

Expanded View Figures

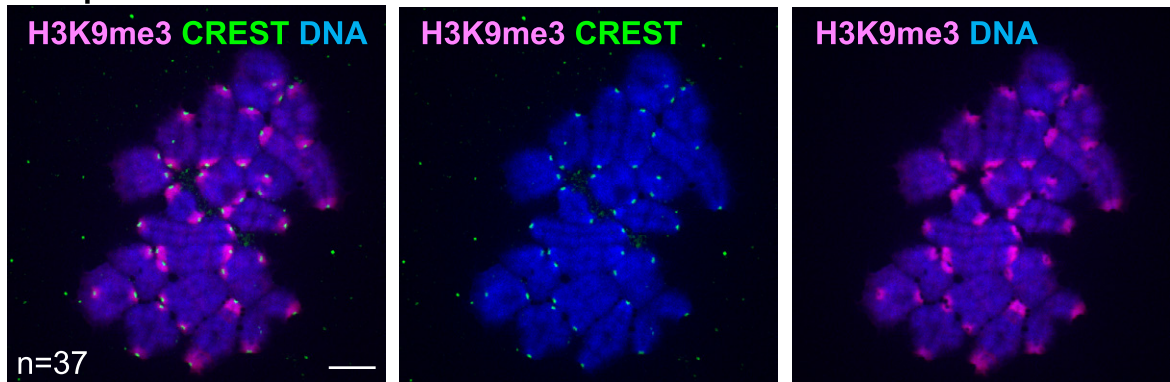
Figure EV1. Pericentromeric heterochromatin staining of mouse oocyte chromosomes in meiosis I and meiosis II (related to Fig 1C).

- A Metaphase I (6 h after GVBD) and metaphase II chromosome spreads were stained with H3K9me3 antibody and CREST serum to reveal pericentromeric heterochromatin (pink) and the centromere (green), respectively. Chromosomes were stained with Hoechst (blue).
- B Schematic representation of the centromere, pericentromeric heterochromatin and chromatid junction in a metaphase II dyad, for quantifications.
- C Metaphase II spreads were stained with either CenPA antibody (green), or CREST serum (green), DNA was visualized with Propidium Iodide (red).

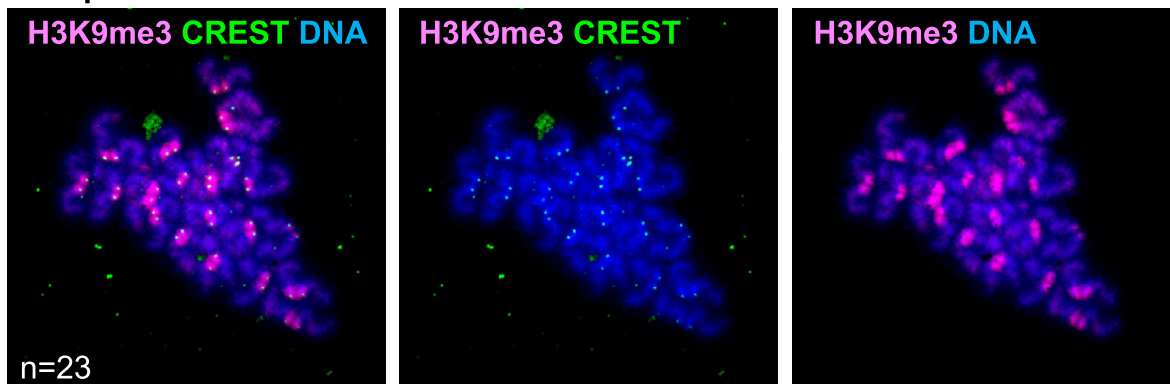
Data information: *n* indicates the number of analyzed oocytes. Scale bars are 10 μm in (A) and 5 μm in (B).

A

Metaphase I

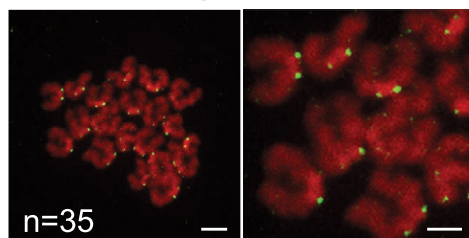


Metaphase II

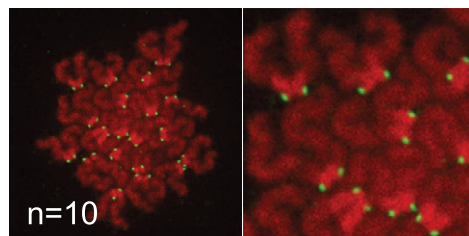


B

CenpA-DNA



CREST-DNA



Metaphase II

Figure EV1.

Figure EV2. Visualization and quantification of centromeric Sgo2 and Sgo2 at the chromatid junction in meiosis II (related to Fig 2).

- A 3D-rendering with Arivis Vision 4-D software of representative stainings of chromosome spreads in Fig 2B. (Chromosomes appear in blue, Sgo2 in red, and CREST in green). In the lower panels Sgo2 staining at centromeres and the chromatid junction are indicated.
- B A representative wild-type control chromosome spread of a dyad in metaphase II from Fig 2B is shown to indicate how the regions for quantifications were defined.
- C, D The graphs show the corresponding quantifications of the centromere signal (blue: overlapping with CREST) and the signal at the chromatid junction (red: not overlapping with CREST), for Fig 2B, per oocyte (C) and per kinetochore pair (D).
- E, F Quantifications from Fig 2G, distinguishing centromere and chromatid junction pools of Sgo2, per oocyte (E), and per kinetochore pair (F).

Data information: In panels (C and D), Sgo2/CREST intensities at the centromere versus the chromatid junction in *Bub1KD*, Reversine-treated and *Mps1ΔN* oocytes were normalized to the mean of Sgo2/CREST intensities in wild-type control oocytes. On each graph, mean is indicated, error bars are \pm SD, asterisks indicate significant difference according to Mann–Whitney *U*-test (* $P < 0.1$; ** $P < 0.01$; **** $P < 0.0001$). *n* indicates the number of kinetochore pairs and number of oocytes analyzed.

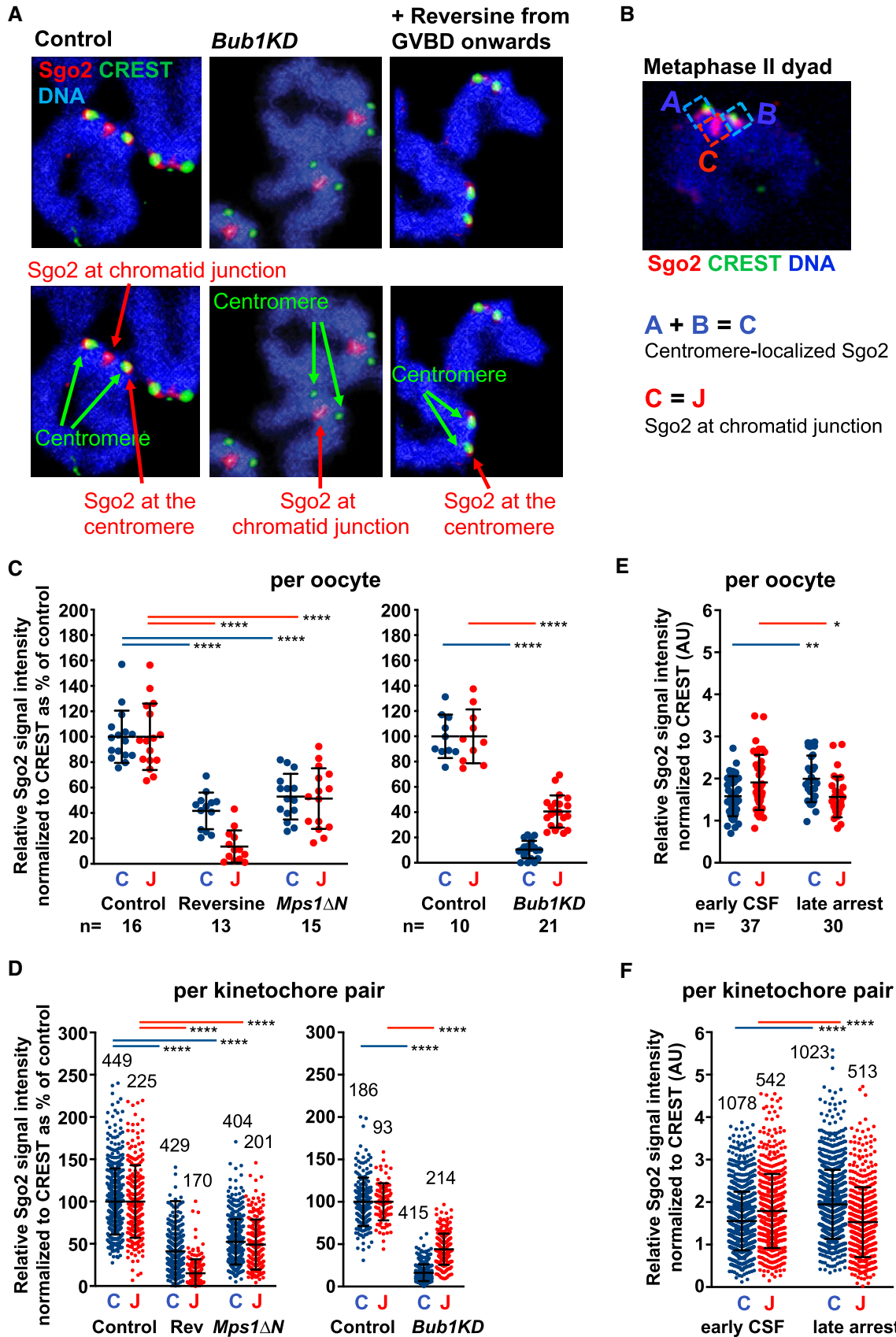


Figure EV2.

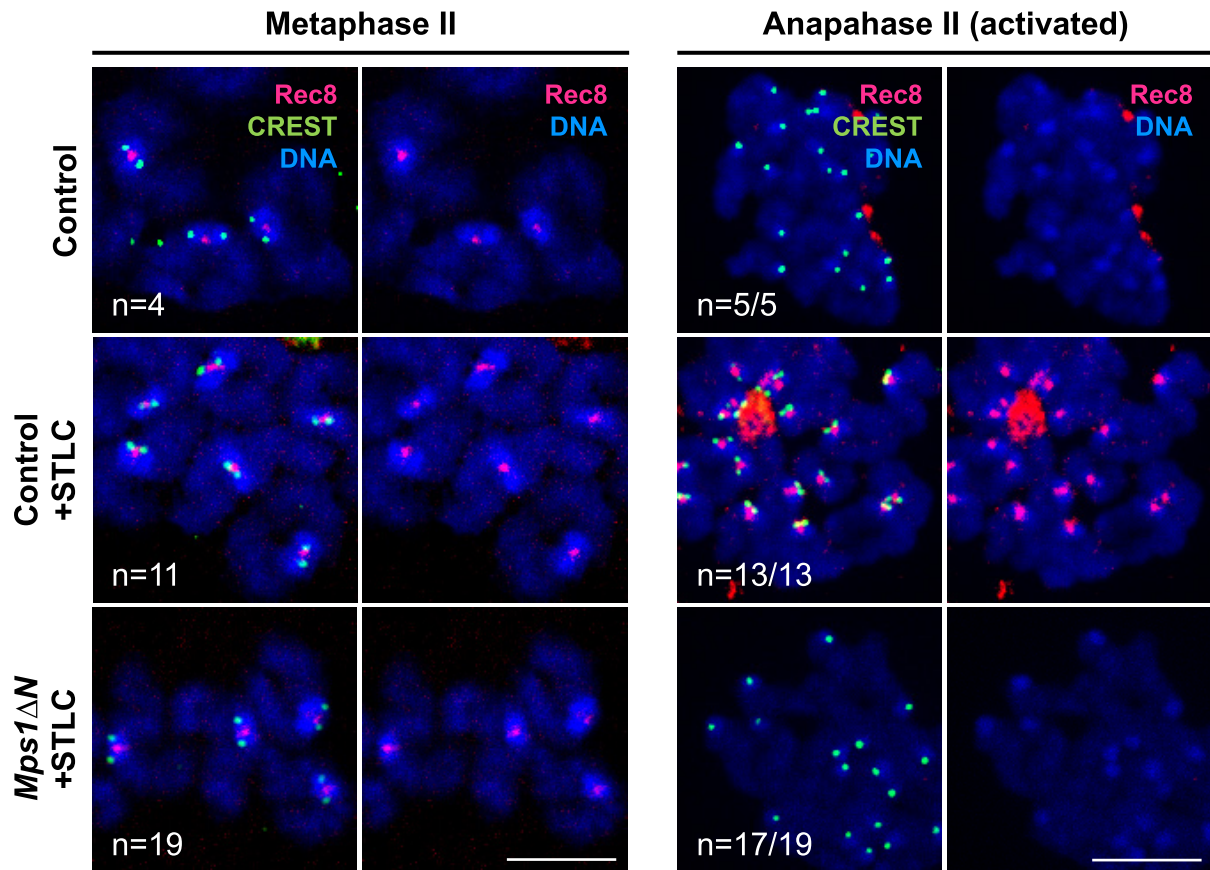


Figure EV3. Inactivation of Mps1 kinase activity is not required for sister chromatid segregation on monopolar spindles (related to Fig 3).

Chromosome spreads obtained from control or *Mps1 Δ N* oocytes *in vitro* matured oocytes, in metaphase II and in anaphase II, in the presence or absence of prior STLC treatment (for 2.5 h), as indicated. Spreads were stained with Rec8 antibody (pink), CREST (green) and Hoechst (blue).

Data information: In all panels, *n* indicates the number of oocytes with the shown phenotype and the total number of oocytes analyzed. Scale bar, 10 μ m.

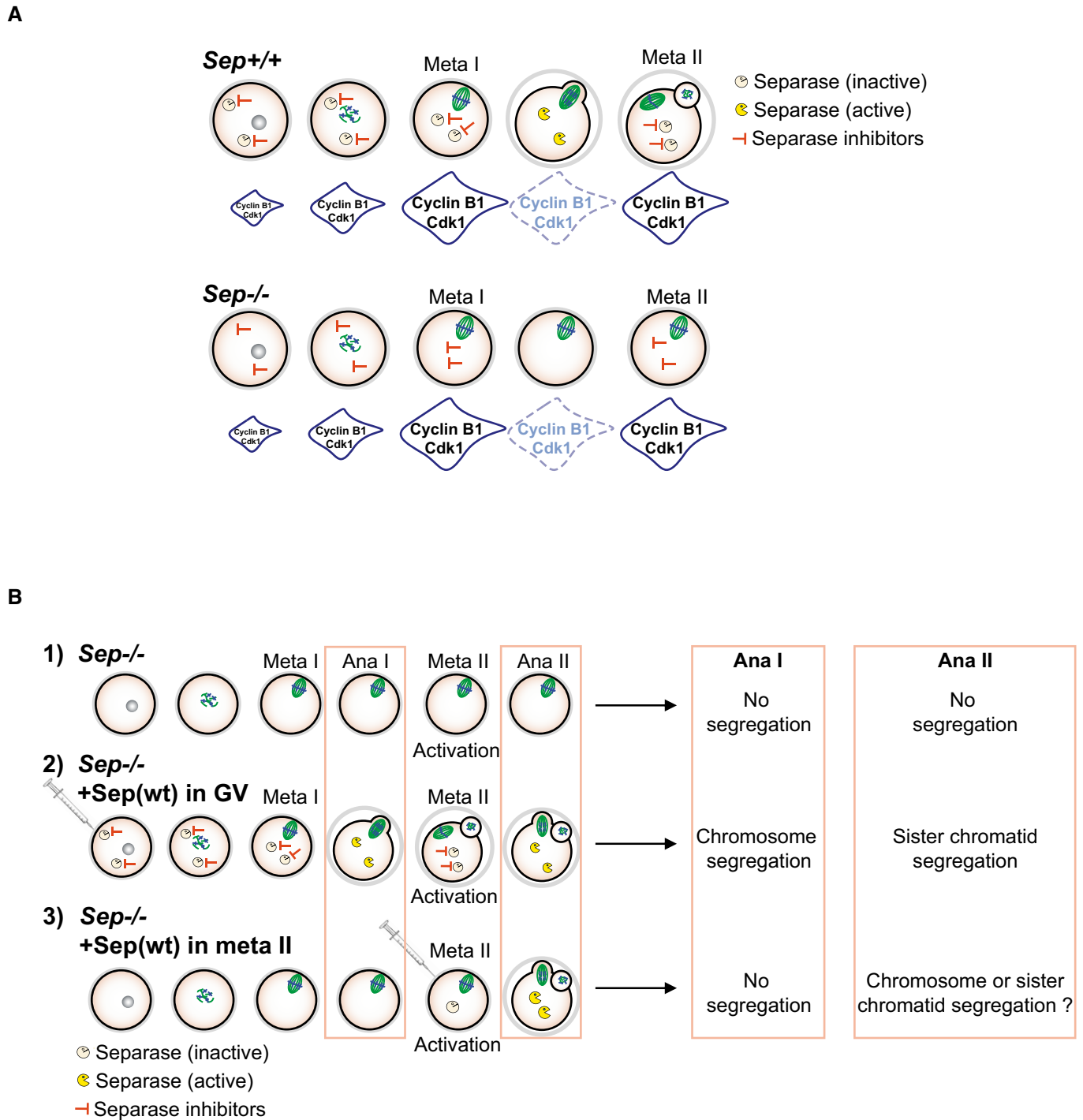


Figure EV4. Schemes of cell cycle progression in oocytes devoid of separase, and rescue experiment performed in this study (related to Fig 4 and 5).

A Scheme summarizing published data on cell cycle progression in oocytes devoid of separase. Cdk1 activity accumulates, disappears, and re-accumulates on time. The APC/C substrate cyclin B1 is degraded on time in the absence of separase, and re-accumulates as oocytes progress into meiosis II, even though no polar body extrusion is observed, due to a role of separase in polar body extrusion, which is independent of its cleavage activity. The SAC is not activated, because chromosomes are correctly attached without separase. See text for references.

B Rescue experiments performed in this study: 1) *Sep*^{-/-} oocytes without rescue. 2) *Sep*^{-/-} oocytes rescued from GV onwards (Fig 4). 3) *Sep*^{-/-} oocytes rescued from metaphase II onwards. The question we asked was whether chromosomes or sister chromatids are separated when separase is absent in meiosis I, and present in meiosis II.

Figure EV5. GFP-Sgo2 overexpressing oocyte progressing through meiosis I into metaphase II (related to Fig 6).

Montage of live imaging movie of control and GFP-Sgo2 expressing oocytes (from GV onwards) undergoing meiosis I. Chromosome movements were followed by staining DNA with SirDNA (far red, shown in red), GFP-Sgo2 appears in green. Shown are the DNA, GFP, and DIC channels, each frame comprised of an overlay of 11 z-sections of 3 μm for GFP and far-red channels, and 1 z-section in DIC. Timepoints were taken every 10 min, shown are selected timepoints such as indicated. For the control, the GFP channel has been omitted, because it is devoid of any signal. For GFP-Sgo2 expressing oocytes, two different settings for brightness are shown, to visualize either meiosis I or meiosis II in an optimal manner, as the signal decreases after anaphase in meiosis I.

Data information: *n* indicates the number of oocytes analyzed. Scale bars, 50 μm .

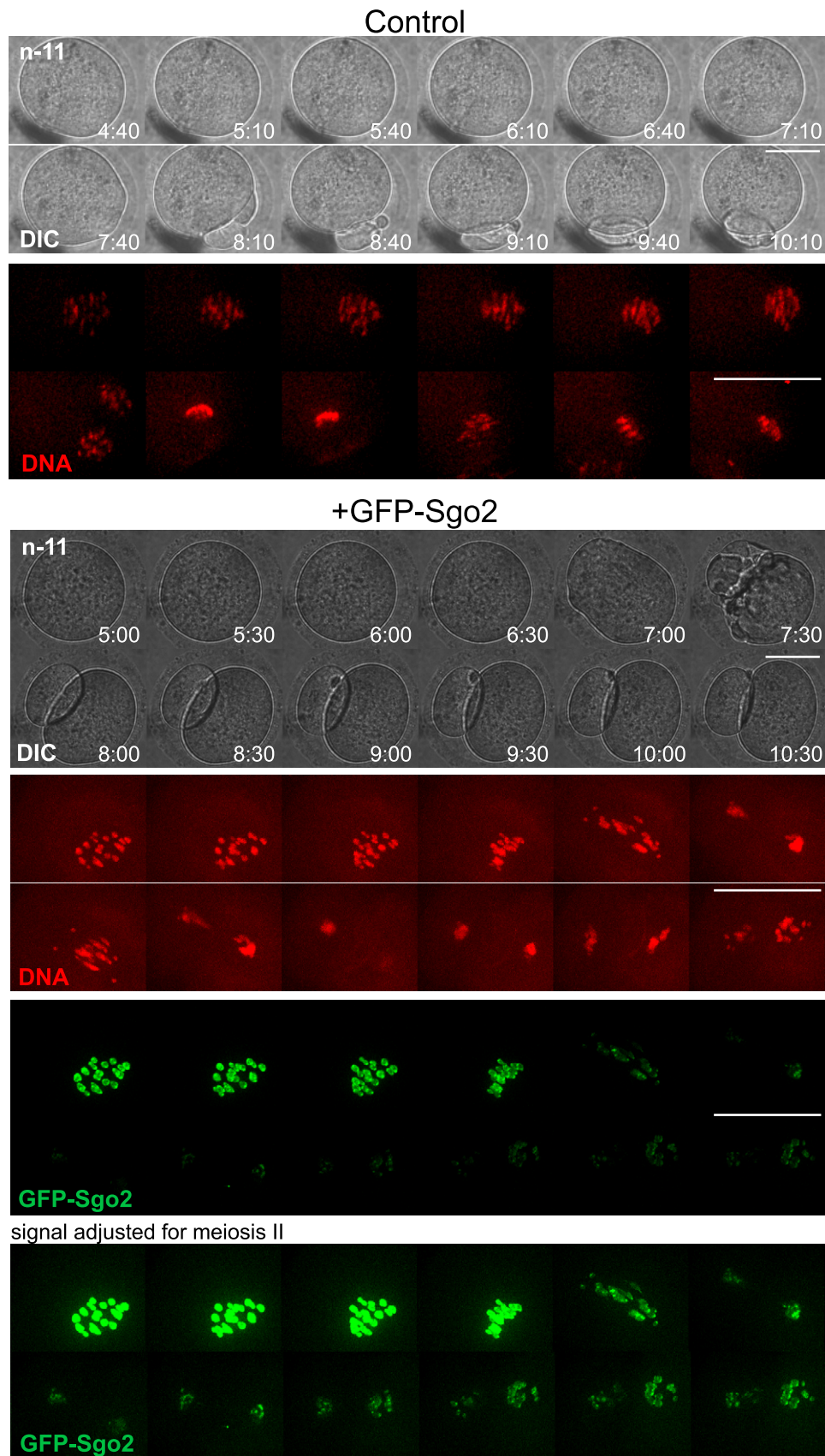


Figure EV5.