CenpH regulates meiotic G2/M transition by modulating the APC/C^{Cdh1}-cyclin B1 pathway in oocytes

Teng Zhang[1,2,*]; Yang Zhou[1,5,*]; Li Li[1,3]; Zhen-Bo Wang[1]; Wei Shen[2]; Heide Schatten[4]; Qing-Yuan Sun[1,2,5,#,]

[1]State Key Laboratory of Stem Cell and Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing, China;
[2]Institute of Reproductive Sciences, College of Animal Science and Technology, Qingdao Agricultural University, Qingdao, China;
[3]Department of Reproductive Medicine, Guangdong Women and Children Hospital, Guangzhou, China;
[4]Department of Veterinary Pathobiology, University of Missouri, Columbia, MO 65211, USA
[5]University of the Chinese Academy of Sciences, Beijing, China

* These authors have contributed equally to this work.
[#]Correspondence to: Qing-Yuan Sun, State Key Laboratory of Stem Cell and Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, #1 Beichen West Rd, Chaoyang, Beijing, 100101, China; Tel./Fax:8610.6480.7050; Email: sunqy@ioz.ac.cn.

Abstract

Meiotic resumption (G2/M transition) and progression through meiosis I (MI) are two critical stages for producing fertilization-competent eggs. Here, we report that CenpH, a component of the kinetochore inner plate protein, is responsible for the G2/M transition in meiotic mouse oocytes. Depletion of CenpH using morpholino injection decreased cyclin B1 levels, resulting in an attenuation of MPF activation, and severely compromised the meiotic resumption. CenpH protects cyclin B1 from destruction by competing actions of APC/C^{Cdh1}. Impaired G2/M transition after CenpH depletion could be rescued by expression of exogenous cyclin B1. Unexpectedly, blocking of CenpH did not affect spindle organization and meiotic cell cycle progression after germinal vesicle breakdown. Our findings reveal a novel role of CenpH in regulating meiotic G2/M transition by acting via the APC/C^{Cdh1}-cyclin B1 pathway.

Key Words: CenpH, G2/M transition, cyclin B1, APC/C^{Cdh1}, meiosis, oocyte

Introduction

Fully-grown mammalian oocytes remain arrested at prophase I within antral follicles before gonadotropin signals induce the resumption of meiosis I (MI) and progression to meiosis II (MII). The prophase I arrest, also termed the germinal vesicle (GV) stage, is the result of low maturation promoting factor (MPF) activity (Adhikari et al., 2012; Jones, 2004). The resumption of meiosis, referred to as the germinal vesicle breakdown (GVBD), is similar to the G2/M transition of mitosis in being associated with activation of MPF (Adhikari et al., 2012; Doree and Hunt, 2002). MPF is a complex of the catalytic subunit cyclin dependent kinase 1 (Cdk1 or Cdc2) and the regulatory subunit

cyclin B1 (Doree and Hunt, 2002). Activation of Cdk1 requires the association with cyclins as well as the dephosphorylation of Thr 14 and Tyr 15 residues by Myt1 and Wee1 protein kinases (Morgan, 1995; Nurse, 1990). Depletion of Myt1 causes partial meiotic resumption in mouse oocytes (Oh et al., 2010). Wee1B, a key Cdk1 inhibitory kinase, is located in the GV stage oocytes and depletion of its expression enhances meiotic resumption. Wee1B inhibits Cdk1 kinase activity, whereas cell division cycle 25 B kinase (Cdc25B) releases Cdk1 activity by dephosphorylating Wee1B phosphorylated Cdk1 (Branzei and Foiani, 2008; Oh et al., 2010). Knock-down of Cdc25B in GV stage oocytes inhibits meiotic resumption and causes low activity of MPF (Lincoln AJ, 2002). Cyclin B1 is continuously degraded by the anaphase promoting complex/cyclosome (APC/C) during the prophase I arrest (Reis et al., 2006). In GV stage mouse oocytes, Cdh1 (also called Fzr1) is required for APC/C-mediated cyclin B1 destruction to arrest at prophase I (Holt et al., 2011; Reis et al., 2006). The early mitotic inhibitor 1 (Emi1), an inhibitor of the APC/C^{Cdh1}, is responsible for cyclin B1 destruction and inactivation of MPF. Reduction of Emi1 can delay resumption of meiosis by preventing the accumulation of cyclin B1 whereas Emi1 overexpression leads to GVBD (Marangos et al., 2007). Interestingly, BubR1 (a spindle assembly checkpoint protein) has been shown to affect prophase I arrest in mouse oocytes. BubR1-depleted oocytes spontaneously underwent GVBD in the presence of IBMX. BubR1 knock-down can decrease the expression level of Cdh1. However, the GVBD rate in the BubR1-depleted oocytes is reduced by injecting Cdh1 cRNA into the oocyte (Homer, 2009). Significantly, a subunit of the Ndc80 complex, Hec1, has also been shown to regulate meiosis resumption in mouse oocytes. Depletion of Hec1 in mouse oocytes severely affects the G2-M transition because of impaired activation of Cdk1. Unexpectedly, impaired meiosis resumption is due to instability of cyclin B2, because Hec1 can protect cyclin B2 from APC/C^{Cdh1}-mediated destruction

(Gui and Homer, 2013).

It is widely known that CenpH localizes to kinetochores in mammals (Alonso A, 2007; Sugata N, 2000; Sugata et al., 1999). The kinetochore plays a fundamental role in accurate chromosome segregation in eukaryotes. It is a multi-protein structure that associates with assembly on centromeric DNA and the binding of spindle microtubules to chromosomes which is required for chromosome movement (Cleveland et al., 2003; Fukagawa, 2004). In particular, the centromere-specific histone H3 variant CenpA forms the platform for kinetochore assembly. Several additional components of the constitutive centromere-associated network, including Cenp-C, -H, -I, and -K to -U, have been identified to associate with CenpA (Foltz et al., 2006; Fukagawa, 2004). In vertebrates, a subgroup of proteins, including Cenp-H, -I, and -K, play essential roles in kinetochore structure and function. The CenpH and -I complex is a direct regulator of kinetochore-microtubule dynamics, and is required for faithful chromosome segregation, and as a marker directing CenpA deposition to centromeres (Amaro et al., 2010; Cheeseman et al., 2008; Okada et al., 2006). Absence of CenpH has been shown to cause severe mitotic phenotypes including misaligned chromosomes and multipolar spindles in human cells (Orthaus et al., 2006). Indeed, CenpH also has at least one other function involving modulation of the cell cycle through an interaction with CenpC (Fukagawa T, 2001.). Interestingly, however, it is not yet known whether CenpH exerts other relevant roles beyond either the binding of spindle microtubules to chromosomes or the chromosome segregation machinery.

Here, we investigated the role of CenpH protein in regulating the meiotic cell cycle in mouse oocytes. Unexpectedly, we show that depletion of CenpH inhibits G2/M transition by continuous degradation of cyclin B1, while the prophase I arrest induced by CenpH knockdown can be rescued by injecting exogenous cyclin B1 mRNA. Finally, we show that CenpH-dependent effects on meiotic

resumption requires the presence of Cdh1, thereby demonstrating that CenpH-dependent regulation of APC/C^{Cdh1} is essential for regulating prophase I arrest.

Results

Expression and subcellular localization of CenpH during oocyte meiotic maturation

To investigate the role of CenpH during meiosis, the expression and subcellular localization were examined. Oocytes were collected after having been cultured for 0, 4, 8 and 12 h, corresponding to GV, GVBD, MI and MII stages, respectively. Immunoblotting analysis showed that CenpH protein was expressed from GV to MII stages (Fig. 1A). As shown in Fig. 1B, CenpH was more concentrated in the germinal vesicle at the GV stage. Shortly after GVBD, clear staining was observed at the kinetochores. When oocytes reached the MI and MII stages, the signals of CenpH were still obvious at the kinetochores of chromosomes. Subcellular CenpH localization during oocyte meiosis was similar to that in mitosis, suggesting that it may contribute to the kinetochore-microtubule attachment in meiosis. **Depletion of CenpH impairs GVBD and Cdk1 activity dependent on cyclin B1**

For depleting CenpH in mouse oocytes, we used a morpholino (MO) antisense microinjection approach. CenpH MO was microinjected into GV stage oocytes followed by a 24 h incubation in IBMX to deplete the protein. We found that 60-70% CenpH protein was knocked down by CenpH MO-injection compared to the control group (Fig. 2A).

Unexpectedly, only ~37% of CenpH-depleted oocytes underwent GVBD by 3 h following release from IBMX. The percentage of oocytes at the GVBD stage was significantly lower in the CenpH knockdown group than in the control or wild-type oocytes (36.96% ± 13.32% vs. 86.15% ± 11.09%; 89.82% ± 4.75%, $P < 0.05$) (Fig. 2B). This indicated that CenpH-depleted oocytes had reduced

capacity for meiotic resumption. Furthermore, we examined Cdc2 activity by examining the phosphorylation state of the Tyr15 of Cdc2 kinase. We found that Cdc2 activity in CenpH-depleted oocytes was less than that of control oocytes by 1 h following release from IBMX (Fig. 2C; D). In mouse oocytes, cyclin B1 is indispensable for Cdk1 activation during the G2/M transition. We therefore examined the cyclin B1 level in CenpH-depleted oocytes and found that it was reduced (Fig. 2A). This suggested that the prophase I arrest following CenpH-depletion was due to reduced cyclin B1 level and MPF activity.

Impaired GVBD after CenpH depletion can be rescued by cyclin B1 overexpression

We next examined whether exogenous cyclin B1 expression could rescue the G2/M transition defect. In line with this, overexpression of cyclin B1-GFP in CenpH-depleted oocytes increased GVBD dramatically after restoring cyclin B1 levels by injecting exogenous cyclin B1 mRNA (Fig. 2B; Fig. S1E). These data suggested that CenpH was required to stabilize cyclin B1, which in turn plays an indispensable role in the G2/M transition.

Loss of CenpH causes decreased cyclin B1 by APC/C^{Cdh1}-mediated destruction

To further confirm that CenpH-depletion caused decreased levels of cyclin B1, we monitored the dynamics of cyclin B1-GFP accumulation after microinjection of exogenous cyclin B1-GFP mRNA in the GV-stage oocytes injected with control or CenpH MO. We found that loss of CenpH caused a two-fold decrease in cyclin B1-GFP accumulation compared to controls (Fig. 3 A; B). Decreased cyclin B1-GFP accumulation in CenpH-depleted oocytes indicates that the effects of CenpH-depletion are caused by cyclin B1 de-stabilization rather than translation. Cyclin B1 translocation into the nucleus is a prerequisite for MPF activation and GVBD occurrence. Live cell imaging following injection of exogenous cyclin B1-GFP mRNA demonstrated the ability of the cyclin B1-GFP to

accumulate in the nucleus. In contrast, in CenpH-depleted oocytes, cyclin B1-GFP failed to accumulate in the nucleus (Fig. 3A; C).

In mouse oocytes, APC/C^{Cdh1}-mediated destruction of cyclin B1 is indispensable for the prophase I arrest. Consequently, alterations in Cdh1 levels characterize the conditions that perturb cyclin B1 accumulation and meiotic resumption. We therefore examined Cdh1 in CenpH-depleted oocytes using an antibody and found that it was increased (Fig. 3D). This suggested that the prophase I arrest following CenpH-depletion was due to increased APC/C^{Cdh1} activity.

CenpH is not necessary for spindle formation and progression through MI

CenpH, as a component of the kinetochore inner plate, contributes to the kinetochore-microtubule attachment in mitosis. As mentioned above, about 37% of CenpH MO injected oocytes underwent GVBD by 3 h following release from IBMX. These oocytes were used for subsequent observation. The observed phenotypes revealed that the CenpH-depleted oocytes by MO injection showed normal spindle assembly, chromosome alignment, and polar body extrusion (Fig. S1A; B). As this may be caused by insufficient knockdown of CenpH protein we thus further examined the effect of blocking CenpH on MI spindle formation and meiotic cell progression after GVBD. Firstly, CenpH antibody was microinjected into GV stage oocytes. We found that blocking of CenpH protein function by antibody injection caused prophase I arrest similar to that in the CenpH MO injection oocytes (Fig. S1C). This result suggested the availability of workable antibody for disruption of protein function to determine the role of CenpH in mouse oocytes. Subsequently, CenpH antibody was microinjected into GVBD stage oocytes. Interestingly, oocytes injected with CenpH antibody extruded the first polar body at a rate similar to those of control oocytes (Fig. S1D). In mouse oocyte, shortly after GVBD, the earliest stage of spindle assembly is characterized by a spherically shaped spindle with clumped

chromosomes. Subsequently, at 4-8h, the spindle becomes molded into a barrel-shaped bipolar structure and chromosomes aligned on the metaphase plate (Fig. 4A). Strikingly, after blocking CenpH, we found that indexes of spindles were not significantly affected after oocyte GVBD (Fig. 4B). Meanwhile, inter-kinetochore distance had no significant difference as revealed by ACA staining between control oocytes and CenpH antibody-injected oocytes (Fig. 4C). Subsequently, we also examined the MII spindle morphology. Similarly, in CenpH antibody-injected oocytes, indexes of MII spindles were not significantly affected compared with control oocytes (Fig. 5A, B). Overall, these data show that, unlike in mitosis, CenpH does not play critical roles in spindle formation and the MI-MII transition during mouse oocyte meiosis.

Discussion

CenpH is a relatively important member of the family of kinetochore proteins that play a critical role in regulating the mitotic cell cycle (Matson et al., 2012; Orthaus et al., 2006; Zhu et al., 2015). Here, we provide the first definitive evidence showing a role for CenpH in regulating meiotic G2/M transition via APC/C^{Cdh1}-cyclin B1 in oocytes, differing sharply from the effects observed in mitosis.

In mouse oocytes, CenpH was expressed throughout meiotic maturation (Fig.1A). Inherently, the subcellular localization of CenpH is not very different from that of mitosis: the clear signal was still obvious at the kinetochores at the meiotic metaphase (Fig. 1B). Furthermore, at the GV stage, it is more concentrated in the germinal vesicle, suggesting that it may play an important role in this stage. We knocked down the expression of CenpH protein in GV stage oocytes by specific MO microinjection. Unexpectedly, ~63% of CenpH-depleted oocytes still maintain prophase I arrest by 3 h following release from IBMX (Fig. 2B). This result indicates that the expression of CenpH at the

GV stage is essential for oocyte meiotic resumption. The mouse oocyte provides an ideal model for studying G2/M transition, since oocyte is arrested at the prophase of meiosis I for a long time before release from the inhibitory micro-environment. It is well known that MPF is a key regulator of G2/M transition in mouse oocytes (Jones, 2004). The Cdk1-activating cyclin at the boundary of G2/M in mouse oocytes is cyclinB1, whose APC/C^{Cdh1}-mediated destruction is indispensable for preventing MPF activation during the G2 arrest (Marangos and Carroll, 2008; Reis et al., 2006). Consequently, alterations in cyclin B1 and/or Cdh1 levels characterize many conditions that perturb Cdk1 activity and meiotic resumption (Holt et al., 2011; Marangos and Carroll, 2008; Reis et al., 2006). Our results show that reduction in cyclin B1 levels following CenpH-depletion was due to increased APC/C^{Cdh1}activity (Fig. 2A; Fig. 3D), indicating that the CenpH-dependent inhibition of APC/C^{Cdh1} in mouse oocytes is important for the control of the G2/M transition. Thus, G2/M transition is critically poised by the competing actions of APC/C^{Cdh1} and CenpH. Analogously, prophase I arrest in mouse oocytes is controlled by Emi1-dependent regulation of APC/C^{Cdh1} (Marangos et al., 2007). In contrast, in mouse oocytes, BubR1 sustains Cdh1 levels in prophase I arrest (Homer, 2009). The physiological regulation of APC/$C^{Cdh1}$with kinetochore proteins that allows for a timely meiotic resumption is a topic for future investigation.

The importance of CenpH in balancing the G2/M transition is shown by the effects of CenpH-depletion on the timing of meiotic resumption. The APC/C^{Cdh1} is apparently the target of CenpH in mediating meiotic resumption because CenpH-depletion leads to the reduction of the APC/C^{Cdh1} substrate cyclin B1 (Fig. 2A). Live cell imaging following injection of exogenous cyclin B1-GFP shows that loss of CenpH caused a two-fold decrease in the rate of cyclin B1-GFP accumulation (Fig. 3A,B), indicating that the effects of CenpH-depletion are caused by cyclin B1

de-stabilization rather than translation. This increase in cyclin B1 instability likely explains the much-attenuated increase in MPF activity during the meiotic resumption progression of CenpH-depleted oocytes. Cyclin B1 accumulation in the nucleus, is a prerequisite for MPF activation and meiotic resumption (Holt et al., 2010). Furthermore, APC/C^{Cdh1} protects the oocyte from precocious GVBD by preventing nuclear accumulation of cyclin B1. In CenpH-depleted oocytes, cyclin B1-GFP failed to accumulate in the nucleus and thus inhibited MPF activity, which explains the severely compromised meiotic resumption, together with an increased level of Cdh1 (Fig. 3C,D). Significantly, impaired GVBD could be restored in CenpH-depleted oocytes by co-expressing exogenous cyclin B1 mRNA (Fig. 2B). A recent observation has showed that APC/C^{Cdh1} is necessary for maintaining cyclin B1 stabilization in mouse oocytes (Reis et al., 2006). Our results show that meiotic resumption is critically poised by the competing actions of APC/C^{Cdh1} and CenpH. Thus, CenpH is required to stabilize cyclin B1, which in turn plays an indispensable role in MPF activation required for the meiotic resumption.

Our observations may have wider implications for the role of CenpH in mammalian somatic cells. CenpH has been shown to contribute to the kinetochore-microtubule attachment and accurate chromosome segregation in the mitotic cell cycle (Matson et al., 2012; Orthaus et al., 2006; Zhu et al., 2015). We found that meiotic resumption was impaired by CenpH antibody injection in GV stage oocytes (Fig. S1C), indicating the availability of workable antibody for inhibiting CenpH protein function. However, in contrast to mitosis, we found that destruction of CenpH protein function in GVBD stage oocytes by antibody injection did not influence the cell cycle progression (Fig. S1D). Interestingly, the early-stage spindle assembly and the MII spindle morphology was not affected by inhibiting CenpH at the GVBD stage (Fig. 4; Fig. 5). Similarly, using MO injection, 37% of

CenpH-depleted oocytes underwent GVBD by 3 h following release from IBMX, and these oocytes could still extrude the first polar body (Fig. S1A). Yet it is not clear if CenpH level is actually reduced in these GVBD oocytes. Subsequently, CenpH-MO was injected into GV stage oocytes followed by 24 h incubation in IBMX to deplete the protein. The CenpH protein level of control oocytes and CenpH-MO oocytes (GV or GVBD) by 2 h following release from IBMX was assessed by Western blot. As shown in Fig. S1F, the protein level of CenpH was significantly decreased in the GV oocytes or GVBD oocytes than in the control oocytes. The data confirmed that CenpH was also depleted in these GVBD oocytes. This result indicates that, unlike mitosis, CenpH is not required for the spindle assembly and meiotic cell cycle progression after GVBD. An alternative interpretation of these results might be that pre-existing trace amounts of CenpH in GVBD oocytes is sufficient for meiotic spindle assembly and further cell cycle progression.

Our data uncover a major feature of CenpH in mouse oocytes not shared with mitosis. It is involved in APC/C^{Cdh1}- mediated cyclin B1 proteolysis during prophase (Fig. 6). CenpH is necessary for cyclin B1 stabilization, which in turn plays an indispensable role in MPF activation. Moreover, APC/C^{Cdh1} is apparently the target of CenpH in mediating meiotic resumption. Thus, CenpH deficiency could have significant consequences for fertility by increasing the prophase I-arrested oocytes.

Materials and Methods

Antibodies

Mouse polyclonal anti-CenpH antibody used for Western blot and immunofluorescence was purchased from Abcam (Cat# ab88593); mouse monoclonal anti-α-tubulin-FITC antibody and mouse monoclonal anti-γ-tubulin antibody were obtained from Sigma-Aldrich Co (Cat# F2168, Cat# T6557);

human polyclonal anti-ACA antibody was purchased from Antibodies Incorporated (Cat# 15-234); mouse monoclonal anti-cyclin B1 antibody was purchased from Santa Cruz (Cat# sc-245); rabbit polyclonal anti-Cdc2 p34 (Tyr 15) antibody was purchased from Santa Cruz (Cat# sc-12340-R); mouse polyclonal anti-β-actin antibody was purchased from Santa Cruz Biotechnology; FITC-conjugated goat anti-mouse IgGand TRITC-conjugated goat anti-mouse IgG were purchased from Zhongshan Golden Bridge Biotechnology Co, LTD.

Oocyte collection and culture

Care and handing of 6-8 week-old ICR mice was conducted in accordance with policies promulgated by the Ethics Committee of the Institute of Zoology, Chinese Academy of Sciences. Oocytes were cultured in M16 medium (M7292, Sigma) supplemented with 200 μM 3-isobutyl-1-methylxanthine (IBMX) to maintain them at the GV stage. After specific treatments, oocytes were washed thoroughly and cultured in M2 medium (M7167, Sigma) to specific stages.

Microinjection of CenpH morpholino (MO) or cyclin B1-GFP mRNA

Microinjections were performed using a Narishige microinjector and completed within 30 minutes. For CenpH knockdown in mouse oocytes, CenpH MO 5'-ACGCCACAGAAAATAACCCAGCAGT-3' (Gene Tools, Philomath, OR) was diluted with nuclease-free water (Sigma) to give a 2 mM stock concentration. The same amount of negative control MO was used as control. Each oocyte was microinjected with 5-10 pL of MO. After microinjection, the GV oocytes were cultured for 24 hours in M2 medium supplemented with 200 μM IBMX for the depletion of CenpH. For cyclin B1-GFP overexpression, 1 μg/μL cyclin B1 mRNA solution was injected into the cytoplasm of the GV oocytes. For cyclin B1-GFP dynamic analysis, 20 ng/μL cyclin B1 mRNA solution was injected into the cytoplasm of the GV oocytes. For protein

expression, oocytes were arrested at GV stage in M2 medium supplemented with 200 μM IBMX for 1 hour. The same amount of GFP mRNA was injected as control. For blocking of CenpH protein function, GV or GVBD stage oocytes were microinjected with 5-10 pL of CenpH antibody (0.5 mg/ml). After antibody microinjection, oocytes were cultured in M2 medium to specific stages.

Immunofluorescence analysis

Immunofluorescence was performed as described previously (Zhang T, 2015;). For immunofluorescent staining, oocytes were fixed in 4% paraformaldehyde in PBS buffer for 30 minutes at room temperature. After being permeabilized with 0.5% Triton X-100 for 20 minutes, they were then blocked in 1% BSA-supplemented PBS for 1 hour at room temperature. For staining of CENP-H, ACA and γ-tubulin, oocytes were incubated overnight at 4°C with anti-CenpH antibody (1:100) or anti-ACA antibodies (1:40) or anti-γ-tubulin antibodies (1:200), respectively. After three washes in washing buffer, oocytes were incubated with FITC-conjugated goat-anti-mouse IgG (1:100) or cy5-conjugated goat anti-human IgG (1:500) or TRITC-conjugated goat anti-mouse IgG (1:100) for 2 hours at room temperature. For α-tubulin staining, oocytes were incubated with anti-α-tubulin-FITC antibodies for 2 hours at room temperature, oocytes were then washed 3 times in wash buffer, co-stained with Hoechst 33342 (10 mg/ml in PBS) for 15 min. These oocytes were mounted on glass slides and examined with a confocal laser-scanning microscope (Zeiss LSM 780 META, Germany).

Immunoblotting analysis

Immunoblotting was performed as described previously (Zhang et al., 2016). Briefly, a total of 100 mouse oocytes were collected in a 7μL 2x SDS buffer and heated for 5 min at 100°C. The proteins were separated by SDS-PAGE and then transferred to PVDF membranes. Following transfer, the

membranes were blocked in TBST containing 5% BSA for 2 hour at room temperature, followed by incubation overnight at 4°C with mouse polyclonal anti-CenpH antibody (1:500); mouse monoclonal anti-cyclin B1 (1:1000); rabbit polyclonal anti-Cdc2 p34 (Tyr 15) antibody (1:1000) and mouse polyclonal anti-β-actin antibody (1:1000). After three washes in TBST buffer, 10 minutes each, the membranes were incubated with 1:1000 HRP-conjugated goat anti-mouse or anti-rabbit or anti-goat IgG, for 1 hour at 37°C. Finally, the membranes were processed using the enhanced chemiluminescence-detection system (Bio-Rad, CA). The CenpH antibody recognized two bands on the western blot. Lower molecular weight band (28kD) is close to the expected molecular weight for CENP-H. Higher molecular weight band might represent a kind of postranslational modification.

Time-lapse live imaging experiments

Cyclin B1-GFP dynamics were filmed on a Perkin Elmer precisely Ultra VIEW VOX Confocal Imaging System. A narrow band passed EGFP filter set and a 30% cut neutral density filter from Chroma. Exposure time was set ranging between 300-800 ms depending on the cyclin B1-GFP fluorescence levels. The acquisition of digital time-lapse images was controlled by IP Lab (Scanalytics) or AQM6 (Andor/Kinetic-imaging) software packages. Confocal images of cyclin B1 in live oocytes were acquired with a 10x oil objective on a spinning disk confocal microscope (Perkin Elmer).

Image analysis

Images were acquired using a confocal laser-scanning microscope (LSM 780; Zeiss) equipped with a C-Apochromat 40 ×water immersion objective. Data analysis was performed using ZEN 2012 LSM software (Zeiss) and ImageJ software.

Statistical analysis

Data (mean ± SE) were generated from replicates that were repeated at least three times per experiment and analyzed by ANOVA using SPSS software (SPSS Inc., Chicago, IL) followed by student-Newman-Keuls test. Difference at $p < 0.05$ was considered to be statistically significant and different superscripts indicate the statistical difference.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

T.Z. Y.Z. L.L. Z.B.W. and W.S. performed the initial experiments. T.Z. H.S. and Q.Y.S. designed the study and prepared the manuscript.

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Figures

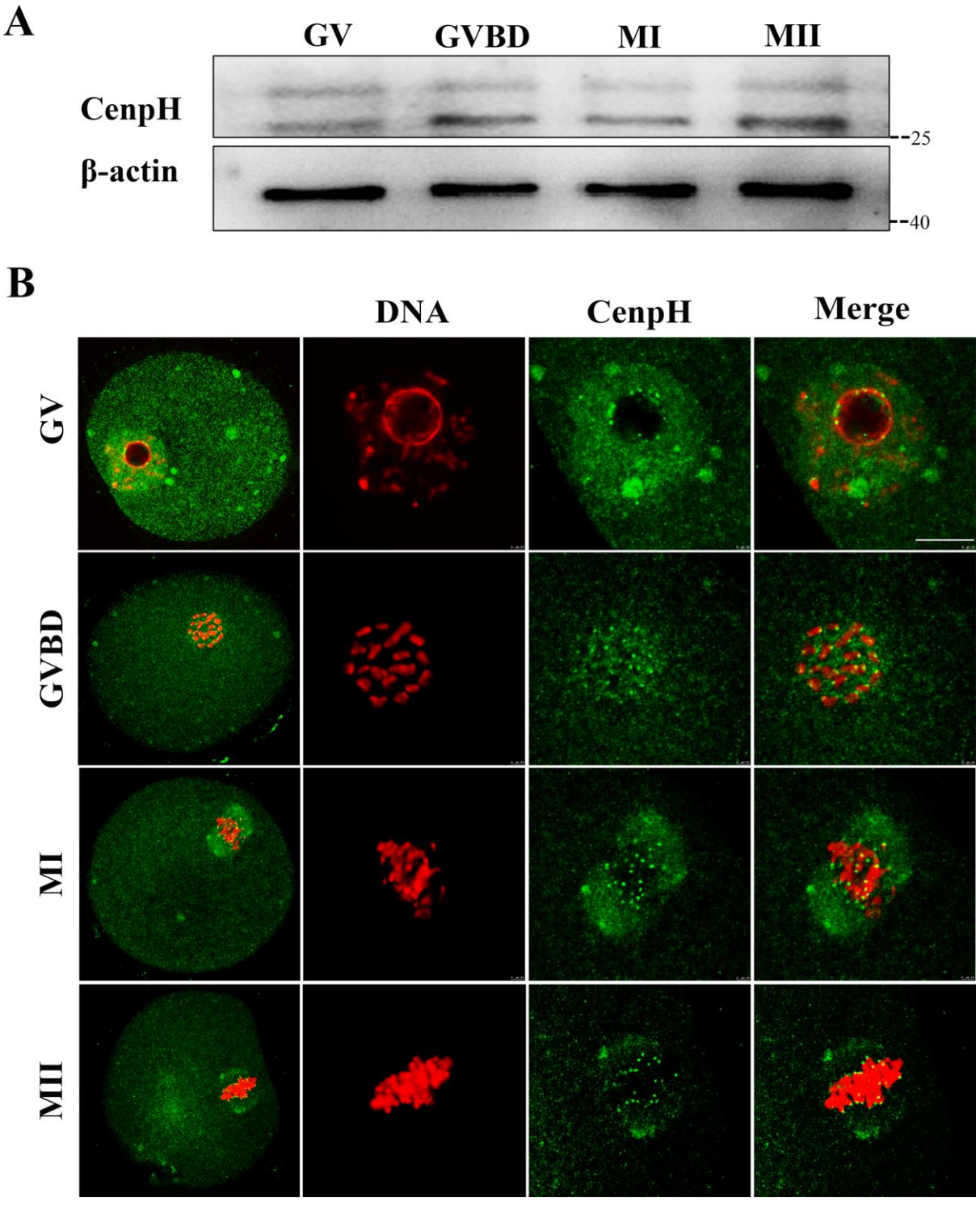

Figure 1. Expression and subcellular localization of CenpH during mouse oocyte meiotic maturation. (A) Expression of CenpH protein as revealed by Western blot analysis. Samples of 200 oocytes were collected after culture for 0, 4, 8 and 12 h, the time points when most oocytes had reached the GV, GVBD, MI and MII stages, respectively. (B) Confocal microscopy showing the subcellular localization of CenpH (green) in mouse oocytes at GV, GVBD, MI and MII stages. Note the localization of CenpH to kinetochores as well as spindle and poles at MI and MII stages. Also note that nonspecific CenpH antibody binding occasionally produces punctuate staining artefacts. DNA (red) was counterstained with Hoechst 33342. Scale bars: 10 μm.

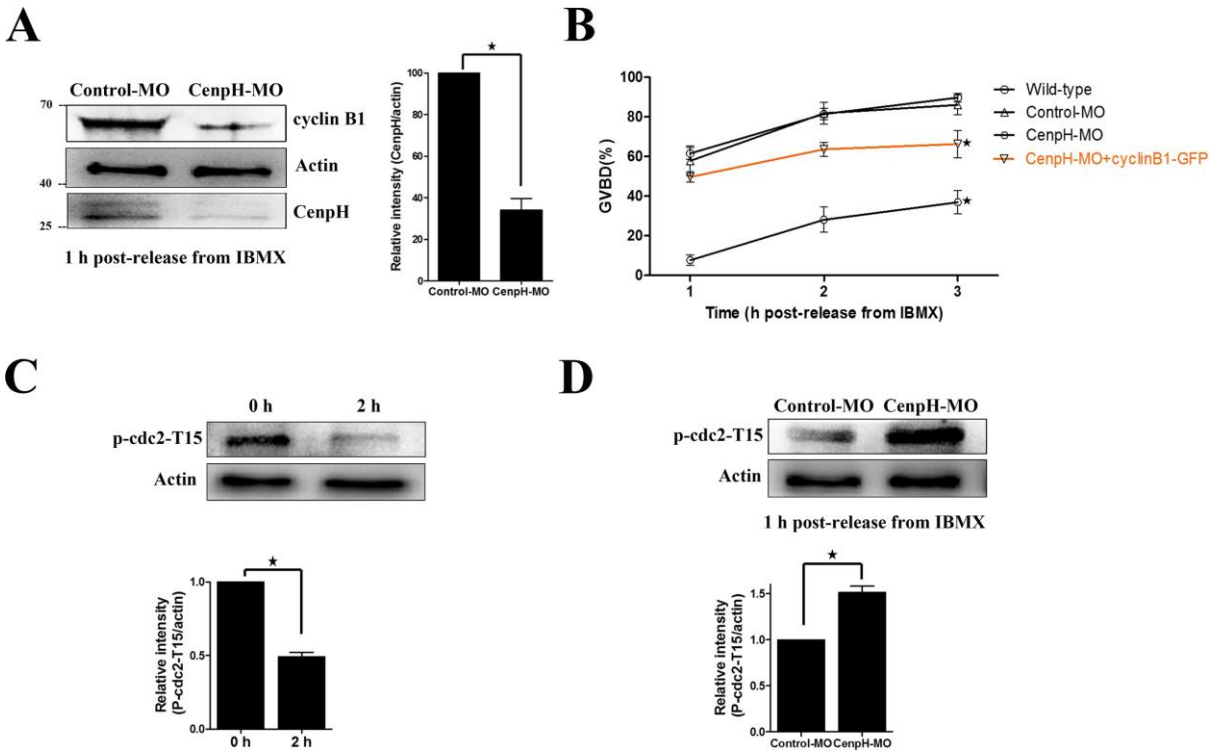

Figure 2. Depletion of CenpH impairs GVBD and MPF activity. (A) Western blotting results for CenpH; cyclin B1 and β-actin in the CenpH-MO and control-MO oocytes by 1 h following release from IBMX (150 oocytes per-sample). CenpH molecular mass is 28 kDa and that of β-actin is 43 kDa and that of cyclin B1 is 55 kDa. The relative staining intensity of CenpH was assessed by densitometry. (B) GVBD rates at 1, 2, and 3 h following release from IBMX for wild-type, control-MO, CenpH-MO, and CenpH-MO + cyclin B1-GFP oocytes. (C) The phosphorylation level of Tyr15 of Cdc2 in normal GV and GVBD oocytes. The relative staining intensity of p-cdc2-T15 was assessed by densitometry. (D) The phosphorylation levels of Tyr15 of Cdc2 in control-MO and CenpH-MO oocytes by 1 h following release from IBMX (150 oocytes per-sample). The relative staining intensity of p-cdc2-T15 was assessed by densitometry. Data are mean ± SEM. * significantly different ($p < 0.05$).

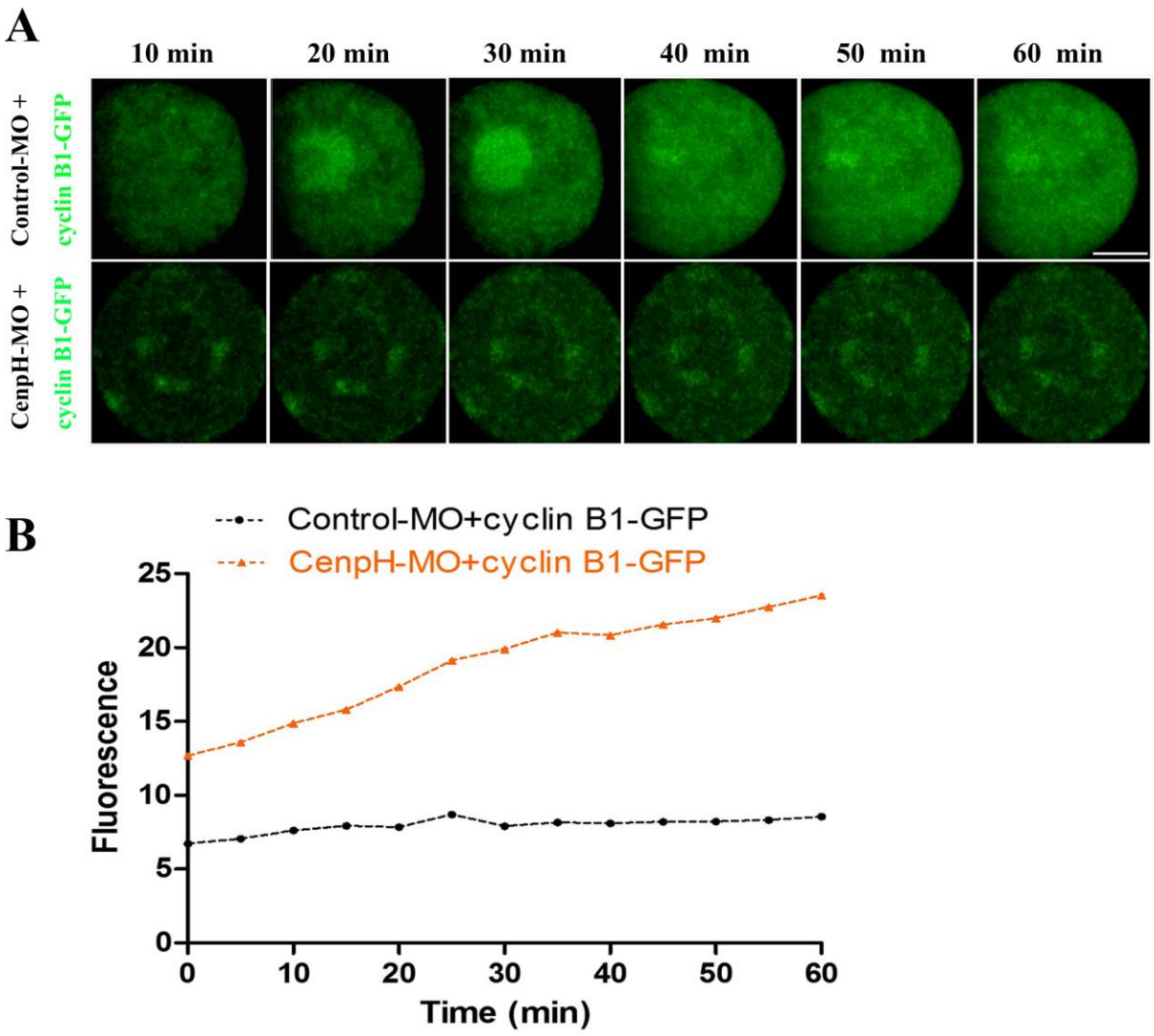

Figure 3. Depletion of CenpH causes increased level and failed nuclear entry of cyclin B1, as well as increased level of Cdh1 protein. (A) CenpH MO or control MO was microinjected into GV stage oocytes followed by 20 h incubation in 200 μM IBMX. Then cyclin B1-GFP mRNA was injected into CenpH-MO or wild-type oocytes. Following microinjection of cyclin B1-GFP mRNA, oocytes were maintained for 15min in 200 μM IBMX. Live cell imaging showed the dynamics of cyclin B1-GFP at 10 min intervals. (B) The relative fluorescence intensity of cyclin B1-GFP was assessed by densitometry. (C) The accumulation pattern of cyclin B1-GFP in the nucleus of CenpH-MO or control-MO oocytes. The boxed area fluorescence intensity analysis of each oocyte. (D) Western blotting results for Cdh1 and β-actin in the CenpH-MO and control-MO oocytes by 1 h following release from IBMX (150 oocytes per-sample). The relative staining intensity of Cdh1 was assessed by densitometry. Scale bars: 20 μm.

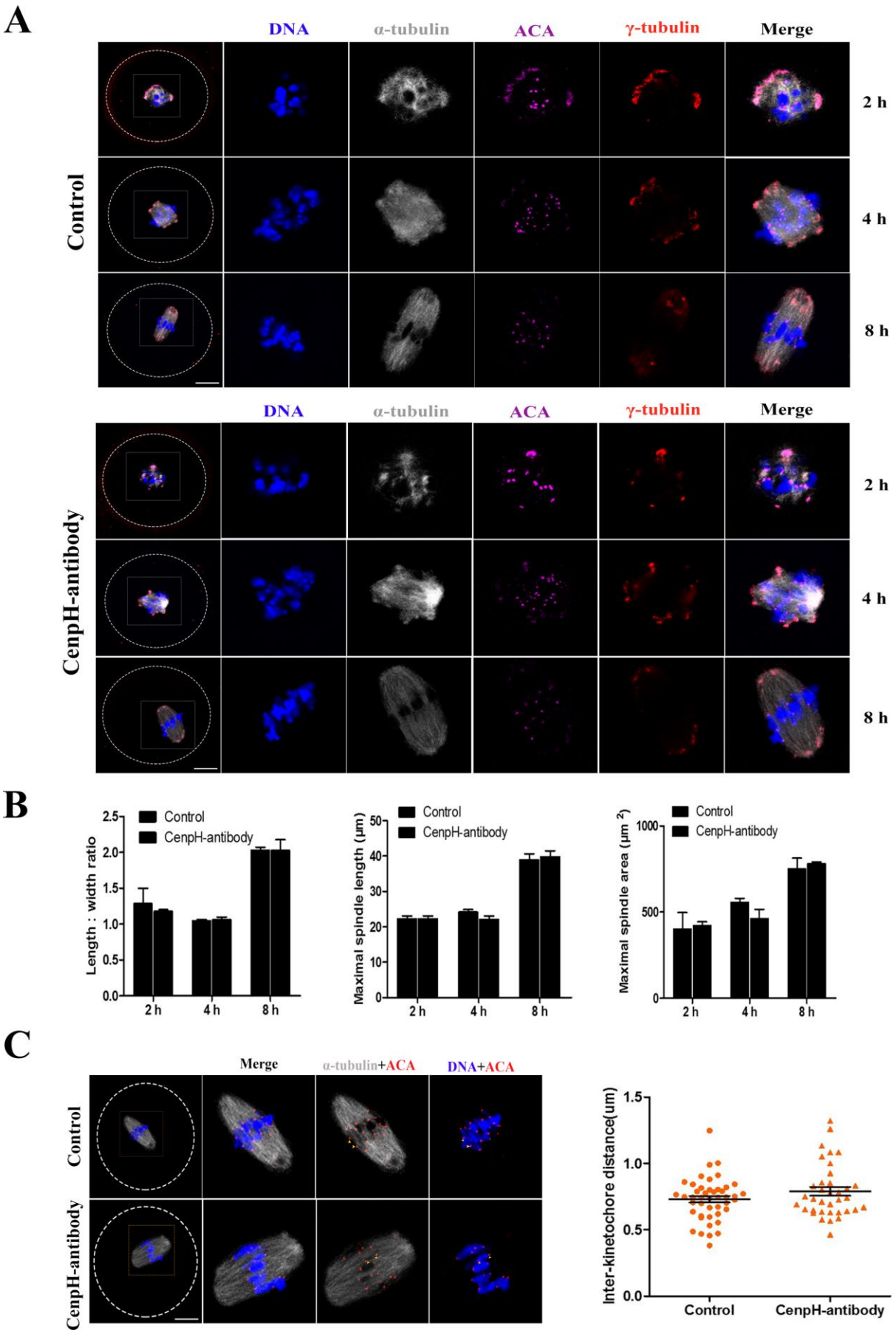

Figure 4. CenpH is not required for spindle assembly. CenpH antibody was microinjected into GVBD stage oocytes. (A) Confocal images of control and CenpH-antibody injected oocytes immunostained for DNA, kinetochores (ACA), microtubule organizing center (γ-tubulin), and microtubules (α-tubulin). (B) Graph showing length: width ratios; maximal spindle lengths and spindle areas for control and CenpH-antibody oocytes at 2 h (n=20 and n=12), 4 h (n=20 and n=14) and 8 h (n=18 and n=14). (C) Confocal images of control and CenpH-antibody oocytes immunostained for DNA, kinetochores (ACA), and microtubules (α-tubulin) at 8h (n=18 and n=14). The inter-kinetochore distance of control and CenpH-antibody oocytes was assessed. Data are mean ± SEM. Scale bars: 20 μm. The total numbers of analyzed oocytes are indicated (n).

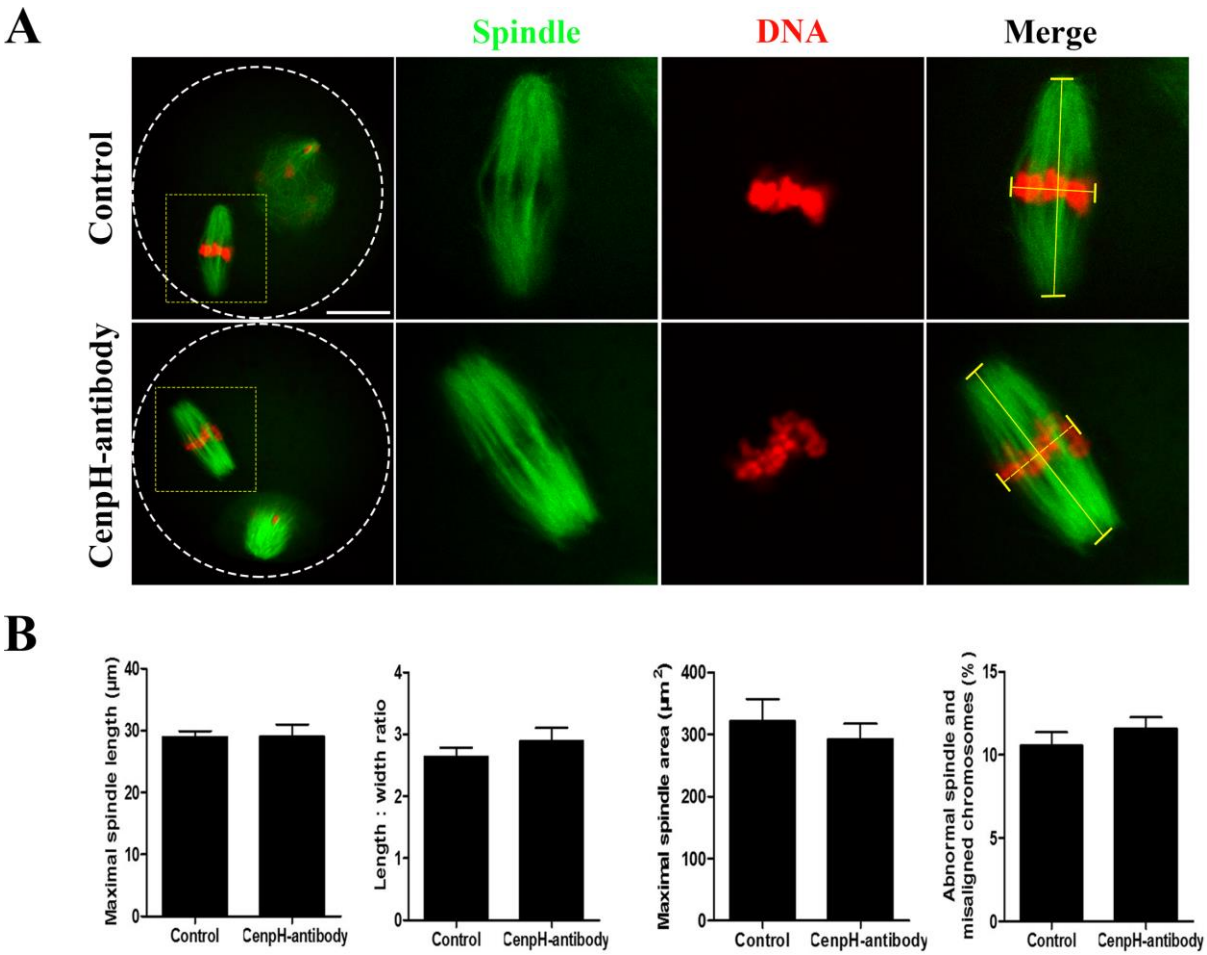

Figure 5. CenpH is not necessary for MII spindle morphology. CenpH antibody was microinjected into GVBD stage oocytes. (A) Confocal images of control and CenpH-antibody oocytes immunostained for DNA, and microtubules (α-tubulin) at 14h. (B) Graph showing maximal spindle lengths; length: width ratios and spindle areas for control and CenpH-antibody oocytes at 14 h (n=20 and n=16). The percentage of abnormal spindle and misaligned chromosomes in control and CenpH-antibody oocytes. Data are mean ± SEM. Scale bars: 20 µm. The total numbers of analyzed oocytes are indicated (n).

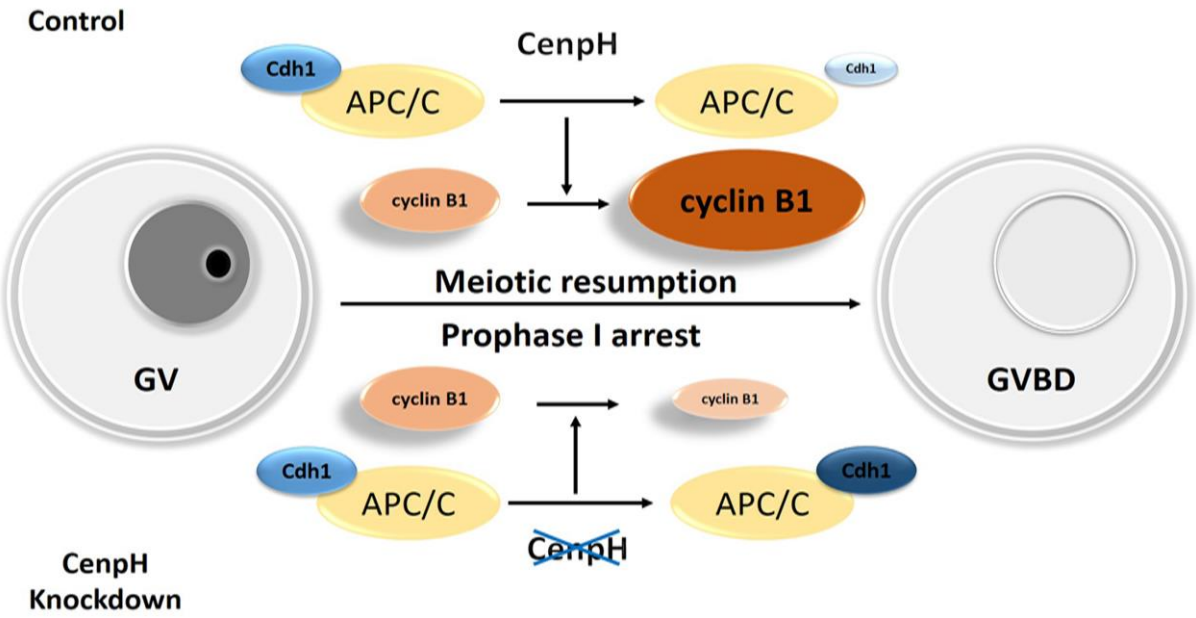

Figure 6. Proposed effects of CenpH depletion on meiotic resumption in mouse oocytes. Depletion of CenpH in mouse oocytes leads to elevated APC/C^{Cdh1}activity and instability of cyclin B1. One consequence is that the APC/C^{Cdh1} is apparently the target of CenpH in mediating meiotic resumption. The other consequence is that increased expression of APC/C^{Cdh1} perturbs cyclin B1 accumulation, resulting in prophase I arrest. Overall, CenpH is very important in balancing the G2/M transition in mouse oocytes.

Supplemental information:

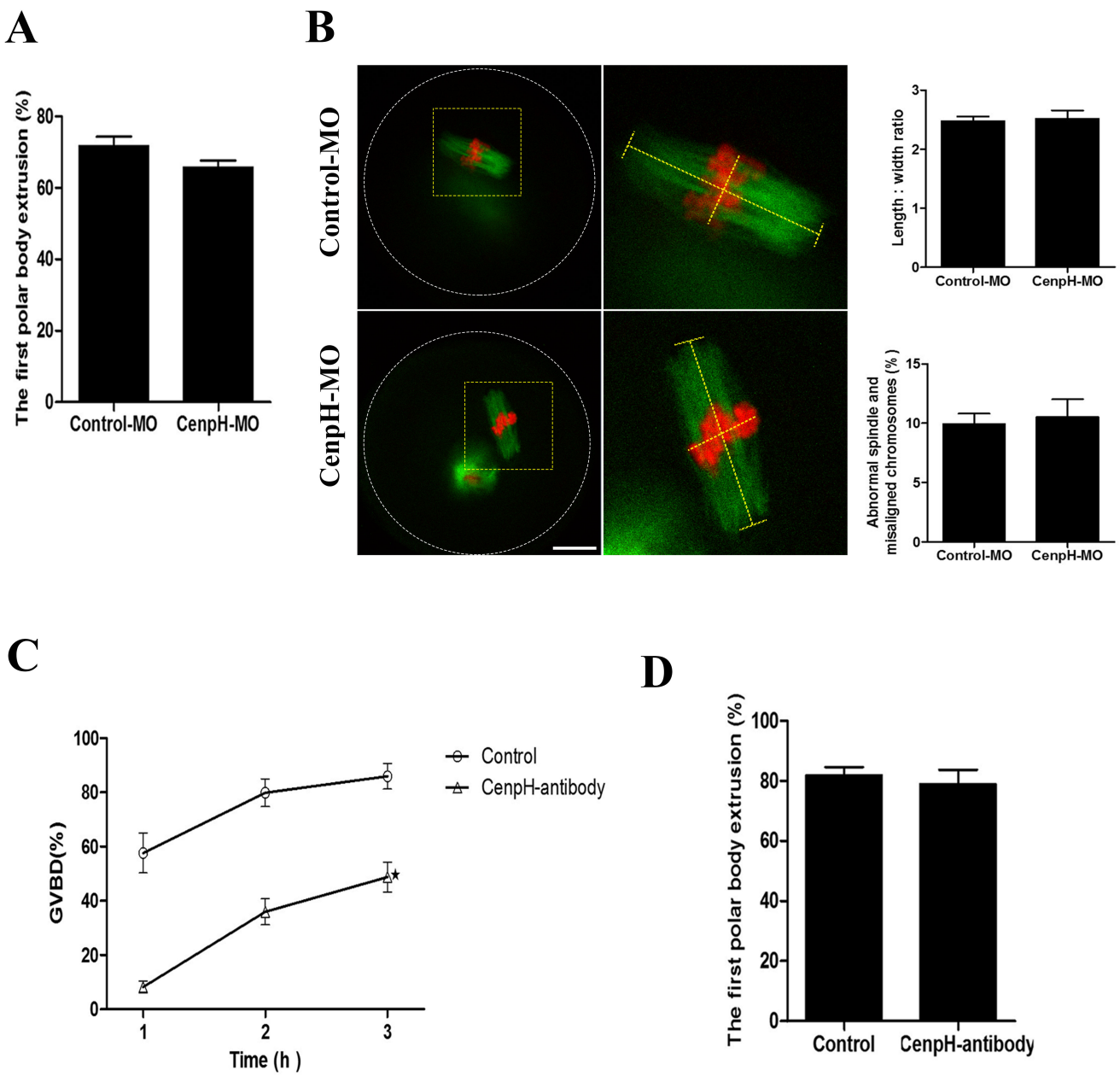

Figure S1. CenpH is not required for polar body extrusion and MII spindle morphology. (A and B) About 37% of injected CenpH MO oocytes underwent GVBD by 3 h following release from IBMX, and these oocytes were used. (A) The percentage of first polar body extrusion at 14 h following release from IBMX in control-MO and CenpH-MO oocytes. (B) Confocal images of control-MO and CenpH-MO oocytes immunostained for DNA, and microtubules (α-tubulin) at 14h. Graph showing length: width ratios; abnormal spindle and misaligned chromosomes for control-MO and CenpH-MO oocytes at 14 h (n=20 and n=18). (C) CenpH antibody was microinjected into GV stage oocytes. The percentages of GVBD at 1, 2 and 3 h following microinjection of CenpH antibody in control and CenpH-antibody oocytes. (D)CenpH antibody was microinjected into GVBD stage oocytes. The percentage of first polar body extrusion at 14 h following microinjection of CenpH antibody in control and CenpH-antibody injected oocytes. (E) CenpH-MO was injected into GV stage oocytes followed by 20 h incubation in IBMX. Subsequently, high-concentration of cyclin B1 mRNA was injected into these GV oocytes followed by 2h incubation in IBMX. The cyclin B1 protein levels of control-MO oocytes and CenpH-MO-cyclin B1-GFP oocytes were assessed by Western blot. (F) CenpH-MO was injected into GV stage oocytes followed by a 24 h incubation in IBMX to deplete the protein. The CenpH protein levels of control-MO oocytes and CenpH-MO oocytes (GV or GVBD) by 2 h following release from IBMX were assessed. Data are mean ± SEM.* significantly different ($p < 0.05$). Scale bars: 20 μm. The total numbers of analysed oocytes are indicated (n).