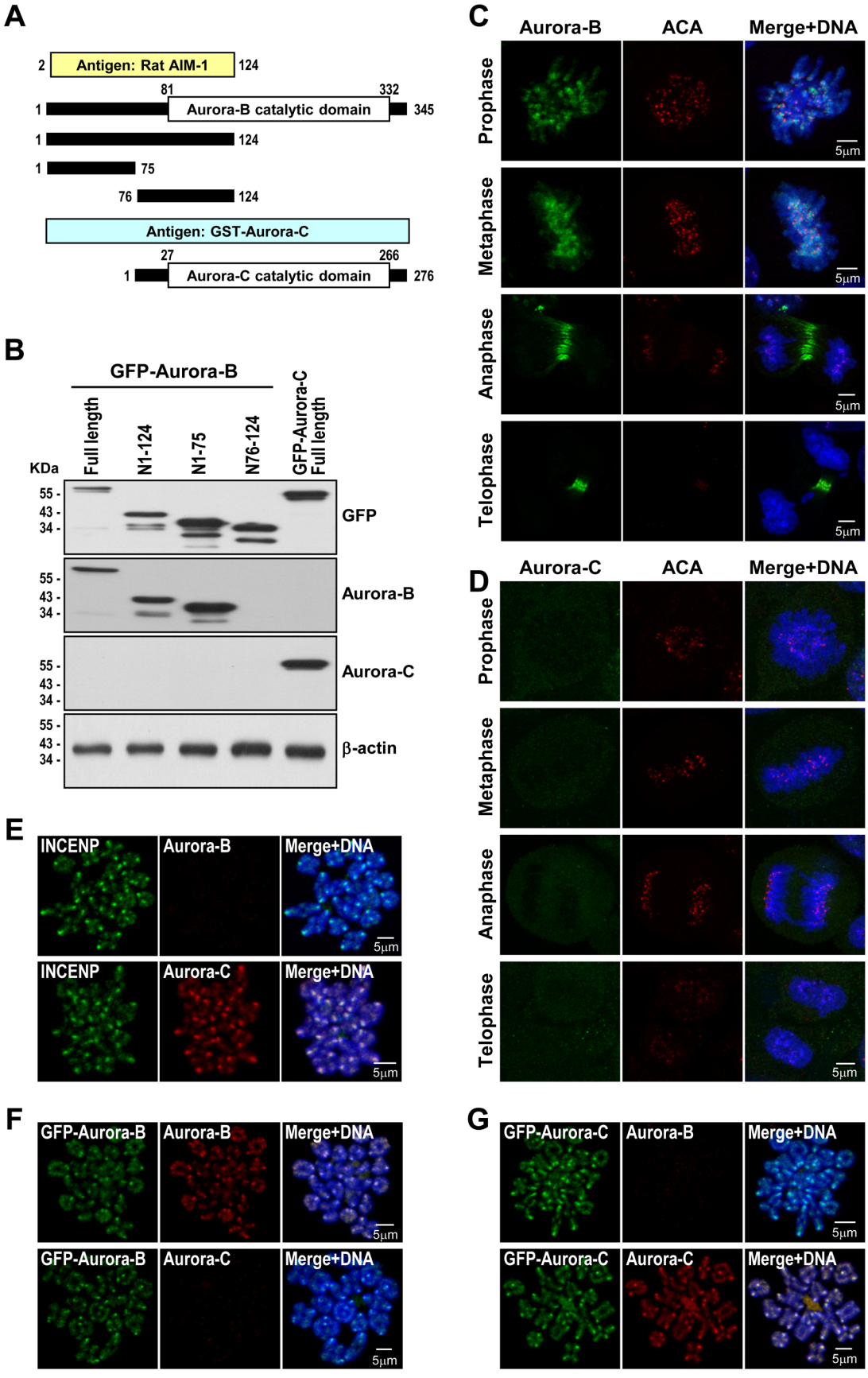
SUPPLEMENTARY FIGURES

Figure S1. (A) cDNA constructs containing various portions of Aurora-B and –C were subcloned into a pEGFP-C1 vector and used for antibody specificity test in B. (B) The cell lysates (20 μg) prepared from mouse N2A cells transfected with indicated Aurora-B and Aurora-C constructs were subjected to immunoblotting using anti-GFP, anti-Aurora-B (AIM-1 antibody, BD, Transduction Laboratories), anti-Aurora-C (Tang et al., 2006), or anti-β-actin antibodies. (C) Immunofluorescence analysis of endogenous Aurora-B and Aurora-C in TM4 (a testis Sertoli cell line) cells during mitosis. Aurora-B was detected at centromeres at prophase/metaphase, and relocated to the midzone/midbody at anaphase/telophase, a pattern similar to a previous report (Terada et al., 1998). No endogenous Aurora-C was detected in TM cells (D). (E) Chromosome spreads prepared from MI oocytes (8 h post-GVBD) were probed with indicated antibodies and analyzed by confocal fluorescence microscopy. (F-G) Chromosome spreads of MI oocytes (8 h post-GVBD) injected with *GFP-Aurora-B* (F) or *GFP-Aurora-C* (G) mRNAs were immunostained with anti-Aurora-B or anti-Aurora-C antibodies and analyzed by LSM510 laser confocal microscopy.

Figure S2. Effects of ZM447439 on meiotic divisions. GV-intact oocytes were treated with ZM447439 (0-8μM) for 1 h in the presence of IBMX. The oocytes were then cultured in IBMX-free medium and matured *in vitro* in the same concentration of ZM447439 for 6 h or 8 h prior to fixation and analyzed by confocal microscopy. (A) Chromosome misalignment induced by ZM447439 treatment. The mitotic spindle was detected using anti-acetyltubulin antibody and DNA was stained by DAPI. Data are shown as mean +/- s.d.. (B) ZM447439 induced premature chromosome separation in a dose-dependent manner. Chromosome spreads prepared from ZM447439-treated oocytes (8 h) were fixed and stained with DAPI. For a better view of chromosome morphology (bivalent *vs*. univalent) in B, the DAPI image was converted to pseudocolor (Black and White). (C) Chromosome spreads prepared from ZM447439-treated oocytes (4μM, 6 h) were fixed and probed

with antibodies against Aurora-C and p-Aurora-C. The large square box in panel C is a blowup of each corresponding smaller dotted box. ZM447439 (4μM) significantly inhibited Aurora-C phosphorylation. The strong red signals detected in ZM447439-treated chromosomes (C-g) are non-specific, since these signals did not localize at centromeres. (D) ZM447439 inhibited 1st PBE in a dose-dependent manner. Number of post-injected GVBD oocytes is shown (n). Grouped data from 3 independent experiments.

Figure S1


A
2
Antigen: Rat AIM-1
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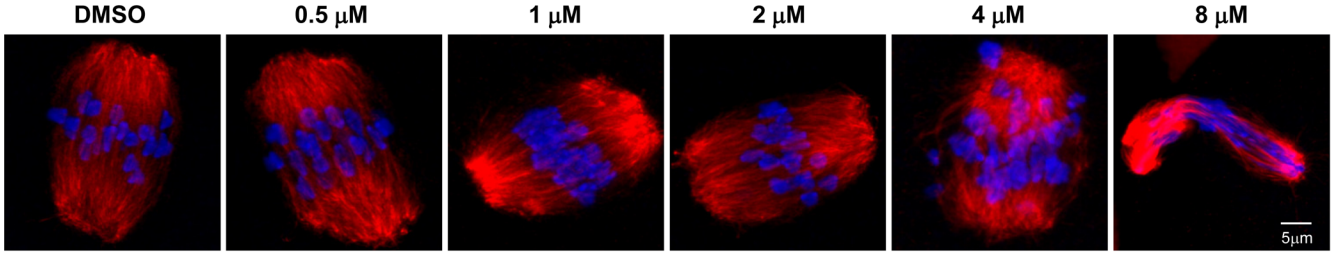
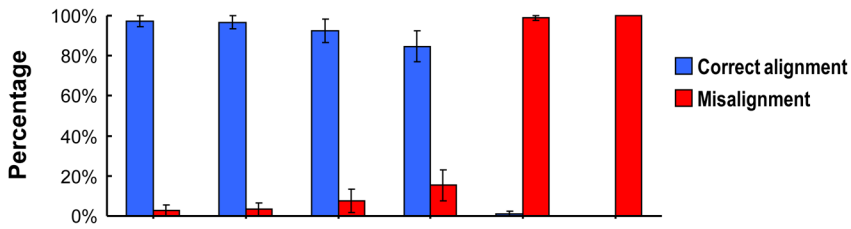
81
332
1
Aurora-B catalytic domain
345
1
124
1
75
76
124
Antigen: GST-Aurora-C
27
266
1
Aurora-C catalytic domain
276
B
GFP-Aurora-B
GFP-Aurora-C
Full length
Full length
N1-124
N1-75
N76-124
KDa
55
43
34
GFP
Aurora-B
Aurora-C
β-actin
C
Aurora-B
ACA
Merge+DNA
Prophase
Metaphase
Anaphase
Telophase
5μm
D
Aurora-C
ACA
Merge+DNA
Prophase
Metaphase
Anaphase
Telophase
5μm
E
INCENP
Aurora-B
Merge+DNA
INCENP
Aurora-C
Merge+DNA
5μm
F
GFP-Aurora-B
Aurora-B
Merge+DNA
GFP-Aurora-B
Aurora-C
Merge+DNA
5μm
G
GFP-Aurora-C
Aurora-B
Merge+DNA
GFP-Aurora-C
Aurora-C
Merge+DNA
5μm

Figure S2

A. ZM447439 induces chromosome misalignment

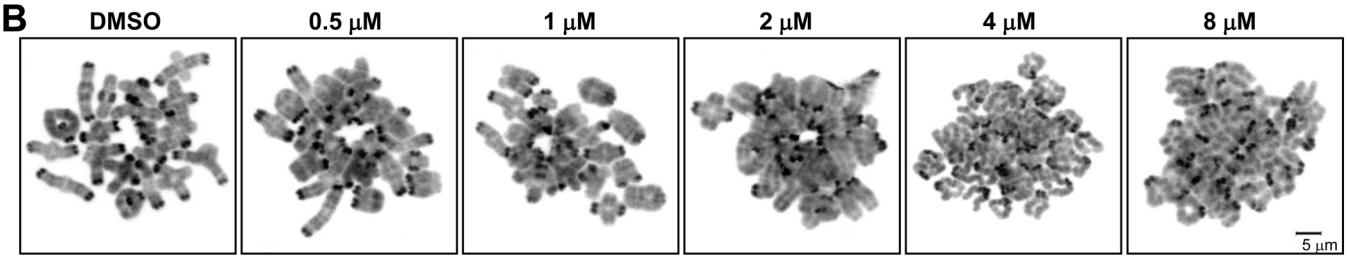


DNA / ac-Tub

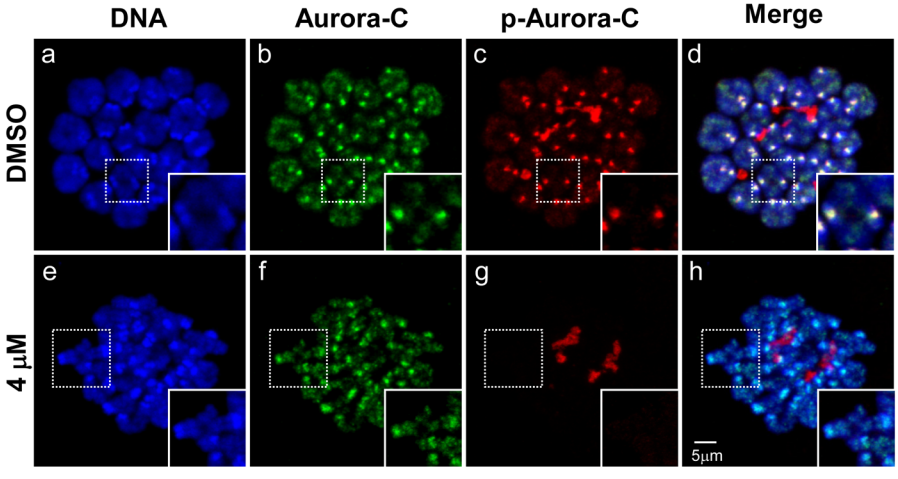


ZM447439 (μM)	DMSO	0.5	1	2	4	8
Correct alignment (%)	41(97.6)	47(95.9)	44(91.7)	37(82.2)	1(1.8)	0(0)
Misalignment (%)	1(2.4)	2(4.1)	4(8.3)	8(17.8)	54(98.2)	50(100)
Total (n)	42	49	48	45	55	50

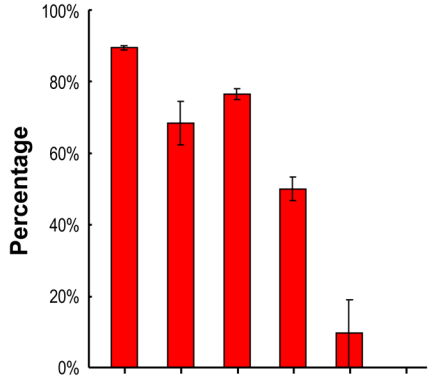
B



C. ZM447439 inhibits Aurora-C phosphorylation



D. ZM447439 inhibits 1st polar body extrusion



ZM447439 (μM)	DMSO	0.5	1	2	4	8
PBE (%)	35(89.7)	40(65.6)	31(77.5)	33(58.9)	2(3.8)	0(0)
Total (n)	39	61	40	56	52	34