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latrunc B

D

colcemid cytochal D latrunc B control

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bam > RedX Green2/3







S8 Fig. Inhibitors of F actin and microtubules do not interfere with chromosome territory formation in controls and with Green2/3 dot stretching in condensin II protein mutants

(A, B) Time lapse imaging of progression through M I confirms inhibitor effectivity. Spermatocytes expressing Cenp-A/Cid-EGFP and His2Av-mRFP were analyzed. Time (min:sec) is given relative to the onset of NEBD I. (A) The F actin inhibitor latrunculin B (latrunc B) results in a complete block of cytokinesis. Green autofluorescent signals primarily from mitochondria, which form a cylinder around the M I spindle, disclose contractile ring activity (yellow arrowheads) in mock treated (DMSO) control spermatocytes during exit from M I, but not in latrunculin B treated spermatocytes (yellow lines). Chromosome segregation, as indicated by the His2Av-mRFP and Cenp-A/Cid-EGFP signals is not affected by latrunculin B. A specific inhibition of cytokinesis was also observed in the presence of cytochalasin D. (B) Time lapse imaging of progression into M I in the presence of the microtubule inhibitor colcemid reveals a complete block of spindle formation. Untreated (control) and treated spermatocytes expressing GFP- β Tub56D were analyzed.

(C) Inhibitors of F actin and MT dynamics do not preclude chromosome territory formation in *Drosophila* spermatocytes. Spermatocytes expressing His2Av-mRFP and Cenp-A/Cid-EGFP were treated with latrunculin B (latrunc B), cytochalasin D (cytochal D) or colcemid and analyzed by time-lapse imaging during the stage of chromosome territory formation. Time (h:min) is indicated.

(**D**) Inhibitors of F actin and MT dynamics do not preclude chromocenter disruption during chromosome territory formation. *bam>RedX Green2/3* spermatocytes were analyzed during the stage of chromosome territory formation in the absence (control, mock treated) or presence of the indicated inhibitors. Time-lapse imaging revealed an efficient splitting of single Green2/3 dots into two also in presence of the inhibitors. Time (h:min) is indicated.

(E) Stretching of 1.686 chromatin in condensin II protein mutants is not affected by inhibitors of F actin and MT dynamics. *Cap-H2* mutant spermatocytes (*Cap-H2*^{cc1}/*Df*) with *bam>Green2/3* and His2AvmRFP were analyzed in the absence (control, mock-treated) or presence of the indicated inhibitors. Time lapse imaging revealed that Green2/3 dot stretching and definitive splitting into two occurs also in presence of inhibitors. Bar diagram indicating the percentage of *Cap-H2*^{cc1}/*Df* spermatocytes, which displayed at least one Green2/3 dot stretching or splitting episode during imaging for 4 hours in the indicated conditions. The analyzed spermatocytes were either at the earliest stages (d < 9 μ M; S1 - S2) or thereafter (d = 9 -12 μ M; S3 - S4). Latrunculin B resulted in an apparent slight reduction of Green2/3 stretching early but not late, whereas cytochalasin D and colcemid had at most very subtle effects. n = 126, 46, 80, 102, 52, 50, 70, 45, 25, 135, 31, 104 from left to right. Time (h:min) is indicated.

Scale bars = $3 \mu m$.