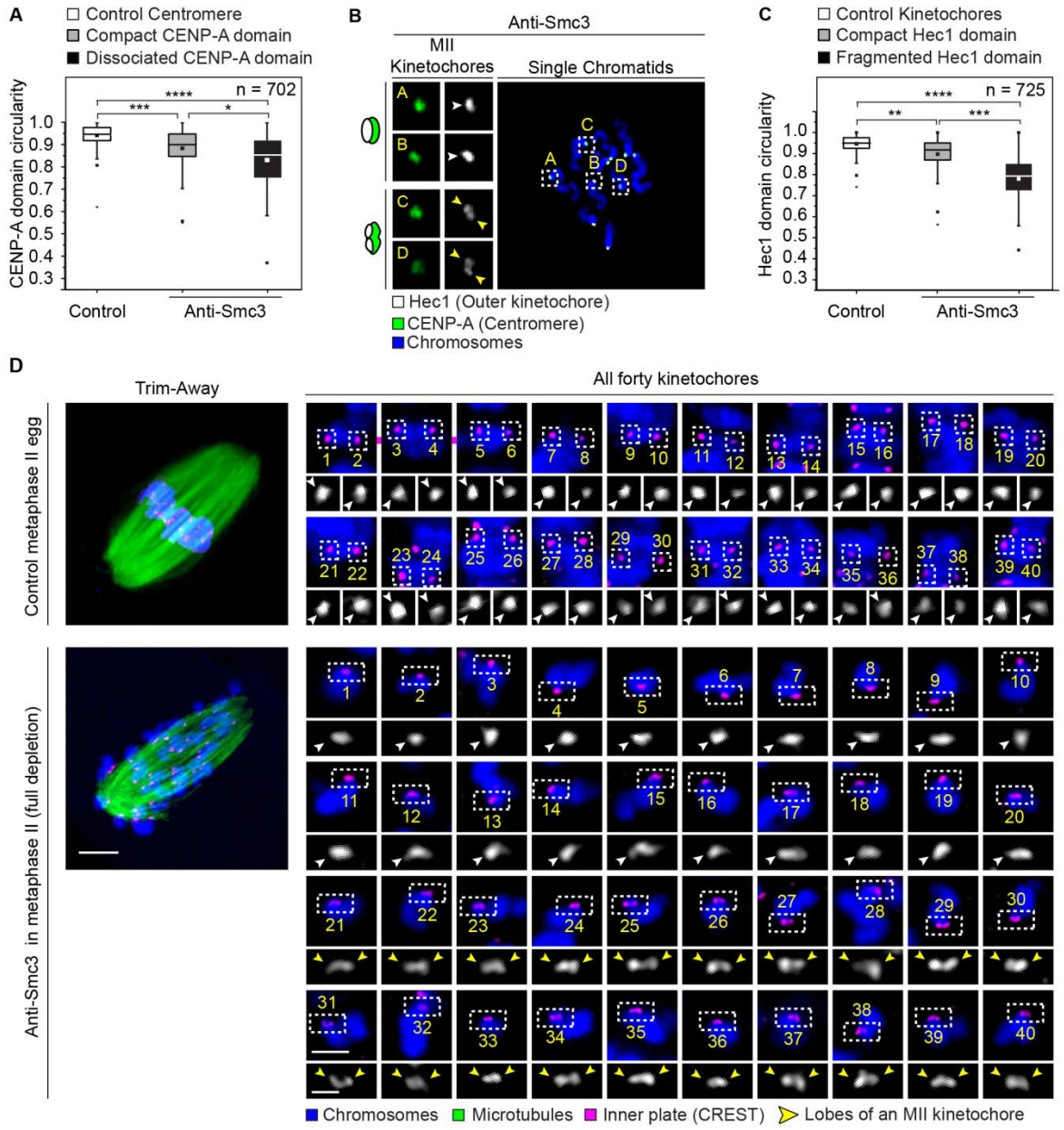

Figure S4| Centromeres decompact and inner/outer kinetochore plates fragment upon cohesin loss, related to Figure 3

(A) Assessment of the circularity (circularity of a perfect circle = 1.0) of the CENP-A domain in control eggs and young MII eggs depleted for Smc3. Centromere circularity and fragmentation status were assessed based on the CENP-A signal. 702 kinetochores from 20 MII eggs were evaluated (2 experiments).

(B) Representative chromosome spread images of single chromatids in Smc3-depleted MII eggs from young mice. Outer kinetochores are labelled in white (Hec1), centromeres in green (CENP-A) and chromosomes in blue (Hoechst). Scale bars: 2 µm in overviews and 1 µm in insets.

(C) Assessment of the circularity (circularity of a perfect circle = 1.0) of the Hec1 domain in control eggs and young MII eggs depleted for Smc3. Both the fragmentation status of a kinetochore and the circularity measurements were based on Hec1 labelling. 725 kinetochores from 20 MII eggs were evaluated (2 experiments).

(D) Representative examples of all 40 kinetochores of 20 chromosomes from a control MII egg (top) and all 40 kinetochores of 40 single chromatids following Trim-Away of Smc3 (bottom). Scale bars: 5 µm for spindle overviews, 2 µm for chromosome overviews and 0.5 µm for kinetochore insets. A projection through z-sections of the same Trim-Away MII egg microinjected with anti-Smc3 is shown in **Video S2.**

P values are designated as *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001. *P* values were compared by one-way ANOVA followed by Turkey's test. Box plots show median (horizontal lines), mean (small squares), 25th and 75th percentiles (boxes) and 5th and 95th percentiles (whiskers). White arrows point to compact kinetochores and yellow arrows point to lobes within a fragmented MII kinetochore.

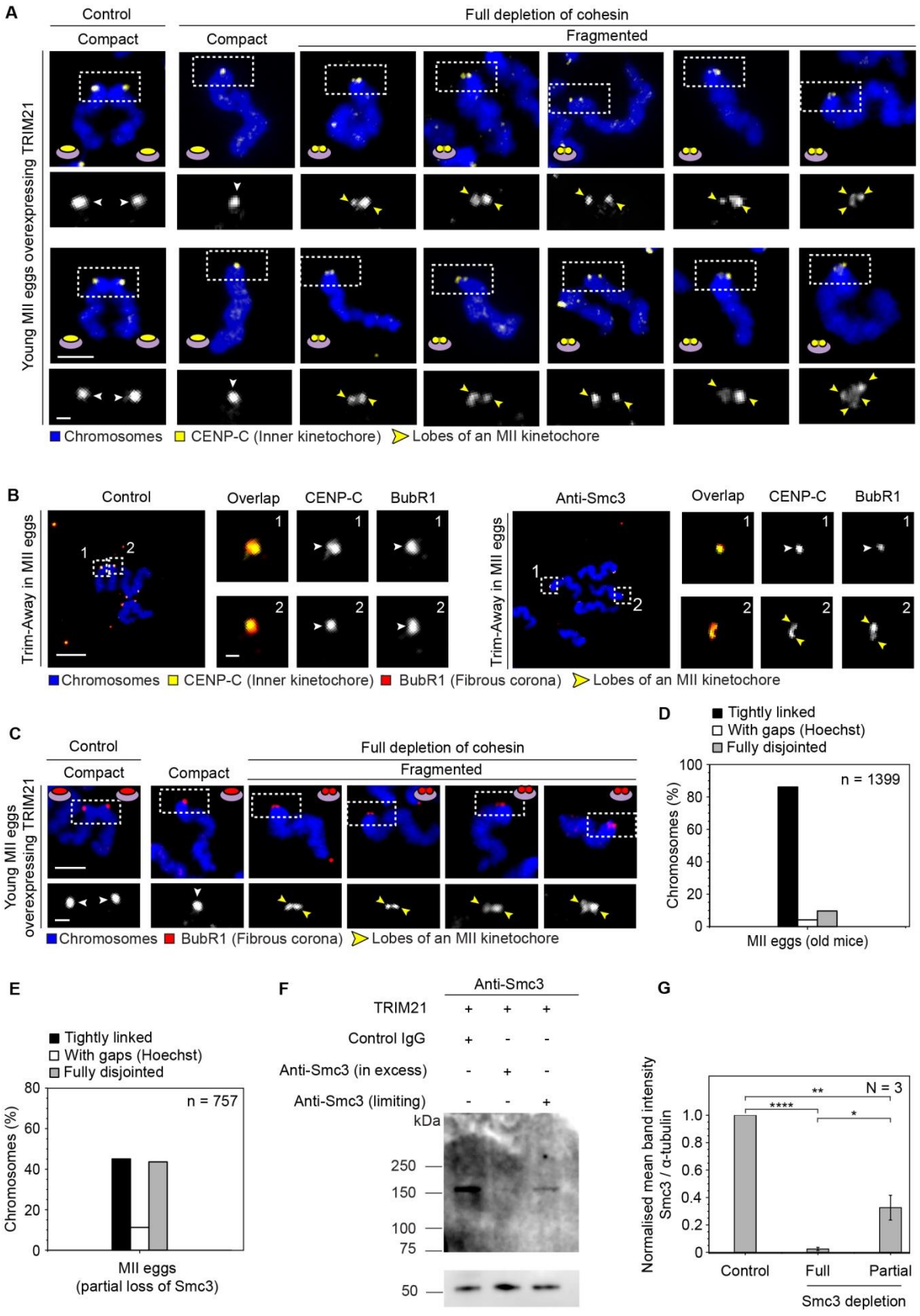

Figure S5| Fragmentation affects all key layers of the kinetochore complex and is linked to chromosome architecture, related to Figure 4

(A) MII kinetochores labelled with inner plate protein CENP-C in metaphase-II arrested eggs from young mice. Images show control chromosomes (left panels; two sister kinetochores) and single chromatids (other panels) induced by a full depletion of Rec8. Chromosomes are labelled in blue (Hoechst) and kinetochore inner plates in yellow (CENP-C). Scale bars: 2 µm in overviews and 0.5 µm in insets.

(B) MII kinetochores in young eggs treated as in **(A)** and co-labelled with anti-CENP-C (yellow) and anti-BubR-1 (red). Scale bars: 5 µm in overviews and 0.5 µm in insets.

(C) MII kinetochores in young eggs treated as in **(A)** and labelled with anti-BubR-1 (red). Scale bars: 2 µm in overviews and 0.5 µm in insets.

(D) Chromosome configurations as in **Figure 4C** and their occurrence in MII eggs from naturally aged mice. Kinetochores were labelled with CREST and the DNA was labelled with Hoechst. “With gaps” refers to no detectable Hoechst signal between sister chromatids that are still linked. *n* refers to the number of chromosomes analyzed. Data from 67 MII eggs.

(E) Chromosome configurations as in **Figure 4C** and their occurrence in MII eggs from young mice with reduced cohesin following partial Trim-Away. Kinetochores were labelled with CREST and the DNA was labelled with Hoechst. “With gaps” refers to no detectable Hoechst signal between sister chromatids that are still linked. Data from 41 MII eggs (4 experiments). *n* refers to the total number of chromosomes analyzed.

(F) Representative anti-Smc3 immunoblot of whole MII egg lysates following microinjection of either a control IgG or an anti-Smc3 antibody (provided in excess or at a rate-limiting concentration) to TRIM21 overexpressing MII eggs from young mice.

(G) Quantification of the relative Smc3 protein levels in **(F)** across 3 experimental repetitions following Smc3 depletion. *P* values are designated as *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001 and were calculated with Student's t-test. Error bars show SEM.

White arrows point to compact kinetochores and yellow arrows point to lobes within a fragmented MII kinetochore. DNA is labelled in all panels with Hoechst (blue).

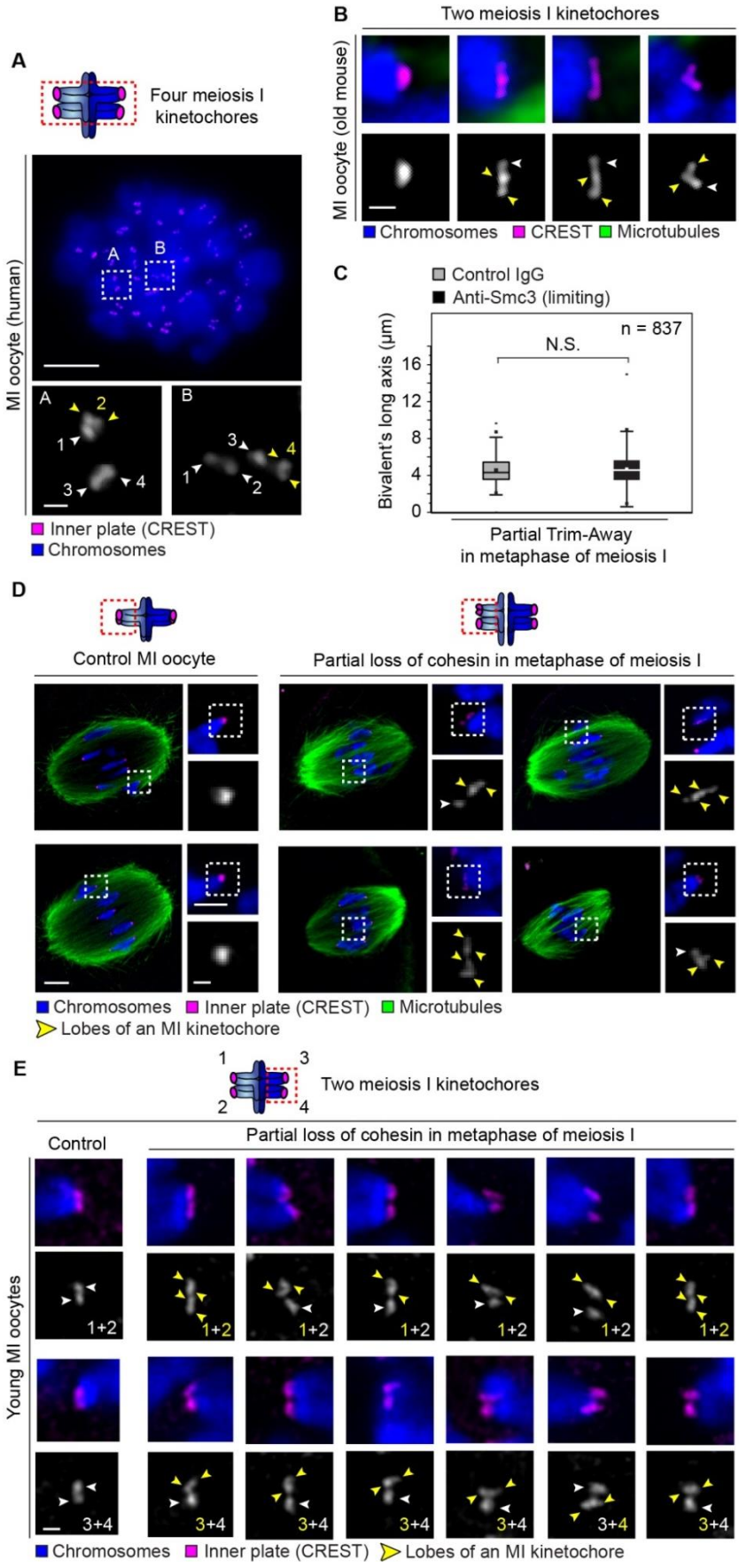

Figure S6| Cohesin loss results in sister kinetochore splitting and fragmentation of MI kinetochores into lobes, related to Figure 5

(A) Representative image of a human metaphase-I chromosome spread from a 25 year old donor. The four kinetochores of two representative bivalents are shown in insets. Scale bars: 10 µm in overview and 1 µm in insets.

(B) Representative images of inner plates of fragmented MI kinetochores in meiosis I oocytes from old mice. Scale bar: 0.5 µm.

(C) Distance between the two sister kinetochore pairs of a meiosis I bivalent in metaphase I (bivalent's long axis). Oocytes have been treated as in **Figure 5B**. Data from 42 MI oocytes from young mice (2 experiments). Box plots show median (horizontal lines), mean (small squares), 25th and 75th percentiles (boxes) and 5th and 95th percentiles (whiskers). *P* values are designated as *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001. *P* value was calculated with one-way ANOVA followed by Turkey's test (N.S.).

(D) Representative images of Trim-Away meiotic spindles treated as in **Figure 5B**. Insets demonstrate chromosome and kinetochore architectures in MI bivalents under these conditions. Scale bars: 5 µm in overview, and 2 µm or 0.5 µm in insets.

(E) Representative immunofluorescence images of two sister kinetochores of a bivalent in control oocytes (left panels) or anti-Smc3 microinjected oocytes in Trim-Away experiments (other panels). Scale bar: 0.5 µm

(**A**, **B**, **D, E**) Chromosomes are labelled in blue (Hoechst) and kinetochores in magenta (CREST). In (**B** and **D**), microtubules are additionally labelled in green (α-tubulin). White arrows point to compact kinetochores and yellow arrows point to lobes within a fragmented MI kinetochore. Numbers in insets refer to sister kinetochores shown (as in the schematic in (**E**)).

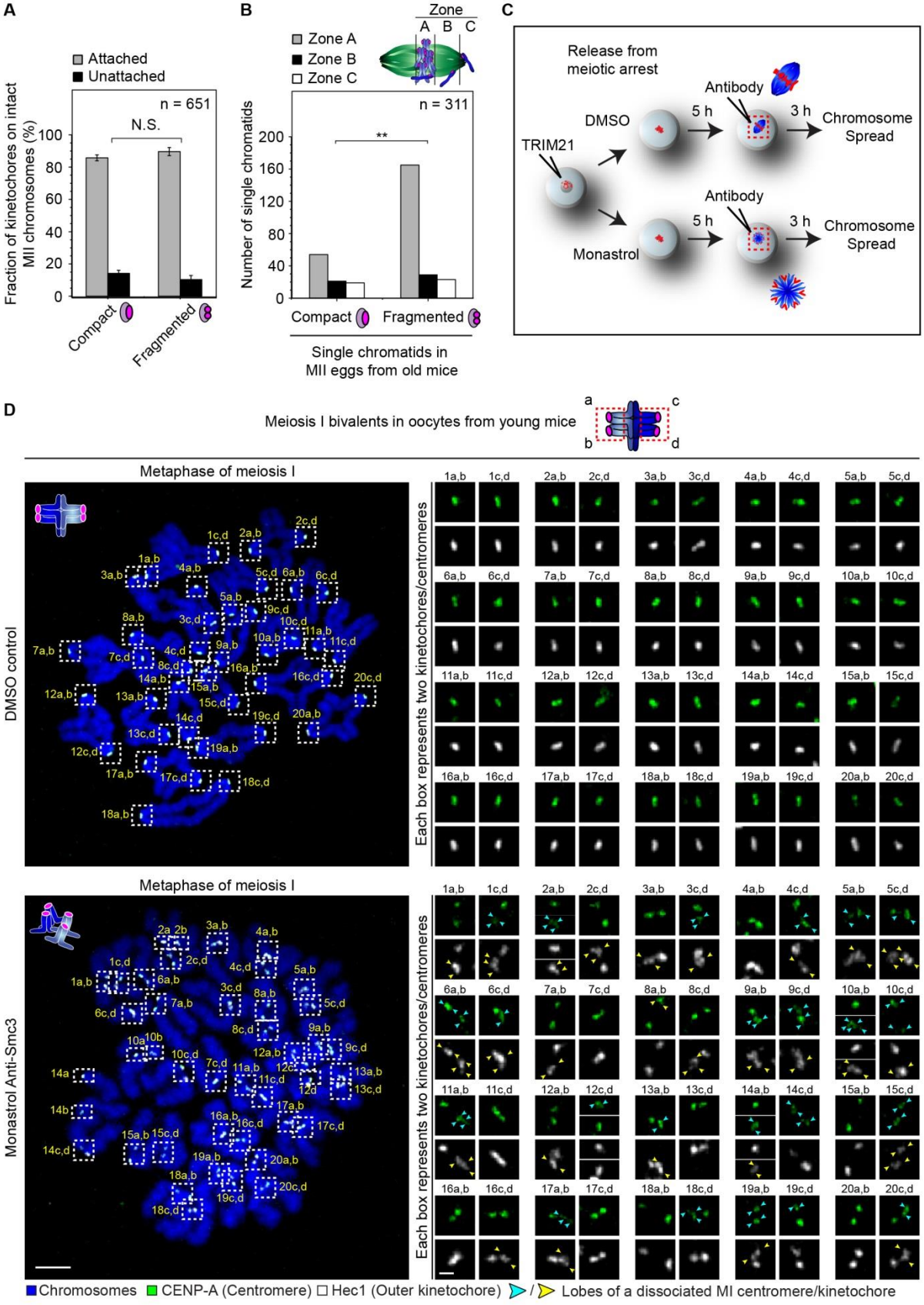

Figure S7| Microtubule pulling shapes kinetochore fragmentation, related to Figure 6

(A) Occurrence of unattached kinetochores, in relationship to their fragmentation status in MII cold-treated eggs from aged mice.

(B) Distribution of single chromatids on the metaphase-II spindle of aged mice. Zone definitions as in the scheme. The location of 311 single chromatids relative to spindle poles was evaluated.

(C) Schematic diagram of Trim-Away experiments to degrade cohesins during meiosis I in control DMSO or monastrol-treated oocytes. Monastrol prevents spindle bipolarization.

(D) Representative examples of chromosome spreads showing all 20 bivalents from a control MI oocyte (top) and a monastrol treated oocyte with cohesion weakened by partial Trim-Away with anti-Smc3 (bottom). Each inset shows a sister kinetochore pair (a, b: one pair and c, d: the other kinetochore pair of the same bivalent). Outer kinetochores are labelled in white (Hec1), centromeres are labelled in green (CENP-A) and chromosomes are labelled in blue (Hoechst). Scale bars: 5 µm in overview and 1 µm in insets. Yellow/blue arrows point to lobes within fragmented MI kinetochores/centromeres, respectively.

(A and **B)** Data from 28 aged MII eggs (3 experiments).

P values are designated as *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001. *P* values were calculated with Fisher's exact test. Error bars show SEM.