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BERNHARDT ET AL., SUPPLEMENTAL DATA

Supplemental Table S1. Zinc is required for successful MI-MII transition.

	n	TPEN-like (telophase I arrest-like)	partial TPEN-like (chromatin mass)	other (>2 chr. masses)	normal MII
Control 14h	29	0%	0%	0%	100%
C 7.5h → T 6.5h	42	30%	51%	19%	0%
TPEN 14h	34	91%	3%	6%	0%

Oocytes treated with TPEN during the MI-MII transition fail to enter MII. Following IVM under the conditions listed in control (C) or TPEN-containing (T) medium, oocytes were stained and imaged as in Figure 1, and their spindle phenotypes were scored. Compiled data from 3 independent experiments is shown. (Related to Figure 1.)

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Supplemental Table S2. Proteasome inhibition partially rescues zinc insufficiency phenotype.

	n	MII	spindle with ≤3 chr. out of place	spindle with chr. scattered along length	persisting midbody or chromatin mass	other	complete TPEN-like
Control 16h	29	100%	0%	0%	0%	0%	0%
T 10h → T + MG132 6h	26	19%	31%	19%	15%	15%	0%
TPEN 16h	13	0%	0%	0%	0%	23%	77%

A majority of zinc-insufficient oocytes treated with MG132 following the first meiotic division transition progress beyond telophase I arrest and form MII spindle-like structures. Following IVM in control (C) or TPEN-containing (T) medium with or without addition of 20 μM MG132 after 10 h, oocytes were stained and imaged as in Figure 2, and their spindle phenotypes were scored. Data shown are compiled from 2 independent experiments. (Related to Figure 2.)

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Supplemental Figure Legends

Supplemental Figure S1. Proteasome inhibition only partially restores CCNB1 levels. Western blots for CCNB1 and EMI2 with oocytes cultured in TPEN containing medium for 10 h and transferred to medium containing both TPEN and MG132 for an additional 6 h. Equal numbers GV stage oocytes, which have low levels of CCNB1 and EMI2 protein, were also included for comparison. (Related to Figure 2.)

Supplemental Figure S2. EMI2 knockdown phenotype resembles zinc insufficiency. (A-F) The majority of oocytes injected with EMI2 MO do not form MII spindles. MO injected oocytes show varying degrees of midbody microtubule retention, and frequently have large first polar bodies. Occasionally oocytes divide incorrectly to produce two chromatin-containing polar bodies with an empty oocyte (E), or progress through an apparent second meiotic division to produce a second polar body (F). MO injected oocytes were held in IBMX containing medium for 5-6 h prior to a 15 h IVM culture; siRNA injected oocytes were held for 24 h prior to IVM. Projections of confocal Z stacks with actin (red), tubulin (green), and DAPI (blue) shown. Bar = 20 μm. (Related to Figure 3.)

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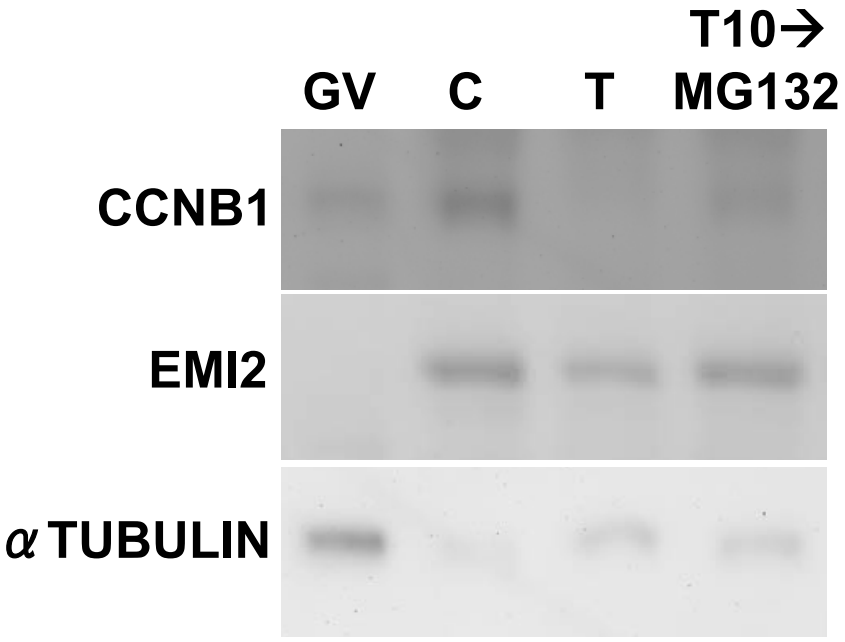
Supplemental Figure S1



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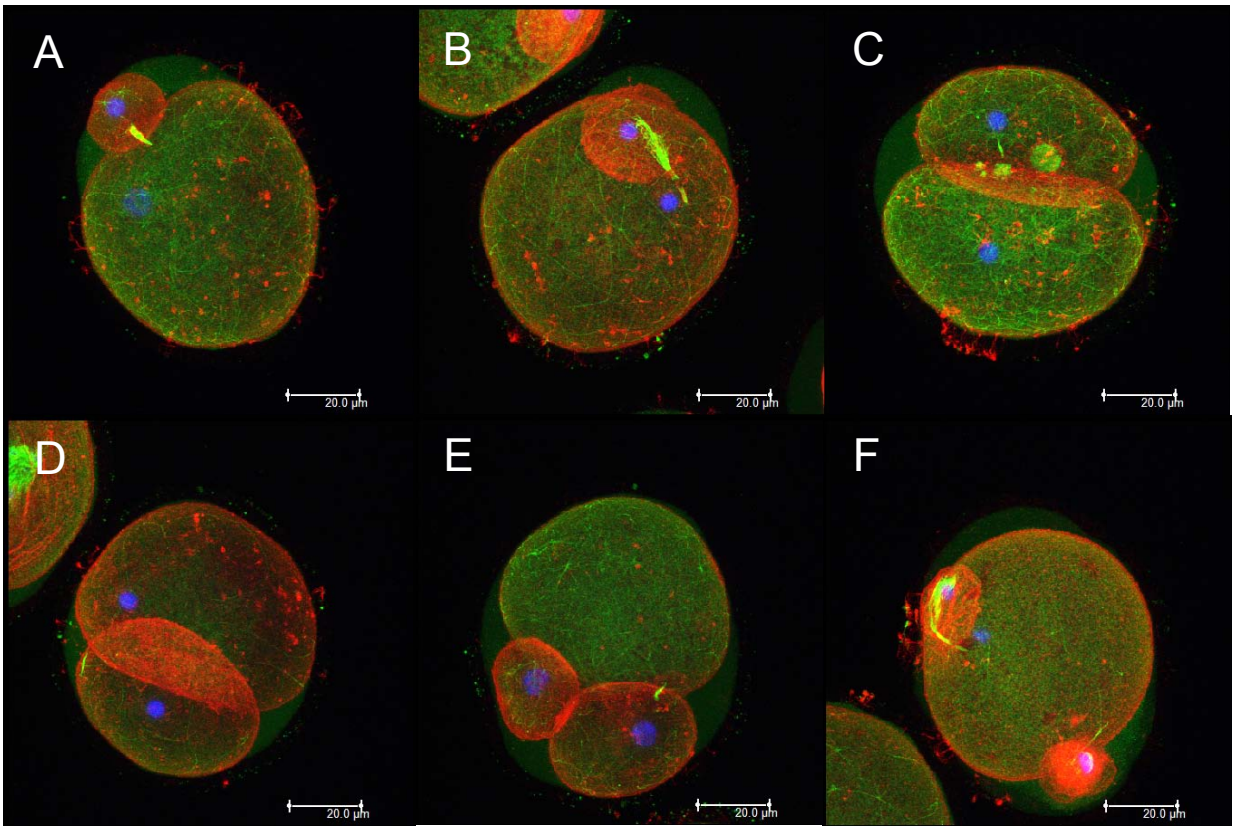
Supplemental Figure S2



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