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Journal of Cellular Biochemistry 116:1888–1897 (2015)

Journal of Cellular Biochemistry

Sirt1 Regulates Microtubule Dynamics Through Negative Regulation of Plk1 in Mitosis

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ABSTRACT

Although loss of Sirt1 leads to chromosome aneuploidy, which accounts for higher tumor susceptibility, the molecular mechanisms remain unclear. Herein, we demonstrate that Sirt1 directly regulates Plk1, of which activity is critical for mitotic progression and spindle dynamics. Depletion or inhibition of Sirt1 significantly perturbs the formation of the mitotic spindle, leading to defective chromosome segregation. Elevated depolymerization of the mitotic spindle following loss of Sirt1 was associated with the deregulation of Plk1 activity. Thus, we conclude that Sirt1 may contribute to a mitotic regulator that controls spindle dynamics through Plk1 activity, resulting in fine-tuning of Plk1 dependent microtubule dynamics. J. Cell. Biochem. 116: 1888–1897, 2015.

KEY WORDS: Sirt1; Plk1; MICROTUBULE DYNAMICS; NOCODAZOLE; MITOTIC ARREST

Silent information regulator 2 (Sir2) is a nicotinamide adenine dinucleotide-dependent histone deacetylase that participates in a number of critical physiological events, including aging, metabolism, and cancer [Saunders and Verdin, 2007]. The role of sirtuin 1 (Sirt1), a member of the Sir2 family, in cancer is controversial because the protein has been described as both a tumor suppressor and tumor promoter in a number of studies [Deng, 2009]. This contradiction arises from the diverse range of deacetylation substrates of Sirt1, which include tumor suppressors such as p53 and the fork-head class O transcription factor, as well as tumor promoters such as β-catenin, survivin, and nuclear factor κB [Deng, 2009]. Recent studies have shown that Sirt1 null mice are susceptible to genomic instability and defects in DNA damage repair, suggesting that Sirt1 functions as a guardian of the genome [Wang et al., 2008]. In addition, embryonic fibroblasts from Sirt1 null mice exhibit defects in mitotic chromosome condensation and segregation [Wang et al., 2008; Fatoba and Okorokov, 2011], which may underlie delayed mitotic progression [Sasaki et al., 2008]. Nevertheless, the molecular mechanisms by which Sirt1 affects mitotic chromosome integrity are not fully understood.

Mitotic chromosome congression and equal segregation are regulated by the timely formation of the mitotic spindle, which is

Jin-Ju Kim and Na-Yeon Gil contributed equally to this work.
The authors declare no competing financial interests.

Grant sponsor: Korea Science and Engineering Foundation (KOSEF) of the Korean government (MOST); Grant sponsor: Scientific Research Center (SRC) program; Grant number: 2011-030043; Grant sponsor: Radiation Research Center; Grant number: 2012055464; Grant sponsor: Medical Science Research Center (MRC) program; Grant number: 20110030700.

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Manuscript Received: 14 October 2014; Manuscript Accepted: 17 February 2015
Accepted manuscript online in Wiley Online Library (wileyonlinelibrary.com): 3 March 2015
DOI 10.1002/jcb.25144 • © 2015 Wiley Periodicals, Inc.

closely associated with maintaining genomic stability [Kops et al., 2005]. Errors in spindle formation can halt mitotic progression to ensure the maintenance of chromosome integrity [Lara-Gonzalez et al., 2012]. Proper formation of the mitotic spindle is determined by the dynamic action of microtubule (MT) polymerases and depolymerases [Gadde and Heald, 2004]; thus, tightly regulated turnover of MTs is critical for placing the mitotic spindle at the mitotic equator. During anaphase, spindle dynamics (active spindle turnover) generate a pulling force across sister kinetochores to promote mitotic segregation [Jang et al., 2008], which is under stringent control by the spindle assembly checkpoint (SAC) [Musacchio and Salmon, 2007]. Once all of the kinetochores are attached to MTs, the MT depolymerase activity increases to provide a driving force for equal chromosome segregation during anaphase [Musacchio and Salmon, 2007]. Polo-like kinase 1 (Plk1), which is critical for normal mitotic progression, regulates the enzymatic activity of kinesin family member 2a, an important mitotic MT depolymerase [Jang et al., 2009]; hence, the regulation of Plk1 activity is crucial for controlling not only mitotic progression, but also mitotic chromosome segregation. However, other than activation of the protein by phosphorylation, little is currently known about the molecular mechanisms underlying the regulation of Plk1 activity [Qian et al., 1998; Jang et al., 2002; Macurek et al., 2008; Tang et al., 2008]. Here, we demonstrate that the Sirt1 expression level is inversely correlated with the sensitivity of cells to nocodazole (Noc), a MT depolymerizing agent. This relationship is a result of the direct regulation of Plk1 by Sirt1 and subsequent alteration of MT dynamics. The data presented here suggest that Sirt1 functions as a novel modulator of Plk1 and MT dynamics, which are important for proper mitotic chromosome congression and segregation.

MATERIALS AND METHODS

REAGENTS AND CELL CULTURE

The Sirt1 (cat#: SC-15404), Plk1 (cat#: SC-5585), ERK2 (cat#: SC-154), HA (cat#: SC-7392), Myc-9E10 (cat#: SC-40) antibodies were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). The anti-phospho-Plk1 (Threonine 210, cat#: ab12157) and pan acetyl lysine (cat#: ab21623) antibodies were obtained from Abcam (Cambridge, UK). The phospho-MEK1/2 (S217/221, cat#: 9121S), antibody was obtained from Cell Signaling Technology (Danvers, MA). The phospho-Histone H3 (cat#: LFPA20214) antibody was obtained from Abfrontier (Seoul, Korea). Anti-β-tubulin E7 monoclonal antibody (mAb) was obtained from the Developmental Studies Hybridoma Bank at the University of Iowa. HEK 293T and HeLa cells were maintained in Dulbecco's Modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum and gentamycin (50 μg/ml). In some experiments, cells were synchronized via a double thymidine block or by mitotic shake-off as described previously [Jeong et al., 2015].

GENE DELIVERY

Sirt1-expressing HeLa and HEK293T cells were generated as previously described [Lee et al., 2012]. siRNAs were synthesized by Dharmacon, Inc. The Sirt1 siRNA sequence was 5′-CUGUGAAAUUA-CUGCAAGATT-3′, and the control siRNA (siGL2) sequence was 5′-CGTACGCGGAATACTTCGATT-3′. siRNAs were transfected into HeLa cells with DharmaFect 1 (Dharmacon, Inc., Lafayette, CO).

IMMUNOBLOTTING AND IMMUNOFLUORESCENCE

Immunoblot and immunofluorescence analysis were performed as previously described [Jeong et al., 2015].

IMMUNOPRECIPITATION AND IN VITRO KINASE ASSAY

Immunoprecipitation was preformed as previously described [Jeong et al., 2015]. In case of in vitro kinase assay, immunoprecipitated Plk1 was washed with kinase buffer (25 mM Hepes pH 7.4, 25 mM $MgCl_2$ and 1 mM DTT). In vitro kinase assays were conducted in a kinase buffer of 25 mM Hepes (pH 7.4), 15 mM $MgCl_2$, 1 mM dithiothreitol, 20 mM unlabeled ATP, and 10 μCi/μl of [γ-^{32}P] ATP using α-casein as a substrate for Plk1. The incorporation of phosphate into substrates was evaluated via PhosphorImager analysis (Storm 840) (Amersham Bioscience, Piscataway, NJ).

LIVE CELL IMAGING

For time-lapse microscopy, HeLa cells stably expressing GFP-H2B were cultured in Leibovitz's L-15 medium (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen) and 2 mM L-glutamine (Invitrogen). Cells were placed into a sealed growth chamber heated to 37°C and observed on a Zeiss Axiovert 200M microscope with a 0.4 NA Achro-plan 20x lens. Images were acquired every 3 min for 5 h with Axiovision 4.0 software (Carl Zeiss, Thornwood, NY).

MT REPOLYMERIZATION ASSAY

The experiment was performed as described previously [Jang et al., 2008]. In more detail, HeLa cells were treated either with 1 μg/ml of nocodazole (Merck Millipore, USA) for 10 min at 37°C or incubated under cold stress condition (4°C for 35 min) for depolymerizing microtubule. Then cells were washed with pre-warmed PBS twice and released into fresh media. MT fluorescence intensity of metaphase cells ($n = 10$, determined by IFC with anti-β-tubulin antibody) was quantified.

STATISTICAL ANALYSIS

The graphical data were presented as mean ± SEM. Statistical significance among groups and between groups were determined using one-way or two-way analysis of variance (ANOVA) following Bonferroni post-test and Student's *t*-test, respectively. Significance was assumed for $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***).

RESULTS

Sirt1 CONTROLS CHROMOSOME CONGRESSION AND SEGREGATION

Loss of Sirt1 results in the formation of chromosome bridges and lagging chromosomes, suggesting that uneven chromosome segregation between the daughter cells is responsible for the chromosome aneuploidy observed in Sirt1 null mice [Wang et al., 2008]. Analogous to the aforementioned report, to address the importance of Sirt1 in controlling chromosome segregation, we depleted Sirt1 using a specific siRNA and examined the mitotic progression of HeLa cells. As reported previously [Haigis and Guarente, 2006], Sirt1 localized to the nucleus in interphase cells, whereas mitotic Sirt1 was distributed diffusely following nuclear envelope breakdown (Fig. 1A and Fig. S1). Immunoblotting confirmed that Sirt1 protein levels were downregulated in HeLa

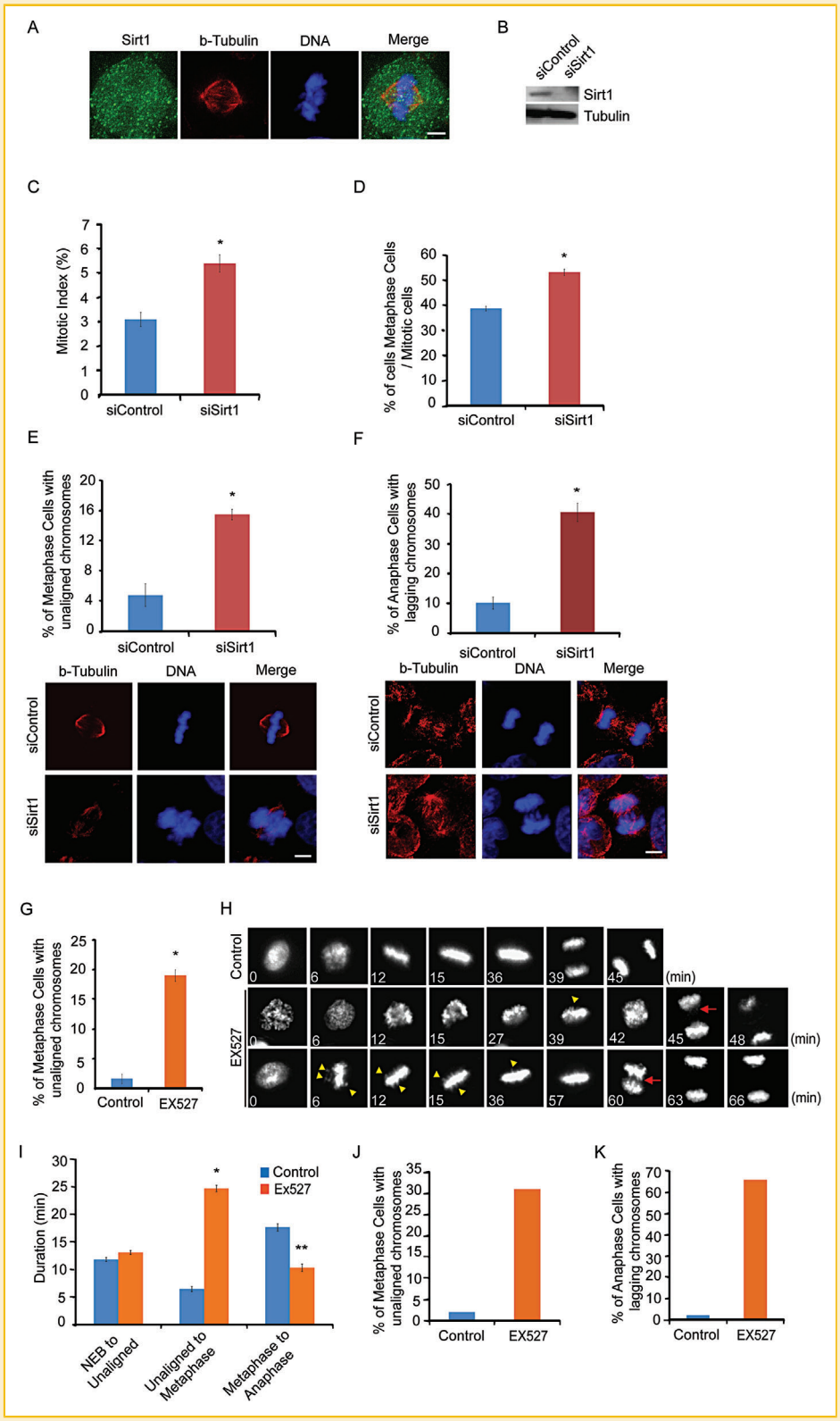

Fig. 1. Sirt1 controls chromosome congression and segregation. A: A mitotic HeLa cell, immunostained with β-tubulin (red) and Sirt1 (green) and counterstained with DAPI (blue). B: HeLa cells were transfected with control (siControl) or Sirt1-specific siRNAs (siSirt1), and analyzed by Western blotting. Tubulin served as a loading control. C: Mitotic index was determined by FACS analysis using an anti-MPM2 antibody that recognizes mitotic phospho-proteins 72 h after siRNA transfection. D: The percentage of metaphase cells over total mitotic cells. E: The percentage of metaphase cells with unaligned chromosomes over total metaphase cells. F: The percentage of anaphase cells with lagging chromosomes were quantified and plotted. G: HeLa cells were treated with 0.5 μM of Ex527 for 5 h at 37°C, and the percentage of metaphase cells with unaligned chromosomes over total metaphase cells were quantified and plotted. H: HeLa/GFP-Histone H2B cells were treated with either vehicle (DMSO: Control, top panel) or 0.5 μM of Ex527 (middle and bottom panels) and imaged for GFP-Histone H2B by time-lapse starting from 2 h post-treatment. Images were captured every three minutes to monitor mitotic progression. Still frames from time-lapse movies of representative cells are shown. Arrowheads point to unaligned chromosomes. I: The duration from nuclear envelop breakdown (NEB) to the formation of a bipolar spindle/metaphase plate ("NEB to Metaphase") and from metaphase to anaphase ("Metaphase to Anaphase") were determined for control and Ex527 treated cells ($n = 40$ cells). J: The percentage of metaphase cells with unaligned chromosomes over total metaphase cells and (K) the percentage of anaphase cells with lagging chromosomes were quantified and plotted. Scale bar, 5 μm, $^{*}P < 0.005$, $^{**}P < 0.001$.

cells transfected with a Sirt1-specific siRNA (Fig. 1B). Consistent with a previous report showing an increased 4N peak after Sirt1 knockdown [Fatoba and Okorokov, 2011], the mitotic index was significantly higher in Sirt1-depleted HeLa cells than HeLa cells transfected with a non-specific control siRNA (Fig. 1C). A closer examination of the mitotic cells revealed that the metaphase population was also increased in the absence of Sirt1 (Fig. 1D). Notably, as reported previously [Wang et al., 2008; Fatoba and Okorokov, 2011], the number of metaphase cells with unaligned chromosomes or a chromosomal bridge was threefold higher in the Sirt1-depleted group than the control group (Fig. 1E,F). Consequently, we propose that Sirt1 is important for proper chromosome congression and segregation during mitosis.

Because Sirt1 displays functions that are independent of its deacetylase activity [Pfister et al., 2008], it is important to determine whether the mitotic role of Sirt1 in chromosome congression and segregation is accomplished via deacetylation of specific mitotic substrates. To this end, we performed experiments using EX527, a specific chemical inhibitor of Sirt1 [Peck et al., 2010]. Similar to the effect of depletion of Sirt1, treatment of HeLa cells with EX527 increased the number of metaphase cells containing unaligned chromosomes significantly (Fig. 1G), suggesting that the deacetylase activity of Sirt1 is required for proper chromosome congression. Time-lapse imaging of HeLa cells stably expressing GFP-tagged histone H2B after double thymidine block and release, demonstrated that mitotic chromosome congression and segregation was delayed in EX527-treated cells compared with control cells (Fig. 1H,I). This delay in the metaphase-to-anaphase transition was accompanied by an increase in the percentage of unaligned chromosomes in metaphase cells (Fig. 1H, red arrows, and Fig. 1J). Consistent with the effect of Sirt1 depletion, there was also a noticeable increase in the number of anaphase cells with lagging chromosomes following Sirt1 inhibition (Fig. 1K), indicating that MT dynamics, which are critical for normal mitotic congression and segregation, can be altered by Sirt1 inhibition.

Sirt1 DOES NOT DEACETYLATE MTs

Although one study demonstrated that loading of histone H1 and the condensin I complex onto the chromosome is disrupted following Sirt1 depletion [Fatoba and Okorokov, 2011], the mechanisms underlying the observed mitotic chromosome anomalies in Sirt1 knockout [Wang et al., 2008] and depleted [Fatoba and Okorokov, 2011] cells are largely unknown. To understand these molecular mechanisms in more detail, we investigated the putative mitotic substrates of Sirt1. Sirt2 functions as a tubulin deacetylase [North et al., 2003] and acetylation is an important post-translational modification of tubulin that increases its stability and sensitivity to MT damaging agents [Piperno et al., 1987]. Because Sirt1 and Sirt2 are both class I sirtuins, we hypothesized that Sirt1 also targets tubulin during mitosis. To test this hypothesis, HeLa cells transfected with a Sirt1-specific or non-specific control siRNA were immunostained with an anti-acetylated tubulin antibody. Although Sirt1 depletion increased the percentage of unaligned metaphase chromosomes (Fig. 1E), it had no effect on the level of acetylated tubulin (Fig. S2). This finding indicates that, unlike Sirt2, Sirt1 does not affect tubulin stability by direct deacetylation.

Sirt1 DELAYS NOC-INDUCED MT DEPOLYMERIZATION

Considering the critical role of MT dynamics in mitotic chromosome congression and segregation, we examined whether Sirt1 expression or enzymatic activity is associated with the cellular response to alteration of MT dynamics. In this experiment, MT stress was induced by treating HeLa cells with Noc, a MT depolymerizing agent, and the level of mitotic arrest was monitored. Following siRNA-mediated depletion of Sirt1, mitotic arrest, indicated by the induction of a 4N peak, was facilitated markedly by Noc treatment (Fig. 2A), while Sirt1 knockdown by siRNA did not noticeably altered cell cycle profile determined by FACS analysis (Fig. S3). Based on this result, we speculated that ectopic expression of Sirt1 would adversely attenuate the mitotic arrest caused by Noc treatment. To test this hypothesis, HeLa cells expressing myc-tagged Sirt1 (Sirt1-HeLa) were generated (Fig. 2B). Unlike Sirt1 depletion, increasing mitotic population (Fig 1C), overexpression of Sirt1 did not affect the mitotic index as well as cell cycle profile (Fig. 2C,D). Additionally, mitotic chromosome congression and/or segregation remained unaltered by Sirt1 ectopic expression (Fig. S4).

However, Noc-induced mitotic arrest was attenuated in Sirt1-HeLa cells for up to 2 h (Fig. 2E). Given that Noc arrests cells in mitosis by interfering with MT polymerization, these results suggest that Sirt1 may be involved in MT polymerization either directly or indirectly.

Sirt1 DOWNREGULATES NOC-INDUCED Plk1 SIGNALING

The relationship between Sirt1 expression and sensitivity to Noc-induced mitotic arrest was confirmed by immunoblot analyses of the well-established mitotic markers phosphorylated histone H3 [Hans and Dimitrov, 2001] and phosphorylated nucleophosmin (NPM) [Cha et al., 2003; Zhang et al., 2004]. Stable expression of Sirt1 (Myc-Sirt1) suppressed Noc-induced phosphorylation of both of these markers in HeLa cells (Fig. 3A). Given that mitotic phosphorylation of NPM is mediated by active Plk1 [Zhang et al., 2004], which is important for proper functioning of the SAC [Seong et al., 2002] as well as normal MT dynamics [Jang et al., 2009], we hypothesized that Plk1 activity might be altered in Noc-treated Sirt1-HeLa cells. As expected, the Noc-induced increases in the levels of phosphorylated (T210) Plk1 and phospho-NPM were smaller in Sirt1-HeLa cells than control cells (Fig. 3B). In an independent experiment, Noc-induced phosphorylation of translationally controlled tumor protein (TCTP), a marker of Plk1 activation [Cucchi et al., 2010], was also attenuated in cells expressing Sirt1 (Fig. 3C). Since both depletion of Sirt1 and inhibition of its enzymatic activity caused defects in mitotic chromosome congression and segregation (Fig. 1), we determined whether Plk1 activity is affected by the deacetylase activity of Sirt1. To this end, we generated a HeLa cell line stably expressing the Sirt1HY mutant (Myc-Sirt1HY), which has defective deacetylase activity [Vaziri et al., 2001]. Noc-induced Plk1 activity, determined by the levels of phospho-NPM and phospho-TCTP, was markedly higher in cells expressing Sirt1HY-HeLa than those expressing Sirt1-HeLa (Fig. 3D).

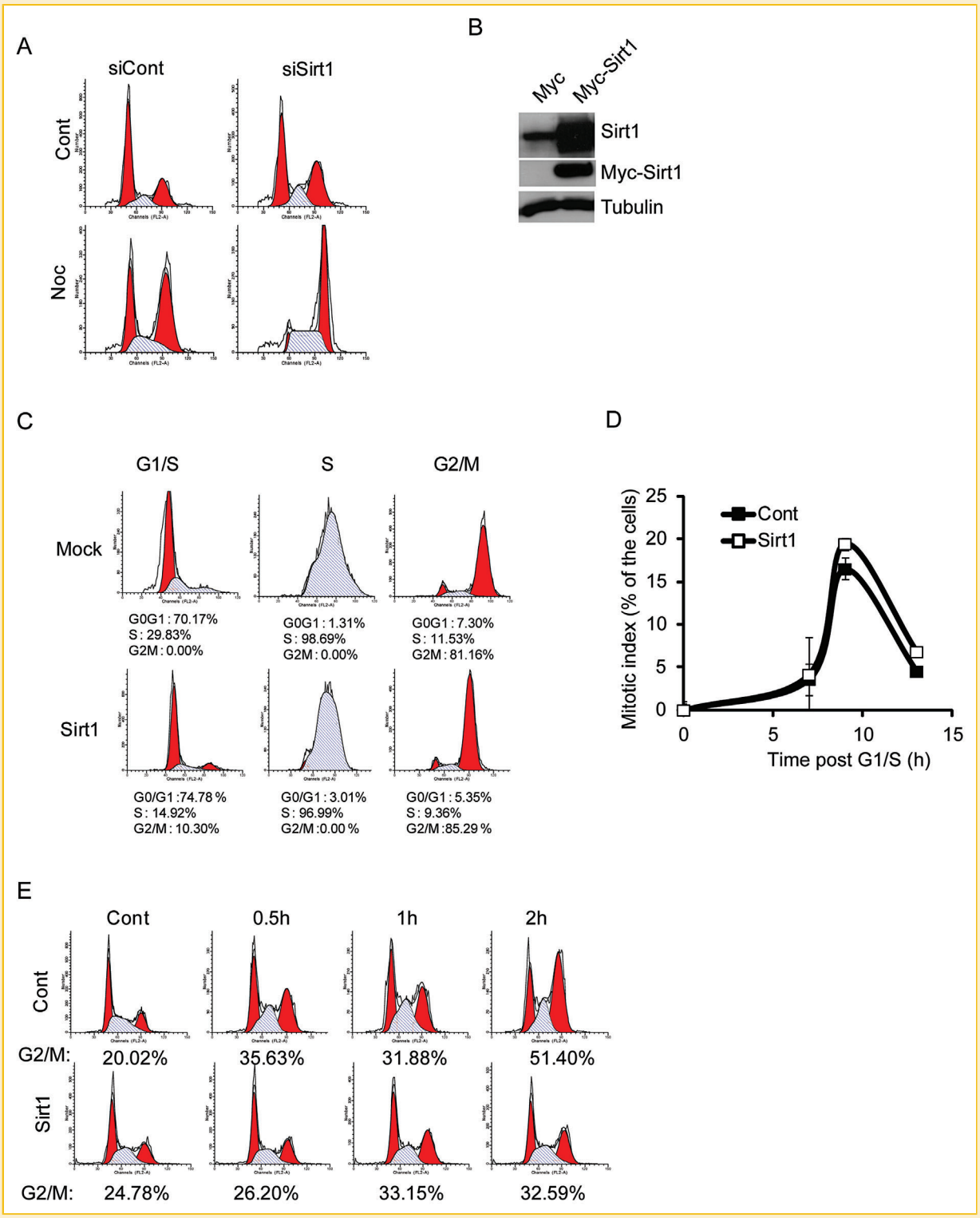

Fig. 2. Sirt1 delays a nocodazole-induced MT depolymerization. A: HeLa cells transfected with either control siRNA or Sirt1 siRNA were treated with or without 0.5 μg/ml of Nocodazole (Noc) and harvested after 6 h for FACS analysis. B: The protein lysate, obtained from Mock-HeLa and Sirt1-HeLa was analyzed by immunoblotting analysis. Tubulin for an equal loading control. C: FACS profile of Sirt1-HeLa and Mock-HeLa at indicative stage of cell cycle was presented. D: Mitotic index (determined by phosphor-Histone H3 staining) of Sirt1-HeLa and Mock-HeLa at indicative time after thymidine release was quantified and presented as a graph. E: Mock-HeLa and Sirt1-HeLa were treated with 0.5 μg/ml of Nocodazole and harvested at the times indicated for FACS analysis. The percentage of 4N population was presented at the bottom of each FACS profile.

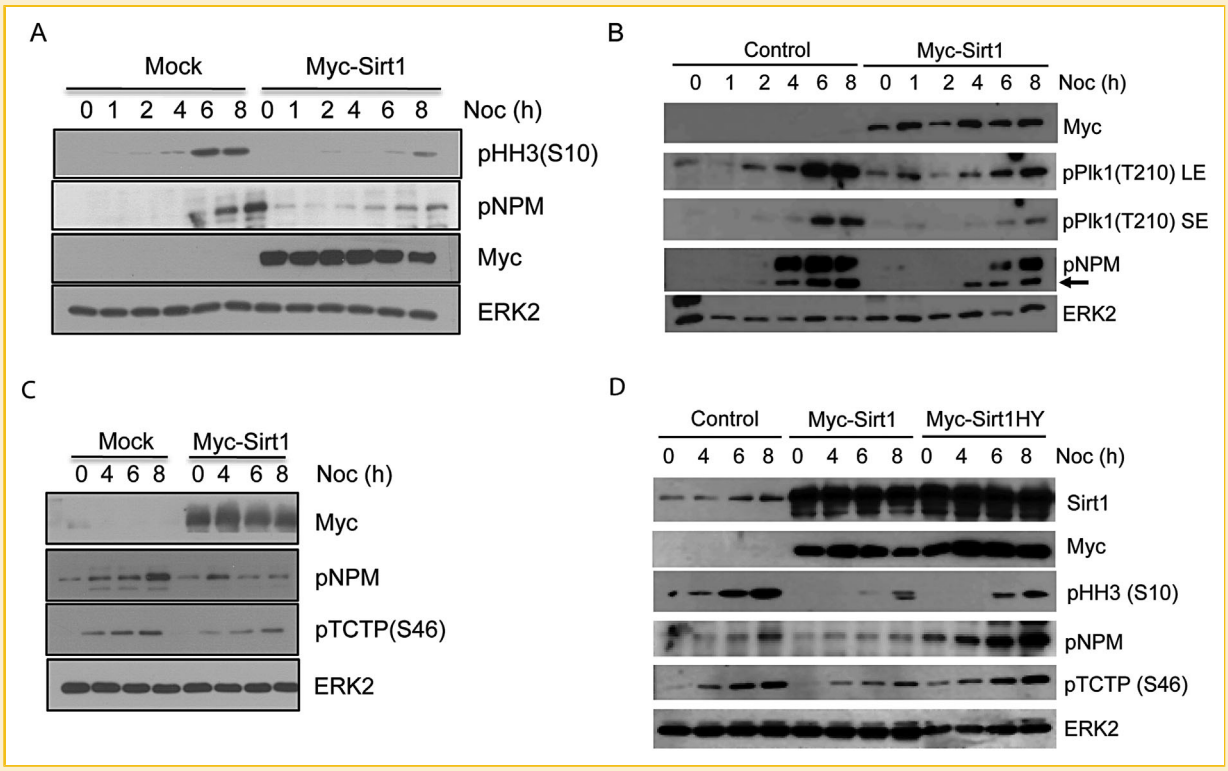

Fig. 3. Sirt1 downregulates nocodazole-induced Plk1 pathway. HeLa stably expressing either control vector (Mock) or Sirt1 (Myc-Sirt1) were treated with or without 0.5 μg/ml of nocodazole and harvested at the indicated times for immunoblotting analysis. A: Level of phospho-Histone H3 (pHH3(S10)) and phospho-NPM (pNPM) and Sirt1 were determined. ERK2 for an equal loading control. B: Levels of Plk1 phosphorylation at T210, and NPM phosphorylation indicated with the arrow, were compared. ERK2 was used for equal protein loading. LE, long exposure. SE, short exposure. C: HeLa cells transfected with the indicated vector were treated with 0.5 μg/ml of nocodazole. Lysates of transfected HeLa cells were analyzed by immunoblotting with the indicated antibodies. D: Myc-Sirt1 WT or HY mutant was transfected into HeLa cells. Lysates of transfected HeLa cells were analyzed by immunoblotting with the indicated antibodies. Endogenous or exogenous Sirt1 level was determined by immunoblotting with αSirt1 and αMyc antibody respectively. ERK2 for an equal loading control.

Sirt1 REGULATES Plk1 ACTIVITY

Sirt1 was identified previously as a component of the Polo-box domain interactome of Plk1 [Lowery et al., 2007]. To examine the possibility that these two proteins interact directly, co-immunoprecipitation analyses were performed using HA-tagged Plk1 and myc-tagged Sirt1. Immunoprecipitation of cells expressing myc-Sirt1 and HA-Plk1 using an anti-HA antibody, followed by immunoblotting using anti-Sirt1 antibodies, revealed a direct interaction between the two exogenous proteins (Fig. 4A). Furthermore, similar experiments revealed interactions between endogenous Sirt1 and exogenous HA-Plk1 (Fig. 4B), as well as endogenous Plk1 and exogenous myc-Sirt1 (Fig. 4C). Next, to test the hypothesis that Sir1 expression affects the kinetic activity of Plk1, which may be involved in the altered sensitivity to Noc (Fig. 2), in vitro kinase assays of Plk1 with or without co-expression of Sirt1 were performed. As shown in Figure 4D, co-expression of Sirt1 noticeably reduced the level of phosphate incorporation of the substrate (α-casein) by Plk1 by in vitro kinase assay, suggesting that Sirt1 expression may interfere with enzymatic activity of Plk1.

Given that Plk1 activity is associated with Noc-induced mitotic arrest [van de Weerdt et al., 2005], we hypothesized that modulation of this activity by Sirt1 may underlie the resistance to mitotic arrest caused by expression of Sirt1, which was accompanied by reduced levels of the mitotic indicators, such as phospho-histone H3 and phopsho-NPM (Fig. 3). To test this hypothesis, the mitotic responses to Noc treatment were compared in control cells and cells expressing Plk1. Ectopic expression of Plk1 increased the 4N peak in both untreated and Noc-treated cells, and this increase was attenuated by concomitant overexpression of Sirt1 (Fig. 4E), suggesting that inhibition of the activity of Plk1 by Sirt1 causes reduced sensitivity to Noc-induced mitotic arrest.

Sirt1 REDUCES MT DEPOLYMERIZATION BY MODULATING THE ACTIVITY OF Plk1

Plk1 activity is crucial for promoting MT depolymerase activity [Jang et al., 2009; Knowlton et al., 2009]; therefore, we speculated that the expression level of Sirt1 determines MT dynamics by controlling Plk1 activity, which leads to altered sensitivity to Noc treatment. To confirm this hypothesis, the effect of Sirt1 on spindle dynamics was determined quantitatively by measuring MT repolymerization after complete MT depolymerization under cold stress conditions, as described previously [Jang et al., 2008]. Notably, MT repolymerization was markedly lower in

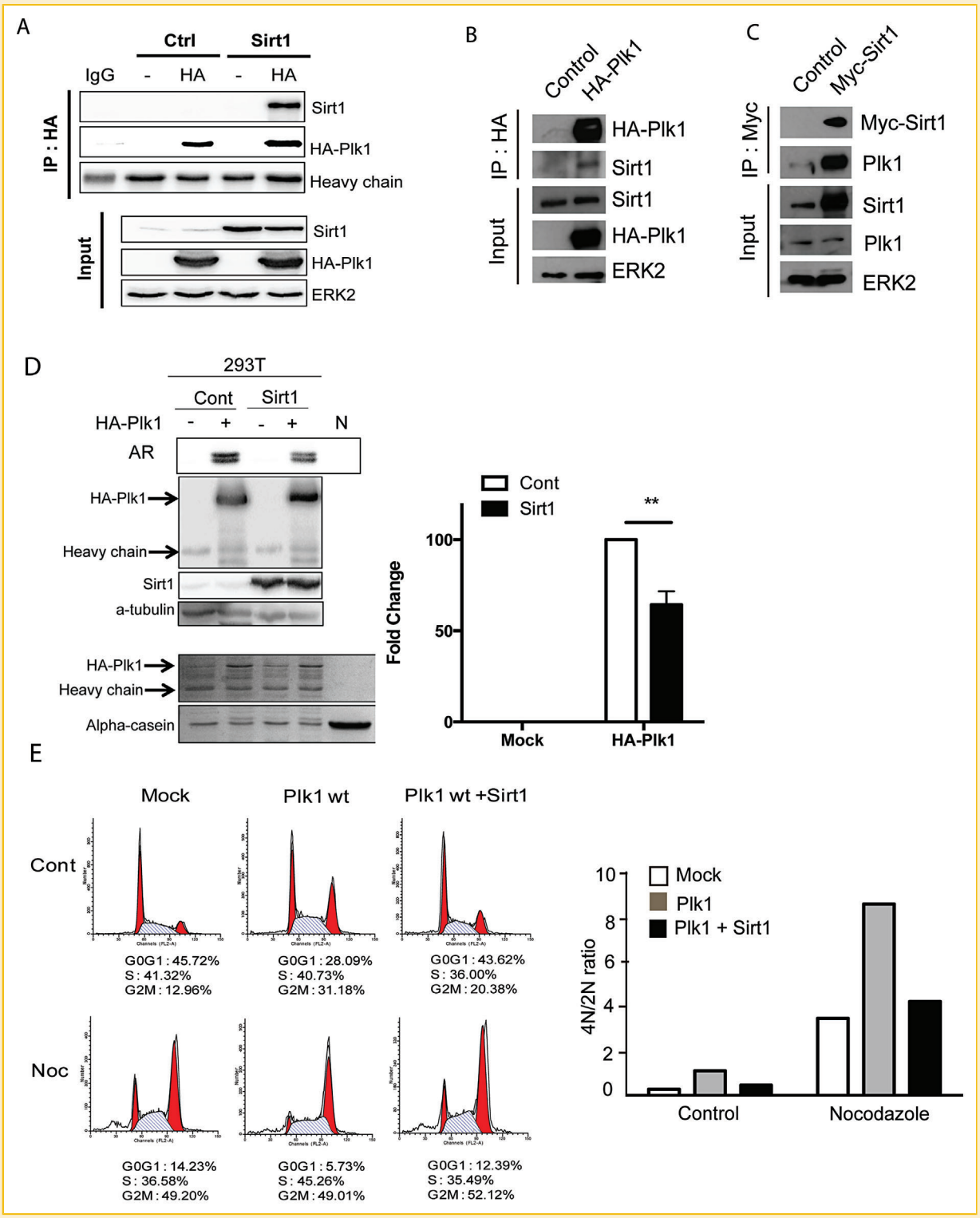

Fig. 4. Sirt1 regulates Plk1 activity by direct interaction. A: Myc-Sirt1 was co-transfected with HA-Plk1 into HEK293T cells. Lysates of transfected HEK293T cells were immunoprecipitated (IP) with anti-HA antibodies, followed by immunoblotting. B,C: Myc-Sirt1 or HA-Plk1 was transfected into HEK293T cells and lysates were immunoprecipitated with anti-Myc or anti-HA antibodies, respectively. D: IPed Plk1 from HA-Plk1 expression in HEK293T cells in the presence or absence of Sirt1 expression was subjected to in vitro kinase assay using α-casein as a substrate. The level of P^{32} incorporation into α-casein was presented as an autoradiogram (AR: top panel). Images of SDS–PAGE after ponceau staining were presented (bottom panel). The intensity of P^{32} incorporation was determined and graphically presented (right panel). $^{**}P < 0.001$. E: HeLa cells transfected with the indicated vector were treated with 0.5 μg/ml of nocodazole. Six hours later, the cells were harvested for FACS analysis. The ratio of 4N versus 2N was calculated for each condition and graphically presented (right panel).

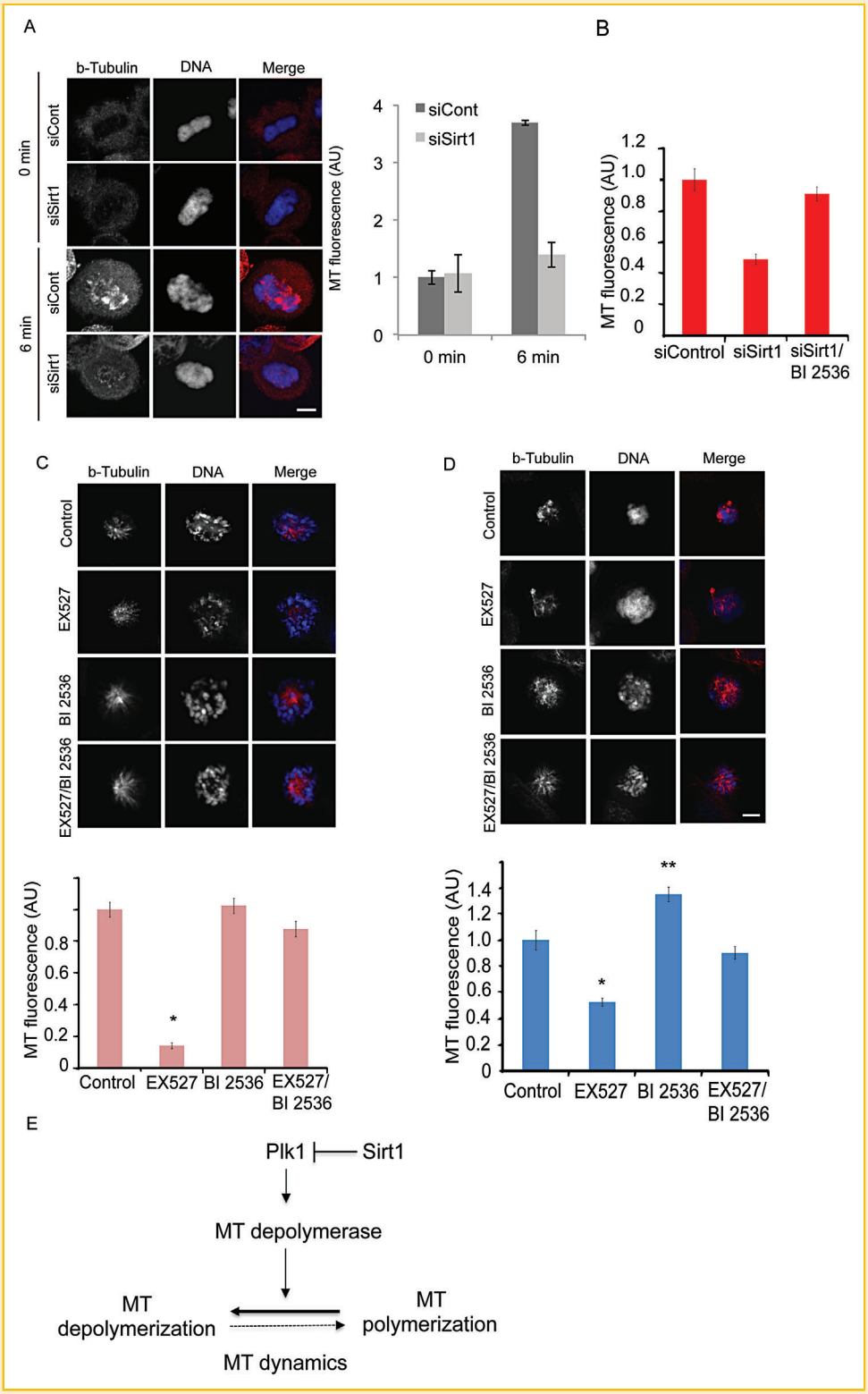

Fig. 5. Sirt1 reduces MT depolymerization by modulating the Plk1activity. A,B: Control or Sirt1-depleted HeLa cells were treated with 1 μg/ml nocodazole for 10 min at 37°C to completely depolymerize the mitotic spindle. Nocodazole-treated cells were washed twice with pre-warmed PBS, released into fresh media, and fixed at 6 min post-release. For Plk1 inhibition, 100 nM of BI 2536 was treated for 2 h prior to the depolymerization. Shown are maximum projections from deconvolved z stacks of representative cells stained for β-tubulin (red) and DNA (blue). Images for β-tubulin were acquired under a constant exposure time. β-Tubulin immunofluorescence intensity on metaphase spindles was quantified and normalized to their respective siControl sample at the 0 min time point (n = 10 cells for each quantification). $^{*}P < 0.05$ (two-tailed t test relative to their respective siControl cells at the corresponding time points). C,D: HeLa cells were treated with 0.5 μM of Ex527 or 100 nM of BI 2536 for 2 h. Mitotic spindle was completely depolymerized with cold stress condition (C) or 1 μg/ml nocodazole (D). The cells were released into fresh media containing one of the following inhibitors: 0.5 μM Ex527, 100 nM BI 2536. Cells were then stained for β-tubulin and DNA and MT fluorescence intensity of prometaphase cells (n = 10 cells for each quantification) was quantified. $^{*}P < 0.05$, $^{**}P < 0.01$. E: Proposed model illustrating the regulation of Plk1 by Sirt1 on MT dynamics.

Sirt1-depleted mitotic cells than control cells (Fig. 5A), indicating that Sirt1 expression promotes MT repolymerization and/or attenuates MT depolymerization. Next, we examined whether the effect of Sirt1 on MT repolymerization is Plk1-dependent. Exposure of cells to BI2536, a chemical inhibitor of Plk1, rescued the attenuated MT repolymerization in Sirt1-depleted cells (Fig. 5B), confirming that the deregulation of Plk1 activity following Sirt1 depletion was responsible for reduced MT polymerization. Simultaneous inhibition of Sirt1 and Plk1 using EX527 and BI2536 also rescued repolymerization of MT after depolymerization under cold stress conditions (Fig. 5C) or after Noc treatment (Fig. 5D). Overall, as summarized in Figure 5E, these data highlight the significance of the kinase activity of Plk1 and the deacetylase activity of Sirt1 to MT spindle dynamics.

DISCUSSION

Regulation of MT spindle dynamics is crucial for the normal congression and timely segregation of mitotic chromosomes; hence, cells have tightly controlled molecular mechanisms, such as the SAC, that halt mitotic progression when the mitotic spindle fails to form normally. Plk1 serves as a multifunctional kinase throughout mitosis [Zitouni et al., 2014] and regulates not only the formation of the mitotic spindle [Sumara et al., 2004; Ahonen et al., 2005], but also the onset of SAC [Seong et al., 2002; van de Weerdt et al., 2005; Wong and Fang, 2007]. However, regulation of the activity of Plk1 for each of its multiple functions is not fully understood. Sirt1 is indispensable for genomic care taking and normal chromosomal segregation [Wang et al., 2008; Fatoba and Okorokov, 2011], but the underlying molecular mechanisms by which Sirt1 serves as a genomic guardian have not yet been determined.

The results presented here show that timely modulation of the activity of Plk1 by Sirt1 is required for accurate mitotic chromosome congression and segregation. A protein interaction between Plk1 and Sirt1 occurs in parallel with the reduction of both the phosphorylation and kinase activity of Plk1 with Sirt1 expression (Figs. 3 and 4). Thus, prolonged activation of Plk1 by Sirt1 depletion or inhibition may disrupt the fine regulation of MT dynamics (Fig. 5), resulting in defective chromosome congression and segregation (Fig. 1). Following Noc treatment to promote MT depolymerization, the extent of Sirt1 expression may determine the level of mitotic arrest, which is an indicator of sensitivity to spindle damage. Sirt1 depletion and ectopic expression clearly increased and decreased the sensitivity of HeLa cells to Noc, respectively (Fig. 2). In addition, MT repolymerization was reduced in vitro following Sirt1 knockdown or enzymatic inhibition, and this effect was abrogated by the inhibition of Plk1 activity (Fig. 5). These results support the notion that Sirt1 contributes to MT dynamics during mitosis and the downstream events are controlled by the Plk1 pathway.

To our knowledge, aside from phosphorylation, no other post-translational modifications of Plk1 that regulate its enzymatic activity have been reported to date. A recent study showed that Plk1 is acetylated by Tip60, although the role of this modification remains unclear [Zhang et al., 2012]. Given the Sirt1 expression reduced Plk1 kinase activity, it would be interesting to determine whether the acetylation status of Plk1 is related to mitosis and/or MT dynamics.

To our knowledge, this study is the first to demonstrate that Sirt1 regulates Plk1 activity during mitosis to control MT dynamics for proper chromosome congression and segregation. Aberrant activation of Plk1 due to loss of Sirt1 likely increases MT depolymerase activity, resulting in impaired mitotic spindle formation, unaligned mitotic chromosomes, and lagging chromosomes. In summary, the data presented here suggest that Sirt1 contributes to proper and timely MT dynamics by regulating the activity of Plk1, which is associated with genomic integrity.

ACKNOWLEDGMENTS

The authors thank Dr. Surabhi Dangi-Garimella for her critical reading of this manuscript. This work was supported by the Scientific Research Center (SRC) program (2011-0030043: H.J.C.), Radiation Research Center (2012055464: H.J.C.) and Medical Science Research Center (MRC) program (20110030700: J.C.Y.) funded by the Korea Science and Engineering Foundation (KOSEF) of the Korean government (MOST).

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