Expanded View Figures

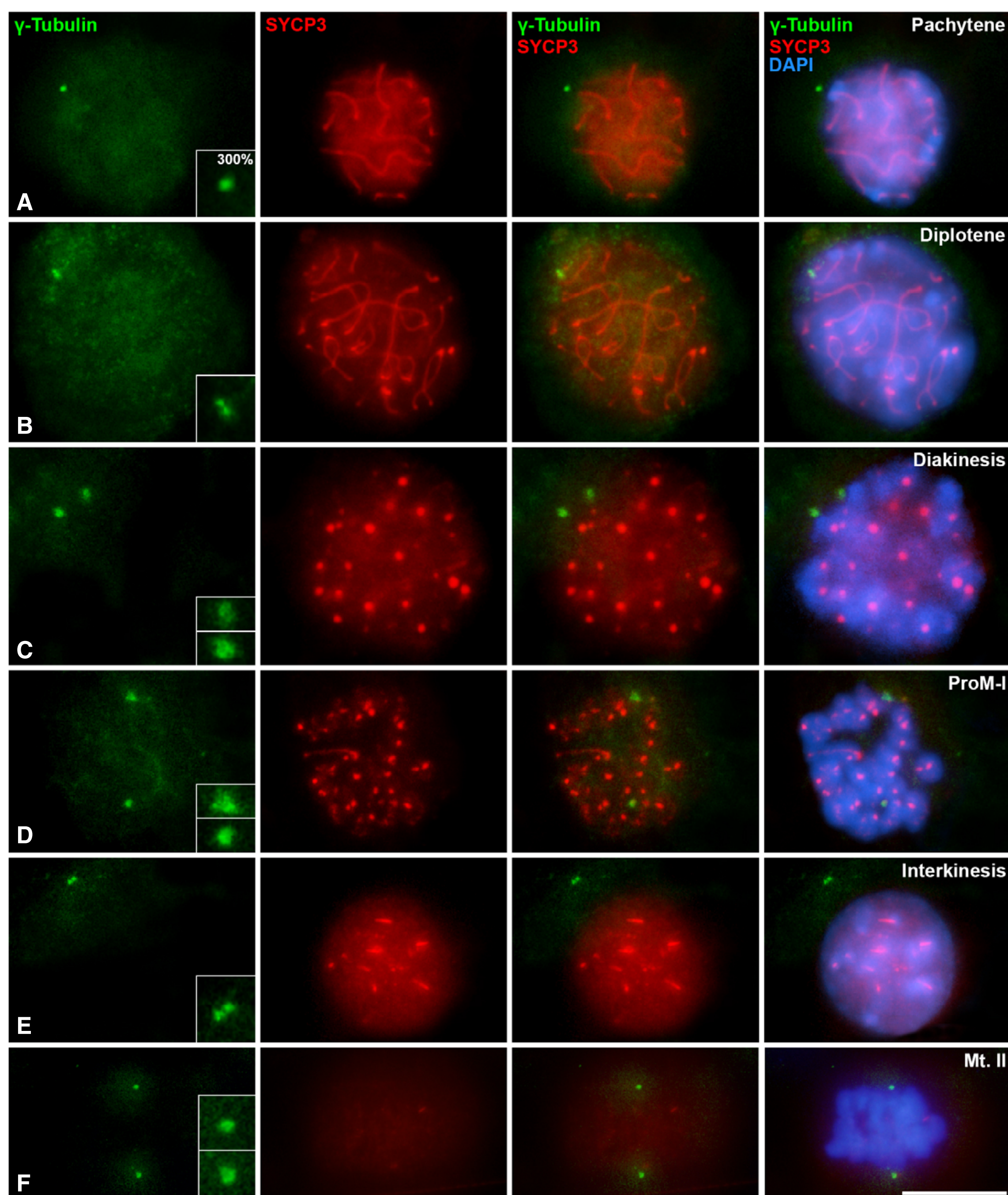

Figure EV1. Distribution of γ-Tubulin and SYCP3 during male mouse meiosis.

A–F Double immunolabelling of γ-Tubulin (green) and SYCP3 (red) on squashed control mouse spermatocytes at (A) pachytene, (B) diplotene, (C) diakinesis, (D) prometaphase I, (E) interkinesis and (F) metaphase II. Chromatin has been stained using DAPI (blue). Insets correspond to centrosomes at 200% magnification. White arrows indicate the location of centrosomes. Scale bar in F represents 10 μm.

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Figure EV2. Distribution of PLK1S137P and PLK1T210P.

A, B Double immunolabelling of PLK1T210P (green) and SYCP3 (red) on squashed spermatocytes at (A) metaphase I and (B) metaphase II. Chromatin has been stained using DAPI (blue).

C, D Double immunolabelling of PLK1T210P (green) and SYCP3 (red) on spread spermatocytes at (C) metaphase I and (D) metaphase II. Chromatin has been stained using DAPI (blue).

E–J Double immunolabelling of PLK1S137P (green) and SYCP3 (red) on spread spermatocytes at (E) metaphase I, (F) anaphase II, (G) interkinesis, (H) prophase II, (I) metaphase II and (J) telophase II. Chromatin has been stained using DAPI (blue). Insets correspond to centrosomes at 200% magnification.

Data information: White arrows indicate the location of centrosomes. Scale bar in D and J represents 10 μm.

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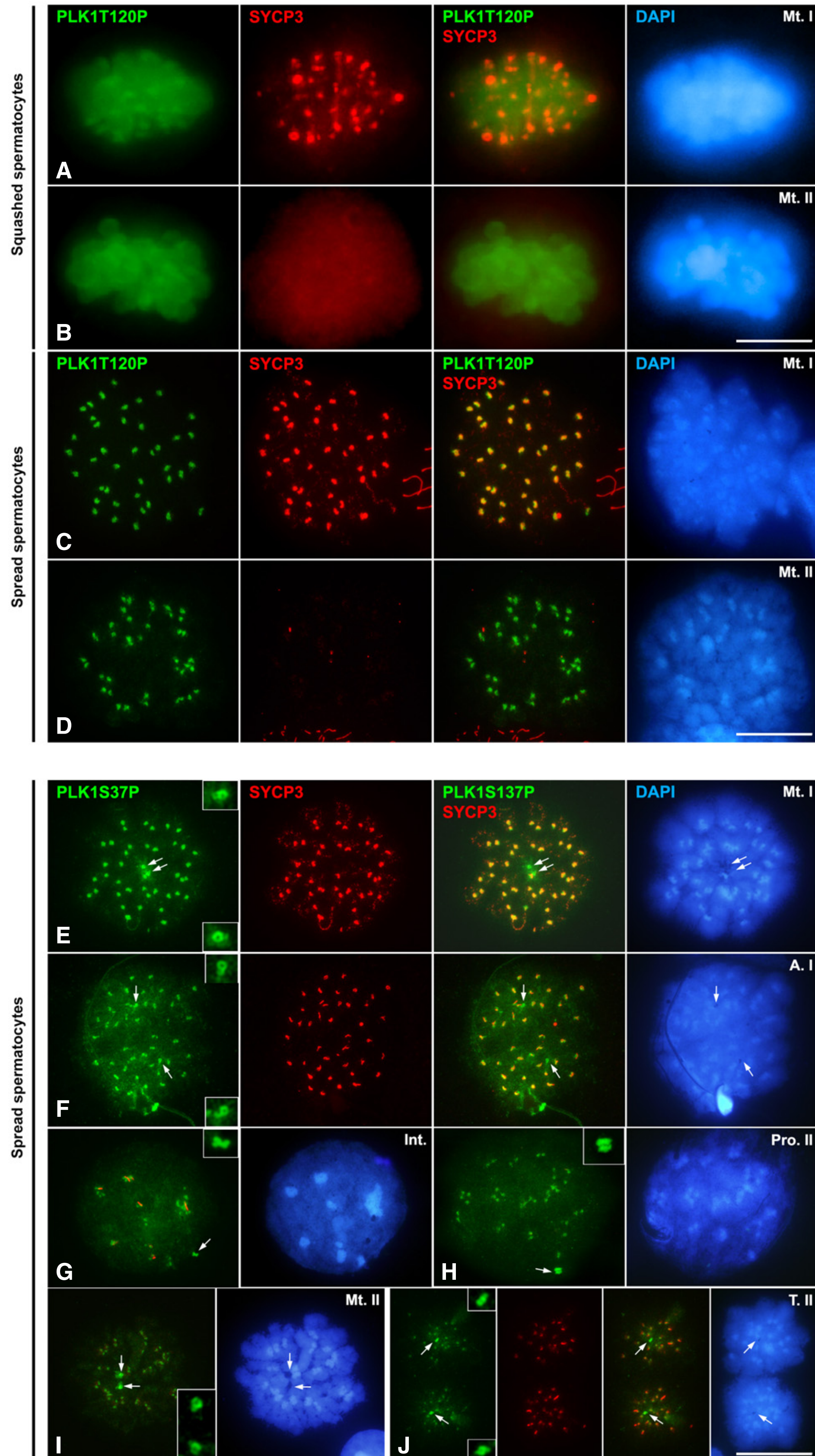

Figure EV2.

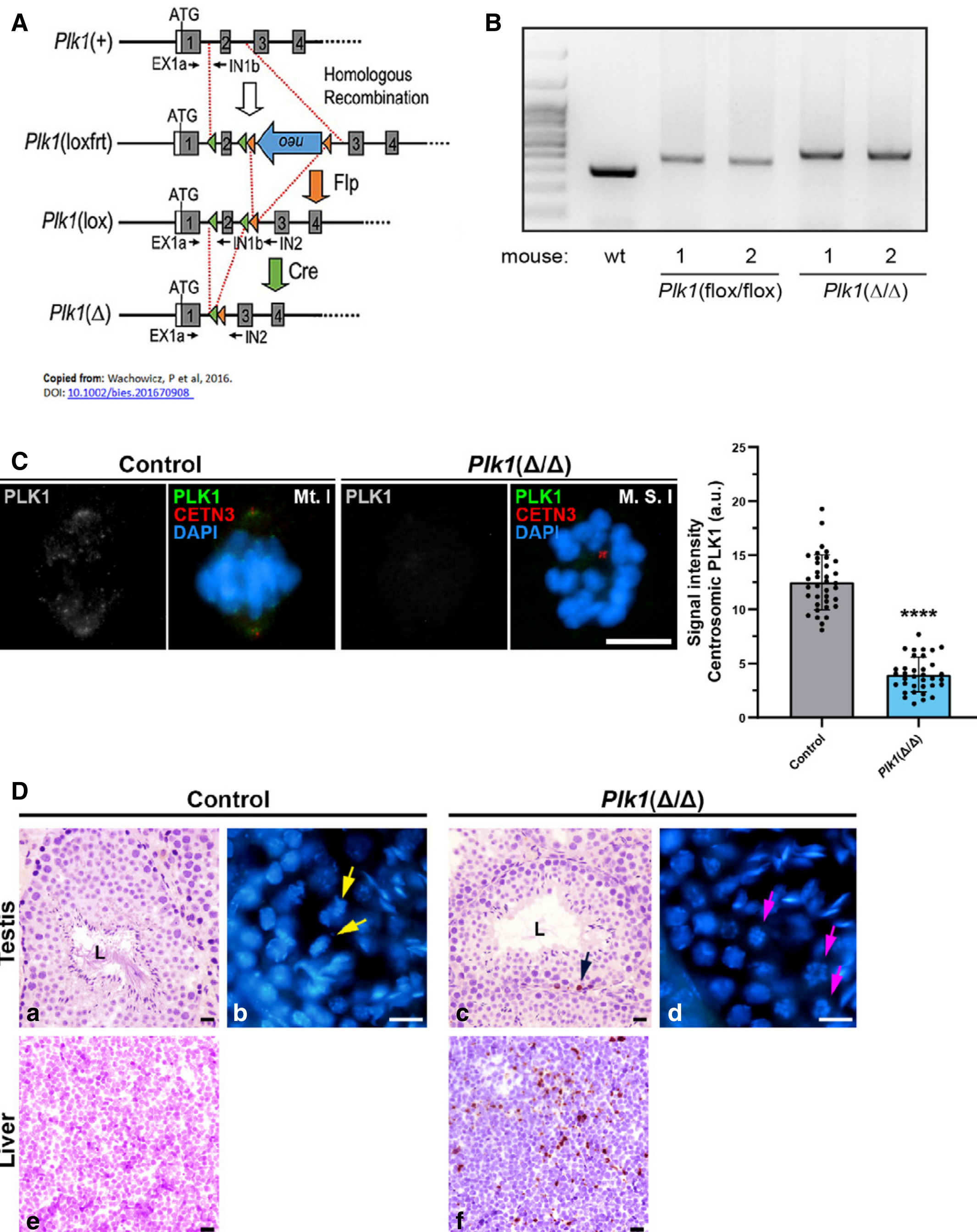

Figure EV3. Gene strategy and PCR analysis for *Plk1*(Δ/Δ) mouse model.

A Schematic representation of the conditional alleles (*loxfrt* or *lox*) and the null allele (–) obtained upon Cre-mediated recombination for *Plk1*, extracted from Wachowicz *et al* 2016.

B Example of genomic genotyping PCRs for *Plk1* alleles (lox and Δ alleles) obtained from testis of the indicated mice.

C Immunolabelling of PLK1 (grey/green), CETN3 (red) and chromatin counterstained using DAPI (blue) in metaphases I from control -*Plk1*(+/+)- and *Plk1*(Δ/Δ) mice. Scale bar represents 10 μm. Experiments were conducted for two biological replicates. Number of cells analysed: control (n = 36), *Plk1*(Δ/Δ) (n = 35). Data are mean ± SD; ****$P < 0.0001$, Student's t-test.

D Histological section of seminiferous tubules stained with H/E staining in control (a) and *Plk1*(Δ/Δ) (c). ["L" indicates the lumen of the seminiferous tubule; black arrow indicates the position of an apoptotic cell]. Histological section of liver stained with H/E staining in control (e) and Plk1(Δ/Δ) (f). Cryosection of testis and counterstaining with DAPI in control (b) and *Plk1*(Δ/Δ) (d). Yellow arrows indicate the presence of aligned metaphases (a). Pink arrows indicate the presence of apparently monopolar metaphases (d). Scale bars represent 20 μm.

Source data are available online for this figure.

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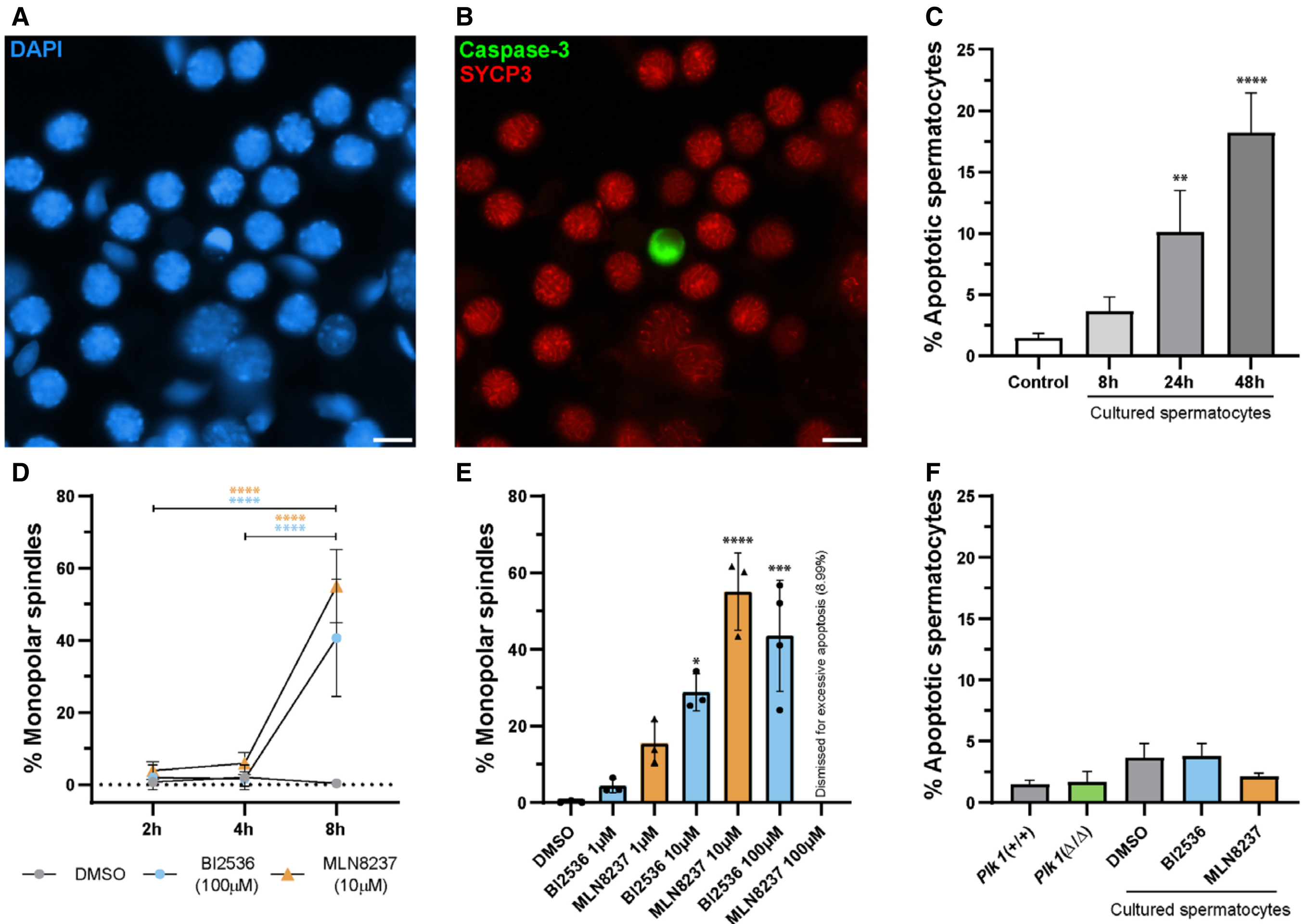

Figure EV4. Optimisation of *in vitro* studies.

A, B Organotypic cultures of seminiferous tubules. (A) X40 microscope field stained with DAPI (blue) and (B) caspase-3 (green) and SYCP3 (red) in control culture of seminiferous tubules in DMSO. Scale bars represent 10 µm.

C Graphic representation of the percentage of apoptotic cells in cultured seminiferous tubule. Caspase-3-positive labelling for the comparison between control (WT freshly squashed spermatocytes) and spermatocytes from different time organotypic control cultures of seminiferous tubules. Three biological replicates were conducted for each condition. Data are mean ± SD; $^{**}P < 0.01$, $^{****}P < 0.0001$, Student's *t*-test.

D Graphic representation of the percentage of monopolar spindles at different timings. Data are presented for 2 h, 4 h and 8 h for control cultures spermatocytes, 100 µM of BI2536 and 10 µM of MLN8237. Three biological replicates were conducted for each condition, except MLN837 (2 h and 4 h) that were conducted in two replicates. Data are mean ± SD; $^{****}P < 0.0001$, Student's *t*-test.

E Graphic representation of the percentage of monopolar spindles in the different conditions in relation to concentration. Data are presented for 8 h for the different concentration trials for BI2536 and MLN8237 treatments used for the optimisation of the methodology. At least two biological replicates were conducted per each condition. Data are mean ± SD; $^{***}P < 0.0001$, $^{****}P < 0.0001$, one-way ANOVA with Turkey's multiple comparisons test.

F Graphic representation of the percentage of apoptotic spermatocytes in the different conditions of this study. Data are presented for 100 µM of BI2536 and 10 µM of MLN8237 for 8-h treatment. Three biological replicates were conducted per each condition. Data are mean ± SD.

Source data are available online for this figure.

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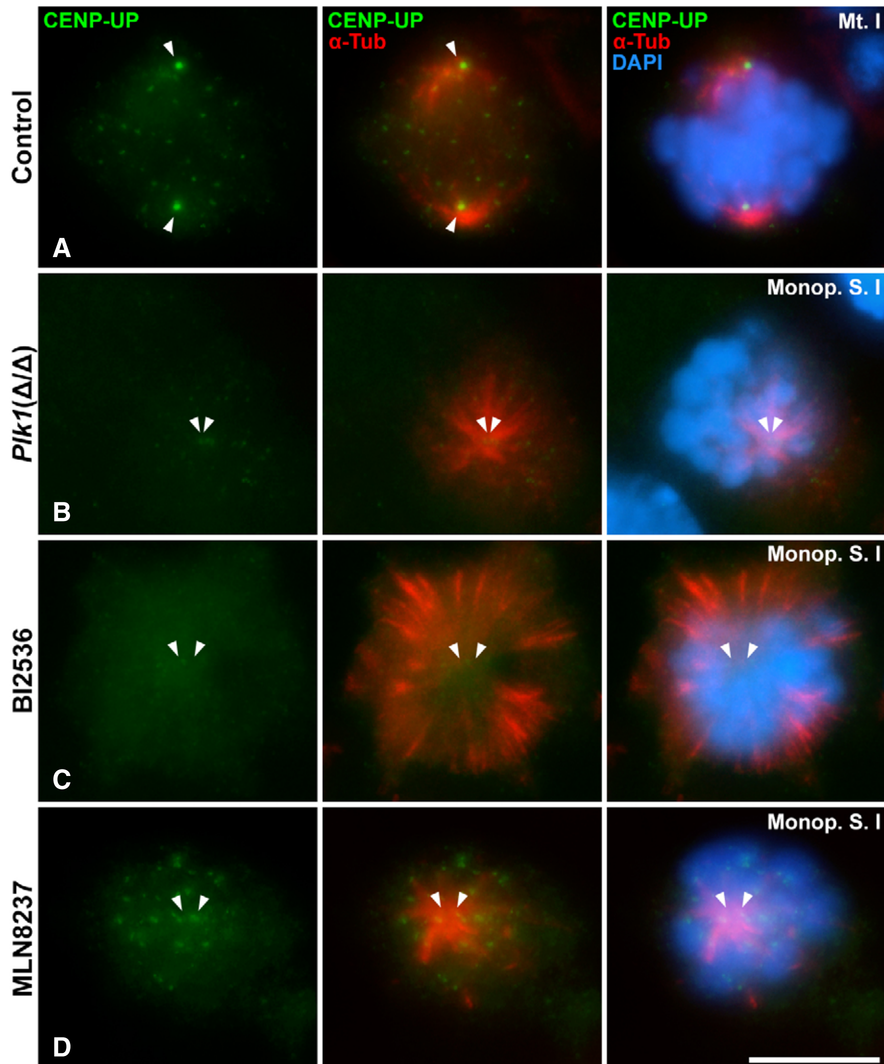

Figure EV5. Distribution of CENP-UP in control metaphases I and monopolar spindles of *Plk1* (Δ/Δ), BI2536-treated and MLN8237-treated spermatocytes.

A–D Double immunolabelling of CENP-UP (green) and α-Tubulin (red) on squashed mouse spermatocytes at (A) bipolar control metaphase I, (B) *Plk1*(Δ/Δ) monopolar spindle I, (C) 100 μM 8-h BI2536-treated monopolar spindle I and (D) 10 μM 8-h MLN8237-treated monopolar spindle I. Chromatin has been stained with DAPI (blue). White arrows indicate the location of the poles. Scale bar in D represents 10 μm.

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