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HP1 links centromeric heterochromatin to centromere cohesion in mammals

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1st Editorial Decision 20 December 2017

Thank you for the submission of your research manuscript to EMBO reports. We have now received reports from the three referees that were asked to evaluate your study, which can be found at the end of this email.

As you will see, all three referees have raised a number of concerns and suggestions to improve the manuscript, or to strengthen the data and the conclusions drawn. As the reports are below, I will not detail them here. Most importantly though, it will be important to address the concerns regarding the use of HeLa cells (point 1 of referee #1, referee #3 in her/his general comment, and point 2 by referee #3), and the specificity of the CRISPR/Cas9 editing system (point 1 referee #2). We also feel that the concerns of referee #2 regarding the discussion of the previous literature should be taken into account during revision. Please describe the previous related literature more completely, point out inconsistencies, compare your data with previous results, clearly highlight the similarities and differences, and then reconcile your data with previous findings.

Given the constructive referee comments, we would like to invite you to revise your manuscript with the understanding that all referee concerns must be addressed in the revised manuscript and in a point-by-point response. Acceptance of your manuscript will depend on a positive outcome of a second round of review. It is EMBO reports policy to allow a single round of revision only and acceptance or rejection of the manuscript will therefore depend on the completeness of your responses included in the next, final version of the manuscript.

Revised manuscripts should be submitted within three months of a request for revision; they will otherwise be treated as new submissions. Please contact us if a 3-months time frame is not sufficient for the revisions so that we can discuss the revisions further.

Supplementary/additional data: The Expanded View format, which will be displayed in the main

HTML of the paper in a collapsible format, has replaced the Supplementary information. You can submit up to 5 images as Expanded View. Please follow the nomenclature Figure EV1, Figure EV2 etc. The figure legend for these should be included in the main manuscript document file in a section called Expanded View Figure Legends after the main Figure Legends section. Additional Supplementary material should be supplied as a single pdf labeled Appendix. The Appendix includes a table of content on the first page, all figures and their legends. Please follow the nomenclature Appendix Figure Sx throughout the text and also label the figures according to this nomenclature. For more details please refer to our guide to authors:
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Important: All materials and methods should be included in the main manuscript file.

Regarding data quantification and statistics, can you please specify, where applicable, the number "n" for how many experiments were performed, the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values in the respective figure legends. Please provide statistical testing where applicable.

We now strongly encourage the publication of original source data with the aim of making primary data more accessible and transparent to the reader. The source data will be published in a separate source data file online along with the accepted manuscript and will be linked to the relevant figure. If you would like to use this opportunity, please submit the source data (for example scans of entire gels or blots, data points of graphs in an excel sheet, additional images, etc.) of your key experiments together with the revised manuscript. Please include size markers for scans of entire gels, label the scans with figure and panel number, and send one PDF file per figure or per figure panel.

In addition I would need from you:
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- two to three bullet points highlighting the key findings of your study
- a schematic summary figure (in jpeg or tiff format with the exact width of 550 pixels and a height of about 400 pixels) that can be used as visual synopsis on our website.

I look forward to seeing a revised version of your manuscript when it is ready. Please let me know if you have questions or comments regarding the revision.

REFEREE REPORTS

Referee #1:

This manuscript addresses the long controversial question of whether HP1 protein contributes to cohesion in mammalian cells. By use of CRISPR knockout cell lines, the authors provide a definitive answer to this question and also address the mechanism. They show that HP1 contributes to the recruitment of Haspin kinase to promotes cohesion in the pericentromere by counteracting Wapl1 (this effect of Haspin on counteracting Wapl was previously shown by this lab and others). The data is of very high quality, well presented and controlled. The manuscript follows a logical progression: with questions raised at each stage addressed in the subsequent figure.

I only have two suggestions, one that is a general comment for the authors to consider in their future work, the other a presentation suggestion to aid the reader to understand the overall conclusions.

1. Although I realise that there is a long tradition of using HeLa cells for this type of work and therefore methodologies and reagents are well established, it is also clear that they are definitely not a good model for studying chromosome segregation as they are highly abnormal, being aneuploid and unstable. Therefore I urge the reader to consider using cell lines with a more typical karyotype for future studies to provide a more accurate view of what occurs in organisms.
2. The model in Figure 5 is not easy to understand. Pds5B is a component of cohesin but is shown at the top of the model, with cohesin shown separately below. Please set this model out in a more logical manner.

Referee #2:

HP1 proteins are required for the protection of cohesin at centromeres in diverse organisms. In mammals, there are three HP1 homologues. In this paper, the authors used CRISPR technology to knockout HP1 alpha and HP1 gamma, and observed a cohesion defect in the double KO. Next, the authors demonstrated that the CSD of HP1 is important and sufficient for protecting centromere cohesion. The authors then showed that Haspin, a kinase that protects cohesion, interacts with HP1 and is delocalized upon HP1 DKO. Moreover, targeting Haspin to centromeres can rescue HP1 DKO, suggesting that HP1 alpha and gamma protects centromere cohesion by recruiting Haspin. Finally, the authors showed that Haspin antagonizes Wapl, a factor that promotes cohesion release, and that Wapl inhibition can also rescue HP1 DKO.
The study used a clean KO system to define the function of HP1 proteins in centromere cohesion in a mammalian system. The data quality is very good. However, my main reservation is that the key points of this study can be inferred from an earlier study in fission yeast (Yamagashi et al. 2010), in which Swi6 (HP1 homolog) is important for Hrk1 (Haspin homolog) localization and that Swi6 CSD interacts with Hrk1. In addition, the role of HP1 alpha and HP1 gamma in cohesion has been reported (Shimura, 2011). There are inconsistencies in the literature regarding the function of HP1 in cohesion and the authors failed to put their results into context.

Referee #3:

Qi Yi and colleagues report that HP1alpha and HP1gamma act redundantly in Human cells to protect mitotic sister-chromatid cohesion through the recruitment of Haspin.
Using immunofluorescence microscopy the authors found that HP1-alpha and HP1-gamma - but apparently not HP1-beta - localized at inner centromeres of mitotic Hela cells. Using CRISPR/Cas9 they generated single KO cell lines and double KO for HP1-alpha and HP1-gamma (DKO). The DKO showed profound mitotic defects including loss of sister-chromatid cohesion, increased inter-kinetochore distance, prolonged mitosis and chromosome mis-segregation. These defects were not seen in the single KO cell lines and were efficiently rescued by stable expression of either HP1-alpha or HP1-gamma, indicating that HP1-alpha and gamma act redundantly to promote sister-chromatid cohesion. Next the authors found that the Chromo-Shadow Domain (CSD) of HP1 was crucial for its cohesion function. CSD mutants of HP1-alpha failed to complement the DKO while a mutant of the chromodomain (CD) that disrupts CD binding to H3K9me2/3 localized normally to mitotic centromeres and restored centromere cohesion in HP1 DKO cells. Consistently, they found that the artificial tethering of the CSD to centromeres (CSD-CB, HP1 alpha CSD fused to the centromeric DNA binding domain of CENP-B) was sufficient to restore HP1 DKO cohesion defects. As previously reported in fission yeast, the authors found that HP1-alpha binds Haspin. They further show that the HP1-alpha CSD binds the N-terminal of Haspin both in vitro and in human protein extracts. The Haspin kinase acts by counteracting the cohesin releasing factor WAPL, suggesting that the cohesion defects in HP1 DKO cells may stem from a failure to recruit Haspin to mitotic centromeres. Consistently, WAPL knock down rescued centromeric cohesion defects in HP1 DKO cells, indicating that the primary defect of HP1 DKO stems from a failure to counteract WAPL. Using Histone H3 threonine 3 phosphorylation (H3T2P) as a read-out of Haspin activity, the authors found that indeed H3T3P was reduced in HP1 DKO cells and was increased by stable expression of CB-CSD, indicating that HP1 alpha and gamma are required for full Haspin recruitment to centromeres and cohesin protection from WAPL. Haspin is thought to counteract WAPL through its kinase activity and through its N-terminal PDS5 Interacting Domain (PIM) which is thought to counteract WAPL by competing for PDS5-B binding. Indeed, the stable expression of the N-terminal of Haspin fused to CB (CB-Haspin-N50-GFP) was sufficient to rescue sister-chromatid cohesion in HP1 DKO cells.
The authors propose a model in which HP1 and PDS5-B are both required for the full recruitment of Haspin to centromeres to inhibit Wapl-Pds5B interaction and Wapl-mediated cohesin release.

This study claims that HP1 proteins at human centromeres are crucial for protecting sister-chromatid cohesion during mitosis. This is potentially novel and of great importance to the field and of general interest to cell biologists. However, data were generated from Hela cells which are known to carry extensive genome alterations, therefore questioning the validity of the conclusions for normal

human cells. Moreover, I'm concerned about the possible genetic heterogeneity of the KO clones. This feeling was enhanced by their stochastic use throughout the study which left me uncomfortable with the conclusions, as detailed below.

Major points
1- Specificity of CRISPR/Cas9-mediated editing of HP1 genes in HeLa cells
I BLASTed the targeted sequences and found that many showed a perfect match (within their entire length) with another human gene (as listed below). As far as I understood, the authors used 2 sgRNAs simultaneously "which would largely reduce potential off-target effects". Was that actually efficient? Does the sequences shown in FIG. EV1 are consistent with a "double nicking event" as expected? Did the authors check that the other genes that showed a 100% match were left intact? Listed below are the targeted sequences that show a perfect match with other genes. The BLAST analyses also uncovered many genes that showed a perfect match over 70-80% of the length of the query. These are not listed here but may be included in the analysis / comments.
For HP1 alpha (clone 1D4) the first targeted sequence (AGCGGACAGCTGACAGTTCT) shows a perfect match with dystrophin and the other targeted sequence (GGATGAGGAGGAGTATGTTG), a perfect match with SMG5. For HP1β KO (clone 2A5), (TCGAGTGGTAAAGGGCAAAG) shows a perfect match with NRP2, and (GGAGTACCTCCTAAAGTGGA), a perfect match with COL5A1. For the single HP1γ KO clone (3C3), the targeted sequence (5'-ACGTGTAGTGAATGGGAAAG-3') shows a 100% match with the NMD gene. Double KO HP1alpha-gamma was obtained by HP1 gamma KO in clone 1D4. Clone 2A4: the targeted HP1gamma sequence (GAAGAATTTGTCGTGGAAAA) shows 100% match with genomics sequences. Clones 3A2 and 4A4: (GAAGAATTTGTCGTGGAAAA, as above) and (CTAGATCGACGTGTAGTGAA) shows 100% match with genomic sequences.

2- Genetic heterogeneity between the DKO clones and consistency throughout the study.
Possibly related to point 1, there is apparently some variability among the 3 independent DKO clones (2A4, 3A2 and 4A4). In Fig. 1C, 3A2 shows a lower rate of cells with lagging chromatids than 2A4 and 4A4, suggesting genetic heterogeneity among the clones (seen also in Fig. 2E). In some Figures, the authors used all 3 clones, in others 2 or even only one and the essential control (rescue by stable expression of HP1-alpha or gamma) was done for 2A4 only (Fig. 2G). In addition, it is difficult for the reader to assess the consistency of the results when different clones are used for different experiments. The study would be largely improved by showing a homogenous data set with well-characterized clones that is, checked for the absence of undesired mutations in other genes, as detailed above, and checked by complementation by stable expression of HP1-alpha & gamma. The reference of the Hela cell line used should be mentioned as well.

Minor points.
1-Quantification of cohesion loss.
It is stated in the text page 4 that "the percentage of cells with cohesion loss, defined as a cell containing at least 26 separated chromatids". First, this should be moved into the Methods section because here, it is unclear whether it is applicable to this particular figure of to the whole study. Second, why "at least 26 separated chromatids"? Why this cut-off? I assume this is just above the (high) rate of sister-cohesion loss in Hela cells? This should be clarified in the methods.

2-Data on HP1 beta KO.
The KO was done and I expected the authors to indicate whether or not chromosome segregation defects were observed. As the authors claimed that HP1gamma was not clearly detectable at centromeres, it was interesting to see whether the KO had consequences on chromosome segregation. In addition, the authors should provide the sequence of the edited locus in clone 2A5, as a reference for future study. FIG EV1: it is difficult to see centromeres. Please provide an enlargement view of the centromere as in Fig1.

3- Fig. 1GH. It is stated in the text that "Similar defects in chromosome alignment and segregation were seen in three independent clones of HP1 DKO cells undergoing the same transient mitotic arrest/release procedure (Figures 1G and 1H)". From Fig. 1GH, the data are from 3A2 only.

EMBOR-2017-45484V2_Response to Referees' comments

Referee #1:

This manuscript addresses the long controversial question of whether HP1 protein contributes to cohesion in mammalian cells. By use of CRISPR knockout cell lines, the authors provide a definitive answer to this question and also address the mechanism. They show that HP1 contributes to the recruitment of Haspin kinase to promotes cohesion in the pericentromere by counteracting Wapl1 (this effect of Haspin on counteracting Wapl was previously shown by this lab and others). The data is of very high quality, well presented and controlled. The manuscript follows a logical progression: with questions raised at each stage addressed in the subsequent figure.
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Response*: Thanks for the suggestion. We are aware of the importance to use chromosomally stable cell lines to study chromosome segregation in mitosis. Though the HeLa cell line used in this study is aneuploid, several lines of evidence indicate that it is a near chromosomally stable cell line. For example, this cell line behaves like the non-transformed retinal pigment epithelial (RPE-1) cells with regard to few segregation errors in anaphase and the proper maintenance of chromosome bi-orientation and sister-chromatid cohesion upon metaphase arrest induced by the proteasome inhibitor MG132 (Appendix Figs S1 and S6; new data). Please see details in our response to the general commentof referee #3. As suggested, wherever possible, we will use the diploid non-cancer cell line RPE-1 and the chromosomally stable, near-diploid cell line HCT116, together with our HeLa cell line for future such studies.*
2. The model in Figure 5 is not easy to understand. Pds5B is a component of cohesin but is shown at the top of the model, with cohesin shown separately below. Please set this model out in a more logical manner.
Response*: Thanks for the suggestion. We have now modified the model by removing Pds5B in the schematic. In the conclusion of the main next, we state that "We propose that, together with Pds5B, HP1 ensures the full occupancy of Haspin at mitotic centromeres, thereby enabling Haspin to prevent Wapl-Pds5B interaction and Wapl-mediated cohesin release (Fig 5M)."*

Referee #2:

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Response*: Thanks for appreciating the experimental system used in this study and the quality of our data. It was reported that the fission yeast HP1 homolog Swi6 interacts with the Haspin homolog Hrk1 (Yamagishi Y et al., Science, 2010). Yamagishi et al. noted that "The interaction of Hrk1 with*

Swi6 might also be important, because Hrk1 localization at the heterochromatic region partly depends on this interaction (fig. S7)". Actually, except for Hrk1 localization, Yamagishi et al. did not address the functional consequence of loss of the Swi6-Hrk1 interaction in fission yeast. This is not surprising since, as the authors wrote, "Hrk1 is dispensable for centromeric cohesion in both mitosis and meiosis (fig. S1, A and B), meaning that Hrk1 is not relevant to cohesion or Sgo1 function [in fission yeast]". Thus, evidence is lacking to support the existence and functional significance of the HP1-Haspin interaction under physiological conditions.
In our study, using CRISPR/Cas9-mediated gene knockout and the rescue experiments with exogenous proteins, we first demonstrate that HP1α and HP1γ are redundantly required for the protection of mitotic centromere cohesion in human cells. We further reveal and dissect the molecular details underlying the interaction between the human proteins HP1 and Haspin. By examining sister-chromatid cohesion in cells in which endogenous Haspin was replaced by an exogenous Haspin mutant defective in binding HP1, we show that the HP1-Haspin interaction is important for centromeric cohesion protection. Moreover, we provide evidence that HP1 protects centromeric cohesion through promoting centromeric localization of Haspin, thereby antagonizing Wapl-mediated cohesin release at mitotic centromeres. Taken together, our study reveals a molecular mechanism by which HP1 links centromeric heterochromatin to the protection of sister-chromatid cohesion at mitotic centromeres of human cells. Thus, it is unfair to conclude that "key points of this study can be inferred from an earlier study in fission yeast (Yamagishi et al. 2010)".
As the referee pointed out, Shimura et al. reported that expression of the HIV-1 encoded protein Vpr displaced HP1, Sgo1 and Scc1 from centromeres of mitotic chromosomes, resulting in premature chromatid separation (Shimura M et al., J Cell Biol, 2011). The authors wrote in the abstract that "[this] study reveals for the first time centromere cohesion impairment resulting from epigenetic disruption of higher-order structures of heterochromatin by a viral pathogen". However, they failed to address the mechanism underlying the cohesion defects upon HP1 displacement from centromeres. Shimura et al. suggested that "hSgo1 acts downstream of HP1-αγ in the maintenance of centromeric cohesin during mitosis". In contrast, we found that Sgo1 localization at inner centromeres was not conspicuously altered in nocodazole-arrested mitotic HP1 DKO cells (Fig EV5C and D), suggesting that HP1 may not play a critical role in localizing Sgo1 at mitotic centromeres of human cells. In line with our results, Kang J et al., reported that a HP1-binding-deficient mutant of Sgo1 was functional in centromeric cohesion protection, and localized normally to mitotic centromeres in HeLa cells (Kang J et al., Mol Biol Cell, 2011).
As suggested, wherever possible, we have now stated in the main text the discrepancy in the literature, and highlighted the similarities and differences compared to our results.

Referee #3:

Qi Yi and colleagues report that HP1alpha and HP1gamma act redundantly in Human cells to protect mitotic sister-chromatid cohesion through the recruitment of Haspin. Using immunofluorescence microscopy the authors found that HP1-alpha and HP1-gamma - but apparently not HP1-beta - localized at inner centromeres of mitotic Hela cells. Using CRISPR/Cas9 they generated single KO cell lines and double KO for HP1-alpha and HP1-gamma (DKO). The DKO showed profound mitotic defects including loss of sister-chromatid cohesion, increased inter-kinetochore distance, prolonged mitosis and chromosome mis-segregation. These defects were not seen in the single KO cell lines and were efficiently rescued by stable expression of either HP1-alpha or HP1-gamma, indicating that HP1-alpha and gamma act redundantly to promote sister-chromatid cohesion. Next the authors found that the Chromo-Shadow Domain (CSD) of HP1 was crucial for its cohesion function. CSD mutants of HP1-alpha failed to complement the DKO while a mutant of the chromodomain (CD) that disrupts CD binding to H3K9me2/3 localized normally to mitotic centromeres and restored centromere cohesion in HP1 DKO cells. Consistently, they found that the artificial tethering of the CSD to centromeres (CSD-CB, HP1 alpha CSD fused to the centromeric DNA binding domain of CENP-B) was sufficient to restore HP1 DKO cohesion defects. As previously reported in fission yeast, the authors found that HP1-alpha binds Haspin. They further show that the HP1-alpha CSD binds the N-terminal of Haspin both in vitro and in human protein extracts. The Haspin kinase acts by counteracting the cohesin releasing factor WAPL, suggesting that the cohesion defects in HP1 DKO cells may stem from a failure to recruit Haspin to mitotic centromeres. Consistently, WAPL knock down rescued centromeric cohesion defects in HP1 DKO cells, indicating that the primary defect of HP1 DKO stems from a failure to counteract WAPL. Using Histone H3 threonine 3 phosphorylation (H3T2P) as a read-out of Haspin

activity, the authors found that indeed H3T3P was reduced in HP1 DKO cells and was increased by stable expression of CB-CSD, indicating that HP1 alpha and gamma are required for full Haspin recruitment to centromeres and cohesin protection from WAPL. Haspin is thought to counteract WAPL through its kinase activity and through its N-terminal PDS5 Interacting Domain (PIM) which is thought to counteract WAPL by competing for PDS5-B binding. Indeed, the stable expression of the N-terminal of Haspin fused to CB (CB-Haspin-N50-GFP) was sufficient to rescue sister-chromatid cohesion in HP1 DKO cells. The authors propose a model in which HP1 and PDS5-B are both required for the full recruitment of Haspin to centromeres to inhibit Wapl-Pds5B interaction and Wapl-mediated cohesin release.
This study claims that HP1 proteins at human centromeres are crucial for protecting sister-chromatid cohesion during mitosis. This is potentially novel and of great importance to the field and of general interest to cell biologists. However, data were generated from Hela cells which are known to carry extensive genome alterations, therefore questioning the validity of the conclusions for normal human cells. Moreover, I'm concerned about the possible genetic heterogeneity of the KO clones. This feeling was enhanced by their stochastic use throughout the study which left me uncomfortable with the conclusions, as detailed below.
Response*: We thank the referee for appreciating the potential novelty and importance of our study. We also agree with the importance of using chromosomally stable cell lines, although a large body of work performed in the cell cycle field and other areas of cell biology had implied that most basic processes observed in non-transformed diploid cells are fully operational in HeLa cells. Indeed, we are aware of the importance to use chromosomally stable cell lines to study chromosome segregation in mitosis. Though the HeLa cell line used in this study is aneuploid, several lines of evidence indicate that it is a near chromosomally stable cell line.*
First, in this HeLa cell line, chromosomes align efficiently at metaphase, segregate synchronously at anaphase with few (1-2%) errors (Fig 1C). The incidence of chromosome missegregation in this cell line is as low as that in the non-transformed RPE-1 cells (Appendix Fig S1A; new data), but is much lower than that (around 30%) in the chromosomally instable human bone osteosarcoma epithelial U2OS cells (see Figure 1C in Bakhoum S et al., Nat Cell Biol, 2008). Second, this HeLa cell line is capable of maintaining proper metaphase chromosome alignment and sister-chromatid cohesion (Fig 2), which is comparable to RPE-1 cells (Appendix Figs S1B, S6C and D; new data) but is in sharp contrast to U2OS cells (Appendix Fig S1C; new data).
The characteristics of this HeLa cell line are reminiscent of a variant HeLa cell line ("HeLa-1") (see Figure 1A in Tanno Y et al., Science, 2015), which is chromosomally stable and shows few errors in anaphase. Moreover, another example of such a cancer cell line is the colon cancer cell line HCT116. Though genetically instable (Lengauer C et al., Nature, 1997), HCT116 cells are chromosomally stable as evidenced by the faithful chromosome segregation with low incidence of errors (Sarah Thompson and Duane Compton, J Cell Biol, 2008). Thus, certain types of cancer cell lines carrying extensive genome alterations can be chromosomally stable.
Moreover, we have shown now that, similar to our HeLa cell line, HP1α and HP1γ, but not HP1β, are enriched at inner centromeres of chromosomes in nocodazole-arrested mitotic RPE-1 cells (Appendix Fig S2; new data). In addition, siRNA-mediated knockdown of HP1α did not compromise metaphase chromosome alignment and sister-chromatid cohesion in both HeLa cells and RPE-1 cells (Appendix Fig S6; new data).
Regarding the referee's concern about the possible genetic heterogeneity and the "stochastic" use of the KO clones, please see below in our response to Major point 2.

Major points
1- Specificity of CRISPR/Cas9-mediated editing of HP1 genes in HeLa cells
I BLASTed the targeted sequences and found that many showed a perfect match (within their entire length) with another human gene (as listed below). As far as I understood, the authors used 2 sgRNAs simultaneously "which would largely reduce potential off-target effects". Was that actually efficient? Does the sequences shown in FIG. EV1 are consistent with a "double nicking event" as expected? Did the authors check that the other genes that showed a 100% match were left intact?
Listed below are the targeted sequences that show a perfect match with other genes. The BLAST analyses also uncovered many genes that showed a perfect match over 70-80% of the length of the query. These are not listed here but may be included in the analysis / comments. For HP1 alpha (clone 1D4) the first targeted sequence (AGCGGACAGCTGACAGTTCT) shows a perfect match with dystrophin and the other targeted sequence (GGATGAGGAGGAGTATGTTG), a perfect match with SMG5. For HP1β KO (clone 2A5), (TCGAGTGGTAAAGGGCAAAG) shows a perfect match with NRP2, and (GGAGTACCTCCTAAAGTGGA), a perfect match with COL5A1. For the

single HP1γ KO clone (3C3), the targeted sequence (5'-ACGTGTAGTGAATGGGAAAG-3') shows a 100% match with the NMD gene. Double KO HP1alpha-gamma was obtained by HP1 gamma KO in clone 1D4. Clone 2A4: the targeted HP1gamma sequence (GAAGAATTTGTCGTGGAAAA) shows 100% match with genomics sequences. Clones 3A2 and 4A4: (GAAGAATTTGTCGTGGAAAA, as above) and (CTAGATCGACGTGTAGTGAA) shows 100% match with genomic sequences.

Response: *We thank the referee for carefully checking potential off-targets of genome editing by the sgRNAs used in our study. It is known that, in the CRISPR-Cas9 system, the target DNA sequence must immediately precede a 5'-NGG PAM (protospacer adjacent motif), and the 20-nt sgRNA base pairs to the opposite strand of the target sequence to mediate Cas9 cleavage at ~3 bp upstream of the PAM (Jinek M et al., Science, 2012; Ran F et al., Nat Protocol, 2013). It seems that the PAM sequences were not included by the referee for BLAST. We apologize that we did not show in the original submission the NGG PAM sequence together with the 20-nt sgRNA sequence, which may have caused the confusion. We have now made the corresponding changes in the Materials and Methods section, and showed more detailed information of the sgRNA sequences in Appendix Fig S3.*

Indeed, though genes mentioned by the referee can be matched to various extent (maximally 18 out of 20 bases, see Appendix Fig S4) to the sgRNA sequences used in this study, no NGG PAM is found directly downstream of the targeting sequences. Thus, Cas9 cannot make a double-strand break (DSB) at these sites theoretically. Consistently, sequencing of the genomic DNA fragments of these genes did not find mutations (Appendix Fig S4). Due to unknown reason, we failed to amplify by PCR the fragments of COL5A1 gene in either HeLa cells or HP1β KO clone 2A5. Note that HP1β KO clone 2A5 is not the focus of this study, and was only used for the study of EGFP-HP1β localization (Fig EV1A) and chromosome missegregation (Fig EV1F).

We note that sequences for sgRNAs targeting HP1α ((5'-AGAACTGTCAGCTGTCCGCTtgg-3' and 5'-GGATGAGGAGGAGTATGTTGtgg-3', for clones 2A4, 3A2 and 4A4), HP1γ (5'-CTAGATCGACGTGTAGTGAAtgg-3', for clones 3A2 and 4A4), and HP1β (5'-CTTTGCCCTTTACCACTCGAcgg-3', for clone 2A5), were selected from the list of genome-wide sgRNA candidates suggested by the Eric Lander Laboratory (Wang T et al., Science, 2014), which presumably have been screened for low off-target potentials.

Moreover, wherever possible, we have utilized the Cas9 nickase mutant with paired guide RNAs to introduce targeted DSBs, which was developed by the Feng Zhang Laboratory and can reduce off-target activity by 50- to 1500-fold (Ran F et al., Cell, 2013). According to this paper, "because individual nicks in the genome are repaired with high fidelity, simultaneous nicking via appropriately offset guide RNAs is required for DSBs and extends the number of specifically recognized bases for target cleavage". Compared to the single sgRNA-mediated double strand bread, double-nicking guided by pairs of sgRNAs tends to cause large fragment indels between the two targeting locus. Indeed, sequencing results (Fig EV1) show such large fragment indels close to the PAMs of the sgRNA in clones 1D4, 3A2 and 4A4, respectively. Besides, we have corrected the mistake in Fig EV1B in the original submission, and now show a 13-base-deletion in clone 1D4 cells (Fig EV1B).

Regardless of any unknown potential off-targets caused by any sgRNAs, it is important to note that we observed similar centromeric cohesion defects in three independent HP1 KO clones derived from cells transfected with different sgRNAs. Moreover, we observed the restoration of proper centromeric cohesion in HP1 DKO cell lines by means of exogenous expression of Flag-tagged HP1α or HP1γ, CENP-B-fused HP1α (full-length or CSD only), CENP-B-fused Haspin-N50, as well as by inhibiting the cohesin-release factor Wapl. All these results support the specificity of the centromeric cohesion defects observed in the HP1 DKO cell lines.

2- Genetic heterogeneity between the DKO clones and consistency throughout the study. Possibly related to point 1, there is apparently some variability among the 3 independent DKO clones (2A4, 3A2 and 4A4). In Fig. 1C, 3A2 shows a lower rate of cells with lagging chromatids than 2A4 and 4A4, suggesting genetic heterogeneity among the clones (seen also in Fig. 2E).

Response: *We understand these concerns. Regarding the genetic heterogeneity between the DKO clones, we have now carried out further genomic DNA sequencing of HeLa-derived clones in which HP1α (clone 1D4, Fig EV1B), or HP1α and HP1γ (clones 2A4, 3A2 and 4A4, Fig EV1C), were knocked out. The genomic DNA PCR fragments were subcloned into plasmid vectors, transformed into E. coli, then certain numbers of bacterial colonies were sequenced. For clone 1D4 cells, all 12 colonies showed insertion of 13 bases. For clone 2A4 cells, all 8 colonies showed insertion of 1 base. For clone 3A2 cells, all 10 colonies showed insertion of 38 bases. For clone 4A4 cells, 7 out of*

12 colonies showed insertion of 22 bases, whereas the rest 5 colonies showed insertion of 57 bases and deletion of 2 bases. Thus, at least at the genomic DNA level, clones 1D4, 2A4 and 3A2, which were mainly used in this study, do not show genetic heterogeneity.
It was pointed out that clone 3A2 shows a lower rate of anaphase cells with lagging chromatids than 2A4 and 4A4 (Figs 1C and 2E). In our understanding, this may not necessary reflect the genetic heterogeneity among the clones. Instead, this may also reflect the normal variation among multiple independent experiments. This is why we usually need to do the same experiments twice to show the mean and range, or more than twice to show the mean and standard deviation. This is also why we usually need to use various independent stable cell lines because probably not every line of stable cells behaves identically. We have now repeated the experiments and showed the mean and range in Fig 1C. Regarding Fig 2E, additional data from similar experiments can now be found for clone 2A4 (Figs 2I, 3D and 3I) and for clone 3A2 (Figs EV3K and 5H).

In some Figures, the authors used all 3 clones, in others 2 or even only one and the essential control (rescue by stable expression of HP1-alpha or gamma) was done for 2A4 only (Fig. 2G). In addition, it is difficult for the reader to assess the consistency of the results when different clones are used for different experiments. The study would be largely improved by showing a homogenous data set with well-characterized clones that is, checked for the absence of undesired mutations in other genes, as detailed above, and checked by complementation by stable expression of HP1-alpha & gamma.
***Response**: We apologize that, due to the amount of additional work required, we did not do all the experiments using all three HP1 DKO clones (2A4, 3A2 and 4A4) throughout the study. However, we realize that most, if not all, of the important experiments were done with at least two HP1 DKO clones. For example, we showed chromosome missegregation in unperturbed clones 2A4, 3A2 and 4A4 (Fig 1C). We did live cell imaging analysis of mitosis progression in H2B-GFP-expressing clones 3A2 and 4A4 (Fig 1E-H). We demonstrated metaphase chromosome alignment defects in fixed cells of clones 2A4, 3A2 and 4A4 (Fig 2A and B), as well as the sister-chromatid cohesion defects in clones 2A4 and 3A2 (Fig 2E and F). We visualized by live cell imaging the metaphase chromosome alignment defects in H2B-GFP-expressing clones 2A4 and 3A2 (Fig 2C and D).*
For the rescue experiments, we showed that exogenous expression of HP1α-Flag or HP1γ-Flag (Figs 2G, 3C-F, EV3D and F), or CENP-B-fused HP1α CSD (Fig 3H-J), rescued centromeric cohesion defects in HP1 DKO clone 2A4 cells. We demonstrated that transient expression of CENP-B-fused full-length HP1α or CSD rescued the centromeric cohesion defects in HP1 DKO 3A2 cells (Fig EV3G and H, L-N). Moreover, stable expression of CB-Haspin-N50-GFP was sufficient to maintain proper metaphase chromosome alignment and sister-chromatid cohesion in HP1 DKO clone 3A2 cells (Fig 5F-H). In addition, Wapl inhibition restored proper centromeric cohesion in HP1 DKO clone 3A2 cells (Figs 5I-L, EV5E and F).
We have now carried out additional experiments and further show that transient expression of HP1α-Flag or HP1γ-Flag rescued centromeric cohesion defects in HP1 DKO clone 3A2 cells (Fig EV2F and G; new data). Moreover, centromeric cohesion defects in HP1 DKO clone 3A2 cells were rescued by stable expression of CB-CSD (Fig EV3I-K; new data).
Regardless of all the data obtained from our HP1 DKO cell lines, we have now obtained important new data to support our model. We identified the PxVxL motifs in the N-terminus of Haspin which is required for the interaction of Haspin with HP1α (Figs 4F and G, EV4C and D; new data). When stably expressed in endogenous Haspin KO cells, Haspin-GFP, but not the Haspin-ΔPxVxL-GFP mutant defective in binding HP1, is able to support proper centromeric cohesion (Figs 4K-M; new data). Thus, the interaction with HP1 is important for Haspin to protect centromeric cohesion in mitosis. Moreover, compared to Haspin-GFP, the Haspin-ΔPxVxL-GFP mutant was around 2.2-fold less concentrated at mitotic centromeres (Fig 5D and E; new data). These results indicate that HP1 directly binds Haspin and promotes its centromeric localization to protect centromeric cohesion.

The reference of the Hela cell line used should be mentioned as well.
***Response:** We thank the referee for reminding the reference of the HeLa cell line used in this study. This HeLa cell line was originally the Laboratory of Dr. Jonathan Higgins at Brigham and Women's Hospital, Harvard Medical School. This cell line has been used for the study of mitotic chromosome segregation in a number of publications (Dai J et al., Gene Dev, 2005, Dev Cell, 2006, J Cell Sci, 2009; Wang F et al., Science, 2010, Curr Biol, 2011, J Cell Biol, 2012; Zhou L et al., EMBO Rep, 2014, Curr Biol, 2017; Liang C et al., EMBO Rep, 2018).*

Minor points.
1-Quantification of cohesion loss.

It is stated in the text page 4 that "the percentage of cells with cohesion loss, defined as a cell containing at least 26 separated chromatids". First, this should be moved into the Methods section because here, it is unclear whether it is applicable to this particular figure of to the whole study. Second, why "at least 26 separated chromatids"? Why this cut-off? I assume this is just above the (high) rate of sister-cohesion loss in Hela cells? This should be clarified in the methods.
Response: *We thank for the suggestion. We have now changed the statement in the text to "For example, after 8 h treatment with MG132, the percentage of cells with cohesion loss increased from 5.7% in control cells to 21.3%-27.4% in HP1 DKO cells (Appendix Fig S5)". Regarding the use of at least 26 separated chromatids as the cut-off, as far as we know, there is no "standard" in the literature that we can simply follow. We counted the percentage of separated sister-chromatid using metaphase chromosome spreads prepared from our HeLa cells which were arrested in mitosis after treatment with MG132 for 1 h, 4 h and 8 h. Based on the results (Appendix Fig S5), we have now described in the Materials and Methods section that "Cohesion loss was defined as over 20% of sister-chromatid pairs in a cell were separated. Since the average number of chromosomes in our HeLa cell line is 62.6, a cell was counted as cohesion loss when at least 26 chromatids (13 pairs of sister-chromatid) were separated (Appendix Fig S5)."*

2-Data on HP1 beta KO.
The KO was done and I expected the authors to indicate whether or not chromosome segregation defects were observed. As the authors claimed that HP1gamma was not clearly detectable at centromeres, it was interesting to see whether the KO had consequences on chromosome segregation. In addition, the authors should provide the sequence of the edited locus in clone 2A5, as a reference for future study. FIG EV1: it is difficult to see centromeres. Please provide an enlargement view of the centromere as in Fig1.
Response: *Using antibodies specific for endogenous HP1β, we did not detect its enrichment at mitotic centromere (Fig 1A). To exclude the possibility that this might be due to the quality of the antibody, we transiently expressed EGFP-HP1β in HeLa cells and found the same result (data not shown). To further exclude the possibility that this might be due to the potential pre-occupation of endogenous HP1β at centromeres, we transiently expressed EGFP-HP1β in HeLa cells in which endogenous HP1β was knocked out (Fig EV1A). In all cases, we did not find the enrichment of HP1β at mitotic centromeres in HeLa cells. We found the similar results in RPE-1 cells (Appendix Fig S2).*
As suggested, the genomic DNA sequencing results of the HP1β KO clone 2A5 are now shown in Fig EV1D. We also carried out the suggested experiments and did not find increased rate of chromosome missegregation in asynchronously growing HP1β KO HeLa cells (Fig EV1F). Also as suggested, we have now shown an enlargement view of the centromere as in Fig EV1A.

3- Fig. 1GH. It is stated in the text that "Similar defects in chromosome alignment and segregation were seen in three independent clones of HP1 DKO cells undergoing the same transient mitotic arrest/release procedure (Figures 1G and 1H)". From Fig. 1GH, the data are from 3A2 only.
Response*: Thanks for pointing this out. We realized that clone 4A4 was mis-labeled as 2A4 in the original Fig 1H. We have now corrected this mistake, and have changed the statement in the main text to "Following transient mitotic arrest and release, similar defects in chromosome alignment (Fig 1G) and segregation (Fig 1H) were observed in HP1 DKO clones 3A2 and 4A4, as revealed by live cell imaging".*

2nd Editorial Decision 26 January 2018

Thank you for the submission of your revised manuscript to our editorial offices. We have now received the reports from the two referees that were asked to re-evaluate your study (you will find enclosed below). Original referee #2 was not able to assess the manuscript again. However, going through your point-by-point response, I consider her/his points as sufficiently addressed. As you will see, referees #1 and #3 now support the publication of your study in EMBO reports. However, both have a few remaining points, we ask you to address in a final revised version of the manuscript.

Further, I also have the following editorial requests:

The Appendix needs a TOC with page numbers. Please add this.

Please mention in the legend of Figure 4 that parts of the images in panel A are show up in panel C (or cut versions of C panels are shown in A).

Could you add more information to the legend of Figure 1G/H (number of cells, replicates, statistics).

Could statistics be added to the diagrams shown in Figs. 2A, 2B, 2G, 2H, 3C, 3F, 3H and S1B?

Finally, the movie legends need to be ZIPped together as text file with the movie itself. Please do that, and remove the legends from the main manuscript file.

We now strongly encourage the publication of original source data, in particular of Western blots, with the aim of making primary data more accessible and transparent to the reader. The source data will be published in a separate source data file online along with the accepted manuscript and will be linked to the relevant figure. If you would like to use this opportunity, please submit the source data (for example scans of entire gels or blots, data points of graphs in an excel sheet, additional images, etc.) of your key experiments together with the revised manuscript. Please include size markers for scans of entire gels, label the scans with figure and panel number, and send one PDF file per figure.

I look forward to seeing a revised version of your manuscript when it is ready. Please let me know if you have questions or comments regarding the revision.

REFEREE REPORTS

Referee #1:

The authors have addressed my concerns satisfactorily. I have one minor comment: what is the prominent band on the gel in Figure 4E? Also, Figures 4E and F would benefit from a more thorough description of what was done in the figure legend.

Referee #3:

The authors have addressed in full the points I raised in the first round of review. I have still a point of concern regarding the quantification of cohesion defects.
It is stated that "Cohesion loss was defined as over 20% of sister-chromatid pairs in a cell were separated. Since the average number of chromosomes in our HeLa cell line is 62.6, a cell was counted as cohesion loss when at least 26 chromatids (13 pairs of sister-chromatid) were separated (Appendix Fig S5)."
The use of an arbitrary threshold erases a large part of the data set. As presented the data are misleading as one can conclude that 95% of HeLa cells have no cohesion defect whereas from Fig.S5, we learn that 80% of the control cells have around 7-8 separated sister pairs and apparently not a single cell with no separated pairs (green bars, 8h MG132 treatment). It would be clearer and more rigorous to show the data as they are (side by side the DKO clones and the wt control) in a graph as in Appendix Figure S5.
I think the authors should make this point clearer. I suggest including the data from the DKO clones in the Fig. S5 graph, side by side with the wild-type control so that the reader can easily see that DKO clones have an increase in the frequency of cells with more than 13 separated sister pairs and state that this value was chosen as a threshold to quantify cohesion loss in all experiments.

Minor points
Appendix Figure S5. I think the Y axis should be labelled as "% of cells" (rather than % of cells with separated chromatids).

Figure EV4-D: I think the labelling on the top of the panel is wrong.

The reference of the HeLa cells line: Although mentioned in the rebuttal letter, I could not find it in the revised manuscript.

2nd Revision - authors' response 28 January 2018

Thank you for the submission of your revised manuscript to our editorial offices. We have now received the reports from the two referees that were asked to re-evaluate your study (you will find enclosed below). Original referee #2 was not able to assess the manuscript again. However, going through your point-by-point response, I consider her/his points as sufficiently addressed. As you will see, referees #1 and #3 now support the publication of your study in EMBO reports. However, both have a few remaining points, we ask you to address in a final revised version of the manuscript. Further, I also have the following editorial requests:

The Appendix needs a TOC with page numbers. Please add this.
Response: We have done now as requested.

Please mention in the legend of Figure 4 that parts of the images in panel A are show up in panel C (or cut versions of C panels are shown in A).
Response: Thanks for the reminder. Given that original Fig 4A was also shown in original Fig 4C, we have now removed the original panel A.

Could you add more information to the legend of Figure 1G/H (number of cells, replicates, statistics).
Response: We have done now as requested. Note that these data are from one time-lapse live cell imaging experiment.

Could statistics be added to the diagrams shown in Figs. 2A, 2B, 2G, 2H, 3C, 3F, 3H and S1B?
Response: We can certainly analyze the statistics for the data shown in these figures. However, given the complex display of the data which include three types of chromosome alignment for each time point of the MG132 treatment, it is infeasible to properly show the statistics in the diagrams. We therefore request for opting this out.

Finally, the movie legends need to be ZIPped together as text file with the movie itself. Please do that, and remove the legends from the main manuscript file.
Response: We have done now as requested.

We now strongly encourage the publication of original source data, in particular of Western blots, with the aim of making primary data more accessible and transparent to the reader. The source data will be published in a separate source data file online along with the accepted manuscript and will be linked to the relevant figure. If you would like to use this opportunity, please submit the source data (for example scans of entire gels or blots, data points of graphs in an excel sheet, additional images, etc.) of your key experiments together with the revised manuscript. Please include size markers for scans of entire gels, label the scans with figure and panel number, and send one PDF file per figure.
Response: We have now uploaded the scans of gels and blots for all the Western blots shown in the manuscript.

Referee #1:

The authors have addressed my concerns satisfactorily. I have one minor comment: what is the prominent band on the gel in Figure 4E? Also, Figures 4E and F would benefit from a more thorough description of what was done in the figure legend.
***Response**: Thanks for pointing this out. The prominent band on the gel is the degraded partial proteins of GST-Haspin, which is quite common in our experience for the purification of bacterially expressed recombinant Haspin (see also Figure 1A in Wang F et al., Current Biology, 2011, 21: 1061–1069). We have now mentioned in the legend that "Note the presence of a significant amount of partial GST-Haspin protein", and have added more details in the legends as suggested.*

Referee #3:

The authors have addressed in full the points I raised in the first round of review. I have still a point of concern regarding the quantification of cohesion defects.
It is stated that "Cohesion loss was defined as over 20% of sister-chromatid pairs in a cell were separated. Since the average number of chromosomes in our HeLa cell line is 62.6, a cell was counted as cohesion loss when at least 26 chromatids (13 pairs of sister-chromatid) were separated (Appendix Fig S5)."
The use of an arbitrary threshold erases a large part of the data set. As presented the data are misleading as one can conclude that 95% of HeLa cells have no cohesion defect whereas from Fig.S5, we learn that 80% of the control cells have around 7-8 separated sister pairs and apparently not a single cell with no separated pairs (green bars, 8h MG132 treatment). It would be clearer and more rigorous to show the data as they are (side by side the DKO clones and the wt control) in a graph as in Appendix Figure S5.
I think the authors should make this point clearer. I suggest including the data from the DKO clones in the Fig. S5 graph, side by side with the wild-type control so that the reader can easily see that DKO clones have an increase in the frequency of cells with more than 13 separated sister pairs and state that this value was chosen as a threshold to quantify cohesion loss in all experiments.
***Response**: Thanks for the excellent suggestions. We agree with the potential confusion that may be caused by the original Fig S5, which has now been replaced with a new one that was prepared as suggested. We have now stated in the Materials and Methods section that "The average number of chromosomes in our HeLa cell line is 62.6. Cohesion loss was defined as over 20% sister-chromatid pairs (>25 separated chromatids) in a cell were separated (Appendix Fig S5).*"

Minor points
Appendix Figure S5. I think the Y axis should be labelled as "% of cells" (rather than % of cells with separated chromatids).
***Response**: Thanks for the suggestion. We have now made the correction.*

Figure EV4-D: I think the labelling on the top of the panel is wrong.
***Response**: Thanks for the suggestion. We have now made the correction.*

The reference of the HeLa cells line: Although mentioned in the rebuttal letter, I could not find it in the revised manuscript.
***Response**: Thanks for the suggestion. We have now stated in the Materials and Methods section that "The HeLa cell line, originally from Dr. Jonathan Higgins laboratory at Brigham and Women's Hospital and Harvard Medical School, has been used in a number of studies for the regulation of mitotic chromosome segregation.*"

Acceptance 5 February 2018

I am very pleased to accept your manuscript for publication in the next available issue of EMBO reports. Thank you for your contribution to our journal.

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REFEREE REPORT

Referee #3:

The authors have now addressed in full the questions I raised during the revision process.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Fangwei Wang
Journal Submitted to: EMBO reports
Manuscript Number: EMBOR-2017-45484

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	n= or >3, and following the convention in our field, in all figure legends
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	NA
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	NA
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	NA
For animal studies, include a statement about randomization even if no randomization was used.	NA
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	NA
4.b. For animal studies, include a statement about blinding even if no blinding was done	NA
5. For every figure, are statistical tests justified as appropriate?	Yes, all statistical tests used are described the figure legends.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Yes, Normality tests (Shapiro-Wilk) were performed for experiments.
Is there an estimate of variation within each group of data?	Each group of data is displayed as the mean +/- standard deviation.
Is the variance similar between the groups that are being statistically compared?	Yes, comparison was made between group with simailar variance.

C- Reagents

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http://www.selectagents.gov/

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	Rabbit polyclonal antibodies H3T3ph (B8633 and B8634) Wapl (A300-268A, Bethyl) Cyclin B1 (clone D5C10, Cell Signaling Technology, CST) GFP (A11122, Invitrogen; sc-8334, Santa Cruz) GST (G7781, Sigma) MBP (E8032, New England BioLabs) GAPDH (14C10, CST) Mouse monoclonal antibodies HP1α (MAB3446 for immunoblotting; MAB3584 for immunostaining) HP1β (MAB3448) HP1γ (MAB3450) α-Tubulin (T-6047, Sigma) Myc-tag (4A6, Millipore) 6xHis (GNI4110-HS, GNI) Flag-tag (M2, Sigma) Myc-tag (4A6, Millipore) Sgo1 (3C11, Abnova)
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	HEK-293T and HeLa cells are all originally from ATCC, U2OS-LacO cells, was kindly provided by Dr. David Spector (Cold Spring Harbor Laboratory, USA). These cell lines were routinely tested for mycoplasma contamination.

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	NA
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	NA
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	NA

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	NA
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	NA
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	NA
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	NA

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	NA
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	NA
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	NA

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	NA