Supplemental Figures

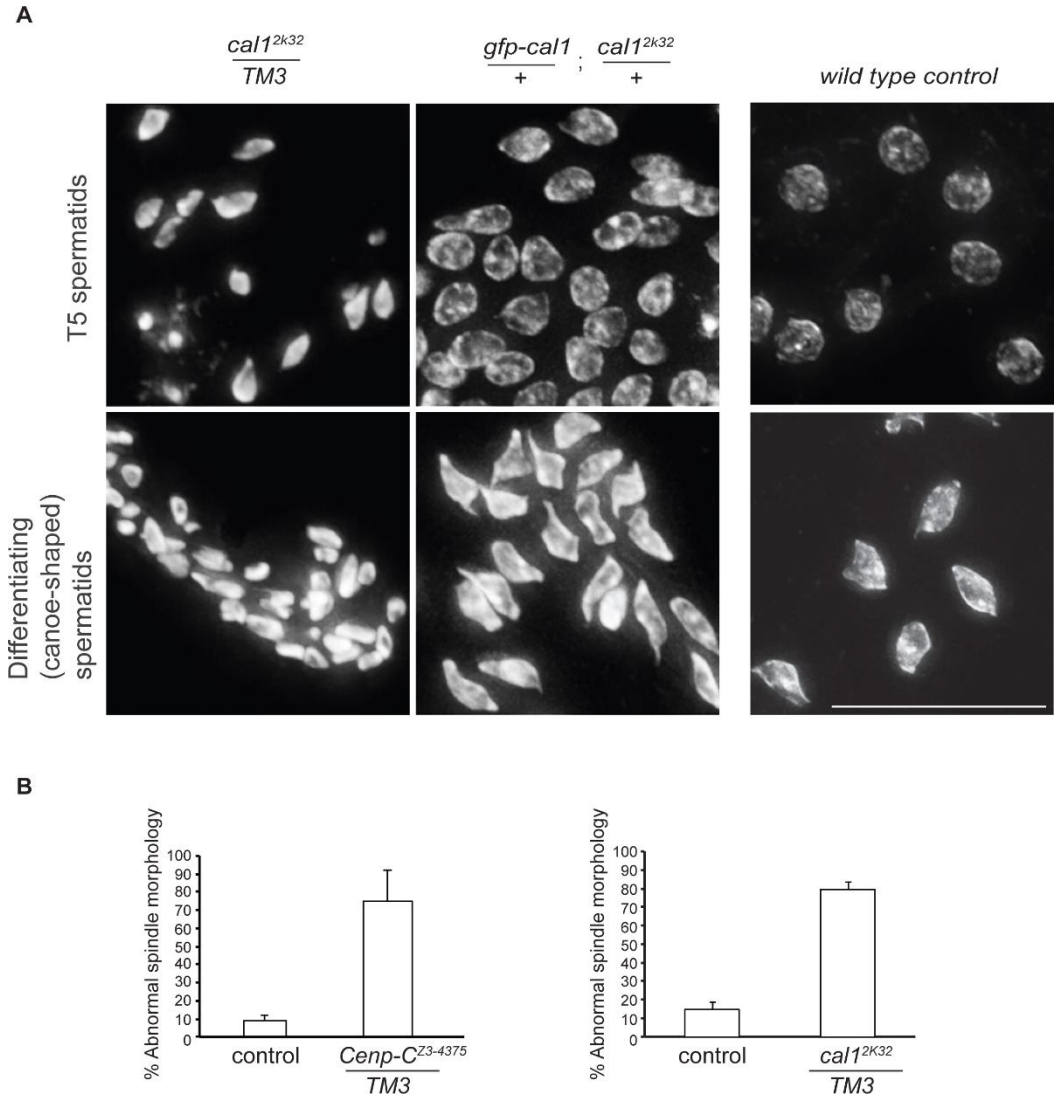

Figure S1 (related to Figure 1):

A: Introduction of GFP-tagged CAL1 into *cal1*^{2k32} mutants rescues defects in spermatid nuclear morphology.

cal1^{2k32} heterozygous adult testes (left) or *cal1*^{2k32} heterozygous adult testes expressing an extra copy of GFP-tagged CAL1 on chromosome 2 (middle), fixed and stained with DAPI showing spermatid nuclei at T5 and canoe-shaped stages (grey). Typical morphologies of wild type control T5 and canoe-shaped spermatids are shown for comparison (right). Scale bar 20 μm.

B: *Cenp-C*$^{Z3-4375}$ and *cal1*^{2k32} mutants have defective meiotic spindle morphology.

Quantitation of control (*wild type*), *Cenp-C*$^{Z3-4375}$*/TM3* (left) or *cal1*^{2k32}*/TM3* (right) spermatocytes with abnormal spindle morphology in meiosis I (prometaphase to telophase, M1 to M5) and II (prometaphase to telophase, M6 to M11), as determined by tubulin staining, expressed as a percentage of total number of nuclei analysed (n=100). Error bars = SD.

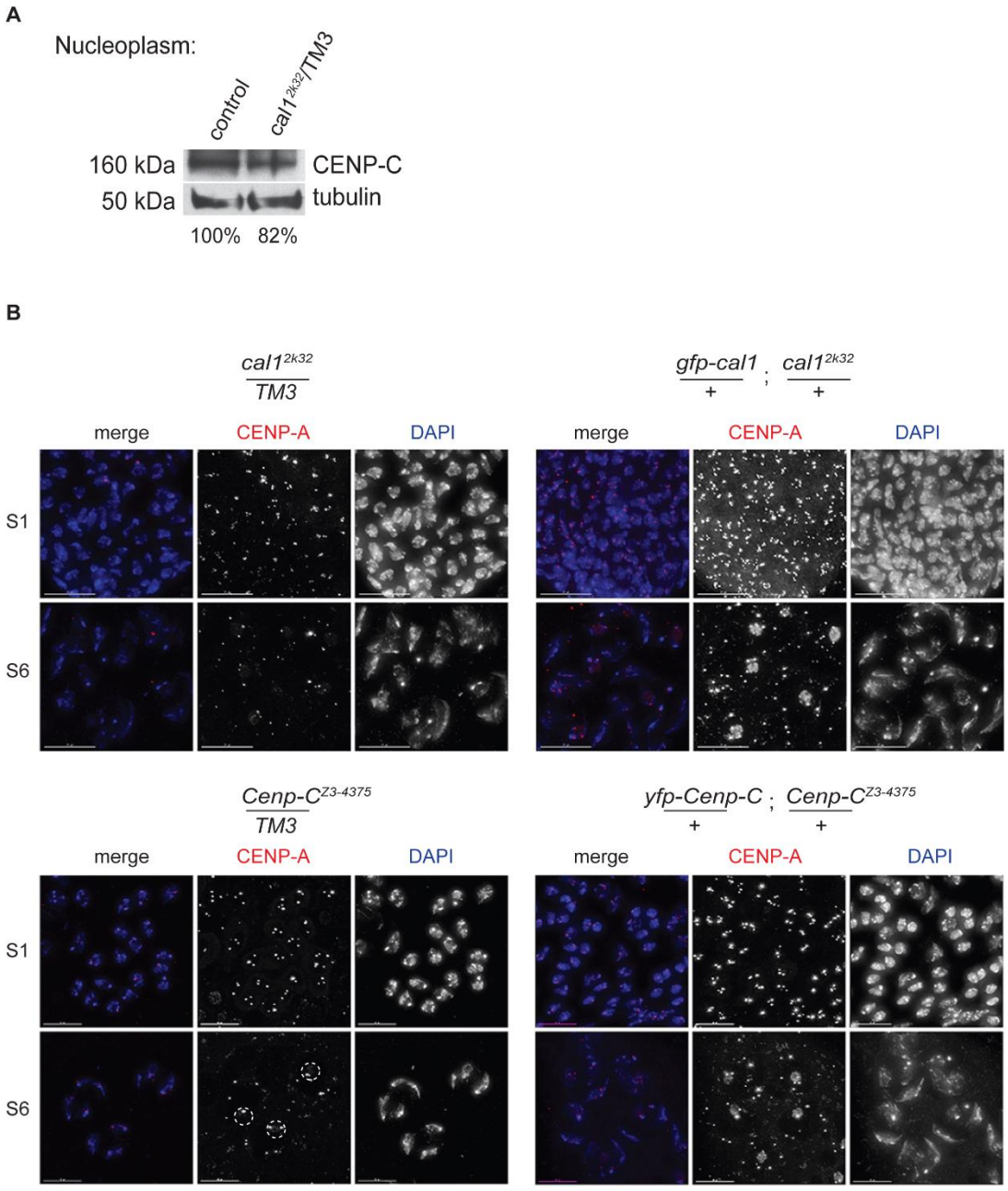

Figure S2 (related to Figure 2):

A: Nucleoplasmic CENP-C level is decreased in *cal1*^{2k32} mutant larval testes.

Western blotting of nucleoplasmic extracts from control (*wild type*) and *cal1*^{2k32}*/TM3* larval testes probed with anti-CENP-C antibody and anti-tubulin as loading control. Molecular weights (kDa) are indicated. Quantitation (normalized to control) shows that CENP-C level in *cal1*^{2k32} mutant testes is 82% of wild type level.

B: Introduction of YFP-tagged CENP-C into *Cenp-C*$^{Z3-4375}$ mutants or GFP-tagged CAL1 into *cal1*^{2k32} mutants rescues defects in CENP-A assembly in meiotic prophase I.

Top: *cal1*^{2k32} heterozygous testes or *cal1*^{2k32} heterozygous testes expressing an extra copy of GFP-tagged CAL1 on chromosome 2, fixed and stained with anti-CENP-A (red) and DNA is stained with DAPI (blue). Individual crosses were performed two times. Representative images from prophase S1 and S6 stages are shown. Scale bar 20 μm.

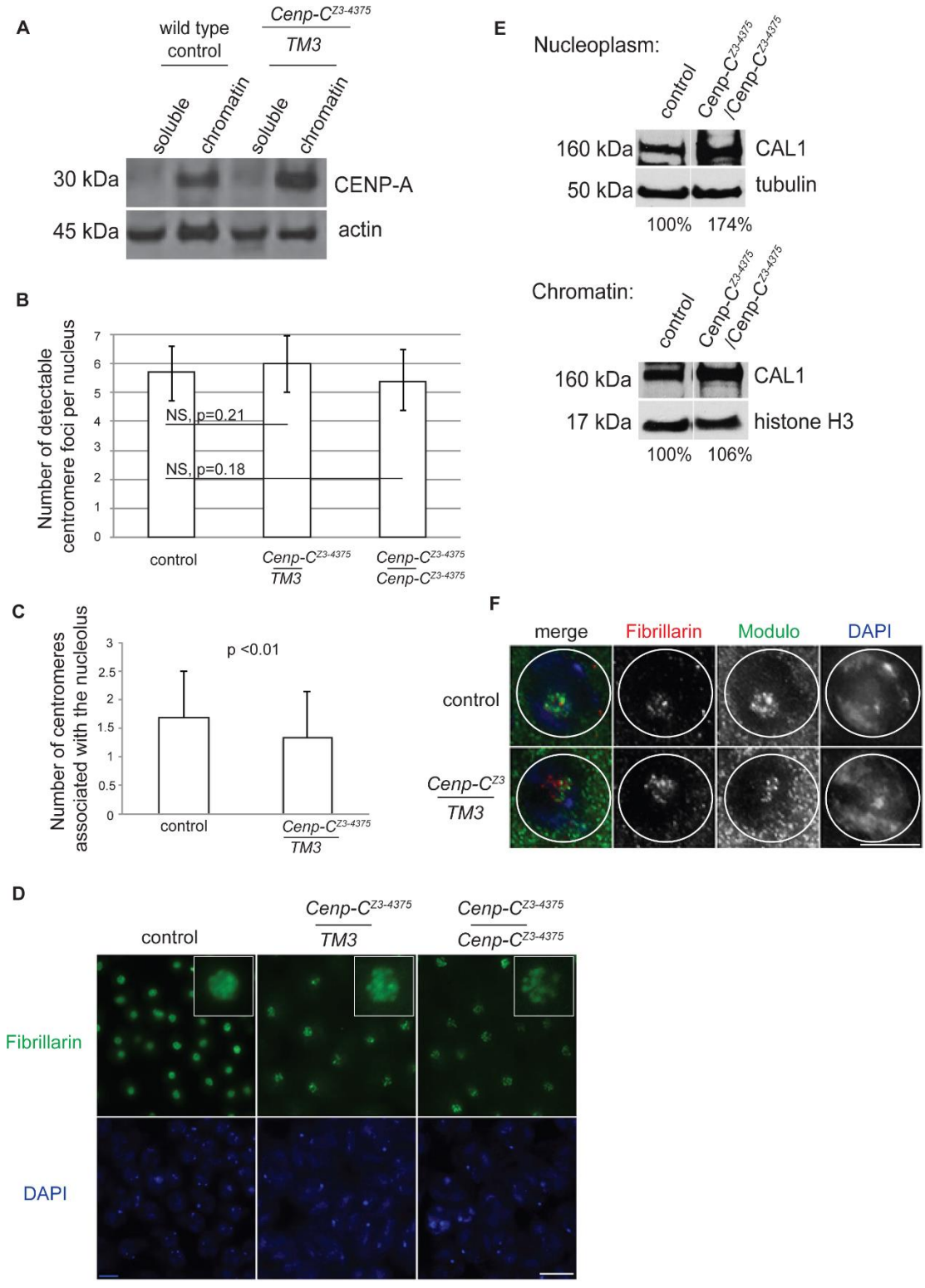

Figure S3 (related to Figure 3):

A: Chromatin-associated CENP-A is increased in *Cenp-C$^{Z3-4375}$* larval testes.

Western blotting of soluble and chromatin-bound extracts from control (*wild type*) and *Cenp-C$^{Z3-4375}$/TM3* larval testes probed with anti-CENP-A antibody and anti-actin antibody as loading control. Molecular weights (kDa) are indicated.

B: No significant difference in the number of detectable centromeric foci in *Cenp-C$^{Z3-4375}$* mutant spermatocytes compared to controls.

Quantitation of number of detectable centromere foci (scored using CENP-A and CENP-C staining) in control (*wild type*), *Cenp-C$^{Z3-4375}$/TM3* heterozygous or *Cenp-C$^{Z3-4375}$/Cenp-C$^{Z3-4375}$* homozygous prophase S6 nuclei (n=30). T test gave no significant difference compared to control (p=0.21 for heterozygotes and p=0.18 for homozygotes).

C: Reduced number of centromeres associated with the nucleolus in *Cenp-C$^{Z3-4375}$* S6 spermatocytes compared to wild type control.

Quantitation of number of CENP-A foci associated with the nucleolar CENP-A pool in control (*wild type*) and *Cenp-C$^{Z3-4375}$/TM3* spermatocytes (n=300 nuclei). T test gave significant difference ($p< 0.01$). Error bars = SD.

D: Fibrillarin staining is disrupted in *Cenp-C$^{Z3-4375}$* heterozygous and *Cenp-C$^{Z3-4375}$* homozygous mutant spermatocytes.

Control and *Cenp-C$^{Z3-4375}$* heterozygous and homozygous S6 spermatocytes stained with antibodies against Fibrillarin (green) and DNA is stained with DAPI (blue). Scale bar 20 μm. Insets show a single nucleolus from control or *Cenp-C$^{Z3-4375}$* mutants, highlighting the typical Fibrillarin staining pattern observed.

E: Chromatin-associated and nucleoplasmic CAL1 levels are increased in *Cenp-C$^{Z3-4375}$* homozygous larval testes.

Western blotting of nucleoplasmic and chromatin-bound extracts from control (*wild type*) and *Cenp-C$^{Z3-4375}$/Cenp-C$^{Z3-4375}$* larval testes probed with anti-CAL1 antibody and anti-tubulin or anti-histone H3 antibody as loading control. Molecular weights (kDa) are indicated. Quantitation (normalized to control) shows that nucleoplasmic CAL1 level in *Cenp-C$^{Z3-4375}$* homozygous mutant testes is 174% of wild type level, whereas chromatin-bound CAL1 level in *Cenp-C$^{Z3-4375}$* homozygous mutant testes is similar to wild type.

F: Both Fibrillarin and Modulo staining is disrupted (reduced or more dispersed) in *Cenp-C* mutant S6 spermatocytes.

Control and *Cenp-C$^{Z3-4375}$/TM3* S6 spermatocytes stained with antibodies against Fibrillarin (red) and Modulo (green) and DNA is stained with DAPI. Scale bar 10 μm.

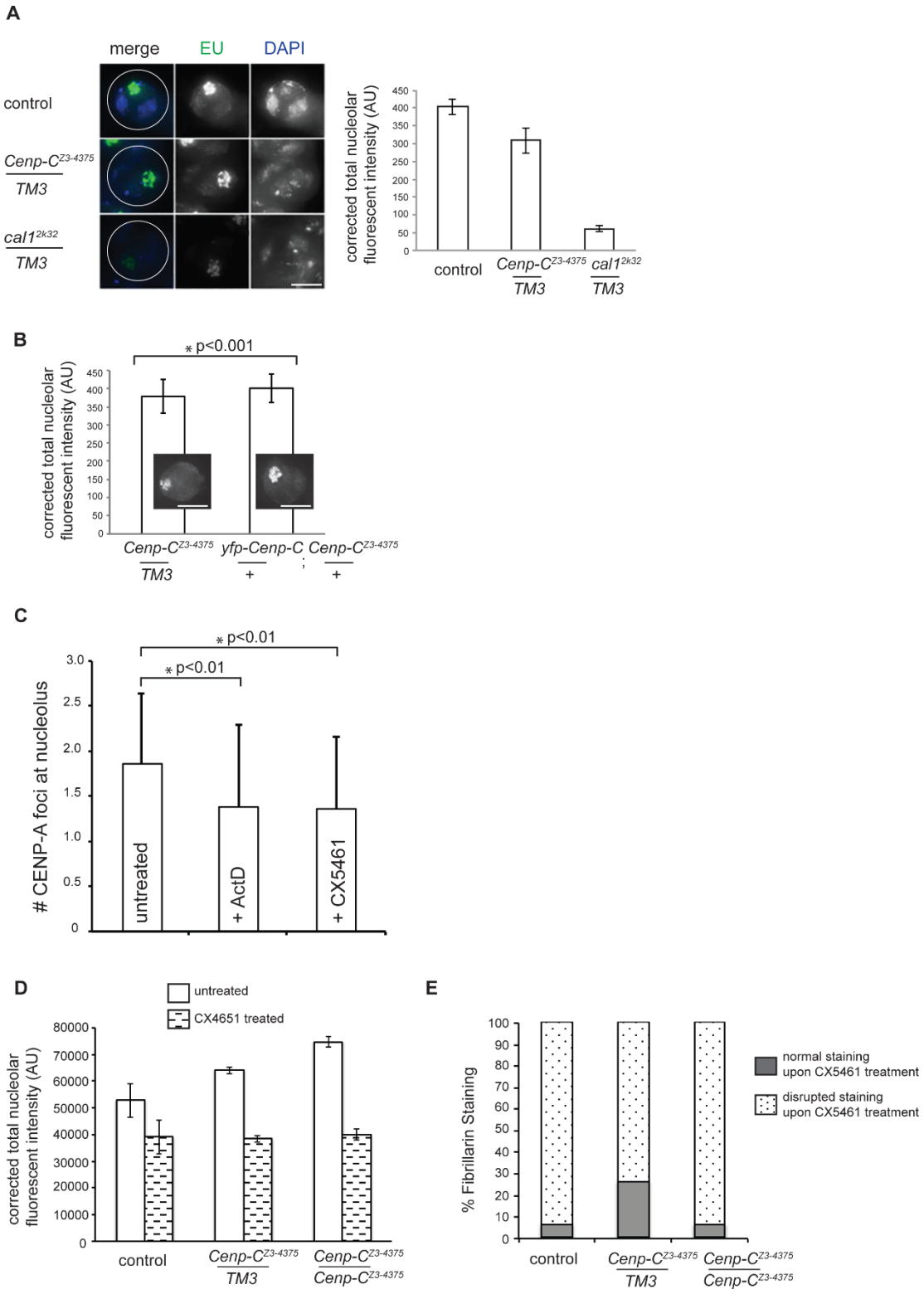

Figure S4 (related to Figure 6):

A: Nucleolar transcription is reduced in *Cenp-C* and *cal1* mutants.

Left: Control, *Cenp-C*$^{Z3\text{-}4375}$*/TM3* and *cal1*^{2K32}*/TM3* S6 spermatocytes pulsed labeled with EU (green), then fixed and DNA is stained with DAPI (blue). Scale bar 10 μM. Right: Quantitation of nucleolar EU incorporation in control, *Cenp-C*$^{Z3\text{-}4375}$*/TM3* and *cal1*^{2K32}*/TM3* prophase I spermatocytes. Error bars = SD.

B: Introduction of YFP-tagged CENP-C into *Cenp-C*$^{Z3\text{-}4375}$ mutants results in a significant increase in EU incorporation rate.

Quantitation of nucleolar EU incorporation in *Cenp-C*$^{Z3\text{-}4375}$ heterozygous testes or *Cenp-C*$^{Z3\text{-}4375}$ heterozygous testes expressing an extra copy of YFP-tagged CENP-C on chromosome 2. T test gave a significant increase ($p < 0.001$), n=50 S6 spermatocytes. Error bars = SD. Representative images of EU incorporation (grey) in spermatocytes of indicated genotypes are shown. Scale bar 10 μM.

C: Reduced number of centromeres associated with nucleoli in wild type spermatocytes treated with Actinomycin D or CX5461.

Quantitation of number of CENP-A foci associated with the nucleolus in wild type S6 spermatocytes untreated or treated with ActD or CX5461 (n=300 nuclei). T test gave a significant difference in the number of centromeres tethered at nucleoli in untreated spermatocytes and those treated with ActD ($p < 0.01$) or CX5461 ($p < 0.01$). Error bars = SD.

D: Intensity of the CENP-A pool is reduced in control and *Cenp-C*$^{Z3\text{-}4375}$ hetero- and homozygous S6 spermatocytes upon treatment with CX5461.

Quantitation of CENP-A pool intensity (corrected total nucleolar fluorescent intensity, AU) in control, *Cenp-C*$^{Z3\text{-}4375}$*/TM3* and *Cenp-C*$^{Z3\text{-}4375}$*/Cenp-C*$^{Z3\text{-}4375}$ S6 spermatocytes (n=50) untreated and after CX5461 treatment.

E: Fibrillarin staining is disrupted in control and *Cenp-C*$^{Z3\text{-}4375}$ hetero- and homozygous S6 spermatocytes upon treatment with CX5461.

Quantitation of normal and disrupted Fibrillarin staining in control, *Cenp-C*$^{Z3\text{-}4375}$*/TM3* and *Cenp-C*$^{Z3\text{-}4375}$*/Cenp-C*$^{Z3\text{-}4375}$ S6 spermatocytes (n=70) untreated and after CX5461 treatment.