Aurora B regulates PP1γ-Repo-Man interactions to maintain the chromosome condensation state

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ABSTRACT

The mitotic kinase Aurora B regulates the condensation of chromatin into chromosomes by phosphorylating chromatin proteins during early mitosis, whereas the phosphatase PP1γ performs the opposite function. The roles of Aurora B and PP1γ must be tightly coordinated to maintain chromosomes at a high phosphorylation state, but the precise mechanisms regulating their function remain largely unclear. Here, mainly through immunofluorescence microscopy and co-immunoprecipitation assays, we find that dissociation of PP1γ from chromosomes is essential for maintaining chromosome phosphorylation. We uncover that PP1γ is recruited to mitotic chromosomes by its regulatory subunit Repo-Man in the absence of Aurora B activity, and that Aurora B regulates dissociation of PP1γ by phosphorylating and disrupting PP1γ-Repo-Man interactions on chromatin. Overexpression of Repo-Man mutants that cannot be phosphorylated or inhibition of Aurora B kinase activity resulted in the retention of PP1γ on chromatin and prolonged the chromatin condensation process; a similar outcome was caused by the ectopic targeting of PP1γ to chromatin. Together, our findings reveal a novel regulation mechanism of chromatin condensation in which Aurora B counteracts PP1γ activity by releasing PP1γ from Repo-Man and provide important implications for understanding the regulations of dynamic structural changes of the chromosomes in mitosis.

Fluctuations in kinase and phosphatase activities throughout the cell cycle ensure that both the initiation and shutdown of phosphorylation proceed normally (1,2). The mitotic kinase CDK1 phosphorylates a number of chromatin-associated proteins as well as many other substrates to promote DNA supercoiling, while the mitotic kinase Aurora B phosphorylates histone H3 to facilitate chromatin condensation (3-8). Aurora B also
stimulates Haspin to phosphorylate histone H3 at the chromosome arms for chromatin condensation (9-12). In contrast, the chromatin-associated protein phosphatase PP1γ dephosphorylates histone H3 as well as other phosphorylated chromatin-associated proteins to facilitate chromosome decondensation (13-16). A recent report analyzed primarily in vitro the global RV[S/T]F motif-containing proteins including Repo-Man that can be regulated by Aurora B and PP1, and revealed that phosphorylation of these proteins by Aurora B may disrupt the binding between PP1 and the RV[S/T]F motifs-containing proteins (17).

Despite these findings, how the cell manages to regulate the process of chromatin condensation in early mitosis remains elusive. The prevailing model of this process asserts that the activities of several mitotic protein kinases, including CDK1 and Aurora B, are most active in early mitosis, whereas the activity of PP1γ is reduced through its phosphorylation at T311 by CDK1/Cyclin B1 and complex formation with Inhibitor-1 (16,18,19). However, several recent lines of evidence support the important role of PP1γ in early mitosis (20-22) and suggest that the enzyme activity of PP1γ is not completely inhibited in early mitosis.

Repo-Man, a nuclear protein and specific regulatory subunit of PP1γ (23), is phosphorylated by CDK1/cyclin B and Aurora B in mitosis (24-26). Repo-Man was found to disperse in the cytoplasm as cells enter prophase and re-localize to chromatin at anaphase onset (15,24,26). Repo-Man also recruits PP1γ to chromatin in anaphase, in which it is responsible for the inactivation of regulator of chromosome architecture (RCA) (27), and in interphase, in which it is responsible for heterochromatin formation (28). In addition, Repo-Man recruits PP2A to chromatin under the regulation of Aurora B (26).

In this work, we report that Aurora B regulates the dissociation of PP1γ from chromosomes to maintain the chromosome condensation state and provide deep insight into the mechanisms of mitotic chromatin condensation and decondensation for duplicated genome separation in mitosis.

Results and Discussion

**PP1γ dissociation from chromosomes in early mitosis is essential for the maintenance of chromosome phosphorylation**

To investigate how protein kinases and phosphatases cooperate to maintain chromosome phosphorylation in early mitosis, we first tested the localization of Aurora B and PP1γ, as it was reported that their localization is dynamic (29,30). By expressing GFP-fused PP1γ and immunostaining for Aurora B, we found that, when the prophase cells went into metaphase, Aurora B accumulated on the chromosomes, whereas PP1γ dissociated from the chromosomes and relocated to the cytoplasm, mitotic spindle and kinetochores (Fig. 1A). When metaphase cells entered anaphase, Aurora B disassociated from the chromosomes
and relocated to the midzone, whereas PP1γ rejoined the separated chromosomes/chromatin (24,27). These results indicate that Aurora B and PP1γ are exclusively localized on chromosomes during mitosis.

It is well known that Aurora B can carry out autophosphorylation at T232 and phosphorylate histone H3 at S10 (31), and inhibition of Aurora B kinase activity resulted in a dramatic decrease in histone H3 S10 phosphorylation (13). In this work, we investigated Aurora B and PP1γ enzyme activities by adding the Aurora B inhibitor ZM447439 (32) and the phosphatase inhibitor okadaic acid (OA) (16) to mitotic cell extracts, followed by western blot analysis using specific antibodies. In the presence of ZM447439, Aurora B was dephosphorylated and lost its enzyme activity, rendering it unable to phosphorylate histone H3 at S10. In contrast, when we introduced OA into the extract, both Aurora B T232 and histone H3 S10 phosphorylation were maintained regardless of the presence of ZM447439 (Fig. 1 B), indicating the phosphatases were able to dephosphorylate histone H3 at S10 and Aurora B. As PP1γ is the major phosphatase responsible for chromosome histone dephosphorylation, we performed an in vitro dephosphorylation assay to test the enzyme activity of PP1γ during the cell cycle using immunoprecipitated GFP-PP1γ fusion protein from interphase or mitotic cells and purified mitotic histones as substrates. We found no difference in the abilities of PP1γ protein from cells at interphase and mitosis to dephosphorylate histone H3 at S10 (Fig. 1 C). In addition, we generated a triple fusion protein GFP-H2B-PP1γ and expressed it in cells (33,34) (Fig. 1 D and Fig. S1 A). As expected, this fusion protein was localized on mitotic chromosomes and did not affect the chromosomal localization of Aurora B (Fig. S1 B). However, the phosphorylation of histone H3 at S10 was significantly reduced in early mitosis when this fusion protein was expressed (Fig. 1 E and Fig. S1 C). In contrast, the phosphatase activity-null mutant GFP-H2B-PP1γ-D64A/H66A (20) had no obvious effect on the phosphorylation of histone H3 at S10 (Fig. 1 E). Together, these results demonstrate that PP1γ is active in early mitosis.

Next, we coexpressed GFP-PP1γ and RFP-H2B in cells. By time-lapse imaging, we found that coexpression of GFP-PP1γ or GFP-H2B-PP1γ-D64A/H66A with RFP-H2B did not affect progression of the cell cycle (Fig. 1 F and G). In contrast, we found that even mild expression of the triple fusion protein GFP-H2B-PP1γ remarkably prolonged the cell cycle and that overexpression of the triple fusion protein resulted in defects in chromosome alignment and cell cycle arrest at prometaphase (Fig. S1 D). Statistically, among mitotic cells overexpressing GFP-H2B-PP1γ, the percentage of prophase-to-metaphase cells greatly increased and the percentage of anaphase-to-telophase cells accordingly decreased (Fig. S1 E). Taken together, these results indicate that catalytically active Aurora B localizes on chromatin/chromosomes, where it
phosphorylates chromatin-associated proteins, and that active PP1γ disassociates from the chromatin to avoid dephosphorylating these chromatin-associated proteins.

**Aurora B antagonizes the chromosomal localization of PP1γ in early mitosis**

Then, we inhibited the activities of CDKs, Aurora A, Aurora B or Plk1 with their respective specific inhibitors. We found that PP1γ was retained on uncongressed chromosomes when the kinase activity of Aurora B was inhibited with AZD1152 (35) or ZM447439 (32). When these inhibitors were washed away, PP1γ was released from the chromosomes and relocated to the spindle and the cytoplasm, and the chromosomes were properly congressed (Fig. 2A and B). In contrast, inhibition of all other examined mitotic kinases (36, 37) or expression of an unphosphorylatable PP1γ mutant (S311A) (6,19) had no obvious effect on the localization of PP1γ to the spindle and the cytoplasm (Fig. S2A). Using purified mitotic chromosomes, we also demonstrated that the amount of chromosome-localized PP1γ increased when cells were treated with ZM447439 (Fig. 2C). Through ectopically targeting Aurora B to mitotic chromosomes by expressing a triple fusion protein GFP-H2B-Aurora B, we found that PP1γ could not be efficiently recruited onto the Aurora B-containing chromosomes in anaphase (Fig. S2B-E). Together, these findings demonstrate that kinase-active Aurora B promotes the release of PP1γ from the chromatin to maintain the phosphorylation states of the chromatin-associated proteins during early mitosis.

**Repo-Man recruits PP1γ to mitotic chromosomes in the absence of Aurora B activity**

It has been known that PP1γ forms complexes with regulatory subunits in a combinatorial manner (38,39), and Repo-Man was reported to bind PP1γ in the nucleus in interphase and to recruit PP1γ onto the chromosomes for chromosome dephosphorylation and decondensation in anaphase (15,23,27,40). We speculated that Repo-Man is involved in the process of PP1γ release from the chromatin under the regulation of Aurora B, as Repo-Man can bind and recruit PP1γ. To test this supposition, we first examined the associations among Repo-Man, PP1γ and Aurora B by sucrose density gradient ultracentrifugation assay, and the results showed that Repo-Man and PP1γ were concentrated in the same fractions in interphase, in which expression of Aurora B was absent (Fig. 3A). In mitotic cells, Repo-Man and Aurora B were localized in the same fractions, and most PP1γ shifted to lower-density fractions, which indicates that Repo-Man associated with Aurora B but disassociated with PP1γ in mitosis. However, when mitotic cells were treated with the Aurora B inhibitor AZD1152, Repo-Man and PP1γ reassociated with each other, regardless of the presence of Aurora B (Fig. 3A). We also knocked down Repo-Man in HeLa cells through RNAi, arrested the cells in early mitosis and
then treated the cells with ZM447339. We found that although Aurora B kinase activity was inhibited in Repo-Man-knockdown cells, the phosphorylation state of the Aurora B substrate histone H3 at S10 was not affected, indicating that Repo-Man depletion eliminated the recruitment of PP1γ onto chromosomes (Fig. S3 A). Given that during the normal cell cycle, PP1γ cannot be recruited onto chromosomes before anaphase (Fig. 1 A), we speculated that the binding of Repo-Man and PP1γ is spatiotemporally regulated. We further tested the expression levels of both endogenous Repo-Man and PP1γ during the cell cycle, and found they were expressed at relatively constant levels (Fig. S3 B). Through immunoprecipitation and pulldown assays, we found that the affinity of Repo-Man and PP1γ was largely enhanced in anaphase compared to in metaphase and after Aurora B inhibition (Fig. 3 B and Fig. S3 C-E), confirming that the binding of Repo-Man to PP1γ is inhibited by the kinase Aurora B during early mitosis.

To further explore the regulatory mechanisms, we then tested how AZD1152 treatment affects the localization of Repo-Man and PP1γ. The results showed that Repo-Man and PP1γ in control cells were largely recruited to the chromosomes in anaphase, as previously reported (15,23). However, when AZD1152 was added, PP1γ was quickly recruited to the chromosomes within 10 min and completely lost its spindle localization within 30 min (Fig. 3 C). Statistical analysis of the relative fluorescence intensity of chromosome-localized Repo-Man and PP1γ showed that both Repo-Man and PP1γ were recruited onto the chromosomes after Aurora B inhibition. Interestingly, we found that there are two stages, around 10 min and 30 min after AZD1152 treatment, during which the chromosome localization of PP1γ significantly increased (Fig. 3 D). By western blotting, we found that Aurora B kinase activity was inhibited at the 10-min timepoint by AZD1152, while CDK1 kinase activity was lost at the 30-min timepoint, as indicated by the degradation of Cyclin B1 (Fig. 3 E), although CDK1 has been reported to play inhibitory roles in Repo-Man and PP1γ recruitment (24,27). Cumulatively, these data strongly argue that the Aurora B has a dominant effect in PP1γ expulsion via regulating Repo-Man compared to CDK1.

We also noticed that although Repo-Man was largely localized on the chromosomes at anaphase, a small pool of Repo-Man was still detected on the chromosomes at early mitosis, and in some cases, staining for this pool was robust in the margin of the chromosomes (Fig. 3 C), a phenomenon also observed in methanol- or trichloroacetic acid (TCA)-fixed cells (Fig. S3 F-H) or in live cells (Fig. S4 A). Furthermore, by expressing truncated Repo-Man proteins, we identified a Del-N fragment that contributed to the localization of Repo-Man to the chromosomes of mitotic cells (Fig. S4 B and C), consistent with the results of a previous report (24). Taken together, these results demonstrate that a fraction of Repo-Man is closely associated...
with chromosomes during the entire process of mitosis.

**Aurora B phosphorylates Repo-Man on chromosomes arms and prevents it from recruiting PP1γ to chromosomes**

Since Aurora B promotes PP1γ dissociation from the mitotic chromosomes, we speculated that Aurora B antagonizes the localization of PP1γ on chromosomes through regulating binding of PP1γ with Repo-Man. To verify this hypothesis, by reciprocal immunoprecipitation assay, we confirmed that both Repo-Man and Aurora B coprecipitated in nocodazole-arrested mitotic cell extract (Fig. S5 A). We further titrated the binding domain of Repo-Man with Aurora B, and found it is in C-terminus (Fig. S5 A). We then co-stained cells for Repo-Man and Aurora B and observed that both Repo-Man and Aurora B colocalized on the condensed chromosomes in prophase (Fig. 4 A); however, in prometaphase and metaphase, although most Aurora B was concentrated at the centromeres, a fraction of Aurora B still colocalized with Repo-Man on the chromosome arms (Figs. 4 A, square 1 and 2). These results imply that Aurora B phosphorylates Repo-Man on the chromosomes.

To determine how Aurora B regulates the affinity of Repo-Man with PP1γ, we performed an *in vitro* assay of Aurora B kinase activity towards PP1γ and Repo-Man and used MCAK as a positive control (42). The results showed that Repo-Man, but not PP1γ, was directly phosphorylated by Aurora B (Fig. S5 B and C), consistent with a previous report (15). Next, we analyzed the sites of Repo-Man phosphorylated by Aurora B. Combining data from bioinformatic analysis and mass spectrometry (Fig. S5 D) and the *in vitro* kinase assay (Fig. S5 E), we identified three potential phosphorylation sites on the Repo-Man molecule (S543, S977 and S981). We generated phospho-null 3A (S543A, S977A and S981A) mutant Repo-Man and expressed it and wild-type Repo-Man in HeLa cells. The cells were then arrested in mitosis by nocodazole, followed by Phos-tag western blotting. We observed that the band for wild-type Repo-Man in mitosis was obviously above the wild-type Repo-Man band in interphase and the band of the 3A mutant. When the cells expressing wild-type Repo-Man or 3A were treated with Lambda-phosphatase or AZD1152, the wild-type Repo-Man upshifted band disappeared (Fig. S5 F and G). This result demonstrates that the three sites of Repo-Man (S543, S977 and S981) are phosphorylated by Aurora B in early mitosis. We noticed that, in addition to S543D mutation of full-length Repo-Man significantly reduced its phosphorylation, the sequence of Repo-Man CT fragment, hosting S977 and S981 resides and also similar to PP1 docking motifs RVxF, could bind with and be phosphorylated by Aurora B (Fig. S5 A and E). These indicate that the binding of Repo-Man with PP1γ might be regulated by Aurora B phosphorylation.

We further found that, although any single mutation of the three sites did not affect the chromosome localization of Repo-Man,
expression of phosho-null 3A but not phosphomimetic 3D mutant Repo-Man clearly promoted recruitment of PP1γ onto the chromosomes (Fig. S5 H, Fig. 4 B). Consistently, the phosphorylation of histone H3 at S10 was reduced in mitotic cells expressing 3A compared to those expressing 3D or wild-type Repo-Man (Fig. 4 C), likely because PP1γ was recruited by chromosome-bound 3A and dephosphorylated histone H3 at S10. Then, we tested the affinities of PP1γ with the 3A and 3D Repo-Man mutants, and the results showed that 3A clearly bound more PP1γ than 3D or the wild-type (Fig. 4 D). Therefore, we conclude that unphosphorylated Repo-Man binds PP1γ and that this binding is negatively regulated by Aurora B through the phosphorylation of Repo-Man. When Repo-Man is phosphorylated by Aurora B on chromosomes in early mitosis, PP1γ is not recruited by Repo-Man to the chromosomes.

**Forced retention of PP1γ on early mitotic chromosome arms prolongs mitotic progression**

By time-lapse imaging, we further found that overexpression of 3A mutant of GFP-Repo-Man also significantly affected the metaphase chromosome alignment and prevented the cell cycle progression (Fig. 4 E and F), consistent with overexpression of GFP-H2B-PP1γ obviously prolonged the cell cycle as mentioned above (Fig. 1 F and G, Fig. S1 C-E). In contrast, overexpression of 3D had only a slight effect on mitotic progression, as 3D could not bind and recruit PP1γ to chromosomes. Together, these results demonstrate that the phosphorylation of Repo-Man by Aurora B is crucial for the timely dissociation of PP1γ from the chromosomes to maintain the phosphorylation states of the chromatin-associated proteins.

In summary, our data suggest a unique model of which Aurora B and PP1γ balance the phosphorylation and dephosphorylation of the chromatin-associated proteins in the process of the chromatin condensation in early mitosis (Fig. 4 G). During the cell cycle, a fraction of Repo-Man is constantly localized on the chromatin/chromosomes. In interphase, this pool of Repo-Man recruits PP1γ to the chromatin by directly binding with PP1γ. From mitotic entry to metaphase, PP1γ remains phosphatase-active but dissociates from the chromosomes. A fraction of Repo-Man localizes at the margin of the chromosomes, where it is phosphorylated by Aurora B at S893 (15) to inhibit its massive chromosome localization and at S543, S977 and S978, which serves as an inhibitory signal for the interaction between Repo-Man and PP1γ, leading to dissociation of PP1γ from the mitotic chromosomes. Therefore, cells maintain chromosomes at highly phosphorylated state, while keep sensitive for the Aurora B kinase activity. If the kinase activity of Aurora B is abnormally reduced or lost, the mitosis progression could not be ensured. From the metaphase/anaphase transition to mitotic exit, due to relocation of Aurora B from the chromosomes to the midzone, the phosphorylated Repo-Man on the chromosomes
switches to a dephosphorylation phase by binding with and recruiting PP1γ to the anaphase chromosomes. Once recruited, PP1γ dephosphorylates the chromosome-associated proteins, including Repo-Man and histone H3 (at S10), and promotes the chromosomes decondensation into the chromatin. In conclusion, our present work uncovers a unique mechanism underlying the chromatin condensation in early mitosis. During the mitotic entry, the mitotic kinase Aurora B dissociates PP1γ from the chromatin/chromosomes by phosphorylating Repo-Man to prevent its untimely recruitment of PP1γ to the chromatin/chromosomes and the premature dephosphorylation of the chromatin-associated proteins.

Experimental procedures
Detailed Experimental procedures are put into the Supplemental Information. They primarily include plasmids and proteins, cell transfection, immunofluorescence, live-cell imaging, kinase inhibitors, antibodies, immunoprecipitation, in vitro kinase and dephosphorylation assays and chromosome purification.

Data availability
All the data described are contained within the article and accompanying supporting information.

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Conflict of Interest
The authors declare that they have no conflicts of interest regarding the contents of this article.

Author contributions
CMZ conceived of the project; GWX, JYF, JL and ZXD performed the experiments; and CMZ, GWX and QJ analyzed the data and wrote the manuscript.

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Figures and Legends

Figure 1. Forced retention of PP1γ on mitotic chromosomes prolongs mitotic progression. (A) HeLa cells expressing GFP-PP1γ were immunostained with antibody against Aurora B. (B) HeLa cells were treated with nocodazole (Noc) and then with Aurora B inhibitor ZM447439 (ZM) or phosphatase inhibitor Okadaic acid (OA) or both for 60 min, followed by western blotting (WB). (C) In vitro dephosphorylation assay. HeLa cells expressing GFP-PP1γ were synchronized at G1/S transition by double thymidine (Inter) or at prometaphase by nocodazole (0 hr) and released to the indicated time points. PP1γ was immunoprecipitated from the cell lysates and incubated with histone proteins purified from mitotic HeLa cells, followed by WB. (D) Diagram of GFP-PP1γ and mutant constructs. (E) HeLa cells expressing GFP-PP1γ or mutants were immunostained with H3-pS10 antibody. (F) HeLa cells expressing GFP-PP1γ or mutants were viewed by time-lapse microscopy. The time point of nuclear envelope breakdown (NEBD) was set as 00:00. (G) Quantification of the duration of mitosis (from NEBD to anaphase II) in (F). Over 30 cells were counted in each of three independent experiments. The data are mean ± s.d, and significance was assessed by performing t tests in GraphPad. **, p<0.01. Scale bars, 10 μm.

Figure 2. Aurora B kinase activity antagonizes PP1γ chromosome localization during early mitosis. (A) HeLa cells expressing GFP-PP1γ were synchronized at mitosis and treated with DMSO (control), Aurora B inhibitors AZD1152 or ZM447439 (ZM) for 60 min, or recovered from ZM treatment by wash-off for additional 60 min (ZM Wash-off), fixed by 10% TCA and stained with DAPI. (B) Quantitation of PP1γ chromosome localization in A. Over 30 cells were counted in each of three independent experiments. The data are mean ± s.d, and significance was assessed by performing t tests in GraphPad. ***, p<0.01. (C) HeLa cells were blocked at mitosis by nocodazole and treated with DMSO (-) or ZM447439 (+) for 60 min, then chromosomes were purified and analyzed by WB. RCC1 serves as marker for both cytoplasm and chromosome, while α-Tubulin as cytoplasm marker. (D) HeLa cells co-expressing RFP-PP1γ with GFP-Aurora B or GFP-H2B-Aurora B were fixed and stained with DAPI. Scale bars, 10 μm.

Figure 3. Repo-Man mediates PP1γ recruitment onto chromosomes via Aurora B inhibition. (A) Repo-Man and PP1γ were present in the same fraction in the absence of the kinase activity of
Aurora B. HeLa cells were arrested in interphase by Thymidine or at mitosis by nocodazole, or arrested at mitosis and treated with AZD1152 for 60 min, followed by sucrose density gradient ultracentrifugation and WB. (B) HeLa cells expressing GFP or GFP-Repo-Man were blocked at mitosis by nocodazole and treated with DMSO (-) or AZD1152(+) for 60 min, and subjected to pulldown assays with purified GST-PP1γ. (C) HeLa cells co-expressing GFP-Repo-Man and RFP-PP1γ were observed by time-lapse microscopy. The timepoint when DMSO (control) or AZD1152 was added was set as 00:00. Pictures were taken every 1 min. (D) Quantification of fluorescence intensity of Repo-Man and PP1γ on chromosomes. The relative fluorescence intensity of chromosome-localized Repo-Man and PP1γ was measured using Volocity software and the curves were fitted by GraphPad. (E) HeLa cells were first blocked by nocodazole, and then treated with AZD1152 for indicated time and analyzed through WB. Note that 10 min and 30 min are the timepoint when p-Aurora B and Cyclin B1 disappeared. Scale bar, 10 μm.

**Figure 4. Aurora B reduces Repo-Man/PP1γ complex affinity through phosphorylating Repo-Man on chromosome arms.** (A) HeLa cells expressing GFP-Repo-Man were immunostained with Aurora B antibody. Arrows indicate the chromosome-localized Aurora B. Squares 1 and 2 in (A) are zoomed into right panels with separated channels. (B) HeLa cells expressing RFP-PP1γ and GFP-Repo-Man WT, 3A or 3D were fixed by 10% TCA and stained with DAPI. (C) Lysates of HeLa cells expressing GFP-Repo-Man WT, 3A or 3D were analyzed with WB. (D) HeLa cells expressing GFP-Repo-Man WT, 3A or 3D were blocked by nocodazole and pulldown with purified GST-PP1γ, followed by WB analysis. (E) HeLa cells expressing GFP-Repo-Man WT, 3A or 3D were observed with time-lapse microscopy. Selected images at indicated time points were shown. The full videos can be seen in Supplementary Movies 1, 2 and 3. (F) Quantitative analysis of mitosis duration (from NEBD to Anaphase II) in (E). All the experiments were repeated at least three times, and more than 100 cells were measured for each sample. The data are mean ± s.d, and significance was assessed by performing t tests between the indicated groups in GraphPad. ***, p<0.01. (G) A model for PP1γ dissociation from the chromatin regulated by Aurora B (see main text for interpretation). Scale bars, 10 μm.
**Figure 4**

**A** Prophase, Prometaphase, Metaphase, Anaphase images with zoomed squares 1 and 2.

**B** GFP-Repo-Man WT, GFP-Repo-Man 3A, GFP-Repo-Man 3D images with PP1γ and Repo-Man.

**C** Western blot analysis for GFP-Repo-Man and H3-pS10 with WT, 3A, and 3D conditions.

**D** Gelatin zymography for GST-Pp1γ with WT, 3A, and 3D conditions.

**E** Time progression of mitosis from Interphase to NEBD, Prophase, Metaphase, Anaphase I, and Anaphase II for GFP-Repo-Man WT, 3A, and 3D.

**F** Bar graph showing mitosis duration time (min) for GFP-Repo-Man WT, 3A, and 3D conditions. NS (p = 0.4474) and ***p < 0.0001.

**G** Diagram of mitotic entry and exit with Repo-Man PP1γ, AurB, and microtubule interactions.
Aurora B regulates PP1γ-Repo-Man interactions to maintain the chromosome condensation state
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