Expanded View Figures

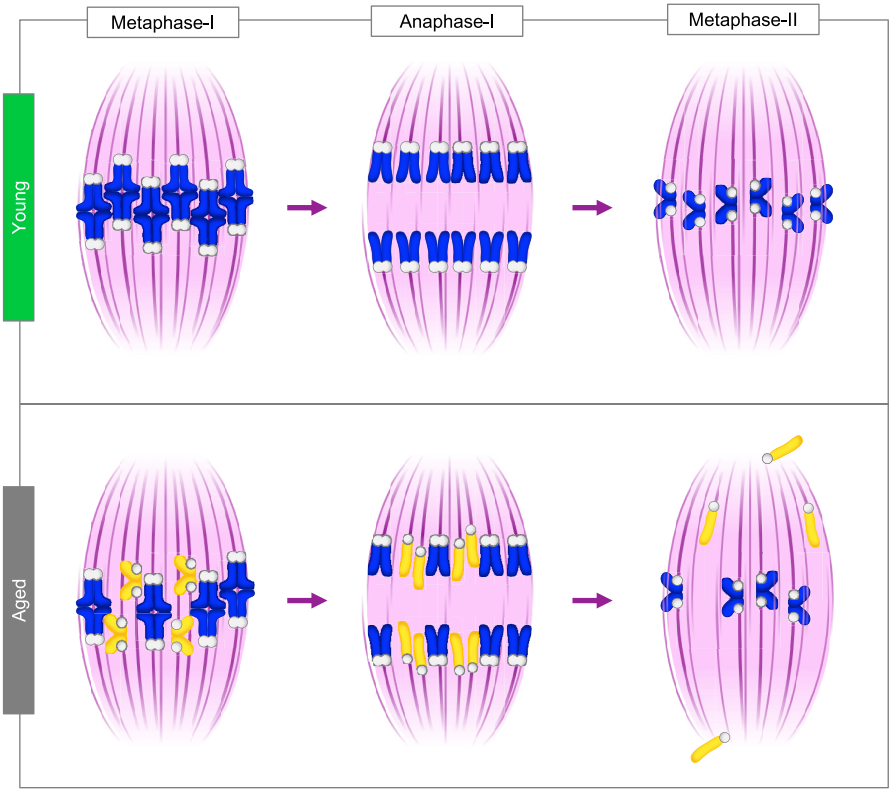

Figure EV1. Age-related cohesion loss leads to chromosome misalignment in meiosis-II.

In meiosis-I, homologous chromosomes (sister chromatid pairs) are held together by the chromosome cohesion. In young oocytes, cohesion is normally intact allowing homologous chromosomes to biorient and segregate towards the opposing spindle poles during anaphase-I. As a result, sister chromatid pairs remain intact in metaphase of meiosis-II, biorient and align. In aged oocytes, cohesion loss leads to premature individualisation of homologous chromosomes and consequent biorientation of sister chromatids (yellow) in meiosis-I. As a result, sister chromatids prematurely separate during anaphase-I and the resulting individualised chromatids randomly misalign at the metaphase-II spindle in aged oocytes.

Figure EV2. Generation of *Kif18a*^{GFP} mouse line and *Kif18a* conditional deletion in mouse oocytes.

A Schematic representation of *Kif18a* alleles. Top: Wild-type *Kif18a* allele (WT). Middle: GFP knock-in/floxed (GFP^{in}) allele with two GFP exons and an artificial intron inserted immediately after the start codon to produce N-terminally tagged fusion protein (GFP-KIF18A). Bottom: *Kif18a* knock-out (KO) allele produced following simultaneous Cre recombinase-mediated excision of GFP- and *Kif18a*-exon 2. Arrows indicate the position of genotyping primers.

B GFP-KIF18A colocalizes with the midzone protein PRC1 in *Kif18a*$^{wt/GFP}$ Met-II eggs (no Cre-recombinase). Chromosomes are labelled with Hoechst33342 (blue), kinetochores with CREST (grey), midzone with PRC1 (magenta) and GFP-KIF18A with chicken-GFP (green) antibodies. '*n*' is the total number of Met-II eggs per group. Scale bars, 10 μm.

C Breeding strategy to generate female littermates with 4 denoted genotypes.

D Graph shows no notable difference in fertility between *Zp3-Cre*$^{Tg/O}$; *Kif18a*$^{GFP/GFP}$ females (KIF18A-cKO oocytes) and controls (*Zp3-Cre*$^{Tg/O}$; *Kif18a*$^{wt/wt}$ [WT] and *Zp3-Cre*$^{Tg/O}$; *Kif18a*$^{wt/GFP}$ [HET]) after 5 generations of pups (one-way ANOVA with multiple comparisons), consistent with our observation that a majority of KIF18A-cKO eggs correctly align their chromosomes.

E PCR genotyping confirms successful conditional *Kif18a* deletion (cKO) in GV-stage oocytes (10 oocytes per group).

F GFP^{in} but not KIF18A-cKO oocytes show significantly increased GFP-KIF18A levels at spindle vs. cytoplasm (paired *t*-test, $P < 0.0001$ and $P = 0.31$, respectively). DNA is labelled with Hoechst33342 (blue) and GFP-KIF18A, kinetochores and microtubules (MTs) are detected with chicken-GFP (green), HEC1 (grey) and β-Tubulin (magenta) antibodies, respectively. Scale bars, 10 μm. Error bars represent SEM. Data from totals of 49 and 44 oocytes across 3 experimental days, for GFP^{in} and KIF18A-cKO groups, respectively.

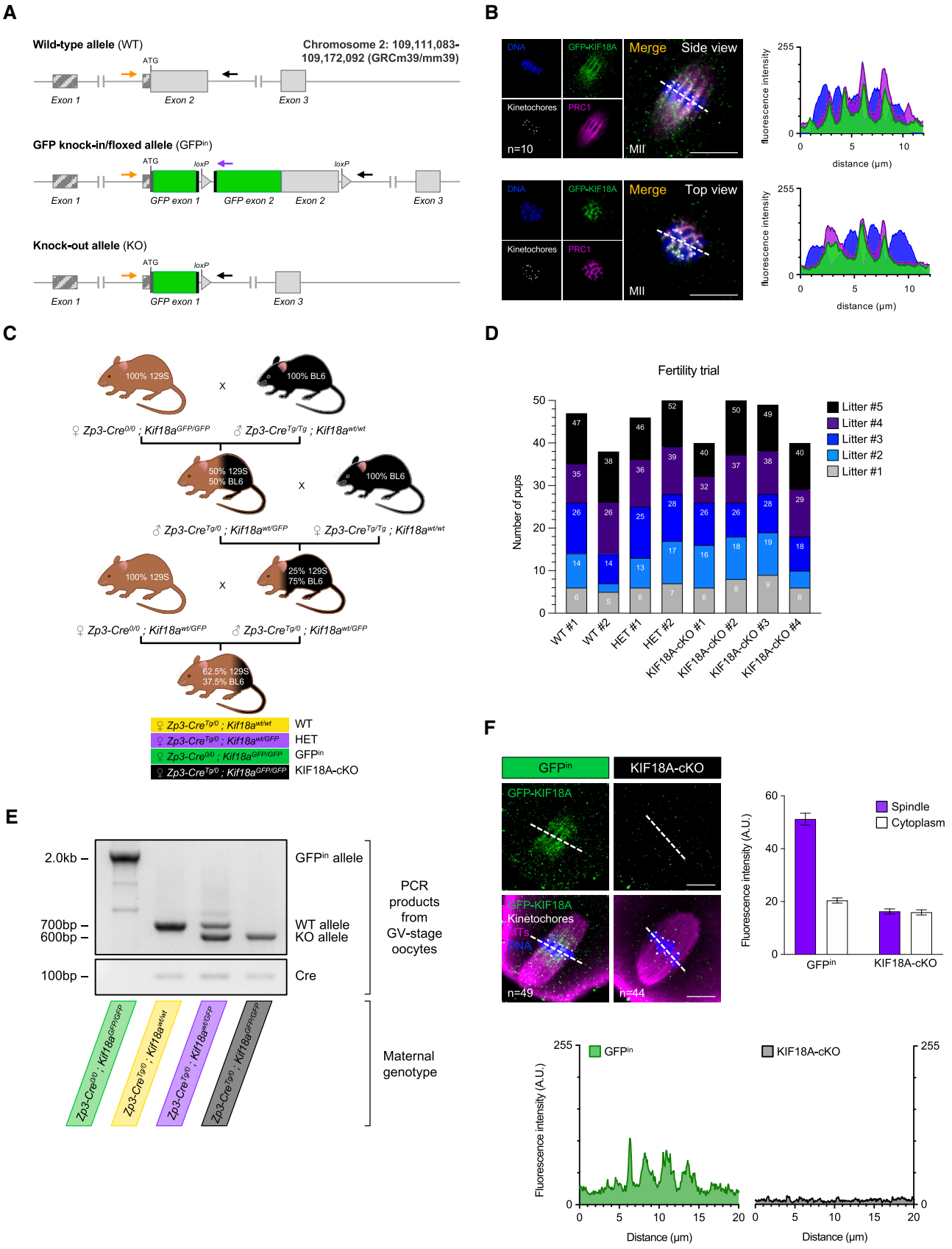

Figure EV2.

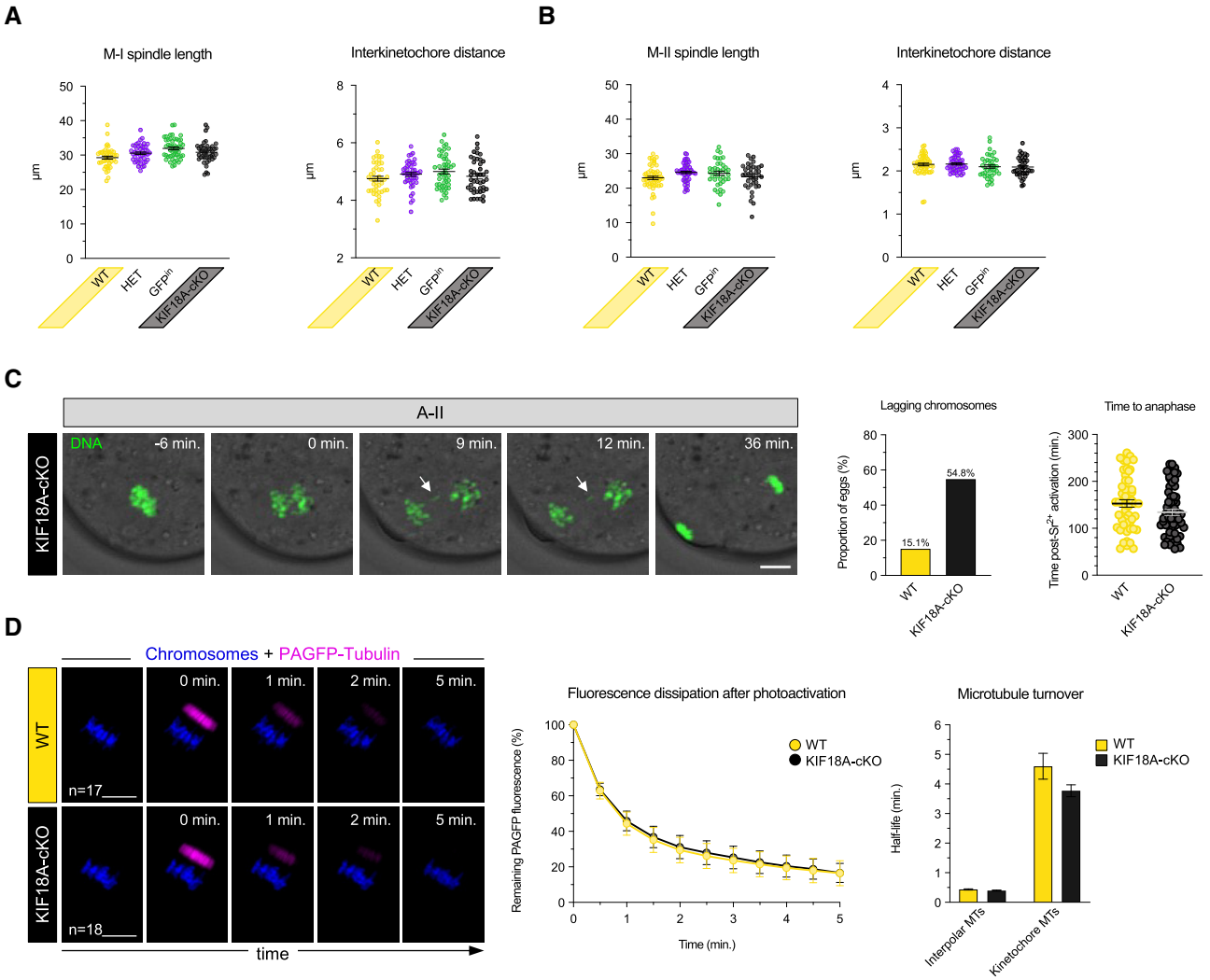

Figure EV3. KIF18A removal has no impact on spindle length and interkinetochore distance in female meiosis, but causes lagging chromosomes in M-II.

A, B Charts show no impact of KIF18a removal on spindle length and interkinetochore distance in late M-I (A) and M-II (B) (one-way ANOVA with multiple comparisons). Error bars represent SEM. For late M-I, data from totals of 45, 46, 49 and 44 oocytes across 3 experimental days, for WT, HET, GFP^{in} and KIF18A-cKO groups, respectively. For M-II, data from totals of 55, 53, 39 and 39 oocytes across 3 experimental days, for WT, HET, GFP^{in} and KIF18A-cKO groups, respectively.

C Time-lapse confocal images show anaphase with lagging chromosome (white arrow) in KIF18A-cKO Met-II egg. Charts show significant increase in lagging chromosomes formation (χ^2-test, $P < 0.00001$) and no difference in anaphase onset timing (unpaired t-test, $P = 0.07$) in KIF18A-cKO ($n = 62$) vs. WT ($n = 53$) Met-II eggs. DNA is visualised with SiR-DNA (green) and indicated time (min) is relative to the anaphase onset. Error bars represent SEM.

D Time-lapse confocal images show dissipation of PAGFP-Tubulin fluorescent signal (magenta) in WT and KIF18A-cKO Met-II eggs. Chromosomes are visualised with H2B-RFP (blue). The '0 min' marks the first frame after photoactivation. The fluorescence dissipation after photoactivation curve shows the average decay rate of PAGFP-Tubulin in WT and KIF18A-cKO Met-II eggs (as mean ± SEM for each time-point). Chart shows no significant difference in the stable/non-stable MT turnover rates between the groups (unpaired t-test, $P = 0.09$ for stable and $P = 0.08$ for non-stable MTs). 'n' is the total number of Met-II eggs per group.

Data information: In (C and D), scale bars, 10 µm.

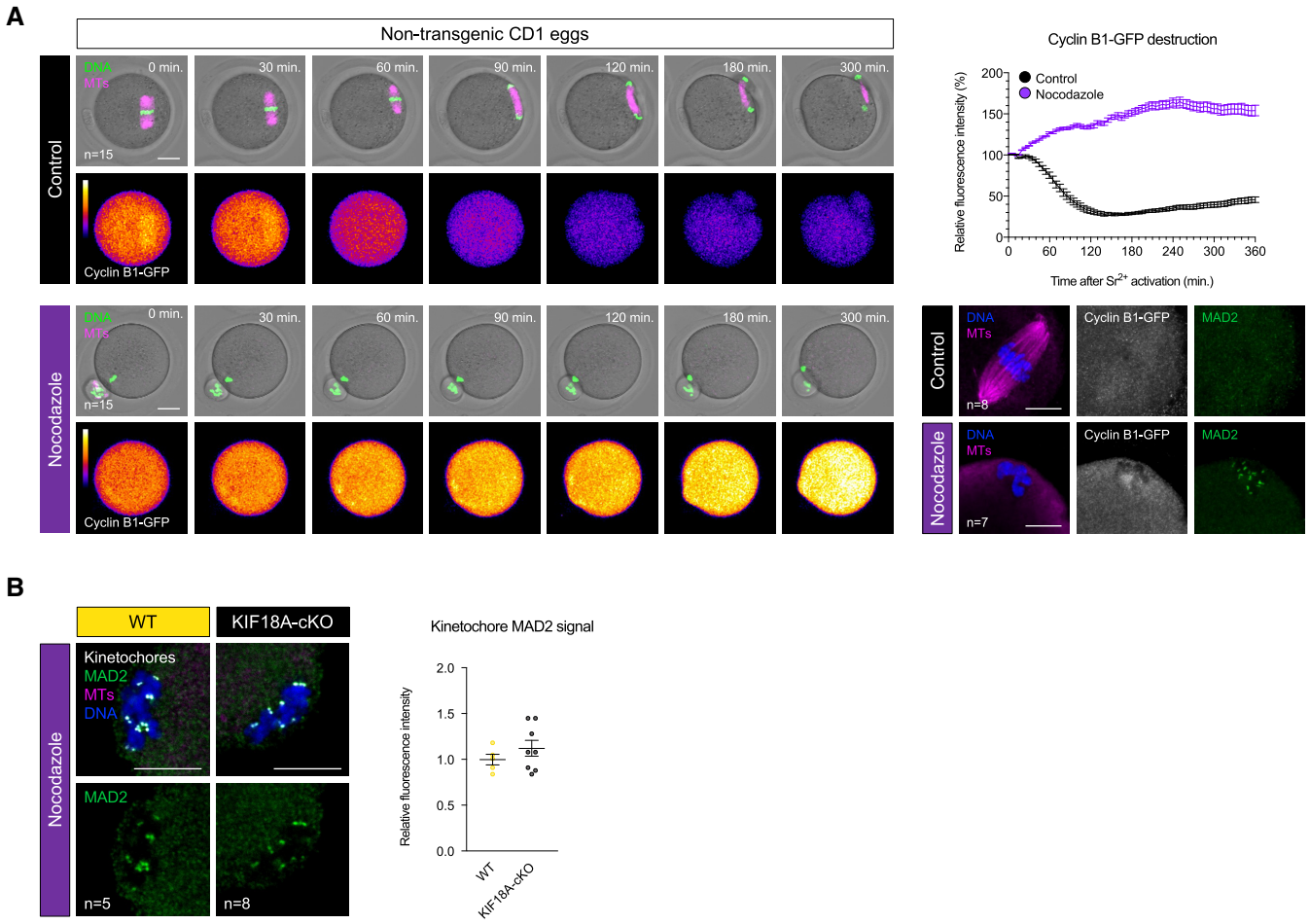

Figure EV4. Complete spindle disruption activates SAC in Met-II eggs.

A Time-lapse confocal images (left) show nocodazole-treated and control non-transgenic Met-II eggs following Sr^{2+} activation. Note the lack of Cyclin B1-GFP destruction and chromosome segregation in nocodazole group. Indicated time (min) is relative to Sr^{2+} addition. DNA is visualised with SiR-DNA (green) and spindle with SPY555-Tubulin (magenta). Scale bars, 20 μm. Graph (top right) shows changes in Cyclin B1-GFP levels over time. Confocal images (bottom right) show kinetochore-MAD2 recruitment following nocodazole addition. Chromosomes are labelled with Hoechst33342 (blue), and Cyclin B1-GFP (grey) and microtubules (magenta) with chicken-GFP and β-Tubulin antibodies, respectively. Scale bars, 10 μm.

B MAD2 immunofluorescence (green) in nocodazole-treated WT and KIF18A-cKO Met-II eggs. Chart shows no difference in kinetochore-MAD2 levels between the groups (unpaired *t*-test, $P = 0.33$). DNA is labelled with Hoechst33342 (blue), and kinetochores (grey) and microtubules (magenta) with CREST and β-Tubulin antibodies, respectively. Scale bars, 10 μm.

Data information: In (A and B), '*n*' is the total number of Met-II eggs per group. Error bars represent SEM.